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Thermal Generation of Aromas

Thomas H. Parliment, EDITOR
General Foods USA

Robert J. McGorrin, EDITOR
Kraft USA

Chi-Tang Ho, EDITOR
Rutgers, The State University of New Jersey

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Thomas H. Parliment, editor; Robert J. McGorin, editor; Chi-Tang Ho, editor

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Foreword

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Preface

THERMALLY GENERATED AROMAS PLAY AN IMPORTANT ROLE in the formation of flavors in many foods. Thermal processes enhance the palatability of foods that otherwise would be less desirable. In addition to ensuring food safety, thermal processing provides an essential function for flavor development and is routinely exploited by food and beverage manufacturers, flavor suppliers, and consumers.

The purpose of this book is to review aromas that are developed by thermal processes. Because the flavor of heated foods is affected principally by aroma, we focus attention exclusively on the volatile flavor constituents of foods. Moreover, we intend this book to complement the 1986 book, *Biogenesis of Aromas*, ACS Symposium Series 317, edited by Thomas H. Parliment and Rodney Croteau. A broader overview of the Maillard reaction was discussed in the 1983 book, *The Maillard Reaction in Foods and Nutrition*, ACS Symposium Series 215, edited by George R. Waller and Milton S. Feather, which addressed the volatile, nonvolatile, and nutritional aspects of the Maillard reaction.

The symposium upon which this book is based was developed to provide an overview of relevant aroma isolation and identification techniques and to describe recent advances in the thermal, microwave, and extruded generation of aromas. Contributors from academia, government, and industry were carefully chosen to provide different insights and areas of expertise in these fields. Through corporate sponsorship, we were pleased to provide an opportunity for 10 leading international scientists to present their research. Interactions among participants during the symposium helped stimulate numerous discussions and promote useful linkages.

The first two sections of this book provide the reader with a background on the thermal generation of aromas. Included in these sections are perspectives on the regulatory aspects and the analytical methodologies at the forefront of aroma research. Subsequent sections present original research on aromas derived from various food sources. In addition, we have included a section on mechanistic studies to provide insights into aroma formation through thermal decomposition of lipid, carbohydrate, and amino acid precursors. The final section is entirely

devoted to extrusion and microwave-generated aromas. We have attempted through this book to construct a useful reference to researchers in the food and flavor industry.

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THOMAS H. PARLIMENT
General Foods USA
Tarrytown, NY 10591

ROBERT J. MCGORRIN
Kraft USA
Glenview, IL 60025

CHI-TANG HO
Rutgers, The State University of New Jersey
New Brunswick, NJ 08903

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Chapter 1

Thermal Generation of Aromas

An Overview

Thomas H. Parliment

General Foods USA Technical Center, 555 South Broadway,
Tarrytown, NY 10591

Uncooked foods commonly possess only weak aromas and desirable aromas are generated upon cooking. For example, unroasted coffee beans possess a weak green, bean character while raw beef possesses a weak, slightly sweet, and bloody character. Numerous chemical reactions occur upon roasting and the typical, highly desirable aroma of roasted coffee and roasted beef are generated. Large numbers of volatile aroma chemicals are produced during these thermal reactions. For example, as of 1987 there were approximately 750 volatiles identified in roasted coffee and 700 identified in cooked beef. Aromas are generated in foods primarily by three processes:

1. Enzymatic and microbial processes which liberate low molecular weight volatile chemicals. Biological reactions are particularly important in the aromas of fruits and vegetables, berries, fermented dairy products, and alcoholic beverages. A recent ACS Symposium reviewed these processes (1).
2. The second route to aromas involves the production of chemical precursors during a fermentation step. Subsequent heating generates aromas from these biologically-derived precursors. Cocoa and bread aroma are two examples of these types of reactions.
3. The third path to aromas are non-enzymatic processes resulting from thermal treatment such as cooking and roasting. These reactions typically include thermal decomposition of lipids, carbohydrates and proteins, and are responsible for the aroma of foods such as coffee, meat, nuts, cereals, and also contribute to the aromas of heat processed foods and vegetables.

Aromas generated by thermal processes are the subject of this book.

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The Maillard or non-enzymatic browning reaction was first described by L. C. Maillard in 1912 (2) in his classic study covering the reaction of sugars and amino acids. The wide variety of aromas generated in the Maillard Reaction results from the large number of reactants which can combine during the thermal reaction. Hodge et al (3) have classified the flavors produced in this reaction in four groups.

1. Caramelized sugar
2. Roasted, toasted, baked, nutty
3. Corny and amine-like
4. Burnt aromas/bitter taste

A summary of the Maillard Reaction is given in Scheme 1 (4). The first step involves the condensation between the carbonyl group of a reducing sugar, and the free amino group of an amino acid or peptide to produce a N-glycosylamine or fructosylamine. These glucosyl- or fructosyl- amines can rearrange to produce amino-deoxy-aldoses or ketoses via Amadori (from glucose) or Heyns (from fructose) rearrangements. Structures of these two important intermediates are shown in Figure 1.

Quantitatively, the major path of degradation of the Amadori or Heyns intermediate is a dehydration reaction which yields furfural and hydroxymethylfurfural. Of greater flavor significance are the minor pathways which can result in both aromatic products as well as reactive intermediates. These intermediates can undergo a retro-aldolization reaction to produce alpha dicarbonyl compounds, such as pyruvaldehyde and diacetyl as well as reactive monocarbonyls, such as glycolaldehyde and glyceraldehyde.

The variety of aromas which can arise from the Maillard Reaction is large. Lane and Nursten (5) reacted more than 400 mixtures of amino acids and carbohydrates at elevated temperatures. They classified the aromas in 14 groups as shown in Table I.

TABLE I. GROUPING OF AROMA DESCRIPTORS USED

<u>Group</u>	<u>Descriptor(s)</u>
1.	Sweet, boiled sugar, caramel, toffee
2.	Chocolate, cocoa
3.	Bread, crusty, biscuits, cakes, toast
4.	Meaty, beefy
5.	Potato, potato skins, potato crisps
6.	Fruity, aromatic ester
7.	Celery, chicory, leeks, Brussels sprouts, turnips
8.	Puffed Wheat, Sugar Puffs
9.	Nutty
10.	Floral
11.	Ammoniacal
12.	Unpleasant, 'caused coughing'
13.	Aldehydic
14.	Burnt, charred, scorched, acrid, potato crisps, toast, smoky

In addition to the products generated by the Maillard Reaction other routes exist for the formation of aromas via thermal processes.

When lipids are heated a large variety of compounds are formed. Hydrolysis of the ester linkage occurs, liberating glycerol and volatile fatty acids. It is well known that thermal decomposition of triglycerides generates a series of compounds including alkanals, alkenals, alkadienals, methyl ketones, lactones, and hydrocarbons.

Heating of carbohydrates produces a number of aromatic compounds including aldehydes, ketones, and dicarbonyls as well as oxygen containing heterocyclic compounds such as furans, dihydrofuranones, and pyrans through caramelization and dehydration reactions.

Finally, heating of amino acids can produce volatiles including aldehydes, amines and hydrogen sulfide. One minor, but important, flavor generating pathway involves the Strecker degradation of an amino acid as shown in Figure 2. In this reaction, an alpha amino acid reacts with an alpha dicarbonyl at an elevated temperature to produce an aldehyde (one carbon less than the amino acid) as well as an alpha amino ketone. These products can react further to yield important heterocyclic aroma chemicals such as pyrazines, thiazoles, and dihydrofuranones.

ANALYTICAL METHODOLOGY

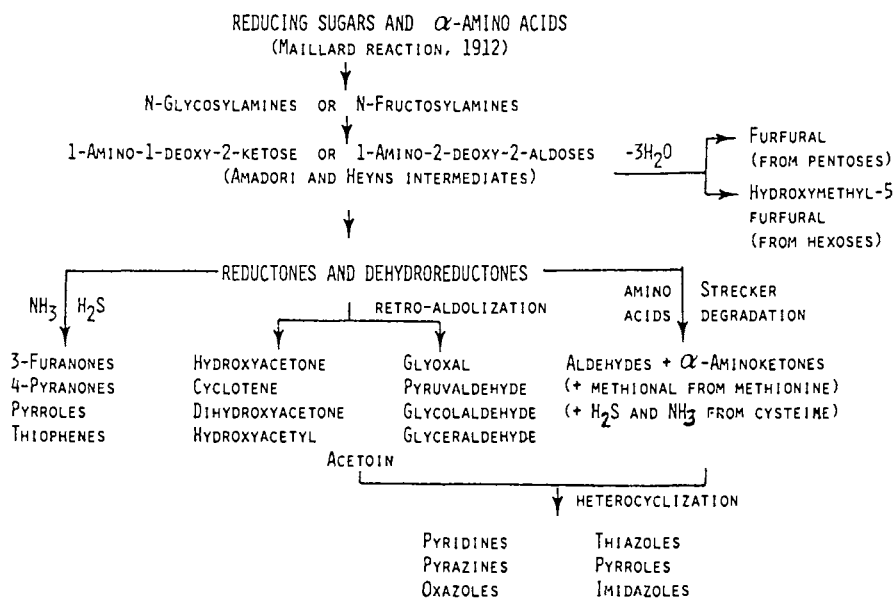
Isolation, concentration, separation, and identification of thermally-derived aromas is an important area. These aromas are frequently associated with other non-volatile products including pigments, fats, carbohydrates, and proteins. Techniques are required to separate the volatiles from the non-volatiles without causing chemical deterioration.

Volatiles in thermally-derived aromas are frequently present at levels of parts per million. It is generally necessary to concentrate the aroma by many orders of magnitude prior to identification.

An isolation and concentration scheme should be appropriate for the sample under investigation. As an example of this, a distillation technique would not be satisfactory for separating caramel aroma compounds such as Maltol, Cyclotene, or Furaneol which possess an enolone structure and do not steam distil.

The great complexity of thermally-derived aromas was previously mentioned. The presence of hundreds of volatile components requires that the separation scheme, typically gas chromatography, possess high resolving power. Fused silica capillary gas chromatographic columns of 0.25, 0.32, or 0.53 mm i.d. are useful since these columns possess 3,000-5,000 plates per meter and since column lengths longer than 100 meters are available. Frequently, aroma chemicals decompose in the presence of hot metallic surfaces. Fused silica columns, in addition to being robust and easy to handle, are inert.

Finally we come to the subject of identification techniques. Historically GC/MS has been useful in the aroma field. As structures of isolated aroma chemicals became more complex, additional techniques, such as GC/FTIR and NMR were required. The first section of this book addresses the important areas of isolation, concentration, and identification.



Scheme I. Formation of heterocyclic compounds in food products. (Reprinted with permission from ref. 4. Copyright 1982 Ellis Horwood Limited.)

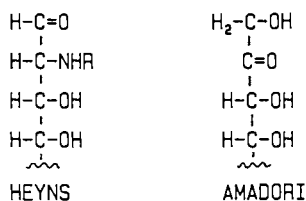


Figure 1. Amadori and Heyns intermediates.



Figure 2. Strecker degradation of an amino acid.

ISOLATION AND IDENTIFICATION STUDIES

There are two procedures which are followed in aroma research. One involves isolation and identification of a food aroma, while the other involves model system studies.

In the first method, volatile compounds are removed from a food, concentrated, separated, and identified. More than 700 coffee volatile chemicals have been identified by this technique, but it can be very laborious and time consuming.

A number of chapters in this book discuss current research involving specific food flavors. Schieberle has presented some provocative work on wheat bread flavors. Bread flavors result from a combination of both biochemical and thermal processes. Yeast metabolism of the bread ferment produces a series of volatile and non-volatile metabolites. These compounds can react during the baking of the bread to produce surface browning, crust formation, and desirable aroma generation. Through the use of aroma extract dilution analysis, Schieberle was able to demonstrate the importance of 2-acetyl-1-pyrroline in bread crust aroma. He related this chemical to the presence of yeast cells in the dough. In the absence of yeast cells, as in chemically leavened bread, only low levels of 2-acetyl-1-pyrroline are generated. Studies such as this suggest procedures to develop higher quality aromas in food products.

Other chapters in this book deal with meat flavors and demonstrate that exciting work continues in this area. Buckholz and Bailey presented two overviews on these topics. Approximately 680 compounds were identified as of 1988, including a large number of heterocyclic compounds possessing meaty aromas. The importance of water soluble, low molecular weight, non-volatile precursors in beef flavor was emphasized. In one experiment, beef was taken, the fat removed, and the lean residue was ground and water extracted. The extract was dialyzed, and the diffusate MW < 15,000 was freeze dried to give a powder. When this material was heated, a good meat aroma was detected. A number of heterocyclic compounds were identified. An interesting comment by Bailey was that these Maillard Reactions are useful for preventing warmed-over flavor, which is a rancid, metallic flavor in beef.

New compound identifications are also presented. Werkhoff et al described studies involving reaction mixtures of cysteine, thiamine, glutamate, and ascorbate. Their study produced a large variety of unique mixed heterocyclic mono- and disulfides possessing roasted and meaty character. Many of these compounds were subsequently identified in actual meat aroma systems.

MODEL SYSTEM FLAVOR STUDIES

The other technique employed in aroma research involves model system studies. In this case, we investigate the volatiles formed when precursors react. Among the advantages of model system studies are:

1. The reaction mixtures are simpler - the number of volatile chemicals produced is smaller than found in a typical roasted food.

2. Significant quantities of product are generated which makes identification easier.
3. One may be able to predict the structure of the products.
4. It can give us insights into reactions which occur in aroma generating reactions.
5. The reaction can be used to produce natural aromas as flavorants when proper ingredients and conditions are chosen.

The topic of Maillard Reactions was the subject of a recent American Chemical Society Symposium (6). The wide variety of reactions that can occur in simple model systems can be extraordinary. For example, Shibamoto commented in this symposium that a simple rhamnose/ $\text{NH}_3/\text{H}_2\text{S}$ reaction mixture generated 1,000 gas chromatographic peaks. A number of presentations in this book cover model systems of various compositions. Some of the mixtures studied in great detail and reported herein include:

Glucose/cysteine	Shibamoto
Serine/glucose	Baltes
Lysine/glucose	Leahy
Proline/rhamnose	Shaw
Cysteine/Furaneol	Shu
Cysteine/ribose/phospholipid	Mottram

Baltes concluded that temperature is a major factor in his reaction system; higher temperatures favor pyrazine formation and reduce furan production. Leahy stressed the effect of A_W , and concluded that maximum pyrazine formation occurs at A_W on the order of 0.75-0.84. The work of Shu related interesting meaty, roasted odors to reaction parameters. The best reactions occurred at 75% water level and a temperature of 160°C. Under these conditions, trithiolanes, thiophenones, and diones predominate. Mottram stressed that the presence of phospholipids in a reaction system increased the amount of meaty character.

In the future, we can expect to see further investigations of model systems, for both knowledge building as well as practical uses.

Model reaction flavor systems have been used in flavors for many years. Four decades ago, Unilever researchers received a patent describing a reaction system which generated a meaty aroma and flavor (7). That invention described the reaction of a monosaccharide with a sulfur containing amino acid in aqueous media. A decade later, Giacino (8) received a patent for a reaction flavor which consisted of a protein hydrolysate and a sulfur containing reagent. The sulfur species was described as either organic or inorganic, and included cysteine, methionine, alkyl mercaptans, hydrogen sulfide, sodium sulfide, etc. The product of these reactions was described as "meat-like." These and many other patents demonstrate the importance of reaction chemistry in the generation of desirable food aromas.

EXTRUSION FLAVORS

Food extruders are devices which accomplish several operations in one apparatus:

1. Mixing
2. Heating
3. Energy transforming
4. Conveying
5. Shaping
6. Reacting

Cooking is accomplished at the same time that the product is shaped and extruded. Generally, moistened starch and/or proteinaceous materials are converted to a thick dough and cooked in the extruder. Cooking is accomplished by steam injection, by steam jackets, and by frictional forces. In the course of cooking extrusion, starch is gelatinized and protein is denatured. Once cooked, the product is forced through a die at the discharge end of the barrel. The product expands and loses moisture due to rapid decrease in pressure. After drying, the extruded product develops a rigid yet porous structure. The subject has been well covered by Harper (9). A typical food extruder is shown in Figure 3.

Currently, many types of extruded food products are produced (9) and include ready to eat (RTE) cereals, snack foods, beverage bases, as well as soft-moist and dry pet foods.

From an aroma viewpoint, the cooker extruder possesses distinct disadvantages. The residence time of product in the barrel is short and ranges from 10 to 120 seconds, and is generally less than 45 seconds. Temperatures may be as high as 180°C but are frequently significantly lower. The pressure may reach 200 psi. Moisture levels in the feed can range from 12% to 55% - a typical moisture level for a RTE cereal is 16%.

As a result of these parameters - short time, modest temperature, and moderate moisture level - the product has a bland taste since the desirable browning reactions do not occur.

Two solutions to this problem have been proposed:

1. Spraying or dusting a flavor on the surface of the product after extrusion.
2. Addition of a flavor precursor system to the ingredients prior to extrusion. After extrusion, the product can be dried and toasted so the desirable flavors to develop.

Several discussions of extruder generated flavors are included in this book. We can expect to have this topic covered in greater depth in the future.

MICROWAVE GENERATED FLAVORS

Microwave flavor generation is an area which has not yet received much attention. It is estimated that microwave oven penetration in US households is now approximately 75%. A few years ago, food processors simply added microwave instructions to their regular line of products. More recently, microwave specific products have arrived and include popcorn, pizza, cake and brownie mixes, stuffing mixes, and main meals. Major food processors will continue to design

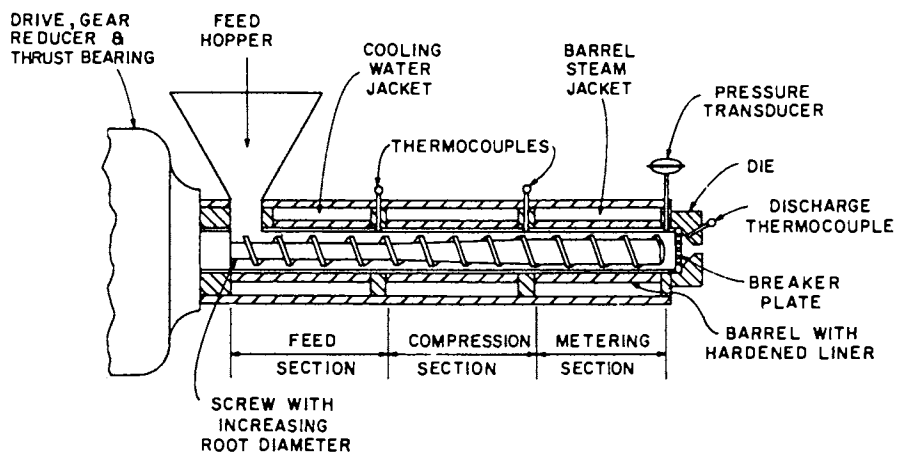


Figure 3. Cross section of a typical food extruder. (Reprinted with permission from ref. 10. Copyright 1978 Institute of Food Technologists.)

microwave-specific products, in an attempt to overcome the perceived limitations of the microwave oven.

Microwave energy is composed of high-frequency electromagnetic radiation which causes heating when the microwaves impinge upon the food. The microwave energy alternates in direction 2,450 million times per second. This rapidly alternating field causes polar molecules, such as water, to attempt to align with the field, and frictional heat is generated.

In a typical convection oven, high temperatures dehydrate the surface of the food, producing drying and desirable Maillard Reactions. In a microwave oven such is not the case; rather, the surface of the food remains cool, frequently no higher than the temperature in the oven cavity. Surface heating and dehydration do not occur, and desirable browning reactions are retarded. In addition, since the microwave energy heats the interior portion of the food rapidly, the total cooking time of a microwave food is reduced compared to a conventionally prepared food, and less time is available for heat generated aroma reactions to occur.

The problem of microwave-derived aromas is just beginning to be addressed by the food industry. Several contributions to this book cover this topic and will open the doors to solving the microwave browning problem.

CONCLUSION

Foods with heat generated aromas have been consumed for eons. The Maillard or non-enzymatic reaction is important in developing the desirable aromas of roasted and baked foods such as coffee, meat, nuts, chocolate and breads. These aromas arise primarily from the reaction of non-volatile carbohydrates and amino-containing groups. The precursors may be present in the food before heating, may be generated by heating, or may arise from biochemical processes. In addition, thermal processes play a role in the development of flavors in cooked fruits and vegetables.

Recent research has identified numerous aroma chemicals in roasted foods, such that hundreds are known. Current work is devoted less to identification of aroma chemicals, but more to identification of specific flavor notes of significant importance, and to maximizing the production of these chemicals.

Two areas where we can expect to see advances made are in extruded flavors and microwave flavors. These two technologies do not lend themselves to browning reactions due to the time/temperature/moisture relationships. Thus these areas remain an area of active research.

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Chapter 2

Progress in the Science of Thermal Generation of Aromas

A Review

Charles H. Manley

Takasago International Corporation (USA), 100 Green Street,
Teterboro, NJ 07806

For many thousands of years man has developed desirable flavors by the use of the art of cooking. Only in the last hundred years has man used heat to create aromas which have been found to be useful specifically as food flavorings. At first the practice related to the hydrolysis of proteins to yield products which contained both flavor and taste enhancing properties. The first person to approach the problem scientifically was Dr. Louis Camille Maillard. The discoveries he made on the non-enzymatic browning reactions of proteins/amino acids with polysaccharides/reducing sugars still bears his name and have been the focus of many research papers and symposia. The Maillard Reaction was, indeed, the chemical platform for producing many of the aromas currently used as meat flavors. During the 1950's and 1960's significant discoveries by natural product chemists contributed further to establishing the science of thermally created aromas. The oxidation of fat, the reactions of sulfur and the degradation of sugars are only a few examples of the chemical mechanisms which are used today in directing precursor material to produce usable aromas.

Written history did not record the advent of the first thermal developed aroma used as a food product. An early cave man probably determined from some accident that food which fell into a fire was more desirable either from an organoleptic aspect, ease of digestion or a higher degree of preservation of the food. Whatever prompted the observation that food materials exposed to heat became more desirable has long been forgotten but the use of cooking has become a basic human experience.

The skillful use of ingredients exposed to the proper amount of heat gave rise to schools of the fine culinary arts.

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The aromas that are developed by the heat induced reactions between components found in the food stuff is, indeed, one of man's unsung major accomplishments.

Over \$120 billion dollars of food stuffs which are thermally treated in one way or another was produced in the USA in 1987. Scientific efforts are commensurate to the economic significance of the products. In the last 15 years over 800 citations covering the Maillard Reaction as well as over 100 citations covering meat and coffee aroma have been recorded by Chemical Abstracts.

Early History.

As we know, the first empirical use of heat to develop aromas came from the great chefs and their skillful use of ingredients and heat. A refinement of the empirical approach no doubt occurred during the war efforts of the 1800's Napoleonic epoch.

Meat extracts satisfied the immediate needs but they became in short supply. A Swiss chemist by the name of Julius Maggi developed a meat type flavoring product based on acid hydrolysis of plant protein. When such materials are neutralized and reduced to paste or powder by heat they acquire a flavor profile useful as a meat extract substitute. Today the market for that product, called Hydrolyzed Vegetable Protein or HVP, is more than \$300 million world wide (1). HVP represents the first modern commercial example of the use of heat to develop a useful material for its use as a flavoring.

The early scientific discoveries relating to heat induced aroma development can be traced to the work of Louis-Camille Maillard at the University of Nancy during the period of 1912 to 1936 (2). He published at least 8 papers on the subject of the reaction of sugars with amino acids. The Maillard Reaction, or so-called, non enzymatic browning reaction chemistry, has become the focus on a great amount of scientific work (3).

The reaction pathways for the Maillard Reactions have been studied and reviewed by many researchers since Dr. Maillard's early work (4-6). These papers give a concise outline of the major chemical pathways identified in the Maillard Reaction Mechanism. In heat treated meat with nearly 75% of those volatiles generated are pyrazines derivatives (7). Those pyrazines have been found to play an important role in developing a roasted flavor in heated products. They will be discussed later.

The initial stages of the Maillard Reaction deals with the condensation of amino acids, peptides or proteins with reducing sugars. The reaction occurs with the application of heat with the formation of an "Amadori compound" (See Chapter 1 of this book).

The Amadori compound from an aldohexose may decompose to the 5-hydroxy methyl-2-furaldehyde through a 1,2 eneaminol. The amino acid may also react, by the Strecker pathway, with a dehydroreductone from a reducing sugar to form an aldehyde or ketone of one carbon less than the amino acid involved in the initial reaction. The Strecker pathway was first proposed in the mid 1800's. The Strecker aldehydes formed from these reactions have been shown to be important flavor compounds (8).

Other pathways have been found to generate aroma compounds associated with heat treatment. These pathways will be reviewed briefly in this paper with implications to overall aroma noted. It is clear that many of the components found in food play important roles in the development of acceptable flavor profiles.

The chemistry of thermogenesis of food aroma can best be reviewed by focusing on those interesting components that have been discovered in popular prepared food products. Needless to say the most significant product to man is meat.

Meat Flavors

From the early works of Bender and Co-workers (9) and that of Hornstein and Crowe (10), it was recognized that water soluble extracts of meat contained some of the precursor material that when heated gave rise to cooked aroma. Their original findings have led to further research and more than 500 research articles and patents. A few review articles are worthy of note (11-13). The heterocyclic chemicals are some of the most interesting of the chemical volatiles .

Heterocyclic compounds. The heterocyclics, particularly sulfur containing ones, are extremely important contributors of roasted meat flavor profiles. For example 4-hydroxy-2,5-dimethyl-3(2H)furanone (Furaneol) and 4-hydroxy-5-methyl-3(2H) furanone (HMF) which were identified as major furanone components in meat (14,15). When these compounds are heated in the presence of hydrogen sulfide they form mercapto-substituted furan and thiophene derivatives. Van den Ouweland and Feer (16), postulated that the replacement of the ring oxygen of the furan compounds by sulfur led to a number of compounds which are significant meat-like aromas. Precursors of these products were identified as ribose-5-phosphate. Other studies indicate that the furanones may also be formed from Amadori products with amine elimination and subsequent dehydration (17-19).

Thiazoles and thiazolines such as 4-methyl-5-(2-hydroxy ethyl)thiazole, 4-ethyl-5-(3-acetoxypropyl) - thiazole, 2 acetyl-2-thiazoline and 2 acetyl-5-propyl-2-thiazoline are of particular note as they have been identified (20-23) and patented for use in meaty-type flavors. These materials, particularly the 2 and 4 acetyl derivatives are very powerful materials with extremely low odor threshold (22,23) values (eg. 1.3 ppb for the 2-Acetyl compound).

The poly-sulfide heterocyclics such as thialdine(5,6-dihydro-2,4,6-trimethyl - 1,3,5 - thiazine) and trithioacetone have been isolated (24,25). Roasted chicken was found to contain 2,4,6 trimethyl-1,3,5 trithiane (26). Trithiane (1,3,5-trithiane) was found to have an odor threshold of 0.04 ppb in water (27). Both the character and strength of these materials make them very important components in the over all flavor profile of roasted products where they occur.

Pyrazine compounds. As mentioned earlier the pyrazines have been found in abundance. As a rule the alkyl derivatives of the pyrazines produce roasted-nut like odor profiles. Fifty pyrazines have been reported to occur in cooked or roasted beef (28).

The odor threshold of the pyrazines range from 0.006 ppb in water for 2-propyl-3-methoxypyrazine (29) to 43 ppm in water for 2,5-dimethyl-3-ethyl pyrazine (30). A excellent review of the pyrazines found in food products has been prepared by Maga (31).

Before leaving our discussion of heterocyclic compounds found in meat, it is of interest to note that investigators have reported that 40% (223/600) of the volatile compounds of processed meat and 10% (18/183) of those volatiles found in cooked chicken (32) are heterocyclic. These heterocyclics represent the major aroma impact components related to basic meat flavor. In the work by Bodrero (33) using response surface methodology in evaluating flavor volatiles, the reaction mixture of hydrogen sulfide with HMF was found to give the highest score related to meat aroma of any of the aroma substances found in cooked meat. Other workers also support this conclusion (34,35).

Lipid decomposition volatiles. Reactions of sugar and amino acids give rise to odor profiles that are, at best, common to all cooked or roasted meats. The water soluble materials extracted from chicken, pork, or beef give reasonably similar meat flavor. To develop a species specific aroma one needs to study the lipid fraction and the volatiles produced from those lipids. The work of Hornstein and Crowe (10) reported that the free fatty acids and carbonyls generated by heating will establish the specific species flavor profiles.

Both intermuscular (depot) lipids and intramuscular (tissue) lipids play important roles. Table I shows the difference in the aldehydes produced from thermally oxidized lipids from meat of different species (36).

Table I. Aldehydes Produced during Heating of Meat

<u>ALDEHYDES</u> (#C)	<u>BEEF</u>	<u>CHICKEN</u>	<u>PORK</u>
C ₅	+	-	-
C ₇	+	+	-
C ₇ (2t 4c)	-	+	+
C ₈	+	-	+
C ₉ (2t 4c)	-	+	+
C ₁₀	-	+	-
C ₁₀ (4c)	-	+	-
C ₁₀ (2t 4c 7c)	-	+	-
C ₁₁	-	-	+
C ₁₁ (2t 5c)	-	+	-
C ₁₂ (2t 6c)	-	+	-
C ₁₂ (2t 6t)	-	+	-
C ₁₃	-	+	-
C ₁₃ (2t)	-	+	-
C ₁₃ (2t 4c)	-	+	-
C ₁₃ (2t 4c 7c)	-	+	-

t = TRANS c = CIS + = PRESENT - = ABSENT

The aldehydes are generated at temperatures as low as 60°C. Of particular note is the oxidation of phospholipids and polyunsaturated fatty acids. It is the oxidative products of arachidonic acid from the phospholipid fraction in chicken, yielding cis-4-decenal, trans-2-cis-5-undecadienal, and trans-2-cis-4-trans-7-tridecatrienal that are responsible for the species identity of chicken. At temperatures of 200' to 300°C the thermal oxidative reactions allow the formation of ketones and lactones as well as aldehydes.

Gamma and delta C₅ to C₁₅ lactones have been identified in beef fat while gamma C₅, gamma C₉ and gamma C₁₂ have been reported in pork (37). Longer chain unsaturated aliphatic monocarbonyl compounds are found in cooked beef aromas (38)

Degradation products of vitamins. Thermal decomposition of certain vitamins is considered another route to the formation of aromas. Of interest are the thermal degradation products of thiamin (Vitamin B₁), which includes thiazoles, thiazolines and 3-mercaptopropanol (39). High amount of thiamin is found in yeast and in pork. Yeast extracts and autolysates of yeast have been used for many years by the food industry as flavor boosters and taste enhancers (40). Over 100 volatile components have been identified in yeast extract including five products of thermally degraded thiamin (formic acid, 2-methylfuran, 2-methyl-3-thiol, 2-methyl thiophene and 2-methyl tetrahydrothiophen-3-one) (41). One interesting compound isolated from a degraded thiamin

is bis (2 - methyl-3-furyl) disulfide (42). This material was found to have an extraordinarily low odor threshold of 2 part in 10^{14} part of water (42).

It is speculated that the reaction product of HMF and hydrogen sulfide as noted earlier is also a major odor compound of yeast extracts (43).

Most published research efforts have been expended in understanding the thermogenesis of aromas in heated meat products, but there are many other food products where the unique chemistry of thermal generation of aroma has been studied.

Chocolate and Cocoa.

One of the world's most popular flavors, chocolate, is a product of both fermentation and roasting. Early studies by Rohan (44-47) pointed out the importance of liberating the precursor materials (amino acid) so that roasting will generate chocolate aroma.

Table II presents examples of some of the free amino acid profiles of unfermented and fermented and roasted cocoa beans (47).

Table II. Some key amino acid profile changes in cocoa beans

Amino Acids	Unfermented	Fermented	Roasted
	(gm/ 100 gm dry bean)		
Leucine	0.45	4.75	2.39
Phenylalanine	0.56	3.36	1.16
Valine	0.57	2.60	1.67
Isoleucine	0.56	1.68	1.26
Proline	0.72	1.97	1.27

Amino acids increase in the order of 1.5 to 10 fold during fermentation. The 7 fold increase of phenylalanine is of particular interest. In the roasting process 65% of the phenylalanine is converted to aroma components, with the major one being 5-methyl-2-phenyl-2-hexenal. That compound is produced via the Strecker degradation of leucine and phenylalanine and the aldol condensation of the two aldehydes produced from the degradation.

Some 350 other volatiles have been identified in chocolate aroma, and about 10% are pyrazines (48-51) and quinoxalines (52). During roasting 49% of the total free amino acids are lost with only 4% of the bound amino acid being lost (53).

Bread and Baked Cereal Products.

Maillard reactions also play an essential role in the development of flavor in one of the most ancient of

man's prepared foods: bread. Research by Hodge and Moser (54) confirmed the contribution of the reaction products of maltol and isomaltol to bread aroma, and other investigators (55-57) have demonstrated the importance of L-proline as a precursor of the bread aroma constituent, 2-acetyl-1-pyrroline. Over 280 compounds have been found in the volatile fraction of wheat bread (58). Further studies on bread aroma will be found in this book.

Coffee.

Coffee represents one of the major beverages consumed in the world. Like cocoa the coffee bean must under-go a fermentation step before the roasting process can develop the fine coffee aroma so cherished by man. Early studies supported the Maillard pathway as the significant producer of volatiles.

More than 650 volatile compounds have been isolated and identified in the aroma of coffee and approximately 260, or 40%, are heterocyclic (59).

Roasted Nuts.

Roasted nuts were one of the first foods in which large amounts and numbers of pyrazines were isolated and identified. The pioneering work on roasted peanuts by Mason (60) in the middle 1960's contributed a great deal to the understanding of the relations of pyrazines, roasting and the development of nut-like flavor. In more recent time some unique pyrazines have been isolated in a number of different roasted nuts(61,62).

Out of 228 compounds found in roasted filberts, 42 were identified as pyrazines (63). Several dihydrocyclopentapyrazines and furyl pyrazines were in that number.

Certainly the occurrence of pyrazines in cooked and roasted foods covers the proverbial "soup to nuts".

Flavor Creation

Chapters that follow will focus on the current state of the science and report the most recent significant findings.

What is the current use of the research results ? Needless to say, the food industry has found use for a great deal of the information. The industry has used the knowledge to control processes and hence to develop better tasting food products. However, one of the most intriguing uses of the information has been in the development of flavorings. The first patent for the use of the Maillard Reaction to create processed flavor, as the Flavor Industry refers to it, was

issued in the late 1950's. It used amino acids, sugars and protein hydrolysates to develop an aroma of value as a flavoring substance (64,65). In the years since then hundreds of companies have developed and patented interesting products. The current annual commercial value of those products can be estimated to be in the range of 300-350 million US dollars.

The common path for flavor development can be seen in Figure 1. Flavors have been created by the thermal processing of foods, ingredients, or by the use of blends of synthetic chemicals and extractives which have been identified as thermogenesis products of foods. Although the scientific knowledge has been growing since the days of Drs. Maillard and Maggi, the practical use of the knowledge is still expanding and of great interest to the Food and Flavor Industry of the world.

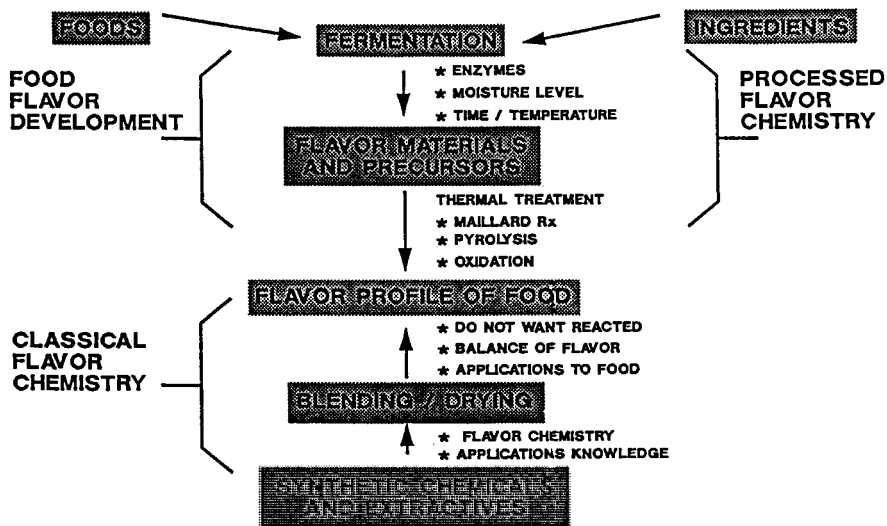


Figure 1. Common path for flavor development.

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Chapter 3

Regulatory Toxicology and Food Flavors

Charles J. Kokoski

Division of Toxicological Review and Evaluation, Center for Food Safety and Applied Nutrition, Food and Drug Administration, 200 C Street, SW, Washington, DC 20204

Flavors constitute perhaps the largest technically functional group of substances and additives used in food. Flavors really cannot be separated from food. This paper will discuss the regulatory toxicology aspects of food safety, with emphasis on the use of flavors in food, and in particular with the recent innovations of flavor product development.

The primary consideration of products intended for human consumption is safety. The Food and Drug Administration (FDA) administers the Food, Drug, and Cosmetic Act, and part of the Act includes the Food Additives Amendment of 1958. This amendment places on the regulated industry the burden of responsibility to demonstrate the safety of food additives. However, for many other ingredients used in food, the burden of demonstrating a lack of safety remains on the government, where it has been for centuries (1). Ideally, we would like everything we eat to be perfectly safe. A point of fact is that since we do not live in an ideal, perfect world, we should not expect our food to be perfect. With everything there are inherent risks. The law, therefore, speaks of reasonable certainty that no harm will result.

Prior to the 1958 Food Additives Amendment, the safety of additives in food was based on a harmless *per se* concept. Regardless of the level of animal testing or exposure, if an additive in food could cause harm or injury, it was considered unsafe. That a substance must be nontoxic at any test level was an absolute and impractical concept. This concept does not recognize that chemicals have thresholds for inducing toxicity.

Section 409 of the Act requires that a food additive be shown to be safe under its intended conditions of use before it is allowed in food. Congress recognized the impossibility of determining with absolute certainty that no harm shall result from the intended use of an additive. Therefore, the legislation did not follow a standard of absolute safety. Congress described the safety standard as follows (2):

"Safety requires proof of a reasonable certainty that no harm will result from the intended use of an additive. It does not -- and cannot -- require proof beyond any possible doubt that no harm will result under any conceivable circumstances...The safety of a given additive involves informed judgement based on educated estimates by scientists of the anticipated ingestion of an additive by man or animals under likely patterns of use."

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In placing the burden of proof of safety through premarket approval on the manufacturer of a new food additive, Congress recognized that it would be impractical to require safety testing of the large number of ingredients that were already in commercial use in 1958 without evidence of adverse health effects. Therefore, Congress specified that "generally recognized as safe" (GRAS) substances were exempt from premarket approval.

According to the food additive procedural regulations established by the FDA (21 CFR 170.30), general recognition of safety may be based only on the views of experts qualified by scientific training and experience to evaluate the safety of substances added to food. This recognition may be based on either 1) a history of safe common use in food prior to 1958, or 2) scientific procedures that demonstrate safety. The concept of general recognition of safety requires common knowledge about the substance throughout the portion of the scientific community that is knowledgeable about the safety of substances added to food.

To affirm GRAS status of a food ingredient on the basis of scientific procedures, the FDA requires that the sponsor provide the same quality and quantity of scientific evidence to establish safety of the intended use as would be required for approval of a food additive. In addition, GRAS status is ordinarily based on published studies which may be corroborated by unpublished information.

FDA regulatory toxicologists evaluate relevant data, including, in large measure, data from animal toxicology testing. The potential for adverse health effects are assessed in order to determine the safety of food additives under conditions of use. Scientific judgment is exercised in determining what specific toxicology studies are recommended to demonstrate the safety of a food additive. The FDA must take into account what is already known about the properties of the additive, its intended conditions of use, and current standards for toxicology testing.

Requirements for Demonstrating Safety of Food Additives

In 1982 the FDA published its guidelines -- Toxicological Principles for the Safety Assessment of Direct Food Additives and Color Additives Used in Food (3). The guidelines in this so-called "Redbook" define a system of recommended tiered testing for additives in food. Not only does the "Redbook" provide testing guidance, it sets out a priority-based ranking scheme for compounds according to level of health concern based on extent of human exposure and assessed or presumptive toxicological effects. In the absence of actual test data, presumptive toxicity also can be anticipated from chemical structure-activity relationships or from the nature of the substance and the known biological activity of substances of the same or similar structural class. This information can provide guidance on how much toxicology testing should be done for certain levels of human exposure.

Tiered Approach to Testing

Determinations of "Levels of Concern" of additives, therefore, are based on:

- Population exposure, and
- Potential toxicity.

Concern levels derived from exposure and chemical structure, are used for determining the amount of toxicology data needed for a direct food additive (Figure 1). The concern levels reflect the potential hazard of specified compounds -- Level I being of least concern, Level II of intermediate concern, and Level III of highest concern. Because there are many exceptions to structure-activity relationships, the FDA gives more weight to exposure data than to chemical structure information in determining the minimum testing level for a food

DEGREE OF CONCERN	STRUCTURE C	STRUCTURE B	STRUCTURE A
HIGHER / \			CL III
		CL III	1.0 ppm
	CL*III 0.25 ppm**	0.5 ppm	CL II
		CL II	0.05 ppm
	CL II 0.0125 ppm	0.025 ppm	CL I
LOWER \ /	CL I	CL I	

* CL = Concern Level

** ppm = parts per million (dietary)

Figure 1. Concern level from exposure and structure for direct food additives. Data from ref. 3.

additive. As seen in Figure 1, there is only a 4-fold difference in breakpoints across structure categories A, B, and C, while there is a 20-fold difference in exposure breakpoints within a given structure category.

Typical Toxicological Test Data Recommended

Substances that fall into Concern Level I require only minimal toxicological data. The recommended tests include:

- A short-term feeding study (usually of 28 days duration) in a rodent, and
- A battery of short-term tests for carcinogenicity potential.

The feeding study is expected to detect acute, life-threatening toxicity and to indicate the target organs and appropriate doses for longer-duration toxicology testing when results of the short-term tests indicate the need.

Concern Level II indicates a need for toxicity tests of intermediate sensitivity, to detect most toxic phenomena other than late-developing histopathological changes. The recommended tests are:

- 90-day feeding study in a rodent,
- 90-day feeding study in a nonrodent,
- 2-generation reproduction study with a teratology phase in a rodent, and
- Battery of short-term tests for carcinogenicity potential.

The short-term tests indicate whether or not chronic testing is necessary. Results of the reproduction study indicate whether there is need for teratology or reproduction testing in more generations or need for a feeding study employing *in utero* exposure.

Direct food additives that fall into Concern Level III require comprehensive, long-term toxicology studies, including:

- Carcinogenicity feeding studies in two rodent species,
- Chronic toxicity feeding study of at least one year in a rodent -- usually undertaken as a combined carcinogenicity/chronic toxicity study,
- Long-term, at least one-year, feeding study in a nonrodent,
- Multigeneration reproduction feeding study in a rodent, carried to a minimum of two generations, with a teratology phase, and
- Short-term tests for carcinogenicity potential to assist in evaluating results of such bioassays.

Although testing in humans is not a requirement for approval of a food additive, availability of human data or experience based on common use in food may reduce the amount of animal safety data that will be required, may signal requirements for special studies, or may influence the safety factor applied in making the ultimate safety evaluation.

What is Involved in the Evaluation of Data Submitted to Demonstrate the Safety of a Food Additive?

First, we recognize that every substance has a toxicity level. Any substance will produce some adverse effect at a high enough test level. Evaluating safety requires that this potential adverse effect be identified and that adequate toxicological data are available to determine the level at which exposure to the substance can be considered safe. This is done by determining a "no-adverse-effect" level in

appropriate animal studies and then applying a safety factor in order to arrive at a level of maximum acceptable daily intake (ADI). The ADI is an official term of the World Health Organization (WHO).

Second, select appropriate dosage levels of test material. Toxicology feeding studies for a food additive should be performed with several dosage levels (usually three) plus a control. The highest dose should be sufficient to stress the animal and ideally produce some toxic response, but not so high as to cause early death and inanition. Multiple dosage levels are used so that a dose-response curve can be constructed. By knowing what the toxic response to a compound is at the high level, one can focus more clearly on the target tissues for a more confident assessment of a "no-adverse-effect" level at the lower test levels.

Third, safety factors are based on a "no-adverse-effect" level. Testing in more than one animal species provides a better reflection of what to expect in the human, and allows extrapolation of safety data from the animal to the human by the use of a safety factor. In determining an ADI for humans, the FDA applies a safety factor to the highest "no-adverse-effect" level determined in an appropriate animal study. The safety factor is intended to account for differences between the animal and human and to provide an adequate margin of safety for the consumer.

Applying Safety Factors

The safety factor itself may vary, depending upon the nature of the test data available and on other judgmental factors. When long-term animal studies are available, the 100-fold safety factor generally is applied. The food additive procedural regulations (21-CFR 170.22) refer to a safety factor of 100 to 1 in applying animal test data to humans. Exceptions to the 100-fold safety factor may be allowed for certain substances and under certain circumstances of use; for example, in the case of micronutrients or macronutrients, or when information on dose-response effects in humans is available.

To estimate the ADI for the human, take the highest amount of tested compound demonstrating a "no-effect" level and divide that by a safety factor (e.g., safety factor of 100), expressed in milligrams of compound per kilogram of body weight of the human. The "no-effect" level may be expressed as mg compound/kg body weight of the animal or as a percentage or ppm (parts per million) of the test diet of the animal. A food additive would be considered safe for its intended use if the probable reasonable human daily exposure to the food additive does not exceed the ADI.

Unique Problems with Evaluating Safety of Flavors

Consideration of flavors used in food presents some unique problems not typically associated with other food additives. For example, there are a vast number -- well over a thousand -- of different substances that are used as ingredients for the single technical purpose of imparting flavor to foods. These, of course, do not include the flavors already naturally occurring in foods themselves. The number of flavor additives alone far exceeds the total number of other direct or intentional food additives used for all other purposes (4).

An ever increasing population, limited supplies, and expensive sources of flavors of natural origin have prompted the chemical and flavor industries to develop many synthetic counterparts. Many synthetic flavors have relatively simple chemical structures and are used in very low amounts. Components of natural foods are being identified chemically in greater numbers today and in many cases are being reproduced synthetically (4). The industry is working hard to develop innovative methods of producing new materials having potential use as

flavors in foods. Evaluating the safety of this vast number of flavoring compounds and substances certainly presents challenges.

It is expected that flavor manufacturers will be making use of new techniques, such as biotechnology, to create new and novel compounds or, alternatively, to find more efficient ways to produce existing compounds. "FDA intends to review new methods of manufacturing for biotechnology products by the same criteria used for products produced by traditional means." (5). This symposium deals with the technological aspects of generating new flavor materials by applying various thermal processes to substances, many of which are commonly found in food. In any safety evaluation of such additives, it is necessary to consider changes in chemical identity, new or altered levels of impurities, and increases in dietary exposure. In this regard, it is possible that even GRAS compounds produced by these new techniques might be considered for evaluation. If the FDA does not agree that the food ingredient manufactured by a new process is GRAS, the ingredient would be subject to the food additive provisions of section 409 of the Act (5).

The law does not preclude an ingredient produced by a novel method from being affirmed as GRAS. "However, to be affirmed as GRAS, the food ingredient must be shown to meet the criteria of a GRAS food ingredient, including a wide recognition of safety based largely on published information concerning the intended use of the ingredient." (5).

Information Required for GRAS Affirmation by the FDA

The information required for both food additive and GRAS affirmation petitions is described in detail in the Code of Federal Regulations (21 CFR 171.1 and 170.35, respectively). It considers the following general areas:

- identity of the ingredient and impurities that may be present,
- safety --- toxicological and/or relevant data to support safety under conditions of use,
- functionality in food --- to show that it serves its intended technical effect,
- analytical method --- to identify and quantify the ingredient in food --- is needed when a tolerance is required to assure safety in use, and
- potential environmental impact of its manufacture and use.

To assess safety even for novel food ingredients or products, such as those from new biotechnology, the agency believes that new laws or regulations are not necessary (5). The Food, Drug, and Cosmetic Act states that GRAS substances are exempt from the premarketing clearance required for food additives. The CFR defines GRAS as the general recognition of safety by experts qualified by scientific training and experience to evaluate the safety of substances on the basis of scientific data derived from the published literature. The CFR also indicates that expert judgment is to be based on the evaluation of results of credible toxicological testing for those substances used in food prior to January 1, 1958, and on a reasoned judgment founded in experience with common food use. The evaluation is to take into account reasonably anticipated patterns of consumption, cumulative effects in the diet, and safety factors appropriate for the utilization of animal experimental data (6,7).

Recognizing the possibility of potential hazard, the FDA studied the toxicity of about 200 of the approximately 1100 flavoring substances in use at that time (8,9). Those selected were most widely used and had structures similar to compounds of known toxicity or were suspected of being toxic. The various oral feeding studies were done in several species for acute to short-term (90 to 120 days) or for chronic testing periods. These studies in part served as a basis for FDA listing a number of

the FEMA (The Flavor and Extract Manufacturers Association) GRAS flavors in the CFR. At that time, a 2000-fold margin of safety, with no other evidence of untoward effects, resulted in permitted use. In many cases, FDA sanction was made without such testing, but was based on reasoned judgment from knowledge of natural occurrence, data on metabolism, and low self-limited use (6). The FDA lists of flavoring substances published in the CFR either on the GRAS list or by food additive regulation have remained essentially unchanged since 1965.

FEMA Approach to GRAS Affirmation Through Expert Panel Review

The Code of Federal Regulations does not identify all GRAS substances such as natural and synthetic flavorings used in foods. The statutes do not specifically state how recognition of safety is to be established or who the qualified experts are to be. The FEMA interpreted the statutes as allowing creation of expert panels outside the FDA or other agencies. On this basis, the FEMA in 1958 selected an Expert Panel to review flavoring materials used as food ingredients (4). Since 1958, the FEMA Expert Panel has periodically surveyed manufacturers and users to obtain information on identity, quantities produced, levels of use, manner of use, and toxicological data related to natural and synthetic flavoring substances. The FEMA Expert Panel developed criteria for evaluating the flavors in an evolutionary manner (6). From this process, the FEMA established a GRAS list of flavors which is continually updated by the FEMA and to which the FDA has not voiced objection.

The FDA, under contract with the National Academy of Sciences (NAS), also monitors market disappearance of food additives, including flavors that the FDA regulates. This is done by surveys of manufacturers. Presently these surveys take place every five years.

A cogent point in the FEMA evaluation process is that relatively small amounts of flavoring substances are generally added to foods. It was reported from 1971 FEMA survey information that 73% of the flavors were used at average levels of less than 100 ppm in food (6). Any one flavoring substance is not used in all foods. Therefore, the overall daily dietary exposure is well diluted. For example, the survey indicated that 71% of the flavors are used industry-wide in amounts of less than 1000 pounds per year. It was estimated earlier that the total quantity of flavoring substances consumed was about 26 grams per person per year (4). This estimate made in 1968 was based on approximately 1100 flavor additives that were used in food. This, of course, excluded flavoring already naturally present in food or generated during cooking.

The guidelines of the FEMA Expert Panel were similar to those developed in 1969 by the Food Protection Committee, NAS National Research Council (10). The NAS expressed the opinion that 1) if a chemical was in use five years or more without evidence of harm, 2) was not a heavy metal, and 3) was not intended for use because of biological activity, dietary exposure at less than 0.1 ppm could be considered toxicologically insignificant. At levels above 0.1 ppm and below 1 ppm in the human diet, use of accumulated scientific evidence and structure analogy to other chemicals of known metabolism or toxicity would be justifiable to arrive at a conclusion of toxicological insignificance.

The FEMA Expert Panel used the following criteria in reaching decisions on the flavoring substances it considered:

- analogy with chemically-related substances with known toxicity or metabolism,
- the nature, level, and volume of use in food,
- toxicological significance of the levels of use,
- available toxicity data,
- metabolic pattern of the compound in the body, and
- natural occurrence of the substance in foods.

Much emphasis was placed on natural occurrence in foods in presumptions of safety. Of course, natural occurrence is not an assurance of safety. Many naturally occurring substances possess toxicity; in fact, some are quite toxic. Chemical analogy also presents some problems in presumptions of safety.

As mentioned earlier, the "Redbook" decision-tree system for determining toxicological testing of food additives falls into three levels of concern (3,11), whereas there are four FEMA concern levels, each determined by combining information about the human exposure levels to a compound with the information about its chemical structure derived from a 33-question "decision tree" (12).

WHO Consideration of Flavors

The Codex Committee on Food Additives of the WHO/FAO has for many years given "temporary endorsement" to a large number of substances, particularly the flavor materials on Codex Lists B1 and B2. This was done for one of the following reasons: the Joint Expert Committee on Food Additives (JECFA) did not allocate ADI levels mainly because of lack of "adequate" classical toxicological or specification data (these were placed on list B1), or the materials were never evaluated by JECFA (these were placed on list B2).

Recently the WHO Codex Committee has been working toward devising a system for priority ranking of the many flavor compounds in order to consider what toxicological data are available and to resolve the long standing "temporary endorsements." The Committee will consider, among others, the FDA "Redbook" process, including the three levels of concern, as well as the FEMA four-concern-level decision-tree approach. The goal is to devise a priority-level ranking system for decisions on final endorsement of ADIs. As yet, no final agreement on the use of any specific approach has been reached by the WHO Codex Committee.

Summary

In summary, safety evaluations of the more than one thousand flavors substances currently in use, and of the many others under development or yet to be envisioned, present unique challenges to the toxicologists not only in industry but also in regulatory agencies. Different approaches have been devised for setting priorities and using available information to make decisions on safe use in food, and for undertaking additional testing when indicated to demonstrate safety under conditions of use. In the regulatory scene under the current food additive law, the approaches of GRAS affirmation and food additive regulation assure safe use of substances added to food.

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Chapter 4

Thermal Decomposition of Carbohydrates

An Overview

Joseph A. Maga

Department of Food Science and Human Nutrition, Colorado State
University, Fort Collins, CO 80523

Carbohydrates ranging from cellulose to simple sugars are subject to thermal alteration. Factors such as temperature, pH, compound concentration, and other reactants present can alter both the rate and complexity of decomposition reactions. Carbohydrate types are reviewed relative to degradation/caramelization pathways and endproducts. Some of the resulting typical food flavors produced are also discussed.

Frequently, simple carbohydrates are considered as a source for sweet pleasant tastes, and complex carbohydrates, such as starch, as thickeners. In actuality, both simple and complex carbohydrates can be heat processed either in the dry or liquid state, alone or in combination with other reactants, to produce a vast array of aromas and flavors. Thus, carbohydrates serve as flavor precursors/components in the commercial formulation of processed foods.

Perhaps one of the most fascinating and complex chemical reactions involving carbohydrates is caramelization. For example, granulated sugar heated at a high temperature in the dry state eventually produces a dark, viscous mass which has a strong and characteristic flavor totally different from the sweet taste of sucrose.

If a nitrogen source is added, or if residual nitrogen-containing compounds are present, classical non-enzymatic browning (Maillard) along with caramelization can occur. Although the Maillard reaction is not the major emphasis of this review, it is difficult not to include it in discussions of carbohydrates and their degradation products.

Throughout the years, flavor chemists have been deeply interested in the thermal reaction of various forms of carbohydrates due to the formation of both desirable and undesirable flavors and colors. Previous reviews covering specific carbohydrates are

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available (1-5). The current review provides an overview of the thermal degradation of carbohydrates ranging from monosaccharides to cellulose.

Caramelization

Caramelization can occur under both acidic and basic conditions. Acids promote dehydration reactions resulting in the formation of furfural derivatives while alkalies favor isomerization and fragmentation reactions (6).

With acid degradation, the first step appears to involve the formation of 1,2-enols from the aldose or ketose (7), followed by a series of dehydration reactions resulting in the formation of 5-hydroxymethyl-2-furfuraldehyde. If the initial sugar is a pentose, the final product is 2-furfuraldehyde.

The resulting furfurals then can undergo a series of complicated polymerization reactions. Hodge (8) reported that these reactions include hydrolytic fission, fission of 2-ketoses, dehydration of triose, dismutation of biose, trioses, and tetroses, self- and cross-condensations of aldehydes and ketones, reversion of aldoses and ketoses to various oligosaccharides, dimerization of monosaccharides, cyclodehydration of aldoses followed by polymerization, and finally, the enolization and dehydration of formed oligosaccharides.

With alkaline degradation, the formation of 1,2-enols is also the initial step. This reaction in turn can produce three-carbon compounds which yield a series of intramolecular reactions involving condensation and polymerization. Both acidic and alkaline caramelization produce numerous volatile and nonvolatile compounds that significantly contribute to aroma, taste, and color.

Thermal Decomposition of Monosaccharides

The thermal degradation products of glucose and fructose have been extensively investigated (9-19). It can generally be concluded that both produce varying quantities of similar compounds.

Heyns et al. (9) have conducted one of the most extensive studies utilizing glucose that was pyrolyzed at 300°C for four hours or at 500°C for three hours under nitrogen. Approximately 130 compounds were observed. They found that the higher pyrolysis temperature resulted in the formation of aromatic hydrocarbons. Other compound classes identified included aliphatic aldehydes and ketones, furans and oxygenated furans, alcohols, lactones, volatile and nonvolatile acids, and oligosaccharides.

Thermal Decomposition of Disaccharides

The major disaccharides that have been evaluated are sucrose (glucose and fructose) and lactose (glucose and galactose). However, from the literature it becomes apparent that temperature and the degree of moisture, if any, associated with the disaccharide

can significantly influence the types and amounts of observed degradation products. The reaction time is also important since many degradation products serve nicely as intermediates, and thus, if the study is conducted for a long period of time, certain intermediates may have already been consumed.

Another key factor to consider is pH since both acidic and alkaline degradation reactions can occur.

Sucrose. Most thermal decomposition studies involving sucrose have been in aqueous models which usually result in the production of acids. For example, Kharin and Palash (20) found that when a 0.5 M aqueous sucrose solution was heated at 90°C for 70 hours, the pH had dropped to 3.93 and that approximately 0.49 mole per liter of sucrose had decomposed. The hydrogen ion source was water, sucrose, and/or its degradation products. It has been shown (21) that the acid hydrolysis of sucrose is bimolecular and that a linear relationship is apparent between the rate constant and hydrogen ion concentration. During the normal processing of cane sugar, natural acid hydrolysis is controlled by the addition of lime to raise pH. However, this in turn can cause reducing sugars that already may be present to undergo alkaline degradation resulting in the formation of acids and brown polymeric products (22).

Color formation during aqueous sucrose degradation appears to be temperature dependent since colored compounds are produced rapidly at 130°C. Color formation is also increased with the presence of impurities such as amino acids (23).

Early researchers (24,25) identified 5-hydroxymethylfurfural from sucrose solutions heated above 150°C. Later reports (26,27) listed a series of aliphatic and aromatic carbonyl compounds identical to those associated with the thermal degradation of monosaccharides.

In an effort to eliminate the role of water during the aqueous thermal degradation of sucrose, Poncini (28), citing unpublished data, has proposed that in dimethyl sulfoxide, sucrose degradation begins with an initial scission of the glycosidic linkage to produce α -D-glucopyranose and a fructose carbonium cation intermediate. This cation can react with an alcohol to produce anomeric fructofuranosides, can internally cyclize to form 2,6-anhydro- β -D-fructofuranose, or can undergo complex but less specific types of degradation to yield hydroxymethylfurfural.

Richards (4) utilized a sucrose melt along with relatively pure crystalline sucrose and attempted to follow degradation as influenced by temperature and the addition of glucose, sodium chloride, and sodium carbonate. He found that crystalline sucrose was stable for several hours at 150°C, but a melt state degraded into glucose, fructose, and trisaccharides quite rapidly. Both glucose and sodium chloride additions were found to enhance degradation whereas sodium carbonate stabilized the melt. He also reported that α -D-glucopyranose was an initial degradation product of sucrose melt at 190°C which eventually anomerized. This first degradation step was very sensitive to catalysis by protonation of the glycosidic oxygen. The fructose carbocation was lost rapidly

by several pathways. It may cyclize to form an anhydride, add a hydroxyl ion to produce fructose, it may aggregate to a trisaccharide or it may undergo non-specific degradations to generate a wide variety of products.

Lactose. Due to its reactivity in dairy products, many researchers have investigated the thermal degradation of lactose (29-36). For example, Hohno and Adachi (31) were able to identify the disaccharides 3-O- β -D-galactopyranosyl-D-glucose, lactulose, 6-O- β -D-galactopyranosyl-D-glucose, and 6-O- β -D-galactopyranosyl-D-galactose along with unreacted lactose, glucose, and galactose when dried -lactose hydrate was heated at 170°C for two hours in a solid state or at 200°C for two hours in a molten state. The molten state produced about four times more monosaccharides than solid state pyrolysis. Based on the relative amounts of disaccharides found, they concluded that lactose had undergone aldose-ketose isomerization, hydrolysis, and condensation of the resulting monosaccharides in the primary stage with no apparent cleavage of the carbohydrate carbon skeleton.

Anhydro sugars have also been identified as pyrolysis products of lactose. These, in turn, can act as intermediates to form other polymers. For example, Urashima et al. (34) have identified 1,6-anhydro-3,4-O-[5-hydroxymethyl-2-furfurylidene]- β -D-galactopyranose which is derived from the reaction of levogalactosan and hydroxymethylfurfural which in turn were formed from lactose and its breakdown product galactose. They also isolated the furfurylidene derivative 1,6-anhydro-3,4-O-furfurylidene- β -D-galactopyranose in both *cis* and *trans* forms. Lactosan (4-O- β -D-galactopyranosyl-1,6-anhydro- β -D-glucopyranose) was found in γ -lactose anhydride that was heated at 180°C for five hours or at 150°C for ten hours under reduced pressure (36). The authors concluded that lactosan may be a precursor of 1,6-anhydroglucose and that it may also polymerize during the heating of lactose.

Another lactose heat-derived compound is lactulose (4-O- β -D-galactopyranosyl-D-fructofuranose) which was first reported by Montgomery and Hudson (37). This compound can be found in heated milk products, heat processed lactose-containing foods, and alkaline lactose solutions via the isomerization of lactose. It is of interest due to claimed beneficial effects on the gut flora of infants. It has also been proposed as an indicator of the severity of heat treatment in processed dairy products (5). Apparently, it can be present in two forms in heated milks, namely in free solution and covalently bound to protein amino groups.

It has been postulated (37) that lactulose is formed from lactose by the Lobry de Bruyn and Alberda van Ekenstein transformation, whereby glucose is isomerized to fructose via an enol intermediate. In turn, two mechanisms have been proposed for the degradation of this intermediate (38). One involves the addition of a proton to the enediol resulting in epimeric aldoses and the original ketose, while the other involves β -elimination to yield galactose and saccharinic acids. The authors' experimental data would tend to better support the second pathway.

From a theoretical standpoint, there are five possible lactulose isomers with the fructose portion providing α - or β -

pyranose, α - or β -furanose or an open chain. However, the β -furanose form seems to predominate.

Thermal Decomposition of Oligosaccharides

The pyrolysis of oligosaccharides such as cellulose and related products has provided a good understanding of how complex carbohydrates degrade thermally (39-49). Interestingly, most of this research was centered around the development of flame retardants for building materials.

Glassner and Pierce (39) verified the belief that levoglucosan is the intermediate through which cellulose is thermally degraded. Byrne et al. (41) concluded that levoglucosan resulted from the rearrangement of hexose units due to breakage of glycosidic bonds in cellulose. Kato and Komarita (47) reported that aside from the breakage of glycosidic bonds simultaneous chemical changes in anhydroglucose units also occurred.

Shafizadeh and Lai (48) have summarized the degradation of oligosaccharides as arising by three types of thermal transformations. At low temperatures, dehydration and melting primarily occur. At temperatures above the carbohydrate melting point, free sugars are produced which can anomerize and participate in inter- and intra-molecular condensations; glycoside and anhydro sugar intermediates can also be generated, which yield inter- and intra-molecular transglycosylation products. The consequence of higher temperatures is thermal decomposition of the sugar moiety. Stahl and Herting (50) demonstrated that cellulose pyrolysis temperature influenced the number of volatiles produced with the higher the temperature, the greater the number of compounds.

Thermally Produced Sugar-Based Foods

Various sugar-containing foods exist, some of which, such as honey, do not normally receive heat processing. However, others can undergo extensive heat treatment, usually in an acid medium, to yield numerous flavoring compounds along with color. Some of these resulting compounds can be derived from the thermal degradation and rearrangement of the carbohydrate moiety itself, whereas a far greater number are formed via the classical Maillard reaction. At this point, some of the more well-known food aromas produced by the thermal degradation of sugars will be discussed.

Molasses. A large number of volatile and nonvolatile compounds have been identified in the flavor fractions of various types of molasses (51-62). Compound classes identified include aliphatic and aromatic acids, aldehydes, phenols, lactones, amines, esters, furans, pyrazines, and sulfides. Most of these compounds can arise from carbohydrate degradation through a number of traditional pathways especially because residual nitrogen-containing sources are present.

Godshall et al. (57) have concluded that typical molasses aroma is composed of two fractions. One is a sweet component that can arise from compounds such as diacetyl and other aldehydes while the other is a strong grassy or green note which they attribute to

dimethyl sulfide. Other potentially important compounds associated with molasses flavor include massoialactone (54), vanillin (56), pyrazines (58), phenols (60), and sotolon (59,60).

Caramel Coloring. These products are manufactured using sugar caramelization techniques in the presence of an acid base and sodium chloride, producing coloring material ranging from very dark brown to light yellow. A typical range of carbohydrate and carbohydrate/nitrogen thermally induced compounds have been reported (63-69).

Since the compound hydroxymethylfurfural is a major product of ketohexose dehydration, its presence can be used as an indication that caramel has been added as a coloring agent in a nonheated food system (66). In contrast, ketopentose dehydration produces furfural. Another dehydration product, bis-5,5'-formylfurfuryl ether, can also be used to confirm the addition of caramel color (66).

Recently, Patey et al. (69) identified three hydroxypyridines and seven hydroxypyrazines from fifteen different caramel colorings.

Ryu and Lee (67) evaluated the role of catalysts on caramel color formation and reported that optimum color was formed at pH 9 in the presence of 0.4% ammonium carbonate and either 0.8% glycine or 0.4% lysine.

Maple Syrup. The typical flavor of maple syrup which originates during the heat concentration of maple sap in the presence of air has also been extensively investigated (70-80). Important sugar-based degradation products present in maple syrup include 3-methylcyclopentane-1,2-dione and 2,5-dimethyl-4-hydroxy-3(2H)-furanone.

It should also be noted that maple syrup contains high molecular weight polysaccharides (80) which probably are also partially degraded during processing and thus can serve as sources for additional flavor compounds.

Conclusions

The thermal degradation of simple and complex carbohydrates either alone or in combination with inorganic or organic catalysts as influenced by pH, time, and temperature can result in a wide array of flavor and color compounds. Many of the important compounds have been identified. However, a better understanding of the numerous chemical reactions should result in the identification and production of even more potent flavoring compounds.

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Chapter 5

Isolation of Thermally Generated Aromas

Sara J. Risch and Gary A. Reineccius

Department of Food Science and Nutrition, University of Minnesota,
St. Paul, MN 55108

The analysis of thermally generated aromas by gas chromatography or gas chromatography/mass spectrometry generally requires that the volatile or aroma compounds be isolated from a food or model system matrix. Many of the techniques that have been used for the isolation of flavors in general are applicable for the isolation of thermally generated aromas. These include solvent extraction, steam distillation, simultaneous steam distillation-solvent extraction, liquid-liquid extraction (dialysis) and both static and dynamic headspace methods. An overview of these methods will be presented with an emphasis on new developments and new applications. Some new developments include a microwave desorption system for dynamic headspace concentration and supercritical fluid extraction. These newer methods are reviewed and the challenges that remain in quantitatively and qualitatively isolating aromas are discussed.

Numerous reviews have appeared in the literature dealing with the isolation of volatile compounds from foods (1-6). Therefore, it appears of greater value to present some of the more recent developments in this area rather than repeat what is currently already reviewed.

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Dynamic Headspace Techniques

The most popular methods for flavor isolation are dynamic headspace concentration and distillation/extraction (1). The dynamic headspace method started with simply passing an inert gas through the sample, collecting stripped organic volatile constituents and water in a cold trap (7). This technique generally required extracting any trapped water with an organic solvent such as diethyl ether or dichloromethane. In an effort to simplify this technique, investigators chose to pass the purge volatiles through a trap which was packed with a material such as Tenax (8,9). Tenax has a weak affinity for water but readily binds organic volatiles. Thus Tenax could be loaded with volatiles and then thermally desorbed to recover the trapped aroma compounds. Since this early development, numerous innovations in methodology have occurred. It is of interest that today the loaded Tenax traps are most often desorbed of volatiles through solvent extraction rather than thermal desorption (10). In the past, the loaded trap was placed in the carrier flow path of the gas chromatograph (GC) and then the trap was heated to desorb the volatiles. The desorbed volatiles would be sent entirely into the GC for analysis. One can readily see disadvantages in the fact that the trap can only slowly be heated (i.e., desorbed) and thus the chromatographic injection is too slow to permit good chromatography of the highly volatile substances. The solution was to cold trap the desorbed volatiles thereby giving excellent chromatography and sensitivity. The disadvantage was, of course, time. A second disadvantage is that the entire sample is sent to the GC, i.e., there is no opportunity for a second analysis of this isolate. Solvent desorption of the loaded traps tends to overcome these two limitations plus permits the use of larger traps, thereby giving greater quantities of sample for repeated analyses.

Simultaneous Distillation/Adsorption

Recently, researchers have published work investigating the different ways to load the trap, different types of trapping, and techniques to unload the traps. In terms of loading the trap, Sugisawa et al. (11) have reported on a technique involving simultaneous distillation and adsorption (Figure 1). In this technique, the sample was placed in a three necked flask, heated to 60-70°C and then steam was introduced to the sample. The distillate was passed through the trapping material (10 mg charcoal) and its flow controlled by the stopcock. This distillation was continued for 30 min and the trap was dried by passing clean nitrogen gas through it. The loaded trap material was extracted with a small volume of solvent (ca. 0.01 ml of dichloromethane or carbon disulfide) for qualitative work while quantitative work involved five extractions with a larger volume of solvent (ca. 1.0 to 2.0 ml each time). Recoveries of model compounds from aqueous solution at 10 ppm ranged from 23-100%.

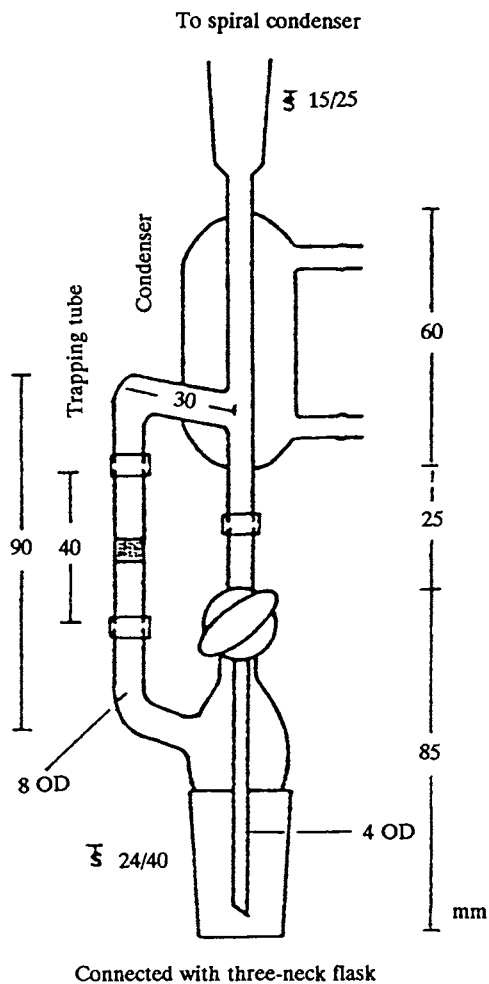


Figure 1. Apparatus for simultaneous distillation/adsorption. (Reprinted with permission from ref. 11. Copyright 1984 de Gruyter.)

Sugisawa et al. (11) demonstrated that this technique could also be used with Tenax as the adsorbent. In this situation, they chose to thermally desorb the Tenax into the GC carrier gas flow. While no quantitative data were provided using Tenax, the authors were quite optimistic about this methodology.

Cold Trapping of Volatiles

Badings and DeJong (12) have chosen a different approach to trap purged volatiles. In this method, stripped aroma compounds are passed through a very small diameter cold trap. In order to minimize trap blockage by frozen water, an initial cold condenser was put in-line. Cold trapping efficiency and thermal desorption were optimized (13). Using this technique, quite good sensitivity and precision have been demonstrated. A major advantage of this technique is the use of cold trapping for sample isolation. This methodology offers the greatest opportunity for the collection of all volatiles (a -100°C cold trap is quite effective), contributes virtually no artifacts, and will readily liberate the trapped volatiles by heating. The primary limitation to this method is that trapping time is restricted due to water freezing in the cold trap. Sample purging may be limited to only 10-20 min which then may limit sensitivity.

Microwave Desorption Techniques

A final innovation in dynamic headspace methodology involves trapping the purged volatiles on a charcoal based trapping material and then desorbing the volatiles using microwave heating (Figure 2). Rapid desorption is the major advantage of this technique. Volatiles are typically desorbed in 4-5 seconds. This rapid desorption negates the need for any cryofocusing of the desorbed volatiles, thereby saving time, decreasing cost, and permitting optimization of chromatography without concern for cryofocusing. The major disadvantages of this technique are a concern for artifact formation on the charcoal based material and trap to trap reproducibility.

Static Headspace

The advent of automated static headspace analysis systems has offered some new opportunities in aroma research via this technique. Wylie (15, 16) has presented some modifications in the Hewlett Packard commercial system hardware as well as operating procedures which have vastly improved the sensitivity of the automated system. Wylie (15, 16) has documented improvements in sensitivity for the analysis of priority pollutants in water using the following modifications: 1. Changing from a 1 ml sample loop to a 3 ml loop; 2. Salting out volatiles by adding inorganic salts. 3. High sample equilibration temperature (ca. 90°C). While artifact formation often limits sample temperature, this parameter substantially influences sensitivity; 4. Cryofocusing of volatiles, thereby permitting multiple injections from the

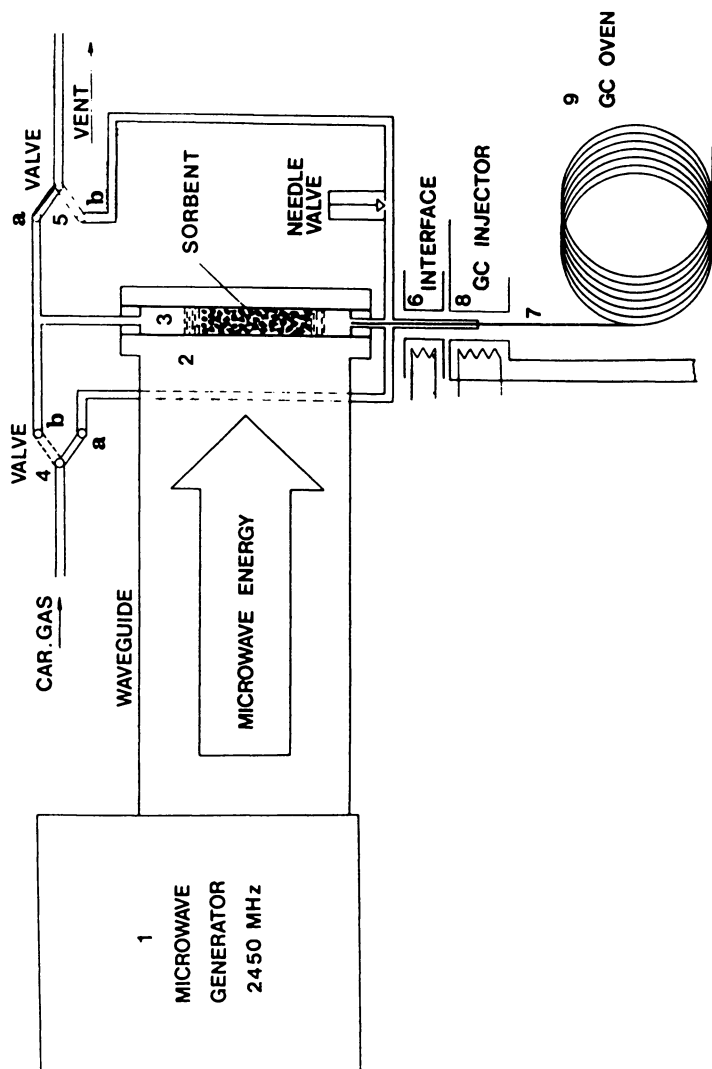


Figure 2. Schematic of a microwave desorption system. (Reprinted with permission from ref. 14. Copyright 1983 Elsevier.)

same sample vial or pooling of injections from different sample vials. Cryofocusing must be efficient, however, or the most volatile substances may break through the trap and give multiple peaks and 5. using splitless injection and cryofocusing to improve chromatography. Wylie (16) demonstrated that static headspace techniques modified as he suggested will approach the sensitivity of the more tedious and less reproducible method of dynamic headspace sampling. While the sample composition may prevent the use of some of the modifications suggested by Wylie (15, 16), other modifications can be used to advantage.

Simultaneous Distillation/Extraction

As was mentioned earlier, distillation and subsequent solvent extraction remains popular in the aroma research area (1). In this method for aroma analysis, the Likens-Nickerson apparatus has been a standard for over 20 years (17, 18). The primary limitation of the Likens-Nickerson distillation/ extraction procedure has been its operation at reduced pressure. It is desirable to operate the system under vacuum in order to reduce the sample boiling point to minimize the formation of thermally induced artifacts. The fact that the solvent side of the distillation-extraction apparatus is also under vacuum makes it difficult to retain the solvent in the apparatus. Even modifications of the apparatus to include a dry ice/acetone condenser followed by a liquid nitrogen trap do not permit easy operation under vacuum. Problems arise in that the solvent or aqueous vapors reach the cryogenic traps, thereby eventually blocking the exit of the condenser. The need to minimize exposure of the sample to heat has resulted in the more frequent use of two step procedures. Very often, the sample is simply placed in a flash evaporator, a certain volume of distillate collected and the distillate is solvent extracted via either separatory funnel or a continuous extractor. In this manner, the distillation process and solvent choice are not conflicting processes.

Dialysis

The availability of and improvement in membranes has rekindled some interest in dialysis in aroma research. Benkler and Reineccius (19, 20) initially published studies on the use of Nafion (Dupont) membranes for the separation of fat from flavor isolates. This would permit solvent extraction to be used in the isolation of aroma compounds from fat containing foods. Chang and Reineccius (21) later used a continuous tubular counter current flow system to accomplish this fat/aroma separation more efficiently. These membranes can be obtained commercially and have been improved in terms of membrane thickness and purity. While the aroma isolate obtained using this membrane may not perfectly reproduce the aroma being studied, this is an alternate technique for aroma isolation.

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Solid Phase Extraction

Solid phase extraction techniques have been applied both in the isolation and clean-up of flavor extracts. As an example, Uhlig et al. (22) extracted celery with dichloromethane and then passed the dichloromethane extract over a LC-Si Supelclean cartridge. The cartridge was flushed with hexane followed by dichloromethane plus 0.5% methanol to desorb the celery aroma components (phthalides).

Bitteur and Rosset (23) have applied solid phase extraction for the recovery of black currant aroma compounds from waste water. They passed solutions of known compounds in water through three different extraction columns, eluted each with either ethanol or dichloromethane and then analyzed the eluant to determine extraction efficiency. While this study was aimed at the recovery of aroma compounds on a commercial basis, their results demonstrated that reverse phase polymers can effectively be employed in the recovery of aroma compounds from dilute aqueous systems.

Heymann, et al (24) have used solid phase extraction techniques for the isolation of pyrazines from wines. In this analysis, it was necessary to first distill the pyrazines from the wine and then pass the distillate through a Sep-Pak C-18 cartridge (Waters Assoc.). To speed the analysis, the distillate was forced through the cartridge at a rate of 30 ml/min. Pyrazines were eluted from the Sep-Pak with 2 x 1 ml. aliquots of methanol. Recoveries of wine spiked with methoxypyrazines in the ppb ranged from 10 to 63%. While recoveries appear low, they were reproducible and quite suitable to address the problem being investigated.

Supercritical CO₂ Extraction

Solvent extraction has also remained a popular technique for flavor isolation. The most recent developments in this area have involved the use of supercritical CO₂ in both flavor extraction and analysis (25-27).

Supercritical CO₂ is a particularly good choice in aroma studies since it has an extremely low boiling point and leaves no off-odor residue to interfere in either analytical work or sensory evaluation. The fact that the solvent strength of a supercritical fluid depends on density is an additional factor which may be useful. One can vary solvent properties by changing density, thereby obtaining an effective extraction of a broad range of aroma compounds.

In retrospect, there are no totally new techniques for the isolation of thermally generated aroma compounds. The developments we have seen in recent years have been modifications of techniques which have existed for several years. As in the past, each method has its own unique strengths and weaknesses. The choice of method is determined by the food product to be analyzed, the volatiles of interest and the analytical methods to be applied.

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Chapter 6

Advances in Gas Chromatographic Analysis of Thermally Generated Aromas

M. J. Feeney and W. J. Jennings

J&W Scientific, 91 Blue Ravine Road, Folsom, CA 95630

The relative importance of column efficiency, retention and selectivity is presented with applications to the analysis of complex, volatile samples. Recent trends in the analysis of aroma samples by high resolution gas chromatography include utilizing specific stationary phases to analyze particularly difficult samples. Important considerations in selecting the optimum column for an analysis include the overall efficiency generated by the column, the partition ratio of the solutes to be resolved and the selectivity of the stationary phase towards the compounds of interest. After comparing the relative contributions these three factors, methods of optimizing stationary phase selectivity will be described.

Gas chromatographic analysis of aromas can be difficult due to the complex nature of the samples and the volatility of the solutes of interest. The presence of a wide variety of chemically active functional groups containing oxygen, nitrogen and sulfur puts additional demands upon the column and chromatographic conditions selected. Since the goal of the analysis is to separate the components of interest, it is important to optimize the chromatographic parameters which determine resolution.

Equation 1 is a fundamental resolution equation (1,2) which contains the measured terms α , k and n . Theoretical Plates (n) are a measure of a column's overall efficiency, the partition ration (k) is a measure of the amount of time a solute spends in the stationary phase relative to the mobile phase and relative retention (α) is a measure of the selectivity of the stationary phase.

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$$R = \frac{1\sqrt{n}}{4} \times \frac{k}{k+1} \times \frac{\alpha - 1}{\alpha} \quad (1)$$

efficiency
retention
selectivity

To a degree, each of these parameters plays a role in the separation process, and through appropriate selection of the column and operating conditions, the analyst can usually obtain the best possible separation.

Efficiency

Gas Chromatographic separation using open tubular columns are often referred to as high resolution gas chromatography (HRGC) because it is possible to generate much larger theoretical plate numbers. From equations by Van Deemter (3) and Golay (4) it is known that column efficiency is inversely proportional to column diameter.

Figure 1 is a comparison of the analysis of a complex mixture using 0.32 and 0.10 mm I.D. columns. In this example, the more efficient 0.10 mm column, produces almost equivalent resolution to the 0.32 mm column in approximately one third the analysis time. Unfortunately there are practical limitations to decreasing column diameter. With diameters equal to or less than 0.10 mm, sample introduction becomes difficult due to high inlet pressures. Sample capacity is limited and signal processing of the fast chromatographic peaks requires specialized A/D conversion.

Another method of increasing column efficiency is to increase column length. The advantages here also are somewhat limited since analysis time is proportional to column length, and the resolution increases only with the square root of column efficiency. Although column efficiency is very important in HRGC, the analyst needs also to consider the roles of k and α in developing a method of separation.

Retention

The partition ratio (k) is defined as the amount of solute in the stationary phase compared to the amount in the mobile phase. The value of k is calculated for each component from the corrected retention time (t'_r) and the gas holdup time (t_m) by the equation:

$$k = \frac{(t_r - t_m)}{t_m} = \frac{t'_r}{t_m} \quad (2)$$

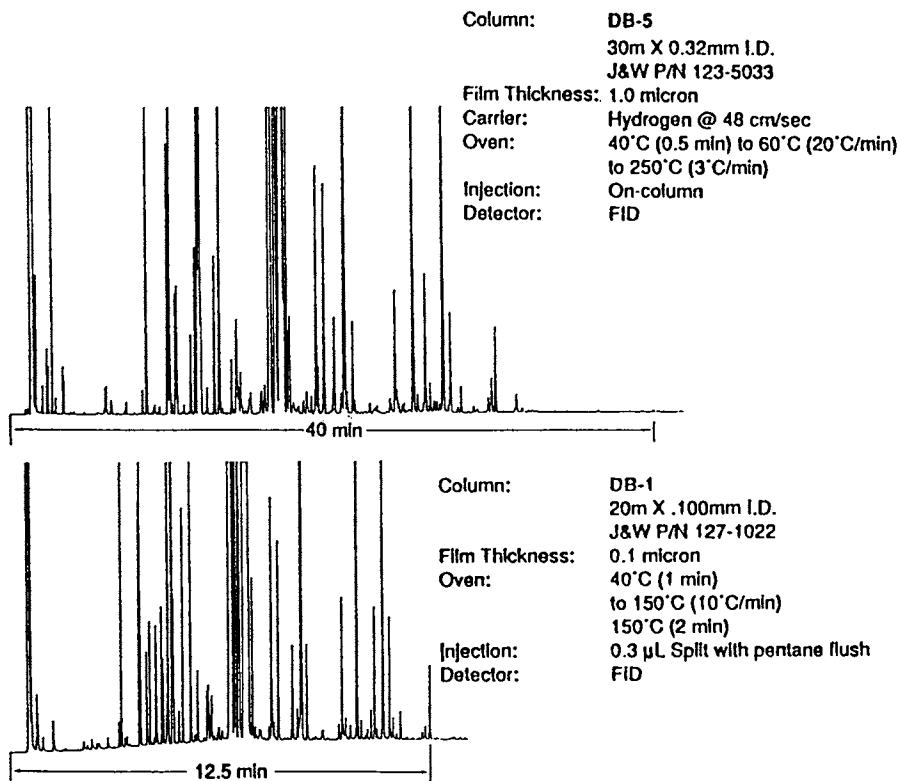


Figure 1. Comparison of peppermint oil separation using 0.32 mm. I.D. and 0.10 mm I.D. columns.

Optimizing the value of k , or more importantly $(k + 1)$ is important for the analysis of volatile compounds such as those in aroma samples. The value of k is strongly dependent upon temperature and the phase ratio (β) of the column. From Equation 3 it is apparent that decreasing temperature shifts the equilibrium constant K_D , and therefore k increases. When k is a small number (i.e. <2), which is often the case with volatile compounds, resolution increases greatly with only small increases of k .

$$K_D = \frac{C_s}{C_m} = k \times \beta \quad \text{where } \beta = (r - 2d_f) \quad (3)$$

There are practical limits to decreasing temperature since most modern gas chromatographs cannot regulate temperature below 35°C without using liquid nitrogen or carbon dioxide as a coolant.

Equation 3 can be utilized in another way to increase retention. The phase ratio can be optimized by selecting a column with a smaller radius (r) or by using a column with a greater film thickness (d_f). As mentioned earlier, there are limits to decreasing the radius of the column. Figure 2 compares the separation of C_1 to C_6 hydrocarbons using a 1 μm and 5 μm film thickness. The resolution between methane and ethane is increased from a merged peak to baseline resolution by changing only film thickness.

Selectivity

There are several possible modes of interaction between the solute and stationary phase in gas-liquid chromatography. The type of interaction is dependent upon the chemical nature of the compound to be separated and that of the phase coating the inside of the column. Figure 3 lists five different types of interaction which include dispersion, induced dipole, dipole-dipole hydrogen bonding and high dipole. In actual practice the interactions are usually a combination of two or more forces. It is important to note that functional groups are primarily responsible for differences in selective retention (selectivity) of a column. For example, Figure 4 shows the difference in resolution between a methyl silicone (DB-1) and a 7% cyanopropyl 7% phenyl methyl silicone (DB-1701). By utilizing a unique ($\underline{5}$) phase of 3% cyanopropyl 3% phenyl methyl silicone (DB-1301) it is possible to resolve all six compounds (Figure 5).

Selectivity Tuning

For the majority of gas chromatographic analyses there is a single column which under certain flow and temperature conditions has the "correct" combination of efficiency, retention and selectivity to resolve the compounds of interest in a particular sample. When faced with a "difficult" sample which no single column can

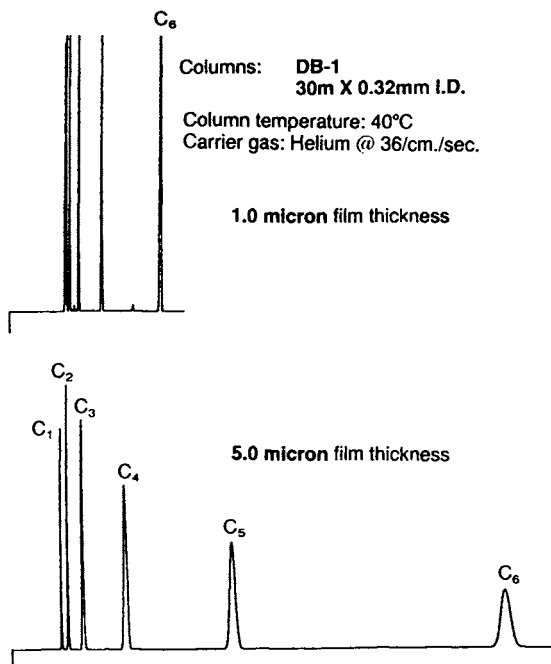


Figure 2. Comparison of C1-C6 hydrocarbon separation using 1 μm and 5 μm film thickness.


Dispersion	— CH_3
Induced Dipole	— 
Dipole-Dipole	$(\text{CH}_2)_n$ — CN
Hydrogen Bonding	PEG
High Dipole Low Bond Polarizability	$(\text{CH}_2)_n$ — CF_3

Figure 3. Modes of interaction of several stationary phases.

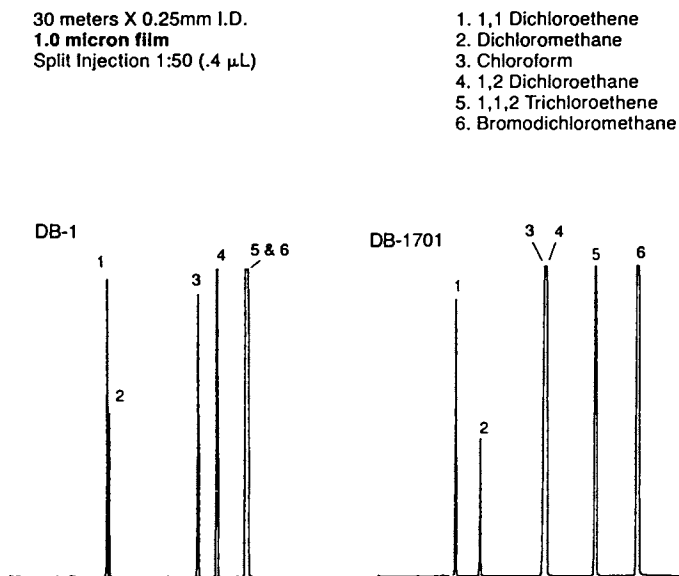


Figure 4. Comparison of relative retentions of halogenated hydrocarbons with DB-1 and DB-1701 stationary phases.

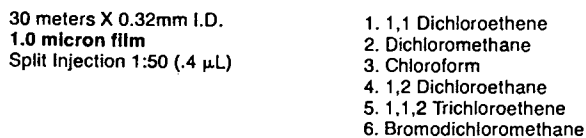


Figure 5. Resolution of six halogenated hydrocarbons using a stationary phase (DB-1301) with polarity between that of DB-1 and DB-1701.

separate, then multi-dimensional chromatography is often the solution to the problem. Multi-dimensional chromatography involves gas chromatographic analysis using columns in parallel (6) or in series (7,8). Still another approach is to use mixtures of stationary phases or some "appropriate" combination of the lengths of two columns connected together (9,10).

In one example of "selectivity tuning" a volatile solvent mixture was analyzed using a 60 meter methyl silicone (DB-1) column (Figure 6) and a 30 meter cyanopropyl phenyl methyl silicone (DB-624) column (Figure 7). The relative retention between components in this example were significantly different with the two stationary phases. A plot of the partition ratios (k) vs stationary phase composition was approximated (Figure 8) by linear interpolation between the values for each solute with the two separate columns. Upon inspection of the plot it appears that there are two possible combinations of the two columns for resolution of all 10 compounds. Choosing to cut only one of the columns, 30 meters of DB-624 were combined with 6.0 meters of DB-5 using a "press fit" connector (11), resulting in the separations shown in Figure 9. Although from the graph the optimum separation would be obtained using a 7.5 meter DB-5 column, in actual practice the front portion of the joined column has a greater effect on the overall separations observed.

Column:	DB-1	1. Acetone
	60m x 0.32 mm I.D.	2. Propylene Oxide
	J&W P/N 123-1063	3. Isopropanol
Film Thickness:	1.0 micron	4. Vinylidene Chloride
Oven:	35° C (Isothermal)	5. Dimethoxy Methane
Carrier:	Helium @ 34 cm/sec	6. Nitromethane
Injection:	1.0 uL Split 1:50	7. Methyl Ethyl Ketone
Detector:	FID	8. 2-Methyl Pentane
		9. Butylene Oxide
		10. 2-Butanol
		11. 1,1,1-Trichloroethane

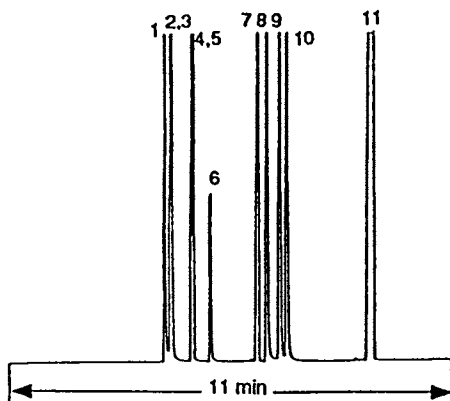


Figure 6. Solvent analysis using a methyl silicone stationary phase.

Column:	DB-624	1. Acetone
	30m x 0.32 mm I.D.	2. Propylene Oxide
	J&W P/N 123-1334	3. Isopropanol
Film Thickness:	1.8 micron	4. Vinylidene Chloride
Oven:	35° C (Isothermal)	5. Dimethoxy Methane
Carrier:	Helium @ 34 cm/sec	6. Nitromethane
Injection:	1.0 uL Split 1:50	7. Methyl Ethyl Ketone
Detector:	FID	8. 2-Methyl Pentane
		9. Butylene Oxide
		10. 2-Butanol
		11. 1,1,1-Trichloroethane

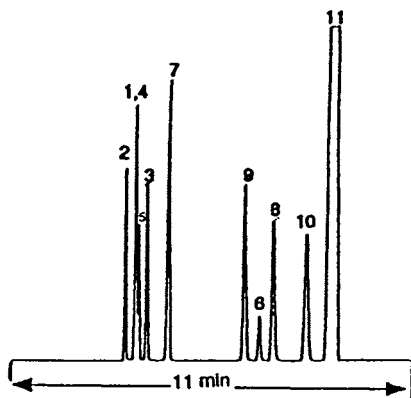


Figure 7. Solvent analysis using a cyanopropyl phenyl methyl silicone stationary phase.

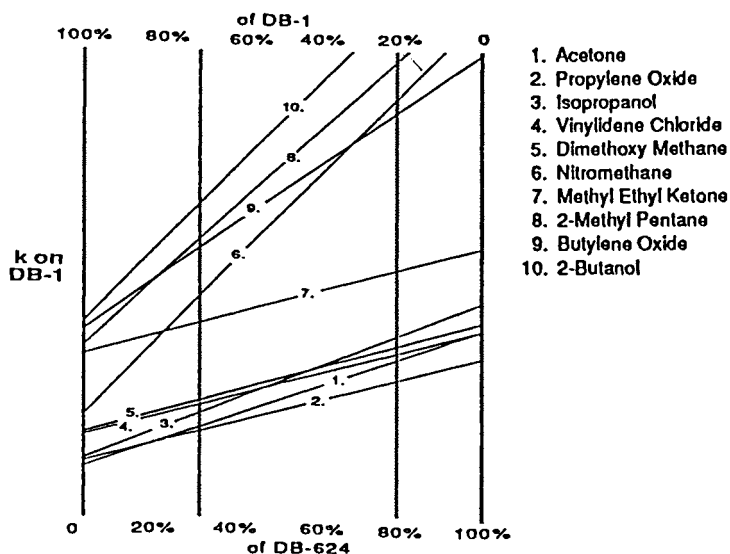


Figure 8. Plot of capacity factors for combination of DB-1 and DB-624 stationary phases.

DB-1 and DB-624 In Series

Column 1: DB-1
6m x 0.32 mm I.D.
Film Thickness: 1.0 micron
Column 2: DB-624
30m x 0.32 mm I.D.
Film Thickness: 1.8 micron
Oven: 35° C (Isothermal)
Carrier: Helium @ 34 cm/sec
Injection: 1.0 uL Split 1:50
Detector: FID

1. Acetone
2. Propylene Oxide
3. Isopropanol
4. Vinylidene Chloride
5. Dimethoxy Methane
6. Nitromethane
7. Methyl Ethyl Ketone
8. 2-Methyl Pentane
9. Butylene Oxide
10. 2-Butanol
11. 1,1,1-Trichloroethane

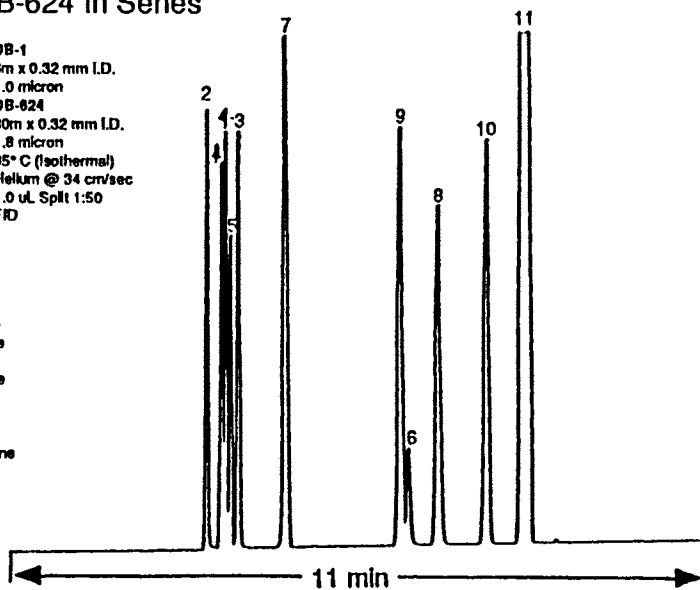


Figure 9. Solvent analysis using a combination of DB-1 and DB-624 columns in series.

Conclusion

The three measured components (n , k , α) play roles in separation process. Although they are not completely independent of one another, the chromatographer can utilize each to varying degrees to resolve the compounds in a particular sample. On occasion, it is necessary to combine more than one column with different stationary phases or a single column of mixed phases to resolve a difficult mixture. Of the various options, combining two columns in series with a low volume connector is often the simplest and most cost effective.

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Chapter 7

Gas Chromatography—Matrix Isolation Infrared Spectroscopy—Mass Spectrometry for Analysis of Thermally Generated Aroma Compounds

William R. Croasmun and Robert J. McGorin

Kraft USA, 801 Waukegan Road, Glenview, IL 60025

An integrated GC/IR/MS instrument is a powerful tool for rapid identification of thermally generated aroma compounds. Fourier transform infrared spectroscopy (GC/IR) provides a complementary technique to mass spectrometry (MS) for the characterization of volatile flavor components in complex mixtures. Recent improvements in GC/IR instruments have made it possible to construct an integrated GC/IR/MS system in which the sensitivity of the two spectroscopic detectors is roughly equal. The combined system offers direct correlation of IR and MS chromatograms, functional group analysis, substantial time savings, and the potential for an expert systems approach to identification of flavor components. Performance of the technique is illustrated with applications to the analysis of volatile flavor components in charbroiled chicken.

Flavor chemists have traditionally relied on mass spectrometry in conjunction with gas chromatography (GC/MS) to identify the structures of volatile flavor components in heated food systems. Mass spectrometry provides the molecular weights of fragment ions, which are useful for deducing molecular structure. The MS detection limit is on the order of 10^{-9} g, however detection limits for target compound analysis or chemical class detection via selected ion monitoring can be much lower. Extensive libraries of mass spectra are available; even so, many new flavor compounds can often not be identified from MS data alone.

Complementary structural information is available from gas chromatography/infrared spectroscopy (GC/IR), which provides functional group or substructure analysis. Recent improvements in light pipe GC/IR (1) and the advent of matrix isolation GC/IR (2-4) have pushed detection limits into the 10^{-8} to 10^{-9} g range. GC/IR allows the generation of a functional group IR chromatogram, which displays infrared absorbance across a selected wavelength region as a function of time. For example, if a $1780\text{--}1680\text{ cm}^{-1}$ range is chosen, the resultant chromatogram displays carbonyl-containing

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components. Because IR spectra are detected by a Fourier transform technique, detection limits are not improved in functional group (versus full-spectrum) chromatograms, as they are with GC/MS. The IR spectrum libraries useful for GC/IR, which contain about 3,000 to 10,000 spectra, are smaller than MS libraries. However, an advantage for IR is that computer library searching of the spectrum of an unknown tends to produce best matches which contain similar functional groups, even though the spectrum of the specific compound is not contained in the library.

The complementary nature of IR and MS data is illustrated in Figure 1, which compares the IR and MS spectra of four isomeric dimethylphenols. The isomers are easily distinguished in the IR, although their mass spectra are similar. Conversely, MS is better suited than IR for distinguishing a homologous series of compounds; whereas IR spectra appear similar for adjacent members of the series, MS can provide a characterizing molecular ion.

GC/IR has previously been utilized in flavor research to analyze aroma components in tropical fruits (5) and apples (6). While a linked GC/IR/MS system was proposed earlier (5), the principal limitation at that time resulted from the inherent lack of IR sensitivity in older light pipe systems.

It is possible to obtain GC/MS and GC/IR data in separate experiments (7-11), but there are powerful reasons for integrating the measurements into a single instrument (1,12-17). Data from two separate experiments can be difficult to correlate, owing to slight retention time shifts and differences in GC-spectrometer interfacing. Separate experiments are unnecessarily time consuming. Often, a flavor sample may be limited in amount or may deteriorate between experiments. Finally, it is necessary to assemble the entire data set in a single data system in order to take advantage of the potential for automated data interpretation of the combined data.

Experimental Methods

Commercial GC/IR/MS instruments are available from Mattson Instruments (using a matrix isolation GC/IR interface) and from Hewlett Packard (using a highly optimized light pipe GC/IR design). Each uses a Hewlett Packard Mass Selective Detector to obtain electron impact MS data. The instrument in our laboratory is a prototype version of the Mattson instrument, built in collaboration with Mattson Instruments.

Three key issues must be faced in constructing an integrated instrument. First, the sensitivity of the IR and MS detectors must be matched. We have chosen to use a Mattson Instruments Cryolect matrix isolation GC/IR interface, which has IR detection limits comparable to full scan electron impact MS. In this system, the effluent stream from a capillary gas chromatograph is doped with 1% argon and deposited on a gold-coated surface of a slowly rotating disk cooled to 12°K. Helium in the effluent stream is removed by vacuum, while the argon forms a solid matrix on the cold surface, trapping the chromatographic eluents into a solid track. IR spectra of individual flavor components or of the entire chromatogram are obtained by reflecting an IR beam off the disk surface. In our

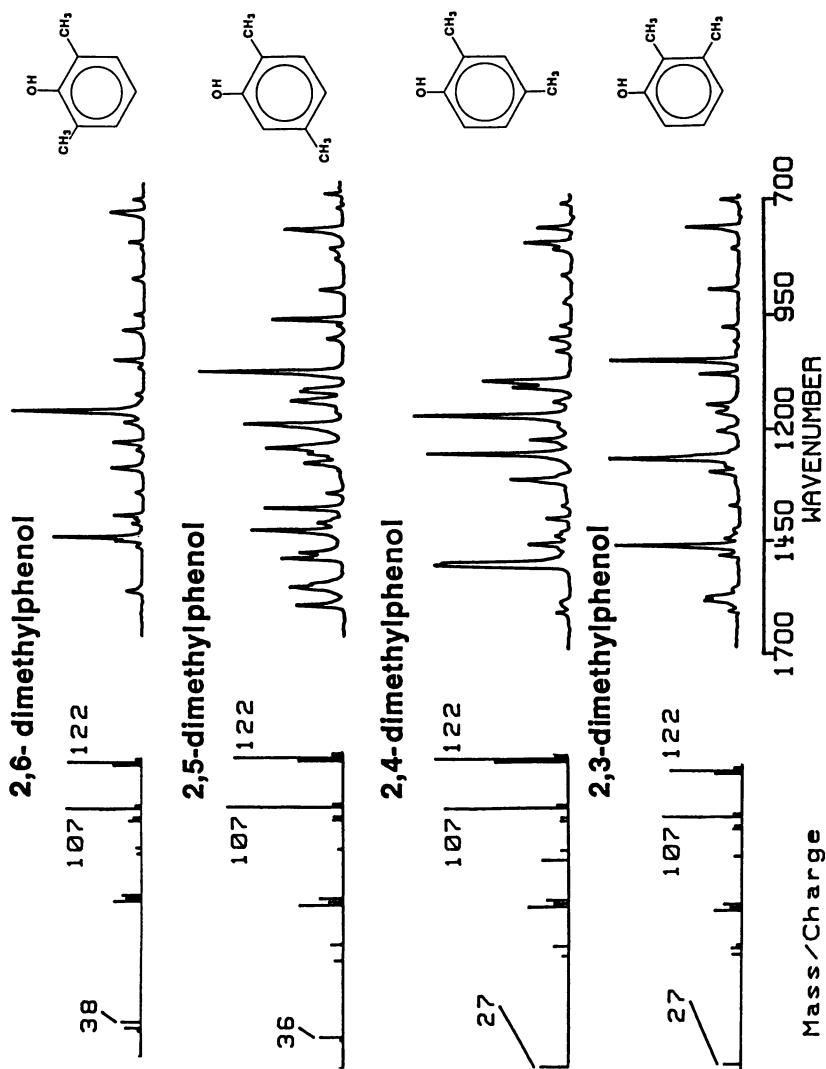


Figure 1. Mass spectra (left) and matrix isolation infrared spectra (right) of isomeric dimethylphenols.

laboratory a Nicolet 7199 FTIR spectrometer is used to acquire and process the IR data.

The second issue is to interface two detectors to a single GC. Since both the MS and the matrix isolation interface are destructive detectors, the sample is split in a 1:1 effluent splitter and half the sample is routed to each detector via a specially-designed open split interface (18).

The third key issue involves data handling. To take full advantage of the complementary nature of the data, it is desirable to handle all the spectral information in a single system. In our lab the IR and MS data are handled separately by the Nicolet and Hewlett Packard computer systems, respectively. Current commercial instruments also handle MS and IR data on separate systems, with only limited communications between the two computers. On our instrument, the following computer-based libraries were used to search unknown spectra: IR - EPA Vapor Phase; MS - National Bureau of Standards, Revision F.

Analysis of thermally generated aromas by GC/IR/MS will be illustrated with data from a charbroiled chicken distillate. The chicken was grilled at 400°F for a total of 36 minutes (18 minutes on each side). The flavor isolate was obtained from seven Likens-Nickerson distillation/extractions using methylene chloride, and was concentrated to 2 ml of solvent. A 2- μ l aliquot of the isolate (~2g chicken) was injected splitless onto a 50m by 0.32 mm OV-1 cross-linked methyl silicone gum GC capillary column (0.52 μ m film thickness) in a Hewlett Packard 5790A GC. The column was held at 10°C for 1 minute, programmed at 15°C/minute to 50°C, held at 50°C for 3 minutes, and heated at 2°C/minute to 220°C. Mass spectra were acquired at the rate of ~2/second, scanning from m/z 35 to 250. Infrared spectra used to compute the IR chromatograms were acquired at 1-second intervals with 8 cm^{-1} resolution; each spectrum was the average of four scans. The IR spectra in Figures 1, 4 (top two spectra) and 6 (bottom spectrum) were acquired at 4 cm^{-1} resolution and are the average of 32 scans.

Analysis of Charbroiled Chicken

IR and MS chromatograms of the distillate are displayed in Figure 2. The IR chromatogram is computed over an absorbance window corresponding to saturated C-H stretch and therefore serves as a nearly universal detector. (Gram-Schmidt chromatograms of matrix isolation IR data yield poor results in our hands, probably due to background water in the matrix.) Note that the chromatograms match well enough that it is straightforward to identify corresponding peaks. Observe also that the signal-to-noise in the two chromatograms is similar.

Figure 3 illustrates that functional group chromatograms can be particularly useful in flavor analysis. The "universal" C-H window (top) is compared to three different windows which detect carbonyl compounds. The 1780-1680 cm^{-1} window picks up most carbonyls, while the narrower 1710-1690 cm^{-1} window detects mostly unsaturated aldehydes in this highly oxidized sample. The 1810-1790 cm^{-1} window shows mainly a homologous series of gamma-lactones. It should be pointed out that IR is not able to produce functional group chromatograms of heterocycles, since they lack useful characteristic

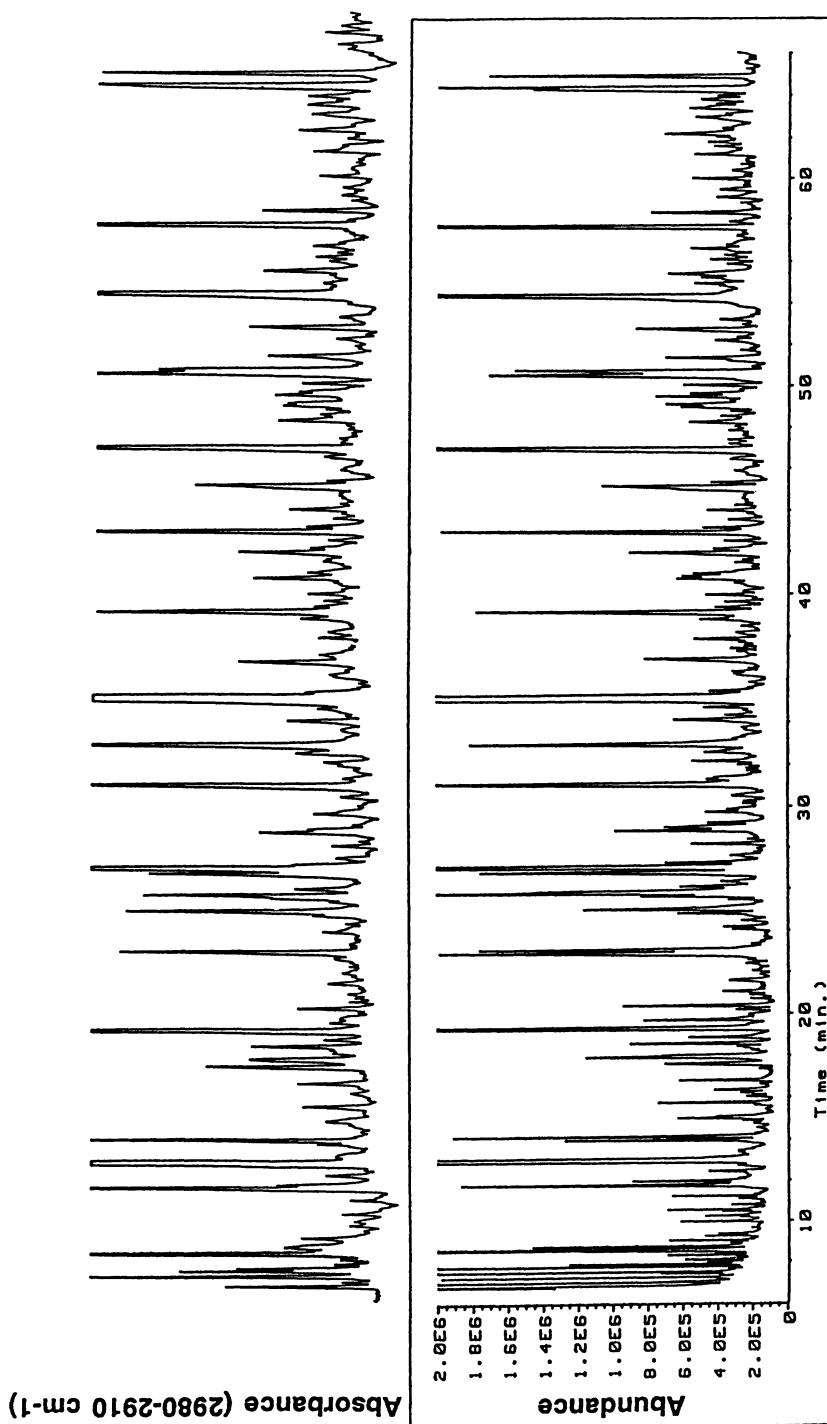


Figure 2. Chromatograms for matrix isolation capillary GC/IR (top) and GC/MS (bottom) of charbroiled chicken obtained by Likens-Nickerson distillation/extraction.

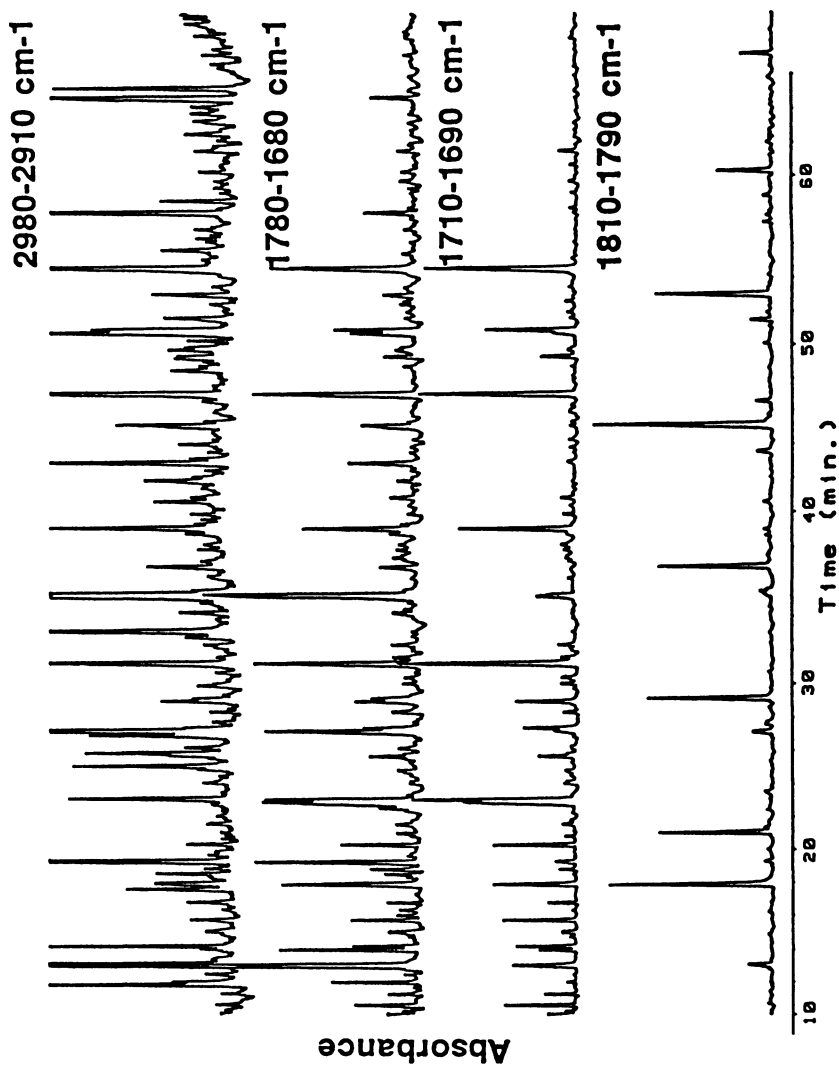


Figure 3. Selected matrix isolation GC/IR absorbance windows for charbroiled chicken isolate (cf. Figure 2).

group frequencies. However, these compounds are easily detected in selected ion MS chromatograms, since they produce intense, characteristic molecular ions.

Consistent with thermal processing of chicken, pyrazine compounds are generated via Maillard browning reactions, and contribute browned, roasted aromas to charbroiled chicken flavor. The utility of IR in resolving pyrazine isomers is illustrated in Figure 4. The peak at 19.685 minutes is easily identified as 2,5- or 2,6-dimethylpyrazine by MS (not shown), but the MS cannot distinguish these coeluting isomers. The IR shows that both are present and indicates the relative proportions; it also detects a coeluting carbonyl compound (bands at 1720 and 1080 cm^{-1}). Only a single ion in the mass spectrum indicates the presence of a third component.

To illustrate the complementary nature of IR and MS data we will focus on three adjacent peaks near 29 minutes (Figure 5, boxed region). Note that the center peak shows a shoulder in the MS. The corresponding infrared and mass spectra are presented in Figure 6.

The peak on the left (28.821 minutes) is an aldehyde by IR ($\text{C}=\text{O}$ at 1700 cm^{-1} and aldehyde C-H stretch at 2700 cm^{-1}). The molecular weight is 124, yielding a molecular formula of $\text{C}_8\text{H}_{12}\text{O}$ and requiring a second double bond or a ring in the hydrocarbon portion. There is no evidence for a ring; the position of the $\text{C}=\text{O}$ stretch implies that a second double bond is not conjugated with the first. The ratio of CH_2 to CH_3 stretching bands (2900-3000 cm^{-1}) suggests a branched structure. The combined data indicate a branched eight carbon doubly-unsaturated aldehyde, with only one double bond conjugated to the carbonyl. This conclusion is consistent with an observed Kovats retention index of about 1000.

The center peak (28.986 minutes) is clearly a mixture of two compounds, as indicated by the shoulder in the total ion chromatogram, by the strong 85 and 91 ions in the mass spectrum, and the presence of two carbonyl bands in the IR. The individual compounds are most easily identified by spectrum subtraction in the IR, yielding resolved spectra of the overlapping components. The first is gamma-hexalactone and the second phenylacetaldehyde (Figure 7).

The third of the three adjacent peaks (29.207 minutes) is readily identified as 2-hydroxybenzaldehyde (salicylaldehyde) by comparison of the MS or IR spectrum with library data. It should be noted that the automated search routine in the MS software picked 3- and 4-hydroxybenzaldehyde as better matches than the 2-hydroxy compound, even though the 76 ion in the spectrum of the unknown is present only in the mass spectrum of the 2-hydroxy compound. However, the IR search routine correctly identified the 2-isomer. This illustrates that casual operators who rely on automated search routines for compound identification are much less likely to make errors when they have access to both IR and MS searching.

Future Directions

From the point of view of aroma analysis, the ultimate objective of developing so-called "multiply hyphenated" instruments is to produce a device which can automatically determine the identity of all of the constituents of a complex volatile mixture. Integrated GC/IR/MS is a step along that path, but a host of crucial issues remain.

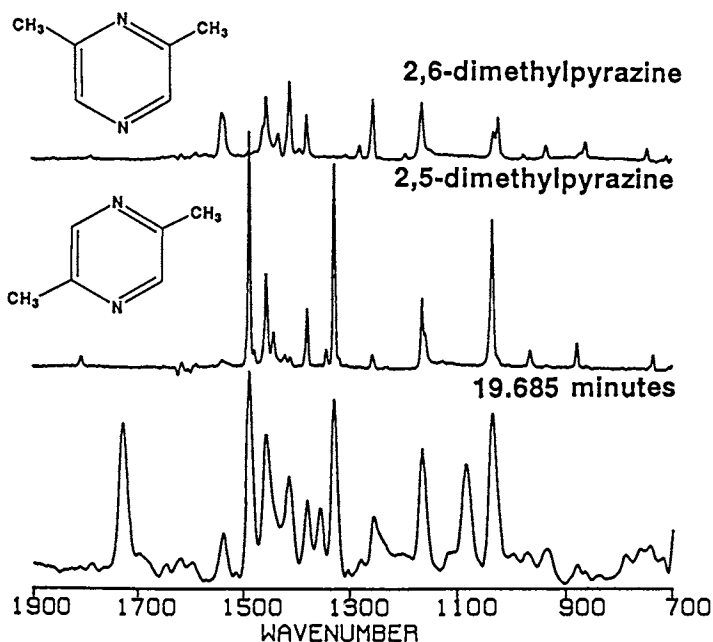


Figure 4. GC/IR spectra of 2,6-dimethylpyrazine (top) and 2,5-dimethylpyrazine (middle) identified in a spectrum of an unknown peak (Rt 19.685 min., bottom) from charbroiled chicken.

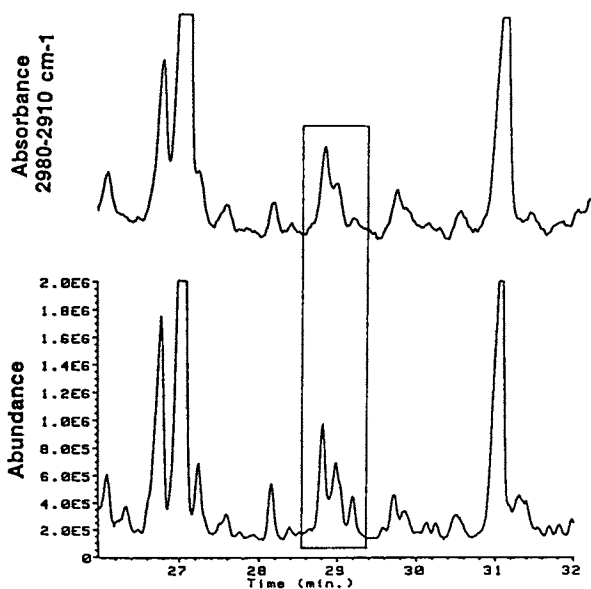


Figure 5. Expanded chromatogram region (26–32 min.) of charbroiled chicken isolate (cf. Figure 2). Top: GC/IR. Bottom: GC/MS.

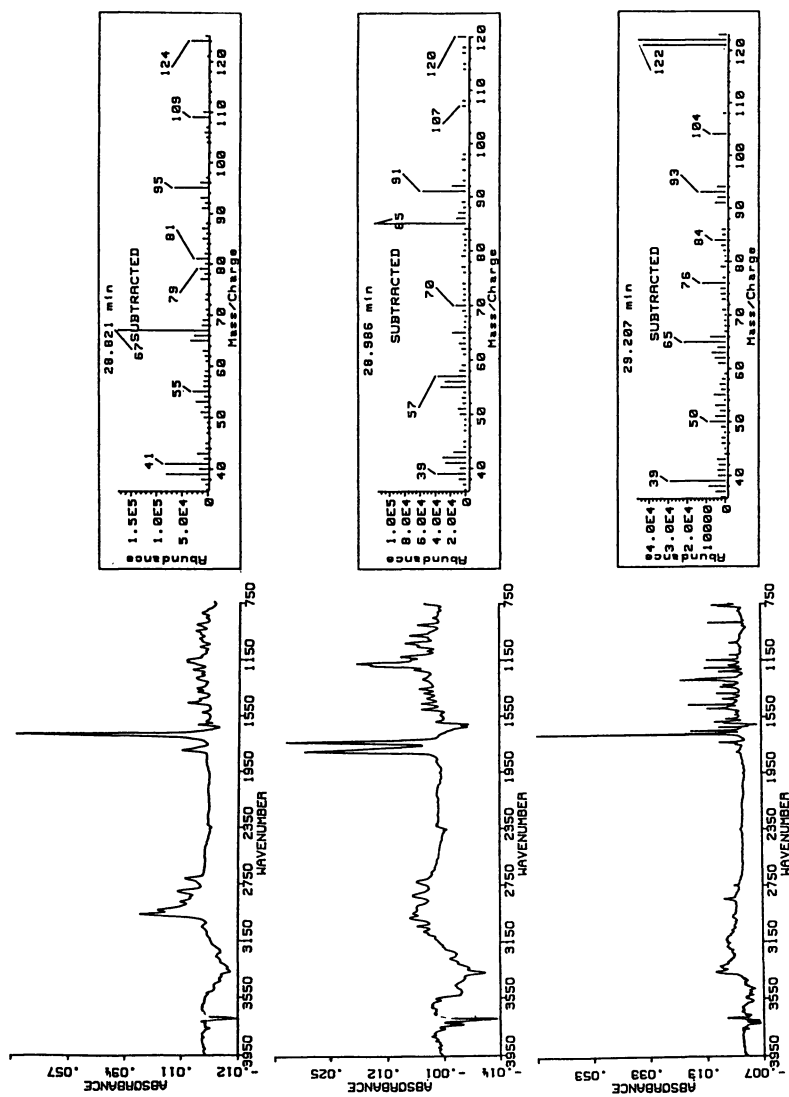


Figure 6. Matrix isolation IR spectra (left) and MS spectra (right) for selected unknowns in charbroiled chicken (cf. Figure 5). Top: Rt 28.821 min., C₈-dienal. Middle: Rt 28.986 min., mixture of γ -hexalactone and phenylacetaldehyde. Bottom: Rt 29.207 min., 2-hydroxybenzaldehyde.

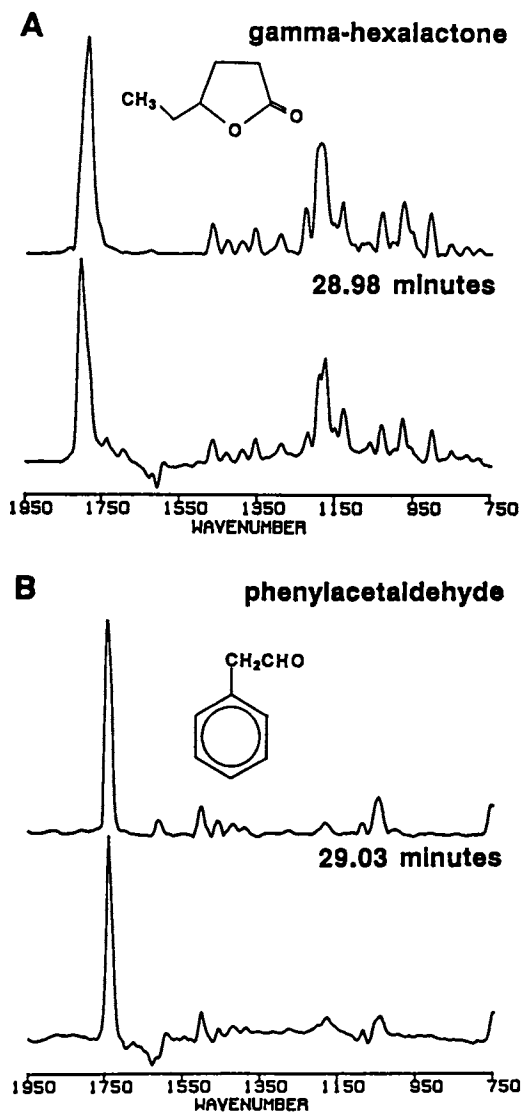


Figure 7. (A) Identification of GC/IR spectrum for unknown peak at 28.98 min. (bottom) as γ -hexalactone (top). (B) Identification of GC/IR spectrum for unknown peak at 29.03 min. (bottom) as phenylacetaldehyde (top).

First, a truly integrated GC/IR/MS instrument needs an integrated data system. The experiment is capable of generating a wealth of data in a relatively short time; efficient data capture, reduction, interpretation, and archiving are crucial to the utility of the method (16). Second, it may very well prove necessary or desirable to use information in addition to MS and IR spectra, e.g. exact mass data, to reliably identify total unknowns (19).

Third, assuming that the required information can be automatically gathered, it will be necessary to use an expert system approach to automate the data interpretation. Such a system will likely employ both library searching and IR and MS spectrum interpreters to analyze the data, making use of intermediate results from one approach to guide the others in the same way that a human operator interprets all of the data in concert. Combined library search approaches have been demonstrated (16,17,19) and a variety of MS (20-22) and IR (23-25) spectrum interpreters are available. Several laboratories have begun to address the need for a combined expert system (16,22). It seems clear that some of the most useful new developments in GC/IR/MS technology will appear in this area.

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Chapter 8

Modern Techniques in Mass Spectrometry for the Analysis of Nonvolatile or Thermally Labile Flavor Compounds

T. G. Hartman¹, Chi-Tang Ho², J. D. Rosen², and R. T. Rosen¹

¹Center for Advanced Food Technology, Rutgers, The State University of New Jersey, New Brunswick, NJ 08903

²Department of Food Science, New Jersey Agricultural Experiment Station, Cook College, Rutgers, The State University of New Jersey, New Brunswick, NJ 08903

In the past several decades the use of combined gas chromatography - mass spectrometry (GC-MS) has produced quantum leaps in the science of flavor chemistry. However, this methodology is unsatisfactory for the analysis of flavor or flavor precursors which are nonvolatile, highly polar, high molecular weight or thermally unstable. In these cases alternate means for mass spectrometric identification and characterization is necessary. The use of desorption ionization techniques such as fast atom bombardment (FAB), desorption chemical ionization (DCI) and HPLC-MS are well suited for these purposes. These techniques often produce mass spectra with intense pseudomolecular ions lacking fragmentation which is desirable for structure elucidation. This shortcoming can be partially remedied by combining these low ionization energy techniques with tandem mass spectrometry (MS-MS). Structural information can be obtained from the daughter ions produced upon collisionally induced dissociation. Experiments involving thermally labile flavor compounds, Maillard reaction precursors and high molecular weight polar molecules will be presented in this discussion to illustrate these techniques.

The science and technology of flavor chemistry has always been greatly influenced by advancements in mass spectrometry. The interface of gas chromatography to mass spectrometry (GC-MS), first with packed columns and then later with capillary GC columns was a milestone in flavor chemistry achievement. With this technology flavor chemists could routinely separate and identify the hundreds of volatile organic compounds present in the aroma of foods. No other technique can rival the detail and volume of information obtainable by GC-MS regarding the composition of highly complex volatile flavor mixtures. Although GC-MS using electron or chemical

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ionization remains the primary tool of the flavor chemist, it has several shortcomings. Thermally labile flavor compounds can decompose at the high temperatures used in GC yielding artifacts which can complicate investigations. Furthermore, the technique is not amenable to highly polar, nonvolatile or high molecular weight compounds. Recently the field of flavor chemistry has expanded to the point where these types of compounds are of great interest. For instance flavor precursors, taste modifiers, sweeteners, sour and bitter components and peptides such as those found to be important in meat and cheese flavor are now the focus of much attention. In these instances alternate means for mass spectrometric identifications are needed. As the science of flavor chemistry began to recognize the importance of these types of compounds, the field of mass spectrometry has also expanded with new ionization techniques which have the potential to accommodate the special needs of the flavor chemist in these instances. The use of desorption ionization techniques such as fast atom bombardment (FAB), desorption chemical ionization (DCI) and HPLC-MS are well suited for applications such as these. These techniques become even more useful when combined with tandem mass spectrometry (MS-MS). Protonated molecular ions can be collisionally dissociated to produce daughter ion spectra rich in fragmentation which is desirable for structure elucidation. In this discussion we will briefly outline these techniques and demonstrate their usefulness in flavor chemistry by using selected examples from our ongoing research projects.

Desorption Ionization Techniques

GC-MS and Electron and Chemical Ionization (EI/CI)-MS rely on the ability of organic species to survive volatilization prior to ionization. In many cases, this requires a degree of heating which often leads to decomposition. In desorption chemical ionization (DCI), field ionization (FI), thermospray (TSP) or fast atom bombardment (FAB) ionization occurs before volatilization, and measurement by the mass spectrometer often occurs before decomposition can result. These techniques have allowed determination of many high molecular weight and polar species, which could not previously be analyzed.

Desorption Chemical Ionization (DCI)

The technique DCI was first described by Baldwin and McLafferty in 1973 (1). These investigators inserted films of polar organic compounds deposited on thin glass rods directly into the ion volume of the mass spectrometer source which was maintained under CI conditions. Bringing the sample into direct contact with the high pressure (1 Torr) reagent gas plasma promoted ionization at greatly reduced temperatures as compared to traditional direct probe CI. Further refinements in the technique included the use of specially designed low thermal mass probe tips which could be ballistically heated (2). It has been postulated that under DCI conditions the rate of thermal desorption and chemical ionization exceeds that of thermal decomposition (2). DCI spectra usually exhibit prominent protonated molecular ions (or M-H ions in negative ionization mode)

and moderate fragmentation. This technique is ideally suited for intermediate polarity and thermally labile organics with molecular weights below 1200. This technique is particularly useful for the analysis of short peptides, oligosaccharides and heat sensitive flavor compounds. We have used this technique extensively on several magnetic sector mass spectrometers by simply evaporating small amounts of sample onto an inverted capillary glass melting point tube then inserting it directly into the CI plasma. We have found that external heating is rarely needed in that the sample rapidly desorbs and ionizes upon contact with the plasma, suggesting that vapor phase conversion may not be necessary. The DCI spectra we obtained on alanyl-histamine dihydrochloride (a compound with no appreciable vapor pressure) illustrates this point. The DCI spectrum was obtained with no heat being supplied to the probe tip and showed a strong protonated molecular ion (minus 2HCL) at mass 183 and a base peak at 112 corresponding to loss of the alanyl residue from cleavage of the amide linkage. Similarly, figure 1 shows the DCI spectra of the substitute sweetener aspartyl-phenyl-alanine methyl ester (Aspartame). The spectra exhibits a protonated molecular ion base peak (295) and numerous fragments including loss of methyl (280), water (277), CO₂ (251) and the aspartyl residue (180).

The following example demonstrates the use of DCI for the analysis of thermally labile compounds. A sample was submitted for analysis which was an acidic fraction from a bacterial fermentation which had been purified by HPLC. The sample was initially analyzed using hot on-column injection GC-MS on a short (10m x 0.32mm i.d. DB-1 with 0.25 μ film) capillary column with electron ionization detection. The injector temperature was 250°C and the column was programmed from 50°C to 280°C at 10°C per minute. The peak on the resulting ion chromatogram exhibited a high degree of tailing after elution and yielded the mass spectra shown in figure 2 with an apparent molecular weight of 148. A computer library search of the spectrum revealed a perfect match with 3,4 dihydrocoumarin. However, the peak shape of this compound on the gas chromatograph would be expected to be sharp and free of tailing and it is a neutral species not an acid as was expected. Since dihydrocoumarin is a lactone we immediately suspected that the original sample contained the corresponding hydroxy acid which was decomposing in the GC injection port or column to form the lactone. The sample was then analyzed by DCI and the spectra obtained (Figure 3) clearly indicates the molecular weight to be 166 ($M+H^+=167$) which is that of the hydroxy acid. The loss of water in the DCI spectra yielding the peak at mass 149 further substantiates our theory that the hydroxy acid dehydrates to yield the stable lactone upon heating. This decomposition artifact formed in the GC despite the inertness of the on-column injection capillary chromatography.

Fast Atom Bombardment (FAB)

The FAB ionization technique was originally described by Barber et al. in 1981 (3). FAB is a direct probe technique suitable for the analysis of high molecular weight polar organic compounds. In FAB the sample is dissolved in a matrix such as glycerol or thioglycer-

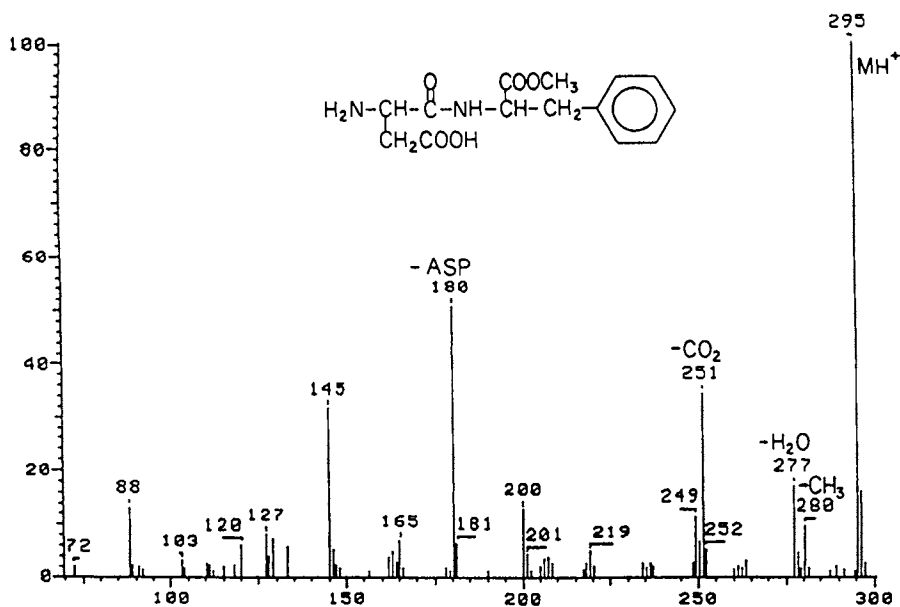


Figure 1. Desorption chemical ionization (DCI) mass spectrum of aspartylphenylalanine methyl ester.

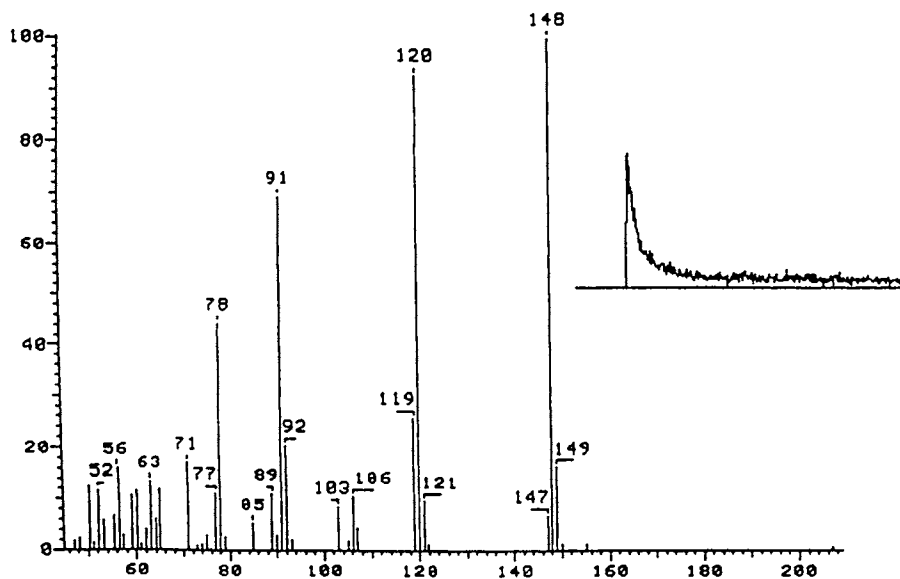


Figure 2. Ion chromatogram (inset) and electron ionization (70 eV) mass spectrum of hydroxyphenylpropionic acid showing decomposition to the lactone (dihydrocoumarin).

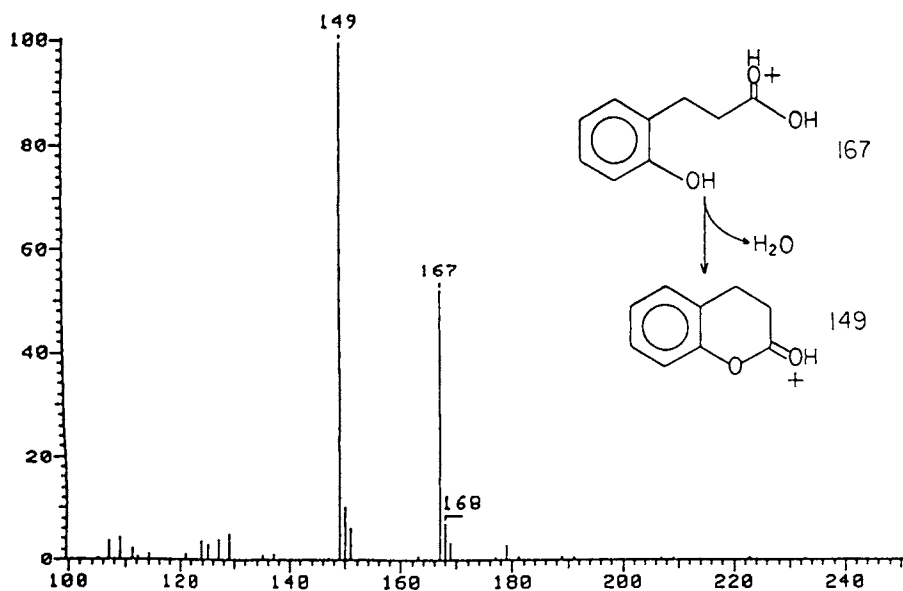


Figure 3. Desorption chemical ionization (DCI) mass spectrum of hydroxyphenylpropionic acid.

ol and placed on a probe tip. The sample is then bombarded with an energetic beam of neutral xenon or argon atoms (8Kv) which promotes the ionization of both the matrix and sample molecules. The ions formed in the matrix solution are then sputtered into the gas phase in the field regions of the mass spectrometer, hence the terms sputter ion or secondary ion mass spectrometry have been used to describe the technique. In practice the fast atom beam was found to contain considerable amounts of ions which did not adversely effect the ionization process. In fact, just recently instrument manufacturers have begun to change from 8Kv Xe fast atom guns to 35Kv Cesium ion guns which improves the high mass sensitivity. When the incident atom or ion beam strikes the matrix much of its energy is transferred to the more abundant matrix molecules causing a fair degree of radiolytic damage. Some of the energy released is then transferred from the matrix to the sample molecules to produce protonated (positive ion mode) or deprotonated (negative ion mode) molecular ions which usually exhibit little fragmentation. We have accumulated evidence which supports the theory that CI plasma-like conditions are created immediately above the matrix during FAB operation and this may further influence the ionization process (4). The presence of trace amounts of salts such as sodium or potassium ions often leads to cationization and $M+Na^+$ and/or $M+K^+$ ions are observed. At high levels these salts are known to suppress ionization and this requires that samples be desalted prior to analysis. Musselman (5) discussed the utility of silver ion attachment in FAB. Molecular ions can then be confirmed as the $M+Ag^+$ adducts which give characteristic doublets at 107 and 109 mass units greater than the molecular weights. Gower reviewed the many different matrices, cosolvents and modifiers which have been described for use in FAB (6). The most popular matrices include glycerine, thioglycerine, m-nitrobenzyl alcohol and Proton Sponge (a strong organic base used for negative ion mode). A matrix which we have found particularly useful is the so called μ magic bullet which is a 3:1 mixture of dithiothreitol and dithioerythritol dissolved in a small amount of methanol. Virtually any matrix can be successful if it meets certain criteria. It must have a low vapor pressure to prevent it from evaporating too rapidly in the high vacuum, it must dissolve the sample and be a good proton donor (or proton abstractor in negative ion mode) to promote ionization. Finally it must be able to conduct any surface charge formed to the probe rod which is maintained at ground potential. Cosolvents such as methanol, dimethylsulfoxide, water and dimethylformamide are often utilized. Modifiers such as trifluoroacetic acid, hydrochloric acid, ammonium hydroxide and triethanolamine may be added in small amounts to the matrix to aid in ionization.

To obtain optimum results in a FAB analysis samples should be relatively pure, though simple mixtures can be tolerated. We routinely perform mixture analysis with FAB, especially in our peptide mapping studies. However, certain shortcomings may arise. Complex mixtures are often plagued by a signal suppression effect where certain mixture components are preferentially ionized at the expense of others. When peptide digests are analyzed the hydrophobic components are ionized with much greater efficiency and the more hydrophilic components are suppressed. Surfactant type

compounds usually give very strong signals in FAB which can obscure other mixture components. It is likely that surfactant like compounds reside at the glycerol matrix surface - vacuum interface region and dominate the ionization process. If ionic species are present in mixtures such as quaternary ammonium compounds they will totally dominate the signal. HPLC is often used to isolate samples for FAB analysis and for these reasons buffer salts and surfactants much be avoided at all costs.

A new and very promising solution to some of these problems is the development of continuous flow FAB (Dynamic FAB). Pioneered by the work of Caprioli and coinvestigators (7) dynamic FAB is an HPLC-FAB interface technique. In this mode of operation the analyte is dissolved in a 5.0 % aqueous solution of glycerine and is then pumped or aspirated through a length of fused silica capillary tubing to a specially designed FAB probe tip. During dynamic FAB operation the probe tip temperature is maintained at 35-50°C to prevent ice formation from the evaporating mobile phase as it is pumped away by the vacuum. Samples can be introduced directly from an HPLC column or in batch mode using loop injections. In the former case it is convenient to add the glycerine post column so as to not effect the chromatography. Almost any solvents are permissible but only volatile buffers or mobile phase modifiers may be used. For optimum performance the flow rate into the probe should be approximately 5 μ l/min, which makes the technique highly compatible with syringe pumps and microbore chromatography. The advantages of dynamic FAB, besides the obvious benefit of HPLC-MS interfacing are numerous. The problem of signal suppression due to complex mixtures is alleviated since HPLC separation of the components is accomplished prior to FAB ionization. With components eluting as sharp peaks higher numbers of ions can be collected in a narrow time window thereby improving the signal strength. In traditional FAB experiments signal averaging techniques are often employed since ion collection occurs over a much longer time frame. An additional advantage is that dynamic FAB exhibits a considerable reduction in matrix derived chemical noise background. This improves the signal to noise ratio and thus increases the overall sensitivity. Routine operation with picomole sample concentrations is now possible. Early problems with the technique included unstable ion beams and memory effects from sample hangup on the probe tip. These problems have been largely eliminated with the use of syringe pumps, careful control of tip temperature and the use of a wicking system to facilitate the removal of mobile phase from the probe tip.

We have used FAB to analyze a series of flavanoids and saponins of botanical origin. Many of these compounds are present in nature as complex glycosides which are normally hydrolyzed to yield the aglucons when analyzed by traditional methods. However, FAB is ideally suited for the analysis of such intact glycosides and it was our intention to obtain molecular weights and fragmentation patterns for a series of these compounds. One such compound is stevioside which is an experimental sweetening agent obtained from the leaves of *Stevia rebaudiana*. This compound is a complex macrocyclic acid (steviol) containing three carbohydrate moieties. The protonated molecular ion is readily discernible at mass 805 and

fragments corresponding to loss of each sugar molecule are present including the aglucon base peak at mass 319. Similar results were obtained on tomatine a bitter tasting saponin found in tomatoes and glycyrrhizin which is an intensely sweet compound found in licorice. Both of these compounds are glycosides containing tetra and disaccharide moieties respectively. Other compounds in this class which we have successfully analyzed include α -solanine and α -chaconine (found in potatoes and tomatoes) and naringin (bitter component from grapefruit). Table I summarizes the data from these experiments.

FAB has been widely promoted as the technique of choice for the analysis of high molecular weight polar compounds. However, we have found it to be equally useful for the analysis of certain types of low molecular weight compounds. We were requested to obtain molecular weight information on a homologous series of gingerols and n-methylgingerols, compounds responsible for the pungent properties in ginger spice. The compounds were extracted from commercial ginger using supercritical CO₂ and were isolated by HPLC (8). It is known that upon heating these compounds rapidly dehydrate yielding the corresponding shoagols or thermally decompose via the retro-Aldol reaction into zingerone and a series of aldehydes (9). We were unable to obtain molecular weights using EI, CI or even DCI but succeeded using FAB (8,10). Interestingly, the FAB spectra for the entire homologous series were found to contain M⁺ ions and not the protonated species which are usually produced in FAB. Other major peaks which were found in all spectra included M+Na⁺ and M⁺-OH ions. We initially postulated that the M⁺ ions were due to charge exchange within the glycerol matrix. To understand this phenomenon we analyzed a series of low molecular weight aromatic alcohols and obtained similar results. We then compared the FAB spectra obtained to that produced by low pressure CI-MS and noted amazing similarities (4). Following additional studies we postulated that ionization and fragmentation can occur in a region a few microns above the actual glycerol matrix surface where low pressure CI plasma-like conditions may exist. Under these conditions charge exchange reactions resulting from collisions with Xe ions (in the not-so-neutral fast atom beam) are believed responsible for the production of M⁺ ions.

We have been involved in an ongoing research investigation to characterize the high molecular weight Maillard reaction products formed in a lysozyme-glucose model system (11). Although these species are not flavor compounds they can be considered a flavor precursor model system and serve to illustrate the usefulness of FAB-MS for the analysis of such compounds. Hen egg white lysozyme is a relatively small protein consisting of 129 amino acid residues (14,305 M.W.) and four disulfide bridges. In the Maillard (non-enzymatic browning) reaction, reducing sugars react with the free amino groups of proteins, peptides and amino acids to form a Schiff base. Being somewhat unstable the Schiff bases rearrange to form more stable structures known as Amadori products. As the reaction proceeds it leads to the formation of increasingly complex advanced glycosylation products many of which are important flavor compounds. It was our intention to study the site specific formation of Amadori products in the early stages of the reaction. In

TABLE I. FAB MASS SPECTRAL DATA OF FLAVANOID-O-GLYCOSIDES AND SAPONINS

Compound	M/Z (Relative Intensity)	Assignment
STEVIOSIDE	805 (2)	MH ⁺
	643 (5)	MH ⁺ - C ₆ H ₁₀ O ₅
	463 (8)	643 - C ₆ H ₁₂ O ₆
	319 (100)	*AH ⁺
	301 (70)	AH ⁺ - H ₂ O
	273 (28)	A ⁺ - COOH
	255 (50)	273 - H ₂ O
TOMATINE	1034 (25)	MH ⁺
	1033 (12)	M ⁺
	854 (4)	MH ⁺ - C ₆ H ₁₂ O ₆
	415 (75)	A ⁺
	397 (100)	A ⁺ - H ₂ O
GLYCYRRHIZIN	823 (8)	MH ⁺
	647 (13)	MH ⁺ - C ₆ H ₈ O ₆
	471 (25)	AH ⁺
	453 (100)	AH ⁺ - H ₂ O
α-SOLANINE	868 (100)	MH ⁺
	867 (8)	M ⁺
	706 (5)	MH ⁺ - C ₆ H ₁₀ O ₅
	397 (50)	A ⁺
	380 (95)	A ⁺ - OH
α-CHACONINE	852 (30)	MH ⁺
	397 (40)	A ⁺
	380 (100)	A ⁺ - OH
NARINGIN	581 (25)	MH ⁺
	273 (100)	AH ⁺

*A = AGLUCON

the case of glucose (180 M.W.) a mole of water is lost during Schiff base formation thus the Amadori adducts add 162 mass units to the protein at each glycosylation site. Native lysozyme and lysozyme glycosylated under various experimental conditions were subjected to tryptic and chymotryptic hydrolysis. The digests (tryptic or chymotryptic peptides) were then analyzed by FAB to produce peptide maps which can be correlated back to the intact proteins. Specific glycosylation sites are then determined by observing 162 mass unit differences in the peptides derived from native and reacted lysozyme. Figure 4 shows the FAB chymotryptic peptide map of native lysozyme obtained by direct FAB analysis of the digest mixture. The map is not complete since several of the peptides are not detected due to the signal suppression effect mentioned previously. Some of the more hydrophilic peptides required HPLC isolation prior to FAB analysis. Note that in these FAB spectra the peptides are detected exclusively as $M+H^+$ ions with no fragmentation. Figure 5 is the FAB chymotryptic peptide map of a glycosylated lysozyme sample. The peptide Amadori adducts are clearly identifiable at 162 mass unit increments above the corresponding peptide. This data allows us to deduce the structure of the original intact Maillard reaction product. This particular structure was found to contain Amadori adducts at amino acid (lysine or arginine) residues # 1,5,21,33 and 128 numbered from the n-terminal of the protein. Additional confirmation of this structure was obtained from the FAB tryptic peptide maps.

Mass spectrometry has continually pushed the molecular weight detection barrier upward. It is now becoming routine to obtain molecular weights of intact proteins up to 20,000 daltons and slightly higher. Proteins which contain hundreds of carbon atoms yield complex polyisotopic molecular ion clusters when analyzed by mass spectrometry. These clusters result since ^{13}C is approximately 1.1% of ^{12}C . The centroid of these clusters relates to the average molecular weight of the protein not the monoisotopic mass which we normally refer to in mass spectrometry. However, to resolve a high mass isotope cluster into individual peaks exceedingly high instrument resolution is required. For instance, it would take an instrument resolution of 20,000 to separate the 20,000 peak from the 20,001 peak and at this resolution the sensitivity would be extremely poor. Therefore, when making high mass measurements on modern high field magnetic sector mass spectrometers it is common practice to operate at very low resolution (500). In this case the polyisotopic cluster is detected as a broad unresolved peak commonly referred to as the molecular ion envelope. The centroid of this peak is then calculated to determine the average chemical molecular weight of the protein. Figure 6 shows the molecular ion envelope of native lysozyme (M.W.= 14,305) obtained using FAB. The FAB mass spectrum of a glycosylated lysozyme is shown in Figure 7 along with cesium iodide cluster ions which are used for mass calibration. The glycosylated sample is actually a mixture of lysozyme Amadori products therefore, the molecular ion envelope is even further broadened. However, at least two peaks are readily discernible which have a center to center spacing of approximately 162 mass units. The centroided masses of these peaks at 16,265 and 16,427 correspond to lysozyme Amadori adducts containing 12 and 13

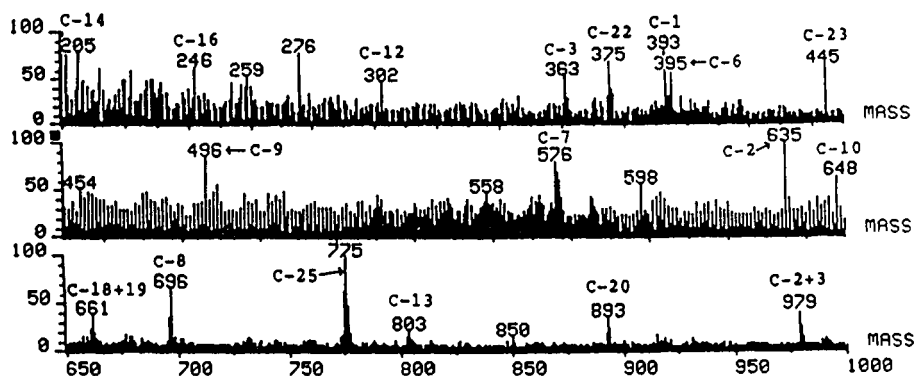


Figure 4. FAB-MS chymotryptic peptide map of native lysozyme. Peptides are labeled in the order of cleavage from the N-terminal of the protein (C-1 through C-23).

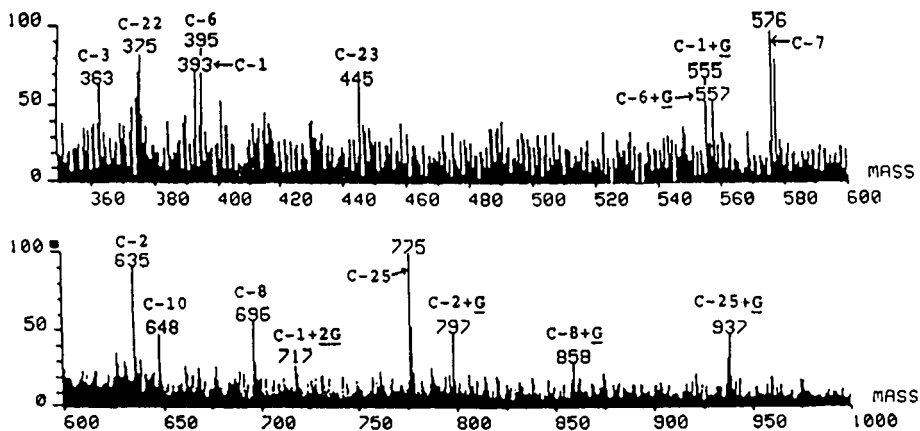


Figure 5. FAB-MS chymotryptic peptide map of glycosylated lysozyme. Glycosylated peptides containing one (+G) or two (+2G) Amadori adducts are found at 162 or 324 mass unit increments above the corresponding native peptide.

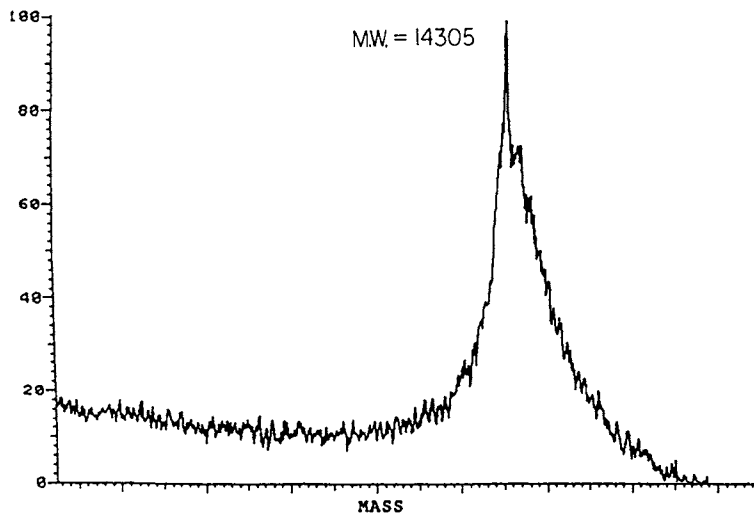


Figure 6. Molecular ion envelope of native lysozyme (M.W. 14,305) obtained using FAB ionization on a high field mass spectrometer.

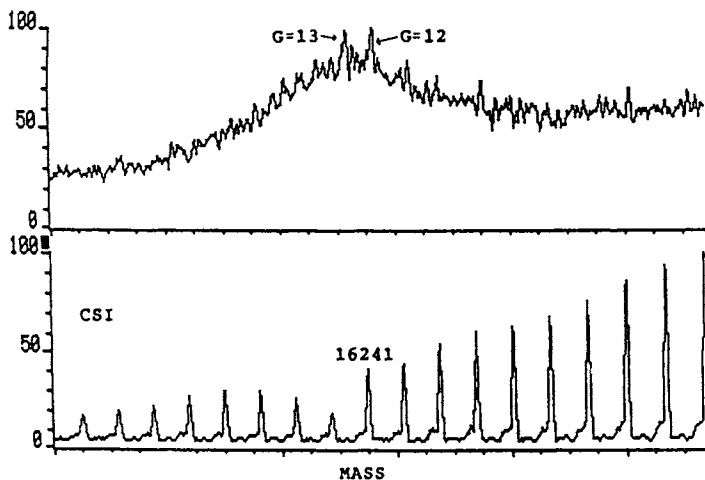


Figure 7. Molecular ion envelope of glycosylated lysozyme Amadori products (top) and cesium iodide reference mass cluster ions (bottom). The centroided masses of the two labeled peaks are 16,265 and 16,427 corresponding to lysozyme Amadori adducts containing 12 (G=12) and 13 (G=13) glucose residues respectively.

glucose residues respectively. We believe this to be the highest molecular weight Maillard reaction product ever to be characterized by mass spectrometry.

Plasma desorption mass spectrometry, also known as fission fragment induced desorption is another technique which is ideal for the analysis of high molecular weight polar compounds such as proteins (12). This technique uses high energy fission fragments emitted from an unstable Californium isotope (^{252}Cf) to ionize molecules deposited as a thin film on an aluminized mylar foil. The molecular ions thus produced are then sorted using a TOF (time of flight) mass analyzer which resolves ions as they drift through a field free region at different rates.

Liquid Chromatography - Mass Spectrometry (HPLC-MS)

The development of an effective interface for combined HPLC-MS operation has proceeded slowly. This is largely due to difficulties in removing a liquid mobile phase and enriching the solute prior to introduction into a high vacuum system. In spite of severe difficulties four systems, direct liquid introduction (DLI), thermospray (TSP), particle beam (PB or MAGIC), thermobeam (TB) and moving belt have been developed. These techniques have been reviewed (13-16). Although each of these systems have advantages and disadvantages we will limit this discussion to the thermospray technique which was pioneered by Vestal, Blakely and coworkers (17). Thermospray ionization is accomplished by vaporizing a high pressure liquid stream as it passes through a narrow diameter (100 micron) resistively heated tube. The vapor jet (containing analyte ions and molecules in aerosol droplets) is then directed through a sampling cone into a high pressure ion source where further desolvation occurs. Evaporating solvent molecules are then pumped away often with the aid of cryotrap. Ions which form in solution or by gas phase chemical ionization type reactions are released from the micro-aerosol droplets and mass analyzed. Initially, the TSP technique referred to the production of ions in solution from proton transfer reactions with ammonium acetate buffer. In this mode of operation primarily protonated molecular ions (or $\text{M}+\text{NH}_4^+$ ions) are produced with little fragmentation. The technique has been modified to include filaments, high voltage discharge electrodes and repellers in the source design. These additions create CI plasma-like conditions in the source which tends to enhance ionization and increase fragmentation which is often desirable for structure elucidation. Commercial TSP ionization sources are available for quadrupole or magnetic sector mass spectrometers. However, quadrupole instruments are favored since they are more tolerant of the high pressures inherent to TSP operation. Even more ideal is the recent introduction of dedicated HPLC-MS instruments which alleviate the set up and optimization problems associated with the need to constantly interchange ion sources on large research grade instruments. TSP ionization works equally well in isocratic or gradient mode and virtually all solvents are tolerated. However, when choosing solvents some familiarity with high pressure CI reactions is needed since cluster ion artifacts are often produced which may interfere with masses of

interest. As is the case with all HPLC-MS techniques only volatile buffers or mobile phase modifiers can be used. Acids such as hydrochloric, acetic and trifluoroacetic work well as does ammonium acetate, ammonium hydroxide and simple alkyl amines. The use of HPLC-MS adds a dimension of specificity and selectivity previously unattainable with UV-VIS detection. Sensitivity, of course will vary depending on the chemistry of the individual species. In general compounds which contain π -bonded heteroatoms such as a carbonyl moiety (good Bronsted bases) will produce excellent TSP spectra. Compounds lacking significant ionizable functionalities such as aliphatic hydrocarbons are invisible in TSP. These differences can often be exploited advantageously when targeting compounds in complex matrices. We routinely perform TSP-HPLC-MS using a UV detector in-line before the vaporizer probe therefore detector response comparisons can be readily obtained. Figure 8 shows the TSP ion chromatogram and spectra of a lysozyme derived tryptic peptide fraction obtained in discharge ionization mode. The HPLC separation was performed using a water-methanol gradient (5% MEOH to 20% in 10 minutes) on a 25cm x 0.45mm i.d. C-18 column. Note the low intensity of the $M+H^+$ ions and the abundant fragmentation as compared to the FAB spectra shown previously in Figures 4 and 5 which contain only protonated molecular ions. These peptide fragmentations which contain numerous primary bond cleavages and proton rearrangements are typical of the CI-like gas phase reactions promoted by discharge ionization. Fenselau *et al.* (18) made a comparison of TSP and FAB ionization using ammonium acetate as the sole source of ion production in TSP. Their conclusion was that the TSP technique does appear to produce more fragmentation and less abundant molecular ion species than does FAB. Another example of TSP is the analysis of thiamine (vitamin B₁) which acts as a flavor precursor in that it decomposes with heat and or acid and alkali conditions to yield alkyl thiazole heterocycle compounds. These compounds are thought to be responsible for slight off-odors in various foods and beverages following thermal processing. Figure 9 is the TSP spectra of thiamine dihydrochloride produced using discharge ionization. The two characteristic ions at 265 and 144 correspond to the $M+H^+$ species (minus $2Cl^-$) and the thiazole fragment respectively. To evaluate the sensitivity of the technique the thiazole fragment ion (144) was monitored as repetitive loop injections containing lesser amounts of the compound were made. With selected ion monitoring the 144 ion was readily observed down to a level of 20 nanograms. In general we have found HPLC-MS to be an order of magnitude less sensitive than GC-MS. However, this is indeed an accomplishment when one considers that this technique opens up a new realm of investigation in the area of nonvolatile or thermally labile compounds.

Tandem Mass Spectrometry (MS-MS)

The roots of tandem mass spectrometry can be traced back to fundamental studies conducted by McLafferty and coworkers in the late 1960's on metastable ion analysis (19-21). Metastable ions are ions which undergo decompositions in the field free regions of mass spectrometers giving rise to unusual diffuse peaks in the resulting

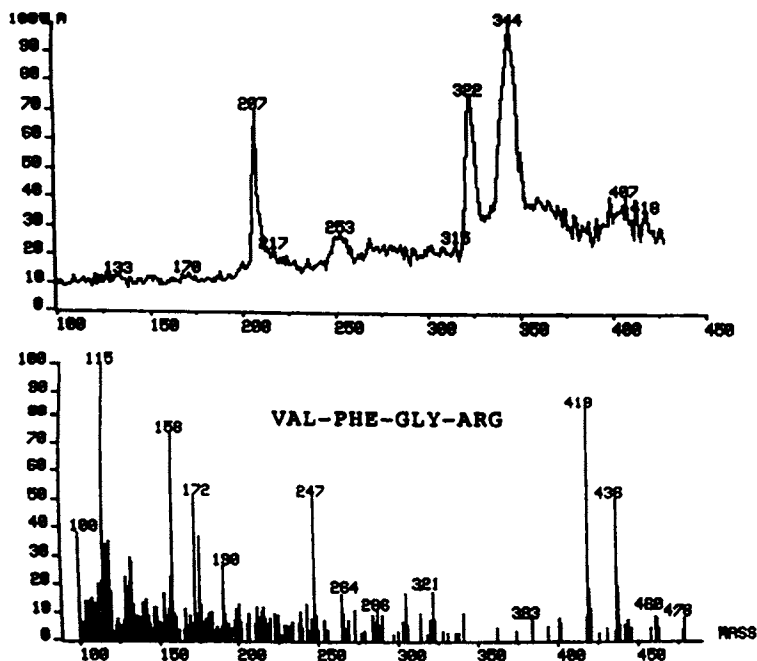


Figure 8. Ion chromatogram (top) of a lysozyme derived tryptic peptide fraction obtained using thermospray discharge ionization HPLC-MS. The resulting mass spectrum of one of the peptides (val-phe-gly-arg) is shown (bottom).

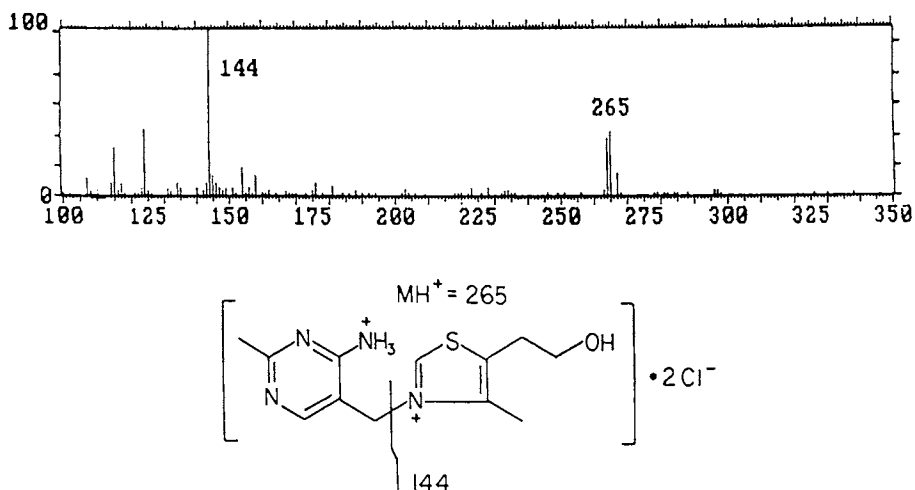


Figure 9. Mass spectrum of thiamine dihydrochloride obtained using thermospray discharge ionization HPLC-MS.

spectra. The study of metastable ions eventually resulted in the development of linked scanning techniques which were first used to determine daughter ions of a parent ion in a mass spectrum. In linked scanning techniques the accelerating voltage, magnetic sector and electrostatic analyzer on a double focusing instrument are independently scanned in various combinations. Different types of linked scans allowed for the determination of daughters, parents, neutral loss or metastable ion compositions. Throughout the 1970's modified instruments began to appear with three and four sector designs. These instruments enabled investigators to conduct true MS-MS experiments with increased sensitivity and resolution. Fundamental to the success of tandem mass spectrometry was the use of collisionally activated dissociation (CAD) to aid in fragmenting a preselected ion into daughters. CAD is a process whereby an ion produced in a mass spectrometer is directed into a collision with a neutral gas in a high pressure region causing dissociation of the incident ion into daughter ion fragments. The daughter ions are then mass analyzed by a second mass spectrometer. The CAD process had long since plagued early mass spectrometers where unexplainable signals arose from ion-gas collisions in instruments with poor vacuum systems. In the early 1980's explosive advancement occurred in the field of MS-MS as investigators realized the potential of this technique for target compound analysis in complex mixtures. In this type of application samples with little or no cleanup are ionized in MS 1 and an ion corresponding to a compound of interest could be subjected to CAD and the resulting daughter ions are then mass analyzed in MS 2. This highly sensitive and selective process is accomplished in a time frame of approximately 10^{-5} seconds. This technique is particularly well suited for FAB ionization since the chemical noise background produced from the matrix is effectively filtered out by MS-1, thereby increasing the signal to noise ratio in MS-2. Instrument manufacturers were quick to respond and various types and combinations of tandem mass spectrometers became commercially available. Basically three types of tandem instruments became popular, triple quadrupoles, hybrid instruments (sector instrument for MS-1 and quadrupole for MS-2) and four sector instruments. Each design has advantages and disadvantages. In triple quads and hybrid instruments the CAD process is carried out in a small quadrupole lens system which provides focusing of ions scattered in the collision process thereby improving transmission into MS-2. However quadrupoles are low energy, low resolution mass analyzers and suffer the disadvantages of low energy collisions (insufficient energy available to induce fragmentation in the CAD process) and poor resolution of daughter ions. The problem of resolution is even more acute in MS-2 because the energy spread resulting from the CAD process can be severe enough to cause incorrect mass assignments. Hybrid instruments have the advantage of high resolving power in MS-1 but share the same problems of low energy collisions and insufficient resolution in MS-2. Four sector instruments have the advantage of high energy collisions and high resolving power in MS-1 and 2. However, the design of the collision cells in these instruments cannot provide for focusing therefore, the acceptance angle of scattered ions is reduced as compared to triple quads or hybrids. This translates into lower ion beam trans-

mission rates from MS-1 to MS-2 and hence a decrease in the sensitivity of daughter ion detection. Despite these problems each instrument design has been found to be useful for certain applications. In general triple quads and hybrids perform well for routine low mass (<1000) applications. When higher molecular weight compounds such as peptides or oligosaccharides are analyzed then the high energy collisions obtainable on four sector instruments is preferred. With four sector instruments mass assignments in MS-2 are more reliable and in theory even high resolution (10,000-25,000) analysis of daughter ions is possible. Regardless of the type of instrument many useful experiments are made possible by MS-MS. The use of tandem mass spectrometry for complex mixture analysis has been highly promoted but this represents only one of the many potential applications. Daughter ion, parents, neutral loss and many other experiments are possible. Tandem MS has been found to be particularly useful for peptide sequencing studies. Indeed MS-MS is ideal for producing structurally significant fragments from pseudomolecular ions formed via the desorption techniques described in this article. We have made extensive use of this technique in our Maillard reaction studies. At one point in the investigation a question arose pertaining to the identity of certain lysozyme derived chymotryptic peptides. Chymotrypsin cleaves peptides at the carboxyl side of hydrophobic amino acid residues (tyr, trp, phe, leu, ile etc.). However, we were observing peptides which were thought to arise from cleavage at asparagine residues. Furthermore, the mass of one such peptide at 893 was similar to one obtained in tryptic digests (894) raising the possibility that tryptic activity may have been present in the chymotrypsin enzyme preparation. To solve the problem we used MS-MS to ascertain the sequences of the various peptides in question. Figure 10 shows the CAD derived daughter ion spectra of the 893 molecular weight peptide fragment. This spectra was generated on a four sector instrument in a high energy collision process. The observed fragmentation pattern is unambiguous and allows for complete sequence determination. In contrast Figure 11 is the CAD daughter ion spectra of the 894 M.W. tryptic peptide obtained using a hybrid MS-MS instrument under low energy collision conditions. Although sufficient fragmentation was induced to establish that the two peptides are not identical it was not enough to characterize the entire sequence. These results are typical of our observations and those of many other investigators. Nonetheless, the answer to our problem was chymotrypsin indeed cleaved lysozyme at asparagine residues although only when next to an acidic or basic residue. A careful review of the literature indicated that this cleavage has been reported despite the fact that it is a rare occurrence.

We hope that this manuscript will encourage flavor chemists to understand and better utilize the modern techniques available in mass spectrometry. In particular we believe these desorption techniques and associated chromatographic interfaces to be especially useful for the is of food derived peptides, amino acids, polyhydroxypyrazines and other polar or high molecular weight components of caramelization and Maillard reactions.

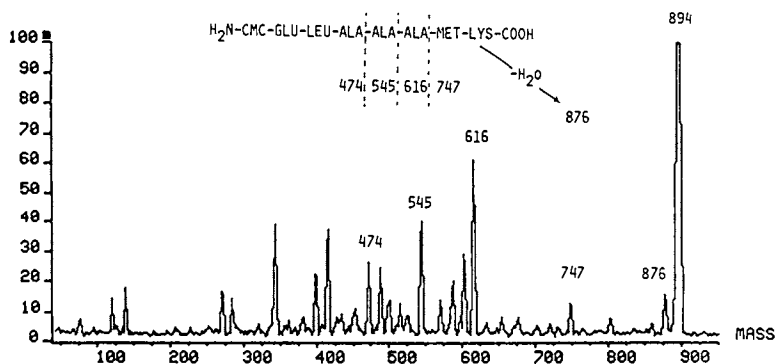


Figure 10. FAB-MS-MS daughter ion spectrum of a lysozyme derived 893 m.w. chymotryptic peptide fragment yielding sequence information. The spectrum was obtained using high energy collision on a four sector instrument.

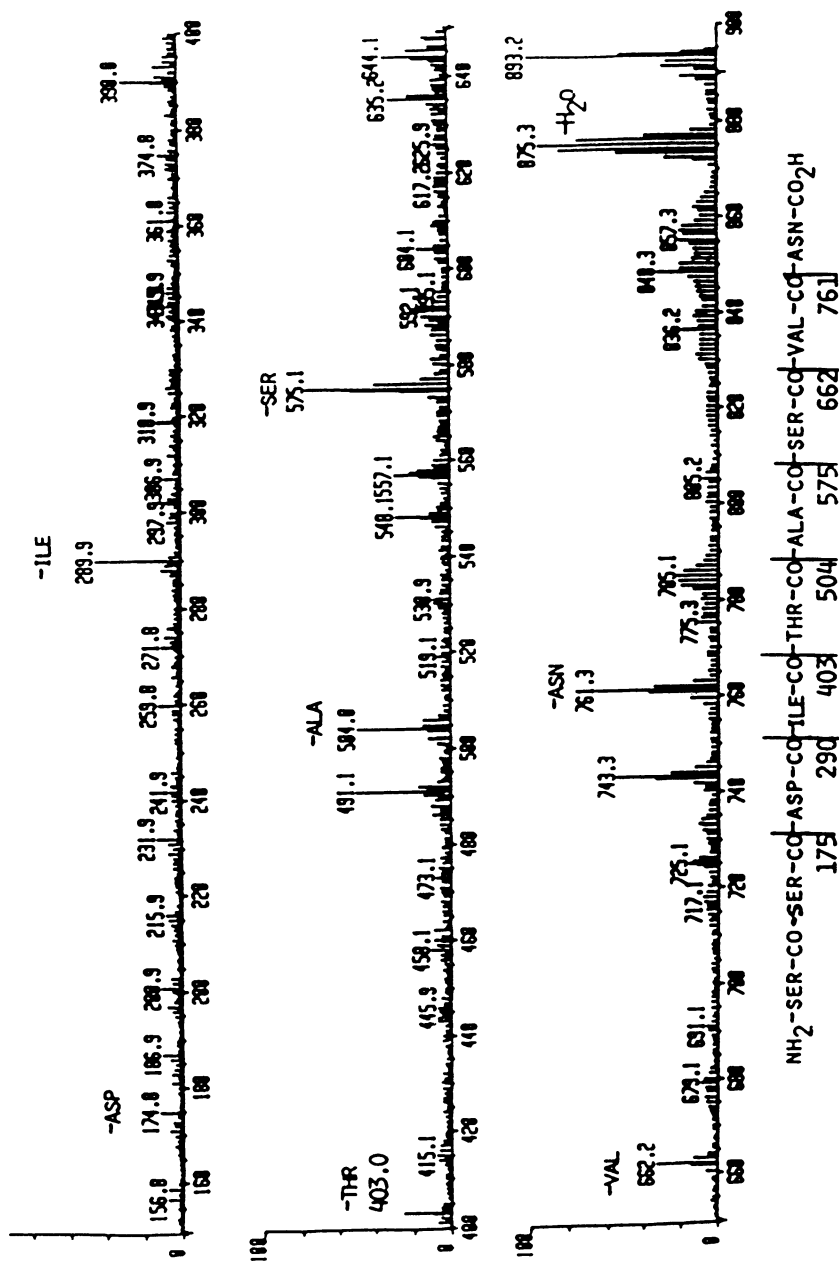


Figure 11. FAB-MS-MS daughter ion spectrum of a 894 m.w. lysozyme tryptic peptide obtained using a hybrid MS-MS instrument under low energy collision conditions.

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Chapter 9

Thermal Decomposition of Lipids

An Overview

Wassef W. Nawar

Department of Food Science and Nutrition, University of Massachusetts,
Amherst, MA 01003

The chemistry of lipid decomposition in foods at elevated temperatures is complicated. Multiple reactions and interactions can occur rapidly and competitively. The rates and pathways of these reactions are influenced significantly by temperature, reaction time, other constituents in the surrounding environment, physical state, and molecular organization.

The basic mechanism of autoxidation at elevated temperatures is similar to that of room-temperature oxidation, i.e., a free radical chain reaction involving the formation and decomposition of hydroperoxide intermediates. Although relative proportions of the isomeric hydroperoxides, specific for oleate, linoleate and linolenate, vary with oxidation temperatures in the range 25°C - 80°C, their qualitative pattern is the same (1). Likewise, the major decomposition products isolated from fats oxidized over wide temperature ranges are those reflecting autoxidation of their constituent fatty acids (2-6). The mechanisms and products of lipid oxidation have been extensively studied. The reader is referred to the numerous monographs, reviews and research articles available in the literature (1,4,7,8,9,10,11).

The bulk of our knowledge regarding thermal oxidation has been derived from studies with model systems of fatty acids and their derivatives, or with individual natural oils (2,3,6,12,13,14,15,16). However, in biological systems as complex as food, lipids usually exist in a complicated environment markedly different from that of the single phase model system. In cell membranes, for example, the lipid molecules are highly ordered, relatively restricted in distance and mobility, and closely associated with different neighboring molecules, e.g., other lipids, protein, cholesterol, water, pro- and antioxidants. What influence does such an environment have on the oxidation of the lipids at elevated temperature? Even in less organized systems, e.g., depot fat from animal or plant, the lipids

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come in contact with other food constituents for extended periods at relatively high temperatures, as in the case of frying, broiling and baking.

In view of the above I have chosen to emphasize, in this article, three areas which in my opinion merit additional focus: (1) unique aspects of high-temperature oxidation; (2) interactive effects; (3) influence of physical state.

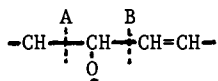
UNIQUE ASPECTS OF HIGH-TEMPERATURE OXIDATION

Studies in which lipid oxidation at different temperatures was compared are limited. However, certain specific differences between high and low temperature oxidations have been observed. The following are some examples:

Hydroperoxide Levels. In thermally oxidized fats hydroperoxides are usually very low. At higher temperatures, oxidation proceeds rapidly and the rate of hydroperoxide decomposition exceeds that of hydroperoxide formation (17,18). For example, when ethyl linoleate was oxidized at 70°C, peroxide content reached a maximum of 1777 meq/kg after 6 hr then decreased to 283 meq/kg after 70 hr. At 250°C, on the other hand, peroxide value reached a maximum of only 198 meq/kg after 10 min, and was zero after 30 min.

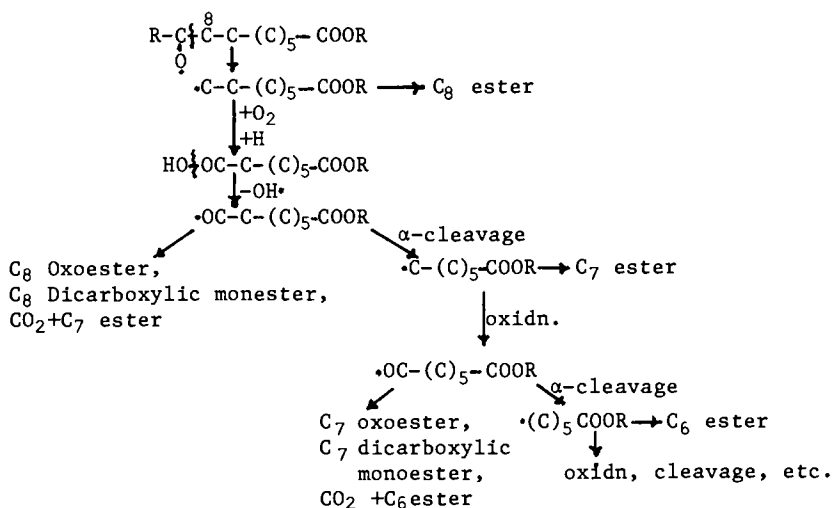
Quantitative Aspects. Although the major products isolated from heated fats are largely typical of lipid autoxidation, and in spite of the fact that the total amount of these products increases significantly with increased temperatures of heating, the flavor qualities of heated and oxidized fats are markedly different. This is most likely due to quantitative differences in the decomposition product patterns rather than to the presence or absence of individual compounds. It should be realized that the amount of a given decomposition product at any given time during the course of oxidation is determined by the net balance between complex reactions occurring simultaneously and competitively. Since the rates of these reactions are influenced differently by temperature, the quantitative pattern of the resulting products will obviously be different at different temperatures.

Aldehyde Formation. Several investigators observed a marked dominance of hexanal in the volatile products of low-temperature oxidation. At the higher temperatures, however, 2,4-decadienal was the major aldehyde formed (19,20,21). Both aldehydes are typical scission products of linoleate hydroperoxides. Swoboda and Lea (20) explained this difference on the basis of a selective further oxidation of the dienal at the higher temperature, while Kimoto and Gaddis (19) speculated that the carbon-carbon bond between the carbonyl group and the double bond (Type B) is the most vulnerable to cleavage under moderate conditions of autoxidation, while scission at the carbon-carbon bond away from the olefinic linkage (Type A) is favored under stress such as heat or alkali.



The 13-hydroperoxide of linoleate would thus produce more hexanal at lower temperatures while 2,4-decadienal from the 9-hydroperoxide isomer predominates at elevated temperatures. Although our quantitative work with propyl linoleate (21) supports this rationale, i.e., a temperature-dependent preferential cleavage, the pattern was not as clear when linoleate was oxidized at three different temperatures (18). The more unsaturated substrate oxidized much faster and many of the oxidation products, themselves polyunsaturated, readily underwent further decomposition. Consequently some of the predicted compounds could not be detected while others were formed which were not expected from direct alkoxy radical cleavage. Furthermore, the amounts of volatiles present varied markedly with heating time. It is not surprising, therefore, that at high temperatures, and particularly with polyunsaturated systems, it has been extremely difficult to establish the nature and extent of preferential hydroperoxide scission.

Secondary Decomposition and Polymerization. Reactions which usually occur in the later stages of autoxidation at room temperature may assume increased significance, and their consequences may be encountered much more rapidly, at elevated temperatures. For example, the series of short chain esters, oxo-esters, and dicarboxylic acids - usually formed in only trace amounts in room-temperature oxidation of oleate, linoleate and linolenate - can be found in significant amounts after heating for 1 hr at 180°C. Possible reactions leading to the formation of such compounds are given below.



The C8 aldehyde ester may be produced by cleavage of the 9-hydroperoxide of ethyl linoleate followed by terminal hydroperoxidation. Further oxidation would produce the corresponding dicarboxylic acid which upon decarboxylation would give rise to ethyl heptanoate. The 8-alkoxy radical may also decompose to give the C7 alkyl radical, which would yield ethyl heptanoate or form a terminal hydroperoxide, and so on. Polymerization, both intra- and intermolecular, is also a major reaction in high temperature oxidation. Combination of alkyl, alkoxy, and peroxy radicals yields a variety of dimeric and polymeric compounds with C-O-C or C-O-O-C crosslinks.

Cyclic Compounds. Certain cyclic compounds identified in heated polyunsaturated esters could be explained only by invoking mechanisms which involve allylic proton abstractions outside the 1,4-pentadiene systems (22).

Thermolytic Reactions. At the higher temperatures, nonoxidative reactions also occur as for example the formation of tetrasubstituted cyclohexenes via Diels-Alder reaction, or the formation of dehydrodimers and mono- or polycyclic dimers via combination of alkyl free radicals or free radical attack on double bonds.

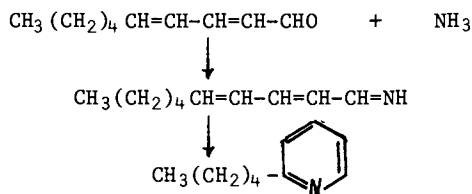
Decomposition of Saturated Fatty Acids. Saturated fatty acids which are extremely stable at low temperatures and rarely contribute to the oxidative decomposition pattern, can play a significant role when temperatures higher than 150°C are used. Non-oxidative reactions of triacylglycerols are responsible for the formation of alkane and 1-alkene series, propene- and propanediol diesters, oxopropyl esters, symmetric ketones and fatty acids (12,16,23). If heated in air at high temperature the saturated fatty acids undergo oxidative decomposition. It is believed that the principal mechanism involves the formation of monohydroperoxides, and that oxygen attack may occur at all methylene groups of the fatty acid chain with locations near the ester carbonyl favored. Products of the saturated fatty acids include methyl ketones, aldehydes, lactones, and hydrocarbons (12,16). Formation of the odorous lactones and methyl ketones typical of dairy products are believed to originate from the saturated hydroxy fatty acids and beta-keto acids, respectively, which naturally occur in milk fat.

THERMAL INTERACTIONS OF LIPIDS AND PROTEINS

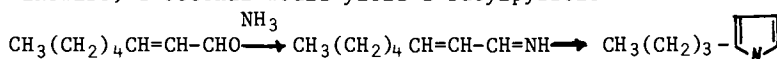
The nature of protein-lipid complexes and the consequences of interaction between lipids and proteins in biosystems have been extensively studied. The reader is referred to the elegant review by Karel (24). It is generally recognized that lipid-protein interactions are involved in a variety of physical and chemical changes which are important to food quality, as for example in the aging of meat or the frozen storage of fish. The reaction of proteins with peroxidizing lipids and/or their breakdown products received particular attention (25-29). However, in spite of the fact that food is frequently subjected to heat at numerous stages in the course of its processing and preparation, reports on lipid-protein

interactions at elevated temperatures are scarce and the specific role of temperature in such interactions is imperfectly understood. We can only give some examples.

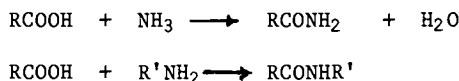
N-Heterocyclics. The reaction of primary amines with the carbonyl products derived from lipid oxidation is a major pathway in lipid-protein interactions. Formation of Schiff's base intermediates followed by cyclization and rearrangement can yield imines, pyridines and pyrroles (5,15,30,31). For example, 2-pentylpyridine may result from the reaction of ammonia with 2,4-decadienal, one of the principle aldehydes from the autoxidation of linoleate (5).



Likewise, 2-octenal would yield 2-butylpyrrole



Amides and Nitriles. Primary amines or ammonia, from the thermal decomposition of amino acids, can be acylated by carboxylic acids to produce amides (30).



The amides can decompose further to give nitriles



Substantial yields of N-substituted amides were observed by Sims and Fioriti (32) when safflower oil or methyl esters were heated with simple alpha-amino acids at temperatures above 150°C. The reaction is believed to involve decarboxylation of the amino acid and displacement of the alcohol moiety of the fatty ester by the amine which is formed. These authors concluded that the ester carbonyl group may participate in the decarboxylation reaction since no CO₂ was evolved and methionine could be recovered quantitatively when it was heated in mineral oil at 200°C for prolonged periods.

Interactions with Histidine. Imidazole lactic acid and imidazole acetic acid were identified as breakdown products when histidine was reacted with methyl linoleate, methyl linoleate hydroperoxide or hexanal for 3 weeks at 25°C and 51°C (33). It was postulated that these compounds were formed via free radical reactions. Two other products were also produced which yielded histidine upon acid hydrolysis. These were thought to be Schiff's base compounds arising

from condensation of the histidyl alpha-amino group with the carbonyl groups of aldehydes from peroxidation of the linoleate.

Interactions with Hexanal and Pentanal. Various compounds identified in French-fried potato flavor, e.g., 2-hexyl-4,5-dimethyloxazole and 2-pentyl-3,5-dibutylpyridine, and in fried chicken flavor, e.g., 3-methyl-5-pentyl-1,2,4-trithiolane (34,35) are believed to arise from the interaction of hexanal or pentanal, both major components of lipid oxidation, with products of protein decomposition.

Lipid-Protein-Carbohydrate Interactions. Evidence for such complex interaction was recently reported by Huang et al (36) who observed that the addition of corn lipids to zein and corn carbohydrates enhanced the formation of alkylpyrazines, indicating that lipid-derived free radicals may accelerate the rate of Maillard reactions. Two of the alkylpyrazines, identified in such mixtures after heating for 30 minutes at 180°C, have 5-carbon alkyl substitution at the pyrazine ring and could only be explained by interaction of lipid or lipid decomposition products. These authors suggested that condensation of amino ketones, formed by protein-carbohydrate interaction, may yield 3,6-dihydropyrazine which would in turn react with pentanal, a lipid oxidation product, to form 2,5-dimethyl-3-pentylpyrazine.

Interactions with Membrane Components. In aqueous systems milk fat globule membrane lipids and the non-lipid membrane solids were found to accelerate the oxidation of milk fat at 50°C, but exhibited antioxidant effects at 95°C (Chen, Z. Y.; Nawar, W. W., University of Massachusetts at Amherst, unpublished data).

Cholesterol Oxidation. In our recent studies on interaction effects in cholesterol oxidation, we observed that various amino acids and phospholipids inhibit, while triacylglycerols accelerate, the oxidation of cholesterol at 180°C. Some phospholipids were protective while others acted as prooxidants at 130°C, and some exhibited acceleration at the beginning of heating followed by protection (Kim, S. K.; Li, Y. G.; Nawar, W. W., University of Massachusetts at Amherst, unpublished data).

EFFECTS OF PHYSICAL STATE

To gain insight into the effect of physical state and/or molecular organization on lipid oxidation, a variety of model systems have been used. These include dispersions, liposomes or vesicles (37,38), monolayers adsorbed on silica (39,40,41), and red blood cell ghosts (42). In most of these studies, oxidation was conducted at relatively low temperatures, i.e., 20 - 40°C. Very little information is available on the effects of physical state on high temperature oxidative reactions or interactions of lipids.

Molecular Order. The autoxidation of linoleic acid in monolayers at 60°C differed from that in bulk in that it involved no lag periods, was considerably faster, and exhibited first order kinetics implying that the overall reaction is not autocatalytic (41). More recently

we compared monolayer and bulk autoxidation at 60°C and 180°C (39). As expected, the rate of oxidation was much faster at the higher temperature for both the adsorbed and the bulk samples. At 60°C ethyl linoleate oxidized faster when adsorbed on silica than when in bulk. On the other hand, oxidation of the bulk samples was faster at 180°C than that of the monolayers (Fig. 1). The faster oxidation of linoleate in monolayers at 60°C is probably due to the larger surface and the more facile exposure to oxygen. This, however, appears to be offset by the higher mobility of the substrate molecules and the faster radical transfer in bulk at the higher temperature.

The products of lipid oxidation in monolayers were also studied. Wu and coworkers (41) concluded that epoxides rather than hydroperoxides might be the major intermediates in the oxidation of unsaturated fatty acids adsorbed on silica, presumably because of the proximity of the substrate chains on the silica surface. In our work with ethyl oleate, linoleate and linolenate which were thermally oxidized on silica, the major decomposition products found were those typical of hydroperoxide decomposition (39). However, the decomposition patterns in monolayers were simpler and quantitatively different from those of bulk samples. For example, bulk samples produced significantly more ethyl octanoate than those of silica, whereas silica samples produced more ethyl 9-oxononanoate than those of bulk. This trend was consistent regardless of temperature, heating period or degree of oxidation. The fact that the same pattern of volatiles was found at both 60°C and 180°C implies that the same mode of decomposition occurs over this temperature range.

Another difference between bulk and monolayer oxidations was observed in the amounts of alkanes and alkenes produced. In bulk, the alkanes in ethyl palmitate heated for 1 hr at 180°C were greater than the 1-alkenes, indicating that hydrogen abstraction is the preferred route for termination of the alkyl radicals which are formed by hydroperoxide scission. The reverse was true in the ordered state. Restriction of the alkyl radicals limits abstraction of hydrogen from other substrate molecules to form alkanes thus favoring the production of 1-alkenes (Hau, L. B.; Nawar, W. W. University of Massachusetts, Amherst, unpublished data).

Although we confirmed the formation of epoxides in the case of monolayers, we suggested that their formation may be the result of a catalytic effect of silica, rather than that of an interaction between the rigidly oriented neighboring molecules as explained by Mead's group. Possibly, hydroperoxide intermediates are the major primary products in the adsorbed phase as well, and the acidic nature of silica favors a selective heterolytic cleavage as proposed by Kimoto and Gaddis (19).

Effect of Melting. Below its melting point pure cholesterol is resistant to oxidation as long as it remains solid. Thus, at 130°C it remains stable up to a point which coincides with visible melting when a sudden rise in oxidation rate occurs (Kim, S. K.; Nawar, W.W., University of Massachusetts at Amherst, unpublished data). This phase transition during oxidation at 130°C is believed to reflect the influence of the trace amounts of oxides formed on the melting point.

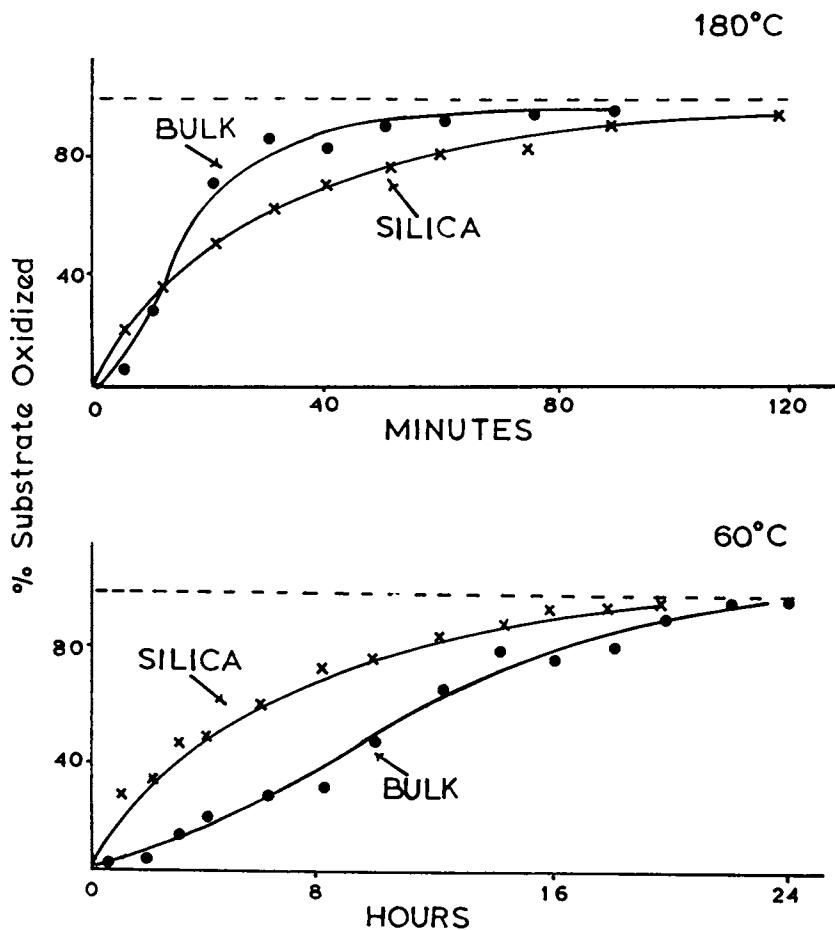


Figure 1. Rate of ethyl linoleate oxidation on silica and in bulk at 60 °C and 180 °C. (Reprinted with permission from ref. 39. Copyright 1988 American Oil Chemistry Society.)

Dry vs. Aqueous States. In the dry state, milk fat globule membrane, the membrane lipids and the non-lipid membrane solids inhibited the oxidation of milk fat at both 50°C and 95°C. In the presence of water, however, all three enhanced the oxidation at 50°C but remained protective at 95°C. Moreover, various amino acids which inhibited the oxidation of milk fat at 95°C in the dry system acted as prooxidants when water was present (Chen, Z. Y.; Nawar, W. W., University of Massachusetts at Amherst, unpublished data). The effects of amino acids on lipid autoxidation in emulsions, in oils, and in freeze-dried systems were also investigated (43,44). The amino acids which were tested were all prooxidants (with the exception of cysteine) in the dried systems. Farag and Osman (44) attributed the prooxidant effect of amino acids in aqueous media predominantly to the presence of the protonated amino nitrogen. Prooxidant/antioxidant effects varied with type of emulsifiers used, pH, and presence of added sugar. Sims et al (45) suggested that the protective effect of added sugar is not due to an actual antioxidant effect but rather to an improved resistance to phase separation resulting in a lower concentration of oxygen in the aqueous phase and a slower diffusion of the gas through the oil-water interface.

Physical Barriers. Wu et al (46) observed that the inclusion of palmitic acid or cholesteryl acetate in linoleic acid monolayers on silica, exerted a protective effect against oxidation. They suggested that these compounds act as a spacer keeping the linoleic acid molecules farther apart while being only slowly oxidized themselves. Similarly, our recent work with cholesterol oxidation appears to indicate that carbohydrates do not change the pathway of cholesterol oxidation but rather act as a physical barrier against the migration of reactive species.

Conclusion

Space does not allow a complete citing of the literature on thermal decomposition of lipids. Nor was it intended to list the hundreds of products which were identified in heated fats and rationalize mechanisms for their formation. The above discussion is an attempt to identify certain factors which influence the fate of lipids in complex biological systems when subjected to high temperature. It should be obvious that the potential for the multiple reactions and interactions which could take place in such systems is enormous. Those which do occur, their rates, and routes, depend on a complicated balance between the influences of many factors. Major among these are temperature, reaction time, other constituents in the environment, physical state, and molecular organization. Indeed, studies with model systems are invaluable, however, perfect simulation of the natural situation is practically impossible. The model and the real systems are still far apart and much more research is required with both to better understand the wide gap in between.

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Chapter 10

Contribution of Lipids to the Formation of Heterocyclic Compounds in Model Systems

Chi-Tang Ho, Linda J. Bruechert, Yuangang Zhang, and E-Mean Chiu¹

Department of Food Science, New Jersey Agricultural Experiment Station,
Cook College, Rutgers, The State University of New Jersey,
New Brunswick, NJ 08903

Heterocyclic compounds are primarily formed through non-enzymatic browning reactions. Recent studies of deep-fat fried food flavors led to the identification of pyrazines, pyridines, thiazole, oxazoles and cyclic polysulfides which had long-chain alkyl substitutions on the heterocyclic ring. The involvement of lipid or lipid decomposition products in the formation of these compounds could account for the long-chain alkyl substitutions. Model systems were used to study the participation of lipids in the formation of pyrazines, pyridines, thiophenes and cyclic polysulfides.

More than 10,000 compounds have been identified as volatiles of foods. Of these, heterocyclic compounds are an important class, because of their exceptional sensory properties (1). Heterocyclic compounds contain one or more heteroatoms (O, S and/or N) in rings or fused ring systems.

The majority of heterocyclic compounds are formed through thermal interactions of reducing sugars and amino acids, known as Maillard reactions. Other thermal reactions such as hydrolytic and pyrolytic degradation of food components (e.g. sugars, amino acids and vitamins) and the oxidation of lipids also contribute to the formation of heterocyclic compounds responsible for the complex flavor of many foodstuffs.

Recent studies in our laboratory show that lipids may be directly associated with the Maillard reaction in the formation of some heterocyclic compounds. The effect of lipids on the formation of heterocyclic compounds in a model Maillard reaction has also been reported by Mottram and Whitfield (2).

This paper discusses model studies which indicate that lipid decomposition products such as 2,4-decadienal and hexanal may react with Maillard reaction intermediates to form heterocyclic compounds.

¹Current address: Development Center for Biotechnology, 81 Chang Hsing Street, Taipei, Taiwan, Republic of China

Lipid Degradation Products and Pyrazines

Alkylpyrazines have been recognized as important trace flavor components of a large number of cooked, roasted, toasted and deep-fat fried foods (3). As a rule, alkylpyrazines have a roasted nut-like odor and flavor. Formation pathways for alkylpyrazines have been proposed by numerous researchers (4, 5, 6). Model studies suggest that they are minor products of the Maillard reaction.

Interest in the influence of lipids on pyrazine formation has recently been generated by the identification of long-chain alkyl-substituted heterocyclic compounds in foods and in model systems. Pyrazines in this category include 2-heptylpyrazine isolated from french fried potato flavor (7), and 2-methyl-3(or 6)-pentylpyrazine and 2,5-dimethyl-3-pentylpyrazine, isolated from extruded zein/corn amylopectin/corn oil systems (8, 9). Only the involvement of lipids or lipid-decomposition products in the formation of these compounds could account for the long-chain alkyl substitution on the pyrazine ring.

The relationship between lipid degradation products and long-chain alkyl substituent of pyrazines was investigated by examining the reaction products generated from the three model systems, acetol/ammonium acetate, acetol/ammonium acetate/pentanal and acetol/ammonium acetate/hexanal. Confirming the result of Rizzi (10), 2,5-dimethylpyrazine and 2,6-dimethylpyrazine were the major products in the reaction of acetol with ammonium acetate. In the acetol/ammonium acetate/pentanal system, 2,5-dimethyl-3-pentylpyrazine and 2,6-dimethyl-3-pentylpyrazine were the major products, and in the acetol/ammonium acetate/hexanal system, 2,5-dimethyl-3-hexylpyrazine and 2,6-dimethyl-3-hexylpyrazine were the major products. This result suggests that 2,5-dihydropyrazine intermediates either undergo dehydrogenation to form pyrazines or they react with aldehydes to generate pyrazines with corresponding alkyl substituents. The competition of pentanal or hexanal for 2,5- or 2,6-dimethyldihydropyrazine might also account for the diminishment of 2,5- or 2,6-dimethylpyrazine in acetol/ammonium acetate systems when pentanal or hexanal is included. Possible formation pathways of 2,5-dimethylpyrazine and 2,5-dimethyl-3-pentylpyrazine in the acetol/ammonium acetate/pentanal reaction are shown in Figure 1.

2,5-Dimethyl-3-hexylpyrazine and 2,6-dimethyl-3-hexylpyrazine were also identified when a solution of hexanal and pyruvaldehyde was heated with various amino acids, including glycine, alanine, valine and leucine, at 180°C.

Interaction of 2,4-Decadienal and Cysteine

The thermal interaction between 2,4-decadienal and cysteine was selected as a model for lipid-protein interaction. 2,4-Decadienal is the major degradation product of linoleic acid and cysteine is a sulfur-containing amino acid in foods. Some heterocyclic compounds identified in the reaction mixture of 2,4-decadienal and cysteine are listed in Table I.

A considerably large number of long-chain alkyl-substituted heterocyclic compounds were detected in this interaction system.

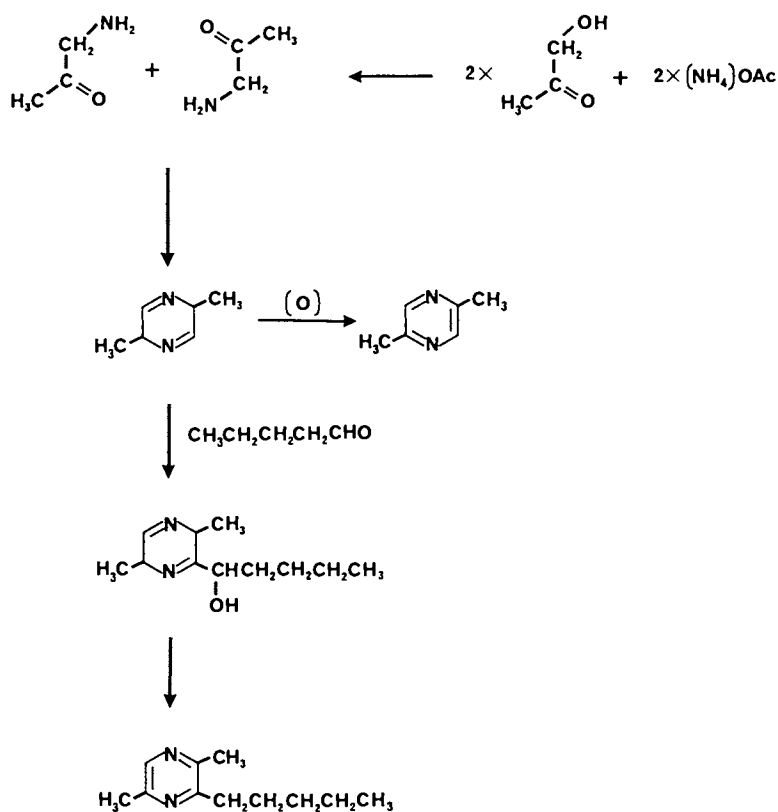


Figure 1. Mechanism for the formation of 2,5-dimethylpyrazine and 2,5-dimethyl-3-pentylpyrazine.

Table I. Some Heterocyclic Compounds Identified from Thermal Interaction of 2,4-Decadienal and Cysteine

Compounds	Amount Produced (mg/mol)
<u>Furans</u>	
2-butylfuran	12.8
2-pentylfuran	6.4
2-hexylfuran	t
<u>Thiophenes</u>	
thiophene	3.5
tetrahydrothiophene-3-one	10.5
2-butylthiophene	57.2
2-formyl-3-methylthiophene	29.8
2-pentylthiophene	13.1
2-hexylthiophene	42.0
2-heptylthiophene	1.8
2-formyl-5(or 3)-pentylthiophene	15.6
<u>Thiazoles</u>	
thiazole	25.6
3-methylisothiazole	2.0
2-acetylthiazole	2.2
<u>Cyclic Polysulfides</u>	
3,5-dimethyl-1,2,4-trithiolane (isomer)	122.8
3,5-dimethyl-1,2,4-trithiolane (isomer)	18.2
3-methyl-5-pentyl-1,2,4-trithiolane	14.3
2,4,6-trimethylperhydro-1,3,5-thiadiazine	828.5
2,4,6-trimethylperhydro-1,3,5-dithiazine	284.2
2,4-dimethyl-6-pentylperhydro-1,3,5-dithiazine	18.9
2-pentyl-4,6-dimethylperhydro-1,3,5-dithiazine	28.7
<u>Pyridine</u>	
2-pentylpyridine	501.5

According to the formation mechanism of heterocyclic compounds from a model system of aldehydes, hydrogen sulfide and ammonia (11), aldehydes such as acetaldehyde and hexanal must have been involved in the formation of heterocyclic compounds identified in this study. As proposed by Josephson and Lindsay (12), under water-mediated conditions, 2,4-decadienal undergoes retro-aldolization to give acetaldehyde and hexanal.

The most interesting compounds identified in the reaction of 2,4-decadienal and cysteine are cyclic polysulfides such as trithiolanes, dithiazines and thiadiazines.

Trithiolanes have received increasing attention since the identification of diastereomeric 3,5-dimethyl-1,2,4-trithiolane in the volatiles of boiled beef (13). The parent 1,2,4-trithiolane is a component of Shiitake mushrooms (14) and red algae (15). In addition to 3,5-dimethyl-1,2,4-trithiolane, Kubota et al. (16) identified 3-methyl-5-ethyl-1,2,4-trithiolane and 3,5-diethyl-1,2,4-trithiolane in both *syn* and *anti* forms in boiled Antarctic Gulls. Both compounds were described as garlicky. Flament and co-workers (17) reported the identification of 3-methyl-5-ethyl-1,2,4-trithiolane and 3-methyl-5-isopropyl-1,2,4-trithiolane in a commercial beef extract.

In addition to 3,5-dimethyl-1,2,4-trithiolane and 3,5-diisobutyl-1,2,4-trithiolane, the two long-chain alkyl-substituted trithiolanes, 3-methyl-5-butyl-1,2,4-trithiolane and 3-methyl-5-pentyl-1,2,4-trithiolane, were reported to be present in fried chicken flavor (11). Along with 3,5-dimethyl-1,2,4-trithiolane, 3-methyl-5-ethyl-1,2,4-trithiolane, 3-methyl-5-propyl-1,2,4-trithiolane and 3-methyl-5-butyl-1,2,4-trithiolane are reported to be important flavor components of Chinese stewed pork (18). In the present study of the interaction between 2,4-decadienal and cysteine, hexanal is involved in the formation of 3-methyl-5-pentyl-1,2,4-trithiolane. 3-Methyl-5-pentyl-1,2,4-trithiolane identified in the volatiles of fried chicken may form through a similar pathway.

In the present study, the two long-chain alkyl-substituted dithiazines, 2,4-dimethyl-6-pentylperhydro-1,3,5-dithiazine and 2-pentyl-4,6-dimethylperhydro-1,3,5-dithiazine were identified in addition to 2,4,6-trimethylperhydro-1,3,5-dithiazine. 2,4,6-Trimethylperhydro-1,3,5-dithiazine, also known as thialdine, has been reported a cooked flavor component of foods (19, 20, 21). Recently, dithiazines containing propyl and butyl substituents such as 2-propyl-4,6-dimethylperhydro-1,3,5-dithiazine and 4-butyl-2,6-dimethylperhydro-1,3,5-dithiazine have been identified in volatile components from cooked *Sakuraebi* (22).

It is also interesting to note that there was no long-chain substituted thiadiazine formed in the reaction of 2,4-decadienal and cysteine although the amount of 2,4,6-trimethylperhydro-1,3,5-thiadiazine was much higher than that of 2,4,6-trimethylperhydro-1,3,5-dithiazine. It was also found that 2,4,6-trimethylperhydro-1,3,5-thiadiazine was unstable and that it disappeared from the gas chromatogram after two weeks, even when the sample was stored in a -40°C freezer. The observed absence of long-chain substituted thiadiazines suggests that the long-chain substituent makes these compounds even more unstable.

Also identified in this system are 2-alkylfurans. 2-Alkylfurans are known thermal and autoxidation products of unsaturated fatty acids (23). 2-Alkylfurans identified in this study are presumably derived by the thermal oxidation of 2,4-decadienal.

Another interesting group of heterocyclic compounds formed in the reaction of 2,4-decadienal and cysteine is the thiophenes. Thiophenes having butyl to heptyl groups substituted at the 2-position were identified. Mechanisms have been suggested for the production of thiophene derivatives by the action of hydrogen sulfide on 1,4-dicarbonyl compounds (24). The exact mechanism for the formation of thiophenes in this system is not clear and deserves more study.

Mechanism for the Formation of 2-Pentylpyridines

2-Pentylpyridine has been identified in both fried chicken and french-fried potato flavors. This compound has a strong fatty and tallow-like odor and was the major product in the volatiles generated from the thermal interaction of valine and linoleate (25). It is postulated to form through the reaction of 2,4-decadienal and ammonia (25). In our study, it was found that at high temperature (180°C) the reaction of 2,4-decadienal with either cysteine or glutathione (γ -glu-cys-gly) in aqueous solution yielded 2-pentylpyridine as the major product. The quantities of five major volatile components, including 2-pentylpyridine, generated for these two systems are listed in Table II.

The amount of 2-pentylpyridine generated in the 2,4-decadienal/glutathione system was greater than that in the 2,4-decadienal/cysteine system. It is known that the formation of dithiazine or thiadiazine requires the presence of free ammonia. The absence of formation of dithiazine or thiadiazine in the 2,4-decadienal/glutathione system indicates that no free ammonia is available. This strongly suggests that the formation of 2-pentylpyridine does not require the presence of free ammonia. It is possible that the amino

Table II. Quantity of Some Major Volatile Compounds Formed in the Interaction of 2,4-Decadienal with Cysteine or Glutathione

Compounds	Amount Produced (mg/mol)	
	With Cysteine	With Glutathione
hexanal	278.6	45.4
3,5-dimethyl-1,2,4-trithiolane	140.9	368.5
2,4,6-trimethylperhydro-1,3,5-thiadiazine	828.5	--
2,4,6-trimethylperhydro-1,3,5-dithiazine	284.2	--
2-pentylpyridine	501.5	1219.0

group from amino acids or peptides condenses directly with the aldehydic group of 2,4-decadienal and is then followed by an electrocyclic reaction and aromatization to form 2-pentylpyridine (Figure 2).

Summary

Heterocyclic compounds, especially those which contain nitrogen and sulfur atoms, possess potent sensory qualities at low concentrations. They are formed in foods by thermal decomposition and interaction of food components. The identification of many long-chain alkyl-substituted heterocyclic compounds suggests that their formation mechanisms directly involve lipids or lipid decomposition products. Model studies indicated that lipid decomposition products such as 2,4-decadienal and hexanal can react with Maillard reaction intermediates to form heterocyclic compounds.

Acknowledgement

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Chapter 11

Processing Parameters and Volatile Compounds from Milk Fat

Y. J. Yoo, R. C. Whiteman, J. K. Dore, M. A. Amer¹, and Wassef W. Nawar

Department of Food Science and Nutrition, University of Massachusetts,
Amherst, MA 01003

Butteroil is known to generate unique flavors when exposed to heat. Not only the quantitative nature of the volatiles produced, but also the relative concentrations of these volatiles are important to flavor. The purpose of this study was to investigate the effect of certain factors on the quantitative pattern of volatiles produced from butteroil by heating. Although the compounds formed reflect thermal oxidation of the major fatty acids present in the butteroil, the relative amounts of these volatiles may vary significantly depending on the concentration of each substrate fatty acid, the surface-to-volume ratio during heating, the heating time and losses by volatilization.

Milk fat is known to generate unique flavor-compounds when exposed to heat (1-5). In a previous report (6), we provided a detailed qualitative and quantitative analysis of the volatile components generated from butteroil by heating for 1 hr at 185 C. More than 200 compounds were detected and, of these, 152 were identified and measured. The major volatile compounds consisted of alkanals, alkenals, methyl and ethyl ketones, alkadienals, alkanes and γ - and δ -lactones. A definite correlation was evident between fatty acid composition of the milk fat and the volatiles expected from simple cleavage of the monohydroperoxides formed by oxidation of the major fatty acids. Thus, 2-decenal and 2-undecenal were the most abundant of the alkenal series, while nonanal and octanal were the most abundant of the n-alkanal series. The 2-ketone found in the greatest amount was the C15 homolog followed by the C13. All members of the γ -lactone series were produced by heating but only the δ -lactones of even carbon numbers were formed in significant quantities.

It is obvious that many of these volatiles contribute to the aroma generated from milk fat by heating. In 1967, Kinsella et al. emphasized the flavor capabilities of milk fat and outlined a scheme for their utilization (7). The lactones and methyl ketones are

¹Current address: The Montreal General Hospital Institute, McGill University, Montreal H3G 1A4, Canada

highly flavorful compounds which contribute significantly to the pleasant flavor attributes of many dairy products. If generated in excessive amounts, however, they can impart off-flavors. The flavor potency of the methyl ketones vary with carbon-chain length, with heptanone having the lowest flavor threshold, i.e. 0.7 ppm in milk (8). Both γ - and δ -lactones impart typical flavors described as buttery, coconut and peach-like. Their flavor threshold value is approximately 5 ppm in butterfat. The alkanals, alkenals and alkadienals have been implicated in a wide range of flavors, both pleasant and unpleasant. "Fruity", "creamy", "cucumber", "beany", "grassy", "fishy", "painty", "tallowy", "cardboard" and "oxidized", are some of the many terms which have been used to describe these flavors. Some unsaturated aldehydes exhibit extremely low threshold values, e.g. 1.5 ppb for 4-heptenal in butterfat (9).

Clearly, the flavor impact of any given compound at any given time depends not only on its chemical nature, but also on its quantitative level, its threshold value and its interaction with the other components present. The purpose of the present work was to study the various parameters which influence the quantitative pattern of volatiles produced from butteroil by heating.

Experimental

Materials: Reagents and authentic compounds for use as standards were purchased in the highest available purity. Butteroil was prepared from unsalted butter by centrifugation at 60 C. Four butteroil fractions of different fatty acid composition, obtained by crystallization at 19 C and 29 C (10), were provided by the Dairy Bureau of Canada.

Heat Treatment: Heating of both 5 g- and 350 g-samples were studied. In the case of the 5 g-samples, the oil was placed in 225 mL round bottomed flasks which were either securely capped or left open. Surface-to-volume ratio for these samples was 22.6/cm. In case of the 350 g-samples, the oil was placed in 1 L stainless-steel beakers with internal diameters of 10.0 cm, providing a surface-to-volume ratio of 0.9/cm. Heating was conducted in a silicone oil bath, maintained at 185 ± 2 C.

Analysis: The volatiles were collected by high-vacuum cold-finger distillation as described previously (11), and fractionated on silica into polar and nonpolar fractions to reduce component overlapping during gas chromatographic analysis (6). Separation was conducted on a 30 m x 0.32 mm i.d. Supelcowax 10 capillary column in a Hewlett Packard Model 5890 chromatograph equipped with a flame ionization detector (FID). Quantitation was done with the aid of two internal standards, one polar and one nonpolar, which were added to the oil sample before volatile collection. A Hewlett-Packard model 5985B chromatography-mass spectrometry (GC/MS) system was used for identification of the volatiles.

Results and Discussion

To examine the effect of fatty acid concentration on the major volatiles produced, five 5 g-samples of different fatty acid

composition (i.e. whole butteroil plus the four butteroil fractions obtained by crystallization) were heated in a closed flask at 185 C for 1 hr, and the volatiles analysed.

A linear relationship was evident when the concentrations of substrate fatty acids were plotted against concentrations of their oxidation products, as for example between oleic acid and decenal, undecenal, nonanal, octanal and nonenal (Figure 1a); palmitic acid and 2-pentadecanone (Figure 1b); and linoleic acid and 2,4-decadienal (Figure 1c).

In another experiment, the effects of heating time and surface-to-volume ratio of the heating oil were studied. Four different heating conditions were compared: 5 g oil, representing a relatively large surface-to-volume ratio, heated for 1 hr in both closed and open systems; 350 g oil, representing smaller surface-to-volume ratio, heated for 1 hr; 350 g oil heated for 37 hr. The quantitative results are given in Table I, where the compounds listed correspond to numbered peaks in Figure 2. It is clear that the surface-to-volume ratio of the oil during heating is an extremely important factor. In general, the amounts of the aldehydes, ketones, and lactones produced were much higher when that ratio was high, that is, when a large surface area exists.

The difference in volatile amounts between the closed and open 5 g-heatings is not surprising. The shorter chain compounds (Peaks 13 - 37) were found in lower quantities in the open system, probably due to losses via volatilization. The aldehydes eluting after peak 37 were found at higher levels in the open system, perhaps due to the result of the greater availability of oxygen. This was not always the case for the ketones which can be produced by non-oxidative mechanisms, and are also more volatile than their corresponding aldehydes.

Comparison between 1 hr and 37 hr-heatings does not provide a consistent trend; some volatiles remaining at the same level, while others increasing at different rates. To clarify the effect of heating time, a new experiment was conducted with more frequent measurements over a period of 5 days. Several samples of butteroil, 10 g each, were placed in special glass tubes to maintain a surface-to-volume ratio similar to that of the 350 g batches of the previous experiment. All samples were heated in the same 185 C oil bath, and tubes taken out for volatile analysis at specified intervals.

Figure 3a shows the quantitative behavior of 2-alkenals. The amount of each compound reached a maximum after 4 hr of heating, then decreased to a plateau. For all four compounds a shoulder was observed between approximately 12 hr and 40 hr. The same trends were observed for the alkanals (Figure 3b), the methyl ketones (Figure 3c), the alkanes (Figure 3d), and the alkadienals (Figure 3e). The behavior of the lactones was not clear (Figure 3f). The 'shoulder' phenomenon, which was observed for most of the compounds tested, can not be explained at this time.

It is clear from the above that while the quantitative nature of the volatiles generated from butteroil by heating is predictable, the relative quantities of such volatiles may vary significantly depending on the concentrations of the different fatty acids in the butteroil, surface-to-volume ratio during heating, heating time, and any factor which may influence losses by volatilization of the different components of the volatile mixture, e.g. vapor pressure,

Table I. Quantitative Analysis of the Polar Components (ug volatiles/g oil) Produced from Butteroil by Heating

Pk no. Fig.2	Compound	Unheated Control	5 g, Heated 185 C, 1 hr		350 g, Heated, 185 C, open	
			Closed	Open	1 hr	37 hr
13	2-heptanone	0.7	12.1	1.1	5.6	0.3
14	heptanal	0.1	40.5	10.8	1.4	2.2
15	t,2-hexenal	tr	9.9	2.5	0.4	0.6
17	2-octanone	tr	4.1	0.9	tr	0.2
18	octanal	tr	41.0	13.3	1.1	2.7
19	t,2-heptenal	tr	32.0	9.2	1.7	2.0
21	2-nonanone	0.3	8.1	1.1	3.6	0.3
22	nonanal	0.2	66.8	30.9	3.2	5.3
23	t,2-octenal	tr	24.4	9.6	1.1	2.1
24	t,c-2,4-heptadienal	0	6.8	3.0	0.4	0.4
27	t,t-2,4-heptadienal	0.3	17.8	7.3	1.9	1.2
28	2-decanone	0.2	2.9	3.7	tr	0.4
29	decanal	0.4	13.0	2.1	0.7	1.2
30	t,2-nonenal	0	26.8	13.3	2.1	2.7
34	2-undecanone	0.3	9.9	4.8	5.8	0.4
35	undecanal	0	10.1	5.9	0.3	0.8
37	t,2-decenal	0.8	77.8	62.3	4.9	10.5
44	2-dodecanone	0	2.1	5.1	tr	0.3
45	dodecanal	0	10.0	5.7	0.5	1.3
46	t,2-undecenal	0	77.8	80.6	4.9	12.7
47	t,c-2,4-decadienal	0	9.0	13.2	0.8	1.2
51	t,t-2,4-decadienal	0	28.7	50.5	3.5	4.9
52	2-tridecanone	0.5	14.0	7.3	11.6	0.9
53	tridecanal	tr	9.4	13.9	0.6	1.2
54	t,2-dodecenal	0	3.4	7.8	tr	0.8
57	γ-octalactone	0	6.8	4.6	tr	0.5
58	2-tetradecanone	0.1	2.9	tr	1.4	0.5
59	tetradecanal	0.5	8.2	8.2	1.2	1.9
60	t,2-tridecenal	0	2.8	3.6	tr	0.8
64	γ-nonalactone	0.3	5.0	4.9	0.3	0.7
65	2-pentadecanone	0.8	35.4	39.1	32.5	5.4
66	pentadecanal	0.3	8.1	16.9	1.4	3.2
70	γ-decalactone	tr	6.2	6.6	0.9	1.9
72	δ-decalactone	4.8	9.2	8.8	8.6	0.6
75	γ-undecalactone	0	2.7	5.7	tr	0.9
76	2-heptadecanone	0	10.2	4.0	3.0	5.6
80	γ-dodecalactone	1.6	5.5	9.8	2.5	1.5
83	δ-dodecalactone	8.2	17.4	41.6	22.5	1.4
85	γ-tridecalactone	0	2.4	tr	tr	1.0
88	γ-tetradecalactone	tr	7.1	16.5	0.7	4.9
89	δ-tetradecalactone	5.8	17.6	57.8	22.3	9.9
94	γ-hexadecalactone	tr	19.6	18.0	3.1	11.9
95	δ-hexadecalactone	7.0	11.9	25.2	7.1	13.6
96	hexadecanoic acid	tr	(4.2)	(40.4)	(9.8)	(10.9)

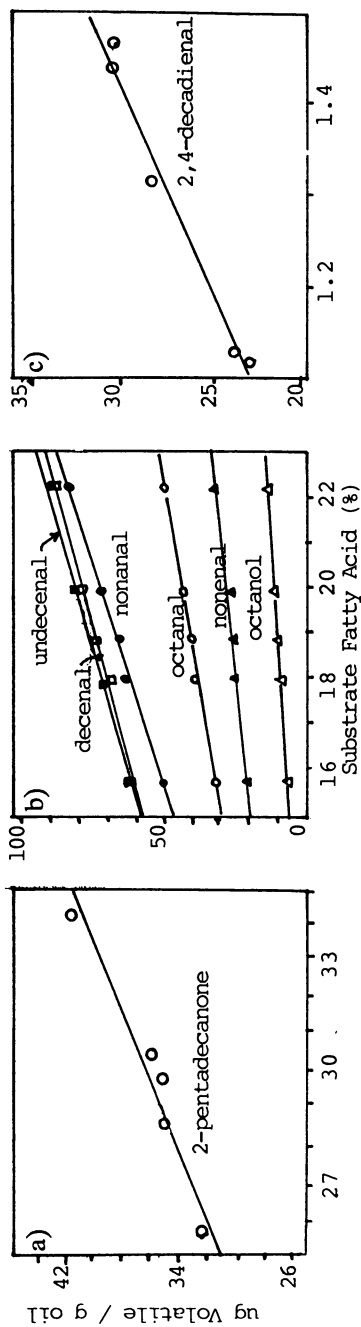


Figure 1. Relationship between concentration of substrate fatty acid and volatiles produced: a) palmitic, b) oleic, c) linoleic.

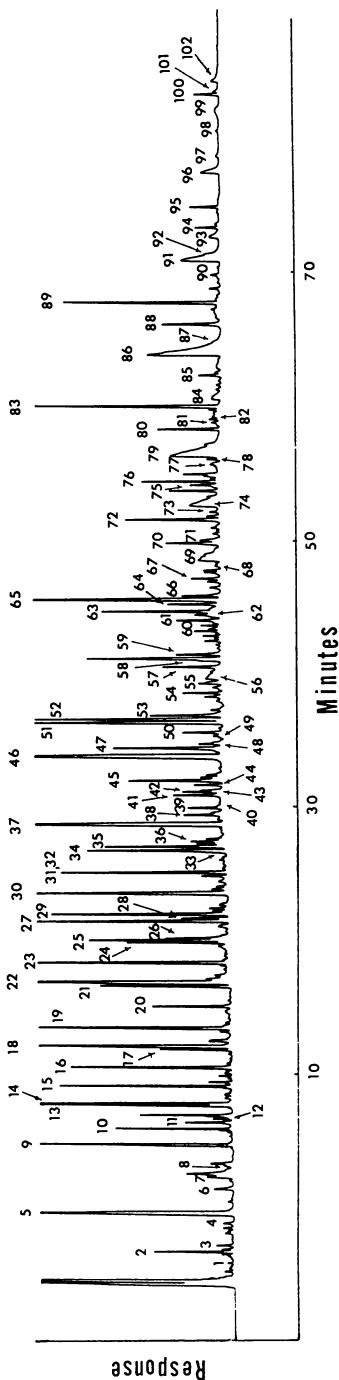


Figure 2. Gas chromatographic analysis of the polar volatile components from butteroil heated for 1 hr at 185 C. Only the relevant peak identifications are given in Table I.

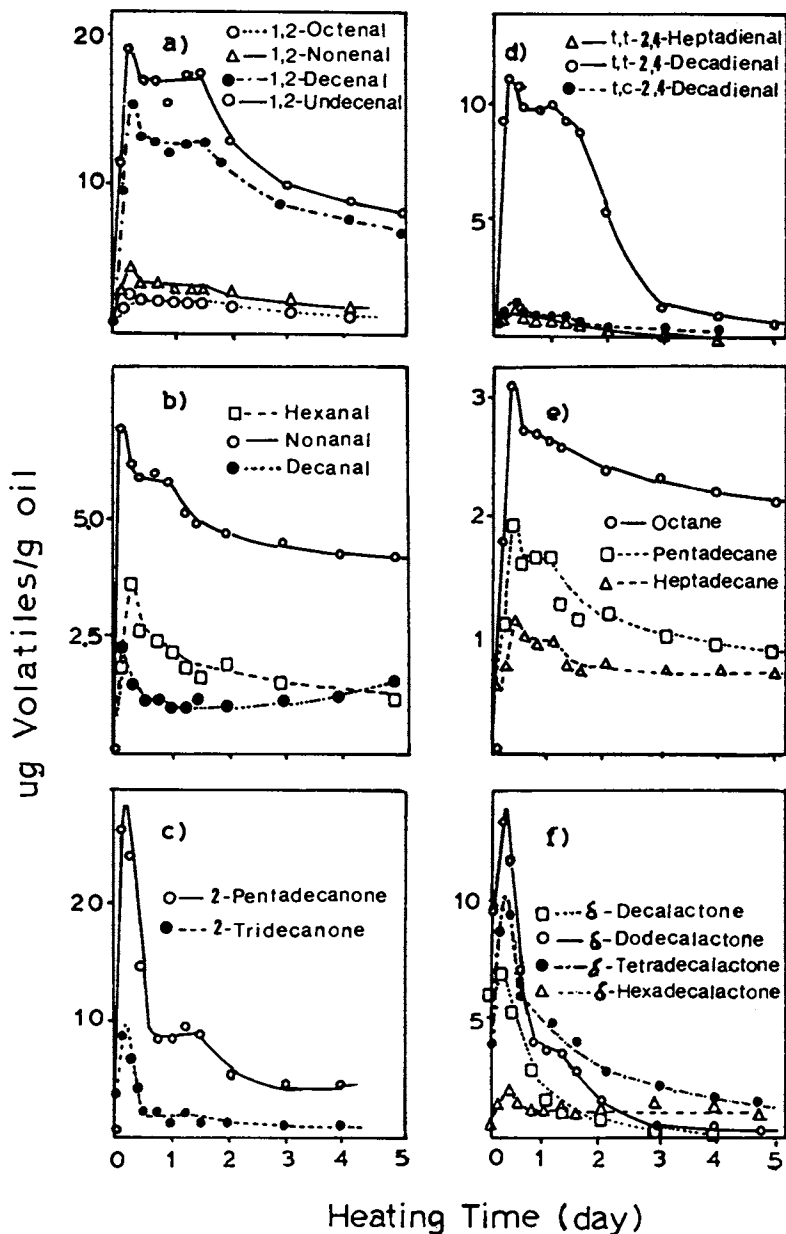


Figure 3. Effect of heating time on the amount of volatile produced: a) alkenals, b) alkanals, c) methyl ketones, d) dienals, e) alkanes, and f) lactones.

solubility in the oil, temperature of heating, etc. Such variations, in turn, would certainly have a definite impact on the final aroma. It is very difficult however to determine the flavor impact significance of individual components, due to the large number of compounds present and the great differences in their relative concentrations and flavor threshold values. In our opinion, an approach similar to that proposed by Ullrich and Grosch (12) is needed to more effectively evaluate the significance of the volatiles generated from milk fat in specific flavors. These authors describe an elegant procedure to determine "flavor dilution factors" by gas chromatographic analysis and effluent sniffing of a dilution series of the original aroma extracts.

Acknowledgments

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Chapter 12

Flavor Composition of Oil Obtained from Crude and Roasted Oats

S. M. Fors¹ and P. Schlich²

¹Department of Food Science, Chalmers University of Technology, c/o SIK, Box 5401, S-402 29 Göteborg, Sweden

²Laboratoire de Recherches sur les Arômes, INRA, 17 rue Sully, BP 1540, F-21034 Dijon Cedex, France

Two oat varieties with different lipid content, Magne (7.4%) and Chiuauhua (8.3%), were studied before and after roasting. Oils were extracted by carbon dioxide and volatile fractions were isolated by molecular vacuum distillation. The samples obtained were analysed by GC-MS and by a panel. The lipid content and the preparation of the oats (heat or no heat, milling before and after roasting), influenced the flavor composition. More than 100 compounds were identified. N- and O-heterocycles were predominant in oils from roasted oats, especially in the Magne variety. Oils from whole roasted oats had larger amounts of volatiles than those from ground roasted oats. Aldehydes were highly correlated to heated oil from crude oats. Alcohols, ketones and hydrocarbons were present in all oils. Association between oils and chemical compounds was visualized by Principal Component Analysis and by using the RV coefficient. Oils from roasted oats had an aroma resembling of sesame oil and Swedish crispbread.

Flavor formation in foods is a very complex process since it results from different phenomena often occurring simultaneously. Especially in heat-treated food, the formation of flavor is often associated with the Maillard reaction. Reducing sugars and amino acids or peptides are constituents involved in this non-enzymatic browning reaction (1-3). The contribution of lipids and their role as flavor generators has also been extensively investigated (4-6). Despite a great deal of research effort, many questions remain concerning the above mentioned flavor precursors.

Oat, *Avena sativa*, is a cereal which has been little studied, at least from a flavor point of view. Only a few investigations have been published, e.g. by Heydanek and McGorin (7, 8). A review concerning oat flavor chemistry has also appeared quite recently (9). However, this cereal has attracted increased interest during the last few years, owing to the health benefits of oat. Examples are its cholesterol-lowering properties, the desirable physiological properties of fibre content and the favourable ratio of polyunsaturated to saturated fats in its lipid content (10).

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New oat varieties are being studied with increasing interest in Sweden, e.g. varieties with a high lipid content. The present study deals with flavor compounds isolated from oils obtained from crude and roasted oats. The influence on the flavor composition of various parameters, such as heat and lipid content in the oat varieties are taken into account.

Experimental Procedures

Materials and Extraction Procedure. Two oat varieties, Magne and Chihuahua, were used. They contained 7.4 and 8.3% lipids respectively. The oil fractions in batches of dehulled crude oats and roasted oats were extracted. Oat samples of 1.5 kg each were placed in glass vessels open to the air and dispersed in a layer 3-4 cm deep. The roasting was carried out in a domestic oven for 2 hours at 200°C, ±5°C. Lipid isolation was performed using supercritical carbon dioxide (SCCO₂). The extraction was carried out at a pressure of 250 bar at 40°C for 5 hours. The oil samples obtained are presented in Figure 1. Details of the oat varieties and the extraction equipment have been given in an earlier paper (Fors and Eriksson, submitted for publication 1988).

Isolation of Volatile Compounds from Oat Oil. Four oil samples were obtained from each oat variety, as shown in Figure 1. Each oil was isolated, in triplicate, for its volatile fraction by means of molecular vacuum distillation. The equipment used has been previously described (11, 12). Aliquots of 5.0 grams were used. The isolation was performed at room temperature and proceeded for 4 hours from the time secondary vacuum was

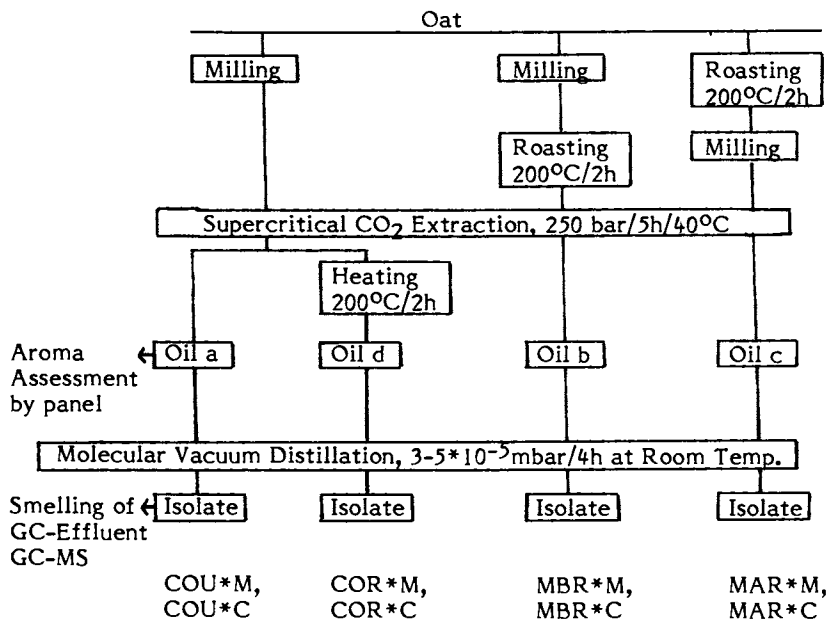


Figure 1. Isolation of volatile flavor compounds from the lipid fraction of dehulled oat seeds.

obtained. Evacuation of the apparatus was carried out in two steps, primary vacuum, 5×10^{-3} mbar, secondary vacuum, 5×10^{-5} mbar. The volatile compounds were condensed and trapped onto a cold finger chilled with liquid nitrogen. The distance between the gently stirred oil sample and the cold finger was about two centimeters. Two additional traps, likewise cooled with liquid nitrogen, were connected after the cold finger. Volatiles which escaped from the cold finger could therefore be retained. The isolates were transferred to deionized water and made alkaline with 2 N sodium hydroxide, PA-quality. The water phase, now at pH 11, was extracted in a separation funnel with distilled dichloromethane (3×15 ml). The first portion of CH_2Cl_2 was used for rinsing the equipment. Two compounds, n-amyl-n-butyrate and ethyl-n-tetradecanoate were added and used as internal standards. The organic solvent extracts were dried with Na_2SO_4 and transferred to a concentration flask (Junk type) connected to a distillation device (13). The glass vessel was kept in a thermostat-controlled water bath, 40°C , until excess solvent removed. The final extract, measuring about $80\text{--}100 \mu\text{l}$, was kept in sealed ampoules at -20°C until analysis.

Chemical Analysis by Gas Chromatography-Mass Spectrometry GC-MS. Volatiles isolated from the oils were separated by GC. A Girdel Series 300 instrument equipped with a FID detector was used for quantification. Column: DB Wax, 0.32 mm id x 25 m. Carrier gas: hydrogen with a flow rate of 3 ml/min. Column temperature: programmed, $40\text{--}220^\circ\text{C}$ at a rate of $3^\circ\text{C}/\text{min}$. The extract was injected, with split, onto the column.

Identification of the volatile constituents was performed by GC-MS. After separation (under similar conditions as those described above) identification was carried out using a Nermag R10-10 mass spectrometer (Rueil-Malmaison, France). Mass spectra were prepared at an ionizing voltage of 70 eV and compared with those included in the NBS library system. The Institute's own collection of reference spectra were also used.

Sensory Analysis. Oat oils and isolates from these oils were sensorially evaluated. Oils from the Magne variety, both crude and heated, were analysed by a panel consisting of 12 to 14 persons who were selected from the laboratory staff. Most of them had previous experience with sensory analysis. Four oat oils, one from crude oats, one heated oil from crude oats and two oils from roasted oats; see Figure 1, were subjected to the triangle test followed by descriptive analysis (14, 15).

Concentrated isolates from the two oat varieties were also studied. The volatile compounds were evaluated by smelling the GC effluent. Each oil was studied in triplicate. The peaks were described and the intensity of the perceived attributes was graded (scale 0-3). One trained person took part in this work.

Statistical Evaluation. Statistical analysis was performed on a Mini 6 Bull computer. The program package used was SPAD, Systeme Portable pour l'Analyse des Données (15). Data from the chemical analyses were evaluated by principal component analysis (PCA) of correlation matrices. PCA was carried out in order to show clearly the association between chemical classes, or compounds (variables), and the isolates studied (individuals). Statistical analysis was also made on the basis of reduced chemical data, by calculating RV coefficients for single variables (17, 18). The number of variables, i.e. chemical compounds in this study, was reduced since only chemical compounds with the highest RV values were selected as representatives of the chemical group.

Results and Discussion

Two oat varieties were studied with respect to their oil content. The composition of these SCCO_2 extracted oils, with regard to fatty acids, free fatty acids, phosphorus and thermal stability has previously been reported (Fors and Eriksson, submitted for publication 1988). Volatile compounds were isolated from the oat oils by molecular vacuum distillation. The fractions obtained were transferred to aqueous alkali and extracted by CH_2Cl_2 . The adjustment in pH was made to remove fatty acids which could otherwise interfere with the later work. Moreover, it is well established that many heterocycles are important flavor compounds in heated food items. These compounds are normally isolated in the basic fraction. The isolates were analysed by chemical and sensory methods.

Analysis by GC-MS. The four GC patterns obtained were each unique in the number of separated peaks as well as their quantity. Crude oat oils (COUM, COUC) differed from similar heated oils (CORM, CORC). For explanation of the abbreviations used, see Legend of Symbols. Oat samples milled after roasting (MARM, MARC) contained more volatile compounds, both with regard to number and quantity, compared with identical samples milled before roasting (MBRM, MBRC). In addition, volatile constituents from the Chihuahuahua variety (MARC, MBRC) were present in lower concentrations than the Magne variety (MARM, MBRM).

More than 100 volatile compounds were separated and identified by GC-MS. Of these compounds 75 were quantified (19). The results are based upon the average value of three replicates of each individual compound, in mg/kg oil (ppm v/v). There were large differences between the samples. Some heterocyclic compounds, such as pyrazines, and derivatives of furan and pyrrole, were only present in samples from roasted oats (MARM, MARC, MBRM, MBRC). Most of the components were present in quantities below 10 ppm (19). These volatile compounds are typical representatives of Maillard-formed flavors (20). On the other hand, the presence of pentylfuran, octylfuran and pentyropyridine, found in all heated samples, can be explained by thermally induced conversion of lipids and degradation products such as decadienal (21, 22). In addition, pentyropyridine requires the presence of a nitrogen source for its formation. Some aldehydes, for example hexanal, nonanal and the 2,4-decadienals, increased markedly in CORM (2ppm, 2ppm and 5-19ppm respectively) and especially in CORC (22ppm, 22ppm and 37-114ppm respectively). The involvement of thermally decomposed oleic acid and linoleic acid is well established (9). Hydrocarbons and ketones were present in all samples. Some alcohols, including n-pentanol, 1-octen-3-ol and n-octanol, were present in all samples but especially in CORM and CORC (up to 22ppm). These alcohols also originate from lipid degradation products (9).

Principal Component Analysis. The chemical data obtained were evaluated statistically by PCA. PCA was performed for individual chemical compounds, group by group, and for all chemical groups together. Results from the latter analysis are presented in Figure 2. In this PCA, the association between individuals (8 extracts, each one in triplicate) and variables (75 volatile compounds classified into 7 chemical groups) was studied. The PCA sample plot along the first two axes is shown in Figure 2, as is the superimposed circle of correlations. These first two principal components accounted for 79% of the total data information. It may be inferred from Figure 2 that the aldehyde group was strongly associated with heated oil from crude oats

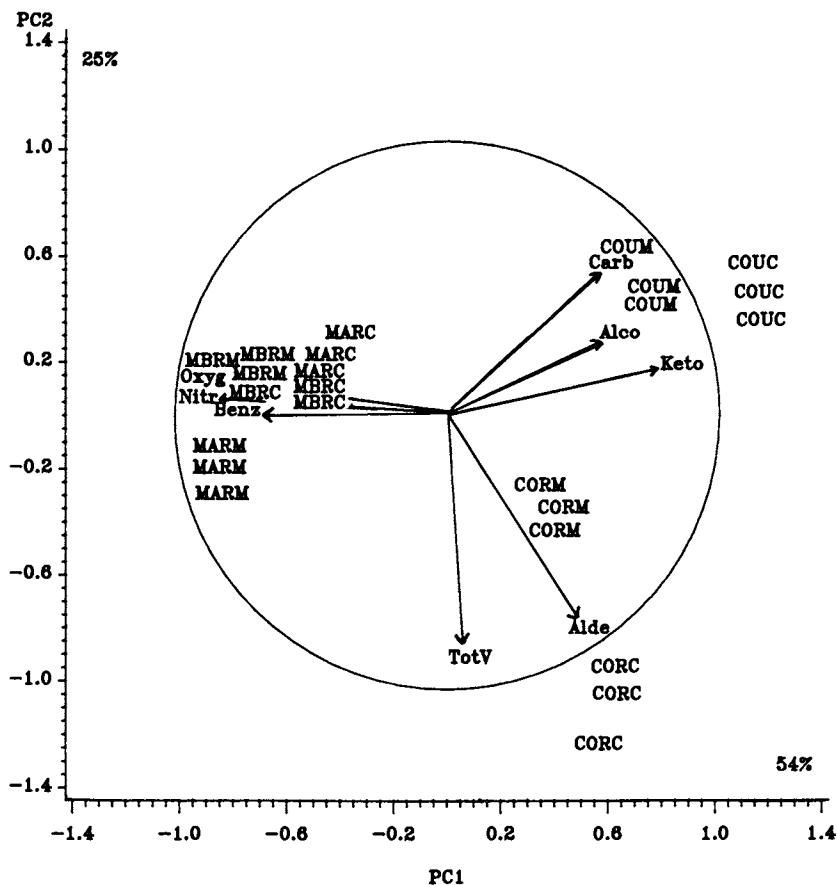


Figure 2. PCA of chemical groups, sample plot, and superimposed circle of correlation.

especially CORC, but also CORM. Alcohols, ketones and hydrocarbons were oriented towards the crude oils (COUM, COUC). Heterocycles containing nitrogen or oxygen were best related to the samples roasted before extraction, MBRM, MBRC and to MARM and MARC. Figure 2 shows that there is a slight difference between samples isolated from oats with lipid content of 7.4% and those containing 8.3% lipids.

PCA of individual volatile compounds, group by group, was performed but will not be visualized due to the large volume of data obtained. It was found that the most of the groups were quite homogeneous, i.e. the chemical compounds within a group behaved in a similar way. Aldehydes (16 compounds) were homogeneous as a group. An information of 85.7% was obtained by the first axis. N-Heterocycles, including 22 compounds, were also homogeneous, 82.4% of information was explained by the first axis. O-Heterocycles were also homogeneous, 77.3% referred to the first axis. Ketones were more divergent as a group. The first axis represented 50.3% while the second and the third accounted for 18.8% and 16.9%, respectively. The first two corresponding values for alcohols were 65.3% and 17.1%. Hydrocarbons needed several axes to reach the same information, axis 1; 43.7%, axis 2; 24.0%, axis 3; 9.7% and axis 4; 7.5%. In other words, large amounts of data were used for PCA. However, the ability to visualize the large data volume in a clear way, as shown in Figure 2, outweighs the disadvantage of the quite cumbersome execution of this statistical analysis.

Moreover, there is a means to reduce the data volume without losing too much important information. This was done by calculating RV coefficients for the variables (chemical compounds). Each chemical group was then represented by one or several compound(s), depending upon the degree of homogeneity within the group. Results are presented in Table 1. The closer the RV value is to one, the better the correlation. It can be seen from Table 1 that aldehydes as a group are well represented by one single compound (instead of 16). 2,4-Decadienal (E, Z) had a RV coefficient of 0.991. Ketones, N-heterocycles, and alcohols were represented by 3 compounds each. O-Heterocycles needed two compounds for illustrating their chemical group. Hydrocarbons were the most complex group as 5 representatives were selected. A new PCA was performed on the reduced data; see Figure 3. Three axes are visualized and together they explained 77% of the information. From this figure it is evident that pyrazines (represented by TMPy, trimethylpyrazine and Cycp, 2-methyl-6,7-dihydro-5H-cyclopentapyrazine), O-Heterocycles (Fume, 2-furanylmethanol) and benzothiazole (Btaz) were strongly associated with MARM but also with MBRM, MBRC and MARC. Pentyfuran (Pfur), 2,4-decadienal E,Z (EZDe), octanol (Octa) and the cyclic ketone (Keto) took the same direction as CORC. Hydrocarbons (Hene, heneicosane and Noda, nonadecane, among others) were related to oils from crude oats. The alcohol 3-penten-2-ol (3Pen), was related to MARC but not to MBRC as could be interpreted by the similar distribution in Figure 3. When the information relies on data near origo it is necessary to be prudent and to check original raw data (numerical values) in order not to draw any incorrect conclusions.

In Figure 3, as in Figure 2, samples isolated from oats with 7.4% and 8.3% lipid content were different with regard to chemical composition. Since the oil itself may play several roles, for example as generator of aroma compounds as well as solvent for other volatile compounds, it is of interest to follow the aroma pattern in the abovementioned samples. The amount of heterocycles decreased in most cases when the initial oat lipid content increased. Compounds such as pyrazine derivatives (methyl, 2,5-dimethyl, 2,6-dimethyl, 2-ethyl-5-methyl, trimethyl, 2,5-methyl-3-ethyl), furfural, 5-

Table 1. PCA, Principal Component Analysis Performed on Chemical Groups. Selection of Volatile Compounds, as Representatives of Each Group

Chemical Group	Subgroup Defined by PCA	Individual*	Selected Representative Compound
Alcohols	Butanol, Pentanol, Heptanol		Octanol (Octa)
	Octanol, 1-Octen-3-ol	CORC ++	(RV = 0.960)
	3-Penten-2-ol	MARC +	3-Penten-2-ol (3Pen)
	Hexanol	COUM +	Hexanol (Hexa)
Aldehydes	Hexanal, 2,4-Decadienal (EZ)		
	Heptanal, 2,4-Decadienal (EE)		
	2-Nonenal, 2-Ethylhexenal		
	2-Hexenal, 2,4-Heptadienal		
	2-Undecenal, 2,4-Nonadienal	CORC ++	Decadienal (EZDe)
	2-Heptenal, Nonanal, Decenal		(RV = 0.991)
	Decanal, 2-Octenal, Octanal		
Ketones	2-Pentadecanone	CORC ++	Cyclic ketone (Keto)
	Dodecadione, Cyclic ketone		(RV = 0.947)
	2-Heptadecanone	COUM, CORM, MBRM +	Heptadecanone (Hede)
	Trimethylpentadecanone		Trimethylpenta- decanone
N-Heterocycles	Pyridine		
	Pyrazines		
	Unsubstituted, 2,3-Dimethyl, 2,5-Dimethyl-3-ethyl, Methyl, 2-Ethyl-6-methyl, 2,5-Dimethyl, 2-Ethyl-5-methyl, 2,6-Dimethyl, Acetylmethyl, 2-(2-Furyl), Ethyl, 2-Methyl-5-(methylethyl), Trimethyl	MARM ++	Trimethylpyrazine (TMPy)
			(RV = 0.989)
	Pyrroles		
	2-Acetyl, N-Methyl-2-furyl, 1-Formyl, 1-Furfuryl-2-formyl, 5-Methyl-2-formyl		
	Dimethylethylpyrazine	MBRM ++	Cyclopentapyrazine (CycP)
	2-Methyl, dihydrocyclopentapyrazine		(RV = 0.954)
	Pentylpyridine	CORC ++	Pentylpyridine (Pepy)
O-Heterocycles	Furfural 2-Furanylethanone		
	Furanmethanolacetate	MARM ++	2-Furanmethanol (Fume)
	Hydroxymethylfurfural		(RV = 0.995)
	2-Methyltetra (or dihydro) furanone		
	5-Methyl-2-furfural		
	2-Furanmethanol		
	Pentylfuran, Octylfuran	CORC ++	Pentylfuran (Pfur)
		(RV = 0.873)	
Hydrocarbons	Dodecane, 2-Ethyl-dodecane	COUM ++	
	8-Methyldecene	CORM ++	Heneicosane (Hene)
	Heneicosane, Hexadecene		(RV = 0.889)
	Octadecane	Opposite Direction as Those Above (Ooda)	
	Dodecene, Pentadecane	CORC +	Dodecane (Doda)
	Hexadecane, Tetradecane		(RV = 0.867)
	Nonadecane	COUC +	Nonadecane (Noda)
	Octadecene	CORM +	Octadecene (Oede)

* ++ = Very Good Association

+ = Good Association

methyl-2-furfural, 2-furanmethanol and 1-formylpyrrol were present in much greater amounts in MARM and MBRM (from oat with 7.4% lipids) compared with MARC and MBRC (from oat with 8.3% lipids). This observation confirms of results obtained in a model system. When the medium was deprived of phospholipids and triglycerides, the amount of heterocyclic compounds increased (21).

The preparation technique of the oats, whether or not heat was used and whether milling was carried out before or after roasting, also influenced the final volatile composition. Isolates from oats milled before roasting (MBRM, MBRC) had volatile compounds in lower concentrations compared with those milled after roasting (MARM, MARC). Despite a larger available surface area promoting the formation of heterocycles, for example, fewer volatiles were found. This was probably due to evaporation from the milled samples MBRM and MBRC. Volatiles formed in roasted whole oat seeds were retained and encapsulated, and gave a higher final amount. This observation concerns samples MARM and MARC which were isolated from oats milled after roasting.

Sensory Analysis. Oils from the Magne variety were evaluated by a profile panel. Oil from crude oats (a), two oils from roasted oats (one part milled before roasting (b) and one milled after roasting (c) , and heated oil from crude oats (d) were subjected to the triangle test and descriptive analysis. No evaluations were performed of oils from the Chihuahua variety, due to lack of material and also to similarities with the various Magne oils.

A triangle test was performed in order to determine whether there was a significant difference between the oil samples studied. Results were as follows:

- * No significant difference was found between b and c (14 answers/+7R)
- * A large difference was found between a and d (12 answers/+12 R)
- * No comparisons were made between a-b and a-c, as there were very clearly marked differences.

Descriptive analysis was made in order to obtain a detailed descriptive evaluation of a sample. A summary of attributes of Magne variety is presented below.

- Sample a: green, rancid, sourish,
chemical (solvent, aldehyde, paint, petrol)
- Sample b & c: roasted (bread, coffee, peanut, walnut, malt), burnt,
popcorn, slightly rancid, heated cooking oil
- Sample d: heated cooking oil, deep-frying fat, solvent, old paint

Since the panel found no difference between samples b and c in the triangle test, it was not possible to ask for two distinct "profiles" in the descriptive test. However, it was evident that samples b and c were the most attractive oils from a flavor point of view. There were similarities with sesame oil and Swedish crispbread.

Isolates from the lipid fraction of the oat varieties were evaluated sensorially by sniffing of the GC effluent. Isolates from crude oil (a) and heated crude oil (d) had a similar aroma. Notes such as herbaceous, green, pungent, plant-like, spicy, green-house and mouldy predominated. Some of the compounds identified as having these attributes belong to the saturated and unsaturated aldehydes. Pentylpyridine, present in heated samples, was described as giving a plant-like, old cookie odor with an intensity of 3. Other easily recognized compounds were 2,4-dienals (E,E and E,Z), which had large peak areas and gave off a strong odor, described as green, pungent, plant-like. The odor of "newly cut grass", found in all samples from crude oats, revealed the presence of n-hexanal.

Isolates from oils from roasted oats (b, c) were quite different in odor compared with those described above. Notes such as roasted, peanut, butterscotch, sesame seeds, creamy, caramel-like were used. Among the compounds identified, furanmethanol (2-furfuryl alcohol), 2-methylfurfural, 2,6-dimethylpyrazine and acetylpyrazine were given these pleasant descriptions. Moreover, the stability of oils extracted from roasted oats were better than that of oils extracted directly from crude oats (Fors & Eriksson, submitted for publication 1988).

Acknowledgments

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Legends of Symbols

E (trans) and Z (cis) used to designate configuration about double bond.

COU Oil from Crude Oats, Unheated

COR Oil from Crude Oats, Roasted

MBR Oil from ground roasted oats, Milled Before Roasting

MAR Oil from whole roasted oats, Milled After Roasting

*M Oats from Magne Variety, 7.4% lipid

*C Oats from Chihuauhua Variety, 8.3% lipid

*M and *C are used in combination with COU, COR, MBR, MAR.

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Chapter 13

Volatile Flavor Chemicals Formed by the Maillard Reaction

Takayuki Shibamoto

Department of Environmental Toxicology, University of California,
Davis, CA 95616

Among the many reactions occurring in heated foods, the Maillard, or browning, reaction of a carbonyl group with an amino group plays the most important role in the formation of flavor chemicals. Simple Maillard model systems consisting of a sugar and an amino acid have been widely used to study the production of flavor chemicals. The major volatile flavor compounds produced by the Maillard reactions are heterocyclic compounds: thiophenes, thiazoles, pyrazines, pyrroles, imidazoles and pyridines. The formation mechanisms of these chemicals have been intensively investigated because of their importance in the flavor of cooked foods.

The Maillard reaction has received much attention since the 1950's as the source of flavor chemicals in cooked foods. Numerous compounds produced by this reaction have been reported in the last two decades. The major flavor chemicals are nitrogen- and sulfur-containing heterocyclic compounds. For example, nitrogen-containing pyrazines contribute a characteristic roasted or toasted flavor to cooked foods. Sulfur-containing thiophenes and thiazoles give a characteristic cooked meat flavor. A striking property of these compounds is their extremely low odor thresholds.

Formation mechanisms of Maillard reaction products are very complex. Many proposed mechanisms have been advanced, but it is still necessary to investigate further to thoroughly understand this important reaction.

Isolation and Identification of Flavor Volatiles Produced by the Maillard Reaction

Even though the occurrence of the Maillard reaction was recognized at the beginning of this century (1), volatile flavor compounds were not isolated and

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identified from the Maillard reaction mixtures until the 1960's. This may have been due to a lack of advanced analytical techniques for volatile chemicals. With the development of gas chromatography, volatile Maillard reaction products began to be isolated and identified. The chemicals identified in Maillard reaction mixtures during the 1960's were mainly sugar degradation products such as aldehydes and ketones. For example, several low molecular weight aldehydes were identified in sugar-amino acid reaction mixtures using a prototype gas chromatograph (2) in 1967. Fifteen volatile chemicals produced by the base-catalyzed degradation of fructose were reported in the late 1960's (3). The products included four furans and ten carbonyl compounds. More recently, ten furans and fourteen carbonyl compounds were reported in a headspace sample collected from heated potato starch (4). Later, some of these sugar degradation chemicals, especially aldehydes and ketones, were also widely used as reactants in Maillard model systems.

The analysis of flavor chemicals formed in browning model systems was a major focus of the study of the Maillard reactions in 1970's and in the early 1980's. The development of high resolution capillary gas chromatography/mass spectrometry (GC/MS) accelerated the identification of volatile chemicals. Heterocyclic compounds give distinctive mass spectral fragmentations. Thus, it is often possible to identify these chemicals by their gas chromatographic retention index and mass spectral fragmentation patterns alone. Consequently, many volatile heterocyclic compounds have been identified in the Maillard reaction mixtures in the last two decades (Figure 1). For example, among sixty volatile compounds identified in a rhamnose/ammonia browning reaction mixture, forty-one pyrazines, seven pyrroles, and four imidazoles comprised 79.84%, 8.38%, and 4.89% of the total gas chromatographic peak area%, respectively (5).

In addition to simple model systems, more complex systems which are closer to actual foodstuffs have been used to investigate the formation of flavor chemicals in the Maillard reaction. Sixty-three volatile chemicals were isolated and identified from starch heated with glycine (4). When beef fat was used as a carbonyl compound precursor in a Maillard model system with glycine, 143 volatile chemicals were identified (6). These included fifteen n-alkanes, twelve n-alkenes, thirteen n-aldehydes, thirteen 2-ketones, twelve n-alcohols, and eleven n-alkylcyclohexanes. Recently, the effect of lipids and carbohydrates on the thermal generation of volatiles from commercial zein was studied (7).

Furans

Furans are the most abundant products of the Maillard reaction and they account for the caramel-like odor of heated carbohydrates (8). Some sugar degradation compounds, such as maltol, isomaltol, 4-hydroxy-5-methyl-3(2H)-furanone, 2,5-dimethyl-4-hydroxy-3(2H)-furanone, and 2-hydroxy-3-methyl-2-cyclopentene-1-one (cyclotene), have odors usually described predominantly

as caramel or burnt sugar (9). The sugar degradation compounds may undergo secondary reactions to yield Maillard reaction products. Therefore, some of these compounds have been used as a carbonyl source in browning reactions. Seventeen volatile compounds were produced from the reaction of furfural with ammonia and hydrogen sulfide (10). Formation of thiophene and pyrrole derivatives in this reaction system suggested that the oxygen atom in the furan ring exchanged with sulfur or nitrogen atoms. In fact, 4-hydroxy-5-methyl-3(2H)-furanone produced numerous thiophene derivatives when reacted with hydrogen sulfide (11).

A maltol-ammonia browning reaction produced thirteen pyrazines, two pyrroles, two oxazoles, and one pyridine (12). The major products of this system were 2-ethyl-3-hydroxy-6-methylpyridine and 2-ethyl-3,6-dimethylpyrazine. It is difficult to construct possible formation mechanisms for these compounds from maltol and ammonia. All the carbon atoms must come from maltol. It is possible, then, that maltol degrades into smaller carbon units and that these fragments recombine to form larger carbon units, producing these compounds. Recently, the formation of thiophenones and thiophenes from the reaction of 2,5-dimethyl-4-hydroxy-3(2H)-furanone and cysteine or cystine was reported (13, 14). All these reaction mixtures were reported to possess a cooked meat-like flavor.

Thiophenes

Thiophenes are widely distributed in vegetables such as onions (15). Maillard model systems consisting of a sugar and a sulfur-containing amino acid produce a large number of thiophenes. Many acetyl- and formyl-thiophenes were produced in a cysteine/cystine-ribose browning model system (16). Glucose produced many thiophene derivatives by the reaction with hydrogen sulfide or with hydrogen sulfide and ammonia (17, 18). The formation of thiophene in a browning reaction is proposed to occur in the early stages of the reaction, as shown in Figure 2.

Many heterocyclic volatile compounds including thiophenes were also identified in an unheated aqueous solution of glucose and cysteine irradiated with 253.7 nm UV light (19). Pyrrole and thiophene, which can be formed without a high degree of sugar fragmentation, were found in relatively high yield in the early stage of irradiation. The yield of heterocyclic compounds such as 2-methylthiazoline and 2-methylthiazole, which require a greater degree of sugar fragmentation to form, increased when the irradiation period was increased. These phenomena are similar to those observed in thermally-activated systems.

Pyrroles

Pyrrole may have been the first individual heterocyclic compound isolated from the Maillard reaction systems. Some pyrroles, such as 1-acetylpyrrole

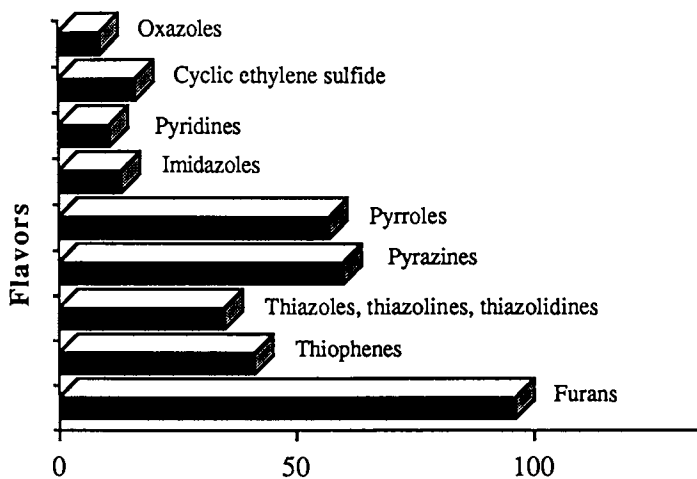


Figure 1. Number of heterocyclic compounds reported in the Maillard reaction mixtures.

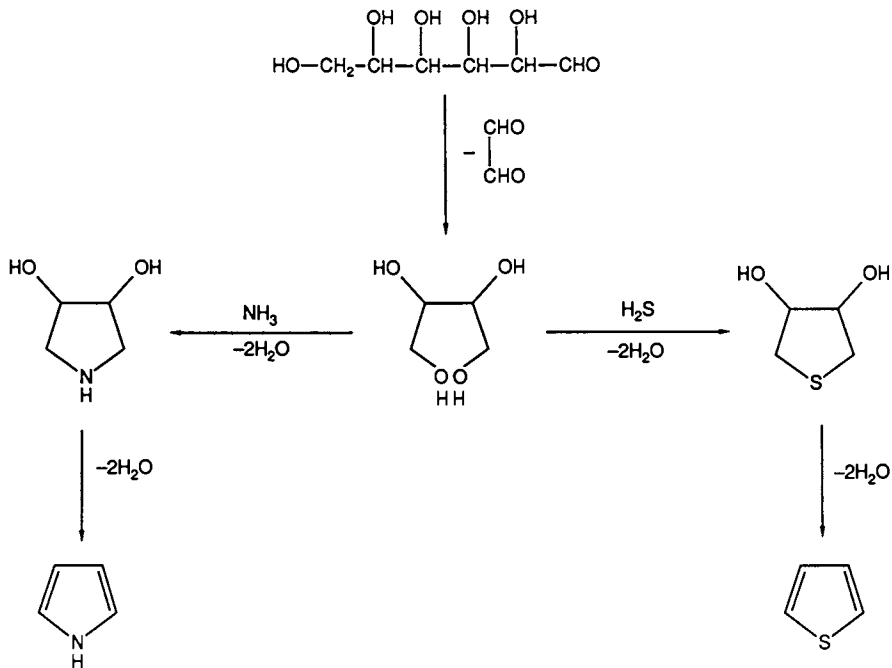


Figure 2. Proposed formation mechanisms of thiophene and pyrrole.

appeared in the reports of Maillard reaction products in the early 1960's (20). Several other pyrroles were also isolated using thin layer chromatography in this period (21, 22).

A simple model system consisting of rhamnose and ammonia produced eight pyrrole derivatives (5). When hydrogen sulfide was added to this model system, acylpyrroles were produced predominantly (23).

Some pyrrole derivatives have pleasant flavor. For example, pyrrole-2-carboxaldehyde gives a sweet and corn-like odor and 2-acetylpyrrole has caramel-like flavor. However, some pyrroles have been found to contribute to off-flavor of food products (24). Pyrroles have not received as much attention as flavor components as other heterocyclic Maillard reaction products such as pyrazines and thiazoles even though the number of derivatives identified is almost the same as that of pyrazines (Figure 1). Proposed formation mechanisms of pyrroles in the Maillard reaction systems are similar to those of thiophenes (Figures 2).

Pyrazines

Pyrazines are the major volatile flavor chemicals produced in Maillard reactions. The discovery of this role of pyrazines was one of the most significant advances in flavor chemistry and two comprehensive reviews of pyrazines have appeared (25, 26). In the 1970's, pyrazines were well-characterized as the compounds which directly contribute to roasted or smoky flavors. Some pyrazines possess an extremely low odor threshold (25, 29). For example, odor threshold of 2-isobutyl-3-methoxypyrazine in water is 0.002 ppb.

Many pyrazines were isolated and identified in cooked foods, especially in cooked meats (27). Pyrazines comprised over 40% of the volatile compounds found in cooked pork liver (28). Two pyrazines, 2-methyl-3(or 6)-pentylpyrazine and 2,5-dimethyl-3-pentylpyrazine were among 52 volatiles identified as lipid-protein-carbohydrate interaction products in a zein regular or waxy corn starch-corn oil model system (7).

It was suggested that α -amino carbonyls such as 3-amino-butane-2-one formed a dihydropyrazine which was subsequently oxidized to a pyrazine (30, 31). The conversion of dihydropyrazine to pyrazine occurs with or without oxygen. There are two possible ways to convert dihydropyrazine into pyrazine without oxygen. One is the disproportionation of dihydropyrazine to give pyrazine and tetrahydropyrazine or piperazine. The other is the dehydration of hydroxy dihydropyrazine (32). Recently, a dialkylpyrazine radical was reported as an intermediate of pyrazine formation (33). However, this simple step from dihydropyrazine to pyrazine is not yet thoroughly understood.

Thiazoles, Thiazolines, and Thiazolidines

Thiazoles are somewhat unique among the Maillard products because they contain nitrogen and sulfur atom in same ring. The presence of thiazole

derivatives in foods has been known for many years. Thiamine, or vitamin B₁, was discovered to contain a thiazole ring in 1935. Thiamin gave three thiazole derivatives by heat-degradation (34). Following a report of 2-acetyl-2-thiazoline in beef broth (35), a number of thiazole derivatives were found in cooked meat in the early 1970's (27). Thiazoles have been considered one of the main constituents which give a meaty flavor and are widely used in imitation meat flavors (36, 37). Many thiazoles were also found in coffee volatiles (38).

Thirteen thiazoles were identified in a reaction mixture of a browning model system consisting of D-glucose, ammonia, and hydrogen sulfide (17). Later, thirty thiazoles and thiazolines were reported in a Maillard reaction mixture of L-rhamnose, ammonia, and hydrogen sulfide (23). Cysteine produced many thiazoles in a reaction with monosaccharide, but the formation of thiazolidine was not observed (16, 39). On the other hand, cysteamine, which is a decarboxylated derivative of cysteine, produced a series of alkylthiazolidines (18). Neither thiazolidine nor its analogues have been found in foods, although alkylthiazolidines have been used as flavor ingredients to create meat-like flavor when mixed with pyrazines and cyclohexenones (40).

The yield of 2-methylthiazole increased most with increased irradiation time in the photochemical reaction mixture of cysteine and glucose (19). Thiazolidine and 2-methylthiazolidine, which give a popcorn-like flavor, were also found in this irradiated sample. There have been no reports of the formation of thiazolidine in thermally-activated Maillard reaction systems except the cysteamine/D-glucose system. It has been proposed that thiazolidines may form from thiazolines, which have been found in various foods and Maillard model systems, via a disproportionation reaction. However, this disproportionation reaction was not observed under a range of conditions in contrast to a disproportionation reaction of dihydropyrazine (Shibamoto, The University of California, Davis, unpublished data). Because thiazolidine forms readily from cysteamine, decarboxylation of cysteine to cysteamine may be required to form thiazolidine. In fact, several thiazolidine derivatives were identified in the photoreaction mixtures of cysteine and D-glucose either upon sunlight or UV irradiation, suggesting that photochemical decarboxylation of cysteine had occurred (19, 41, 42).

Imidazoles

Even though imidazoles comprise the second largest fraction of the volatile products obtained from Maillard reaction after pyrazines, they do not contribute any characteristic flavors to cooked foods (23).

Formation mechanisms of imidazoles in the Maillard reaction are not as well understood as those of other heterocyclic compounds. The role of α -amino carbonyl fragments as intermediates in imidazole formation was suggested in the reaction of sucrose and ammonia (43). In a study of a L-rhamnose/ammonia model system, which produced fifty-two imidazoles, it was proposed that an amino-hydroxy fragment was responsible for imidazole

formation (5). It was generally recognized that an α -amino carbonyl was the major intermediate of pyrazine formation. Therefore, production of tetramethylpyrazine from the reaction of diacetyl and ammonia in high yield was expected. However, 91.6% of the total products from this reaction was 2,4,5-trimethylimidazole (44). 2,4,5-Trimethylimidazole can easily be misidentified as tetramethylpyrazine because their mass spectral fragmentations are almost identical. The proposed formation mechanism of this imidazole is shown in Figure 3.

Pyridines

Many 2-alkylpyridines were isolated and identified in roasted lamb fat (45). 2-Alkylpyridines were proposed to form from the corresponding unsaturated n-fatty aldehyde reacting with ammonia upon heat treatment. Ammonia, arising from glycine, can react with nonanal, arising from beef fat, produced 2-butylpyridine in a beef fat/glycine browning model system (6). The same phenomenon was observed in the formation of 2-pentylpyridine from 1-decanal. Maltol produced 2-ethyl-3-hydroxy-6-methylpyridine as a major product in a reaction with ammonia (12).

Pyridines have a unique odor which plays both positive and negative roles in cooked foods. Pure pyridine possesses a rather unpleasant pungent and diffusive odor. The flavor characteristics of pyridines are, however, dependent upon their concentration. For example, 2-pentylpyridine gives a very pungent and unpleasant odor at high concentration, but an acceptable

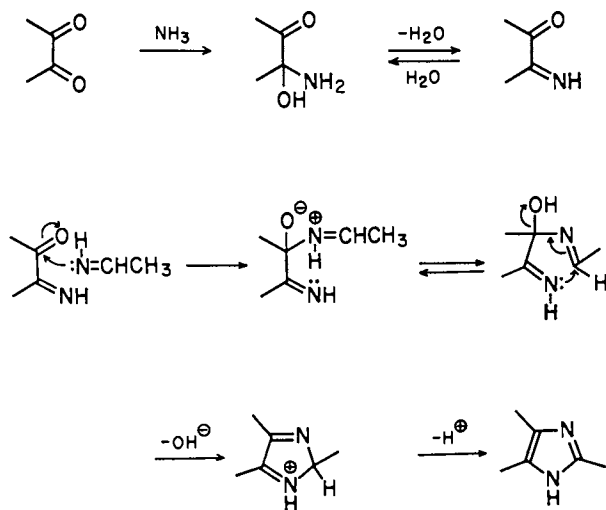


Figure 3. Proposed formation mechanisms of 2,4,5-trimethylimidazole.

fatty or tallow-like odor in dilute solutions. However, the odors of pyridines are generally less pleasant, and the presence of alkylpyridines in cooked lamb may cause its rejection by some consumers (45).

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Chapter 14

Model Reactions on Generation of Thermal Aroma Compounds

W. Baltes, J. Kunert-Kirchhoff, and G. Reese

Institut für Lebensmittelchemie der Technischen Universität Berlin,
Strasse des 17. Juni 135, D-1000 Berlin 12, Federal Republic of Germany

Thermal aromas result from the Maillard reaction. By heating carbohydrates with amino acids degradation is accelerated yielding reactive compounds which, by new reactions with amino acids, are converted to heterocyclic products. Results of model investigations of glucose or its degradation compounds with the amino acids serine and phenylalanine are discussed.

It is demonstrated that a great many flavor compounds are formed in both model systems. On the other hand, phenylalanine formed by aldol condensations some special aroma products. Furthermore, the generation of thermal aroma compounds depend on the pH, the sugar/amino acid ratio and the temperature.

The organization of a special symposium dealing with thermal aromas show the particular importance which they have received in past years. One reason may be the interest of flavor companies producing thermal aromas, which are frequently used in convenience food products. Another reason is their complex composition which challenges scientists to find out suitable ways for their analysis.

We have carried out some model reactions on the formation of thermal aromas in order to test the conditions for the analysis of such aromas and to study the mechanisms of their formation and their dependence on concentration and temperature. Last but not least we were interested to get an overview about the compounds which can be formed by generation of thermal aromas.

The aroma precursors have been selected by taking into account the significant role of the Maillard reaction. Indeed, most aroma compounds of this type are formed by the reaction of amino acids with sugars or their degradation products. So we have obtained roast beef, roast mutton and heated vegetable aromas after having treated a mixture of amino acids and glucose at different temperatures and for varied times.

NOTE: Dedicated to Kurt Heyns on his 80th birthday.

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In this publication we report on the results of reactions between glucose and serine or phenylalanine under the conditions of cooking a soup, roasting of coffee or autoclaving process in water solution at 120°, 150° and 180° C. The experiments have been completed by additional reaction of xylose, fructose and some characteristic sugar degradation products like cyclotene, Furaneol and diacetyl and by thermal decomposition of Amadori rearrangement products. It is well known that sugars can react with suitable amino compounds very easily. In the course of these reactions sugars are mostly decomposed and brown melanoidins are formed. By-products of these melanoidins are many volatile compounds of characteristic aroma properties. They are also responsible for the well known aromas of heated food like meat, coffee and bread. This complex accumulation of reactions is designated as Maillard reaction (1). In the case of aldoses the reaction starts by condensation of the sugar with amino compounds yielding a N-glycoside which can form the corresponding derivative of a ketosugar (1-deoxy-1-amino-ketose, 2) via Amadori rearrangement. This reaction is dependent on the pH. On the one hand, Amadori rearrangement is catalyzed by protons, but on the other side N-glycoside formation will not take place when the reaction medium is too acidic. So we have mostly carried out our experiments at pH values of about 5,5 - 6,2. The mechanism of this arrangement is ascribed to endiol structures. The reverse rearrangement of a N-ketoside forming the corresponding aldose derivative (2-deoxy-2-aminoaldose) is also known: It is the Heyns rearrangement which had been discovered 30 years ago. Some "glucose-amino acids", products of the rearrangement, have been isolated from swine liver (3). Reactions of fructose with amino acids have also been observed after heating of fruits (4). On one hand the browning of a fructose containing reaction medium occurs slower in comparison to glucose (5). The intermediates of fructose degradation after reaction with amino compounds have not been investigated up to now.

On the other hand in 1967 J.E. Hodge (1) has given a description of degradation pathways of glucose after reaction with amino compounds. He had recognized that endiols as intermediates of an Amadori rearrangement are unstable compounds making the elimination of substituents in the allyl-position more easy. This can be a hydroxyl as well as an amino group. Today we distinguish between 2 degradation pathways. The first one contains the 3-deoxyosone as an intermediate. 3-Deoxyglucosone had been isolated from brown fruit pulps and from soy sauce. This degradation will form 5-hydroxymethyl-2-furfural as an end product. This compound is not only a characteristic degradation product of hexoses in weak acidic solutions, moreover it is a constituent of every thermal aroma. In coffee aroma hydroxymethyl furfural is one of the most important compounds (6).

In addition to the "3-deoxyhexosone-pathway" Hodge postulated a 1-deoxyosone as an intermediate of another degradation pathway in 1967 (1). This compound contains carbonyl- and hydroxygroups side by side. It has been recently identified by Ledl (7). As Figure 1 demonstrates this degradation pathway is responsible for the formation of a great many of reaction products from the carbohydrate molecule. It can also be recognized that there are numerous possibilities of keto-enol-tautomerizations. The speed of these reaction steps depends

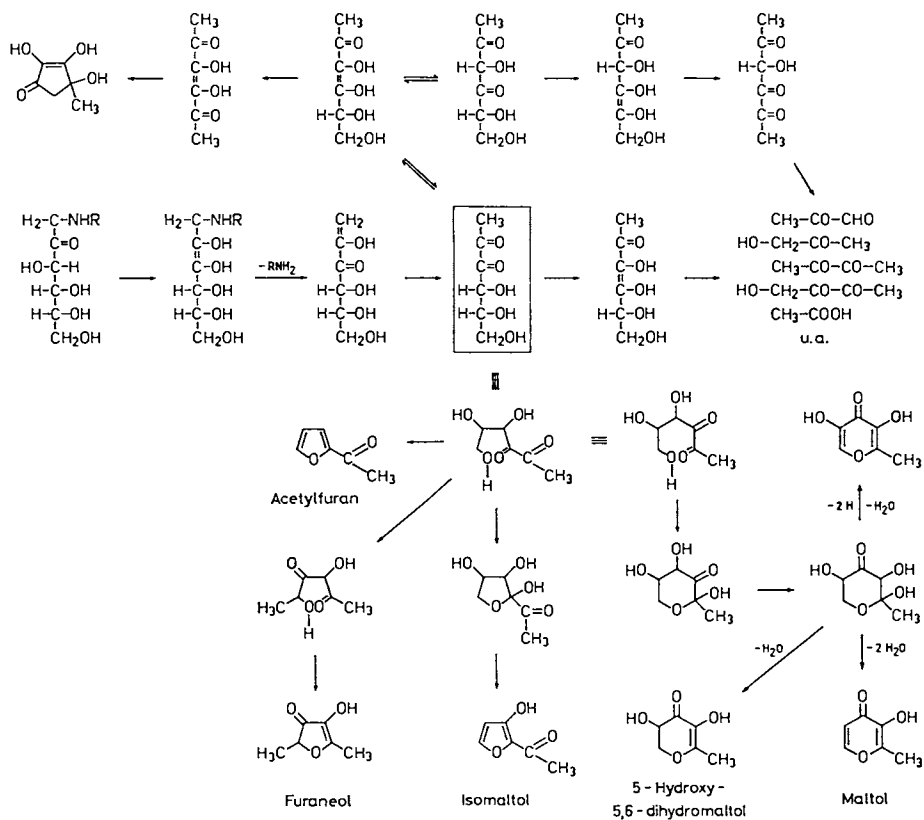


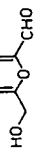
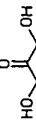

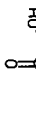
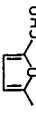







Figure 1: Suggested reaction routes of carbohydrate degradation via the 1-deoxyosone

on the temperature. In the course of these reactions, water eliminations occur and precursors of dicarbonyl compounds are formed via retro aldol reactions. These dicarbonyls show a high reactivity. Also acetic acid may arise from a reaction of this type. Most important compounds of this pathway are pyruvic aldehyde, diacetyl, hydroxyacetone and hydroxydiacetyl which can easily react with amino acids. The Strecker degradation is a reaction where the amino acid is decarboxylated and loses its amino group. Reaction products are the Strecker aldehyde and - as an intermediate - an aminoketone which forms a pyrazine by dimerization. This pathway is considered to be most important for the origin of pyrazines in thermal aromas. However, only limited knowledge is available about the fate of the Strecker aldehydes. As we will demonstrate they are very reactive. In addition, Figure 1 demonstrates that some intramolecular cyclizations are possible. So acetyl-furane will be present in every thermal aroma when the amount of carbohydrate in the reaction medium was high enough. Also maltol, 4-hydroxymaltol, isomaltol and even Furaneol (2,5 dimethyl-4-hydroxy-3[2H]-furanone) the "anas-furanone" can be detected in most cases though their concentrations are lower than the amounts of aliphatic dicarbonyls. But this scheme shows that the formation of maltol doesn't require the substitutions of the 4-position and that the formation of Furaneol is not absolutely dependent upon a 6-deoxysugar. On the contrary the sugar molecule can obviously form suitable precursors for their formation under reaction conditions which are similar to the conditions of industrial aroma processing.

Table 1 shows the most important degradation products of glucose which have been formed in an aqueous reaction medium containing serine as amino acid after heating in an autoclave. These compounds have been quantified after GC/MS analysis of the volatiles by an integration program of our computer. - The compounds cited on the left side are probably formed via the 3-deoxyosone pathway (with the exception of acetyl-furan and acetylpyrrole). The dominant product of this scheme is 5-hydroxymethyl-2-furfural ("HMF") the concentrations of which have been lowered with raising temperature. Furanaldehyde is its dehydration product and 5-methylfurfural probably formed from HMF, too. The most important reaction product of the 1-deoxyosone-pathway is 5-hydroxy-5,6-dihydromaltol. At 120° C this compound which we have identified in most of our model reactions, represents about 30 % of all volatiles in the reaction system serine/glucose. We are just initiating studies dealing with further degradation and reaction pathways of this compound, which is very instable and disappears at higher temperature as well as by treatment on silica gel columns. Simultaneously, the concentrations of cyclotene (2-hydroxy-3-methyl-cyclopenten-2-one) and Furaneol increase. This possible correlation led to the assumption that both compounds are degradation products of the dihydroxymaltol. Therefore it was suggested that cyclotene is formed from this maltol derivative via a hexose reductone and the hydroxycyclotene (9). Another formation pathway of cyclotene is suggested to be a condensation reaction of hydroxyacetone, which can also react as lactic aldehyde. The formation of Furaneol is proposed to happen via ring contraction yielding acetylformoin, which is well known to be a cyclization product of 1-deoxyosone. This compound has been identified via its reaction products

Table 1

Compounds formed in amounts 1% by cooking glucose with serine

	120°C	150°C	180°C		120°C	150°C	180°C
Furans				Pyranones			
Pyrroles				Furanones			
	34,5%	15,4%			32,3%		
	1,0%				1,0%	1,0%	
	0,6%	4,3%			0,9%	11,0%	3,0%
	0,6%	2,2%	1,1%		4,2%	9,0%	1,2%
	2,5%	1,1%	1,1%		1,5%	2,6%	
	3,5%	2,2%		Σ	87,7%	54,4%	9,0%
	5,1%	5,6%	2,6%	Pyrazines	0,5%	22,0%	50,0%

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(10). It is suggested that it forms Furaneol by dehydration and hydrogenation of the exocyclic methylene group.

As was already mentioned, we have reacted our model mixtures under different conditions. Among them, the experiments of cooking and autoclaving a phosphate buffer solution of a sugar/amino acid mixture have been very similar. Only the temperature was different. Furthermore, the roasting experiments have been carried out by heating the sugar/amino acid mixtures on an inert material in the presence of 10 % buffer solution. In the latter we have identified a great many of pseudo-aromatic and aromatic compounds. These are, among others, predominantly different alkyl-substituted furans, pyrazines, pyrroles and benzo-aromatic compounds. This behaviour of the reaction system to form compounds which are stabilized by mesomeric energy seems to be reasonable. When cooking at about 100° C predominantly furans with oxygen containing substituents, furanones, pyranones and aliphatic carbonyl compounds were formed (11). Moreover the number of volatiles in this case are less than in the case of roasting.

By heating the mixture in a buffer solution at greater than 100° C in an autoclave the question about the influence of the reaction medium on number and kind of volatiles should be answered. The answer, indeed, is very simple: most compounds formed in the autoclave have been identical to the volatiles after roasting the mixture. Consequently, the reaction temperature is the most decisive factor for the formation of thermal aroma compounds.

Table 2 shows the relative quantities of component groups in the volatiles after heating mixtures of glucose or fructose with serine at 120°, 150° and 180° C. It shows optimal formation of furans and pyranones at 120° C, whereas furanones possess a maximum at 150° C. Compounds of the other groups are formed preferentially at 150° C, while the formation of pyrazines proceeds better the higher a reaction temperature was chosen. Also pyrroles need higher temperatures for their formation. So we could demonstrate that only acetylpyrrole and 5-methylpyrrole-2-aldehyde has been formed at 120° C whereas many additional pyrroles appear at 150° C or 180°C respectively (12).

By reaction of serine or phenylalanine with fructose instead of glucose we got identical results with the exception of higher pyrazine concentrations (more than 50 % relatively higher!). Main products have been 2,5 and 2,6-dimethylpyrazine which may be formed via the reaction of two C₃-dicarbonyls each. In our opinion these results demonstrate a special ease of fructose to decompose by chain cleavage. As another decomposition product of fructose we have identified furfuryl alcohol after heating a reaction mixture at 120° C. Obviously this C₅-compound has been formed after cleavage of the carbon atom 1 from the sugar molecule.

Carbocyclic Compounds

Ofcourse, the number of components increase with rising temperature. Examples are carbocyclic compounds. Most of them are cycloalkenones and hydroxycycloalkenones. One reason for this fact is their isomerization. As we could demonstrate (12) cyclotene forms at 180° C 3 isomeric compounds and additionally 3 methylcyclopentanones or-pentenones via elimination of one molecule of water (Figure 2). Hydroxycyclopentenones and-hexenones are well known to be important aroma compounds in caramel flavours (13,14).

Table 2: Relative Quantities (%) of Component Groups after Heating of Serine with Glucose or Fructose in an aqueous system

Class	Serine-Glucose %			Serine-Fructose %		
	120°C	150°C	180°C	120°C	150°C	180°C
Furans	45	31	3-4	55	29	2
Furanones	6-7	12	2	6	13	1
Pyranones	33	2	-	23	1-2	-
Carbocyclic Cpds.	1	13	12	1	16	12
Pyrazines	1	14	48	7	22	65
Pyrroles	5	7-8	7	2	5-6	3
Pyridines	1	3-4	9	1	1-2	4
Aliphatic Cpds.	7	12-13	9	5	8	9

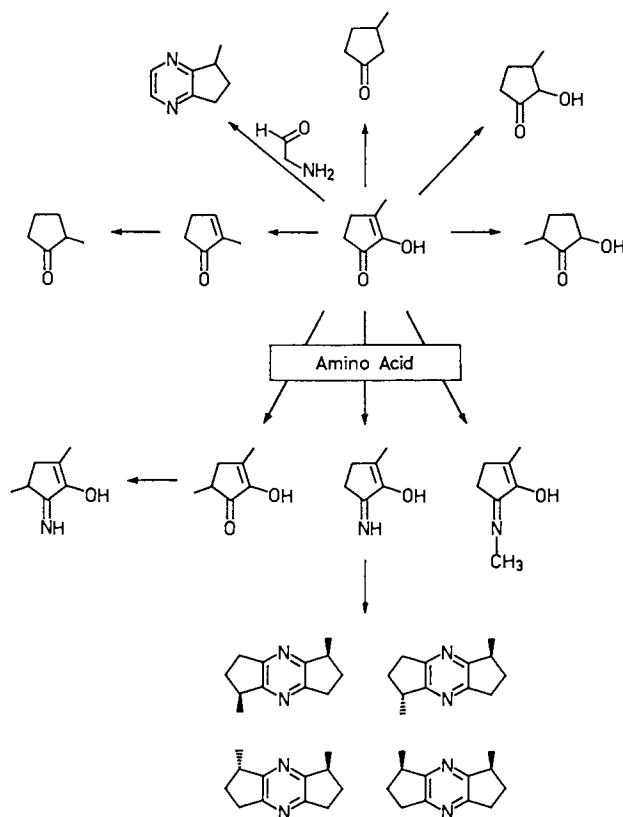


Figure 2: Reaction of cyclotene with amino acids

Pyrazines

The N-heterocyclic compounds which have already been mentioned are formed by reaction of ammonia or amino acids with various carbonyl compounds from the sugar decomposition. Most important in this field is the Strecker degradation of amino acids via reaction with α -dicarbonyl compounds. In this regard every amino acid with primary amino group will probably form compounds of this type. Monocyclic pyrazines are mostly substituted by methyl groups demonstrating the role of diacetyl, pyruvic aldehyde and corresponding decomposition products of the sugars. Other substituents of monocyclic pyrazines are ethyl-, propyl-, vinyl-, allyl- and propenyl groups. Most important examples are 2,5-, 2,6-dimethylpyrazine and 2-ethyl-5-methylpyrazine, whereas alkenylpyrazines mostly appear only in trace amounts. Bicyclic pyrazines like furanyl-, furfuryl-, pyrrolo- and dihydrocyclopentapyrazines respectively tetrahydrochinoxalines mostly require temperatures over 150° C for their formation, and also monocyclic pyrazines are formed in low amounts at 120° C only. Variety and quantity of the pyrazine fraction depend on the amino acid/sugar ratio, too. We have found 10 pyrazines after roasting a mixture of an excess of phenylalanine with glucose at 220° C (11). On the other hand we have obtained 57 mono- and bicyclic compounds after heating this mixture at 180° C in the autoclave, but in a molar ratio of 1 : 1. - The hydroxyamino acids serine and threonine are very important precursors of pyrazines because they form amino-carbonyl compounds alone when heated to temperatures greater than 150° C. So we have identified more than 120 mono- and bicyclic pyrazines (15) after heating mixtures of sugars with these amino acids.

Cyclotene is the precursor of dihydrocyclopentapyrazines after having reacted with ammonia respectively amino acids to form cyclotene imine. Figure 2 demonstrates not only the isomerization and transformation of this compound but also the formula of bis-dihydrocyclopentapyrazines as special reaction products. They are formed by condensation of cyclotene-imine and represent a mixture of 4 diastereomeric compounds. These compounds produce identical mass spectra (Shibamoto et al,16). We have carried out corresponding experiments by heating Furaneol with phenylalanine or serine in an autoclave at 180° C. As Figure 3 demonstrates some monocyclic pyrazines are formed by reaction of the degradation products from Furaneol. The presence of furopyrazines demonstrates that Furaneol can react as an α -dicarbonyl compound. Furopyrazines have also been found at trace levels after heating glucose with amino acids (J. Kunert-Kirchhoff and W. Baltes, *Zeitschrift für Lebensmittel-Untersuchung-Forschung*, in press). After heating Furaneol alone to 180° C 17 degradation products have been identified.

Pyridines and Pyrroles

Pyridines and pyrroles can be formed in different pathways by Mailard reaction. The formation of 5-methylpyrrole aldehyde and 6-methyl-3-pyridinole has been observed by Nyhammar et al (17) by the reaction of isotope labelled 3-deoxyosone with glycine. The 3-deoxyhexosone represents an α -dicarbonyl compound and in this way the Strecker degradation occurs. Another pathway is the reaction of furans with ammonia. Under roast conditions, we have obtained primarily the corresponding pyrrole, whereas we found the corresponding py-

ridinoles after reaction in an aqueous system at 130° - 160° C. (G. Reese and W. Baltes, *Ztschr. Lebensm. Unters. Forsch.*, in press). This reaction requires a carbonyl function in the position 2 of the furan derivative. By ring enlargement the carbonyl group is converted to a hydroxyl group in the β -position, whereas the aliphatic residue (from a ketone!) remains in the α -position of the pyridine ring.

Whereas "normal" α -amino acids give rise to the formation of about 25 different pyrroles under roast conditions, serine and threonine are even more active. We have obtained more than 60 different pyrroles including furfuryl- and furanylpyrroles and dihydropyrrolizines after roasting in the presence of sugars. This variety of pyrroles can be obtained by the reaction of proline or hydroxyproline, which already each contains a pyrrole ring. To our surprise, histidine forms a relatively high number of pyrroles by roasting with sugars. By roasting hydroxyamino acids we have also obtained more than 40 different pyridines, whereas the reaction of phenylalanine yielded only about 20 derivatives. Most products possessed acetyl- or hydroxyl groups in addition to methyl- and ethylradicals, when the mixture was heated in an autoclave.

During our experiments of heating phenylalanine with glucose in an aqueous solution at 180° C we obtained a compound possessing an uncommon structure which is represented in Figure 4. It is an α -N-hydroxymethylpyrrolyl-propionic lactone. Two compounds of this type have been isolated by Dickerson et al (18) from flue cured tobacco. The authors described their flavor as spicy and peppery. After roasting different amino acids with glucose at 200° C (18-20) some compounds of this type have been isolated. We reacted HMF with phenylalanine in an autoclave at 180° C. Under these conditions the pyrrololactone did not arise. That led us to the conclusion that the precursor of this compound might be the 3-deoxyosone occurring, which after reaction of the carbon atom 2 with the amino acid should yield the substituted ketimine.

Oxazoles and Oxazolines

In our experiments oxazoles and oxazolines were found only rarely. After roasting serine and threonine we have found traces of 20 different oxazoles which were mostly substituted by methyl groups. Nine of these oxazoles we found in coffee aroma for the first time (21, 22). About 30 oxazoles have been described to be present in thermal aromas of coffee, cocoa and meat. It has been suggested that hydroxyamino acids are their precursors. In our experiments with serine and threonine only traces of these compounds have been formed, hence we are not in agreement with this mechanism. On the contrary, we assume a reaction pathway consistent with the suggestion of Vitzthum (23) or Rizzi (24). The latter obtained substituted oxazolines via the Strecker degradation of amino acids where a cyclization occurred after decarboxylation. On the other hand, Vitzthum et al have assumed an acetylation of the intermediate amino enol formed by the Strecker degradation. In earlier experiments we have identified di- and trimethyloxazoles after reaction of diacetyl with ammonia or amino acids (25).

Steps in thermal aroma formation

The most important steps of thermal aroma formation via the Maillard reaction are:

1. The first step of the reaction is the condensation of amino acids to carbon atom 1 of aldoses (or C-2 of ketoses) and the rearrangement to the keto (aldo)-sugar (Amadori or Heyns-rearrangement). The intermediate endiol structures give rise to a facile sugar decomposition by which different aliphatic or cyclic mono- or dicarbonyl compounds are formed. For Amadori rearrangement products two reaction pathways are known: the 1-deoxy or the 3-deoxyosone pathways.
2. α -Dicarbonyl compounds react with amino acids to yield the Strecker aldehydes and aminoketones which can be converted via dimerization to yield pyrazines.
3. Sugars are the main precursors of α -dicarbonyls. The amino acid/sugar ratio is decisive for the proportion of pyrazines, furans, furanones, pyranones in the volatile fraction.
4. Sugars as well as amino acids are decomposed by heat treatment. The final reaction products from sugars are often identical with the products formed by the Maillard reaction.
5. Temperature is most influencing factor in the composition of thermal aromas.

This means that most α -amino acid/sugar-models will basically form similar compounds in the volatile fraction at the same temperature. Consequently, the predominant primary sugar degradation products consist of furans, furanones, pyranones, cyclopentenones and cyclopentenones with or without hydroxylgroups plus some aliphatic carbonyls as well as aromatic compounds. By reaction with ammonia a great variety of pyrroles, pyrazines, pyridines, pyridinols and oxazoles are formed. In the presence of sulfur containing amino acids, thiazoles, thiophenes and compounds with more than one S-atom (e.g. trithiolanes, triphanes) are formed. On the other hand a great many of special reaction products are formed when secondary amino acids are reacted with sugars in order to produce thermal aromas.

Reaction of Strecker aldehydes

The question of the fate of the "Strecker" aldehydes requires an answer. By converting the amino acid phenylalanine to yield aroma compounds, phenylacetaldehyde is liberated. Because of its phenyl ring it is a good detector compound. We were able to establish some of its reaction products. For example, we have identified, among others, phenylethylpyrazine, phenylfuran, phenylethylpyrrole and phenylpyridine. We assume that aldol condensations are responsible for the formation of these compounds. Figure 5 illustrates our assumption. We have identified several compounds the structures of which make probable an aldol condensation (3-(2'-furyl)-2-phenyl-2-propenal, phenylhydroxyketones) likely. This assumption is supported by the identification of pyrazines with up to 5 carbon atom side chains in other experiments.

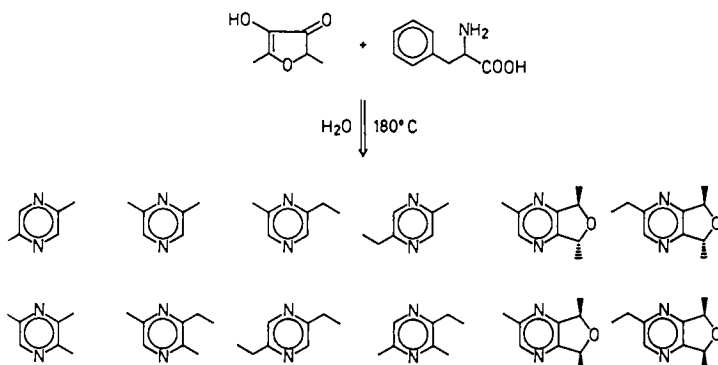


Figure 3: Reaction of Furaneol with phenylalanine

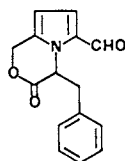
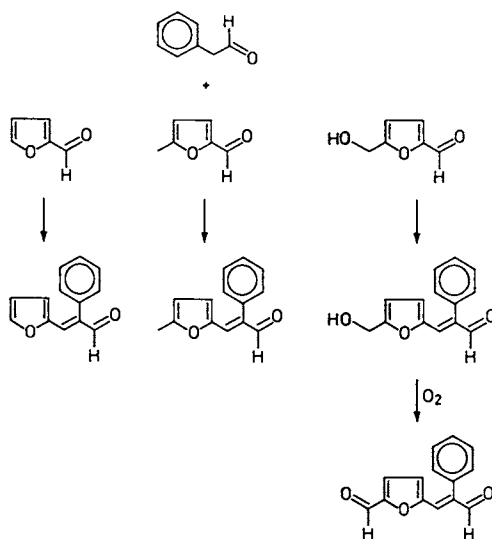
Figure 4: α -N-Hydroxymethylpyrrolyl-phenylpropionic lactone

Figure 5: Condensation products of phenylacetaldehyde

We are just beginning to understand the formation and composition of thermal aromas, but undoubtedly there is still a lot of work to do until we will be able to control all influences.

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Chapter 15

Formation of Amino Acid Specific Maillard Products and Their Contribution to Thermally Generated Aromas

R. Tressl, B. Helak, N. Martin, and E. Kersten

Institut für Biotechnologie, Forschungsinstitut für Chemisch-technische Analyse, Technische Universität Berlin, Seestrasse 13, D-1000 Berlin 65, Federal Republic of Germany

In model experiments L-proline, hydroxy-proline, cysteine and methionine were heated with monosaccharides for 1 - 1.5 h at 150 °C. Amino acid specific Maillard products were isolated from the extracts by preparative GC or HPLC and identified by MS-, IR-, ¹H- and ¹³C-NMR-spectroscopy. Proline derived components are important constituents in bread, malt and beer. More than 120 proline specific Maillard products were characterized. Cysteine and methionine derived components were predominant in roasted coffee and meat flavors. Thirty cysteine- and twenty methionine-specific Maillard products were identified for the first time.

Proline Specific Maillard Products

As demonstrated in previous publications, model reactions of L-proline and monosaccharides result in complex mixtures of proline specific compounds (1-4). During the Maillard reaction of L-proline and reducing sugars more than 120 proline specific compounds were identified by MS-, IR-, ¹H- and ¹³C-NMR-spectroscopy (among them: 2,3-dihydro-1H-pyrrolizines, pyrrolidines, 2-acetyl- and 2-furylpiperidines and -piperideines, di- and tetrahydro-1H-azepines). The reaction conditions determine the spectrum of proline specific compounds. We attempted to select conditions, so we could trace the routes by which important constituents are formed without splitting C-chains of the sugars by pyrolysis. Under mild reaction conditions (100 °C; H₂O; pH 3 - 7; 1 - 2 h) only a few compounds are formed (Figure 1). It can be seen that all constituents (except 25) contain six C-atoms from glucose. In 1982 we isolated 60 as a main component from malt and named the new tricyclic compound maltoxazine (1).

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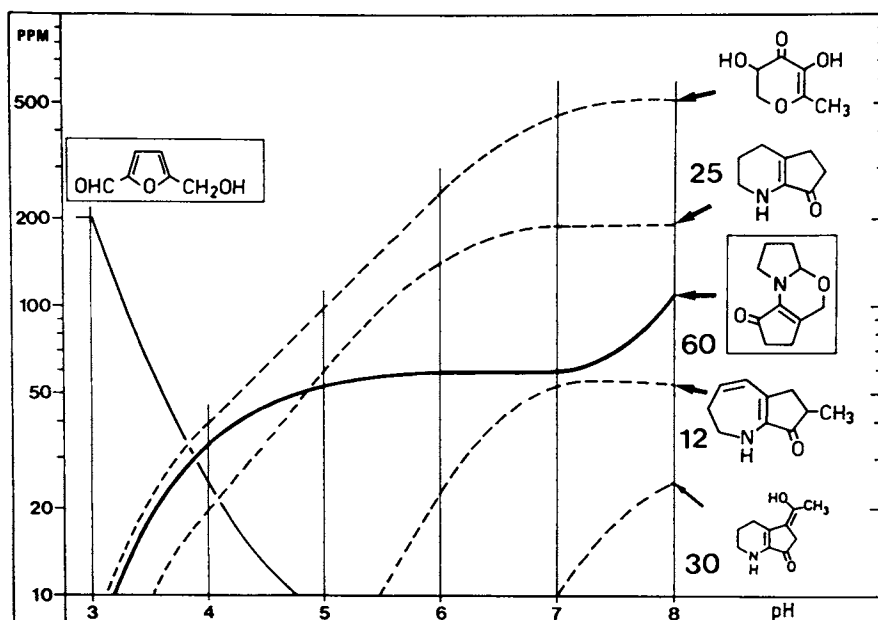


Figure 1: Main components from proline/glucose model system, depending on pH-value

Components 25 and 12 were first detected in wort, which was heated above 140°C . Beer, produced by this process, possessed a bitter aftertaste. Compound 30 was recently identified as a reactive intermediate, which decomposed very soon even by storage at -20°C . On heating L-proline and monosaccharides at 150°C for 1.5 h all compounds increased ten to fiftyfold and 2,3-dihydro-1H-pyrrolizines and di- and tetrahydro-1H-azepines were characterized as major components. On roasting L-proline and monosaccharides (or sucrose) pyrrolidines and azepinones predominate among the Maillard products. These compounds were also formed by heating pyrrolidine and glucose at 100°C . Azepinones and certain pyrrolin-derivatives possess extreme bitter taste and thresholds of 5 - 10 ppm (3,5).

The significant role of L-proline in the formation of specific products can be explained by its Strecker degradation (3). As shown in model experiments, L-proline reacts with α -dicarbonyls to yield 1-pyrroline and α -hydroxyketone, pyrrolidine and α -diketone. Hydroxyproline is transformed into pyrrole and diketone and pyrrolyl derivatives, depending on the sugar and reaction conditions. Primary amino acids form pyrazines and pyrroles which are not produced in the proline system. Under elevated temperatures retro aldol reactions predominate forming α -dicarbonyls and α -hydroxyketones containing two to four C-atoms. In a model experiment of erythrose (glucose) and L-proline, 5-(3-hydroxypropionyl)-2,3-dihydro-1H-pyrrolizine and 1,2,3,4,5,6-hexahydrocyclopenta(b)pyridine-7(1H)-one were identified. Both compounds are formed by 3-deoxyosones and their retro aldol products, respectively (2). During this reaction 2-acetyltetrahydropyridines are formed as flavor components possessing cracker-like aromas and thresholds at 1 to 2 ppb. Azepinone-derivatives are formed under elevated temperatures in proline/monosaccharide experiments. As demonstrated, cyclic α -dicarbonyls act as precursors increasing the products 1000 fold compared to monosaccharides. Figure 2 presents a scheme which may explain the reaction which forms components with extreme bitter taste qualities. In malt and beer L-proline is the major free amino acid and is transformed into flavor contributing compounds during kilning, wort boiling, pasteurization, and elevated storage conditions. Figure 3 shows a pathway which is operative under mild reaction conditions and leads to maltoxazine.

The products found in model experiments and in foodstuffs are not comparable. The concentrations of individual constituents vary considerably between both systems. Figure 4 summarizes proline specific components determined in wort and beer produced under different reaction conditions. Beer I represents a German pale beer, produced from pale malt by a conventional wort

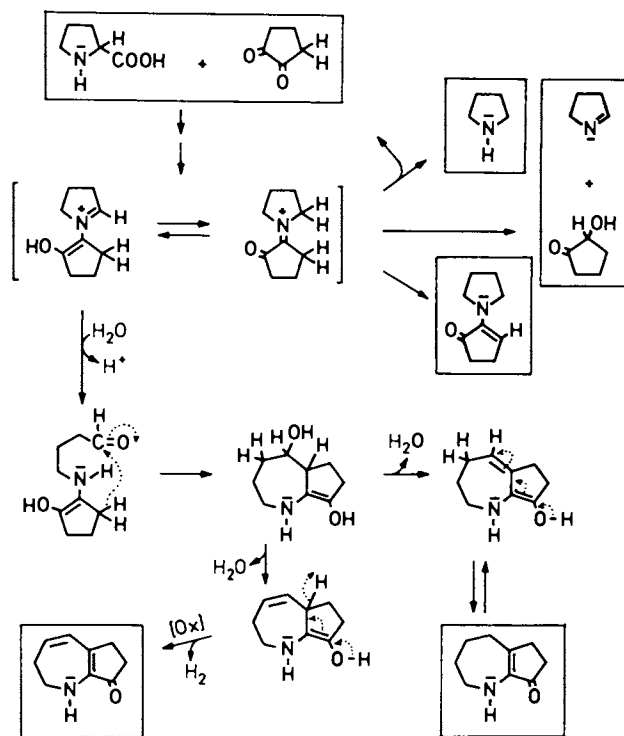


Figure 2: Reaction scheme of hexa- and tetrahydro-1H-azepinones from proline and cyclic α -di-carbonyls

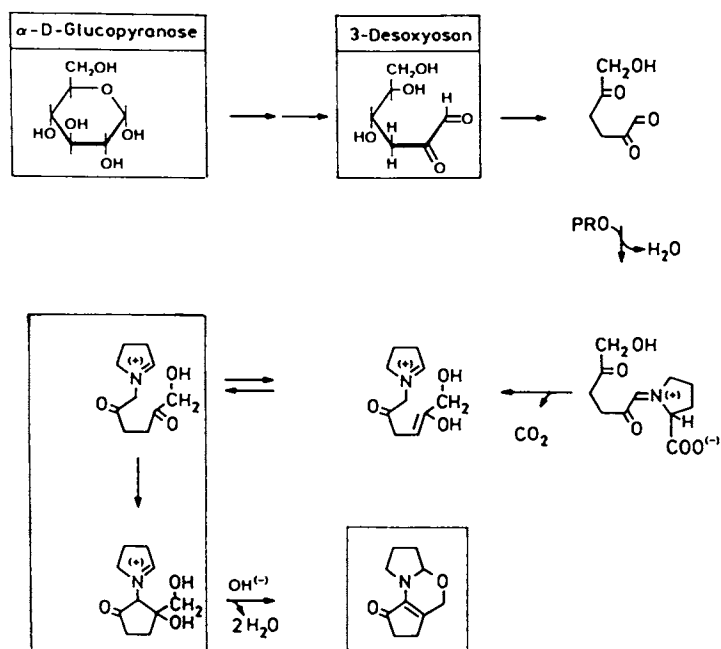


Figure 3: Pathway leading to maltoxazine from 3-deoxyglucosone and proline











			120°C 80 min		120°C 3 min		140°C 3 min		
	Würze I	Bier I	Würze II	Bier II	Würze III	Bier III	Würze IV	Bier IV	
60		170	600	1130	1300	240	220	180	170
		0,5	1	2	3	0,1	2	4	2
		1	4	9	10	0,2	5	11	4
		5	35	10	180	39	73	43	50
25		7	4,5	150	100	4,6	7,6	5,4	4,1
		0,8	0,6	32	35	0,1	2	0,1	2
11		5	5	11	9	14	18	15	13
12		4	3	4	70	6	76	6	70
9		-	-	3	+	6	+	5	+
10		1	4	18	32	14	11	26	15

Figure 4: Typical proline specific constituents in wort and beer depending on different boiling conditions

boiling process, which possessed desirable flavor. Beer II was produced from the same malt by a wort boiling process of 120 °C. This beer possessed a strong bready off flavor and an undesirable bitter aftertaste. It can be seen that some tetrahydropyridines and azepinones increased during this process (6). The concentrations of flavor contributing components were not comparable to our model experiments. Recently we demonstrated that the compounds with high flavor values are methylene active constituents and react with carbonyls to form cyclopenta-(b)pyridinone-derivatives which are less volatile and possess high thresholds. By this reaction, flavor contributing proline derivatives may be bound to the matrix of melanoidines. Azepinones with exocyclic double bonds are transformed into nonvolatile compounds and may cause the bitter aftertaste of the products. Finally, we identified 3-furylidene-1-pyrrolines and 3-furfurylpyrrols by heating L-proline with furanaldehydes and monosaccharides (7, 8).

Cysteine Specific Maillard Products

Figure 5 presents typical Strecker degradation products from cysteine, which were identified in a xylose model system. During Strecker degradation the labile mercaptoacetaldehyde is further decomposed to acetaldehyde and H₂S. 3,5-Dimethyl-1,2,4-trithiolanes and thialdine are formed by this reaction as well as 3,6-dimethyl-1,2,4,5-tetrathiane. 1,2,3-Trithia-5-cycloheptene, first identified by Shu et al. (9), is obviously formed by condensation of mercaptoacetaldehyde and acetaldehyde. Mercaptoacetaldehyde is transformed into 1,2,4-trithiane and 2-methyl-1,3-dithiolane. Thiazolidines are formed from the Strecker amine which undergoes further reactions with carbonyls. 3-Methyl-1,2-dithiolan-4-one, a main product in tetrose model systems, is obviously formed from the corresponding 1-deoxytetrosone (also detected in glucose/cysteine experiments). Similar results were obtained by Shu et al. (9) on heating cystine at different pH-values.

Cysteine/Pentose Model System

Main components in the cysteine/xylose system (180 °C; H₂O / pH - 5) are 2- and 3-mercaptopropionic-, furan-, and thiophene carboxylic acids, which amount to 40 to 80 % of the volatile Maillard products. The furan- and thiophene carboxylic acids are formed via 3-deoxyosone and the mercaptoacids via 1-deoxyosone (Figure 6). By dehydration, addition of H₂O (or by Strecker degradation of Cys) and reduction of the carbonyl group, furfurylmercaptan (the impact component of roasted coffee) results. 2-Hydroxymethyl-4-thiolanone, which may be formed by the same route, was identified for the first

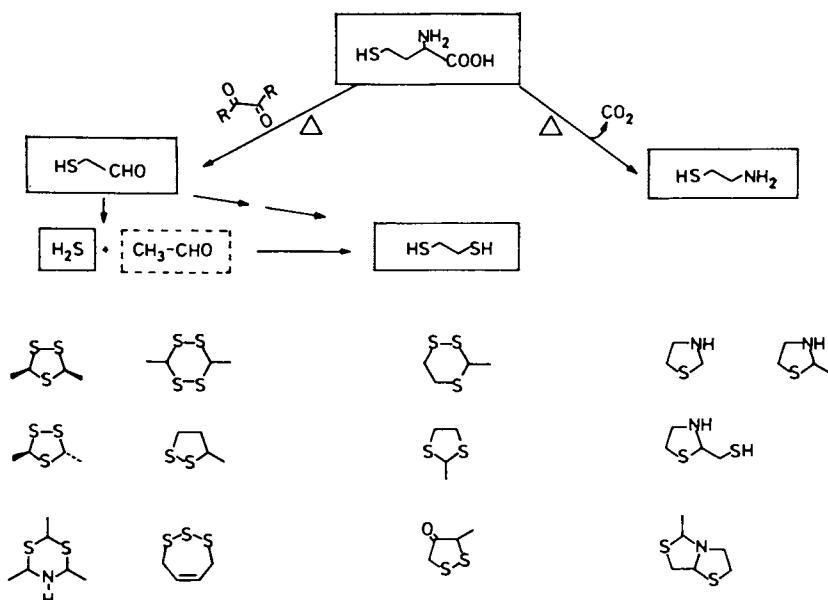


Figure 5: Strecker degradation products of cysteine formed from acetaldehyde, H_2S and cysteamine

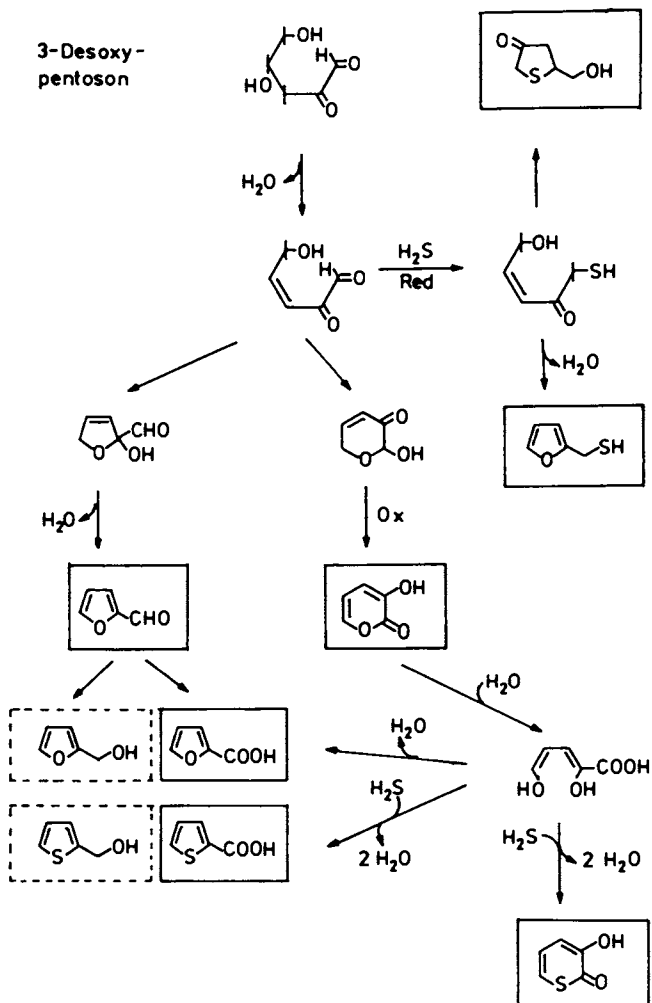


Figure 6: Main components of the cysteine/xylose model system formed via 3-deoxypentose

time as a cysteine-specific Maillard product. It was isolated from the model experiment by preparative GC and identified by IR- and $^1\text{H-NMR}$ -spectroscopy. The ratios for furfuryl alcohol to furancarboxylic acid (and the corresponding thiophenes) were detected at 90 ppm to 1300 and 80 ppm to 2100, respectively. Therefore, disproportionation of furfural can be eliminated. As a possible intermediate we identified 3-hydroxy-2-pyranone which is obviously transformed into the corresponding acids. In the cysteine/rhamnose model experiments all homologous components were identified in a similar range.

At pH-values of 5 - 7 typical cysteine/pentose products are formed via 1-deoxypentose (Figure 7). 2-Methyl-3-thiolanone is a flavor contributing component in roasted coffee (with a threshold of 50 ppb) and 2-methyl-3-mercaptothiophene is a component of roasted/cooked beef (10). Two thianones were identified for the first time as cysteine specific Maillard products. 2-Methyl-4-thianone and 4-thianone (not shown in Figure 7) were also detected in the glucose, erythrose and ascorbic acid experiments.

Figure 8 outlines possible routes leading to cysteine specific products via 1-deoxypentose. Under the chosen reaction conditions, (180 °C; H_2O) norfuraneol is further transformed via 4-hydroxy-5-methyl-3-(2H)-thiophenone and 2-methyl-3-(2H)-thiophenone, into 2-methyl-3-mercaptothiophene and 2-methylthiophene, respectively. Norfuraneol is a methylene active compound, which undergoes aldolcondensation with carbonyls forming colored non-volatile products. 2-Mercaptopropionic acid may be formed from the mercaptoketone by oxidative cleavage. By this route 2-methyl-3-thiolanone and 2-methyl-4,5-dihydrothiophene may result.

Cysteine/Hexose Model System

In the cysteine/glucose model experiments glucose reductone, acetylformoin, furaneol and cyclotene were detected as major constituents indicating that the 1-deoxypentose route is operative at pH-values of 5 to 7. As main component we identified by MS, IR- and $^1\text{H-NMR}$ -spectroscopy 4-hydroxy-2,5-dimethyl-3(2H)thiophenone. Obviously this compound is less reactive than the corresponding norfuraneol derivative. Thiofuraneol is also formed from glucose/reductone, acetylformoin and furaneol as demonstrated in model experiments. Surprisingly, it is not formed in cysteine/rhamnose experiments, where furaneol is a major compound. In addition 2-acetyl-4-thiolanone and 5-hydroxymethyl-2-methyl-3-thiolanone were identified for the first time as cysteine specific products. 2-Acetyl-4-thiolanone is formed at pH 3, indicating 3-deoxyhexosone is a possible precursor. Under this

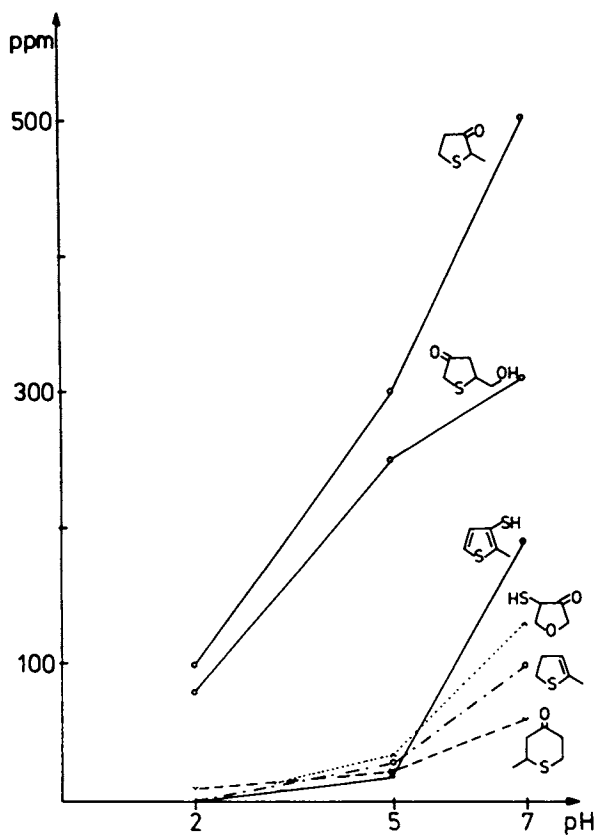


Figure 7: Formation of typical cysteine/pentose products via 1-deoxyosone depending on pH-value

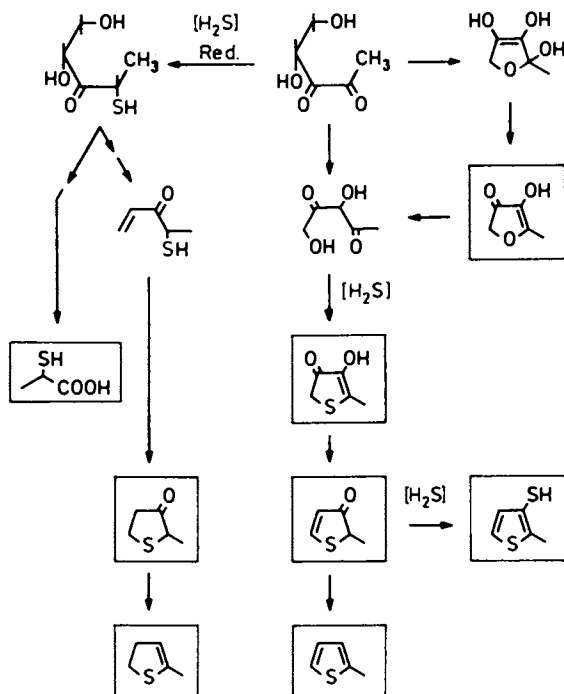


Figure 8: Possible routes leading to 2-mercaptopropionic acid, and several thiophenones and thiophenes via 1-deoxyxylulose

condition 2-(1-hydroxyethyl)-4-thiolanone could also be characterized. These compounds are formed analogously to the cysteine/xylose system. 5-Hydroxymethylfurfurylmercaptan could not be detected in our model experiments.

Figure 9 presents routes leading to major products in the cysteine/glucose experiments at pH values of 5 - 7. Thiofuraneol and 2,4-dihydroxy-2,5-dimethyl-3(2H)-thiophenone were formed as major components at a ratio of 10 : 1. All other thiophenes were minor constituents.

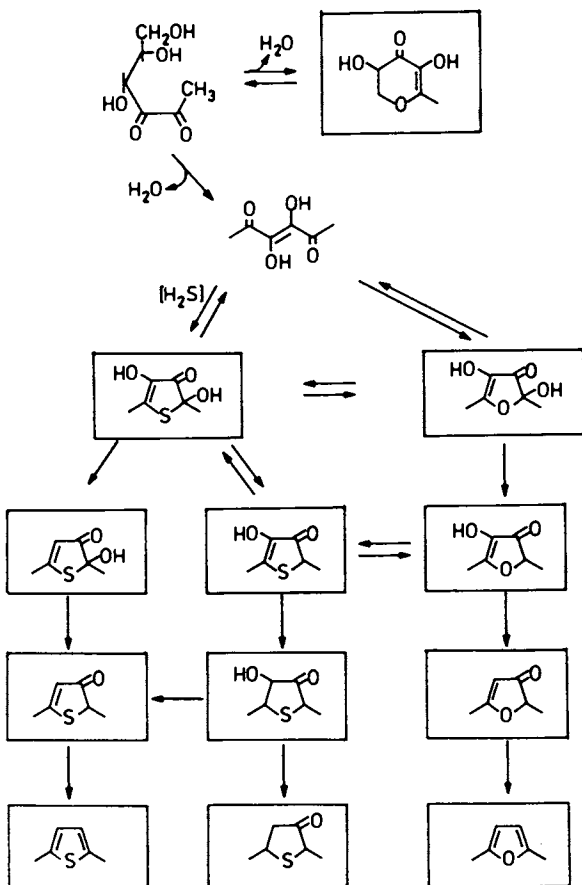


Figure 9: Reaction scheme leading to the major products of the cysteine/glucose model experiments

In the cysteine/rhamnose system 2-hydroxy-2,5-dimethyl-3(2H)-thiophenone and 2,5-dimethyl-3-thiolanone were characterized as major compounds. On heating cysteine and furaneol, products were formed comparable to those found by Shu et al. (11) under milder reaction conditions.

Experimental conditions and spectroscopic data of the new characterized cysteine/sugar reaction products will be published in detail.

Methionine Monosaccharide Model Systems

Maillard products which were identified in methionine/reducing sugar model experiments, result predominantly from the Strecker aldehyde (methional) and methylmercaptan, respectively. Figure 10 summarizes compounds

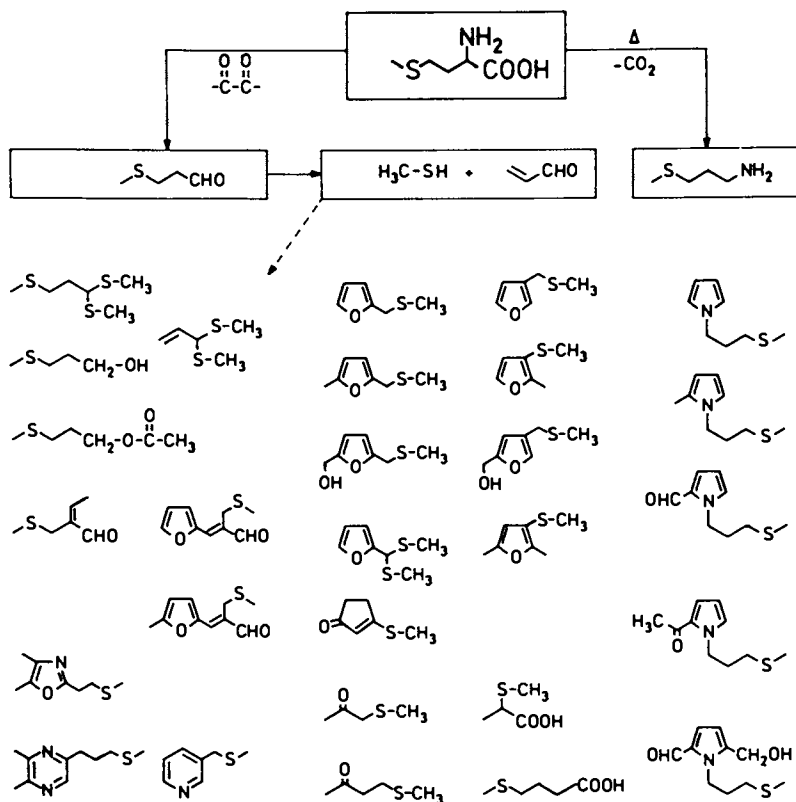


Figure 10: Strecker degradation products identified in methionine/reducing sugar model systems under elevated temperatures

characterized in methionine/reducing sugar model experiments (reaction conditions: 180 °C; H₂O, pH 5; 1 1/2 h). Similar results were obtained by Rijké et al. (12). 3-Methylthiopropional is more stable than the corresponding mercaptoacetaldehyde from cysteine. It is obviously further degraded to methylmercaptan and acrolein. The mercaptals from 3-methylthiopropional and acrolein were characterized as major compounds. Methional undergoes aldol condensation with furan aldehydes and is transformed into 3-methylthiomethylpyridine as well as into series of alkylsubstituted 3-(thiabutyl)- and 4-(thiapentyl)pyrazines which were identified for the first time as methionine specific products. The presence of furfurylmethylsulfides indicate that these compounds are formed from 3-deoxyosones as well as from aldol reactions. Methylmercaptan is the reactive intermediate as demonstrated with the corresponding precursors. The Strecker amine is transformed into pyrroles as specific compounds. More than 20 compounds were identified for the first time in this study. The results will be published in detail.

Acknowledgments

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Chapter 16

Heat-Induced Flavor Formation from Peptides

George P. Rizzi

Miami Valley Laboratories, The Procter & Gamble Company,
Cincinnati, OH 45239-8707

Peptides can degrade during food processing to form novel taste compounds like diketopiperazines (DKPs) or react with reducing sugars to produce volatile Maillard products. Eleven DKPs were detected in commercial cocoas and model studies substantiated a DKP formation mechanism involving intramolecular cyclization of linear peptide precursors. Model Maillard reactions of peptides and fructose generated Strecker degradation products from amino acids with blocked amine and carboxyl functionalities.

Peptides have long been recognized as important flavor compounds in processed foods. The taste of peptides *per se* is well known in cheeses (1), meat (2), hydrolyzed vegetable protein including soy products (3), cocoa (4, 5) and to a lesser extent in roasted malt (6), corn steep liquor (7) and aged sake (8). Structurally, food peptides can occur as linear protein fragments (1-3, 5), cyclic dimers (diketopiperazines, DKPs) [4, 6-8] and cyclic trimers (7).

Peptides formed enzymically or by mineral acid hydrolysis or thermal degradation of higher molecular weight protein can also serve as flavor precursors in thermally induced reactions. The reactivity of peptides is evidenced by their behavior during pyrolysis/GC (9), heating in air (10), reactions with mono- (11), and dicarbonyl (12, 13) compounds and reactions in hot acetic acid (14). The types of reactions observed for peptides include: side-chain thermolysis, fragmentation into amino acids, DKP formation and Maillard reaction with ambient carbohydrates.

Besides their general flavor forming potential peptides are also reported to be unique precursors of composite food aromas. Peptides formed in the fermentative stage of cacao processing have been linked to roast generated chocolate aroma (5). Also, a methionine rich polypeptide has been associated with roasted peanut volatiles (15).

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The purpose of this work was to investigate the occurrence and heat-induced origin of cyclic dipeptides (DKPs) in cocoa; and separately, to study the mechanism of DKP formation in simplified reaction systems. Also, the formation of volatiles was measured in a series of model Maillard reactions of peptides, amino acid mixtures and fructose.

Experimental Part

Amino acids and peptides were analytical grade commercial samples obtained from Sigma Chemical Company, St. Louis, MO. Solvents and chemical reagents were all commercial, analytical grade materials. Commercial cocoa powders were defatted with hexane at room temperature and air dried before analysis. Dried, unroasted cocoa beans were extracted with methanol-water according to Rohan and Stewart (16) and aliquots of freeze-dried bean extracts were sealed into glass vials and roasted in a thermostated oil bath. Prior to DKP analysis cacao products were cleaned up to remove theobromine, polyphenols and fiber by the method of Pickenhagen et al. (4), but omitting the silica-gel chromatography step. DKPs were analyzed by high resolution gas chromatography (HRGC) on a Restek (RTX-5) 60 M X 0.32 mm i.d. fused silica column (1u DB-5 film) interfaced to a Finnegan model 4500 mass spectrometer with an INCOS data processing system. Mass spectra were obtained at 1 sec. scan speed with nominal mass resolution of 1.0 amu. Inlet and transfer line temperatures were 250 and 300°C respectively. A typical separation of DKP homologs and *cis/trans* isomers is shown in Figure 1. DKPs were quantitated using peak area integrals and reference to a complete set of external standards. Standard DKPs with two ring substituents were mixtures of *cis/trans* isomers thereby allowing quantitation of isomeric DKPs in cocoa. Model reactions involving peptides and amino acids in acetic acid were worked up by diluting the products with water and removing unchanged starting materials with Bio-rad AG 50W-X8 cation exchange resin (50-100 mesh, H⁺-form). Aliquots were analyzed for DKPs after adding c(Ala-Gly) as an internal standard. DKP standards were synthesized by refluxing 1.3 M solutions of dipeptides in acetic acid for two hours. Treatment with acetic acid led to partial racemization of enantiomerically pure peptides. After removal of unchanged starting materials with cation exchange resin, products were isolated by vacuum evaporation and purified by recrystallization. Recrystallization provided analytically pure products, but DKPs with two ring substituents still contained mixtures of *cis/trans* isomers. Structures of purified products were verified by elemental analysis and by reference to published MS data (17). For Maillard reactions, aqueous solutions of amino acids, peptides and fructose were freeze-dried in 50 mL serum bottles and septum-sealed before incubating in a thermostated oil bath. Cooled mixtures were treated with water to make 10% w/v mixtures and equilibrium headspaces were sampled at 50°C for HRGC analysis according to procedures developed by Haynes and Steimle (18).

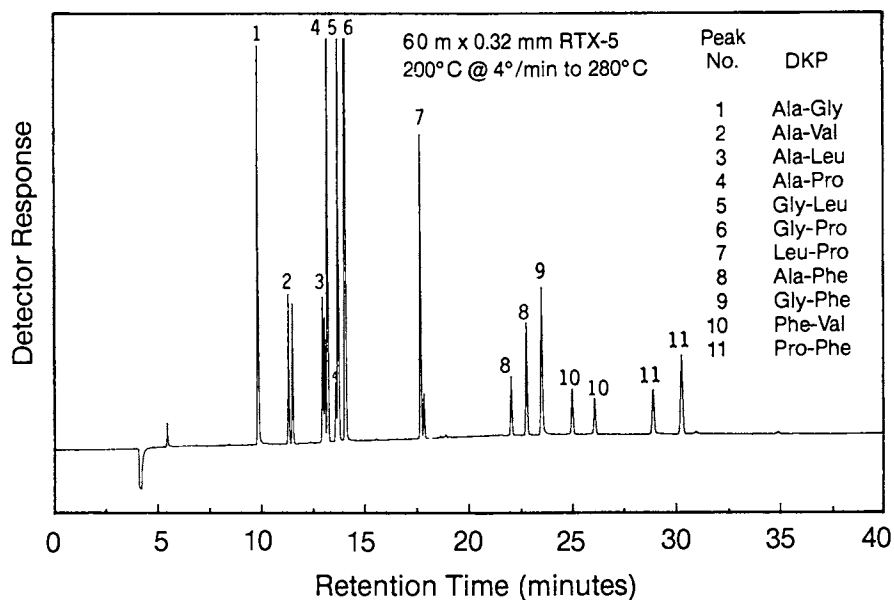


Figure 1. HRGC Chromatogram of Synthetic DKPs Showing Separation of Cis/Trans Isomers

Discussion of Results

Occurrence and Formation of DKPs in Cacao Products. Thermal degradation of peptides (or proteins) during cocoa bean roasting is evidenced by the presence of DKPs in cocoa powder (4, 17). Cocoa DKPs are important flavor components that have been shown to produce a characteristic bitter taste in conjunction with naturally occurring theobromine (4). In this study we utilized HRGC analysis coupled with a sensitive nitrogen selective detector and a mass spectrometer to compare types and amounts of DKPs in variously processed cocoas. Similar analytical technique was used to follow the formation of DKPs during in vitro roasting of chocolate flavor precursors isolated from unroasted beans.

Quantitative data ranges for DKPs found in four cocoas are expressed in mg of DKP/Kg of fat free-cocoa as follows: c(Gly-Leu), 0-25; c(Gly-Pro), 2-7; c(Gly-Phe), 0-11; c(Ala-Gly), 0-12; c(Ala-Val), 56-143, c(Ala-Leu), 22-69; c(Ala-Pro), 19-44; c(Ala-Phe), 2-19; c(Leu-Pro), 39-115 and c(Phe-Pro), 0-26. Toward the end of the study an eleventh DKP was qualitatively identified in cocoa and showed to be c(Val-Phe). Our results confirmed the DKPs found by previous authors (4, 17) except for c(Asp-Pro) and c(Asp-Phe) which were apparently not amenable to direct GC analysis. Two new DKPs, c(Gly-Leu) and c(Ala-Leu) were observed for the first time in cocoa. Cocoas contained similar kinds of DKPs, however quantitative differences suggested that DKP profiles obtained by HRGC might serve as a sensitive fingerprint technique. Also, dutched cocoas typically had lower levels of DKPs compared to ordinary processed cocoas.

HRGC provided a second analytical dimension by resolving cis/trans DKP isomers in compounds containing two ring substituents. For six of the DKPs found in cocoa pairs of compounds corresponding to geometric isomers were observed and they were confirmed to be isomers by their identical mass spectra. It was interesting that c(Ala-Val), c(Ala-Leu), c(Ala-Pro) and c(Phe-Pro) occurred as single geometric isomers in some cocoas, a result that is indicative of stereoretentive protein degradation during roasting. In dutch process cocoa the latter DKPs appeared as mixtures of isomers apparently due to partial racemization caused by more alkaline process conditions.

Heat Induced DKP Formation in Cocoa Bean Extracts. Extracts of dried, unroasted cocoa beans were prepared by the method of Rohan and Stewart (16), roasted at 120°C for 30 minutes and analyzed by HRGC to provide the data in Table I. DKPs were found for the first time in unroasted beans, possibly as a result of heat or enzyme action during fermentation or drying. In general, DKP levels increased after roasting and more DKPs were produced in extracts of the more highly fermented Ghana and Bahia varieties. It was curious that c(Ala-Phe) and c(Phe-Pro) disappeared during Ghana extract roasting but evidently survived a similar treatment in the Bahia extract.

Table I. Heat Induced DKP Formation in Cocoa Bean Extracts

DKP Observed	Bean Variety							
	Arriba		Sanchez		Ghana		Bahia	
	U	R	U	R	U	R	U	R
c(Ala-Val)	0	0	0	470	450	940	480	1560
c(Ala-Leu)	0	0	0	270	0	560	0	880
c(Ala-Pro)	42	125	116	430	450	730	480	1160
c(Gly-Leu)	0	0	0	0	0	69	0	120
c(Leu-Pro)	63	146	190	470	800	1110	1000	1720
c(Ala-Phe)	0	0	0	0	104	Trace	Trace	280
c(Phe-Pro)	0	0	0	0	240	Trace	Trace	480
Totals	105	271	306	1640	2040	3410	1960	6200

U = Unroasted, R = Roasted at 120°C for 30 min.; data in mg/kg of unheated extract rounded to three significant figures

DKP Formation in Model Peptide Systems. The fact that DKPs are formed at relatively low temperatures suggested that catalyzed peptide decomposition occurs in the weakly acidic milieu produced by cacao fermentation. Reactions of simple peptides were followed at 0.02 M (initial concentration) in refluxing acetic acid (120°C) to simulate the interior of a bean during roasting (Table II). Under these conditions Val-Ala was almost quantitatively converted into c(Val-Ala) according to the postulated reaction mechanism (4), Figure 2 whereas an equimolar mixture of valine and alanine failed to react. The intramolecular nature of DKP formation was further established by reactions of tripeptides. Each tripeptide studied produced a single DKP that had to result from attack of the N-terminal amino group according to Figure 2. DKP formation by

Table II. DKP Formation in Model Systems

Reactant(s)	Initial	Time, hrs	Product	% Yield
	Conc.(M)			
Phe-Val	1.9	2.0	c(Phe-Val)	74
Phe, Val	1.9	2.0	c(Phe-Val)	0.076
Val-Ala	0.020	0.50	c(Val-Ala)	94
Val, Ala	0.020	0.50	No Reaction	0
Gly-Leu-Ala	0.020	0.50	c(Gly-Leu)	7.1
Ala-Leu-Gly	0.020	0.50	c(Ala-Leu)	20
Leu-Gly-Phe	0.020	0.50	c(Leu-Gly)	23
Pro-Gly-Gly	0.020	0.50	c(Pro-Gly)	15

Solvent = Acetic Acid, Temperature = normal reflux

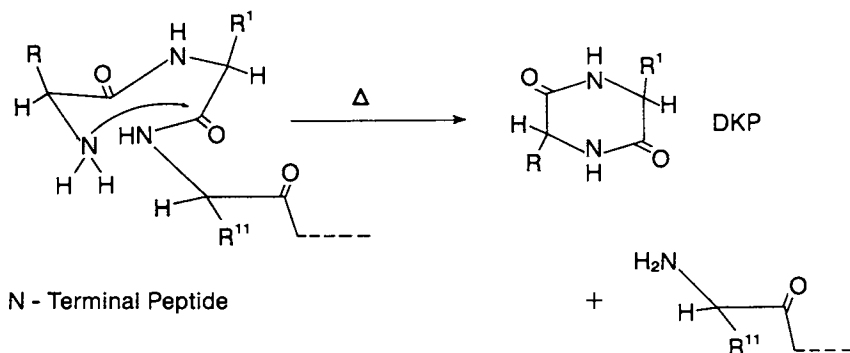


Figure 2. DKP Formation Mechanism

intermolecular reaction of amino acids was detected for a mixture of phenylalanine and valine, but only under exaggerated reaction conditions of 2 hrs. reflux and initial concentrations of 1.9 M.

Stereochemistry of DKP Formation. Cyclization of peptides that contain chiral carbon atoms can yield cis/trans DKP isomers which are separable by HRGC. Enantiomerically pure peptides will theoretically form single geometric isomers, i.e. D(L)-D(L) dipeptides ==> cis DKPs and D(L)-L(D) dipeptides ==> trans DKPs. According to our observations some cocoa DKPs were present as nearly pure, single geometric isomers. Our results suggested a stereoretentive cyclization of peptides containing optically pure (presumably L) amino acids during bean roasting. An attempt to duplicate stereoretentive DKP formation in a model system was only partially successful. Cyclization studies on L-Phe-L-Ala in acetic acid strongly suggested that the L-Ala moiety had undergone partial racemization. The total DKP product (61% yield) was a 91/9 isomeric mixture containing mostly cis c(L-Phe-L-Ala) as evidenced by its optical rotation and melting point (19). The high optical purity of the enriched cis product precluded the presence of a significant amount of c(D-Phe-D-Ala) which would have resulted by partial racemization of both L-Phe and L-Ala. The trans isomer observed by HRGC was therefore c(L-Phe-D-Ala) (19) resulting from partial racemization of alanine prior to or during cyclization. In a separate experiment c(L-Phe-L-Ala) did not isomerize under cyclization conditions. The high degree of isomeric purity for some cocoa DKPs is not yet explained but it may be a result of precursors that contain several contiguous optically pure amino acids.

Formation of Volatiles in Peptide/Fructose Reactions. Typical Maillard reaction volatiles were observed when various food proteins were roasted at 250°C (20) or autoclaved in water at 121-140°C (21, 22). Since protein is known to break down into amino acids and peptides under similar conditions (23), it seemed reasonable that peptides could be reactive intermediates in the Maillard process. In view of the latter possibility experiments were designed to test the extent of Strecker degradation for specific amino acids contained within small peptides. Mixtures containing fructose and equimolar amounts of peptides or mixtures of corresponding free amino acids were roasted at 120°C for 30 minutes and headspace volatiles were analyzed by HRGC (Table III). In general, di- and tripeptides reacted differently than mixtures of amino acids as evidenced by total FID areas and volatile profiles. Dipeptides containing valine or leucine produced significant amounts of Strecker aldehydes, 2-methylpropanal and 3-methylbutanal in spite of blocked amino or carboxyl groups. Also, 3-methylbutanal was a major product in reactions of tripeptides containing leucine as the central amino acid. Pyrazines were minor reaction products that were more easily

Table III. Headspace Analytical Data for Reactions of Fructose Plus Peptides or Amino Acids

Peptide or Amino Acids	Total FID Area/1000	% of FID Area		
		Strecker 2-MP	RCHO 3-MB	Major Pyrazines 2,5 + 2,6-DiMe
Val,Gly	41.0	81	1.2	0.1
Val-Gly	14.9	16	2.4	Trace
Gly-Val	83.0	10	Trace	Trace
Leu,Gly	890	Trace	93	0.001
Leu-Gly	343	Trace	97	Trace
Gly-Leu	134	Trace	90	Trace
Ala,Leu,Gly	710	Trace	88	0.005
Ala-Leu-Gly	600	1.2	4.3	0.01
Gly-Leu-Ala	12.0	Trace	69	0.2

2-MP = 2-methylpropanal, 3-MB = 3-methylbutanal, FID = flame ionization detector

measured by a nitrogen selective detector (Table IV). Peptide reactions led to different pyrazine distributions for each substrate suggesting that particular peptides could, at least in theory serve as unique precursors of composite food aromas. Chemically, it was interesting that the peptides Gly-Val, Gly-Leu and Ala-Leu-Gly produced relatively more pyrazines than respective amino acid mixtures. Based on the extensive Strecker activity it would appear that fructose reactions facilitate peptide hydrolysis, a normally slow process at 120°C. Paper chromatographic analysis of peptide/fructose reaction mixtures showed only traces of unreacted starting material and no evidence for free amino acids. Also, control reactions in which sorbitol was substituted for fructose led to no evidence for Strecker degradation.

Table IV. Headspace Analytical Data for Reactions of Fructose Plus Peptides or Amino Acids

Peptide or Amino Acids	Total NPD Area/1000	Alkylpyrazines (% of NPD)					
		Me	DiMe(3)	Ethyl	TriMe	TetraMe	Total
Val,Gly	4.8	5.1	12	0.3	1.1	0.8	19
Val-Gly	2.4	0	2.7	0	0	0.4	3
Gly-Val	4.2	3.8	25	1.3	10	3.1	47
Leu,Gly	5.7	4.1	11	0.2	7.6	2.5	31
Leu-Gly	2.0	0	5	0	1.3	0.5	9
Gly-Leu	4.0	3.7	27	1.3	9.4	0.9	46
Ala,Leu,Gly	8.5	4.6	15	0	9.6	0	38
Ala-Leu-Gly	10.5	3.5	57	0	13	0	84
Gly-Leu-Ala	2.8	0	27	0	14	0	44

NPD = Nitrogen-phosphorus detector

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Chapter 17

Mechanistic Studies of the Maillard Reaction with Emphasis on Phosphate-Mediated Catalysis

R. P. Potman and Th. A. van Wijk

Unilever Research Laboratorium Vlaardingen, P.O. Box 114, 3130 AC
Vlaardingen, Netherlands

The Maillard reaction, which is basically the reaction of amino acids with carbohydrates during heating, is responsible for the characteristic flavour of many processed food products.

It is known from the literature and own work, that the addition of inorganic phosphate to the reaction mixture increases of the reaction rate. Up to now this has mostly been described through an enhanced rate of browning of the reaction mixture. Research employing modern analytical techniques, e.g. high performance liquid chromatography, may give more insight into the mechanism of the phosphate catalysis, leading to a better understanding of it.

In the early stage of the Maillard Reaction, viz. the conversion of the starting materials into the so-called Amadori Rearrangement Product, phosphate increases the rate of reaction. The phosphate catalysis was found to be dependent on the concentration of inorganic phosphate in the reaction mixture. It may be described as general base catalysis, following approximately first-order kinetics. We suggest that phosphate catalyzes the conversion of the glycosylamine into the Amadori Rearrangement Product.

The term "Maillard reaction" is used to characterize a group of chemical reactions involving amino and carbonyl functions present in foodstuffs, leading to flavour production and browning. The reaction is named after the French chemist Louis Maillard (1), who first described the formation of brown pigments when heating a solution of glucose with lysine. Due to its interest for the food industry the Maillard reaction has been the subject of numerous articles during the past decades. Recently some extensive review articles have

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appeared covering many aspects from a mechanistic (2,3,4) or a more applied (nutritional) point of view (5,6,7).

This article describes a study on the catalytic role of phosphate in the Maillard reaction focussing on the first steps of the cascade of reactions, i.e. the conversion of the starting materials, monosaccharide and glycine, into the so-called Amadori Rearrangement Product (ARP).

The mechanism of the early stage of the Maillard reaction

The mechanism of the Maillard reaction has been described extensively in the literature (3,4,5). In this article focus will be on the so-called "early phase" of the cascade of reactions.

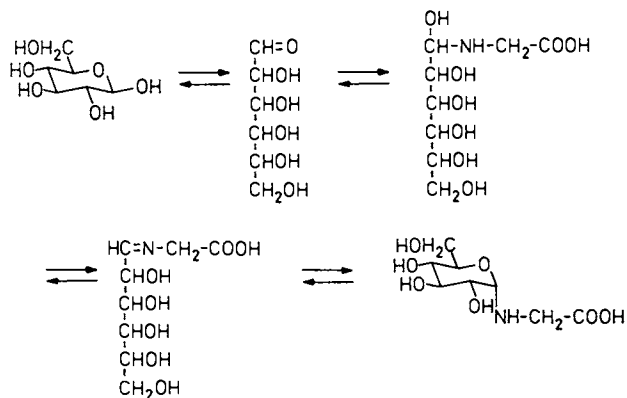
In the "early phase" the starting materials, e.g. glucose and glycine, react leading to a N-glycosylamine, which is not stable in this case, but directly rearranges via the Amadori rearrangement into the Amadori Rearrangement Product (ARP), N-(Carboxymethyl)-1-amino-1-desoxyfructose. The Amadori rearrangement is catalyzed by the amino acid carboxyl moiety.

In the case of aliphatic and aromatic amines as the starting material, where the carboxyl group is absent, the isolation of the corresponding glycosylamines has been reported (3). Structure analyses revealed the compound to be a β -pyranoid having a 4C_1 (D) conformation.

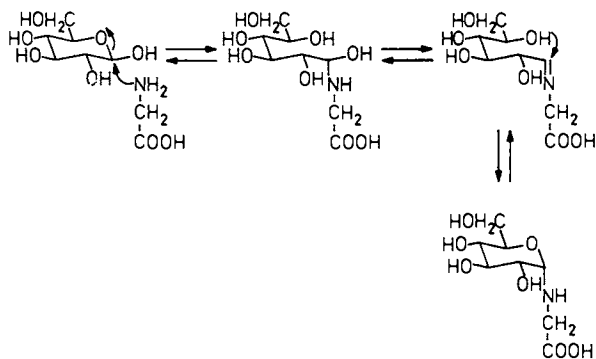
In the literature some disagreement exists about the mechanism of the formation of N-glycosylamine. Some authors suggest the opening of the cyclic form of the sugar, giving a free aldehyde group, prior to the attack by the amino acid (Scheme 1)(2). A better proposition, however, is the attack of the amino acid at the anomeric carbon atom of the carbohydrate in the cyclic pyranose conformation (8), which is the most stable, and therefore the most abundant conformation of the aldohexoses (95%) in solution (3). This attack may follow an S_N1 -pathway, in which either the ring oxygen is the leaving group, leading to ring-opening (in which case there is only a formal difference with the mechanism in Scheme 1), or the anomeric hydroxy moiety is the leaving group, leaving a cyclic carbonium ion; attack of the amino acid in a second step then leads to formation of the N-glycosylamine.

Alternatively the reaction follows an S_N2 -pathway, in which either the ring oxygen functions as the leaving group, making a subsequent substitution of the anomeric hydroxy group, leading to ring closure (Scheme 2), necessary, or a direct substitution at the anomeric centre, leaving the carbohydrate ring intact.

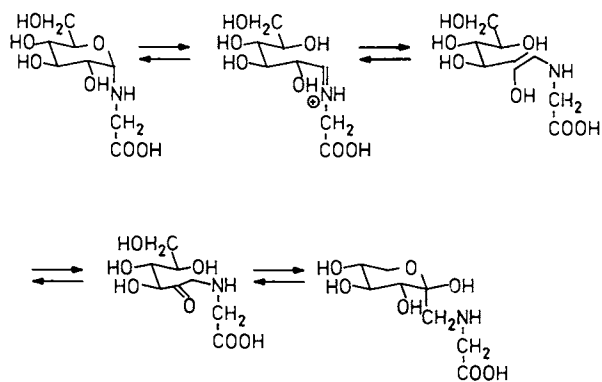
Some uncertainty exists whether the Amadori Rearrangement follows a concerted pathway, in which case no formal charge is formed during the reaction, or whether its course runs via the formation of a carbonium ion (Scheme 3). The latter proposition is commonly accepted in the literature, but the actual mechanism probably is a mixture of the two proposals depending on the reaction conditions. The Amadori Rearrangement is thought to be the rate-determining step in the process, as shown by the isotope effect on the rate of reaction when the hydrogen at C-2 is replaced by deuterium. The rearrangement is acid and base-catalyzed. In the first case the acid donates a proton to the accepting amino group of the amino acid, rendering it a better leaving-group, in the latter case the abstraction of the proton at C-2 of the carbohydrate is enhanced (9).



Scheme 1 Formation of glucosylglycine



Scheme 2 Formation of glucosylglycine



Scheme 3 Amadori Rearrangement

In the advanced stage, subsequently the ARP opens, via 1,2- or 1,3-enolisation, depending on the pH of the medium, leaving di-carbonyl-compounds, the so called reductones (10) after elimination of the glycine residue. These highly functionalized compounds are very reactive towards nucleophiles, like the amino acids, or may give rise to the formation of condensation products.

Upon reaction with amino acids, in the Strecker degradation, unstable imines are formed, which may easily decarboxylate, leaving an enamine, which upon hydrolysis yields an aldehyde from the amino acid and an α -aminoketone from the di-carbonyl-compound. The α -aminoketones are important for the formation of heterocyclic compounds via condensations with the reductones or with themselves. The aldehydes mentioned, and the heterocyclic compounds are the actual flavour components.

The effect of phosphate on the Maillard Reaction

The preparation of reaction flavours is known to be improved by the presence of phosphate in the reaction mixture. The enhancing effect of phosphate on the rate of browning during the reaction of glycine with several hexoses has already been shown by Swimmer *et al* (11). The authors found a 12-fold enhancement of the brown colour (420 nm) of the reaction medium. The reactions, however, were not performed at the same pH. Therefore the quantification by the authors is not very useful, because the formation of brown colour is known to be dependent on the pH; the higher the pH, the higher the rate of the colour formation. The same authors reported the use of sugar phosphates, in which the phosphate is covalently bound to the sugar moiety, also leading to a more rapid browning.

A more detailed study of the catalytic role of phosphate was reported by Reynolds (12), who reacted glucose with glycine, in mixtures containing 75-80% solids, in the presence of phosphate. He also started a kinetic study of the phosphate effect, but as the author stated "further investigation was deferred until methods more suitable for detailed studies could be developed". He reported, however, that the determination of free inorganic phosphate showed that no phosphate was consumed during the course of the reaction indicating that no stable intermediate containing covalently bound phosphate was formed to a measurable amount.

Saunders *et al* (13) reinvestigated the role of buffer salts in the non-enzymic browning, which term is also in use for the Maillard reaction. He used spectrophotometric measurement of the browning, the evolution of carbon dioxide (resulting from the Strecker degradation), and the change in the pH of the medium as the analytical techniques. The authors confirmed the findings of Swimmer and Reynolds.

It is clear that the analytical techniques presently available are superior to those used in the past, and therefore may enable more detailed insight into the mechanism of the phosphate effect. It was decided therefore to start a reinvestigation into the phosphate effect using the latest techniques, e.g. High Performance Liquid Chromatography (HPLC).

Introduction to the experiments

To obtain more insight into the nature of the phosphate catalysis model studies were carried out using glycine and a number of

monosaccharides. The reactions were performed in water at 100 C. Samples were taken from the mixture at regular intervals after the starting-time (t_0), which was chosen as the time when the temperature of the reaction-mixture reached 90 C, in which case it was shown that no reaction had occurred.

Analyses were performed by HPLC using a newly developed method. The method enabled the simultaneous measurement of the concentration of monosaccharide, glycine, and the related ARP, using a refractometer as detector. An external standard method was used to correlate the obtained refractive indexes of the components of the samples with reference materials.

Additionally the concentration of the amino acid has been determined by a spectrophotometric method using trinitrobenzenesulfonic acid as a reagent for the free amino group.

The concentration of the reaction-mixture was chosen in the magnitude of 30-40% dry matter, unless stated otherwise. At lower concentrations study of the Maillard reaction is hampered through competition by base-catalyzed decomposition of the carbohydrate starting material. It was shown (see below), that in the experiments described the contribution of the base-mediated decomposition could be neglected.

The density of the reaction media was determined to ensure correct measurement of the reaction orders. It appeared that when the total mass of the reaction mixture was brought to 100 g, the density was 1.00 ± 0.08 . Therefore it is a good approximation to consider the density as 1.0.

As shown above, glycine is formed again in the course of the Maillard reaction, during the conversion of the ARP into the reductones. Eventually it may react with these reductones or other electrophiles. Therefore the rate of the decrease of the concentration of the amino acid is not similar to the rate of the reaction with the sugar (Figure 1). The rate of consumption of monosaccharide, however, is a good measure of the reaction rate.

Therefore in all kinetic experiments the rate of consumption of monosaccharide was taken as the reaction rate.

As a result of the conversion of the basic glycine the acidity of the reaction mixture increases, leading to a change in the reaction conditions. To minimize the influence of the pH-change the rate of conversion of the starting materials at $t=0$ may be taken as a reasonable measure of the reaction rate.

Experimental

Reaction conditions

Determination of the pH-dependence of the phosphate-effect

Glucose (23.2 g, 0.12 mol), glycine (8.8 g, 0.12 mol), phosphate ($\text{NaH}_2\text{PO}_4 \cdot 1 \text{ aq}$) (0 g or 16.1 g, 0.12 mol), and water were mixed to a total amount of 100 g. The pHs of the reaction-mixtures were adjusted, either with concentrated hydrochloric acid or concentrated sodium hydroxide solution to pH=3, 5.6, 7, or 9, compensating for the amount of solvent added. The reactions were carried out at 100°C in a 100 ml round-bottomed three-necked flask equipped with a reflux condenser. An oil bath equipped with a magnetic stirrer was applied as heating system. The start of the reaction ($t=0$) was defined as the

instant when no conversion of the starting materials had occurred and the desired end temperature of 100°C had almost been reached. This point of time happened to be the moment when the temperature of the reaction-mixture reached 90°C. At this time a sample of about 1 ml was taken from the solution and cooled immediately in ice/water. At t=30; 60; 180 and 300 minutes four more samples were taken likewise.

Determination of the order of the phosphate-effect

Glucose or rhamnose (7.9 g, 0.04 mol), and glycine (3.0g, 0.04 mol) and phosphate ($\text{NaH}_2\text{PO}_4 \cdot 1 \text{ aq}$) (0 mol, 0.01 mol, 0.02 mol, 0.03 mol, 0.04 mol, and 0.05 mol) were mixed with water to a total amount of 100 g. The pH of the reaction mixture was adjusted to pH=5.6 compensating for the volume added. The reactions were performed as mentioned above.

Determination of the order of the phosphate-effect

Xylose was mixed with glycine and phosphate as described above. In this case the concentration was halved, due to the higher reactivity of the pentose.

Determination of the order in glycine

Glucose, glycine, phosphate ($\text{NaH}_2\text{PO}_4 \cdot 1 \text{ aq}$) and water were mixed in the amounts listed in Table I. The pH of the reaction mixture was adjusted at pH=5.6. The reactions were performed as mentioned above.

Analytical methods

High performance liquid chromatography

The qualitative and quantitative analyses of monosaccharide, glycine and ARP were performed on a Waters HPLC-system equipped with a Nucleosil 5-NH₂ (aminopropylsilica) HPLC-column using acetonitrile/phosphate-buffer (pH=3 75/25 (v/v)) as eluent. An external standard method was used to determine the mono- saccharide-, glycine-, and the ARP-intermediate concentrations (reference compounds were available).

The TNBS-method

The TNBS-method (TriNitroBenzeneSulfonic acid) was used to determine the content of "free amino groups" in the sample solutions. The samples from the reaction mixture were diluted with a phosphate buffer (0.01 M; pH=8), so that a glycine concentration was obtained in the order of $1.10^{-2} \text{ g.l}^{-1}$. In an autoanalyzer the solutions were mixed with the TNBS-solution (0.3% w/w) and a phosphate "reaction" buffer (0.1M; pH=8). The colour of the yellow compound formed was measured using a spectrophotometer at 427 nm. To enhance the colour formation and to stabilize the bubbles, which were formed in the apparatus to separate the samples, sodium sulphite and Brij Wetting Agent (ex. Merck) were added to the phosphate "reaction" buffer.

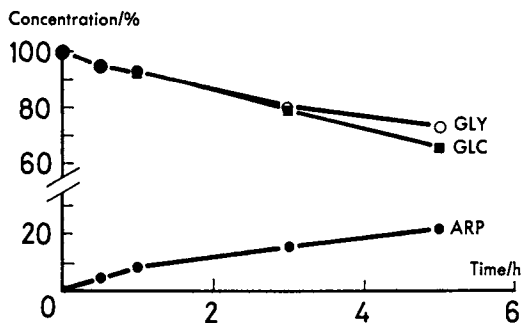


Figure 1. The concentrations of glucose, glycine and the ARP as a function of time (pH 5.6)

Table I. Reaction conditions for the determination of the order of glycine at pH= 5.6

glycine		glucose		phosphate		water	
grams	M	grams	M	grams	M	grams	M
1.87	0.25	19.8	1.00	0	0	0	78.3
3.75	0.50	19.8	1.00	0	0	0	76.5
5.63	0.75	19.8	1.00	0	0	0	74.6
7.50	1.00	19.8	1.00	0	0	0	72.7
9.38	1.25	19.8	1.00	0	0	0	70.8
0.75	0.10	7.90	0.40	5.50	0.40	85.8	
1.50	0.20	7.90	0.40	5.50	0.40	85.1	
2.25	0.30	7.90	0.40	5.50	0.40	84.4	
3.00	0.40	7.90	0.40	5.50	0.40	83.9	
3.75	0.50	7.90	0.40	5.50	0.40	82.8	

With the help of a calibration curve obtained for known glycine concentrations the amount of glycine in the reaction mixture was calculated.

Results

pH-dependence of the phosphate catalysis

The overall reaction rate may be described by the formula:

$$\begin{aligned} v_{\text{obs}} = & k_1 [\text{msacch.}]^{x_1} [\text{glycine}]^{y_1} [\text{PO}_4^{3-}]^{z_1} \\ & + k_2 [\text{msacch.}]^{x_2} [\text{glycine}]^{y_2} [\text{HPO}_4^{2-}]^{z_2} \\ & + k_3 [\text{msacch.}]^{x_3} [\text{glycine}]^{y_3} [\text{H}_2\text{PO}_4^-]^{z_3} \\ & + k_4 [\text{msacch.}]^n [\text{glycine}]^m \end{aligned} \quad (1)$$

where k_1 through k_3 are the rate constants for the catalyzed reaction and x , y , and z are the reaction orders; k_4 is the rate-constant for the uncatalyzed reaction and n and m are the corresponding reaction orders.

The pH-dependence of the phosphate effect was derived by studying the reaction rate at different pH-values. The results (Figure 2) show that the apparent reaction rate is the highest for the higher pH-values. In this case the conversion of the starting materials in the first hour of the reaction was taken as a measure of the efficiency of the reaction. However, as can be seen from the formula the uncatalyzed reaction will always have a certain influence on the overall rate. The apparent reaction rates of the catalyzed reactions (Figure 3) were divided by the rates of the uncatalyzed reactions, giving the rate enhancement by phosphate catalysis. The phosphate effect on the reaction rate was the highest at pH-values between 5 and 7.

Considering the dissociation of phosphate in the pH range investigated ($pK_1 = 2.15$, $pK_2 = 7.21$, $pK_3 = 12.67$) it becomes clear, that the concentrations of the phosphate, and the monohydrogen phosphate ions are too low to give a substantial contribution to the apparent reaction rate as shown in the formula. The formula may therefore be simplified to:

$$\begin{aligned} v_{\text{obs}} = & k_{\text{obs}} [\text{msacch.}]^x [\text{glycine}]^y [\text{H}_2\text{PO}_4^-]^z \\ & + k_4 [\text{msacch.}]^n [\text{glycine}]^m \end{aligned} \quad (2)$$

Determination of the order of the phosphate catalysis

The conversion of the single starting compounds was studied under the reaction conditions, revealing that at pH=7 and higher the monosaccharide was partially consumed by alkali-mediated decomposition, also shown to be enhanced by phosphate catalysis. Therefore all further experiments were performed at pH=5.6, which is the pH obtained by mixing the starting monosaccharides and glycine.

To obtain more insight into the nature of the phosphate effect, it was decided to perform a series of reactions in which the

concentration of phosphate was changed without altering the further reaction conditions. Thus equimolar amounts of monosaccharide and glycine were heated at 100°C with varying amounts of inorganic phosphate. The starting pHs of the reaction mixtures were corrected to 5.6. The reactions were not performed at 30-40% dry matter, because then the reaction rates were too high to visualize the differences obtained by the phosphate catalysis, but at 0.4 M (xylose at 0.2 M). Samples were taken from the mixtures at regular intervals, and analyzed as described.

The rate of conversion of monosaccharide appeared to be dependent on the amount of phosphate added (Figure 4); the higher the phosphate concentration, the higher the reaction rate. To obtain more precise insight into the order of the phosphate effect it is necessary to determine the rate of consumption of the monosaccharide at the start of the reaction ($t=0$), as mentioned above. In this instance the influence of the change of the pH, due to the consumption of glycine, and the change of the glycine-concentration itself may be neglected, allowing a more correct description of the reaction kinetics.

The rate of monosaccharide consumption ($d[\text{msacch.}]/dt$) is easily determined when the concentration as a function of the time is described via a curve-fitting procedure. Use of the statistical programme SAS (running at the URL-Vlaardingen VAX) showed that the concentration may be described by the function:

$$[\text{msacch.}]_t = [\text{msacch.}]_\infty + ([\text{msacch.}]_0 - [\text{msacch.}]_\infty)e^{-k_{\text{obs}}t} \quad (3)$$

where $[\text{msacch.}]_0$ is determined by the amount which was added to the reaction. $[\text{msacch.}]_\infty$ was found to be dependent on the amount of phosphate. We were not able to give a good explanation for this effect which indicates that incomplete conversion has taken place. The obtained function shows that the reactions follow first-order kinetics in monosaccharide. From formula 2 it is clear that the observed reaction rate is a combination of the rates of conversion of the catalyzed and the uncatalyzed reactions. Therefore the function obtained from the uncatalyzed reaction was subtracted from the overall function leaving the net catalyzed reaction. The reaction rate is obtained by taking the first derivative at $t=0$ of the function describing the net catalyzed reaction.

Because of the compensation for the uncatalyzed reaction the phosphate-mediated reaction may be described as:

$$v_{\text{der}} = k_{\text{der}} [\text{msacch.}]^x [\text{glycine}]^y [\text{H}_2\text{PO}_4^-]^z \quad (4)$$

Taking the logarithm of this function leads to:

$$\begin{aligned} \log v_{\text{der}} &= \log k_{\text{der}} [\text{msacch.}]^x [\text{glycine}]^y [\text{H}_2\text{PO}_4^-]^z \quad (5) \\ &= \log k_{\text{der}} + x \log [\text{msacch.}] + y \log [\text{glycine}] + z \log [\text{H}_2\text{PO}_4^-] \end{aligned}$$

Because at $t=0$ the concentration of glycine and monosaccharide may be taken as a constant, formula 5 may be written as:

$$\log v_{\text{der}} = C + z \log [\text{H}_2\text{PO}_4^-] \quad (6)$$

Plotting $\log v_{\text{der}}$ against $\log [\text{H}_2\text{PO}_4^-]$ gives the order of the

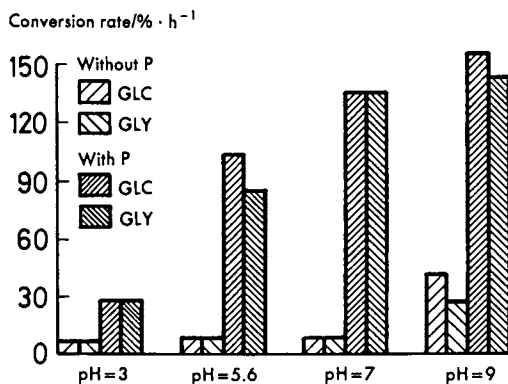


Figure 2. Influence of the pH and the presence of phosphate on rate of conversion of starting materials in the first hour of the reaction

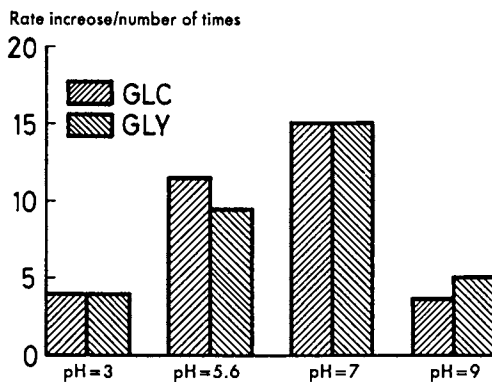


Figure 3. Increase of the rate of conversion of the starting materials due to phosphate

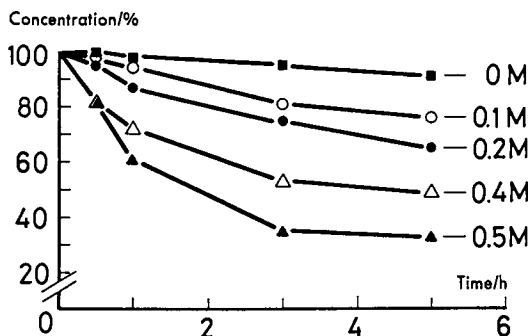


Figure 4. Decrease of the concentration of glucose in the time as a function of the phosphate concentration (pH 5.6; 0.4 M)

phosphate effect (z) as the slope, and C as the intercept (Figure 5). The determined slopes were approx. 1, (glucose: 1.34; rhamnose: 1.32; xylose: 1.09) so it may be concluded that the phosphate catalysis follows approximately first-order kinetics.

For the mechanism of the phosphate catalysis this means that phosphate acts as a base, which abstracts a proton in the rate-determining step of the reaction, i.e. the Amadori rearrangement, or as a nucleophile, which reacts with an intermediate giving a phosphate-containing intermediate, which reacts in a rapid subsequent step, e.g. through the formation of the unstable glucose-1-phosphate from glucose. To differentiate between these two possibilities further experiments were necessary, regarding the kinetics of glycine in the phosphate-mediated reaction.

Determination of the order of the reaction in glycine

From the order of the phosphate effect alone it was not possible to obtain clear insight into the mechanism of the phosphate-catalysis (see above). To differentiate between the two mentioned possibilities measurements regarding the order of the reaction in glycine were carried out in the presence and absence of phosphate. The concentration of glycine was varied in a series of reactions at pH=5.6, keeping the concentrations of glucose and the eventually equimolarly added phosphate constant. The decrease of the glucose concentration was taken as a measure of the reaction rate (Figures 6 and 7). The rate was determined as mentioned using a curve-fitting procedure. It was found that the order of the reaction for glycine, the slope of the line obtained by plotting the logarithm of the reaction rate against the logarithm of the glycine concentration, did not differ measurably in the presence or absence of phosphate (Figure 8). In either case an order slightly larger than unity was obtained, due to the catalytic effect of the amino acid carboxyl moiety.

It may be concluded that the mechanism of the "early phase" of the Maillard reaction is not dramatically changed by the addition of phosphate. It is therefore clear that the phosphate did not act as a nucleophile in the reaction, giving a reactive intermediate, in the rate-limiting step of a typical phosphate-dependent mechanism, but acts as a basic catalyst during the Amadori rearrangement.

The influence of inorganic phosphate and the pH on the concentration of the Amadori Rearrangement Product.

The concentration of the ARP present in the samples taken from the reactions mentioned above appeared to depend on the concentration of inorganic phosphate and the pH of the reaction media.

Variation of the pH of the reaction mixture of glucose and glycine, in the absence of phosphate, showed that at a higher pH the formation of the ARP is enhanced, but that the highest overall concentration is reached at the lowest pH, where the conversion of the ARP is relatively low.

In the presence of an equimolar quantity of inorganic phosphate it was found that the formation and the conversion of the ARP are enhanced, resulting in a relatively low overall concentration of the ARP, and consequently in a high yield of Maillard reaction products, i.e. the results mentioned above showed a high conversion of the starting materials at higher pH-values.

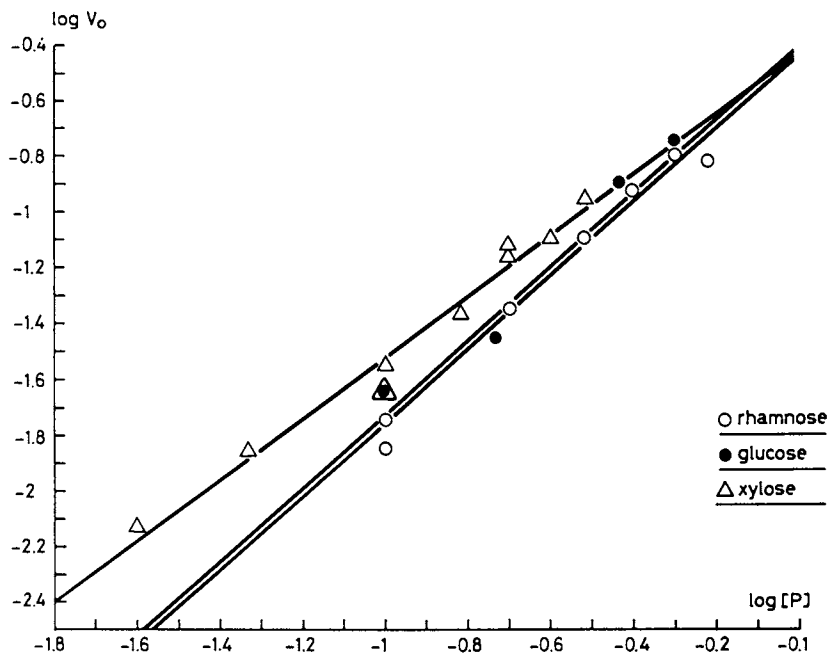


Figure 5. The order of phosphate catalysis. Plotting of the logarithms of the rate of conversion of the monosaccharides against the concentration of phosphate

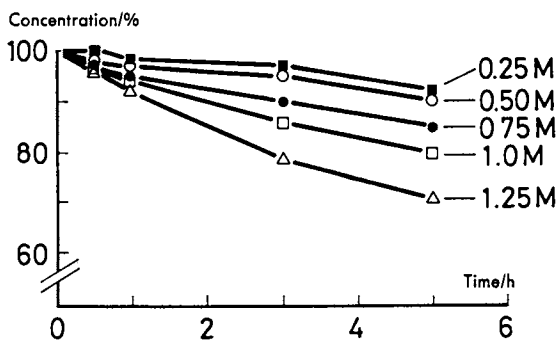


Figure 6. Decrease of the concentration of glucose in the time as a function of the concentration of glycine (pH 5.6; 1 M)

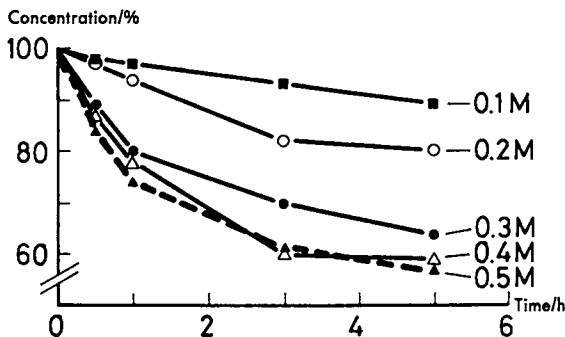


Figure 7. Decrease of the concentration of glucose in the time as a function of the concentration of glycine in the presence of an equimolar quantity of phosphate (pH 5.6; 0.4 M)

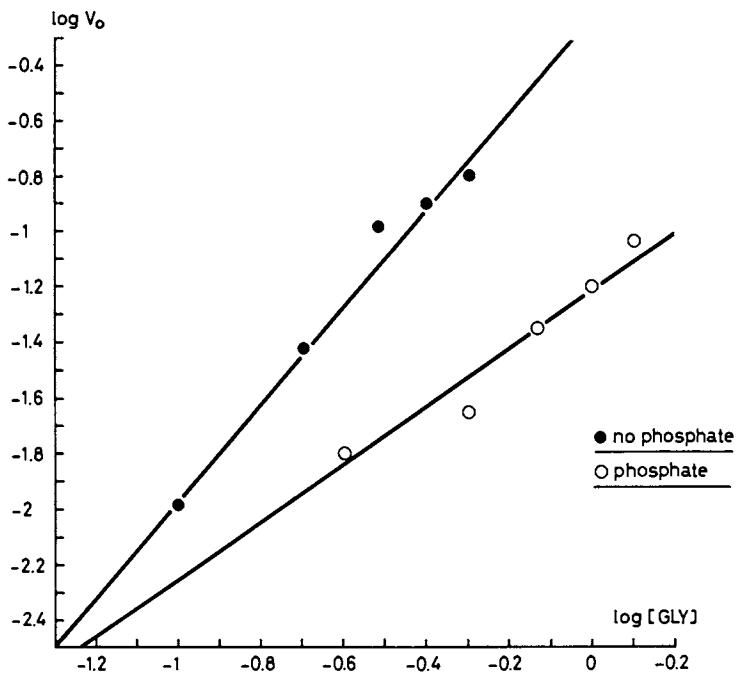


Figure 8. The order of the reaction in glycine. Plotting the logarithms of the rate of conversion of glucose against the concentration of glycine in the presence/absence of phosphate

Conclusion

The results discussed in this article showed that the effect of inorganic phosphate on the course of the Maillard reaction may be described by general base catalysis following approximately first-order kinetics. The effect is optimum in the pH-range between 5 and 7. The catalytic species is presumably the dihydrogen phosphate ion, which acts as a base abstracting a proton during the Amadori rearrangement, resulting in an increase of the rate of conversion of the starting materials.

Additionally the conversion of the Amadori Rearrangement Product is enhanced, resulting in the increased formation of reaction products, which are the actual flavour materials. The highest yields of ARP based on the starting materials were therefore obtained in the absence of phosphate.

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Chapter 18

Kinetics of the Formation of Alkylpyrazines

Effect of pH and Water Activity

M. M. Leahy¹ and Gary A. Reineccius²

¹Ocean Spray Cranberries, Inc., One Ocean Spray Drive, Lakeville-Middleboro, MA 02349

²Department of Food Science, University of Minnesota, St. Paul, MN 55108

Pyrazines are heterocyclic, nitrogen-containing compounds important to the flavor of many foods. Part One of this study reported on the effects of type of amino acid and type of sugar on the kinetics and distribution pattern of pyrazines formed. This study investigates the effect of pH and water activity on the kinetics of alkylpyrazine formation. One-tenth molar lysine/glucose solutions buffered at pH 5.0, 7.0 and 9.0 were heat-processed. Rates of pyrazine formation, as well as number of types of alkylpyrazines detected, were found to increase with pH and temperature. For the water activity (a_w) study, the effect of increasing product a_w over a range of 0.32 to 0.85 on the rate of pyrazine formation was investigated. Nonfat dry milk (NFDM) was chosen as a model system for this study. Rates of formation were found to increase with a_w up to 0.75. In both cases, samples were analyzed using a headspace concentration capillary gas chromatographic technique with nitrogen-selective detection.

The importance of Maillard reaction products to the flavor of foods has received considerable attention. One group of Maillard products, the alkylpyrazines, are thought to contribute roasted, toasted and nutty flavor notes to a variety of foods. Several reviews have detailed the presence of pyrazines in a wide variety of foods (1-7). Considerable work has previously focused on mechanisms of formation and the effects of various parameters on pyrazine formation (8-17). Part one of this study reported on the effects of type of amino acid and type of sugar on the kinetics and distribution pattern of pyrazines formed (18). The current study investigates the effect of pH and water activity on the kinetics of alkylpyrazines formation.

Prior studies relating to pyrazine formation have focused on the effect of drastic extremes in pH. For example, Koehler and

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Odell (19) reacted equal equivalents (0.1 mole) of sulfuric acid or sodium hydroxide in a reaction system of 0.1 mole D-glucose and L-asparagine in diethylene glycol. A yield of practically zero resulted upon addition of the acid, while adding base increased methylpyrazine yield tenfold and dimethylpyrazine fivefold relative to the system without added acid or base. Shibamoto and Bernhard (14, 15) also found that addition of NaOH increases total yield up to a point. Acidity/basicity effects were also studied by Koehler and Odell (19). They reacted glucose with asparagine, aspartic acid and aspartic acid with NaOH added. Yields with aspartic acid were lowest, with addition of base bringing yields almost up to those obtained with asparagine.

Our current study focused on a range of pH (5-9) which one could expect to find under normal food processing conditions. The reactants lysine and glucose were chosen for this study; lysine was chosen because it contains two amino groups available for reaction and glucose because it is a common sugar in foods.

Little previous work has focused directly on the impact of water content on the formation of pyrazines. Koehler and Odell (19) specifically chose a solvent system of lower water content for their study; Maga and Sizer (20) investigated the effects of initial moisture content and temperature of extrusion on the formation of pyrazines in potato flakes.

Our study also investigated the effect of water activity (a_w) on the kinetics of the formation of pyrazines. Water activity is defined as the ratio of partial pressure of water in a food to the vapor pressure of pure water at a given temperature. Nonfat dry milk (NFDM) was chosen as a model system for this study since NFDM and lactose/casein systems which had undergone nonenzymatic browning were found to contain pyrazines (21, 22). The current study investigates the effect of increasing product a_w over the range of 0.32 to 0.85 on the rate of formation of pyrazines.

Experimental Section

For the pH study, ten ml of buffered solutions at pH 5.0, 7.0 and 9.0 of 0.1M L-lysine monohydrochloride and 0.1M D-glucose (Sigma Chemical Co., St. Louis, MO) were heated in teflon-capped 25 mm (o.d.) x 150 mm Pyrex test tubes in a water bath at 75, 85, and 95°C for up to 24 hr. Citrate-phosphate buffers (0.1M) were used to achieve a pH of 5.0 and 7.0 and borate buffer (0.1M) was used for a pH of 9.0 (23). Samples were taken at 7 to 8 time intervals. Eighteen to 22 total samples per temperature and pH were analyzed.

After heat treatment each sample was adjusted to pH 9.0 with 0.1N NaOH. One ml of a solution containing 2-methoxy-pyrazine in distilled water (2 ppm) was added as an internal standard. Final sample volume was 15 ml. Pyrazines were then isolated, separated and quantified using an automated headspace concentration sampler (Hewlett Packard 7675A Purge and Trap) coupled to a Hewlett Packard 5880A gas chromatograph with nitrogen-phosphorus detection. Specifics of sample preparation and chromatographic analysis have been described previously (18). Quantification of the pyrazines was accomplished using 2-methoxy-pyrazine as an internal standard. Pyrazine peak identification was initially accomplished by

cochromatography with standards, (Pyrazine Specialties, Atlanta, GA) then further confirmed by gas chromatography/mass spectrometry.

For the water activity study, fresh NFDM was obtained from Maple Island, Inc. (Stillwater, MN). Five 30 g samples were held for 2 weeks at room temperature in evacuated desiccators over saturated salt solutions. The salts used were magnesium chloride, sodium bromide, sodium chloride and potassium chloride (Mallinckrodt, St Louis, MO) to achieve the water activities of 0.32, 0.58, 0.75 and 0.85, respectively (24). After storage, a_w was measured using an electric hygrometer (the Kaymont-Rotronics Hygroskop DT, Kaymont Instrument Corp., Huntington Station, NY). Triplicate 5 g samples in 75 mm (o.d.) x 150 mm Pyrex culture tubes with Teflon-sealed caps were heat-processed in a 95°C water bath for five sampling times, ranging from 0.5 to 3 hr. then cooled to room temperature. Prior to analysis, the sample cake in the test tube was broken up into a powder with a glass rod or spatula, vortexed, and analyzed using the automated headspace concentration GC technique.

True quantification of pyrazines in the powdered samples presented some difficulty. Due to sensitivity problems in sampling reconstituted samples, the decision was made to purge the powdered NFDM. An internal standard could not be easily added to a dry sample, so quantification was accomplished calculating concentrations relative to response factors determined for external standard solutions. Standardized concentration units were determined using the following relationships:

$$RF = \frac{\text{concentration } (\mu\text{g/ml})}{\text{integrated peak area}} \quad (1)$$

where relative concentration = sample area x RF

$$\text{Standardized Concentration Units} = \frac{\text{relative concentration}}{\text{initial dry wt. of sample}} \times 100$$

Since 5 g (wet weight) samples had been taken at each a_w , standardized concentrations relative to the initial dry weight of the sample were determined. Moisture analysis was accomplished using the GC method of Reineccius and Addis (25).

Results and Discussion

Kinetic Studies. The kinetics of the formation of pyrazines were determined using the basic equation for the rate of change of A with time:

$$\frac{dA}{d\phi} = kA^n \quad (2)$$

where A = concentration of pyrazine (ppm)
 ϕ = time (hr)
 k = rate constant
 n = reaction order

Integrating this equation between A_0 , the concentration A at time zero, and A, the concentration of A at time 0 yields

$$A = A_0 + k_0 t \quad (3)$$

for a zero order reaction. This implies that the rate of formation of A is constant with time and independent of the concentration of reactants. For a first order reaction this yields the relationship:

$$\ln A = \ln A_0 + k_1 t \quad (4)$$

In this case, the rate of formation of A is dependent on the concentration of reactants remaining. Reactions in foods generally have been found to follow either pseudo zero or first order kinetics (26).

One is generally safer when discussing reaction orders in foods in using the term "pseudo", due to the complexity of the system. Pseudo reaction orders in foods are generally assigned because a high correlation (r^2) for a mathematical relationship between formation of product and time exists.

The formation of pyrazines fit a zero order reaction. Plotting concentrations of pyrazines formed versus time of reaction gave the better fit of the line, usually with a coefficient of determination (r^2) of greater than 0.95. For a pseudo first order reaction, a curve rather than a line would be obtained. General least squares analysis of the data was used to compute rate constants (27). Two zero points were used for each regression. Duplicate samples were tested at the early sampling times vs. triplicate samples at later times. Each data point collected was treated separately in the regression analyses.

Activation energies for the formation of pyrazines were calculated using the Arrhenius relationship, which relates the rate constant, k , to temperature:

$$k = k_0 e^{-E_a/RT} \quad (5)$$

where k_0 = pre-exponential (absolute) rate constant
 E_a = activation energy in kcal/mole
 R = gas constant, 1.986 cal/mole °K
 T = temperature in °K

Linear regression data summarizing the effects of pH and temperature on the formation of pyrazines is found in Table I. An increase in rates of formation occurred with an increase in either temperature or pH. Coefficients of determination were quite high for a pseudo zero order fit of the data. This suggests that the rate of formation of pyrazines is independent of reactant concentration. Although the rate of formation must be a function of reactant concentration, it may not be apparent due to the relatively high reactant/product ratio, multiplicity of steps in pyrazine formation and competing side reactions. Pigment formation in the Maillard reaction, which is also a multi-step process, has also been shown to exhibit pseudo zero order kinetics by many researchers, including Labuza et al. (28) and Warmbier et al. (29). Loss of reactants (29) as well as formation of Amadori compounds (30) has generally been shown to exhibit first order kinetics.

Table I. Regressions for the formation of pyrazines (0.1M lysine-glucose systems)

Compound	Temperature	k (ppm/hr)	intercept	n*	r ²
PYRAZINE					
pH 9.0	95°C	3.596	0.0596	22	0.994
	85°C	0.490	0.458	22	0.960
	75°C	0.214	0.279	20	0.965
pH 7.0	95°C	1.346	0.762	19	0.962
	85°C	0.159	0.609	22	0.899
	75°C	0.0957	-0.0014	22	0.989
pH 5.0	95°C	0.0938	-0.0647	22	0.984
	85°C	0.0232	-0.0586	22	0.966
	75°C	0.00356	-0.0185	17	0.928
2-METHYLPYRAZINE					
pH 9.0	95°C	2.837	-0.104	22	0.995
	85°C	0.422	0.142	22	0.967
	75°C	0.159	0.091	20	0.941
pH 7.0	95°C	1.367	-0.070	19	0.981
	85°C	0.276	0.204	22	0.967
	75°C	0.0945	0.028	22	0.981
pH 5.0	95°C	0.00636	-0.00212	22	0.890
	85°C	0.00279	-0.00430	22	0.912
2,5-DIMETHYLPYRAZINE					
pH 9.0	95°C	0.186	-0.0457	20	0.976
	85°C	0.0247	-0.00604	22	0.985
	75°C	0.00668	-0.00536	16	0.942
pH 7.0	95°C	0.0630	-0.0051	19	0.965
	85°C	0.00949	-0.00229	22	0.974
	75°C	0.00209	-0.00574	14	0.857
2,3-DIMETHYLPYRAZINE					
pH 9.0	95°C	0.0229	-0.0057	16	0.948
	85°C	0.00309	-0.00017	18	0.978
	75°C	0.000677	-0.00086	12	0.958

*n = number of data points

Activation energies for alkylpyrazine formation were calculated from the slope of Arrhenius plots, ranging from 33 to 45 kcal/mole (see Table II). Activation energies for pyrazine and 2-methylpyrazine formation were approximately 35 kcal/mole, while those for the dimethylpyrazines were slightly higher. Quantitatively, dimethylpyrazine production was also lower, suggesting fewer available carbonyl fragments or less desirable conditions exist for formation of dimethyl versus unsubstituted or methyl pyrazine.

Table II. Activation energies for formation of pyrazines (0.1M lysine-glucose systems)

PYRAZINE	(E _a in kcal/mole)
pH 9.0	35.8
pH 7.0	33.4
pH 5.0	41.8
<hr/>	
2-METHYLPYRAZINE	
pH 9.0	36.7
pH 7.0	34.0
<hr/>	
2,5-DIMETHYLPYRAZINE	
pH 9.0	41.9
pH 7.0	43.7
<hr/>	
2,3-DIMETHYLPYRAZINE	
pH 9.0	44.8

It is interesting to note that for pyrazine, the activation energy is lowest at pH 7.0 at 33 kcal/mole. The rate of aminocarbonyl reactions has previously been shown to exhibit pH dependence, with the maximum occurring at some intermediate pH. Since rate constants were determined at only 3 temperatures, only 3 data points were used to determine activation energies. Data at other temperatures is necessary to make any further comparisons among activation energies.

Activation energies have been reported for other aspects of the Maillard reaction. The activation energy for browning as measured for pigment production ranges from 15.5 kcal/mole for a glycine-glucose system (31) to 33 kcal/mole for a solid intermediate moisture model food system (29). The current research indicates that the activation energies for pyrazine formation are higher, suggesting a different rate-controlling step.

pH Studies - The effect of pH on the formation of pyrazines may be seen in Tables I and II. Rate constants for the formation of

pyrazines increased with pH, along with the number of substituted pyrazines formed.

The relationship between pH and the rate of formation of pyrazines is graphically depicted in Figure 1. Regressing the rate constants for pyrazine and 2-methylpyrazine formation on pH gave a good fit of the line, with r^2 values of 0.974 and 0.999 respectively. Unfortunately, quantification of the dimethylpyrazines could only be accomplished at pH's greater than 5.0. One might anticipate an increase in the rate of formation of pyrazines with increasing pH for two reasons. At high pH a nucleophilic attack of the amino acid on a carbonyl is favored and an increase in the rate of sugar mutarotation will occur (32).

Table III lists the total concentrations of pyrazines produced under these conditions, as well as the percentage of each pyrazine of the total amount. Effect of pH on yield was great, with a total of 13,000 ppb pyrazines produced at pH 9.0 and only 24 ppb at pH 5.0. The differences in pyrazine distributions at various pH's do not appear to be great as shown in Table III.

Table III. Effect of pH on distribution pattern of pyrazines - 0.1M lysine-glucose, 2 hr, 95 C

	pH 5.0	pH 7.0	pH 9.0
		%	
pyrazine	63.2	58.7	55.8
2-methylpyrazine	36.8	39.6	41.5
2,5-dimethylpyrazine	----	1.7	2.4
2,3-dimethylpyrazine	----	----	0.3
TOTAL (ppm)	0.0239	6.19	13.1

Since the effect of pH on the formation of pyrazines was the focus of this study, efforts were made to standardize all other variables. Buffered solutions were used to resist large changes in pH during thermal processing. Citrate-phosphate buffers were used to achieve a pH of 5.0 and 7.0, while a borate buffer was chosen to achieve a pH of 9.0. Previous work has demonstrated buffers accelerate the non-enzymatic reaction. Spark (33) demonstrated that browning of glycine-glucose was accelerated in the presence of a buffer (phthalate, pH 6.2). Burton and McWeeney (35) found phosphate accelerated initial browning regardless of any buffering effect and proposed that phosphates acted to decrease the initial stability of the aldose sugar. Although both phosphate and borate buffers were used in the current study, it was the case that a change in buffer type did not affect pyrazine formation.

Water Activity Study. Pyrazine and 2-methylpyrazine were identified in the heat-processed, humidified NFDM samples by retention times

and cochromatography with standards (Pyrazine Specialties, Atlanta, GA) using nitrogen-phosphorus detection. Pyrazine and 2-methylpyrazine have previously been identified in a lactose-casein system held at 75% relative humidity (21) and the latter in a 2-year-old NFDM sample (22).

Initial water activities of the humidified NFDM samples were determined to be 0.319, 0.583, 0.747 and 0.841. Rate constants were determined for a pseudo zero order reaction for the formation of pyrazine and 2-methylpyrazine with time at each of these a_w s (Table IV). Figures 2 and 3 depict the relationship of concentrations of pyrazine and 2-methylpyrazine as a function of time at each a_w . Reaction rates increased with a_w over the range of 0.32 to 0.75 and a maximum occurred at a_w of 0.75. Labuza et al. (28) attributed the reaction rate decrease at higher a_w s to a dilution of reactants while relating the decreased reaction rate at low a_w s to an increasing diffusion resistance which lowers the mobility of reactants. This a_w maximum at an intermediate moisture content also explains the results of Maga and Sizer (20) who found yields of pyrazines were greatest in the extrusion of potato flakes in the potato slurries containing the lowest amounts of added water (25% versus 48%). In that study, the a_w at which maximum rate of pyrazine formation resulted corresponded to an intermediate versus high initial moisture content.

Table IV. Regressions for the formation of pyrazines in NFDM as a function of time (ppm/hr) at 95°C at a_w s 0.32, 0.58, 0.75 and 0.84

PYRAZINE					
a_w 0.32	$y = 0.324x + 0.00856$	$n^* = 17$	$r^2 = 0.895$		
a_w 0.58	$y = 0.681x - 0.110$	$n = 17$	$r^2 = 0.930$		
a_w 0.75	$y = 1.371x - 0.102$	$n = 17$	$r^2 = 0.971$		
a_w 0.84	$y = 1.318x + 0.0307$	$n = 18$	$r^2 = 0.940$		
2-METHYLPYRAZINE					
a_w 0.32	$y = 0.271x - 0.0199$	$n = 17$	$r^2 = 0.922$		
a_w 0.58	$y = 0.626x - 0.135$	$n = 17$	$r^2 = 0.899$		
a_w 0.75	$y = 0.886x - 0.133$	$n = 17$	$r^2 = 0.975$		
a_w 0.84	$y = 0.718x - 0.0838$	$n = 18$	$r^2 = 0.955$		

*n = number of data points

A further relationship between reaction rate and a_w has been proposed by Labuza. A linear relationship between the logarithms of reaction rate constants and a_w was found to exist for many foods within a range of a_w 0.3 to 0.8. Figure 4 depicts this relationship for the rate of formation of pyrazine and 2-methylpyrazine as a function of a_w . Table V lists the regressions determined for the three rate constants which fell within this range. Correlation coefficients (r^2) for these regressions were quite high, at 0.99 for both pyrazine and 2-methylpyrazine.

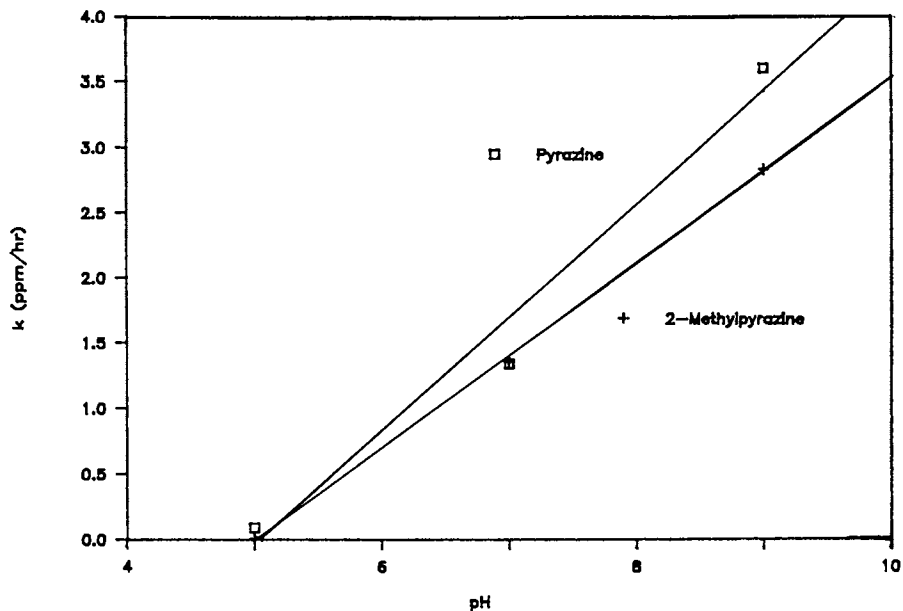


Figure 1. The influence of pH on the rate of formation of pyrazine and 2-methylpyrazine, 0.1M lysine-glucose at 95°C.

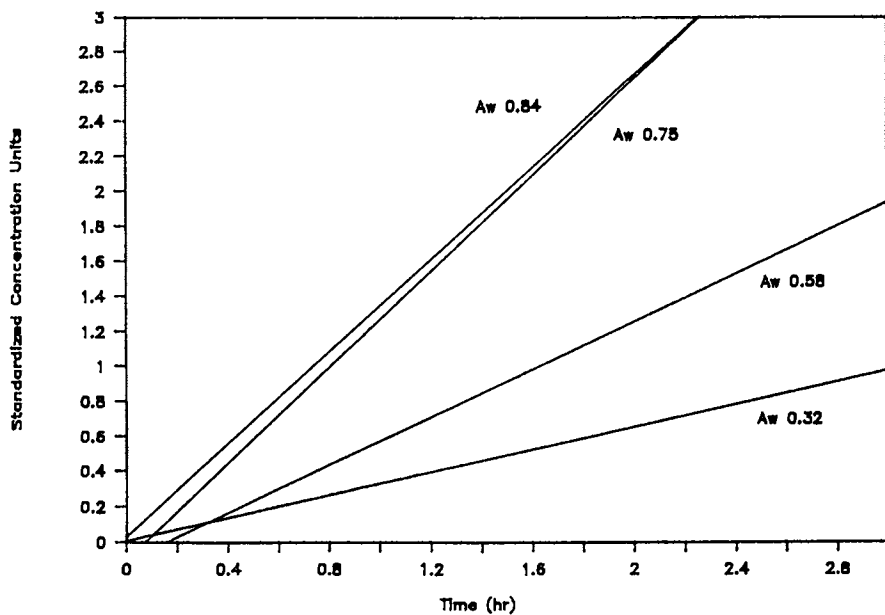


Figure 2. Effect of water activity on the formation of pyrazine at 95°C in NFD.

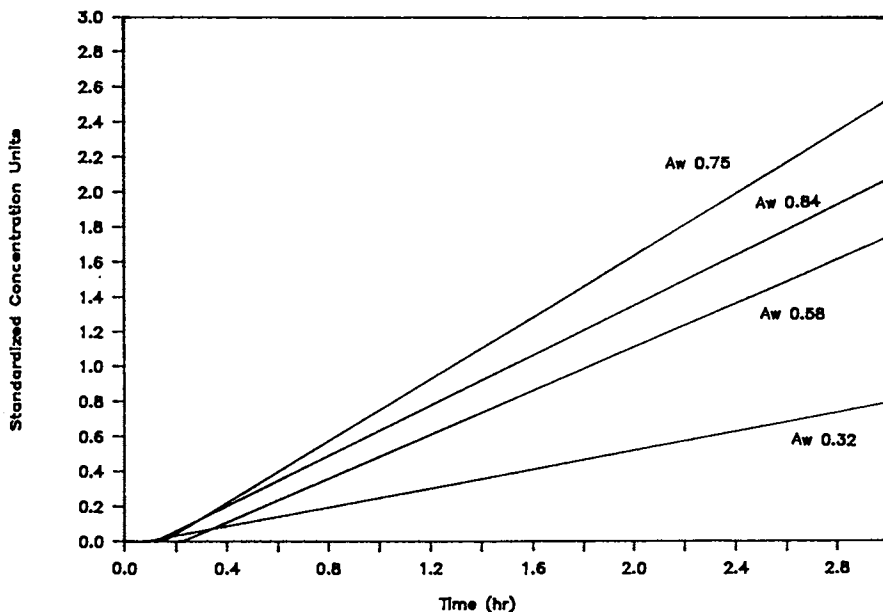


Figure 3. Effect of water activity on the formation of 2-methylpyrazine at 95°C in NFDM.

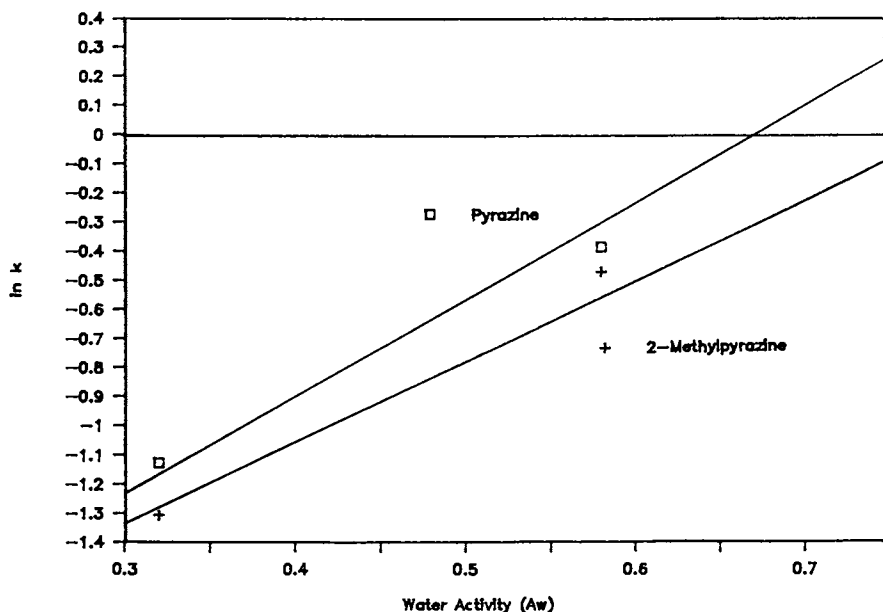


Figure 4. Natural logarithm of rate constant for the formation of pyrazine and 2-methylpyrazine with time vs. water activity.

Table V. Regressions of \ln reaction rates (ppm/hr) for the formation of pyrazine and 2-methylpyrazine at 95°C in NFDM as a function of water activity

PYRAZINE			
\ln	$y = 3.318x - 2.222$	$n = 3$	$r^2 = 0.986$
2-METHYLPYRAZINE			
\ln	$y = 2.806x - 2.174$	$n = 3$	$r^2 = 0.990$

Although a maximum rate of formation of pyrazines occurred at approximately a_w 0.75 in the NFDM samples in this study, one cannot assume that this would be the case in all types of foods or reaction systems. Addition of glycerol and hydrophilic polymers to sugar/amino acid systems has been demonstrated to shift the a_w at which visible browning will occur (29, 34). It is more likely that the rate of pyrazine formation increases up to a maximum at an intermediate a_w range and then decreases again, but not necessarily at a_w 0.75.

Summary

The effects of pH and water activity on the kinetics of the formation of pyrazines were investigated. For the pH study, rates of formation of pyrazines were investigated in lysine-glucose systems. Rates of formation of pyrazines, as well as the number of types of alkylpyrazines detected, were found to increase with pH and temperature over the range of pH 5.0 to 9.0. Coefficients of determination were quite high, usually greater than 0.90, for a pseudo order fit of the data. Activation energies, determined using Arrhenius plots, were approximately 35 kcal/mole for pyrazine and 2-methylpyrazine, except in the case of pyrazine formation at pH 5.0. Quantitatively, dimethylpyrazine production was also lower, suggesting fewer available carbonyl fragments and mechanistically less desirable conditions exist for formation of dimethyl versus unsubstituted or methyl pyrazine. A linear relationship of high correlation was found to exist between pH and the rate of formation of pyrazines, with r^2 values of 0.974 and 0.999 for pyrazine and 2-methylpyrazine, respectively. Effect of pH on yield was investigated under standard conditions (2 hr heat treatment at 95°C). pH was found to have a great effect on yield, with a total of 13,000 ppb pyrazines produced at pH 9.0 and only 24 ppb at pH 5.0. The differences in pyrazine product distributions were not great.

The effect of water activity on the rate of formation of pyrazines was investigated in NFDM systems over the a_w range of 0.32 to 0.84. Rates of formation were found to increase with a_w , up to a_w 0.75. A linear relationship was found to exist for the logarithms of the rate of formation of these pyrazines versus a_w .

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Chapter 19

Sugar-Derived Deoxy-Dicarbonyl Intermediates as Precursors of Food Flavors and Aromas

Milton S. Feather

Department of Biochemistry, University of Missouri, Columbia, MO 65211

The pathway to those food flavor and aroma compounds that are derived from the carbon atoms of sugars appears to involve deoxy-dicarbonyl sugar derivatives ("deoxyosones"). There is abundant evidence (sometimes indirect in nature) that deoxyosones are produced from the sugar as initial dehydration products, and in foods probably arise from the degradation of Amadori compounds (1-amino-1-deoxy-D-ketoses). The formation of 3-deoxyosones explain the presence of 2-furaldehydes frequently found in acidic food preparations such as citrus juices. Conversely, the formation of 1-deoxyosones, which are also produced from Amadori compounds, appear to be the precursors of many of the methyl-containing furanones and pyrones that contribute to food flavor and aroma. Parameters for the formation of these deoxyosones, particularly the 1-deoxyosones are discussed in this chapter.

There is a large body of evidence that suggests that deoxy-dicarbonyl compounds ("deoxyosones") are produced as initial intermediates during the degradation of sugars (1). The compounds (shown below as derivatives of hexoses) are isomeric with one another and represent dehydration products resulting from the parent sugar having lost one molecule of water. For purposes of this discussion, 1 is referred to as 3-deoxyosone, 2 as 4-deoxyosone and 3 as 1-deoxyosone (Scheme 1). Although not often isolated or even detected, there are a number of end products found in degradation reactions that are consistent with having been formed via these intermediates. Collectively, the deoxyosones are thought to be produced as a result of treatment of sugars with base as well as with acid, and, during the degradation of 1-amino-1-deoxy-D-fructose derivatives (Amadori compounds).

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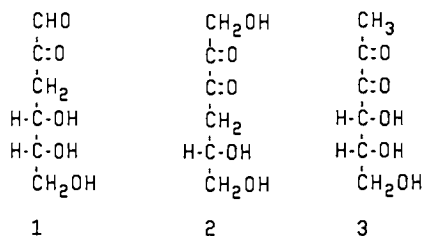
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The 4-deoxyosone (2) is thought to be a precursor of "isosaccharinic acid", produced during the treatment of sugars with base and of hydroxyacetyl furan (2), an acid-catalyzed degradation product of hexoses. This compound has been reported isolated by two different groups (3,4), in both cases by treatment of a disaccharide with base, and was reported to give "isosaccharinic acid" on further treatment with base. Unfortunately, neither group examined whether it gave hydroxyacetyl furan on acid treatment.

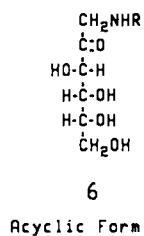
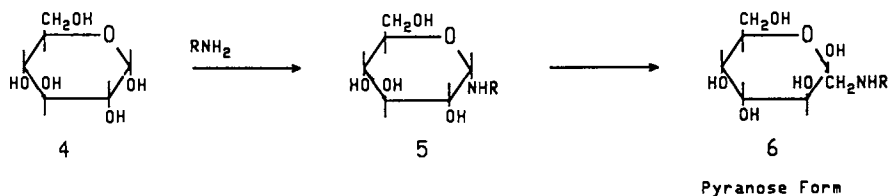
The 3-deoxyosone (1), is thought to be a precursor of "metasaccharinic acid" in basic solution and of 5-(hydroxymethyl)-2-furaldehyde (hereafter referred to as HMF) in acid solution. Compound 1 represents the intermediate about which the most is known. It was originally isolated as a result of the treatment of 3-O-substituted glucose derivatives with base (5) and was found to give "metasaccharinic acid" on further treatment with base. 3-Deoxyosone has been isolated in quantity by the degradation of "difructose glycine" in aqueous solution at pH 4.0 by Anet (6), who also has shown that it is converted to "metasaccharinic acid" in base and to HMF in acid solution (7). Since Anet's original isolation, compound 1 has been prepared by Kato (by treatment of glucose with N-butyl amine) (8) and in our laboratory (9) by transhydrazolation of the bis-benzoylhydrazone, which can be prepared from glucose in a one step reaction in crystalline form. It is noteworthy that Anet, during his lifetime, produced a large body of publications dealing with the formation and further degradation of 3-deoxyosones and studied the relevance of this compound to carbohydrate degradation reactions in general.

The 1-deoxyosone represents the most elusive of the three intermediates, but also the most important from the standpoint of food flavor and aroma production. A large number of methyl-containing furanones, pyrones and related compounds are found in food preparations that are consistent with having been formed from this intermediate. A synthesis of this material was reported some years ago by Isuzu, et al. (10), but the yields were very low and the product was not well characterized. The workers reported that the compound gave one of the two reported isomers of "saccharinic acid", the expected degradation product in alkaline solution, along with fragmentation products. This material will be addressed (vide infra) in a later section.

Amadori compounds (1-amino-1-deoxy-ketose derivatives) represent an important class of compounds that are formed during the thermal treatment of foods, and appear to degrade via deoxyosone intermediates. As shown in Scheme 2, glucose (4) reacts with an amine to give a glycosylamine (5) which rearranges to an Amadori compound (6). Such reactions can be expected to occur in any food systems that contain carbohydrate and an amine (amino acids or protein). The formation of Amadori compounds is an important reaction in food processing, because, compared to glucose, Amadori compounds are much less stable (11) and tend to easily degrade to a variety of compounds at very mild conditions. Thus, when foods are heated, Amadori compounds are produced and undergo degradation almost as fast as they are formed. This reaction, therefore, allows for glucose (normally a stable sugar) to undergo rapid and extensive degradation via its conversion to an



Scheme 1



Scheme 2

Amadori compound and the subsequent decomposition of this compound.

It appears that both 1 and 3 are produced as intermediates during the decomposition of an Amadori compound. There is little evidence, based on end product isolation that the 4-deoxyosone (2) is produced to any extent in these reactions. An early isolation of 1, by Anet (6), was accomplished by decomposing an Amadori compound ("difructose glycine") in aqueous solution. Subsequent studies have shown that Amadori compounds are easily converted to HMF in dilute acid solution as well. Furthermore, Kato's (8) published preparation of 3-deoxyosone, in which glucose is reacted with N-butyl amine almost certainly involves the intermediate formation of an Amadori compound and its decomposition in situ. Thus, it can be reasonably concluded that 3-deoxyosones are produced from Amadori compounds during their degradation.

1-Deoxyosone appears never to have been isolated or detected in a food system. Ledl and his group (12) have recently shown that it is produced during the degradation of an Amadori compound. In these studies the products were isolated and identified as quinoxaline derivatives. It is generally thought that methyl containing flavor and aroma constituents such as maltol and isomaltol, as well as the five carbon 4-hydroxy-5-methyl-3(2H)-furanone probably arise from 1-deoxyosone intermediates, although for most of these cases, direct experimental proof is lacking.

The issue of what factors control the formation of 1-deoxyosone vis-a-vis 3-deoxyosone from the same Amadori compound is an interesting and important one to food chemists. Anet (7) originally suggested that the formation of these two materials may be a function of the basicity of the Amadori compound, which is another way of saying that the reaction may be pH dependent. At acidic conditions, where the Amadori compound is fully protonated, the compound may undergo 1,2-enolization, resulting in the formation of 3-deoxyosone, while at more mild conditions (where the Amadori compound is unprotonated) a 2,3 enolization may ensue, resulting in the formation of 1-deoxyosone. This represents a reasonable scenario for explaining the reactions but clearly much work is needed in this area. If this scenario is correct, however, it would be predicted that the pathway for the degradation of an Amadori compound is pH dependent and would allow certain end products to be produced by altering the pH at which food is treated.

The furan isomaltol (7) and the pyran maltol (8) (Scheme 3) are consistent with having been formed via a 1-deoxyosone intermediate. Cyclization of such an intermediate and the loss of 2 additional moles of water allow one to arrive at these structures. Data which supports this is found in papers by Hodge and his group (13), who synthesized 1-deoxy-1-piperidino-maltulose (9, Scheme 4) and showed that on further heating, it was converted to galactosyl isomaltol (10). This is convincing evidence that isomaltol is sugar derived and is produced from an Amadori compound. Furthermore, the structure is wholly consistent with the formation of a 4-O-substituted 1-deoxyosone as an intermediate in the reaction.

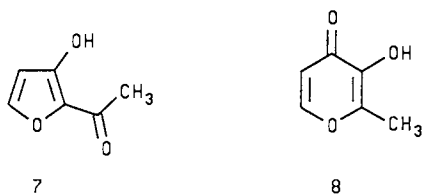
Peer and his group (14) showed that 4-hydroxy-5-methyl-3(2H)-

furanone (11, Scheme 5) can be produced from pentoses by treatment with amine salts.

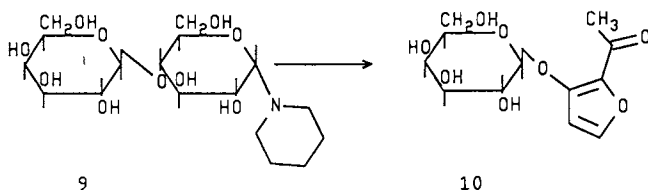
Isomaltol (7) is a prominent component of cooked beef flavor (15) and, is converted to the corresponding thiofuranone when treated with hydrogen sulfide. The thiofuranone is also found in beef broth and arises (presumably) by reaction of the furanone with endogenous hydrogen sulfide during cooking. In subsequent experiments in our laboratory, we were able to demonstrate that the furanone is produced from Amadori compounds derived from either xylose or glucuronic acid (16,17). The latter compound decarboxylates during the conversion, a common reaction of hexuronic acids. Furthermore, isotopic tracer studies clearly showed that the methyl group of the furanone corresponds to carbon 1 of the sugar from which it is derived (17). This provides convincing data in favor of a 1-deoxyosone intermediate in the reaction, since the reaction involves the degradation of an Amadori compound.

The finding that the furanone is produced from an Amadori compound was interesting in that it permitted us to test, for the first time, the effect of pH on the products of the reaction. This is not possible using a hexose-derived Amadori compound, because the end products are not stable. The product of 1,2-enolization (3-deoxyosone intermediate) is HMF, which is stable, but the product derived from 2,3-enolization (1-deoxyosone intermediate), maltol and isomaltol are not stable in aqueous solution and thus cannot be detected and measured accurately. In our experiment (Scheme 6), we prepared 1-deoxy-dibenzylamino (13), and -benzylamino (12) D-fructuronic acids, as well as 1-benzylamino-1-deoxy-D-xylulose (14) (17). Treatment of all three of these compounds with 2 N hydrochloric acid at 100° C gave 2-furaldehyde as the major identifiable product. This is to be expected, since in strong acid the Amadori compounds would undergo 1,2-enolization which would result in the formation of 3-deoxyosone intermediates and the formation of a 2-furaldehyde derivative. When the reaction was repeated at pH 7.0, the end products were found to be the furanone (11). While not a comprehensive study, this experiment provides convincing evidence that the end products of the dehydration reaction can, in fact, be controlled by the pH of the reaction media.

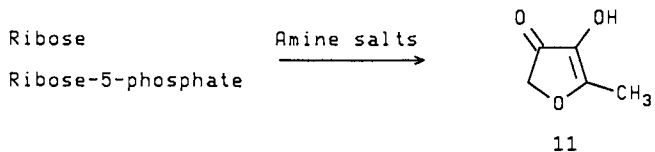
It would be possible to examine many facets of food flavor and aroma production if the 1-deoxyosone were available in quantities for experimental testing. In addition, such a compound would be useful as a flavor precursor in food preparations, since it would be predicted to undergo degradation in a matter of minutes at elevated temperatures. To that end, we have begun a project aimed at the synthesis of such compounds, and have succeeded (18) in synthesizing the 4-O-acetyl-5,6-O-isopropylidene derivative of compound 3. The synthesis is outlined in Scheme 7, below. The starting materials are 2,3-O-isopropylidene-D-glyceraldehyde (16), which is condensed with propynyl magnesium bromide (17) via a Grignard reaction to give the 6 carbon hexyne derivative (18). This reaction proceeds in quantitative yield and requires no chromatographic purification. After protection of the hydroxyl function of 18 as the acetate derivative (19), the latter can be



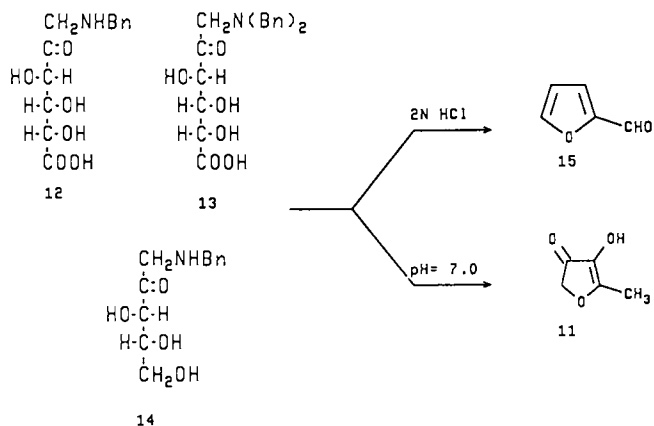
Scheme 3



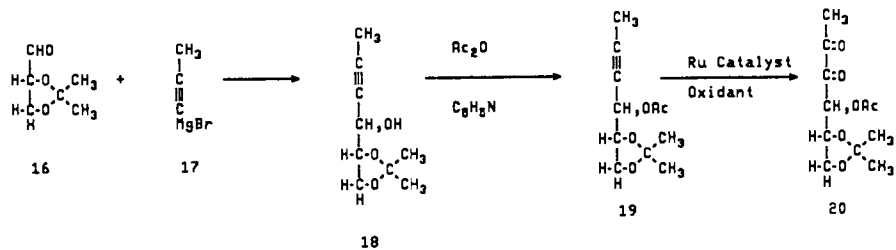
Scheme 4



Scheme 5



Scheme 6



Scheme 7

converted to the 1-deoxy dicarbonyl derivative (20) by oxidation with dichloro (tristriphenylphosphene) Ruthenium^{II} using iodosyl benzene as the oxidant. This reaction also proceeds in near quantitative yield. This general reaction sequence can be used to synthesize 1-deoxyosone derivatives containing any number of carbon atoms with various protecting groups.

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Chapter 20

Effects of Temperature, pH, and Relative Concentration on the Reaction of Rhamnose and Proline

James J. Shaw¹ and Chi-Tang Ho²

¹Warner-Lambert Company, Morris Plains, NJ 07950

²Department of Food Science, New Jersey Agricultural Experiment Station, Cook College, Rutgers, The State University of New Jersey, New Brunswick, NJ 08903

Response Surface Methodology (RSM) was used to investigate the effects of temperature, pH and relative concentration on the quantity of selected volatiles produced from rhamnose and proline. These quantities were expressed as descriptive mathematical models, computed via regression analysis, in the form of the reaction condition variables. The prevalence and importance of variable interaction terms to the computed models was assessed. Interaction terms were not important for models of compounds such as 2,5-dimethyl-4-hydroxy-3(2H)-furanone which are formed and degraded through simple mechanistic pathways. The explaining power of mathematical models for compounds formed by more complex routes such as 2,3-dihydro-(1H)-pyrrolizines suffered when variable interaction terms were not included.

The reaction of rhamnose and proline produces many pleasant aromas which are important to the food industry; among them are bready, cracker-like and roasted aromas. These aromas are greatly influenced by changes in reaction conditions such as temperature, pH and the relative concentration of reactants (1,2). The effects of changes in reaction conditions may be additive or synergistic. Research was undertaken to determine the prevalence and importance of synergies (variable interactions) between common reaction condition parameters for the reaction of rhamnose and proline.

Response Surface Methodology (RSM) is a statistical method which uses quantitative data from appropriately designed experiments to determine and simultaneously solve multi-variate equations (3). In this technique regression analysis is performed on the data to provide an equation or mathematical model. Mathematical models are empirically derived equations which best express the changes in measured response to the planned systematic

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change of independent variables. These equations are often expressed in the form of Taylor polynomial series.

The information obtained from a given investigation can be increased by the use of a planned experimental design (4). Factorial designs, for example, are of great economy because the controlled variables are studied simultaneously as opposed to one-at-a-time. The variables under investigation are studied in a precise, logical fashion, individually and in concert with each other. Measured responses can be isolated for each variable and interactions between variables, if present, can be identified. A proper design is essential to perform statistical evaluations on the data.

A variable interaction or synergy occurs when the effect on the response caused by one variable can be changed by varying the level of a second variable. RSM provides an estimate of the effect of a single variable at selected fixed conditions of the other variables. If the variables do act additively, the factorial (experimental design) does the job with more precision. If the variables do not act additively, the factorial, unlike the one-factor-at-a-time design, can detect and estimate interactions that measure the nonadditivity (5).

The greater the number of experimental variables, the greater the need for an efficient planned experimental design, due to the exponential increase in the experimental points needed to properly study the test variables without the design.

EXPERIMENTAL.

A three factor central composite design was used to study the volatiles formed as functions of the three independent variables: temperature, rhamnose/proline ratio and pH. In this type of design the variables are changed both simultaneously and one-at-a-time. The specific experimental points are listed in Table I.

Solutions of rhamnose and proline (150 ml.) in deionized water were prepared according to the experimental design. The pH was adjusted with blends of sodium phosphate, mono-, di- and tri-basic buffers.

The reactions were carried out in 300 ml. stainless steel Whitey reaction vessels heated for 30 minutes in a constant temperature oil bath. The come-up time to reach the oil bath temperature was shortened by preheating for 10 minutes in a 70°C water bath. After heating, the vessels and contents were cooled rapidly to about 5°C. A 100 ml aliquot of each reacted sample was adjusted to pH 10 according to Tressl et al. (6) and extracted four times with 100 ml portions of methylene chloride. The extracts were combined and concentrated to approximately 5 ml. at ambient temperature. One ml of 0.30% v/v cyclohexanone in methylene chloride internal standard was added, the samples were brought to 10.0 ml and were then filtered through 0.5 μ Teflon cartridge filters.

The volatiles were analyzed with a Perkin-Elmer Model Sigma 2 capillary gas chromatograph with a 60 m fused silica 0.25 mm i.d. 0.25 μ m film thickness Supelcowax 10 bonded phase capillary column. The temperature program consisted of an 8 minute hold at

Table I. Experimental Design Description

Design Point	Temp °C	pH	Rhamnose Conc (m)	Proline Conc (m)	Ratio Rham/Pro
1	190.00	6.30	0.10	0.10	1.00
2	180.00	6.30	0.10	0.10	1.00
3	152.50	6.30	0.10	0.10	1.00
4	139.00	6.30	0.10	0.10	1.00
5	152.50	2.90	0.10	0.10	1.00
6	152.50	9.70	0.10	0.10	1.00
7	152.50	6.30	0.20	0.00	
8	152.50	6.30	0.03	0.17	0.20
9	170.00	4.60	0.05	0.15	0.33
10	170.00	8.00	0.05	0.15	0.33
11	170.00	8.00	0.15	0.05	3.00
12	170.00	4.60	0.15	0.05	3.00
13	139.00	4.60	0.05	0.15	0.33
14	139.00	8.00	0.05	0.15	0.33
15	139.00	8.00	0.15	0.05	3.00
16	139.00	8.00	0.15	0.05	3.00
17	130.00	8.00	0.15	0.05	3.00
18	160.25	5.45	0.13	0.08	1.67
19	145.75	7.15	0.13	0.08	1.67
20	160.25	5.45	0.08	0.13	0.60
21	145.75	7.15	0.08	0.13	0.60

60°C. followed by 4°C. / minute ramping to 250°C. and a 10 min. final hold. The injection port (splitless) was 175°C, detector was FID and H₂ (8 ml/minute) was used as carrier gas. The G.C. signal was collected and intergrated by a Nelson Analytical Model 3000 Chromatography Data System.

Volatiles were analyzed in triplicate. Chromatographic peak areas were normalized based on internal standard response to account for variation in injection size. Chromatographic peaks were grouped by relative retention time for further statistical analysis.

A data base of area count response was prepared for 23 selected volatiles using Lotus 123 spreadsheet software. Regression analysis for model determination was done with SAS software.

A Finnegan MAT Model 8230 magnetic sector mass spectrometer interfaced with a Varian model 3400 gas chromatograph was used for the identification of volatile compounds. On-line spectra matching was done by spectral purity against a computerized National Bureau of Standards data base. Major peaks were subsequently checked against the MSDC Eight Peak Index and the results of Tressl et al. (6-10).

Mathematical models were prepared for the quantities of 23 compounds according to the polynomial equation $Y = \beta_0 + \beta_1T + \beta_2T^2 + \beta_3C(r) + \beta_4C(r)^2 + \beta_5pH + \beta_6pH^2 + \beta_7C(r)T + \beta_8pHT + \beta_9C(r)pH$. The dependant variable Y is the quantity of volatile (ppm) while β 's are assumed constants. T, T², C(r), C(r)², pH, pH², C(r)T, pHT and

C(r)pH are derived from the independent variables (temperature (T), rhamnose concentration C(r) and pH. Thus the model is composed of a constant, 3 linear, 3 quadratic and 3 variable interaction terms. The models were refined by eliminating those terms which were not statistically significant. The resulting mathematical equations may be graphically represented as a response surface as shown in Figure 1.

RESULTS and DISCUSSION.

Rhamnose and proline were reacted under a wide range of reaction conditions with the expectation of producing volatiles of differing type and ratio. Such large differences were desired to give the best opportunity for the empirical models to account for and explain the variation. If valid, the model terms would be expected to account for differences in product composition and perhaps provide insight into the reaction pathways. Some of the 23 volatiles modeled including 2,3-dimethyl-4-hydroxy-3(2H)-furanone (DMHF), 2-acetoxy-3-pentanone, and four 2,3-dihydro-(1H)-pyrrolizines will be discussed below.

Volatiles Produced. Table II lists the quantity of selected volatiles produced at various points of the experimental design. The points were chosen to show the effects of reaction conditions being varied one-at-a-time. DMHF is strongly temperature dependant in agreement with Shu et al. Its subsequent hydrolysis accounts for most of the carbohydrate fragmentation products produced (11). DMHF content roughly follows the concentration of rhamnose. Thus under the reaction conditions employed the catalytic action of proline does not determine the amount of DFHF present. Surprisingly, the hydroxy-pentanone isomers were not found under caramelization conditions though 3-hydroxy-2-pentanone is a known DMHF decomposition product. 2-acetoxy-3-pentanone (from 2-hydroxy-3-pentanone and acetic acid) is found in larger quantity than the change in rhamnose concentration would indicate.

The 2-3-dihydro-(1H)-pyrrolizines have two responses to reaction temperature: increasing with rising temperature or reaching a maxima at 152.5°C. These are compounds in which proline plays a structural as well as a catalytic role. The concentration of these compounds do not follow proline content as would be expected if the structural role of proline was determining. It appears that the concentration of carbohydrate fragments determines the pyrrolizine content.

Figure 2 illustrates the formation of 5-acetyl-7-methyl-[iv], 5-acetyl-6-methyl-[v], 7-formyl-5-methyl-[vi] and [vii] 7-acetyl-5-methyl-2,3-dihydro-(1H)-pyrrolizines after Tressl et al. (5). The 2,3-dihydro-(1H)-pyrrolizines require both carbohydrate fragmentation products and proline for their formation. Both the 5-acetyl- pyrrolizines [iv] and [v] increased in quantity as the reaction temperature increased while [vi] and [vii] were found at maximum quantity at 152.5°C. The first pair are formed through an iminium carboxylate intermediate which is decarboxylated into an exocyclic iminium ion which then undergoes an aldol

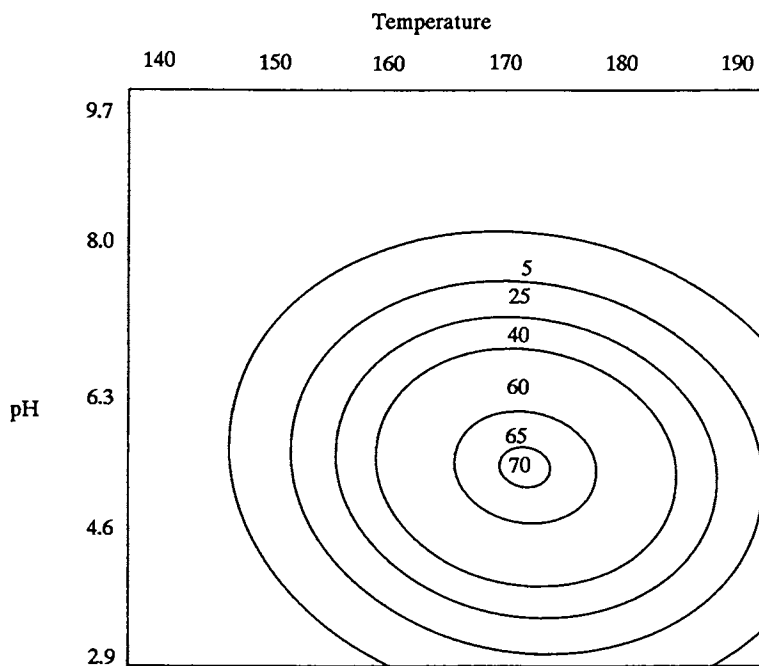


Figure 1. Temperature/pH response surface at 0.033 m rhamnose and 0.167 m proline. Graphical representation of the quantity (ppm) of 6-methyl-2,3-dihydro-(1H)-pyrrolizine.

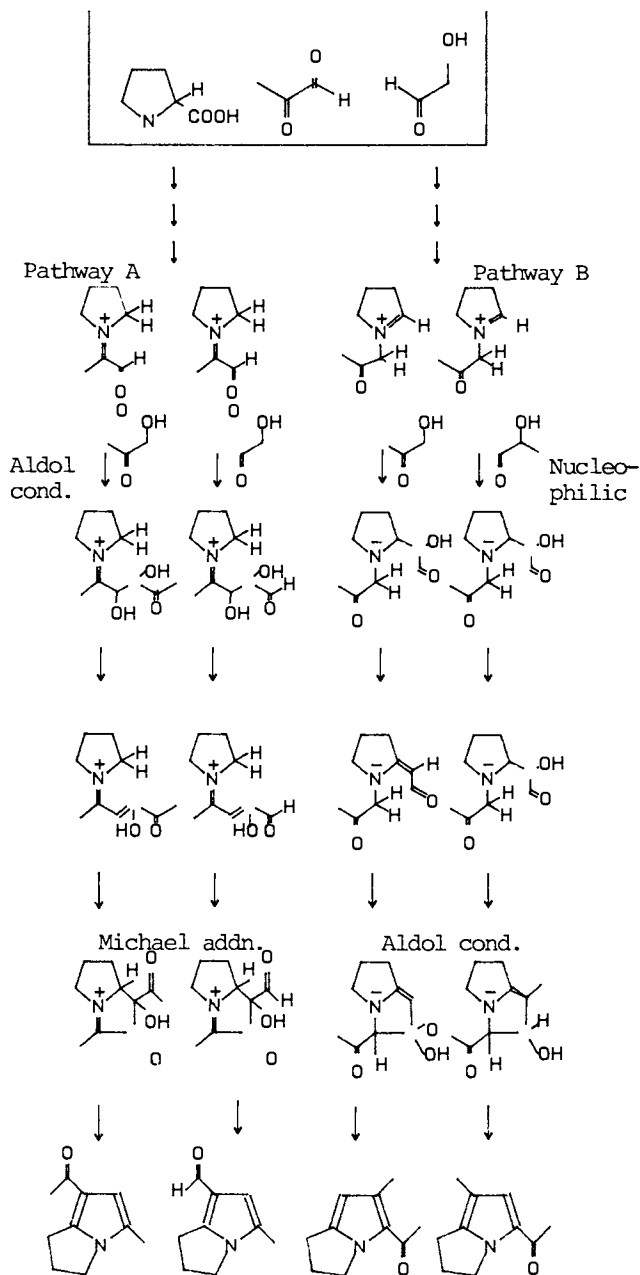


Figure 2. Formation of 5-acetyl-7-methyl, 5-acetyl-6-methyl, 7-formyl-5-methyl, and 7-acetyl-5-methyl-2,3-dihydro-(1H)-pyrrolizines. (Modified from ref. 7.)

Table II. Effects of Changes in Reaction Conditions on the Quantity of Selected Volatiles (ppm)

	[i]	[ii]	[iii]	[iv]	[v]	[vi]	[vii]
<u>Temp.</u>							
190.0	42.3	310.3	22.7	381.9	123.3	287.7	101.9
180.0	100.6	267.5	21.0	363.0	119.4	288.9	112.4
152.5	871.4	63.7	87.0	191.8	75.7	374.1	171.0
139.0	718.4	0.0	0.0	27.8	0.0	79.2	29.9
<u>R/P ratio</u>							
0.20	445.5	40.3	11.7	69.7	35.8	151.8	63.6
1.00	871.4	63.7	87.0	191.8	75.7	374.1	171.0
0.2 Rh	1434.5	0.0	318.3caramelization.....			
<u>pH</u>							
9.7	61.6	54.2	52.2	125.5	0.0	122.8	43.4
6.3	871.4	63.7	87.0	191.8	75.7	374.1	171.0
2.9	25.1	0.0	0.0	35.2	154.3	0.0	0.0

Temperature series conducted at pH 6.3, rhamnose and proline 0.10m each. Rhamnose:proline series at 152.5°C and pH 6.3. pH series at 152.5°C and rhamnose and proline 0.10 m each.

[i]	DMHF
[ii]	sum of 2-,3- and 3-hydroxy-2-pentanone
[iii]	2-acetoxy-3-pentanone
[iv]	5-acetyl-7-methyl-2,3-dihydro-(1H)-pyrrolizine
[v]	5-acetyl-6-methyl-2,3-dihydro-(1H)-pyrrolizine
[vi]	7-formyl-5-methyl-2,3-dihydro-(1H)-pyrrolizine
[vii]	7-acetyl-5-methyl-2,3-dihydro-(1H)-pyrrolizine

condensation followed by Michael addition for ring closure. This pathway yields the 5-acetyl-7-methyl [iv] or 5-acetyl-6-methyl [v] isomers depending if the aldol reactant is $\text{CH}_3\text{CH}(\text{OH})\text{CHO}$ or $\text{CH}_3\text{COCH}_2\text{OH}$ respectively.

The 7-formyl- and 7-acetyl- pyrrolizines are formed by an iminium carboxylate intermediate followed by decarboxylation to the cyclic iminium ion which underwent nucleophilic addition followed by aldol ring closure. This pathway accounts for the 7-formyl-5-methyl [vi] and 7-acetyl-5-methyl [vii] depending if the iminium addition is by OHCCH_2OH or $\text{CH}_3\text{COCH}_2\text{O}$.

Table III shows the statistical significance of model terms for the 4 pyrrolizines as reflected by the model's Prob >T values. Thus for Pathway A, T and pH were the determining factors responsible for explaining the quantity of 7-formyl-5-methyl [vi] found, while C(r) was only somewhat significant in interaction with pH. The situation for 7-acetyl-5-methyl [vii] was different. Here T, pH² and the three interaction terms were significant in explaining the quantity found over the experimental space.

Table III. Statistical Significance of Model Terms for 2,3-dihydro-(1H)-pyrrolizines

	[iv]	[v]	[vi]	[vii]
Intercept	0.0001	0.0001	0.0001	0.0001
T	0.0001	0.0001	0.0004	0.0010
T ²	*	0.0001	*	*
C(r) ²	*	0.0001	*	*
pH	0.0001	*	0.0001	*
pH ²	*	0.0004	*	0.0001
C(r)T	*	*	*	0.0010
C(r)pH	0.1134	*	0.1401	0.0050
pHT	*	0.0253	*	0.0005

* represents model terms which did not significantly contribute

In Pathway B, 5-acetyl-7-methyl [iv] was best described by T and pH, and C(r) only slightly in conjunction with pH. 5-acetyl-6-methyl [v] was best described by T, T², C(r)², pH² and pHT.

From this it could be seen that for 5-acetyl-6-methyl [v] and 7-acetyl-5-methyl [vii] the role of C(r) was important while for the other two pyrrolizines temperature, pH and their interaction were sufficient to describe the process.

Thus the two mechanistic pathways were not dependant on C(r) for their descriptions. The similarity in model terms suggest that the two pathways were quite similar to the effects of temperature and pH.

Both 5-acetyl-6-methyl [v] and 7-acetyl-5-methyl [vii] compete for CH₃COCH₂OH in their formation. This is a decomposition product of DMHF which is strongly C(r) dependant. Thus it is the authors opinion that the competition for a common precursor is the underlying reason for the differences in model terms for the group of pyrrolizines.

Mathematical Models. Secondary variable interactions quantify the synergies which are common in food chemistry. These interactions cannot be computed from pooled primary variable/sequential design studies and interpolations from such pooled data would lack the information given by the secondary interaction terms. Prob > t is an estimate of the relative importance of each model term. Terms with the lowest Prob > t could well be the driving force of the reaction processes accounting for the quantity of the volatiles found. From Table IV, about 25% of the model terms present at >0.05 Prob > t are seen to be interaction terms.

The model's R-Square value expresses the percentage of total variation explained by the mathematical model. The determined values which range from about 0.35-0.65 are indications of a linkage or relationship of the experimental variables to the quantity of volatiles. Thus although the models indicate relationships among the variables, large sources of unexplained variation remain. This is indicative of the dynamic nature of Maillard reaction studies. Although useful for the diagnostic

results reported here, higher R-Square values would be required for a truly predictive model.

Table IV. Terms of Statistical Significance to Mathematical Models

	T	T ²	C(r)	C(r) ²	pH	pH ²	C(r)T	pHT	C(r)pH
[i]		x	x		x	x			
[iii]		x	x		x	x		x	x
[iv]	x				x			x	
[v]	x	x		x		x		x	
[vi]	x				x				
[vii]	x	x				x	x	x	x
[viii]	x		x	x	x	x		x	x
[ix]	x	x	x	x	x	x	x	x	x
[x]	x	x	x		x	x	x		x
[xi]		x	x	x	x	x			

[viii] 2,5-dimethyl-3(2H)-furanone

[ix] 2-methoxy-5-ethylfuran

[x] 1-formyl-pyrrolidine

[xi] 1-acetyl-pyrrolidine

x represents terms of significance based on Prob > t of 0.05 or less in the final computed models.

Table V shows the relative importance of the model terms, by improvement with interaction terms. It is seen that interaction terms are frequently more important than primary independent variables to the nature of the model.

Table V. The Improvement of Model R-Square Value by Variable Interaction Terms

Volatile	R-Square without	R-Square with	Improvement (%)
[i]	0.5380	0.5395	0.28
[iii]	0.3689	0.5192	40.77
[iv]	0.6468	0.6673	3.17
[v]	0.5034	0.5902	17.24
[vi]	0.3261	0.3493	7.11
[vii]	0.4118	0.5567	35.14

The Model for 2,3-dimethyl-3(2H)-furanone (DMHF).

The empirical equation which best described the content of DMHF (ppm) found throughout the experimental space was $Y(\text{DMHF}) = -1670.5 + 24.10 T - 0.08 T^2 - 258.25 C(r) + 95.99 \text{ pH} + 6.58 \text{ pH}^2 + 3.03C(r)T$. The importance of the model terms as expressed by the Prob > t value are:

intercept	0.0001	C(r)	0.0032
T	0.1080	pH	0.0006
T ²	0.0003	pH ²	0.0002

Thus the contribution of these terms as related by their significance from Prob > t has T^2 , pH and pH^2 as the most important with a lesser importance to $C(r)$, T and $C(r)T$ are of much less significance comparatively. Interactions of the reaction condition variables were not important to describing the content of DMHF.

The Model for 2-acetoxy-3-pentanone. This compound is formed by a condensation of acetic acid and 2-hydroxy-3-pentanone, common carbohydrate fragmentation products. As such it would be expected that $C(r)$ would be important (from the carbohydrate source) and pH since its precursors are affected by both acid and base. The most significant model terms according to Prob > T were $C(r)$ (0.0002) and pH (0.0024) with $C(r)pH$, T^2 , pH^2 , pHT and T all showing p values about 10 times larger.

The reaction conditions where the interaction terms are required to explain the 2-acetoxy-3-pentanone content occur in portions of the experimental space at low rhamnose concentration and at temperatures where the combination-heterocyclic compounds are not formed in large quantity. This also represents experimental points where DMHF had greater stability and thus the pool of retro-aldol fragments was lower.

CONCLUSIONS.

This study evaluated changes in the quantity of volatiles formed from rhamnose and proline as functions of three reaction conditions: temperature, pH and relative concentration. The objective was to identify and quantify the presence of interactions between dependant variable reaction conditions. That is, for example, the effect of temperature on trends brought about by pH. Such interactions cannot be estimated when variables are studied one-variable-at-a-time. A further benefit would be attained if the models could provide insight into the chemical processes involved.

The research has shown that interactions among reaction conditions are plentiful although of varying magnitude. These interactions are expressions of synergistic or antagonistic effects of the reaction conditions in concert affecting the quantity of selected volatile compounds. More importantly this research has quantified these interactions as a whole and separated them into their individual components. Besides providing better predictability within the experimental region studied, this may lead to a better understanding of the processes involved. It is seen in cases where the interaction terms are important up to a 30-40% increase in model explaining power can be achieved when the interaction terms are included. This is a dramatic improvement over the results of a one-variable-at-a-time approach for which the interaction terms can not be expressed.

Linkage of reaction conditions to the quantity of volatiles found was made for most of the components studied over the experimental region. The quantity of some low molecular weight compounds formed via retro-aldolization or other carbohydrate

fragmentation pathways could not be adequately modeled as functions of the independent variable reaction conditions despite good quantification. Methyl acetate, acetic acid, 3-hydroxy-2-pentanone and 2-hydroxy-3-pentanone are examples of these compounds. This may indicate a lack of independence for these compounds or discontinuity of the response function.

Interaction terms were found to be very important for volatiles whose formation precursors were limited due to competition for precursor, stability of precursor, or because the designed experiments purposefully limited selected precursors. The 2,3-dihydro-(1H)-pyrrolizines fall into this category.

Thus reaction flavor generation may well be suitably investigated by systematic changes in all of the reaction condition variables in addition to the one-variable-at-a-time approach which is commonly employed. Such systematic change requires a suitable design such as the central composite factorial design which was used for the estimation of both primary, quadratic and variable interaction terms.

Variable interaction terms do not aid in the understanding of DMHF content within the experimental space studied because the primary variable effects are very strong. This is reasonable for a compound which is both easily formed and readily degraded. Variable interaction terms are more important in understanding the formation of 2,3-dihydro-1H-pyrrolizines. These compounds are formed through more complicated mechanistic pathways. Where the interaction terms are important, a 17% and 35% improvement in model fit as expressed by R-Square value was obtained when the interaction terms are considered.

In summary, reaction flavors are complex systems and are strongly influenced by changes in reaction conditions. As the number of reaction condition variables increases so does the possibility of variable interactions or synergies. Without appropriate experimental designs it is not possible to assess the contribution of each variable singly and in concert. Response Surface Methodology is another tool for understanding the effects of reaction conditions on Maillard type flavors.

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Chapter 21

Parameter Effects on the Thermal Reaction of Cystine and 2,5-Dimethyl-4-hydroxy-3(2*H*)-furanone

Chi-Kuen Shu¹ and Chi-Tang Ho

Department of Food Science, New Jersey Agricultural Experiment Station,
Cook College, Rutgers, The State University of New Jersey,
New Brunswick, NJ 08903

The thermal reaction of cystine and DMHF is important for the generation of meat flavors. The products of this reaction, their flavor compounds, aroma profiles and yields, however, vary according to the reaction parameters. This study focused on determining the effect of the reaction medium, duration, water content, temperature, pH and oxygen on the products of this reaction.

The Maillard reaction commonly occurs in food products and during food processing. A typical or pure Maillard reaction is simply the reaction of a sugar and an amino acid. Strictly speaking, the sugar must be a reducing carbohydrate and the amino acid can be either free or bound, as a peptide or protein. The reaction generates not only volatile compounds, which provide odor, but also odorless nonvolatile compounds, some of which are colored.

The Maillard reaction is a complex system. Sugars and amino acids first condense into Amadori or Heyns compounds, which then, through a series of β -elimination and enolization can be converted into α -dicarbonyls. These α -dicarbonyls play the key role in further reactions to generate the different types of flavor compounds. The sugar and amino acid react not only together, but also separately and individually. The thermal degradation of sugar, or caramelization, generates α -dicarbonyls and, subsequently, flavor compounds. With amino acids various active primary products are formed (1-4). These primary products react further to produce a large number of additional flavor compounds.

The yield of flavor compounds from the Maillard reaction is very low compared to the yield of nonvolatiles. Therefore, it is very difficult to use the typical/pure Maillard reaction to synthesize flavor compounds for commercial use or to study its chemistry.

¹Current address: R. J. Reynolds Tobacco Company, Bowman Gray Technical Center, Winston-Salem, NC 27102

To overcome the problems associated with the inherently low yield amino acid degradation products, α -dicarbonyls have been used in place of amino acids and sugars, respectively (5-10).

It is useful to categorize the Maillard reactions into pure Maillard reactions and modified Maillard reactions. The former includes only sugar and amino acid; the latter includes (a) sugar and amino acid degradation products, or (b) α -dicarbonyls and amino acid or (c) α -dicarbonyls and amino acid degradation products.

The thermal reaction of cystine and 2,3-dimethyl-4-hydroxy-3(2H)-furanone (DMHF), a modified Maillard reaction is important for the generation of meat flavors. The reaction products, their flavor compounds, aroma character and yield vary, according to the reaction parameters. These parameters include the reaction medium, duration, water content, temperature, pH and presence or absence of oxygen.

Cystine and other sulfur-containing amino acids are recognized as important precursors of food flavors, especially meat flavors (3, 11-12). DMHF, a cyclic- α -dicarbonyl, possesses a sweet, caramel and fruity aroma (13). It is found in many food sources (14-17) and is used extensively in many flavor applications (18-19). DMHF can be formed from sugar via either sugar enolization (caramelization) by a Maillard reaction then cyclization (20).

Recently, we reported on the thermal degradation of cystine (21) and also the thermal degradation of DMHF (22) as background for the title reaction.

Experimental Section

Materials Employed. 2,5-dimethyl-4-hydroxy-3(2H)-furanone (DMHF) of high purity was provided by International Flavors and Fragrances (IFF). L-cystine, injectable grade, was purchased from Ajinomoto Company, Tokyo, Japan. Reagent grade methylene chloride was purchased from Aldrich Chemical Company, and was freshly distilled prior to use. Spectral grade glycerol was obtained from a commercial source.

Sample Preparation. A mixture of cystine, DMHF (0.05 mole each), and 500 g of distilled water or glycerol was placed in a 2-liter Paar Bomb (Paar Instruments Co., Moline, IL) equipped with a magnetic stirrer, an internal cooling coil and a temperature controller. The reaction mixture was heated to the specific temperature and held at that temperature for a specific time for each experiment. After cooling to room temperature, a reaction mass was obtained. The reaction time and temperature for each parameter studied is outlined below.

The procedures used for the isolation, extraction, analysis and identification of volatiles have been described elsewhere (21).

Specific Conditions for Parameter Study

1. Medium Effect: Cystine and DMHF were reacted in both aqueous and nonaqueous solvents. Glycerol was chosen as the nonaqueous medium for two reasons: (i) glycerol has a high boiling point so that it was not readily distilled under the conditions of vacuum steam distillation and (ii) glycerol is miscible with water so that

an investigation on the effect of the water content was possible. Reactions studying the effect of the reaction medium were carried out at 160°C for a half hour.

2. Duration Effect: The reaction was carried out in water and in glycerol at 160°C and stopped after one half, one and two hours.

3. Water Content Effect: Water and glycerol were combined to prepare reaction mixtures containing 10%, 25%, 50% and 75% water. These reactions were run for one half hour at 160°C.

4. Temperature Effect: Three temperatures (100°C, 160°C and 200°C) were selected to represent boiling, roasting and frying conditions. The temperature studies were run for a half hour in 75% water.

5. pH Effect: Reactions were carried out at three different pH's: at below and above the isoelectric point of cystine. The pH was adjusted with 10% Na₂CO₃ and 1% HCl to the pH values of 2.4, 4.7 and 7.0, which were chosen for the study.

6. Oxygen Effect: The reaction of cystine and DMHF was studied in the presence and absence of oxygen. Prior to starting the reaction in the absence of oxygen, the reaction mixture in the Paar Bomb was degassed by a stream of nitrogen. Both reactions in this study were carried out at 160°C for a half hour.

Results and Discussion

The volatile components identified from the reaction of cystine and DMHF in aqueous medium are shown in Table I. 2,4-Hexanedione, 3,5-dimethyl-1,2,4-trithiolanes and thiophenes are the major compounds. The mechanistic relationship of the three thiophenones produced has been postulated (23). The major groups of volatile components identified from the reaction in the glycerol medium are 1,3-dioxolanes and thiazoles (Table II). 1,3-Dioxolanes are formed by the reaction of glycerol and the degraded carbonyls by ketal or acetal formations. Comparison of the reaction of cystine and DMHF in water and in glycerol is outlined in Table III.

The effect of reaction time on the major components of the reaction of cystine and DMHF in water is shown in Table IV. It is noteworthy that amounts of 2,4-hexanedione, 3,5-dimethyl-1,2,4-trithiolanes and thiophenones were found at a maximum after one hour. It was also found that the amount of 2-acetylthiazole increased with time and that acetol acetate decreased with time as expected. In the glycerol medium, the effect of reaction time on the major components is shown in Table V. Apparently, the 1,3-dioxolane, which is a ketal formed from glycerol and acetone, decreased over time. Also, long reaction time favors the formation of cyclic compounds, including 2,5-dimethyl-2-hydroxy-3(2H)-thiophene, cyclopentenones and 4,5-dimethyl-1,2-dithiolenone.

The samples were reacted for one half, one and 2 hours and yielded 392, 585 and 565 mg volatiles, respectively. The aroma was judged best after one half hour where roasted, pot-roasted and burnt notes were produced. After one hour, the aroma was burnt, roasted and biting, while after two hours, the aroma was considered biting, burnt and phenolic. In the glycerol medium the yield was 310, 410 and 438 mg after one half, one and 2 hours. In contrast to the aqueous medium where the best aroma was produced after one half

Table I. The Volatile Components Identified from the Reaction of Cystine and DMHF in Water

Compounds	GC Area, %
<u>Aldehydes/Ketones</u>	
Acetaldehyde	T
Acetone	T
Methyl ethyl ketone	1.2
2-Pentanone	1.5
3-Hexanone	T
1-Hydroxy-2-butanone	T
2,4-Pentanedione	T
3-Hydroxy-2-pentanone	4.2
2-Hydroxy-3-pentanone	1.3
2,4-hexanedione	16.4
2-Ethyl-5-methyl-2-cyclopenten-1-one	T
<u>Esters</u>	
Acetyl acetate	1.1
2-Oxobutyl acetate	T
<u>Furanones</u>	
2,5-Dimethyl-4,5-dihydro-3(2H)-furanone	T
2,5-Dimethyl-4,5-dihydro-3(2H)-furanone	T
2,5-Dimethyl-3(2H)-furanone	T
2,4,5-Trimethyl-3(2H)-furanone	T
2,4-Dimethyl-5-ethyl-3(2H)-furanone	T
<u>Thiolanes/Thianes</u>	
3,5-Dimethyl-1,2,4-trithiolane	3.7
3,5-Dimethyl-1,2,4-trithiolane	4.9
3-Methyl-1,2,4-trithiane	T
4,6-Dimethyl-1,2,3,5-tetrathiane	T
4,6-Dimethyl-1,2,3,5-tetrathiane	T
3,6-Dimethyl-1,2,4,5-tetrathiane	T
2-Methyl-1,3-dithiolane	T

Continued on next page

Table I. Continued

Compounds	GC Area, %
<u>Thiazoles/Isothiazole-Thiazoline</u>	
Thiazole	T
3-Methylisothiazole	T
2,5-Dimethylthiazole	T
2-Acetylthiazole	1.9
2-Thiazolyl ethyl ketone	1.0
2-Methylthiazole	T
2,4,5-Trimethylthiazole	T
2-n-Propylthiazole	T
2,4-Dimethyl-3-thiazoline	T
2,5-Dimethyl-4-ethylthiazole	T
2-Methyl-5-ethylthiazole	T
<u>Thiols</u>	
1-Mercapto-2-propanone	T
3-Mercapto-2-pentanone	T
<u>Pyrazine</u>	
2,5-Dimethylpyrazine	T
<u>Thiophenes</u>	
2-Acetyl-5-methylthiophene	T
3-Methyl-2-(2-oxopropyl)thiophene	T
4,5-Dimethyl-2-acetylthiophene	T
2-Acetylthiophene	T
3-Methyl-2-thiophene carboxaldehyde	T
<u>Thiophenones</u>	
2,5-Dimethyl-4-hydroxy-3(2H)-thiophenone	
2,5-Dimethyl-2-hydroxy-3(2H)-thiophenone	22.5
2,5-Dimethyl-2,4-dihydroxy-3(2H)-thiophenone	

T = trace, less than 1%

Table II. The Volatile Components Identified from the Reaction of Cystine and DMHF in Glycerol

Compounds	GC Area, %
<u>Thiazoles/Isothiazoles</u>	
Thiazole	T
2-Methylthiazole	2.5
3-Methylisothiazole	T
5-Methylthiazole	T
2,4-Dimethylthiazole	T
2-Ethylthiazole	1.3
2,5-Dimethylthiazole	1.4
2-n-Propylthiazole	1.1
2,4,5-Trimethylthiazole	1.5
4-Methyl-5-ethyl-thiazole	T
2,5-Dimethyl-4-ethyl-thiazole	2.1
2,4-Dimethyl-5-ethyl-thiazole	3.0
4,5-Dimethylthiazole	T
2-(2' Thienyl)thiazole	T
2-Methyl-5-ethylthiazole	2.6
<u>1,3-Dioxolanes</u>	
2,2-Dimethyl-4-hydroxymethyl-1,3-dioxolane ^{a,b}	6.1
2-Methyl-2-ethyl-4-hydroxymethyl-1,3-dioxolane ^a	2.0
2,2-Diethyl or (2-methyl-2-propyl)-4-hydroxymethyl-1,3-dioxolane ^{a,b}	1.9
2-Methyl-4-hydroxymethyl-1,3-dioxolane ^a	T
<u>Aldehyde/Ketones</u>	
Acetaldehyde	T
Acetone	T
Methyl ethyl ketone	T
2-Pentanone	4.0
2,3-pentanedione	1.4
3,4-Dimethyl-2-cyclopenten-1-one	T
2-Ethyl-5-methyl-2-cyclopenten-1-one	2.4
3-Hexanone	3.4
<u>Other Compounds</u>	
2,5-Dimethylpyrazine	T
2,6-Dimethylpyrazine	T
2-Ethyl-3,6-dimethylpyrazine	T
2,4-Dimethyl-5-ethylpyrrole	T
Ethyl disulfide	1.6
2,5-Dimethyl-2-hydroxy-3(2H)-thiophenone	6.9
4,5-Dimethyl-1,2-dithiole-3-one	2.3
2-Acetyl-4,5-dimethylthiophene	T
γ -Thiovalerolactone	T

^a Formed from the reaction between glycerol and degraded carbonyls.

^b Glycerol impurity.

Table III. Comparison of the Reaction of Cystine and DMHF in Water and in Glycerol

Characteristics	in Water	in Glycerol
<u>Components</u>		
Trithiolanes	+	-
2,4-Hexanedione	+	-
Thiophenones	++	+
Furanones	++	+
Thianes	++	+
Thiazoles	+	++
Pyrazines	+	++
<u>Aroma</u>		
Roasted/Meaty	++	+
Burnt	+	++
Biting	+	++
Strength	++	+
Smoothness	++	+
<u>Yield</u>	++	+

Table IV. The Effect of Duration on the Major Components of the Reaction of Cystine and DMHF in Water

Compounds	(mg)		
	1/2 Hr.	1 Hr.	2 Hr.
Methyl ethyl ketone	4.7	7.6	9.6
2-Pentanone	5.9	8.2	7.9
3-Hydroxy-2-pentanone	16.5	17.0	10.2
2-Hydroxy-3-pentanone	5.1	8.2	12.4
2,4-Hexanedione	64.3	80.1	66.7
Acetolactate	4.3	1.2	<1
2-Acetylthiazole	7.4	13.5	15.3
3,5-Dimethyl-1,2,4-trithiolane	33.7	36.3	23.2
2,5-Dimethyl-4-hydroxy-3(2H)-thiophenone			
2,5-Dimethyl-2-hydroxy-3(2H)-thiophenone	86.6	129.9	73.5
2,5-Dimethyl-2,4-dihydroxy-3(2H)-thiophenone			

Table V. The Effect of Durations on the Major Components of the Reaction of Cystine and DMHF in Glycerol

Compounds	(mg)		
	1/2 Hr.	1 Hr.	2 Hr.
2-Pentanone	9.6	3.7	17.5
3-Hexanone	4.0	5.3	14.9
2-Ethyl-5-methyl-2-cyclo-penten-1-one	0.9	4.5	10.5
2-Methylthiazole	7.1	9.8	11.0
2-Ethylthiazole	5.6	5.7	5.7
2,5-Dimethylthiazole	5.9	7.4	6.1
2,4,5-Trimethylthiazole	5.6	10.3	6.6
2,5-Dimethyl-4-ethylthiazole	9.0	12.7	9.6
2,4-Dimethyl-5-ethylthiazole	10.9	14.8	13.1
Ethyl disulfide	1.6	4.9	7.0
2,5-Dimethyl-2-hydroxy-3(2H)-thiophenone	12.1	24.2	30.2
4,5-Dimethyl-1,2-dithiole-3-one	<0.5	6.2	10.1
2,2-Dimethyl-4-hydroxymethyl-1,3-dioxolane	44.6	36.9	26.7
2-Methyl-5-ethylthiazole	7.8	9.8	11.4

hour, here the preferred aroma was formed after two hours. The aroma after one half hour was roasted and biting and after one hour was burnt and biting. After two hours, the aroma was judged to be cracker-like, roasted and burnt.

The effect of water content on the reaction yield is shown graphically in Figure 1. The sample prepared at 75% water content provided the highest yield. It also had the most balanced, meaty aroma. In contrast, the samples with 0%, 10% and 25% water had a biting aroma. The 50% water sample had a blended onion aroma; at 75% water the aroma was pot-roasted, roasted, meaty and clean, while the 100% water sample was roasted and pot-roasted but also burnt.

The water content significantly affects the formation of some compounds. Figure 2 shows that the formation of thiazoles decreases as the water content increases. Figure 3 shows the relationship between water content and the formation of a 3,5-dimethyl-1,2,4-trithiolane, 3-hydroxy-pentanone and 2,4-hexanedione. The highest level of trithiolanes was obtained from the sample prepared with 75% water. Figure 4 shows that these three thiophenones were also produced at maximum at 75% water medium.

Collectively, the sample produced in the 75% water medium had more trithiolanes and thiophenones and a better aroma. Therefore, the aroma properties could be considered to be proportional to the contents of these two types of compounds.

Temperature has a great effect on the yield and aroma of the title reaction. The higher the reaction temperature the higher the yield. For example only 55 mg of volatiles were formed in the 100°C sample while 567 and 752 mg of volatiles were produced by heating to 160°C and 200°C. At 100°C, the reaction mixture is dominated by esters and furanones and one thiazole (2-acetylthiazole); while at

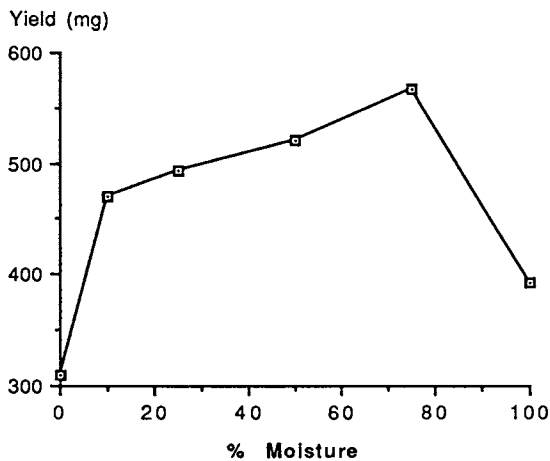


Figure 1. The effect of water content on the yield from the reaction of cystine and DMHF.

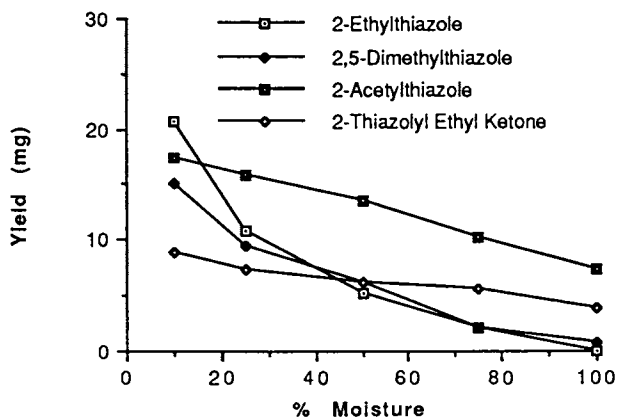


Figure 2. The effect of water content on the formation of thiazoles from the reaction of cystine and DMHF.

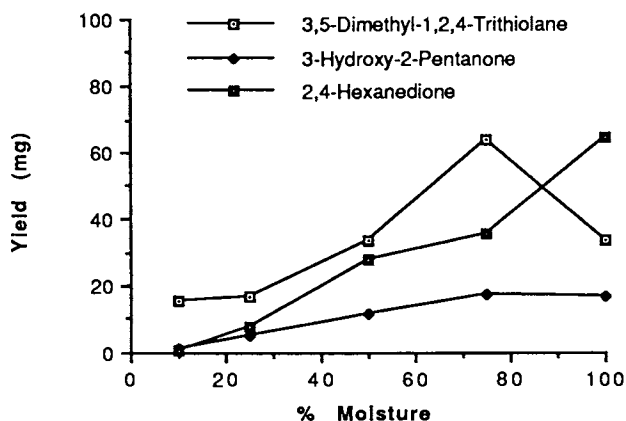
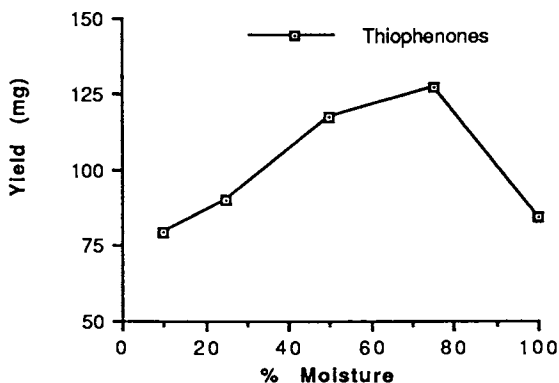


Figure 3. The effect of water content on the formation of 3,5-dimethyl-1,2,4-trithiolane, 3-Hydroxy-2-pentanone and 2,4-Hexanedione from the reaction of cystine and DMHF.



Thiophenones Include:
 2,4-Dimethyl-4-Hydroxy-3(2H)-Thiophenone
 2,4-Dimethyl-2-Hydroxy-3(2H)-Thiophenone
 2,4-Dimethyl-2,4-Dihydroxy-3(2H)-Thiophenone

Figure 4. The effect of water content on the formation of thiophenones from the reaction of cystine and DMHF.

200°C, no esters and furanones are found, but thiazoles, cyclopentenones and other heterocyclic compounds dominate. These data imply that esters and furanones are stable at mild temperatures while the formation of thiazoles, cyclopentenones and other heterocyclic compounds require a higher temperature. Also at 160°C, trithiolanes, thiophenones and 2,4-hexanedione predominate, indicating that formation of such compounds is favored by a medium temperature. Bread, crusty and caramel aromas were found in the 100°C sample, pot-roasted, roasted, meaty and clean aromas were found at 160°C, and roasted, roasted-meat, chemical and off-notes were produced at 200°C.

pH has a less dramatic effect on the yield and aroma from the reaction. The highest yield was obtained at pH 4.7 (523 mg) compared to 392 at pH 2.4 and 329 mg at pH 7.0. The best aroma was found at pH 2.4 where it was roasted, burnt and biting. At pH 4.7, the aroma was toasted, onion-like while at pH 7.0 the aroma was cooked onion and sulfury. Table VI shows that the major components formed from the reaction vary quantitatively at the different pH values. The greatest quantity of trithiolanes, thiophenes, 2-hydroxy-3-pentanone and 3-hydroxy-2-pentanone is formed at pH 4.7, which is the isoelectric point of cystine. Formation of 2,4-hexanedione sharply decreased with increased pH.

Table VII addresses the effect of oxygen on the yield and aroma of the reaction of cystine and DMHF. In general, oxygen affects the yield rather than the types of volatiles generated. A higher yield of volatiles was obtained in the absence of oxygen. It may be that oxygen promotes polymerization of volatiles into non-volatile compounds. This may be applicable to increasing the yield of other model systems. It is noteworthy that the level of the trithiolanes formed was higher when oxygen was absent. The presence or absence of oxygen did not greatly influence the aroma character of the reaction products.

The optimal conditions for generating the major products formed from cystine and DMHF are as follows: 3,5-dimethyl-1,2,4-trithiolane, thiophenones and 2,4-hexanedione are all found preferentially in an aqueous medium heated to 160°C. The trithiolane and thiophenone are optimized at 75% H₂O and pH 4.5, while 2,4-hexanedione formation is better at 100% H₂O and lower pH. Thiazoles, on the other hand, require a higher temperature and a nonaqueous medium.

Table VI. The Effect of pH on the Major Components from the Reaction of Cystine and DMHF

Compounds	(mg)		
	pH=2.4	4.7	7.0
3-Hydroxy-2-pentanone	16.5	27.2	8.2
2-Hydroxy-3-pentanone	5.1	8.9	4.3
2,4-Hexanedione	64.3	31.9	4.3
2-Acetylthiazole	7.5	14.1	24.7
3,5-Dimethyl-1,2,4-trithiolane	33.7	50.7	33.9
Thiophenones*	101.0	104.6	23.7

*Including 2,5-Dimethyl-4-hydroxy-3(2H)-thiophenone
2,5-Dimethyl-2-hydroxy-3(2H)-thiophenone
2,5-Dimethyl-2,4-dihydroxy-3(2H)-thiophenone

Table VII. The Effect of Oxygen on the Major Components from the Reaction of Cystine and DMHF

Major Peaks	In Presence of Oxygen	In Absence of Oxygen
	mg	mg
3-Hydroxy-2-pentanone	16.5	10.1
2,4-Hexanedione	60.1	74.6
3,5-Dimethyl-1,2,4-trithiolane ^a	35.7	80.0
Thiophenones ^b	101.5	98.1
<u>Yield</u>	392	533
<u>Aroma</u>	Roasted, Burnt Biting	Roasted, Burnt, Warmed

^a - cis/trans Isomers

^b - Including 2,5-Dimethyl-4-hydroxy-3(2H)-thiophenone
2,5-Dimethyl-2-hydroxy-3(2H)-thiophenone
2,5-Dimethyl-2,4-dihydroxy-3(2H)-thiophenone

Conclusions

As a result of the reaction parameter study, the products, aromas and chemistry of the reaction between cystine and DMHF is more clearly understood. The major volatile components generated by this reaction are 3,5-dimethyl-1,2,4-trithiolanes, thiophenones, thiazoles and 2,4-hexanedione. The first three groups of compounds contribute greatly to the quality of the overall aroma which is roasted, meaty and burnt. Further variations in aromas may depend on the proportions of these components present as reaction products. Optimal conditions for producing the major components of interest from the title reaction have been determined.

It is hoped that the data presented may prove useful as a guideline for other reaction flavor studies where maximization of flavor compounds is desired.

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Chapter 22

Formation of Influential Flavor Components Through Water-Mediated Retro-Aldol Conversions of α,β -Unsaturated Carbonyls

David B. Josephson and Jerome Glinka

Fries & Fries, 110 East 70th Street, Cincinnati, OH 45216

Aqueous solutions of unsaturated carbonyls are hydrolyzed under alkaline conditions to produce additional carbonyl-containing compounds. Alkaline conditions traditionally used to accelerate this reaction cascade can be replaced with elevated temperatures and pressures without greatly affecting the overall hydrolysis of these unsaturated carbonyls. Water-mediated retro-aldol degradation of alpha/beta unsaturated carbonyls appears to be significant as a means to thermally-generate flavor-active carbonyls, as well as lead to deterioration of character-impact compounds possessing these features.

Addition of water to alpha/beta unsaturated carbonyls results in the formation of the corresponding aldol. Dehydration of the aldol regenerates the alpha/beta unsaturated carbonyl. However, a reverse or retro-aldol reaction fragments the beta-hydroxy carbonyl to two additional carbonyls, each possessing lower vapor pressures than the parent precursor. Figure 1 illustrates the water-mediated retro-aldol cascade as it occurs for aldehydes and ketones containing at least one carbon-carbon double bond conjugated to the carbonyl group. In this reaction cascade ethanal and a series of methyl ketones are formed in addition to an aldehyde.

Base-catalyzed hydration of conjugated carbonyls, followed by retro-aldol fragmentation has been a common strategy for studying the reaction cascade (1-4). The kinetically important step in the base-catalyzed hydration of an alpha/beta unsaturated carbonyl is similar to a nucleophilic substitution reaction at carbon 3. The reaction cascade proceeds rapidly from the conjugated carbonyl through its hydration and subsequent fragmentation.

In two recent reports on retro-aldol degradation of (E,Z)-2,6-nonadienal to (Z)-4-heptenal and ethanal (5), and (E,Z)-2,4-decadienal to (Z)-2-octenal, hexanal and ethanal (6), it was demonstrated that this reaction could be thermally-driven at neutral pH.

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Similarly, a recent patent for the commercial manufacture of benzaldehyde from cinnamaldehyde and 6-methyl-5-hepten-2-one from citral under neutral conditions has been issued (7).

Table 1 lists important precursors of conjugated carbonyls which would be expected to undergo some degree of water-mediated retro-aldol degradation to form volatile flavor compounds under elevated thermal conditions. Previously, it was demonstrated that N-shogaol found in ginger can be degraded through gingerol to form zingerone and an n-alkanal (8). Additionally, fructose can undergo thermally-catalyzed dehydration and tautomerization reactions followed by a retro-aldol condensation to form 4-hydroxy-5-methyl-3-(2H)-furanone and methanal (formaldehyde; 9).

Table 1. Some Important Precursors of Alpha/Beta Unsaturated Carbonyls in Food and their Retro-Aldol Degradation Products

Precursor	Alpha/Beta Unsaturated Carbonyl	Retro-Aldol Products
n-6 Polyunsaturated Fatty Acids (e.g., Linoleic Acid)	2-4 Decadienal	2-Octenal + Ethanal Hexanal + Ethanal
n-3 Polyunsaturated Fatty Acids, e.g., Linolenic Acid)	2,4-Heptadienal	2-Pentenal + Ethanal Propanal + Ethanal
	2,6-Nonadienal	4-Heptenal + Ethanal
Sugars (fructose)	Reaction Intermediate	4-Hydroxy-5-Methyl-3-(2H)-Furanone+ Methanal
Essential Oils	Cinnamic Aldehyde	Benzaldehyde + Ethanal
	Citral	6-Methyl-5-Hepten-2-one + Ethanal
	Chalcone	Substituted Aromatics
Carotenoids	Beta-Ionone	Beta-Cyclocitral + 2-Propanone
Botanicals (e.g., Ginger)	N-Shogaol	Zingerone + n-Alkanal

A substantial number of possibilities for water-mediated retro-aldol degradation chemistry to occur during thermal processing are briefly illustrated in Table 2. Although lipids provide the majority of naturally-derived alpha/beta unsaturated carbonyl precursors, essential oils and sugars also contribute as likely sources of these conjugated carbonyls. Overall thermally derived flavors from retro-aldol degradations have been noted in systems such as boiled potato (10), some seafood flavors (5), commercial benzaldehyde manufacture (4,7), and in situations where losses of pungency in ginger occur (8). However, this reaction cascade can be expected to contribute to many other flavor systems.

Table 2. Terminating Retro-Aldol Derived Volatile Flavor from Various Alpha/Beta Unsaturated Carbonyl Generating Precursors



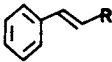
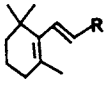


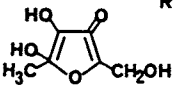
<u>Precursors</u>	<u>Key Functional Group</u>	<u>Terminating Retro-Aldol Derived Carbonyl</u>
n-3 Polyunsaturated Fatty Acid		Propanal
n-6 Polyunsaturated Fatty Acid		Hexanal
Aromatic-Nucleus		Benzaldehyde
Ionone-Nucleus		Beta-Cyclocitral
Aldehyde		Ethanal
Methyl Ketone		2-Propanone
Sugar		Methanal

Figure 2 illustrates the thermal generation of two alpha/beta unsaturated carbonyls which are subsequently susceptible to further flavor deterioration under conditions suitable for water-mediated retro-aldol condensation. In this pathway (E,Z)-2,4-heptadienal and (E,Z)-3,5-octadien-2-one would be generated from autoxidizing linolenic acid, and each would further degrade to (Z)-2-pentenal and then to propanal. Although neither of the conjugated carbonyls are viewed as principle character-impact compounds in foods, in situations where conjugated carbonyls with substantial character-impact are present, deterioration of these compounds would be expected to lead to flavor quality losses.

Overall, this paper provides a basis for water-mediated retro-aldol degradation chemistry contributing to thermally-induced volatile flavors. Although lipids provide the principle starting materials for the generation of alpha/beta unsaturated carbonyls in

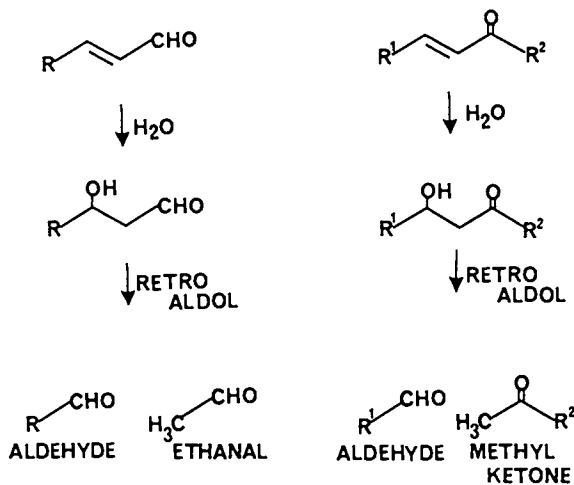


Figure 1. Retro-Aldol Degradation of Alpha/Beta Unsaturated Carbonyls.

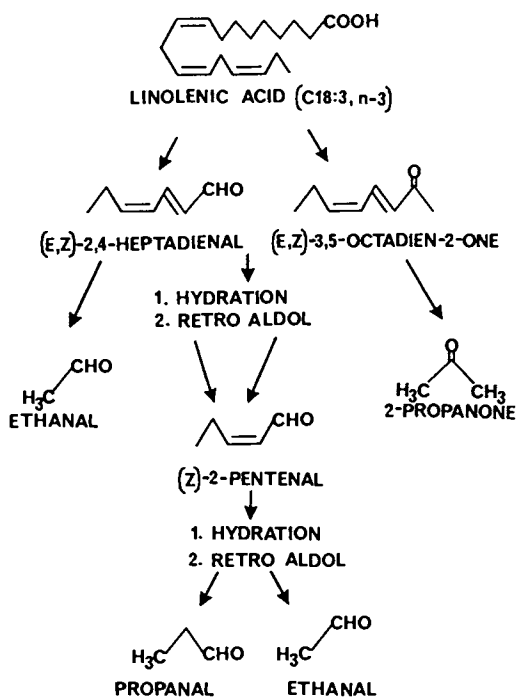


Figure 2. Mechanism for the Formation of 2-Pental, Propanal, 2-Propanone and Ethanal from the Thermal Degradation and Retro-Aldol Condensation of Linolenic Acid.

food systems, essential oils and carbohydrates also provide precursors to this reaction cascade.

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Chapter 23

Volatile Thermal Decomposition Products of β -Carotene

Philip N. Onyewu¹, Henryk Daun, and Chi-Tang Ho

Department of Food Science, New Jersey Agricultural Experiment Station,
Cook College, Rutgers, The State University of New Jersey,
New Brunswick, NJ 08903

The volatile decomposition products of carotenoids formed during the processing of various foods were studied in a model system. Time and temperature parameters were employed to simulate different food processes. β -Carotene in glycerol was heated for 4 hrs. and 1 hr. at 210°C and 155°C, respectively. A continuous stream of nitrogen was maintained through the reaction vessel throughout the heating period. The collected volatiles were analyzed by GC-mass spectrometry. Among the compounds found include β -ionone and its 5,6-epoxide; β -ionol; β -cyclocitral; 3-methylcyclohexane-1,2-dione; 2,6,6-trimethylcyclohexanone; 2,6,6-trimethyl-2-hydroxycyclohexanal; 3-(2,6,6-trimethyl-1-cyclohexenyl)-2-propenal; dihydroactinidiolide; dihydroactinidol; desoxyxanthin; 3-(1,3-butadienyl)-2,4,4-trimethyl-2-cyclohexene-1-one; ionene; 2,6-dimethylnaphthalene; ethyltoluene; 4-hydroxy-2,5-dimethyl-3(2H)-furanone; 2-pentyl-2-nonenal; 2-hexyl-2-decenal; retinal; 5,6,7,7-tetrahydro-4,4,7-trimethyl-2(4H)-benzofuranone.

Carotenoids are one of the most important groups of natural pigments. They are found in a wide variety of foods such as carrots, tomatoes, eggs, seafoods, corn, spinach, berries, mushrooms, oranges, apples, vegetable and fruit oils (1-2). The importance and uniqueness of the pigments lie not only in their function as precursors of vitamin A compounds (3), but also their role in light energy absorption, O₂ transport, protection against photosensitized oxidation, singlet oxygen quenchers, as regulators of plant growth (4-5), as food colorants (6), as colorants for sugar-coated tablets (7) and in their recently found role as potential inhibitors of chemical carcinogenesis (8).

Carotenoid pigments can be extracted from natural sources or synthesized and are used to fortify and color foods. During the

¹Current address: Bristol-Meyers, Evansville, IN 47721

course of food processing, the pigment undergoes changes which influence the color, as well as the nutritive value. Studies have been conducted to determine the thermal degradation products (TDP) of β -carotene--the most important carotenoid. The nonvolatile TDP of carotene have been reported (9-16). Studies involving analysis and identification of TDP of canthaxanthin have been conducted (17). Only five of these studies were conducted in a food system which employed time and temperature parameters that were representative of various food processes; and the nonvolatile TDP were identified (12-14, 16,17). The nonvolatiles have been addressed in previous publications by the authors. Therefore, this report will focus on the volatile TDP.

Many studies have been conducted on the volatile thermal degradation products of carotenoids. Several authors have reported the formation of toluene and xylene as TDP of bixin and capsanthin (18) and β -carotene (10, 11, 18-25), as well as canthaxanthin (26). In addition, 2,6-dimethylnaphthalene was reported to be formed from thermally treated carotenoids (11, 17, 20-29). The formation of ionene as a TDP of β -carotene has been reported (11, 19, 22). The presence of α - and β -ionones have also been reported (24, 25). Among the other reported volatile TDP of β -carotene include β -cyclocitral, 5,6-epoxy- β -ionone and dihydroactinidiolide (25). These compounds were also found by Isoe et al. (30, 31), Wahlberg et al. (32) and Kawakami and Yamanishi (33) as photo-oxygenation products of β -carotene. Volatile thermal degradation of carotenoids has been extensively studied, mainly in nonfood systems. Hence, the objective of this study was to identify the volatile components of the TDP of β -carotene formed in a food model system.

Experimental

A previously developed model system was employed (13, 14, 34). Ten grams of β -carotene (Hoffman LaRoche, Nutley, NJ) and 50 ml. of glycerol were placed in a 3-neck flask which was connected in series with an empty flask and four coiled vacuum traps. The coiled traps were immersed in thermo or dewar flasks filled with dry ice. The 3-neck flask containing β -carotene and glycerol was placed in a high temperature oil bath and heated for 4 hours and 1 hour, respectively, at 210°C and 155°C. A stream of nitrogen was continuously flushed through the reaction flask to sweep the volatiles to the cold traps. After heating, the flask containing the β -carotene nonvolatile degradation products and glycerol was removed from the oil bath and allowed to cool before extraction. The traps, which contained the volatile TDP, were rinsed with diethyl ether then the extracts were transferred to an amber glass vial and stored in a -80°C chamber until they were ready to be analyzed.

GC-Mass Spectrometry. The ether extracts were concentrated using an Oldershaw column with 30 theoretical plates followed by a 200-plate spinning band distillation apparatus (Kontes Glass Co., Vineland, NJ). Two microliters of the respective samples were injected (the solvent was vented) into a 50 m x 0.32 mm OV-1 methyl silicone GC capillary column. The GC was programmed from an initial temperature

of 60°C with 2°C/min. to 225°C. Mass spectra were recorded using a Kratos MS25 double-focusing mass spectrometer. The ionization was at 70 eV and the ion source was operated at a temperature of 200°C.

Results and Discussion

GC-MS Identifications.

Treatment 1: 210°C, 4 hours. While more than sixty compounds were observed, only ten were identified by GC-MS using computer library searches as shown in Table I. The identified compounds include ionene, 2,6-dimethylnaphthalene, ethyltoluene, tetrahydro-dimethylnaphthalene, butadienyl-trimethylcyclohexenone, dihydroactinidiolide, β -cyclocitral, trimethyl-cyclohexenyl-propenal, retinol and tetrahydro-trimethylbenzofuranone.

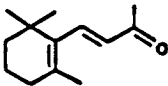
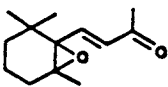
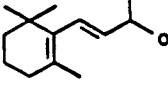
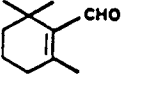
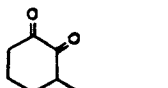
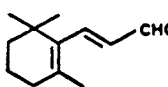
Treatment 2: 210°C, 1 hour. As with treatment 1, many compounds were observed; however, only seven were identified (Table I). These include methylhexanedione, retinol, butadienyl-trimethylcyclohexenone, ethyltoluene, ionene, dimethylnaphthalene and tetrahydrodimethylnaphthalene. Many of the compounds formed during heat Treatment 2 were low molecular weight aromatic hydrocarbons; there were fewer oxygenated volatiles produced than in Treatment 1.

Treatment 3: 155°C, 4 hours. Unlike the preceding experiments, most of the volatiles observed in this treatment were oxygenated compounds. Among them include β -ionone, β -ionone epoxide, β -ionol, dihydroactinidiolide and acetaldehyde (Table I). It is interesting to note that many of the oxygenated compounds observed in this treatment (as well as preceding treatments) were also found in the polar fraction of the nonvolatile TDP from Treatment 3. The relative intensities for the fragment ions from selected compounds observed in the polar fraction of the nonvolatile TDP of Treatment 3 are shown in Table II. The compounds identified are shown in Table III; the predominant compound was dihydroactinidiolide. Others include trimethyl-hydroxycyclohexanone, β -ionone, desoxyanthin, dihydroactinidol and β -cyclocitral.

Treatment 4: 155°C, 1 hour. Compounds identified from this treatment are similar to those from Treatment 3, as shown in Table I. All of the compounds were oxygenated products, namely ionone series compounds and some aliphatic aldehydes.

Mechanism. The mechanism for the formation of the low molecular weight aromatic hydrocarbons, namely ionene and the dimethylnaphthalene compounds can be explained by the scheme of Edmunds and Johnstone (22), advanced by Vetter et al. (35). The mechanism involves cyclization with twelve electron systems followed by rearrangement to a four-ring intermediate, which leads to the formation of dimethylcyclodecapentaene. This leads to the expulsion of ionene and dimethylnaphthalene from the carotene molecule as volatiles and the resulting nonvolatile component has been reported (13).

Table I. Volatile thermal degradation products of β -carotene

Compounds	Heat Treatments			
	1	2	3	4
 β -Ionone	-	-	+	+
 β -Ionone Epoxide	-	-	+	+
 β -Ionol	-	-	+	+
 β -Cyclocitral	+	-	-	-
 3-Methyl-1,2-cyclohexanedione	-	+	-	-
 3-(2,6,8-Trimethyl-1-cyclohexenyl)-2-propenal	+	-	-	-

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Table I. Continued

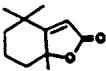
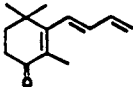

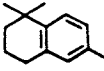
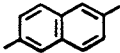

Compounds	Heat Treatments			
	1	2	3	4
5,6,7,7a-Tetrahydro-4,4,7a-trimethyl-2(4H)-benzofuranone	+	-	+	-
 Dihydroactinidiolide	+	-	+	-
 3-[1,3-Butadienyl]-2,4,4-trimethyl-2-cyclohexenone	+	+	-	-
 Ethyltoluene	+	+	-	-
 Ionene	+	+	-	-
 2,6-Dimethylnaphthalene	+	+	-	-
 1,2,3,4-Tetrahydro-1,1-dimethylnaphthalene	+	+	-	-
Acetaldehyde	-	-	+	+
Ethanol	-	-	+	+
4-Hydroxy-2,5-dimethyl-3(2H)-furanone	-	-	-	+
2-Pentyl-2-nonenal	-	-	-	+
2-Hexyl-2-decenal	-	-	-	+
Retinol	+	-	-	-
Retinal	-	+	-	-

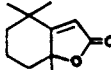


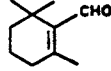
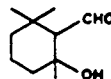
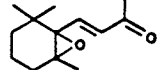
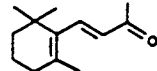
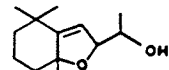
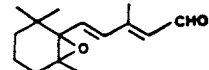
Table II. Characteristic MS Fragment Ions for Selected Compounds in the Polar Fraction of Nonvolatile TDP from Treatment 3 (155°C, 4 hr.)

Compound	Molecular Ion	Fragment Ion	(Relative Abundance %)
1	180	(M ⁺ , 19.7)	111 (100), 137 (35.9), 109 (35.2), 110 (21.9)
2	141	(M ⁺ , 2.9)	82 (100), 69 (28.9), 140 (27.9), 56 (19.7)
3	156	(M ⁺ , 4.1)	71 (100), 95 (71.8), 110 (43.2), 128 (38.9)
4	152	(M ⁺ , 87)	137 (100), 123 (71.6), 109 (70.4)
5	170	(M ⁺ , 2.2)	109 (100), 69 (91.4), 43 (64.3)
6	208	(M ⁺ , 3.8)	123 (100), 121 (57.2), 93 (44.5), 136 (24.3)
7	192	(M ⁺ , 5.7)	123 (100), 177 (91.1), 43 (26), 135 (23)
8	210	(M ⁺ , 1.4)	97 (100), 43 (95), 98 (72), 165 (57), 180 (7.7), 193 (30.3), 109 (69.6), 137 (44.2), 111 (42)
9	234	(M ⁺ , 0.8)	109 (100), 43 (95.3), 123 (56.2), 97 (35.4), 69 (31), 165 (14.1), 217 (3.3)

The formation mechanism of the oxygenated products, namely ionone series compounds and the lactones--dihydroactinidiolide can be said to be very similar to those of Isoe et al. (30), as well as the dioxethane mechanism by Ohloff (36). They proposed that singlet oxygen is involved by direct cyclo-addition to the double bond. Thus, oxygen attack at the terminal 5,6-double bond position, followed by the formation of a peroxy epoxide and cleavage of the C-C and O-O bonds, resulted in 5,6-epoxy- β -ionone, while rearrangement of the 5,6-epoxy derivative, followed by reduction and oxidation, resulted in the formation of dihydroactinidiolide. Furthermore, a peroxy derivative was formed and cleaved to form β -ionone, which then led to the formation of dihydroactinidiolide as a secondary oxidation product.

Flavor Implications. In this study, four experiments were conducted under different time and temperature conditions. The loss of carotene and the generation of volatiles observed in the heat treatments at 210°C were depictive of events occurring during deodorization of edible oils and deep-fat frying of various foods. Nonvolatiles produced during frying already have been reported (13, 14); studies at lower temperature (155°C) would be analogous to the fate of carotene in foods subjected to baking, cooking and frying, especially using crude, red palm oil (37). The formation of oxygen-

Table III. Thermal degradation products of β -carotene from the polar fraction of heat treatment 3 (155 °C, 4 hr)

Compounds #	Compound
1	 Dhydroactinidiolide
2	 2,6,6-Trimethylcyclohexanone
3	 2,6,6-Trimethyl-2-hydroxycyclohexanone
4	 β-Cyclocitral
5	 2,6,6-Trimethyl-2-hydroxycyclohexanal
6	 5,6-Epoxy-β-ionone
7	 β-Ionone
8	 Dhydroactinidol
9	 Desoxyxanthin

ated volatile compounds predominates at the temperatures employed in this study. Most of these have been reported to be important flavor compounds, especially the ionone compounds and dihydroactinidiolide. Many of the ionones and lactones have been observed in cooked corn and alcoholic beverages (24), in tea and tobacco during processing (30-32, 36, 38-39), and in milk (40). These oxygenated compounds were also found in the nonvolatile fraction of β -carotene, some of which have been previously reported (14).

The presence of oxygenated compounds in both the volatile and nonvolatile fractions of TDP of carotene suggests the significance of their contributions to the flavor of foods. Extensive studies on the structural elucidation of dihydroactinidiolide and related compounds have been conducted (35, 41-48). Besides the ionone compounds and dihydroactinidiolide, aldehydes and ketones were also observed. Examination of all the oxygenated products indicates the uniqueness of thermal oxidation of the carotene molecule at the terminal double bond. Because in such a conjugated system, the highest electron density is found in the terminal double bonds of the molecule (49). Thus, as the central double is approached, the electron density depletion becomes progressive. Hence, these reactions requiring high electron density would occur at the terminal double bonds.

Singlet oxygen plays an important role in the formation of the oxygenated products observed in this study; however, the mechanism for generating singlet oxygen under the thermal oxidation of β -carotene is not known at this time. Since many oxygenated volatiles were observed as TDP of β -carotene, the question arises as to the source of oxygen. The experiments were designed to minimize light and oxygen; however, residual oxygen could induce reactions that lead to the formation of oxygenated products under the heating conditions employed. While glycerol is relatively stable at the temperatures employed in this study (155°C, 210°C), it is a good oxygen trapper. Other possible sources of residual oxygen could be from nitrogen used to sweep volatiles to the cold traps, or trace amounts dissolved in β -carotene.

The second class of volatile products observed were hydrocarbons, namely the ionene compounds. The formation of these hydrocarbons during heating is also reflective of deodorization and frying conditions. The formation of low molecular weight aromatic hydrocarbons results from fragmentation of the carotene molecule. The losses of toluene and ionene compounds from β -carotene yield dodecahexaene and octatetraene, respectively. These nonvolatile degradation products have been previously reported in our laboratory (13, 14).

Conclusion

Heat treatment of carotene under conditions which simulated several food processes led to the formation of aldehydes, ketones and low molecular weight aromatic and short-chain oxygenated hydrocarbons, many of which have been reported to be important flavor attributes of some foods, alcoholic beverages and tobacco.

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Chapter 24

Bread Flavor

Peter Schieberle and Werner Grosch

Deutsche Forschungsanstalt für Lebensmittelchemie, Lichtenbergstrasse 4,
D-8046 Garching, Federal Republic of Germany

The composition of the volatile fraction of bread depends on the bread ingredients, the conditions of dough fermentation and the baking process. This fraction contributes significantly to the desirable flavors of the crust and the crumb. For this reason, the volatile fraction of different bread types has been studied by several authors. Within the more than 280 compounds that have been identified in the volatile fraction of wheat bread, only a relative small number are responsible for the different notes in the aroma profiles of the crust and the crumb. These compounds can be considered as character impact compounds. Approaches to find out the relevant aroma compounds in bread flavors using model systems and the odor unit concept are emphasized in this review. A new technique denominated "aroma extract dilution analysis" was developed based on the odor unit concept and GC-effluent sniffing. It allows the assessment of the relative importance of the aroma compounds of an extract. The application of this technique to extracts of the crust of both wheat and rye breads and to the crumb of wheat bread is discussed.

During the last four decades, economic pressures have forced changes in bread manufacture and distribution. Hence, as a consequence of the sensitivity of bread flavor to changes in technological procedures, the bread produced in a modern way is often somewhat deficient in odor and taste compared to bread produced and distributed by traditional methods.

To improve the quality of bread produced by modern methods, it is necessary to understand the chemistry of bread flavor. Therefore many studies have been undertaken to clarify the composition and formation of the flavors of different types of bread (1-5).

Since the last reviews published by Maga (4) and Rothe (5), the methodology of flavor analysis has been further developed. Examples

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are the improvement of the separation of aroma compounds by the use of HPLC as well as the evaluation of odorants within the volatiles of a food by the combination of high resolution gas chromatography with sniffing techniques. The latter technique has focused aroma analysis on the detection and identification of odorants which contribute significantly to the flavor of a food.

The following review is limited to the discussion of those studies which have been carried out to find sensorially relevant volatile compounds.

Addition of Aroma Compounds

One approach to evaluate the contribution of a compound to a particular food flavor is to judge the changes in the overall food flavor after addition of the compound under investigation. Such experiments have been carried out with certain identified bread aroma compounds.

Maltol and isomaltol (1 and 2 in Figure 1) enhanced the fresh bread character of yeast rolls (6) when added at a level of 0.1 %. Other workers (7) showed that 2-acetyl-1,4,5,6-tetrahydropyridine (3 in Figure 1) restores a cracker-like aroma to bread when added at a level of 6 mg/kg. In the pure state this chemical displays a cracker-like aroma and an odor threshold of 1.6 µg/kg (8).

Flavor Unit Concept

A more general approach to estimate the importance of a flavor compound in a particular food is the calculation of the ratio of its concentration to its flavor (odor and taste) threshold (9) or to its odor threshold (10, 11). The result is denoted "aroma value" (9), "odor unit" (10) or "odor value" (11); the higher the value or unit, the more intensely this component contributes to the flavor or odor of the food.

The critical points and the limitations of the flavor unit concept have been discussed by Rothe (12), Frijters (13) and Meilgaard and Peppard (14). However, these authors agreed that the method can help establish the aroma compounds that contribute significantly to the flavor of a food.

Rothe (5, 9, 15) calculated the aroma values of some volatiles identified in the crumb of wheat bread and the crust of rye bread. The data listed in Table I indicate that ethanol, isobutanol, isopentanol, diacetyl and isopentanol contribute with high aroma values to the aroma of the wheat bread crumb. During baking of rye bread, the two Strecker aldehydes, isobutanol and isopentanol, increased so much in the crust that they showed the highest aroma values of the volatiles investigated.

The first comprehensive investigation of the volatiles of wheat bread was carried out by Mulders *et al.* (11, 16-18). After a qualitative analysis (16-18) which led to the identification of 90 compounds, the authors attempted to get an insight into the sensory relevance of the major components which were found in the headspace extract of white bread. A mixture of the main compounds identified was prepared in water to match the gas chromatogram obtained from the bread sample (11). The odor of the synthetic mixture resembled that of the fermented dough but not that of wheat bread.

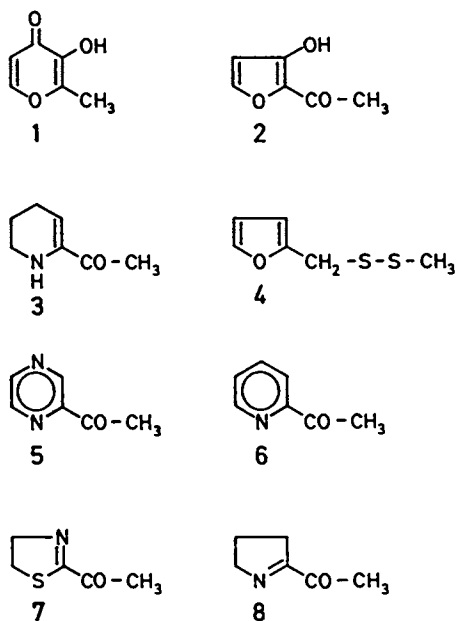


Figure 1. Proposed heterocyclic key compounds of bread flavor

Table I. Aroma Values of Some Volatiles Identified in the Crumb of Wheat Bread and in the Crust of Rye Bread (5, 9, 15)

Compound	Aroma value	
	Crumb of wheat bread	Crust of rye bread
Ethanol	390	110
Isopentanol	47	--
5-Hydroxymethylfurfural	2	60
Isobutanol	1	--
Acetaldehyde	4	25
Propanol	<1	--
Isopentanal	200	1900
Furfural	8	300
Pyruvaldehyde	1	18
Isobutanal	300	6000
Acetone	<1	<1
Acetoin	<1	<1
Diacetyl	50	330

To get an insight into the compounds of the synthetic mixture that cause the fermentation odor, Mulders (11) calculated their odor values. Acetaldehyde showed the highest odor value followed by dimethyl disulfide, ethanol, 2-methylpropanol and 3-methylbutanol.

Since in particular the cracker-like crust odor note was lacking in the synthetic mixture, it was concluded that the character impact compound for this odor note occurs in a concentration in the bread too low to be detected by headspace analysis. Later on the basis of its aroma quality and its very low odor threshold of 0.04 ppb (water), Mulders *et al.* (19) proposed 2-[(methylthio)methyl] furan (4 in Figure 1) as the compound which should be responsible for the "golden brown" crust aroma of white bread.

Sizer *et al.* (20) observed that the compounds causing the pleasant odor which resembled bread crust occur in the basic volatile fraction of white bread. Identification experiments yielded the five pyrazines listed in Table II. A comparison of the odor threshold of each pyrazine in water to its concentration found in bread indicated that 2-ethyl-3-methylpyrazine and 2-methyl-6-propylpyrazine were present at concentrations above their odor thresholds. The authors described the odors of these two pyrazines as "butterscotch, nutty" and "burnt, butterscotch" notes (Table II).

GC-Effluent Sniffing

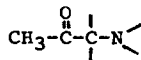
Sensory examination of the effluent from the column of a gas chromatograph by "nasal appraisal" has been introduced into flavor analysis by Fuller *et al.* (22). Since that time this technique known as "GC-effluent sniffing" has been used in aroma analysis to locate the positions of odorants in a gas chromatogram. It was first

Table II. Concentrations and Sensory Characteristics of the Pyrazines found by Sizer et al. (20) in Wheat Bread

No. Compound	Concentration in bread (mg/kg)	Odor	
		Threshold ^{a)} (mg/kg)	Description
1 Methylpyrazine	6.0	60	Burnt, roasted
2 Ethylpyrazine	0.21	6	Buttery, rum
3 2,3-Dimethylpyrazine	0.03	2.5	New leather, linseed oil
4 2-Ethyl-3-methyl- pyrazine	0.29	0.13	Butterscotch, nutty
5 2-Methyl-6-propyl- pyrazine	0.26	0.1	Burnt, butterscotch

a) Threshold in water according to Guadagni et al. (21).

applied in bread aroma analysis for the identification of 2-acetyl-1,4,5,6-tetrahydropyridine (7). In a more extensive study, Folkes and Gramshaw (23, 24) used this method to analyse the neutral volatiles which had been isolated by extraction and vacuum distillation from the crust of white bread. They found twelve GC-fractions associated with strong bread-like odor notes and speculated that heterocyclics with the structural feature,



where the nitrogen atom and the adjacent carbon atom form part of the ring structure, exhibit biscuit- or cracker-like odors. Based on this hypothesis, it was expected that acetylpyrazine, 2-acetylpyridine and 2-acetyl-2-thiazoline (5, 6 and 7 in Figure 1) contributed to the crust aroma. The authors identified components 6 and 7 in addition to 168 other compounds. Sensory significance of these compounds to the bread crust flavor was not determined.

Key Compounds of the Bread Crust Odor

GC-effluent sniffing of wheat bread aroma concentrates has shown the presence of low level volatiles that smell like the fresh bread crust. As discussed in the preceding sections, these compounds (3-7 in Figure 1) were proposed to be responsible for this odor note.

The techniques used for the separation of volatiles have been further improved. HPLC and high resolution gas chromatography (HRGC), the latter in combination with effluent sniffing, have been introduced into aroma analysis of bread (25).

The HRGC chromatogram of the neutral/basic volatiles obtained from wheat bread crust showed a region with an intense cracker-like,

sweet odor (25). The corresponding compound was enriched by silica gel chromatography and HPLC to yield a small peak, which had a strong crust-like, sweet odor during effluent sniffing. Its mass spectrum agreed with that reported by Buttery *et al.* (26) for 2-acetyl-1-pyrroline (8 in Figure 1) found in a special rice variety (27).

The low odor threshold (0.1 µg/kg; water) of this compound and its odor description as "popcorn-like" (27) agrees with its strong crusty character. Furthermore, the statement of Buttery *et al.* (27) that "2-acetyl-1-pyrroline seems to be the most potent of the cracker-like group of odor compounds" (which includes 3, 5 and 7 in Figure 1) underlines its importance for the flavor of the white bread crust.

Aroma Extract Dilution Analysis

The drawback of GC-effluent sniffing is that it does not allow a differentiation between those odor compounds that contribute intensely to a flavor of a food and those which are only components of the background flavor.

The important odor compounds can be evaluated by the GC-effluent sniffing of a series of dilutions from the original aroma extract. Two variations of this technique were developed by Acree *et al.* (28, 29) and by us (30-37).

Acree *et al.* (28, 29) used a video-terminal in addition to the gas chromatograph. They calculated CHARM-values on the basis of the duration of the sensory responses which were maintained during the GC-effluent sniffing of three-fold dilutions of the original extract. CHARM-values are directly proportional to odor units.

We used a simpler method for the determination of the relative odor importance of the volatile compounds (30-37). The aroma extract is stepwise diluted with a solvent until no odor-active region is detected in the GC-effluent. The highest dilution at which a substance is still smelled is its flavor dilution factor (FD-factor). The undiluted sample has then, by definition, an FD-factor of one. For example, an FD-factor of 20 for a substance means that one volume of the initial concentrate was diluted by 19 volumes of the solvent, and that this was the highest dilution at which the substance was still smelled during the gas chromatographic procedure. The FD-factor of a flavor compound is proportional to its odor unit and the flavor compounds occurring in an aroma extract are ranked according to their odor units. This new technique which was named "aroma extract dilution analysis" is less time-consuming than the calculation of odor units from concentration and odor threshold data. The determination of FD-factors indicates the potent odor compounds which should be identified, while the knowledge of the chemical structures of the volatiles is a prerequisite for the calculation of their odor units.

Important Odorants of Wheat and Rye Bread Crusts

The aroma extract dilution analysis of concentrates prepared from the crusts of wheat and rye breads revealed forty-three odorants in rye and thirty-two in wheat extracts (37).

In the case of wheat bread, 2-acetyl-1-pyrroline appeared with the highest FD-factor, followed by 2(E)-nonenal, 3-methylbutanal, diacetyl and 2(Z)-nonenal. These results confirm that the 2-acetyl-1-pyrroline is the "character impact compound" of the wheat bread crust odor.

Compounds 3, 5 and 6 shown in Figure 1 were detected in the aroma extracts of the wheat and the rye bread crusts, but on the basis of their relatively low FD-factors we concluded these compounds contribute only to the background flavors of both bread types. Furthermore, there was no indication that the sulfur-containing heterocyclics 2-[(methylthio)methyl]furan and 2-acetyl-2-thiazoline (4 and 7 in Figure 1) were of significance to the flavor of the wheat bread crust.

In comparison to the aroma of the wheat bread crust, that of the rye bread crust was more complex (37). Four aroma compounds appeared with the highest FD-factors: 3-methylbutanal, 2,6-dimethyl-3-ethylpyrazine, 2(E)-nonenal as well as methional (Schieberle, P.; Grosch, W.; unpublished results) with a sweet, potato-like aroma note. In addition, diacetyl, 1-octen-3-one, phenylacetaldehyde, 2(Z)-nonenal and 2-ethyl-3-methylpyrazine contributed significantly to the rye bread crust flavor. This indicates that compounds with malty, green, tallowy, sweet and potato-like odors are most significant in the aroma concentrate of the rye bread crust. Odorants showing roasty notes, among which 2-ethyl-3-methylpyrazine was the most important, appeared with lower FD-factors. 2-Acetyl-1-pyrroline was also found in the rye bread crust but its FD-factor was 32 times lower than in the wheat bread crust.

It should be mentioned that the quantitative results are only an approximation of the real values, since the FD-factors of the aroma compounds were not corrected for differences in the yields obtained by the isolation procedure (38).

Quantitative Analysis

If more exact data are desired, the results obtained by aroma extract dilution analysis must be complemented by quantitative measurements. Quantification of odorants is a difficult task, since the concentration of the odorants showing high FD-factors can be extraordinarily low.

The use of an isotope dilution assay is the best method to quantify labile and low level odorants. We applied this technique to the determination of 2-acetyl-1-pyrroline and 2-methyl-3-ethylpyrazine, the two compounds which showed the highest FD-factors among the compounds with roasty odor notes in extracts from wheat or rye bread crust, respectively (37, 38). The results are summarized in Table III. The high level of the acetylpyrroline in the crusts of the wheat breads was striking compared to the level in the rye breads. These quantitative data confirm that 2-acetyl-1-pyrroline is a character impact odor compound of the wheat bread crust.

A comparison of the wheat breads showed that the pyrazine but not the acetylpyrroline increased when the bread was prepared by addition of a baking helper (Table III).

Two types of rye breads investigated differed in the odor intensity. As expected the odor of the bread prepared by a one-stage

Table III. Concentration ($\mu\text{g}/\text{kg}$) of the 2-Acetyl-1-pyrroline and 2-Methyl-3-ethylpyrazine in the Crusts of Wheat and Rye Breads

Compound	Wheat bread ^{a)}		Rye bread ^{b)}	
	W 1	W 2	R 1	R 2
2-Acetyl-1-pyrroline	78	72	1	4
2-Methyl-3-ethylpyrazine	66	75	41	94

a) Wheat bread prepared without (W 1) and with (W 2) a baking helper.

b) One-stage (R 1) and three-stage (R 2) sourdough process.

SOURCE: Data are from ref. 38.

sourdough process was weaker than that obtained by a three-stage process. The data shown in Table III reflect this difference.

Odorants of Wheat Bread Crumb

The aroma extract dilution analysis was applied to extracts obtained from the crumb of wheat bread. Twenty nine odorants were detected and the flavor compounds responsible for the odor notes identified (Schieberle, P.; Grosch, W. in preparation). The 12 aroma compounds having the highest FD-factors are presented in Table IV. In particular, 2(E),4(E)-decadienal and 2(E)-nonenal, which coeluted with 2(E),6(Z)-nonadienal on the capillary column OV-1701 were the most important flavor compounds in the crumb extracts, followed by 2(Z)-nonenal and diacetyl.

A comparison of the most important aroma compounds present in the wheat bread crust (37) with those identified in the crumb (Table IV) revealed two striking differences: 2-acetyl-1-pyrroline and 3-methylbutanal, which appeared as potent flavor compounds respectively responsible for the roasty and malty aroma note in wheat bread crust, showed low FD-factors in the wheat bread crumb and are not listed in Table IV. On the other hand, carbonyl compounds with fatty aroma notes like 2(E),4(E)-decadienal, 2(E)-nonenal and 2(Z)-nonenal predominated in the crumb (Table IV).

In the case of 2-acetyl-1-pyrroline, a quantitative determination established the difference between the crust and the crumb which was found by the aroma extract dilution analyses; only 2.5 $\mu\text{g}/\text{kg}$ 2-acetyl-1-pyrroline were present in the crumb compared to 78 $\mu\text{g}/\text{kg}$ in the crust (Schieberle, P.; Grosch, W.; in preparation).

Conclusion

The review shows that the improvement of the methodology of flavor analysis has led to a systematic evaluation of the volatile neutral and basic key compounds of the flavors of wheat and rye breads. The

Table IV. Important Odorants (FD \geq 32) of Wheat Bread Crumb; Results of an Aroma Extract Dilution Analysis and Identification Experiments (Schieberle, P; Grosch, W. in preparation)

No.	Compound	RI ^{a)}	Odor quality ^{b)}	FD-factor
1	2(E)-Nonenal	1258	green, tallowy	} 512
	2(E),6(Z)-Nonadienal	1260	cucumber-like	
2	2(E),4(E)-Decadienal	1434	fatty, waxy	512
3	2(Z)-Nonenal	1241	green, fatty	128
4	Diacetyl	672	buttery	128
5	Methional ^{c)}	1037	sweet, potato-like	64
6	1-Octen-3-one	1058	mushroom-like	64
7	2(E),4(E)-Nonadienal	1339	fatty, waxy	64
8	unknown	1279	flowery	64
9	unknown	1535	green, metallic	64
10	Phenylacetaldehyde	1156	sweet, honey-like	32
11	unknown	1181	sweet	32
12	γ -Nonalactone ^{c)}	1559	coconut-like	32

a) Retention index of the odorant by HRGC on capillary OV-1701.

b) Description of the odors recognized during GC-effluent sniffing of the crumb extract.

c) The structure was not established by MS.

chemical structure of the majority of these compounds have been identified.

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Chapter 25

Formation of 2-Acetyl-1-pyrroline and Other Important Flavor Compounds in Wheat Bread Crust

Peter Schieberle

Deutsche Forschungsanstalt für Lebensmittelchemie, Lichtenbergstrasse 4,
D-8046 Garching, Federal Republic of Germany

We showed that 2-acetyl-1-pyrroline is an impact compound in the crust of a "baguette"-type wheat bread. To gain an insight into its formation during baking, the content of the 2-acetyl-1-pyrroline was quantified in model experiments and in breads prepared by different dough formulas (e.g., addition of amino acids or omission of yeast) using a stable isotope dilution assay. The results revealed that heat treatment of a ground yeast/sucrose mixture is an important source of the 2-acetyl-1-pyrroline formed during the baking process. Furthermore, model experiments suggested that the reaction between 1-pyrroline, the Strecker degradation product of proline, and pyruvaldehyde is responsible for the formation of 2-acetyl-1-pyrroline during bread crust formation.

In addition, the influence of baker's yeast on the composition of other crust flavor compounds was followed by an aroma extract dilution analysis.

Shortened fermentation or baking steps during bread making often result in bread of poor flavor quality. To improve the flavor of bread produced by modern technology it is necessary to gain information about precursors and reaction routes leading to important bread flavor compounds.

It is generally accepted (1) that volatile compounds present in the flour are of minor importance to the aroma of bread. Prerequisites for formation of the desired crust flavor compounds are the dough fermentation and, especially, the baking steps (2, 3).

We recently identified 2-acetyl-1-pyrroline (Acp) with a cracker-like odor as the most intense flavor compound of wheat bread crust (4). Tressl *et al.* (5) reported that small amounts of this compound were formed when model mixtures containing proline and monosaccharides were heated.

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To gain an insight into the origin of the Acp in wheat bread crust, two different experiments were carried out. First, the formation of crust odorants from yeast and from chemically leavened doughs was compared and, secondly, model experiments on the formation of the Acp were undertaken.

Comparison of Crust Flavor Compounds from Yeast and Chemically Leavened Wheat Flour Doughs

A model wheat bread was prepared by a straight dough procedure from wheat flour, salt and water using glucono- δ -lactone as leavening agent (Schieberle, P., in preparation). The bread crust showed an odor note reminiscent of day-old bread and, specifically the cracker-like, roast odor note was lacking.

Crust volatiles were isolated immediately after baking by extraction with dichloromethane and sublimation in *vacuo* (4). Application of aroma extract dilution analysis (6) to the acid-free crust extract led to the detection of 31 odorants. After separation and enrichment, these compounds were identified by comparison of the MS/EI, MS/CI and retention data on two columns of different polarity to reference compounds. Aroma quality was also assessed. The results of the identification experiments (Table I) revealed that 2(E)-nonenal (No. 1), followed by 2(E),4(E)-decadienal (No. 2) and 3-methylbutanal (No. 3) showed the highest FD-factors in the crust of the chemically leavened bread. Additionally 1-octen-3-one, 2(Z)-nonenal, 2(E),4(E)-nonadienal and an unknown compound with a metallic odor contributed high FD-factors to the overall flavor (For a discussion of FD-factors, see Chapter by Schieberle and Grosch, this book).

The flavor compounds of the crust from the chemically leavened model bread were then compared to those recently identified (6) in the crust of a standard wheat bread which was leavened by addition of yeast (Table I). One striking difference was that Acp (No. 16), which showed the highest FD-factor in the yeast-leavened bread showed a very low FD-factor in the chemically leavened bread. This indicated, that the flour contained only minor amounts of the precursor(s) for the formation of Acp. On the other hand, 2(E),4(E)-decadienal, 2(E),4(E)-nonadienal, 1-octen-3-one and 2(Z)-nonenal, which are undoubtedly formed by a heat-induced oxidative degradation of the flour lipids, became predominant odorants in the chemically leavened compared to the yeast-leavened bread.

Labeling Experiments

Recent studies (8) using various amino acid and sugar model mixtures showed that Acp is formed only from proline when heated in the presence of different sugars. To gain a more detailed insight into the formation mechanism of the Acp, two labeling experiments were performed (Table II). In the first, proline in which the carbonyl group was labeled with carbon-13 (1-¹³C-proline) was reacted with unlabeled glucose. In a second experiment, unlabeled proline was heated in the presence of unique carbon-13 labeled glucose (U-¹³C-glucose). The carbon isotope distribution of the Acp formed was determined by MS/CI of extracts obtained by simultaneous distillation/extraction of the reaction mixtures suspended in water.

Table I. Comparison of the Important Neutral/Basic Volatile Crust Flavor Compounds of a Chemically Leavened Wheat Bread (CL-WB)^a With Those of a Yeast-Leavened Wheat Bread (YL-WB)^b

No.	Compound	Odor Description	RI ^c)	FD-factor	
				CL-WB	YL-WB
1	2(E)-Nonenal	green, tallowy	1508	512	256
2	2(E),4(E)-Decadienal	fatty, waxy	1778	256	32
3	3-Methylbutanal	malty	915	256	128
4	1-Octen-3-one	mushroom-like	1281	128	32
5	2(Z)-Nonenal	green, fatty	1480	128	64
6	2(E),4(E)-Nonadienal	fatty, waxy	1669	128	16
7	Unknown	metallic	n.d.	128	<1
8	Diacetyl	buttery	968	64	64
9	2(E)-Octenal	fatty, nutty	1393	64	4
10	Unknown	boiled apple	1786	64	16
11	Hexanal	green	1064	32	8
12	4(Z)-Heptenal	biscuit-like	1214	32	32
13	1,5(Z)-Octadien-3-one ^d)	green, geranium-like	1353	32	64
14	2(E),6(Z)-Nonadienal	cucumber-like	1557	32	32
15	Phenylacetaldehyde	honey-like	1600	32	32
16	2-Acetyl-1-pyrroline	roasty	1299	4	512

- a) A dough prepared from 500 g wheat flour (type 550), 12 g glucono- δ -lactone, 10 g salt and ca. 270 ml of tap water was baked for 30 min at 220°C.
- b) The dough was prepared according to (4).
- c) The retention index (RI) was determined on a 30 m x 0.32 mm fused silica column (Supelcowax 10; Supelchem; Germany).
- d) Structure not established by MS.

The isotope distribution in the Acp formed from labeled 1-¹³C-proline (A, Table II) agreed with the data for synthetic Acp (C, Table II). Because no upward shift of the M+1-ion (m/z 112 \rightarrow m/z 113) was observed, it can be concluded that the carbon atom of the carboxyl group from proline is absent in the Acp formed.

In contrast, when the Acp is formed in the presence of U-¹³C-glucose (B, Table II) the M+1-ion of the major isotope (m/z 114) shows a shift of 2 compared to the major isotope of the synthetic Acp (m/z 112). This indicates that two carbon atoms in the Acp originate from glucose. In the MS/EI of the Acp formed from U-¹³C-glucose (data not shown) the fragment of the acetyl group (m/z 45) appeared two masses higher than in the synthetic Acp (m/z 43) indicating that both carbon atoms are derived from glucose.

The results clearly indicate that the formation of Acp in thermally degraded proline and glucose mixtures proceeds via decarboxy-

Table II. Carbon Isotope Ratio in the Acp Formed from 1-¹³C-Proline/Unlabeled Glucose (A) or Unlabeled Proline/U-¹³C-Glucose (B)^{a)}

m/z	Isotope Distribution (%) ^{b)}		
	A	B	C (synthetic Acp)
112 ^{c)}	91.9	0.8	92.4
113	6.9	3.6	6.2
114	1.2	74.8	1.4
115	<0.4	20.1	<0.4
116	<0.4	1.5	<0.4

- a) 4 mM of proline, 4 mM of glucose and 3 g of silica gel (10 % H₂O) were heated for 30 min at 170°C.
 b) Values were determined by mass chromatography (MS/CI).
 c) m/z 112 is the M⁺+1-ion of unlabeled 2-acetyl-1-pyrroline.

lation of proline, rearrangement of the pyrrolidine ring to a pyrroline ring and addition of a two-carbon fragment from the sugar.

The Role of Free Proline in Wheat Dough

Additional experiments should indicate the role of free proline for the formation of the Acp in wheat dough.

A wheat bread was prepared from a dough in which the free proline content, which was determined by amino acid analysis, increased during fermentation from 12 mg/kg (in the flour) to 32 mg/kg (in the dough) (8). Analysis of this bread showed the production of 34 µg/kg Acp. Addition of proline to the dough at levels of 120, 200, 500, 2000, 10000 mg/kg flour enhanced somewhat the formation of Acp but the increase ranged only between 4.2 and 6.4 µg per 100 mg proline added. This relatively small increase in Acp, which was found to be dependent on the amount of proline added, suggests that the reaction conditions for the formation of Acp were not significantly altered by the increase in proline concentration. Obviously, a relatively small but nearly constant fraction of the proline added was always converted into Acp during the baking process.

In the original dough 32 mg of free proline resulted in the formation of 34 µg of Acp. There were no indications that the reaction route was changed by the addition of proline. The small increase in Acp which was observed after addition of proline, suggests that during baking of the original dough (no additives) another more effective way exists to form Acp than the reaction between proline and sucrose or glucose initiated by high temperatures in the crust.

Yeast as a Source of the 2-Acetyl-1-Pyrroline

To reveal the contribution of yeast in the formation of the Acp the amount of Acp in a bread crust from yeast-fermented dough was compared to the amount present in the crust of a chemically leavened bread. The production of Acp was reduced from 34 $\mu\text{g}/\text{kg}$ to 9.6 $\mu\text{g}/\text{kg}$ flour when the yeast was replaced by a commercial leavening agent.

Yeast as a source of precursors in the formation of the Acp was then studied in model experiments summarized in Table III. Only low concentrations of the Acp were present (No. 1) in an extract of yeast cells which were ground with silica gel. The amount was slightly enhanced (No. 2) when the yeast/silica gel mixture was boiled for 2 hours.

Higher amounts of Acp were formed in the presence of sucrose (No. 3). A further increase in the sucrose level led to an increase in the Acp formed (No. 4 and 5), but this increase was not proportional to the amount of sucrose added. Replacement of sucrose by fructose

Table III. Formation of 2-Acetyl-1-Pyrroline from Ground Baker's Yeast Cells and Sugar Mixtures

No.	Additive to 30 g of yeast and 23 g of silica gel ^{a)}	2-acetyl-1-pyrroline ^{b)} (μg)
1	none	<0.1 ^{c)}
2	none ^{d)}	1.5
3	1 g Sucrose	6.3
4	10 g Sucrose	10.3
5	30 g Sucrose	20.3
6	10 g Fructose	26.9
7	10 g Glucose	5.1
8	10 g Sucrose ^{e)}	0.6
9	10 g Sorbitol	1.2
10	10 g Fructose ^{f)}	1.4

a) The mixture was ground in a mortar for 10 min.

b) The compound was determined by an isotope dilution assay (7) in an extract which was obtained by simultaneous distillation/extraction of the reaction mixture for 2 h.

c) The compound was determined by an isotope dilution assay in an extract obtained by extraction of the reaction mixture with diethyl ether and sublimation in vacuo (4).

d) Boil 2 hours.

e) The grinding procedure was omitted.

f) 10 g of ammonium sulfate were added for protein precipitation.

enhanced the Acp production (No. 6), whereas glucose had the opposite effect (No. 7).

Omission of the grinding procedure significantly lowered the amount of Acp formed (compare No. 4 to No. 8). The same result was obtained when either the yeast protein was precipitated by addition of ammonium sulfate (compare No. 6 to No. 10) or when the sugar was replaced by the sugar alcohol sorbitol (No. 9). These results suggest that glycolytic enzymes liberated by the grinding process are involved in the formation of precursors for Acp.

To localize the precursors of Acp, the low molecular weight compounds present in yeast cells were isolated by cell disruption, centrifugation and ultrafiltration (Schieberle, P., in preparation). Boiling and continous extraction of a phosphate buffer solution containing the compounds of a molecular weight lower than 1000 produced substantial amounts of Acp. Furthermore, the free proline content of the yeast used in these experiments was analysed and calculated to be more than 200 mg/kg yeast.

Model System Studies on Formation of 2-Acetyl-1-Pyrroline

To test whether Acp was formed at such proline concentrations, dilute aqueous model solutions of proline were boiled in the presence of various sugars or phosphorylated compounds known to be metabolites of carbohydrate degradation in yeast (9). The results, summarized in Table IV showed that neither heating of proline alone (No. 1) nor in the presence of glucose, fructose or sucrose (No. 2-4) resulted in Acp formation after boiling. On the other hand, heating of a proline and dihydroxyacetone phosphate mixture (No. 5) yielded significant amounts of Acp. In the presence of 3-phosphoglyceraldehyde (No. 6) low amounts of Acp were formed, while phosphoenolpyruvate (No. 7), glycerophosphate (No. 8) and fructose 1,6-diphosphate (No. 9) were inactive.

The formation of the Acp after cleavage of fructose 1,6-diphosphate with aldolase (No. 10) corroborates the suggestion that distinct metabolites of the glycolytic pathway are precursors of Acp.

Heating of dihydroxyacetone in the presence of phosphate ions is known to produce substantial amounts of pyruvaldehyde (10). Furthermore pyruvaldehyde is assumed to catalyze the formation of 1-pyrroline by Strecker degradation of proline (11). To study the role of 1-pyrroline and pyruvaldehyde in Acp formation, three additional experiments were conducted.

Proline (2 mM) and dihydroxyacetone (DHA; 1 mM) were combined in 100 ml of 0.1 mol/l phosphate buffer (pH 7.0) and boiled at back-flush for 2 hours. The volatiles were isolated by ether extraction and Acp was found to constitute only 0.1 % of the volatile fraction.

A similar experiment was conducted wherein pyruvaldehyde (0.1 mM) replaced the DHA. In this case the Acp increased slightly to 0.3 % of the volatile fraction.

In the third experiment, 1-pyrroline (2 mM) and pyruvaldehyde (0.1 mM) were combined and reacted. A very significant increase in Acp was observed; it represented 72 % of the volatile fraction and amounted to 1140 µg.

Table IV. Formation of 2-Acetyl-1-Pyrroline (Acp) by Reaction of Proline and Sugars or Phosphorylated Sugar Degradation Products in Dilute Aqueous Solution^{a)}

No.	Additive (2 mM)	Acp ^{b)} (μ g)
1	none	<0.1
2	Glucose	<0.1
3	Fructose	<0.1
4	Sucrose	<0.1
5	Dihydroxyacetone phosphate ^{b)}	13.6
6	3-Phosphoglyceraldehyde	1.0
7	Phosphoenolpyruvate ^{c)}	0.3
8	D,L- α -glycerophosphate ^{c)}	<0.1
9	Fructose 1,6-diphosphate ^{c)}	<0.1
10	Fructose 1,6-diphosphate ^{c)d)}	11.2

- a) A mixture of 2 mM (230 mg) of proline and 2 mM of various sugar or phosphate ester mixtures dissolved in 400 ml of distilled water was continuously steam distilled and extracted according to (7).
- b) The Acp was determined by a stable isotope dilution assay.
- c) The compounds were used as their sodium or potassium salts.
- d) 4 mM of fructose 1,6-diphosphate were incubated for 20 min at 25°C with 200 units of aldolase prior to heating.

Conclusions

The results reveal that baker's yeast is a potent source for precursors of 2-acetyl-1-pyrroline. It appears likely that the flavor compound is formed in the yeast cells from proline and dihydroxyacetone phosphate via 1-pyrroline and pyruvaldehyde. This is corroborated by the results of ¹³C-labeling experiments which showed that the acetyl group in the Acp stems from a sugar degradation product and that the pyrroline ring was derived from proline.

Furthermore, the data presented indicate that a thermally induced rupture of the yeast cells during crust formation is an important step to liberate precursors of the Acp or to liberate the Acp already formed in the interior of the yeast. This would also provide an explanation for the fact that much higher amounts of Acp are present in wheat bread crust compared to the crumb (see chapter by Schieberle and Grosch, this book).

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Chapter 26

Aroma Chemistry of Crackers

L. F. M. Yong¹, T. E. Acree², E. H. Lavin², and R. M. Butts²

¹Department of Biochemistry, Faculty of Medicine, National University of Singapore, Republic of Singapore

²New York State Experiment Station, Cornell University, Geneva, NY 14456

Crackers are thin low moisture biscuits consumed in many parts of the world. They are industrial products made from wheat flour, fat, water, leavening and sugar. The aroma of crackers is derived from the chemical interaction of these ingredients during leavening and baking. A problem with the study of cracker aroma or any fat-containing baked goods is separation of aromatic volatiles from a heterogeneous mixture of polar and lipid components. However, once the aroma volatiles have been extracted into an appropriate solvent for gas chromatography then the problem of characterizing the odor active components is fairly routine. This paper will review the present knowledge of the flavor chemistry of crackers and outline steps appropriate for the complete characterization of cracker aroma.

Crackers are generally subdivided into three basic categories: saltines or soda crackers (also known as cream crackers in the United Kingdom), sprayed crackers, and savory crackers (Hoseney, R.C.; Wade, P. & Findley, J.W. 1988 "Soft wheat products" in press). They are a class of baked product with a unique flavor and texture. Crackers are usually made of wheat flower, water, fat, yeast and salt by a process that combines fermentation, baking and dehydration to yield a thin low moisture product. Saltines are the simplest cracker with a typical "cracker-like" aroma. This paper reviews the basic flavor chemistry of saltine crackers and presents preliminary data on the extraction of volatile compounds from these crackers.

INGREDIENTS

Although saltine cracker formulas have never been standardized they are very similar. Martz (1) summarized six published formulae for soda crackers showing the average range for each ingredient used. A typical formula for the production of saltine crackers is shown in Table 1 (Hoseney, *et al.*, 1988). The uniqueness of crackers is due to the use of a two dough system one of which is called a sponge.

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Table 1: Cracker formula described by Hosene, Wade & Finley 1988. Ingredients based on weight of flour with 14 % moisture

Ingredient	Sponge (%)	Dough(%)
Flour	67	76
Water	27	2
Yeast	0.02	-
Shortening	4	15
Salt	-	4
Soda	-	2

While several reviews on the role of ingredients are available, none of these describe the flavor chemistry of cracker sponge and dough (2, 3, 4, 5). However, some insight into the chemistry of cracker aroma can be obtained from examination of products that have a "cracker-like aroma" such as white bread crust.

MANUFACTURING PROCESS

Conventionally, crackers are prepared by a sponge and dough process that takes approximately twenty-four hours. Flour, water and salt are combined with yeast and allowed to ferment to yield a sponge (also called sour dough).. More flour, water and salt are combined with the sponge to yield a dough that is leavened, formed and baked into a cracker. A prolonged sponge fermentation is thought to be required to bring about modification of flour gluten and the changes that give saltine crackers their special textural and flavor properties (2, 6). Leavening can be accomplished by chemical means but the yeast fermentation produces a far better product. Though the cracker sponge fermentation is important, it is also a process that requires about eighteen hours, or approximately seventy-five percent of cracker production time.

Most yeast-leavened crackers are based on the sponge-and-dough fermentation process outlined in Figure 1.

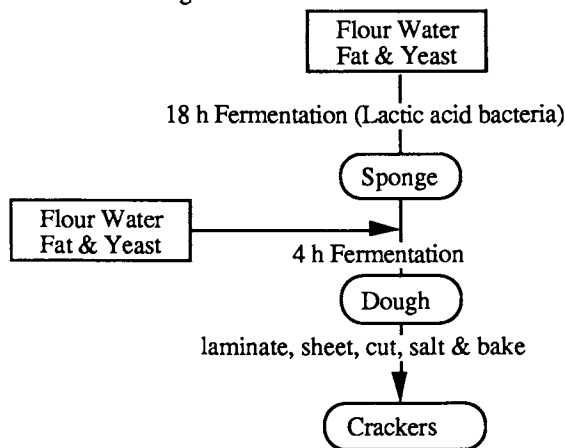


Figure 1. Flow diagram for the typical soda cracker process.

Johnson and Bailey (7), in a fundamental study of crackers involving investigation of the physical and chemical changes in dough during fermentation, had questioned the necessity of prolonged sponge fermentation in order to degrade gluten proteins and saturate the dough with carbon dioxide. As appreciable dry matter is lost during fermentation, they suggested that other, less wasteful, means might cause the same changes that lengthy fermentation accomplishes, and proposed that further research be directed toward a procedure to degrade flour proteins with suitable proteolytic enzymes. To a limited extent, proteolytic enzymes already are used to improve cracker quality and flavor.

Fermentation brings about many changes responsible for the unique aroma and other eating properties that characterize commercial saltine crackers. Precise levels of flakiness, crispness, tenderness, bloom, spring, and flavor are required in a quality saltine cracker. It is generally believed that the prolonged sponge fermentation is required to produce quality crackers despite the material losses, additional equipment, space, time and labor costs. This was somewhat contradicted by Wade (8) in his work on the role of fermentation in the manufacture of saltine crackers. Although he did find that doughs with short fermentation times produced little aroma after the crackers had been cooled, packed, and stored for twenty four hours taste panels were unable to distinguish between those made from doughs with short fermentations and control doughs made by a process that included a long fermentation.

It has long been desired to eliminate or, at least, substantially reduce such a long fermentation period without losing the rich flavor and good physical properties of the final product (9, 10). Micka (11) reported that efforts to shorten the fermentation time resulted in products with atypical flavor. His attempts to produce crackers using a higher percentage of yeast and shorter sponge time have not been successful. The finished product was different in texture and flavor from those made by the traditional procedure.

MICROBIOLOGY OF CRACKER PRODUCTION

Sugihara (12, 13) studied the involvement of bacterial fermentation in cracker production and concluded that bacteria play a vital role in the eighteen hour sponges used in the manufacture of soda crackers. In fact the lactobacillus population at the end of the fermentation was greater than the yeast population. This explains the observation by Faridi & Johnson (10) that the major acid present in the dough was lactic acid. However, the precise role these acids and bacteria play in the development of cracker flavor is still not clear. Pizzinatto and Hosenev (14) have demonstrated that yeast is an essential ingredient in any cracker formula. Yeasts not only adjust pH to allow enzymatic conditioning of the dough but also improve both texture and flavor of the finished crackers.

CRACKER FLAVOR

Literature on the cause and control of the flavor of crackers is scant. Faridi and Johnson (10) studied the chemical changes related to flavor precursor components (organic and amino acids) during the twenty-five hour fermentation period. They measured the total soluble nitrogen, peptides, primary amines, and ammonia formed in cracker doughs as a result of the lengthy fermentation. All components increased

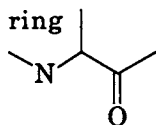
significantly with a twenty hour sponge fermentation. Lactic acid was predominantly produced, followed by acetic and smaller amounts of propionic, butyric, valeric, and isovaleric acids. This is consistent with the observation that lactic acid bacteria are active in the process (12,13)

Faridi and Johnson (15) have reported on a cracker flavor enhancer mixture to be used to produce a saltine cracker with a two-hour fermentation and equivalent texture, flavor, and taste to commercial saltine crackers produced by twenty four-hour fermentations. The cracker flavor enhancer mixture was formulated from a gluten hydrolysate (Promate - 200), organic acid salts, and starch as the carrier. The flavor enhancer mixture was used to replace the free amino acids and the seven organic acids produced during a twenty-five hour fermentation. Johnson (16) has patented a combination of gluten amino acids and a mixture of organic acids which could function as a fermentation compensator, that is, the combination could induce changes in the dough that normally require a long fermentation to achieve.

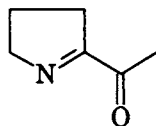
Laboratory production of crackers with the same flavor quality as commercial crackers has been difficult. Micka (11) found that when crackers are produced in a laboratory and no starter sponge is kept, and equipment is kept sterile, fermentation is generally retarded and the resulting dough has a high pH and the crackers have an undesirable flavor. Dynn (17) attempted to develop a procedure for the production of experimental crackers to test flour quality. He found that the crackers made from the same batch of flour varied widely in flavor quality and concluded that commercial crackers could not be produced in a laboratory. However, Pizzinatto and Hosenev (4, 14) have recently developed a procedure for the production of satisfactory experimental saltine crackers under laboratory conditions.

CRACKER-LIKE AROMAS

Teranishi, *et al.* (18) observed "cracker-like" odors in compounds such as 2-acetyl-1,4,5,6-tetra hydroypyridine, 2-acetylpyrazine, and 2-acetyl-2-thiazoline. From this, Folkes and Gramshaw (19, 20) speculated that heterocyclics with the following structural formula,



where the nitrogen atom and the adjacent carbon atoms form part of the ring structure, exhibit biscuit- or cracker-like odors. Based on this theory, it could therefore be expected that acetylpyrazine, 2-acetylpyridine, and 2-acetylthiazoline have a cracker-like aroma. Schieberle and Grosch (21) reported that the most intense odor, i.e. the component with the highest flavor dilution value, in white bread crust was



or 2-acetyl-1-pyrroline. This compound was also isolated from cooked 'scented' rice and the *Pandanus amaryllifolius* Roxb. plant by Buttery, *et al.* (22, 23, 24) who described it as "popcorn-like". The similarity in aroma between the crust of white bread and crackers makes this compound an excellent candidate for cracker aroma. It is possible that 2-acetyl-1-pyrroline is formed by sugar-amino acid reactions (25), however, recent work implicates yeast as its source in bread (Schieberle, *ibid*).

Wiseblatt & Zoumut (26) reacted proline and dihydroxyacetone to yield a cracker-like aroma, but the separation and identification of the component(s) responsible for the odor was not reported. However, in similar experiments, Kobayashi and Fujimaki (27) generated a cracker-like aroma from a reaction of proline and glucose in the presence of pyrrolidine and pyruvaldehyde. They identified the compound responsible for the odor as N-acetyl pyrrole. From a reaction mixture comprising proline and glycerol Hunter *et al.* (28) prepared aroma concentrates which upon separation by gas liquid chromatography gave rise to three main peaks having a cracker- or bread-like aroma. Hunter, *et al.* (29) later reported obtaining a pentane extract from a reaction mixture of proline, dihydroxyacetone, and sodium bisulfite with a strong odor reminiscent of freshly-baked soda crackers. A compound identified in the mixture, 1,4,5,6-tetrahydro-2-acetylpyridine, was synthesized by Buchi and Wuest (30) and described as having a cracker-like aroma. Schieberle and Grosch (31, 32, 33) were able to isolate this compound from rye and wheat bread crust and describe it as having a bread crust aroma with cracker-like notes.

SEPARATION OF VOLATILES FROM CRACKERS

The large amount of fat in saltine crackers causes a problem during the extraction of volatiles because of the solubility of most odor-active volatiles in fatty substances. The simplest solution is steam distillation (34) or co-distillation with solvents such as ethanol (35) followed by extraction of the distillate with non-polar solvents. Steam distillation and simultaneous extraction with the Likens-Nickerson apparatus is based on the same chemistry (37). When the distillation is done under reduced pressure (38, 39) traps cooled with liquid nitrogen are required. For example, Slott and Harkes (40) used low temperature vacuum distillation followed by solvent extraction to separate the volatile components of Gouda cheese. So called "headspace techniques", like that used by Lin (41) to separate the alkylpyrazines in processed American cheese, used nitrogen gas passed through a bed of ground cheese into tubes of Tenax and Porapak Q. The volatiles were desorbed from the tubes into a gas chromatograph. Generally these techniques remove only a few percent of the volatiles from the sample especially when the samples are high in fat, however, they tend to be free of any non-volatile contaminants.

Selective solvent extraction of volatiles will remove volatiles with very high yields although the extracts are always contaminated with non-volatile components. For example, acetonitrile extraction followed by co-extraction with pentane was used by Vernin (38). In our experiments the direct extraction of crushed crackers with Freon 113 or ethyl acetate contained too much residual lipid. Distillation of the solvent yielded a lipid concentrate low in aroma volatiles. Attempts to use gel filtration (Bio-Beads S-X12 from Bio-RAD) to remove the lipids but retain the odorous substances were also unsuccessful.

Schreier *et al.* (42) used aqueous media to extract aroma volatiles from solid substances. We applied this approach to the isolation of volatiles from crackers and obtained a lipid-free extract with a convincing cracker-like aroma. In the procedure, 1.0 kg of crackers was blended with 5.0 L of distilled water containing 250 g of sodium chloride while nitrogen gas was passed into the mixture. After standing four to five hours, it was centrifuged (Sorvall) at 2,000 rpm (4°C) for 20 minutes. The supernatant was filtered through a cheese cloth and extracted with Freon™ 113 and followed by ethyl acetate. Because the residual crackers still had a strong cracker-like aroma after extraction it was apparent that a more polar solvent was required.

Figure 2 outlines a procedure that yielded a more complete extraction of cracker-like aromas.

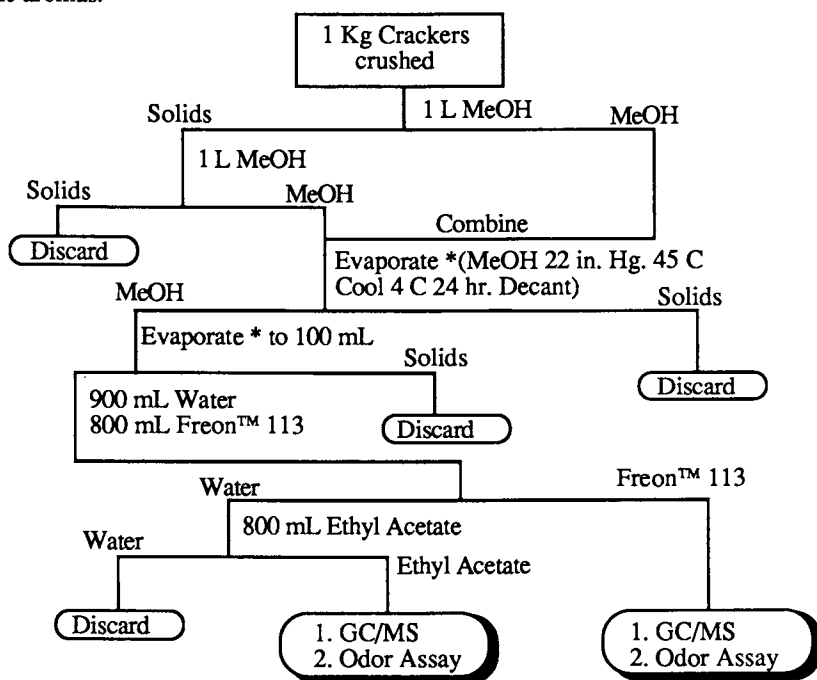


Figure 2. Cracker extraction procedure developed by the authors to yield both polar and non-polar volatiles.

The methanol at room temperature extracts both fats and volatiles. Cooling the methanolic extract desolubilized most of the fat retaining most of the volatiles in solution. The alcoholic solution was then diluted with nine volumes of water. Back extraction of the aqueous solution with Freon™ 113 followed by ethyl acetate yielded a non-polar and a polar fraction free of many contaminating non-volatiles. In this procedure acetonitrile, but not isopropyl alcohol, was a good replacement for methanol and pentane is a good replacement for Freon™ 113. The idea is similar to the estab-

lished procedure used to separate essences from pomades in the production of fragrances.

Both fractions obtained from crackers extracted less than one week after baking had strong cracker-like aromas. The Freon™113 fraction had a cracker, roasted grain, cooked rice aroma while the ethyl acetate fractions had a cracker, sweet baked good, burnt butter aroma. A preliminary analysis of the two fractions by gas chromatography-mass spectrometry on a 25 m by .22 mm methyl silicone column between 700 and 1800 retention indices showed the compounds listed in Table 2.

Table 2: Compounds detected (by MS) in MeOH&water extracts of saltine crackers and their n-paraffin retention indices (RI) on a 0.22 mm by 25 m OV101 (0.33 mm thick) column

Compound	RI	Compound	RI
2-acetylfuran	876	hexadecanoic acid	1917
2-acetylpyridine	995	1(H)-indole-3-ethanol	1672
2-acetylpyrrole	1028	2-methylpyrazine	811
benzacetalddehyde	1001	3-methylthiopropanal	868
benzaldehyde	928	maltol	1070
2,3-dimethyl pyrizine	894	methyl cinnamate	1096
2,4-dimethylheptane	824	2-phenylethanol	1080
2,5-dimethyl pyrizine	888	phenylacetaldehyde	1004
2,6-dimethyl pyrizine	889	tetradecane	1400
ethyl pyrizine	890	undecane	1100
furfural	800	vanillin	1348
furfuryl alcohol	834		

All of these compounds have been reported in similar products and are not unique except for the absence of 2-acetyl-1-pyrroline. To be sure that the system could detect 2-acetyl-1-pyrroline an authentic sample was analyzed by gas chromatography-mass spectrometry on a system that could easily detect 1 pg of hexane and yielded identical spectra and retention index as a component isolated from fragrant rice according to the method of Buttery, et al. 1983. However, analysis of the cracker extracts using both electron impact and mass fragmentography showed no convincing evidence for the presence of 2-acetyl-1-pyrroline at greater than 1 picogram per gram of cracker.

CONCLUSIONS

Many aroma compounds have been identified in crackers but which ones are the most important has still not been established. Further studies of these extracts should involve the use of odor assays to sort out to aroma important compounds in crackers from the unimportant aroma compounds present. For example, the method used by Shieberle & Grosch (33) to describe the odor-active components in bread in terms of their 'flavor dilution values' and the technique called charm analysis (43, 44) both concentrate chemical investigations at retention indices with odor activity.

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Chapter 27

Formation of Flavor Components in Roasted Coffee

R. Tressl

Technische Universität Berlin, Seestrasse 13, D-1000 Berlin 65, Federal Republic of Germany

More than 700 constituents have been identified in aroma extracts of roasted coffee. Heterocyclic aroma components represent the greatest amount of the steam volatile aroma complex (80 - 85 %) which amounts to 700 - 900 ppm in medium roasted Arabica coffees. The concentration of individual components varies depending on coffee varieties and roasting conditions. Typical components are formed by thermal degradation of free and bound amino acid and chlorogenic acid precursors. Compared to other roasted food-stuffs, sulfur containing constituents and phenols are formed in high amounts and contribute to desirable coffee flavor or off-flavor.

Numerous reviews on coffee flavor have been published over the past few years (1, 2, 3). So far, more than 700 components have been characterized in roasted coffee. Therefore, approximately 20 % of the 4000 chemicals reported in the "List of Volatile Compounds in Food" edited by TNO (4) may be consumed by drinking coffee. This list of volatile constituents continues to grow as demonstrated by Baltes and coworkers (5) Improved analytical instruments and data bases of thousands of mass spectra contributed to the detection of volatile constituents in model reactions as well as in roasted food-stuffs. New food constituents are more and more difficult to locate, isolate and identify.

In a contribution presented at the first Weurman Symposium, Rijkens and Boelens (6) estimate that foods can contain between 5000 to 10000 constituents. This

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seems a realistic forecast, but many of these constituents may be present in very low concentrations and may have no organoleptic or toxic effect.

In a recent review on coffee flavor Clarke (3) reported that there are many publications on qualitative data, but information on their actual quantities in coffee is difficult to find. Many of the coffee aroma components are present in high concentrations and these data were used to predict so called consumption ratios for heterocyclic components (7, 8). According to data presented by these authors the consumption of pyrazines from coffee is 380 fold higher compared to pyrazines added as flavoring substances to all other foodstuffs. On the other hand, there are numerous chemicals disclosed in patents which may be used to modify and reinforce the aroma and flavor of coffee and beverages. Better understanding of precursors and routes by which important and typical aroma and flavor components are formed is essential to decrease undesirable, non contributing components and improve the acceptance and wholesomeness of this attractive beverage.

Formation of Phenols by Degradation of Chlorogenic Acids

Compared to other roasted foodstuffs, green coffee beans contain high amounts of chlorogenic acids which are degraded during roasting. Coffea Arabica possesses lower concentrations of 5-caffeoyl- and 5-feruloyl quinic acids than Coffea Robusta as shown in Figure 1. During a medium roast 60 to 77 % of these precursors, which amount 30 to 40 g/kg are degraded and the corresponding phenols are formed in the high ppm range. Only 2 to 5 % of the degraded precursors are detectable as phenols and the rest are transformed into so called high molecular huminic acids (4).

The degradation of chlorogenic-, caffeic-, ferulic- and quinic acids was studied in model experiments and the phenols were characterized and quantified in roasted coffee (9, 10, 11). The phenols derived from caffeic acid possess emetic effects and their concentrations in roasted coffee change according to variety, roasting level and treatment of green coffee beans. In dark roasted blends, catechol and guaiacol increase significantly, and the corresponding 4-vinyl derivatives decrease. Guaiacol and 4-vinylguaiacol possess low thresholds and are contributing flavor components. Chlorogenic acid precursors and their corresponding phenols are also influenced by decaffeination processes (Figure 2). During decaffeination (I = ethylacetate, II = supercritical CO₂) 5-caffeoyl- and 5-feruloylquinic acids decrease significantly and are transformed into 4-vinylcatechol and 4-vinylguaiacol, respectively. The increase of 4- and 5-caffeoylquinic acids can not be explained by transesterification. Obviously, these precursors are







	I	II		I	II
5-CQA			5-FQA		
green	37.5	37.5	green	4.9	4.9
roasted	15.4	1.9	roasted	3.8	1.2
	80	570		2.1	14.7
	19	110		1.0	10.3
	53	33		21	22

Figure 1: Content (g/kg) of 5-caffeoyl- and 5-feruloyl-quinic acids in coffee Arabica and Robusta and their corresponding phenols (ppm) formed during roasting







	I		II			I		II	
green 5-CQA	44	25.2	42	35.4	green 5-FQA	5.5	2.9	3.6	1.7
4-CQA	8.7	15.7	12.3	19.6					
3-CQA	6.6	15.2	8.6	15.7					
roasted 5-CQA	10.6	10.8	16.3	25	roasted 5-FQA	1.6	1.5	1.5	1.6
4-CQA	5.6	7.5	11.7	15.8					
3-CQA	4.5	5.0	7.8	11.3					
	100	96	85	45		6.5	6.0	4.0	1.1
	23	23	31	19		3.9	3.7	2.8	0.6
	50	44	62	78		30.2	28.5	64	41

Figure 2: Chlorogenic acid precursors (g/kg) and their corresponding phenols (ppm) formed by decaffeination process (I ethylacetate, II supercritical CO₂, before and after decaffeination, respectively)

bound as glycosides. These data demonstrate that decaffeinated coffees possess lower amounts of phenols, (when roasted under comparable conditions) than caffeinated blends. Similar results were presented by König et al. (9) for so called stomach friendly coffees where chlorogenic acid precursors are partially degraded by washing coffee beans under elevated temperatures.

Carbohydrates and Amino Acid Precursors

Sucrose, reducing sugars, free and peptide amino acids are other important precursors which determine aroma and flavor of roasted coffee. Feldman et al. (12) investigated these precursors in green Robustas and Arabicas and quantified their changes during roasting. Arabicas possess higher amounts of sucrose than Robustas and lower amounts of reducing sugars. During roasting sucrose, glucose, fructose and galactose decrease below 0.5 % and arabinose and rhamnose can be detected after roasting which indicates that polysaccharides are also degraded (13).

Walter et al. (14) demonstrated that green coffee beans possess approximately 5 to 10 % of peptide amino acids as free amino acids. Meichelbeck and Zahn (15) showed that coffee proteins contain 0.47 - 0.56 % cystine and only 0.02 % cysteine by polarographic methods. They could not find differences between Arabicas and Robustas. Tressl et al. (13) showed differences in the amounts of free amino acids of Arabicas, Robustas and decaffeinated blends. Robustas possess higher amounts of γ -amino-butyric acid, Strecker-active, and basic amino acids and lower amounts of glutamic acid than Arabica. These results were confirmed by Trautwein (16) who investigated more than 50 samples. There are little or no significant differences in the peptide amino acids between the two varieties. During roasting free amino acids react via Maillard Reaction and protein bound Cys, Lys, Arg, Ser, Thr decrease considerably (16). Strobel (17) showed significant differences in the concentration of free cysteine between Arabicas and Robustas and blamed sulfur constituents for the less desirable "robusta taste" (rubbery note).

Aroma Perspective

Aroma and flavor components of roasted coffee were investigated by numerous authors. In 1926 Reichstein and Staudinger (18) characterized furfurylmercaptan (2-furyl-methanethiol) as an important aroma constituent of roasted coffee which is a character impact component.

At the turn of this century, only 13 components were known. This number increased to 60 in the following fifty years. From 1965 to 1975 the number of coffee constituents increased dramatically to more than 600. Stoll et al. (19), Goldman et al. (20) and Vitzthum et

al. (21, 22) contributed most to this development. In the last ten years the identification of new coffee constituents plateaued at over 700 (23, 24). Most of the compounds were reported without presenting quantitative data, flavor, aroma qualities or threshold values. Therefore, the importance of individual aroma and flavor compounds among the 700 constituents in roasted coffee is still an open question.

Furans and Reductones

Furans and reductones are major components in roasted coffee as shown in Figure 3. Arabicas possess higher amounts of furanaldehydes and Furaneol than Robustas, when roasted under comparable conditions. The aldehydes and reductones are Strecker-active components and further transformed into typical aroma and flavor compounds as demonstrated in model experiments.

By applying the concept of odour values (25) to this class of compounds only Furaneol and Maltol contribute to coffee flavor. Both constituents are known as important flavor compounds and are used as nature identical flavorings in many foods. Furaneol contributes a fragrant caramel note to coffee and is known as important compound in pineapple and strawberries. The consumption of Furaneol in the USA in these products is respectively 41,800 to 2,650 to 1,100 kg (per year) compared to 2,718 kg of synthesized Furaneol used in nature identical flavoring. Similar figures were presented for Maltol and other coffee compounds by Stofberg and Grundshober (8).

Roasting conditions (blend I = medium and blend II = dark roast) influence the spectrum of furans and reductones considerably. Reactive aldehydes and enolones decrease and furfuryl alcohol, furancarboxylic acid and Maltol increase significantly. Components 1 to 5 are formed via 3-deoxy- and 6 to 11 via 1-deoxyosones. In addition, caramelization may also be involved.

α -Dicarbonyls, Furanones and Esters

Figure 4 presents some typical α -dicarbonyls, furanones and esters of roasted coffee. Diacetyl and 2,3-pentanedione add a buttery top note to coffee and are important precursors for contributing pyrazines. Components 4 and 5 were identified as possessing roasty, sulfury notes by sniffing techniques. 4 and 5 were isolated and identified by MS- and $^1\text{H-NMR}$ -spectroscopy (13). In model experiments we could demonstrate, that 6 (acetol) is an important precursor in the formation of these components. It was also shown that 5-methyl-2,3-heptanedione is formed by aldol condensation from acetol and 2-methylbutanal, 4 by condensation of acetol and glyoxal, respectively. It is obviously an analogous reaction to the formation of kahweofuran from mercaptoacetone during roasting of green

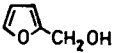
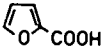

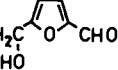
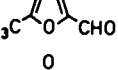
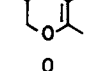
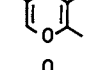
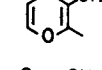
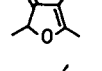
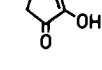
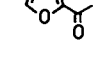
		Robusta	Arabica	Blend	
				I	II
1		250	450	400	710
2		40	55	45	95
3		34	50	58	12
4		18	45	15	-
5		25	35	35	10
6		4	15	15	-
7		4	10	6	-
8		32	37	30	75
9		17	32	28	13
10		9	13	10	20
11		10	7	7.5	10

Figure 3: Furans and reductones in different coffee varieties formed by roasting (units in ppm)







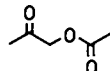
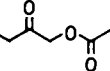
		Robusta	Arabica	Blend		Blend Jetzone
				I	II	
1				n.d.		
2		6	12	13	5	5.4 5.5
3		8	18	16	12	22 42
4		1.5	2	2	1.5	1.5 1.4
5		2	2.5	2.5	2	2.5 6
6				n.d.		
7		3	3.5	3	2.5	2.8 4.4
8		3.5	4	3.5	3	3.4 3.3

Figure 4: Typical α -dicarbonyls, furanones and esters in roasted coffee (n.d. = not determined) (units in ppm)

coffee beans. Roasting level and roasting technology influence the spectrum of this class of compounds more than the variety of the beans.

Pyrroles and Pyridines

Many pyrroles have been identified in roasted coffee. Regarding their formation in coffee pyridine, pyrrole and N-methyl-2-formylpyrrole are trigonellin derivatives; 3 and 4 typical pyrroles from primary amino acids; and 6 to 8 hydroxyproline derived Maillard products (26).

Pyridine and pyrrole (Figure 5) increase according to the roasting level of the beans and are higher in Arabicas than in Robustas under comparable conditions. 5 to 8 are contributing aroma components according to their low threshold values (27).

Pyrazines

More than 50 pyrazines have been identified in roasted coffee (Figure 6). They are formed during Strecker degradation of primary amino acids with α -dicarbonyls, cyclic enolones, furanaldehydes and range from 180 to 220 ppm in medium roasted coffee. They possess roasted nutty, green, cereal and earthy aroma qualities and their thresholds vary in a wide range. From the Flavor Unit data, it can be seen that 11 and 12 which are formed from 2,3-pentanedione, are contributing compounds whereas the more concentrated 1 and 4 possess less significance. Roasting technology and roasting level determine the spectrum of pyrazines by increasing the amounts of methyl- and ethylpyrazines. The consumption of pyrazines from roasted coffee is 600 to 700 fold higher compared to all the synthesized pyrazines added as flavoring materials to foodstuffs.

The odor of green coffee is predominantly determined by 2-methoxy-3-isobutyl- and isopropylpyrazines (28). The concentration of 2-methoxy-3-isobutylpyrazine varies in roasted coffee from 10 to 50 ppb and is therefore a compound with a high flavor value (> 1000 F.U.) in roasted coffee. Nitz et al. (29) demonstrated that the "peasy" off-flavor in certain African coffee beans is caused by a 5 to 20 fold increase of 2-methoxy-3-isopropylpyrazine, obviously formed by fungi.

Sulfur Containing Furans

Sulfur containing compounds play an important role in the flavor spectrum of roasted coffee. They amount 5 to 15 ppm and are influenced by variety, roasting level and storage conditions. More than 100 sulfur compounds were characterized in roasted coffee (24).

Figure 7 shows sulfur containing furans and their range in roasted coffee. It can be seen that Robustas




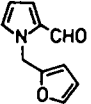




		Robusta	Arabica	Blend	
				I	II
1		26	46	32	190
2		5.3	7.5	6.5	10
3		3.8	4.3	4.2	6
4		3.5	3	4	8
5		3	4	2.5	10
6		2.5	1.4	1.5	2.8
7		1.5	1	1	3
8		2.9	2	2.5	4.4

Figure 5: Pyridine and typical pyrroles in roasted Robusta and Arabica coffees (units in ppm)















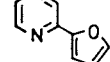
	Range	Blend		Flavor Units
		I	II	
1 	30 - 60	45	130	0.1
2 	15 - 20	20	45	1
3 	18 - 23	22	45	9
4 	10 - 12	7	15	0.2
5 	4 - 6	3.5	16	0.2
6 	6 - 8	9	17	4
7 	8 - 10	6	15	5
8 	6 - 8	6	14	
9 	0.2 - 0.4	0.5	0.7	
10 	0.5 - 0.7	0.8	0.9	
11 	0.7 - 0.9	1.2	1.1	110
12 	2.0 - 2.2	2.4	1.8	125
13 	0.8 - 1.0	1.0	1.3	8
14 	0.4 - 0.6	0.4	0.5	
15 	0.6 - 0.7	0.5	0.7	

Figure 6: Range of typical pyrazines identified in roasted coffee (units in ppm)

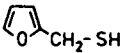
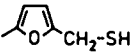
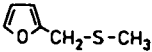
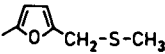
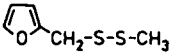
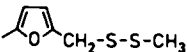
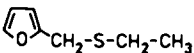
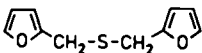
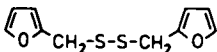
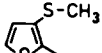
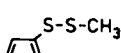
	Range	Arabica	Robusta
	500 - 4000	1000 - 2000	2000 - 3800
	100 - 200	150 - 200	100 - 150
	200 - 1000	250 - 500	500 - 1000
	20 - 50	30 - 50	10 - 20
	200 - 600	200 - 400	400 - 600
	1 - 10		
	20 - 30		
	10 - 70		
	+		
	1 - 10		
	1 - 5		

Figure 7: Sulfur containing furans characterized under different roasting conditions (units in ppm)

possess higher amounts of furfurylmercaptan, furfurylmethylsulfide and furfurylmethyldisulfide and Arabicas more of the corresponding 5-methyl derivatives (at similar roasting levels). Furfurylmercaptan possesses a threshold of 5 ppt. From 0.1 - 1 ppb furfurylmercaptan has an aroma like fresh brewed coffee, while from 5 - 10 ppb it is perceived with a sulfury, mercaptan-like note comparable to staled coffee. 5-Methylfurfurylmercaptan has a threshold of 50 ppt and delivers a sulfury note above 5 ppb. Both constituents are formed during roasting and increase 5 to 10 fold during storage of roasted coffee beans as demonstrated by Silwar et al. (30). Therefore, both constituents determine the aroma and flavor of fresh roasted coffee and are also responsible for the undesirable aroma of staled coffee.

The concentrations of furfurysulfides and -disulfides which possess toasted, bread- and meat-like aromas do not change during storage of roasted coffee. Sulfur containing furans are formed by heating furanaldehydes with cysteine and methionine, respectively (24).

Thiolanones

In roasted coffee similar reactions seem to be involved forming methylmercaptan by the Strecker degradation of free methionine and forming H_2S from peptide cysteine. Figure 8 presents additional flavor contributing constituents of roasted coffee. 3-Thiolanone 6 and 2-methyl-3-thiolanone 7 were identified by Stoll et al. (19) and patented as coffee flavors. The two thiolanones 6, 7 are formed as major constituents in erythrose and xylose/cysteine model systems, respectively.

Kahweofuran

Kahweofuran (2-methyl-3-oxa-8-thiabicyclo(3.3.0)-1.4-octadiene, 8) was first isolated by Stoll et al. (19) and its structure later confirmed by synthesis (31). This compound possesses a roasty/sulfury-note as do the related ethyl- and dimethyl derivatives are unique to coffee. So far, the ethyl and dimethyl compounds have not been detected in the roasted foods or in monosaccharide/cysteine model experiments.

A synthesis of kahweofuran from 3-thiolanone (acetylation, methylation, alkylation) yielded only 0.7%. Rewicki (Rewicki, D.; Gorczynski, M. Synthesis, in press.) synthesized homologous compounds of kahweofuran and we identified, quantified and determined their threshold in coffee. The results show that kahweofuran and the ethyl derivative are higher in Arabica, and the dimethyl compound in Robustas. The thresholds of kahweofuran was 0.5 ppb and at 10 to 50 ppb possessed a roasty sulfur note.

Ethylkahweofuran 9 has a sulfury, meaty aroma and a threshold of 5 ppb. The dimethyl derivative was perceived

with a sulfury mushroom-like aroma and the threshold was determined as 0.5 ppb. The formation of kahweofurans is an analogous reaction to the transformation of acetol into 2-methyl-3(2H)-furanones. Recently we identified mercaptoacetone which is a significant precursor of kahweofuran (Figure 9). More than 50 % of the precursor could be transformed into kahweofuran as demonstrated in model experiments.

Thiophenes and Thiazoles

Figure 10 presents major thiophenes and thiazoles identified in roasted coffee. Robustas which possess rubbery notes contain higher amounts of these constituents which are formed in cysteine monosaccharide model experiments via 3-deoxyosones or by aldol condensations. During roasting of coffee beans terpene alcohols may also be transformed into sulfur containing constituents. 3,3'-Dimethyl-1,2-dithiolane was characterized as well as the corresponding 4-oxo-derivative at 10 - 50 ppb in roasted coffee. By this reaction prenyl mercaptan (which was characterized as compound responsible for the so called "sunstuck" flavor of beer) which possesses a threshold at 5 ppt may be formed (24). During aging of roasted coffee mercaptans play an important role together with other non detectable sulfur compounds possessing low thresholds (24).

Organoleptic Importance of Identified Compounds

Coffee flavor is a complex mixture of compounds belonging to many classes in distinct concentration ratios. Flament (2) listed 17 typical constituents of coffee aroma with buttery, woody, green, earthy caramel, burnt, smoky, roasted and sulfury notes, aroma and flavor qualities. On applying the concept of odor units with our current knowledge we can designate 15 to 20 compounds with high aroma values which only amount to 10 - 20 ppm.

Furfurylmercaptan with an odor unit of 10 - 20000 seems to be the most important compound, 5-methylfurfurylmercaptan with 100 - 200 o.u. is obviously less effective. Furfurylmethyldisulfide (300 - 600 o.u.) and kahweofurans (50 - 100 o.u.) are also contributing compounds to roasted, sulfury aroma qualities. Among pyrazines ethyldimethyl- and acetylpyrazines possess the highest odor units (100 - 120 and 5 - 10, respectively) which are perceived with roasted nutty and earthy notes. Smoky burnt notes as guaiacol (50 - 150 o.u.) and 4-vinylguaiacol (10 - 15 o.u.), buttery, caramel aroma qualities from Furaneol (20 - 30000) and 2,3-pentandione (5 - 10) are also essential for this aroma complex. There are also constituents from green coffee beans transferred to the roasted product, which possess high aroma values: e.g. 2-methoxy-3-isobutylpyrazine (600 - 1200 o.u.) green,







		Range	Arabica	Robusta
1	$\text{H}_3\text{C-S-S-CH}_3$	200 - 600		
2	$\text{H}_3\text{C-S-S-S-CH}_3$	20 - 100		
3	$\text{H}_3\text{C-S-(CH}_2)_2\text{-CHO}$	80 - 120		
4	$\text{H}_3\text{C-S-(CH}_2)_2\text{-CH}_2\text{OH}$	80 - 200		
5		50 - 100		
6		100 - 200		
7		1500 - 2000		
8		1000 - 2500	1500 - 2500	1000 - 1800
9		300 - 500	400 - 500	300 - 400
10		500 - 1000	500 - 800	600 - 1000

Figure 8: Methionine derived components and other flavor contributing sulfur constituents of roasted coffee (units in ppm)

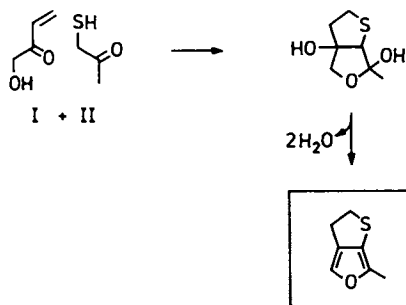


Figure 9: Reaction of mercaptoacetone (II) and a possible intermediate (I) to kahweofuran



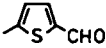


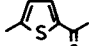

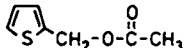



	Range	Arabica	Robusta
	1000 - 2000	1000 - 1500	1200 - 1800
	50 - 100		
	100 - 200		
	500 - 1000	400 - 600	600-1000
	300-800	150 - 300	300 - 500
	300 - 500		
	600 - 1000		
	50 - 100		
	100 - 200		
	500 - 1500	500 - 1000	800 - 1500
	200 - 300		

Figure 10: Predominant thiophenes and thiazoles identified in roasted coffee (units in ppm)

bell pepper, (E)-2-nonenal (200 - 400 o.u.) woody note in coffee 1-octen-3-ol (odor unit mushroom earthy) and 3-/2-methylbutanal (500 - 1000 o.u.) malty, burnt note. All these constituents possess low thresholds and are detectable in the low ppm- or ppb-range in roasted coffee.

The more concentrated di-, triphenols, furanaldehydes and -alcohols, pyrazines and pyrroles possess high thresholds and their contribution to aroma and flavor is obviously less important. Therefore, it should be possible to lower their concentrations by selective methods and make this beverage more stomach friendly, increase its physiological wholesomeness without changing its attractive aroma and flavor.

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Chapter 28

Influence of Nonvolatile Compounds on Coffee Flavor

Sara J. Risch and Yue Mei Ma

Food Science and Nutrition Department, University of Minnesota,
St. Paul, MN 55108

Solvent extraction (diethylether) and vacuum distillation were evaluated as techniques to remove aroma constituents from brewed coffee. Despite multiple extractions or repeated distillations, the aroma constituents of coffee could not be entirely removed. The treated coffee contained a woody, heavy, burned aroma. Results demonstrate that it is difficult to effectively separate the volatile aroma constituents from a food product which obtains its flavor from Maillard reactions and thus the relative flavor contribution of volatile vs non-volatile components is difficult to access.

The science of flavor chemistry has concentrated on volatile compounds for two main reasons. First, volatile compounds are primarily responsible for aroma which is often the component of flavor of greatest significance. Second, the techniques developed for the separation and identification of individual components of a complex natural product are much better for volatile compounds than for non-volatile compounds. For example, gas chromatography (GC) is a far more effective method of separation than high pressure liquid chromatography (HPLC), the best method for separation of non-volatile compounds. Therefore, the literature shows much less published information on the presence of non-volatile compounds in foods than it does for volatile compounds. (1-5).

This paper describes methods for separating the volatile and non-volatile fractions of coffee and the sensory descriptors associated with each fraction.

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Materials and Methods

Fresh coffee was brewed in a drip coffee maker (Mr. Coffee) using 40g Folgers regular grind coffee and 400 ml water. This yielded 300 ml brewed coffee. A 10 ml aliquot was used for purge and trap analysis to establish the volatile profile of the fresh coffee. The sample was purged for 10 minutes with 50 mL/min nitrogen at 60 C into a charcoal filled trap. The trap was put into the desorption chamber of a Rektorik Microwave desorber (J. Rektorik, Switzerland) coupled to a Hewlett Packard model 5890 GC and desorbed for 4 seconds using a "desorption power" of 3. (6).

The chromatography was accomplished on a 30m x 0.25mm DB-1 column (J & W Scientific, Folsom, CA) with Helium at 15 psi using a flame ionization detector. The column was held at 40 C for 1 min. and then increased at 5 C/min. to 80 C and then 10 C/min. to 250 C and held for 15 min.

Two different techniques were used to remove volatile compounds from the coffee. The first was vacuum distillation in a rotary evaporator. A 20 mL aliquot of coffee was placed in a round bottom flask and then it was attached to a Roto-vap and held in a water bath at 60 C during rotation with a vacuum applied from a water aspirator. When 15 mL had been distilled into the collection flask, it was removed and 20 mL distilled water was added to the 5 mL of retentate and the process of rotary evaporation repeated to yield a total of four distillates. Gas chromatographic analyses were performed using purge and trap as described above, with a purge time of 15 minutes.

The second procedure for removal of volatile compounds was a liquid-liquid extraction using ether. A 20 mL sample of the freshly brewed coffee was extracted 5 times with 50 mL distilled diethyl ether. The ether extracts were pooled, dried with anhydrous MgSO₄, filtered and concentrated to ca 0.1 mL under a stream of nitrogen. The retained coffee was analyzed using purge and trap with a 15 minute purge time. The ether extract was also analyzed by GC using the same GC conditions described above with a 1 uL injection and a split of 1:20. Sensory analysis involved five regular coffee drinkers who were asked to sniff each sample and describe it.

Results and Discussion

A. Sensory

As shown in Table 1, the two methods for removal of volatile compounds from coffee gave different results. The rotary evaporation technique yielded a distillate with an aroma that

Table 1 Aroma character of each coffee fraction

Fraction	Aroma Descriptors
Distillation	
Distillate	toasted, roasted, cereal, burnt, coffee roasted, sweet, caramel, light coffee nearly odorless, very weak coffee odorless
Retentate	burnt coffee, woody, molasses
Ether extraction	
Extract	Caramel, burnt, unbalanced coffee, ashtray-like
Residue	sweet, slight caramel

changed with each successive distillation. Both the strength and character of the aroma changed. By the fourth distillation, the distillate was odorless at room temperature. However, the retentate still had a distinct aroma indicating the presence of volatile compounds. Similarly, the coffee which had been extracted with ether also had a significant aroma. Neither distillation nor extraction with diethyl ether could remove all the coffee aroma components. Therefore, it appears that very polar compounds with poor volatility in water contribute to the aroma of brewed coffee.

B. Chromatography

The gas chromatogram of a freshly brewed sample of coffee is shown in Figure 1. Compared to the chromatogram from the three successive distillates shown in Figure 2a-c, the fresh coffee showed a large number of volatiles which elute over a broad temperature range. However, the chromatogram of the retentate (Figure 3) showed a large number of compounds which elute from the CC at temperatures above 120 C. After ether extraction, the coffee gave a chromatogram (Figure 4a) with a wide range of elution temperatures as did the ether extract itself (Figure 4b). It was not possible to obtain a sample from which all of the volatile compounds had been removed by either of the two methods.

The complex aroma flavor of coffee cannot be separated simply by distillation or extraction. However, the distillates of brewed coffee obtained using rotary evaporation had a much more balanced coffee aroma than the ether extract.

This research does not provide a definitive answer to the question of the influence of non-volatile compounds on the flavor of coffee, however, it does indicate their importance. Rotary evaporation can give two distinct fractions with the

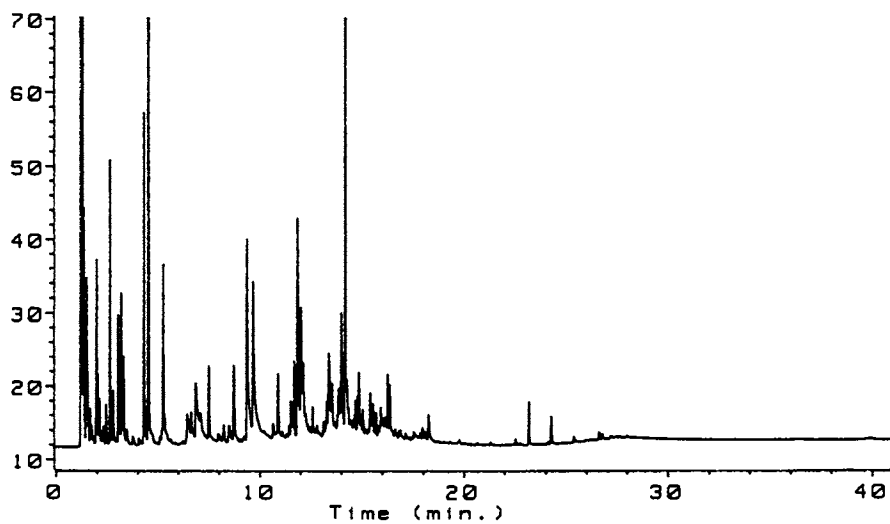


Figure 1. GC profile of fresh coffee.

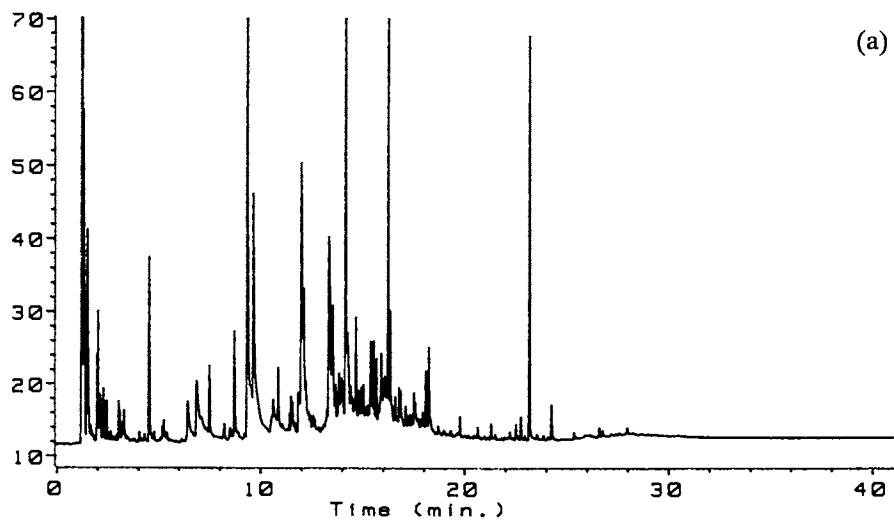


Figure 2a. GC profile of first distillate.

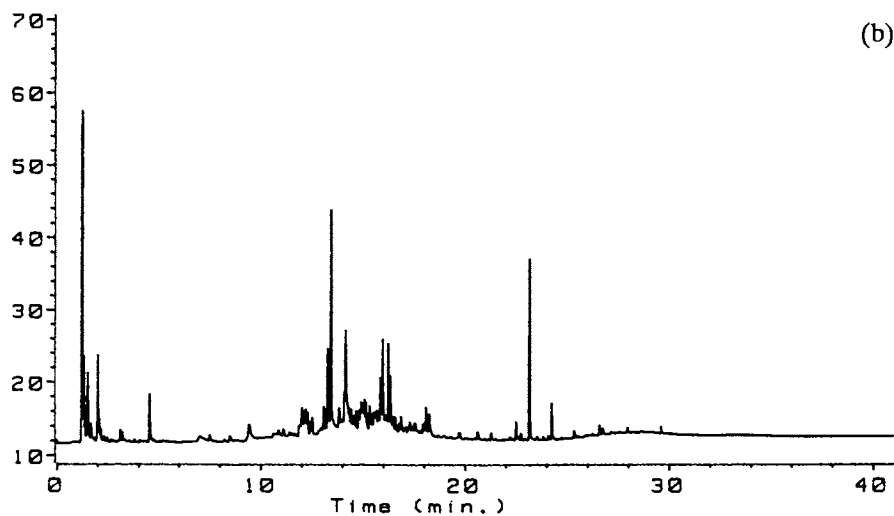


Figure 2b. GC profile of second distillate.

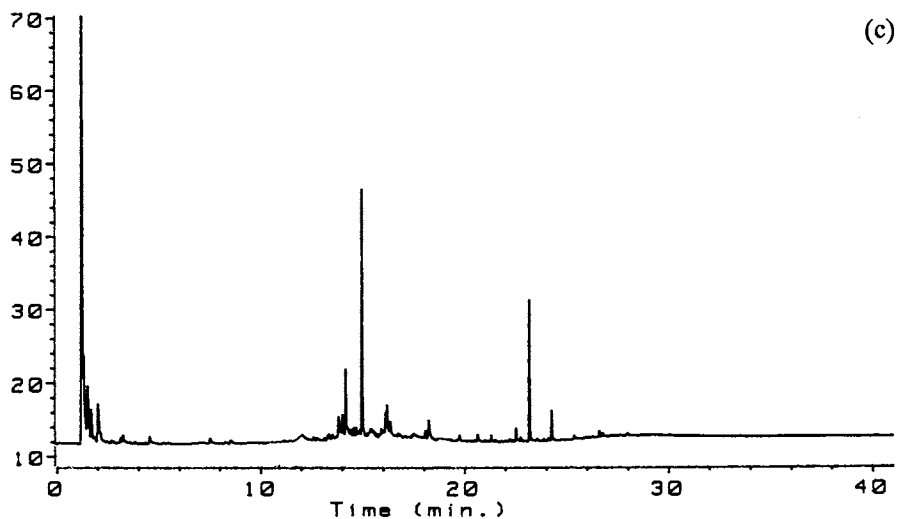


Figure 2c. GC profile of third distillate.

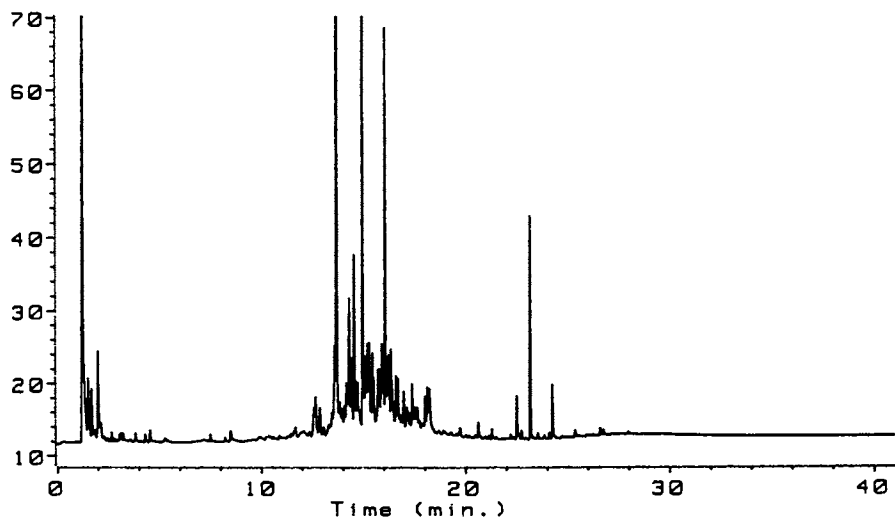


Figure 3. GC profile of retentate from distillation.

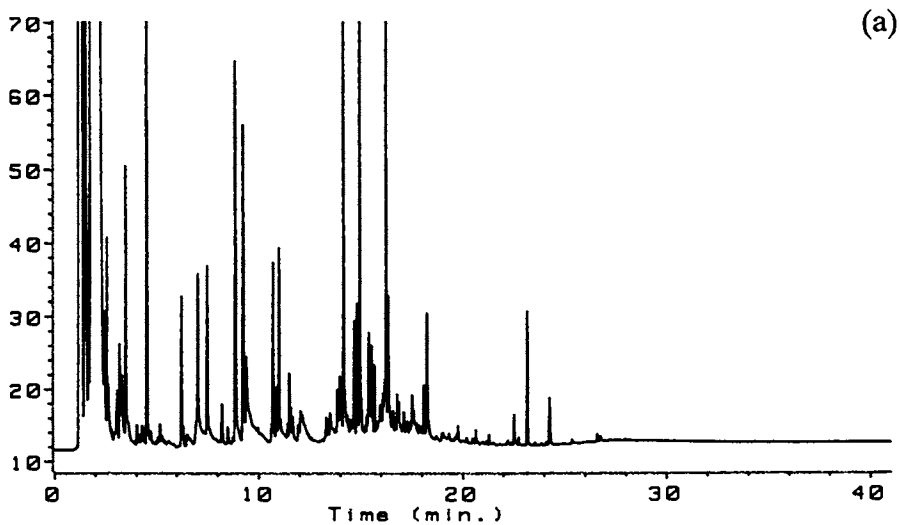


Figure 4a. GC profile of coffee after ether extraction.

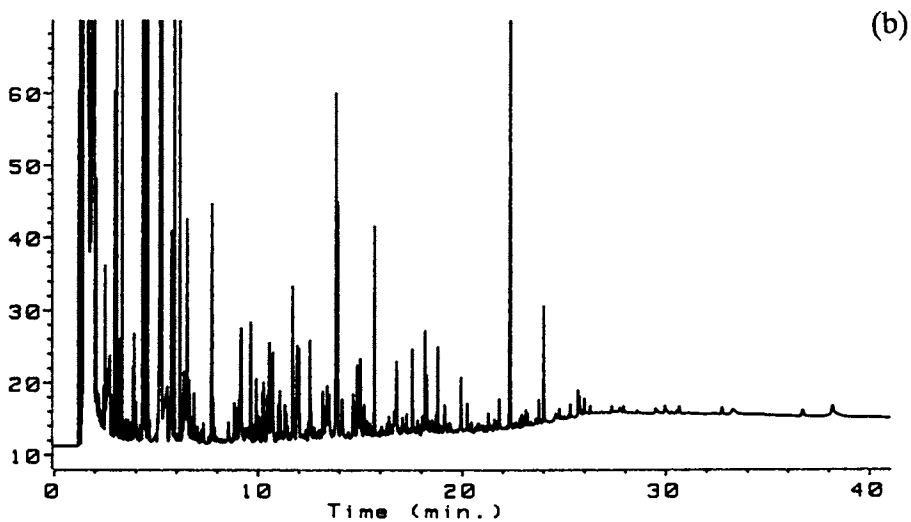


Figure 4b. GC profile of concentrated ether extracts.

distillate having a balanced coffee aroma and the retentate having a more burned, heavy aroma. This suggests that the less volatile compounds contribute the woody or burned notes and may influence overall coffee flavor.

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Chapter 29

Thermal Generation of Aroma Compounds from Tea and Tea Constituents

Tei Yamanishi¹, Michiko Kawakami¹, Akio Kobayashi¹, Tsuyoko Hamada¹,
and Yulina Musalam²

¹Ochanomizu University, Ohtsuka, Bunkyo-ku, Tokyo 112, Japan

²Research Institute for Tea and Cinchona, Gambung, Bandung 40001
Indonesia

The heat processing of tea leads to many complex chemical changes in tea. Tea's taste and aroma is affected by heat in at least three ways: by reducing the content of bitter soluble catechins, by the development of roast aromas and by the thermal degradation of β -carotene. Studies pertaining to the heat-induced changes in tea and appropriate model systems are reviewed.

Additional investigations are required to more fully understand the thermal generation of aroma compounds from tea.

Thermal processing is a part of tea manufacture, whether for green, oolong, black or other types of tea. The aroma of tea is greatly influenced by the type of heat treatment the tea receives. The chemical changes occurring in tea during heat processing are very complex and not fully understood. In addition, tea aroma may be formed through more than one route.

This paper describes some of the chemical changes resulting from heating tea and two model systems that may be important in the generation of tea aroma.

Comparison of Chinese vs Japanese Tea Processing

Tea is made from the tender young leaves of Camellia sinensis. The young leaves are called "tea flush" or "tea shoot tips". The production of tea from the tea flush is outlined in Figure 1.

In the processing of green tea, the tea flush is first steamed in the case of Japanese "sen-cha" or pan-fired to produce Chinese "kamairi-cha". This heat treatment inactivates enzymes in the tea leaves. Steaming produces fewer chemical changes than pan-firing.

The heating conditions in the final drying and refining stages influence the flavor of the finished green tea product. As the moisture content of the tea leaves decrease, more significant chemical changes, both qualitatively and quantitatively, occur.

No pyrazines or pyrroles are found in the aroma concentrate of

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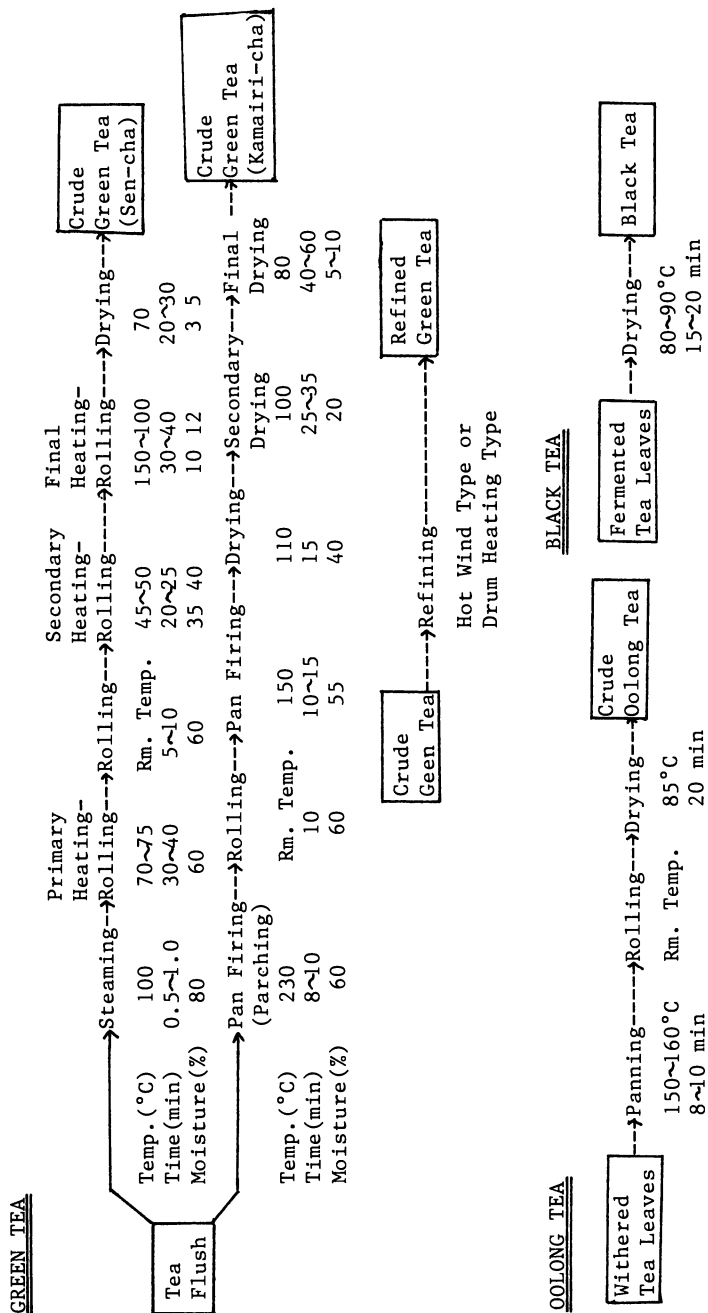


Figure 1. Heat Treatment during Tea Manufacturing.

the steam processed sen-cha (1). In pan-fired kamairi-cha, however, several of these roasty flavored compounds have been detected.

Figure 2 compares gas chromatograms of the aroma concentrates from pan-fired Chinese green vs Japanese tea. The Japanese tea was from Kumamoto Prefecture in southern Japan while the Chinese was a Longjing style tea. As seen from the chromatograms, more volatiles are formed in the Chinese Longjing tea. The Longjing tea was browner in color indicating that more strenuous heating conditions are used for the Chinese tea compared to those used for Japanese kamairi-cha. As seen in Table I, the concentration of pyrazines, pyrroles and ionone related compounds (from β -carotene) were greater in Longjing than in Japanese kamairi-cha (2).

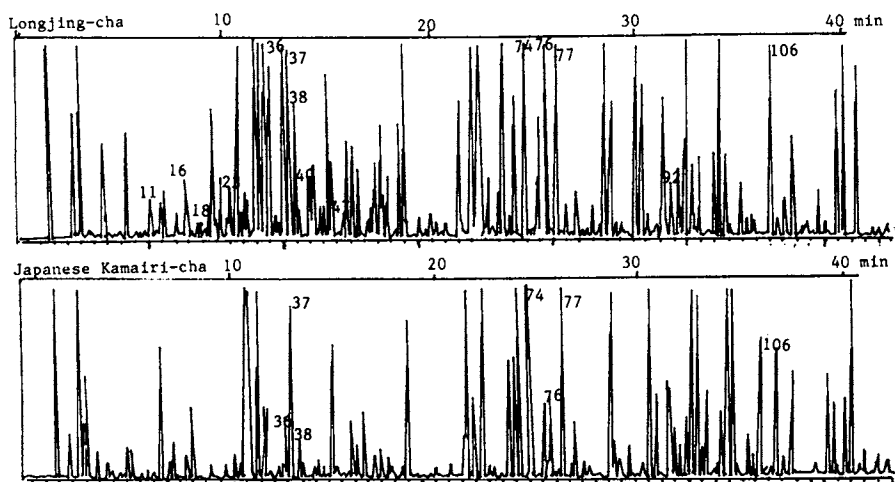


Figure 2. Gas Chromatograms of the Aroma Concentrates from Chinese Longjing Tea and Japanese Tea.

Green tea is usually made from Camellia sinensis var. sinensis (small leaf type). In China, jasmine tea is made from common kamairi-cha (from var. sinensis) while var. assamica (large leaf type) is used for Indonesian jasmine tea. The Indonesian jasmine tea has a stronger roast aroma than Chinese jasmine tea (3). The var. assamica tea leaf also contains a much higher level of polyphenolic catechins than var. sinensis.

Catechins are bitter and astringent and thus a high content is unacceptable. To reduce the concentration of soluble catechins, the pan-fired green tea is subjected to a re-firing prior to scenting with jasmine flowers i.e. Jasminum sambac. As a result, the re-fired green tea has additional heat generated volatiles. Table II shows some of the heat generated aroma compounds that have been identified (3).

Table I. The Composition of Heat Generated Aroma Compounds from Chinese Longjing and Japanese Kamairi Tea

Peak No. in Fig.2	Compound	Peak Area % in Aroma	
		Longjing	Japanese
<u>Pyrazines</u>			
11	2,5-Dimethylpyrazine	0.3	-
16	2-Methyl-5-ethylpyrazine	0.1	-
18	Trimethylpyrazine	0.1	-
23	2,5-Dimethyl-3-ethylpyrazine	0.3	-
<u>Pyrroles</u>			
37	1-Ethyl-2-formylpyrrole	1.8	1.8
40	1-Ethyl-2-acetylpyrrole	0.5	-
76	2-Acetylpyrrole	3.0	1.1
<u>Ionone Related Compounds</u>			
36	2,6,6-Trimethyl-2-OH-cyclohexanone	1.9	0.8
38	β -Cyclocitral	1.1	0.5
47	2,6,6-Trimethylcyclohex-2-1,4-dione	0.2	-
74	β -Ionone & (cis-jasmone)	3.5(1.2)	2.2(3.8)
77	5,6-Epoxy- β -ionone	2.2	2.1
92	Theaspirone	0.5	-
106	Dihydroactinidiolide	1.7	1.4

Adapted from Kawakami, M. and Yamanishi, T. (1983)

Table II. Components of Indonesian Pan-fired Green Tea

<u>Pyrazines</u>		
2-Methyl-	2,5-Dimethyl-	2,6-Dimethyl-
2-Ethyl-	2,3-Dimethyl-	2-Methyl-5-ethyl-
2-Methyl-6-ethyl-	2,3,5-Trimethyl-	2,3,5,6-Tetramethyl-
<u>Pyrroles</u>		
1-Ethyl-2-formyl-	2-Acetyl-	1-Ethyl-2-formyl(isomer)
<u>Furans</u>		
Furfural	5-Methylfurfural	Furfuryl alcohol
<u>Acids</u>		
Hexanoic	Octanoic	
<u>Ionone related Compounds</u>		
α -Ionone	β -Ionone	Dihydroactinidiolide
2,6,6-Trimethylcyclohex-2-enone		trans-Geranic acid

Products of Heated Tea

In Japan, a lower grade of green tea, "ban-cha" is roasted to make its flavor more acceptable. Roasted ban-cha is called hoji-cha. The optimum temperature for roasting is about 180°C. The aroma concentrate from hoji-cha has a strong characteristic roast aroma. Hoji-cha produced about 3 times the aroma concentrate than the original ban-cha. From the basic fraction which comprised 29% of the aroma concentrate, 19 different pyrazines were identified. The neutral fraction which was 47% of the aroma concentrate contained furans and pyrroles along with the original tea aroma. In addition, ionone related compounds such as theaspirone, dihydroactinidiolide and a large amount of β -ionone were found in the neutral fraction. Table III shows the increase of furan and pyrrole content due to roasting of the ban-cha (4).

Pyrazines and pyrroles are generated from amino acids and sugars by heating.

β -carotene, another important component of tea leaf is the precursor of pleasant aromatic compounds. It is present the var. *sinensis* leaves at about 21.7 mg/100 g dry weight.

To clarify the role of β -carotene to the aroma of roasted green tea, β -carotene was heated in a pyrolyzer at 180°C for 6 minutes. The reaction was carried out under air with and without catechin gallates, a component of tea leaves. The volatile products were trapped in a precolumn cooled by dry ice/acetone. The precolumn was then connected to a GC capillary column and the volatiles then analyzed by GC-MS.

Table III. Increase of Furans and Pyrroles during Roasting of Ban-cha to Produce Hoji-cha

	Area % in Aroma Concentrate	
	Ban-cha	Hoji-cha
<u>Furans</u>		
Furfuryl alcohol	0.5	13.7
Furfural	-	9.3
2-Acetylfuran	-	4.7
5-Methylfurfural	0.2	2.9
<u>Pyrroles</u>		
2-Acetylpyrrole	0.9	5.4
2-Formylpyrrole	-	2.6
1-Ethyl-2-formylpyrrole	0.9	4.7
a Pyrrole derivative	0.9	9.1

Ten volatile compounds were produced from the pyrolysis of β -carotene. Among them, toluene, xylene, β -cyclocitral, ionene, β -ionone, 5,6-epoxy- β -ionone and dihydroactinidiolide were identified. The addition of catechin gallates reduced the quantity of the ten

volatiles by about two thirds (5). Ionone related compounds such as β -ionone, 5,6-epoxy-ionone and dihydroactinidiolide were also identified in the aroma concentrate from sen-cha (1).

In another study, β -carotene was heated in aqueous medium at 90°C, 120°C and 150°C. More than 40 different compounds were found in the ether extracts by GC-MS as shown in Figure 3. Dihydroactinidiolide (sweet peachy aroma) was found in highest concentration at all temperatures studied. At 90°C, 5-6-epoxy- β -ionone (sweet, violet-like) was found in second highest quantity, while at 150°C, 2,6,6-trimethyl-2-hydroxy-cyclohexanone (green, citrusy) and 2,6,6-trimethyl-2-hydroxy-cyclohexan-1-aldehyde (floral, geraniol-like) were found in large quantity. At 120°C, these compounds were more evenly balanced than at 90°C or 150°C. A balance of ionone related compounds seem to contribute to an attractive green tea flavor. This data is outlined in Table IV (6).

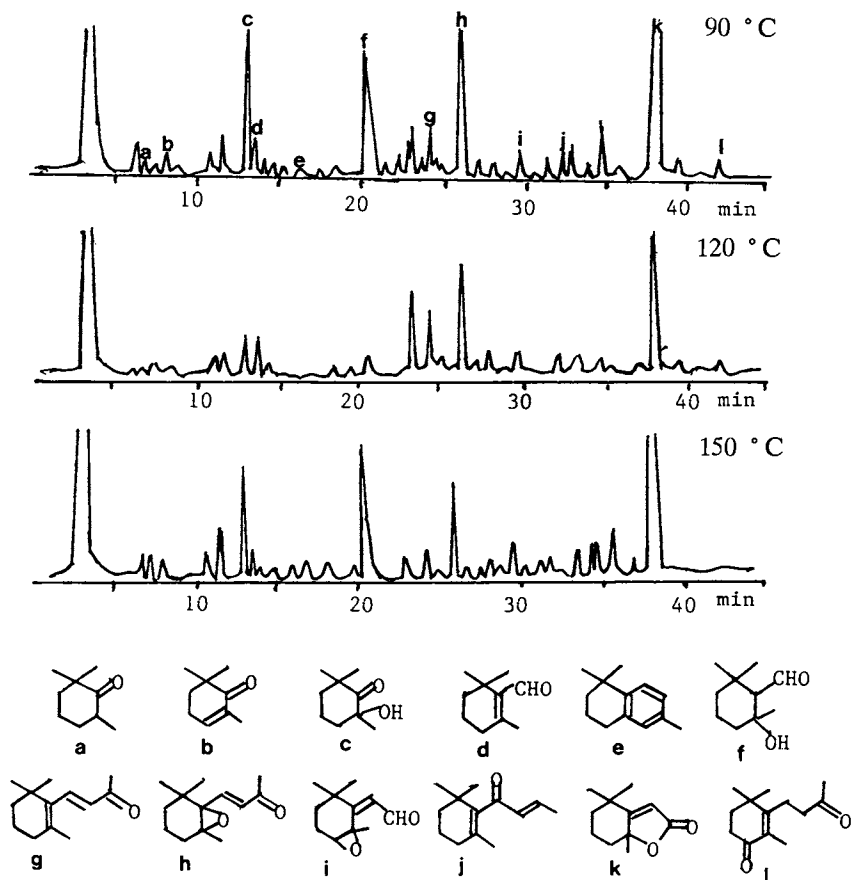


Figure 3. Products of the thermal degradation of β -carotene in aqueous medium. (Data are from ref. 6.)

Table IV. Ionone Related Compounds Identified in the Thermal Degradation of β -Carotene

	90°C	Peak 120°C	Area 150°C	%
Dihydroactinidiolide*	35.5	42.2	45.4	
5,6-Epoxy- β -ionone*	24.3	17.1	9.0	
2,6,6-Trimethyl-2-hydroxy-cyclohexane-1-aldehyde (tentative)*	4.6	2.2	14.9	
2,6,6-Trimethyl-2-hydroxy-cyclohexanone	3.8	3.5	9.2	
β -Ionone*	2.5	7.9	1.5	
2,6,6-Trimethyl-2,3-epoxy-cyclohexliden-1-acetaldehyde	2.5	2.0	2.0	
4-Oxo- β -ionone	1.4	1.5	0.2	
β -Cyclocitral*	0.8	2.9	1.0	
Ionene*	0.2	-	0.5	
β -Damascone	0.2	-	0.5	
2,6,6-Trimethyl-cyclohex-2-enone	0.4	0.1	0.5	
2,6,6-Trimethyl-cyclohexanone	0.2	0.1	0.5	

* Found in pyrolyzed β -Carotene (5)

SOURCE Data are from ref. 6.

The Role of Catechins

Catechins are the most abundant components in tea flush. As previously mentioned, catechins have a bitter, astringent taste. The concentration of individual catechins in tea flush are shown in Table V. The most abundant catechin is (-)-epigallocatechin gallate.

Table V. Catechins in Tea Flush

(-)-Epigallocatechin gallate	(i)	10.7	14.4	% Dry Wt.
(-)-Epicatechin gallate	(ii)	3.3	4.3	
(-)-Epigallocatechin	(iii)	3.2	2.9	
(-)-Epicatechin	(iv)	1.2	1.0	

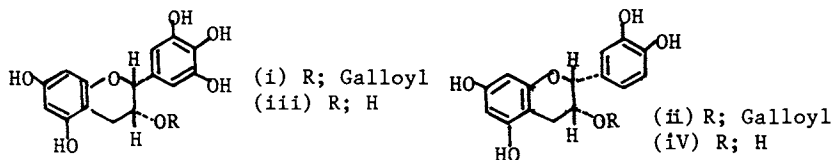


Table VI shows the decrease of catechin by heating as reported by Anan and Kato 1984 (7). The loss of catechins at 70°C is greatly influenced by the addition of amino acids.

Table VI. Changes in Catechin Content During Heating of Catechin-Amino Acid Blend and Catechin Alone

Sample	Time (min) at 150°C	Loss of Catechin (%)			
		(i)	(ii)	(iii)	(iv)
A	30	11.8	36.5	11.7	28.3
	70	61.2	90.4	52.4	80.0
B	30	4.6	19.2	3.8	14.5
	70	6.5	26.9	2.8	38.4

Sample A: Mixture of crude catechin, theanine, glutamic acid, arginine and serine (20:3:1:1:1)

Sample B: Crude catechin alone

Adapted from Anan, T. and Kato, H. (1984) (7)

L-Theanine is the most abundant amino acid in tea flush. Volatiles produced by pyrolysis at 180°C of (A) L-theanine, (B) (-)-epigallocatechin gallate and (C) a mixture of (A) and (B) were examined. The procedure was the same as that reported earlier for the pyrolysis of β -carotene. The results of the GC-MS analysis are shown in Figure 4. From L-theanine alone, a large amount of N-ethyl-formamide was formed, along with ethyl amine, propyl amine, 2-pyrrolidone, N-ethyl-succinimide and 1-ethyl-3,4-dehydro-pyrrolidone.

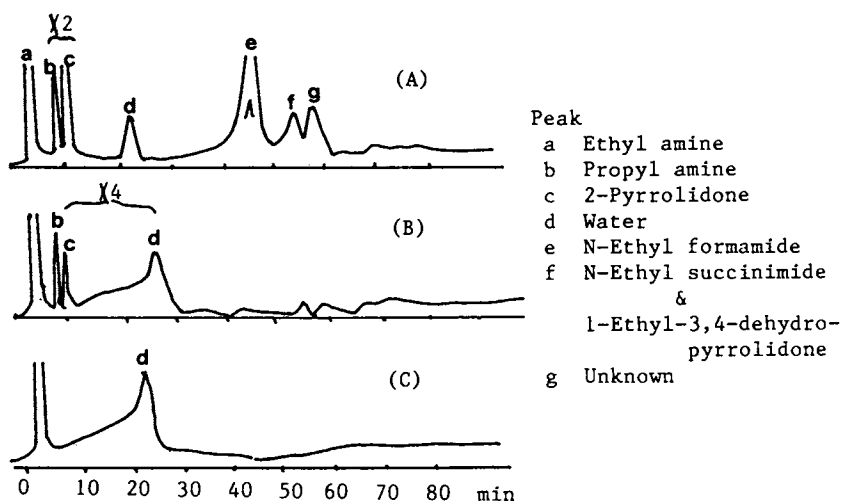


Figure 4. Gas Chromatograms of Thermal Degradation Products from (A) Theanine, (B) Theanine and Epigallocatechin Gallate, and (C) Epigallocatechin Gallate.

GC Conditions; Column SE 30 SCOT, 15 m X 0.5 mm i.d., Column Temp., 30°C(10 min hold) 170°C(3°C/min), Carrier Gas, Helium 3.8 ml/min

The addition of catechins (sample B) greatly reduced these products. Thus catechins show a similar effect on the pyrolysis of β -carotene and L-theanine. (Yamanishi, T. & Hamada, T. unpublished.)

Hara in 1981 (8) reported on volatiles produced by roasting L-theanine and glucose at about 150°C for one hour. 1-Ethyl-3,4-dehydropyrrolidone was the main product. Five pyrroles, three alkyl pyrazines and four furans which were identified by GC-MS and NMR are shown in Table VII. With the exception of 1-ethyl-3,4-dehydropyrrolidone these products are quite different than found from L-threonine alone. Surprisingly, 1-ethyl-3,4-dehydropyrrolidone has never been found in tea aroma.

Table VII. Volatile Compounds Identified from Roasting L-Theanine and D-Glucose

Compounds	Peak Area %
1-Ethyl-3,4-dehydropyrrolidone	41.1
1-Ethyl-5-methylpyrrole-2-aldehyde	9.8
5-Methyl-2-furfuryl alcohol	9.0
2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	8.7
a Pyrrole derivative	5.1
1-Ethylpyrrole	4.0
a Pyrrole derivative	3.6
a Pyrrolidone derivative	3.4
a Pyrrole derivative	2.1
Methylpyrrole	1.5
1-Ethyl-2-acetylpyrrole	1.1
2-Acetylfuran	1.0
5-Methyl-2-furaldehyde	1.0
2,5- (or 2,6)-Dimethylpyrazine	0.8
Trimethylpyrazine	0.4
2-Acetylpyrrole	0.3
2-Furaldehyde	0.3
2-Methylpyrazine	0.2

Adapted from Hara, T. (1981) (8)

CONCLUSIONS

It has been shown that the aroma of tea is affected by the heat treatment received. Tea aroma and flavor are greatly influenced by catechins and proceeds by more than one pathway. The catechins influence tea aroma and flavor in three ways. First, catechins have a bitter, astringent taste. Second in order to reduce the level of soluble catechins (i.e. in var. assamica), a second heat treatment is required. This re-firing produces a stronger roast aroma. Third catechins strongly influence the pyrolysis of β -carotene and L-theanine.

A tea of good quality possesses a balance of tea aroma compounds. This requires control of the heating process. Because

of its complicated nature, further detailed research on the thermally generated aroma of tea is necessary.

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Chapter 30

Natural Precursors of Thermally Induced C₁₃ Norisoprenoids in Quince

P. Winterhalter and P. Schreier

Lehrstuhl für Lebensmittelchemie, Universität Würzburg, Am Hubland,
8700 Würzburg, Federal Republic of Germany

High resolution gas chromatographic (HRGC) and spectroscopic (MS; FTIR; ¹H-NMR) studies of quince fruit constituents revealed the occurrence of several free and glycosidically-bound precursors, which generate C₁₃ norisoprenoids upon thermal treatment. 4-Hydroxy-7,8-dihydro-β-ionol was identified as a natural precursor of the isomeric theaspiranes, the major volatile constituents in quince fruit juice. Four thermally-induced megastigma-6,8-dien-4-ones were identified, and 4-hydroxy-β-ionol was established as their natural precursor. Sugar conjugates that play a principal role as antecedents of C₁₃ norisoprenoids include glycosidically bound 3-oxo-β-ionol, which thermally produces megastigmatrienones. In addition, heat treatment of the conjugate of 3-hydroxy-β-ionol yields bicyclo[4.3.0]nonanes and 3,4-didehydro-β-ionol. The glycoside of 7,8-dihydrovomifoliol was previously substantiated to be thermally degraded to theaspiranes.

Many interesting norisoprenoid aroma compounds have been identified in fruits, vegetables and in particular, tea (1) and tobacco (2). The formation of these flavor-significant components has been attributed to the degradation of higher molecular weight terpenoids, such as carotenoids, by biochemical and nonenzymic reactions in plant tissues (3). These degradations involve cleavage of C₉-C₁₀, C₈-C₉, C₇-C₈ and C₆-C₇ bonds of the polyene chain to produce compounds containing 13, 11, 10, and 9 carbon atoms, respectively. However, our knowledge about the immediate precursors of norisoprenoids and the reactions by which they are formed is rather scarce.

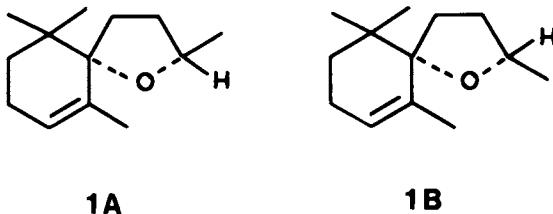
Several volatile C₁₃ norisoprenoids have previously been identified in steam-distilled quince fruit oil, in which they are regarded to contribute to the overall flavor impression. These include isomeric theaspiranes, various bicyclononane derivatives, 3,4-didehydro-β-ionol, and isomeric megastigmatrienones and theaspiranes (4,5). This report concerns the identification of additional norisoprenoids and their natural precursors in quince fruit.

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Isomeric Theaspiranes and their Natural Precursors

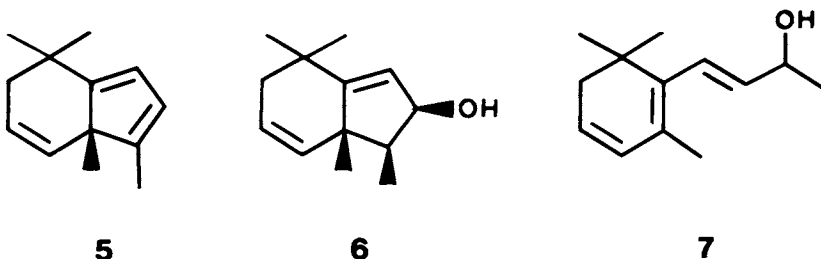
The spiroethers **1A** and **1B** are well-known constituents of several fruit aromas (6-12) and are widely used in the flavor industry (13).



They were identified among the main volatile constituents of quince fruit juice after careful isolation at its natural pH (3.7), employing high-vacuum distillation/solvent extraction (HVD/SE) at 40°C. However, using fruit juice neutralized to pH 7.0 for flavor isolation, HRGC and HRGC-MS revealed only traces of these components (14). These results demonstrate that spiroethers **1A/1B** were originally not present in the intact fruit, but were formed at the natural pH of quince fruit juice after mild heat treatment from an unstable, less volatile precursor. This precursor was identified as 4-hydroxy-7,8-dihydro- β -ionol (**4**). Its synthesis from 4-oxo- β -ionol **2** as outlined in Figure 1, showed coincidence of HRGC and spectral data (MS, FTIR) with those of the constituent isolated from natural quince fruit (Winterhalter, P.; Schreier, P. *J. Agric. Food Chem.*, in press). Prior to this, diol **4** had not been described in the literature. The mechanism of theaspirane formation from the natural precursor **4** can be considered to occur by prototropic dehydration of the corresponding allyl-1,6-diol, as previously described for monoterpene diols by Ohloff *et al.* (15), giving rise to tetrahydrofuran derivatives (Figure 2).

Bicyclo[4.3.0]nonanes, 3,4-Didehydro- β -ionol and their Natural Precursors

Upon employing the more rigorous simultaneous distillation-extraction (SDE) technique (100°C; pH 3.7) to isolate the quince fruit volatiles, the resulting aroma composition distinctly differed from that obtained by HVD/SE. After SDE the hydrocarbon **5**, the bicyclic alcohol **6** and 3,4-didehydro- β -ionol (**7**) were identified as



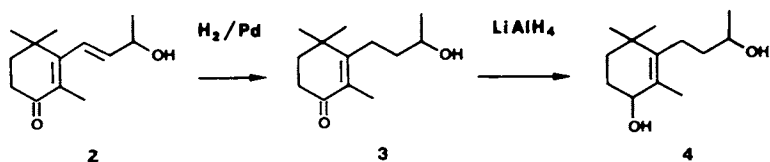


Figure 1. Synthesis of 4-hydroxy-7,8-dihydro-β-ionol (4) from 4-oxo-β-ionol (2) via 4-oxo-7,8-dihydro-β-ionol (3).

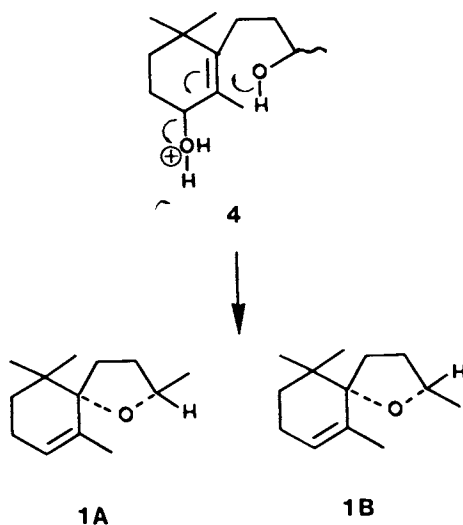
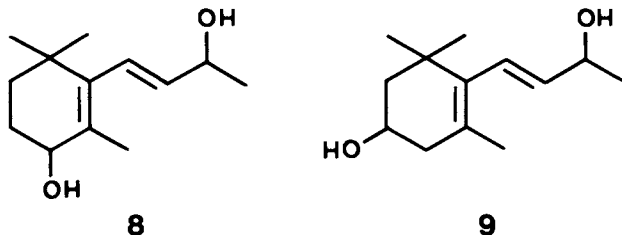


Figure 2. Proposed mechanism for theaspirane 1A/1B formation by prototropic dehydration of 4-hydroxy-7,8-dihydro-β-ionol (4) according to Ohloff *et al.* (Ref. 15).

major volatiles. In smaller amounts, several isomers of 5 with MW 174 and an isomer of alcohol 6 with MW 192 were detected.

Recently, Japanese researchers have demonstrated that the C₁₃ norisoprenoid alcohol 7 has a key role as a flavor intermediate, but information about its natural precursor was not provided (4,5). Potential structures for the precursor of 7 comprise diols 8 and 9; in either case, simple dehydration can afford a double bond in the 3,4-position. Furthermore, the hydroxyl group of 7 could conceivably be glycosidically-bound.



Diols 8 and 9, identified by us in quince fruit for the first time, were synthesized and subjected to thermal processing in model reactions (Winterhalter, P.; Herderich, M.; Schreier, P. *J. Agric. Food Chem.* in press). Accordingly, 4-hydroxy- β -ionol (8) was subjected to thermal degradation under SDE conditions (100°C; pH 3.7), and the results are outlined in Figure 3. In these model reactions, besides a minor quantity of previously-known norisoprenoids 5, 10A/10B and 11A/11B, the majority of degradation products (72%) consisted of the isomeric megastigma-6,8-dien-4-ones 12A-12D. These latter norisoprenoids have not been reported as yet in the literature. Isomer 12B, isolated in purified form by MPLC, showed a weak, long-lasting tobacco note with a cooling effect.

Dienones 12A-12D were also detected as trace components in quince fruit volatiles after SDE sample preparation. However, as shown in Figure 3, except for the low amount of hydrocarbon 5, the distribution of thermal degradation products from 8 did not correspond to the composition of the major C₁₃ norisoprenoids 5-7 obtained after SDE of quince fruit juice. Consequently, diol 8 had to be excluded as their precursor.

In a further series of experiments, model reactions to thermally-degrade 3-hydroxy- β -ionol (9) were carried out. The results of these studies are represented in Figure 4. In these model reactions, compounds 5, 6 and 7 as well as unidentified isomers of 5 and 6 were all found in amounts very similar to the natural quince flavor composition obtained by SDE conditions. However, as shown in Figure 4, additional products were found comprising the megastigmatrienols 13, 14 and the tentatively-assigned bicyclic alcohol 15. These latter compounds were not detectable in quince fruit juice. Thus, the diol 9 came under question as a possible precursor.

One explanation for this surprising result is that the diol 9 is present in quince fruit in both the free and bound forms. To verify this, the glycosides in quince fruit were isolated by XAD

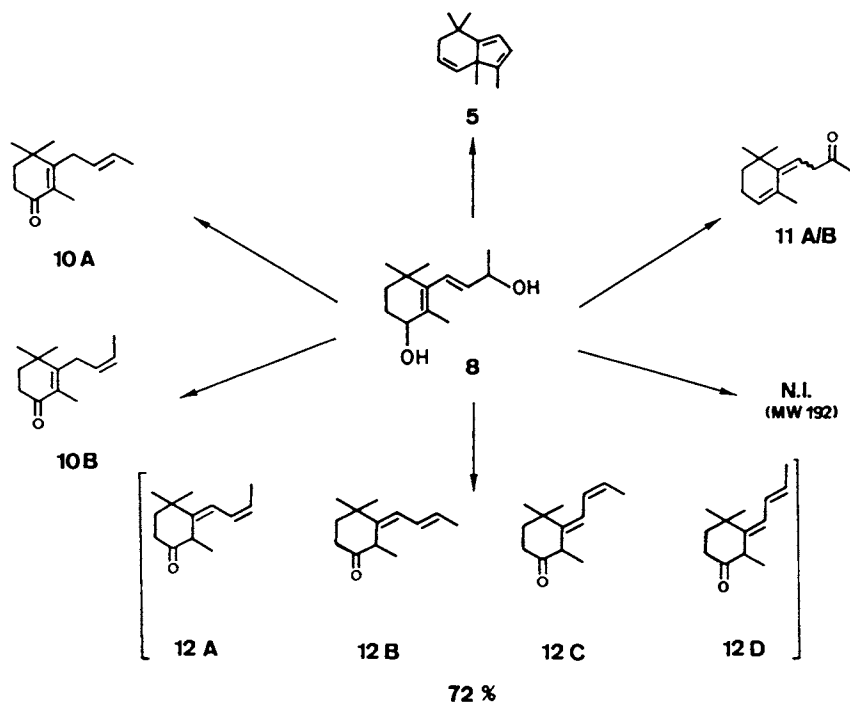


Figure 3. Structures of thermal degradation products of 4-hydroxy- β -ionol (8) under SDE conditions (100°C; pH 3.7). 5 = 2,2,6,7-tetra-methylbicyclo[4.3.0]nona-4,7,9(1)-triene; 10A/10B = E- and Z-megastigma-5,8-dien-4-ones; 11A/11B = isomeric retro- α -ionones; 12A-12D = isomeric megastigma-6,8-dien-4-ones; N.I. = not identified.

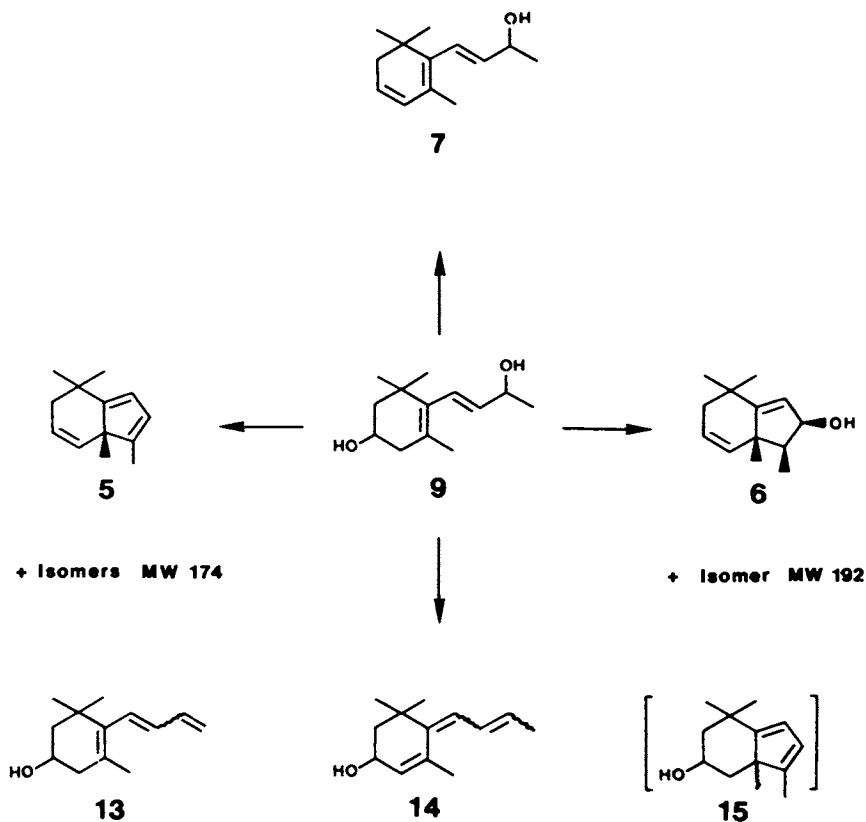


Figure 4. Structures of thermal degradation products of 3-hydroxy- β -ionol (9) under SDE conditions (100°C; pH 3.7). 5 = cf. Fig. 3; 6 = 2,2,6,7-tetramethyl-bicyclo-[4.3.0]nona-4,9(1)-dien-8-ol; 7 = 3,4-didehydro- β -ionol; 13 = megastigma-5,7,9-trien-3-ol; 14 = megastigma-4,6,8-trien-3-ol; 15 = 2,2,6,7-tetramethyl-bicyclo[4.3.0]nona-7,9(1)-dien-4-ol.

adsorption and methanol elution. The glycosidic extract was then subjected to SDE (100°C; pH 3.7) and the volatiles formed were analyzed by HRGC and HRGC-MS. The results obtained in this experiment are represented in Figure 5. First, it has to be emphasized that a similar composition of C₁₃ norisoprenoid products was obtained as found after SDE treatment of quince fruit juice. In addition, marmelo ether (16) and marmelo lactone (17) were also identified. These results suggested that the thermally-induced C₁₃ norisoprenoids found in quince fruit originated from a sugar conjugate of diol 9 as a precursor. To confirm this hypothesis, the glycosidic extract of quince fruit was further subjected to enzymatic hydrolysis using commercial emulsin as a glycosidase. This led to liberation of 3-hydroxy- β -ionol (9) as the major aglycone. In addition to 9, other glycosidically-bound C₁₃ norisoprenoids were identified, including 3-hydroxy- β -ionone (18), 3-oxo- α -ionol (19), 3-hydroxy-7,8-dihydro- β -ionol (20), vomifoliol (21) and 7,8-dihydrovomifoliol (22) (Figure 6).

Megastigmatrienones and their Natural Precursors

Among the aglycones shown in Figure 6, 3-oxo- α -ionol (19) played a role as a precursor of other C₁₃ norisoprenoids detected in quince fruit after SDE sample isolation. As outlined in Figure 7, the keto-alcohol 19 is known to be degraded to the isomeric megastigmatrienones 23A-23D and 24A/24B (16,17) after thermal treatment under acidic conditions.

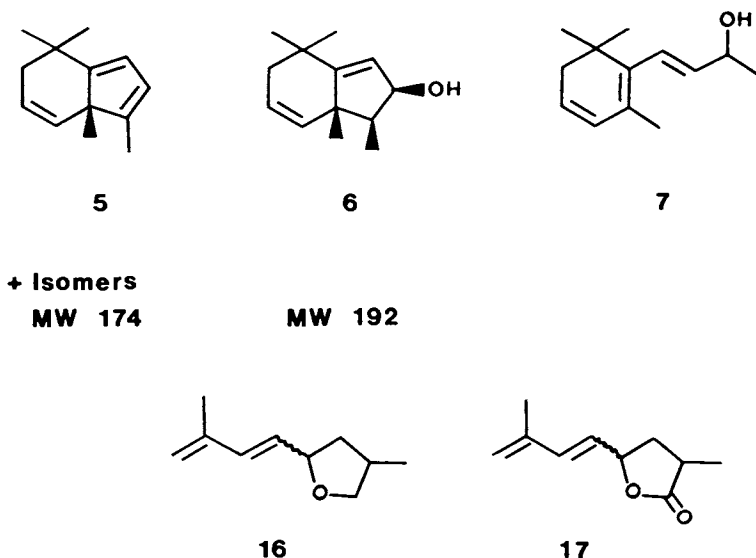


Figure 5. Major volatiles formed from glycosidic extract from quince fruit after SDE treatment (100°C; pH 3.7). 5, 6, 7 = cf. Fig. 4; 16 = marmelo ether; 17 = marmelo lactone.

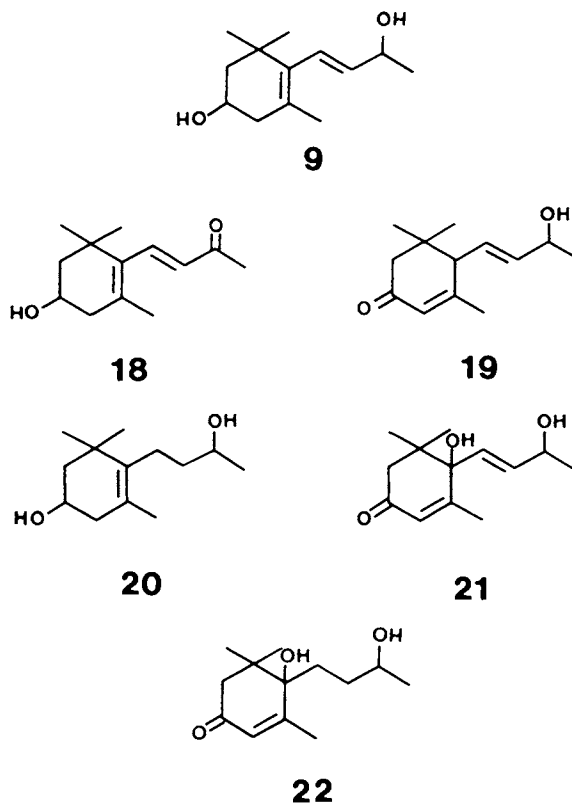


Figure 6. Structures of aglycones released from quince fruit extract after glycosidase (emulsin) treatment. 9 = 3-hydroxy- β -ionol; 18 = 3-hydroxy- β -ionone; 19 = 3-oxo- α -ionol; 20 = 3-hydroxy-7,8-dihydro- β -ionol; 21 = vomifoliol; 22 = 7,8-dihydrovomifoliol.

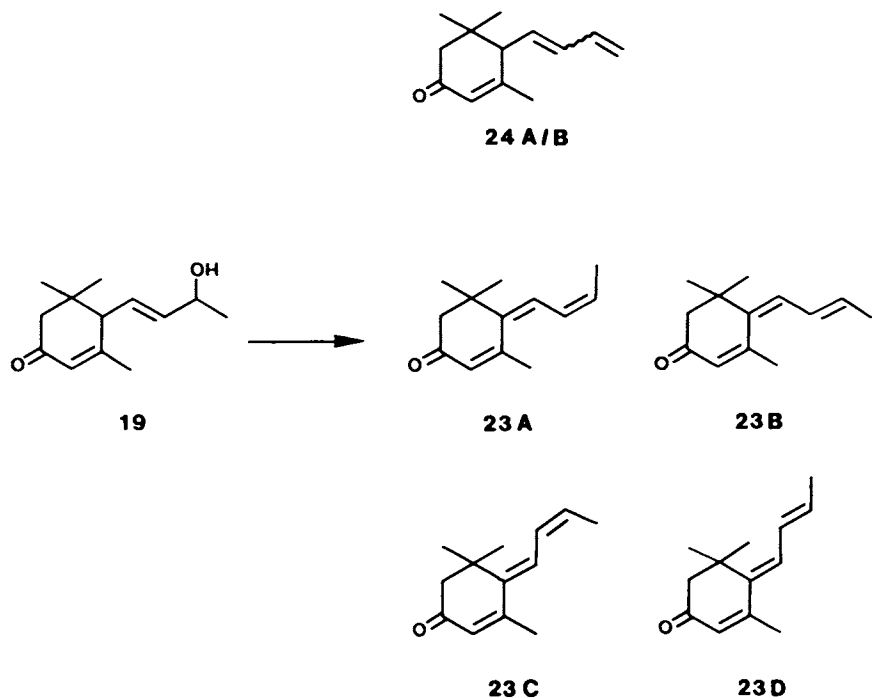


Figure 7. Structures of thermal degradation products of 3-oxo- α -ionol (19) under SDE conditions (100 °C; pH 3.7). 23A–23D = isomeric megastigma-4,6,8-trien-3-ones; 24A/24B = isomeric megastigma-4,7,9-trien-3-ones. (Redrawn from ref. 16 and 17.)

Isomeric Theaspirones and their Natural Precursors

Another C₁₃ norisoprenoid aglycone in quince fruit, 7,8-dihydrovomifoliol (22) (cf. Figure 6), can be considered to be the precursor of theaspirones, which were previously found in steam-distilled quince fruit oil (4). As outlined in Figure 8, a synthetic sequence from α -ionone via dehydrovomifoliol (25) and vomifoliol (21) leads to 7,8-dihydrovomifoliol (22), from which the isomeric theaspirones 26A/26B are formed after thermal treatment (18).

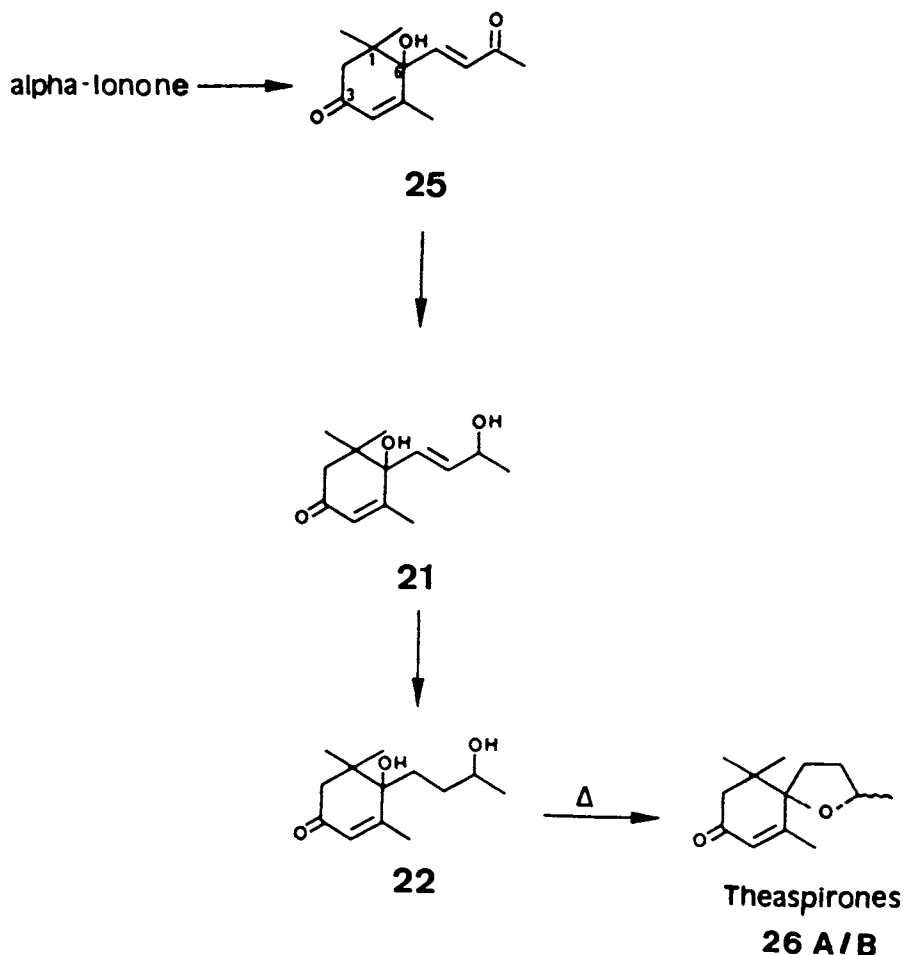


Figure 8. Synthesis of theaspirones 26A/26B from α -ionone via dehydrovomifoliol (25), vomifoliol (21) and 7,8-dihydrovomifoliol (22). (Redrawn from ref. 18.)

Acknowledgments

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Chapter 31

Thermally Degraded Flavors in Citrus Juice Products

S. Nagy¹, R. L. Rouseff², and H. S. Lee¹

¹Citrus Research and Education Center, Florida Department of Citrus,
700 Experiment Station Road, Lake Alfred, FL 33850

²Citrus Research and Education Center, University of Florida,
700 Experiment Station Road, Lake Alfred, FL 33850

Undesirable flavors in citrus juice products are produced by thermal processing and prolonged product storage. Substrates responsible for thermally degraded flavors are essential oils, phenolic acids, sugars, lipids, ascorbic acid and sulfur-containing compounds. Identified detrimental flavor compounds originating from these substrates include 4-vinyl guaiacol, α -terpineol, 1,4-cineole, p-cymene, p-cymen-8-ol, α ,p-dimethylstyrene, 2,5-dimethyl-4-hydroxy-3(2H)-furanone, dimethyl sulfide and trans, trans-2,4-decadienal. Heated and cardboard (oxidized) flavors have been recognized in single-strength and concentrated juices, but the causative agents have not been identified.

Flavor degradation is the single most important factor in quality loss of citrus juice products. The overall flavor degradation is a complex series of chemical reactions involving different substrates, reaction rates, z-values and pH. Changes occurring in citrus juice during thermal processing and storage may be considered as loss of original flavor and development of flavors foreign to freshly expressed juice (1). During the past 40 years, several theories have been proposed to explain origins and causes of thermally degraded flavors in citrus juices: (a) changes in essential oil constituents, (b) changes in lipid constituents, (c) formation of phenolic and sulfur-containing compounds, and (d) by-products of nonenzymic browning from sugars, ascorbic acid and amino acids (2). Additionally, cardboard off-flavor (COF) and

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heated off-flavor have been recognized for many years; however, the compounds responsible for these off-flavors remain to be identified.

Substrate Categories Producing Off-flavors

Essential Oils. Boyd and Peterson (1) stated that the presence of volatile oils in orange juice imparted a pleasant aroma and contributed to flavor. However, under certain conditions, the presence of small amounts of oil yielded objectionable off-flavors during prolonged storage. Blair et al. (3) suggested that the acid of the juice promoted a series of hydration-dehydration reactions with peel oil terpenes. d-Limonene, the principal component of peel oil, was thought to undergo these reactions to produce hydrocarbons, alcohols, ethers, polyhydroxy compounds and other products. The constituent oxygen of these newly formed oxygen-containing compounds was derived from water by chemical addition, and not from dissolved oxygen by oxidation.

Table I is a summary of thermally degraded compounds derived from essential oil constituents. Off-flavor notes derived from some of the compounds have generally been described as "terebinthine" (terpentine-like) or "terpeney." When Askar et al. (8,9) added limonene and linalool to a model juice, decreases of both these compounds were temperature dependent. Both compounds degraded to α -terpineol and cis-1,8-p-menthane-1,2-diol, while linalool also degraded to nerol and geraniol (Table I). Although juice contains much less linalool relative to limonene, the linalool molecule is more reactive and produces most of the α -terpineol. Tatum and coworkers (4) showed that 2.5 ppm α -terpineol imparted a stale, musty or piney note when added to freshly squeezed orange juice. Cis-1,8-p-menthane-1,2-diol forms from α -terpineol by acid-catalyzed hydration (4); it, in turn, undergoes further transformations to form 1,8-cineole and 1,4-cineole. Blair et al. (3) attributed the terebinthine odor to the cineoles and secondarily to terpene hydrocarbons isomeric with limonene. Since 1,4-cineole is produced at high acidity and increased storage temperatures, it is thought to be responsible for the typical pungent, camphoraceous off-flavor of storage-abused orange juices. Citral (mixture of neral and geranial) in lemon and lime juice beverages undergoes a series of acid-catalyzed hydration-dehydration reactions leading to the formation of an isomeric mixture of p-mentha-1,5-dien-8-ol (6,7) and p-mentha-1(7),2-dien-8-ol. These two compounds further degrade by rearrangement and dehydration to three malodorous compounds, namely, p-cymen-8-ol, p-cymene and α ,p-dimethylstyrene (7). Additionally, Ikeda et al. (10) and Shaw (11) found that in lime and lemon oil, γ -terpinene is converted to the off-flavor compound p-cymene. Structures of selected off-flavor notes are presented in Figure 1.

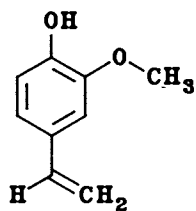
Lipid constituents. Early studies on off-flavor development in citrus products suggested that off-flavors might be related to the degradation of lipids (12-14). Curl (15) compared the flavors before and after storage of whole tangerine juice and tangerine

Table I. Thermally Degraded Compounds Derived from Essential Oil Constituents

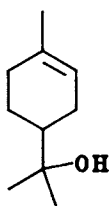
Precursor	Derived Compounds	Flavor Response	Reference
d-Limonene	α -terpineol	stale, musty, piney	<u>3,4</u>
Linalool	α -terpineol	stale, musty, piney	<u>5,8,9</u>
	nerol	sweet, rose fruity	
	geraniol	sweet, floral rose	
α -Terpineol	cis-1,8-p-menthane-1,8-diol	sweet, camphoraceous	<u>3,5</u>
Cis-1,8-p-menthane-1,8-diol ^a	1,8-cineole	pungent, camphoraceous	<u>3,4</u>
	1,4-cineole		
Citral ^b	p-mentha-1,5-dien-8-ol	not characterized	<u>6,7</u>
	p-mentha-1(7),2-dien-8-ol	not characterized	
	<u>cis</u> -p-mentha-2,8-dien-1-ol	not characterized	
	<u>trans</u> -p-mentha-2,8-dien-1-ol	not characterized	
p-Mentha-1,5-dien-8-ol		nonspecified	
+	p-cymen-8-ol	off-flavor	<u>6,7</u>
p-Mentha-1(7),2-dien-8-ol	p-cymene	terpene off-flavor	
	α -p-dimethylstyrene	terpene off-flavor	
γ -Terpinene	p-cymene	terpene off-flavor	<u>10,11</u>

^a Also known as cis terpinol

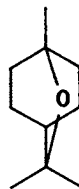
^b Isomeric mixture of neral and geraniol



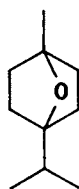
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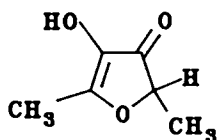
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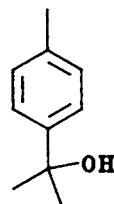
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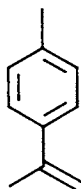
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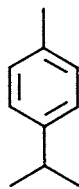
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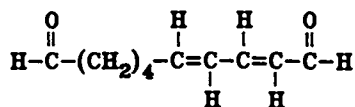
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I

Figure 1. Chemical structures of selected off-flavor notes of citrus juice products. (A) 4-vinyl guaiacol, (B) α -terpineol, (C) 1,8-cineole, (D) 1,4-cineole, (E) 2,5-dimethyl-4-hydroxy-3(2H)-furanone, (F) p-cymen-8-ol (G) α ,p-dimethylstyrene, (H) p-cymene, (I) trans,trans-2,4-decadienal.

juice from which the suspended matter, which includes the lipids, had been removed. He concluded that the suspended matter was responsible for much of the characteristic flavor but also contained precursors of the storage-developed off-flavors. Nagy and Nordby (16) studied the effects of storage temperatures and duration on the different lipid classes of single-strength orange juice, and found extensive degradation of phospholipids. Examination of TLC profiles of the phospholipid fraction did not show formation of any lyso-derivative. They concluded, therefore, that both fatty acids association with the phospholipid molecule were split off.

Table II. Weight of Free Fatty Acids as a Function of Storage Time and Temperature

Time (mo.)	Weight (mg/15 g dried citrus juice powder)							
	4 °C				29 °C			
	16:0 ^a	18:1	18:2	18:3	16:0	18:1	18:2	18:3
0	1.3	1.3	1.1	0.6	1.3	1.3	1.1	0.6
3	2.0	1.9	1.7	0.9	4.5	4.1	4.3	2.4
5	2.3	2.0	2.0	1.1	5.7	5.3	5.7	3.3
11	3.3	2.7	2.7	1.4	8.7	7.6	9.1	4.5
15	3.5	3.4	3.0	1.6	9.1	8.8	10.1	5.6
16	4.0	3.3	3.4	1.8	9.1	8.8	10.2	5.8

^a Number of carbons in chain: number of double bonds
Source (16)

Table II lists the increases for the four major fatty acids in the free fatty acid fraction. Collectively, palmitic (C_{16:0}), oleic (C_{18:1}), linoleic (C_{18:2}) and linolenic (C_{18:3}) comprise greater than 88% of all fatty acids in orange juice (17). For 4°C stored juice, the four acids increased about three-fold. In juice stored at 29°C, free palmitic and oleic acids increased about seven-fold, whereas linoleic and linolenic increased about nine-fold. Fatty acids from the phospholipid fraction are not off-flavored as such but are thought to constitute an unstable system which ultimately leads to the production of substances imparting a rancid note to juice flavor.

Free, long-chain unsaturated fatty acids, as found in stored citrus juice, are important precursors of many volatile off-flavor compounds, namely, alk-2,4-dienals, 2-octenal and n-hexanal (18). Askar and co-workers (8) showed that storage of orange juice resulted in an increase in n-hexenal, n-hexanal, n-octanal and n-decanal. Moshonas and Shaw (19) isolated trans, trans-2,4-decadienal in citrus oils and suggested that it might have been produced by oxidative breakdown of long chain, unsaturated fatty acids. Although some products of lipid

oxidation contribute to flavor deterioration, Nagy (20) concluded that these compounds were not the major off-flavor components formed during short-term, high temperature storage of citrus juice products.

Phenolic compounds. Thermal degradation of various types of phenolic compounds has been shown to produce undesirable off-flavor compounds. Tatum et al. (4) identified 4-vinyl guaiacol as the most detrimental off-flavor compound in aged orange juice. When added to freshly expressed orange juice at a level of 0.075 ppm, it imparted an old fruit or rotten flavor. Substantial amounts of 4-vinyl guaiacol, from 0.6 to 1.6 ppm, were found in orange juice stored for 12 weeks at 35°C by Tatum et al. (4).

4-Vinyl guaiacol can be formed by thermal decarboxylation of ferulic acid (21,22). Naim et al. (23) incubated orange juice with added ferulic acid and noted an accelerated production of 4-vinyl guaiacol concomitant with a reduction in aroma quality. In freshly expressed orange juice, minor amounts (about 0.20 ppm) of free ferulic acid were found (23). This minor amount, however, is probably sufficient to produce 4-vinyl guaiacol at levels above its sensory threshold of 0.05 ppm (4). Most ferulic acid in orange juice is found in bound forms, such as glycosides, esters and amides (23-25). Acidity, thermal processing and subsequent high-temperature storage provide ideal conditions for the release of ferulic acid from its bound forms. Naim et al. (23) and Peleg et al. (Proc. Int. Soc. Citriculture, in press) clearly demonstrated the release of ferulic acid from its bound forms in model systems of orange juice, and its contribution to the production of the objectionable 4-vinyl guaiacol.

In storage tests with commercially bottled orange juice, Lee and Nagy (unpublished data) detected amounts of 4-vinyl guaiacol up to 0.90 ppm. The concentration fluctuated, however, and appeared to form and degrade with increasing storage temperature (Figure 2). In hermetically sealed containers, 4-vinyl guaiacol might undergo polymerization rather than oxidatively degrade to other compounds. Klaren-Dewite and co-workers (26) showed formation of 4-vinyl guaiacol oligomers during thermal decarboxylation of ferulic acid.

Sulfur-Containing Compounds. Volatile sulfur compounds have only attracted minor attention as possible off-flavor compounds in heated citrus products. Hydrogen sulfide, methanediol, sulfur dioxide, carbonyl sulfide, dimethyl sulfide and dimethyl disulfide have been detected in headspace gases of citrus juices (27-29). Sawamura et al. (29) attributed the characteristic off-flavor of mandarin juice to dimethyl sulfide. Shaw and Wilson (28) suggested that the high levels of dimethyl sulfide found in commercially canned orange and grapefruit juices might be an important contributor to off-flavor. Hydrogen sulfide, present in fresh citrus juice (27), is increased by heating and storage and tends to react with aldehydes to produce thioaldehydes. Reaction with n-hexanal, 2-hexenal, 2-octenal, 2-nonenal, neral and

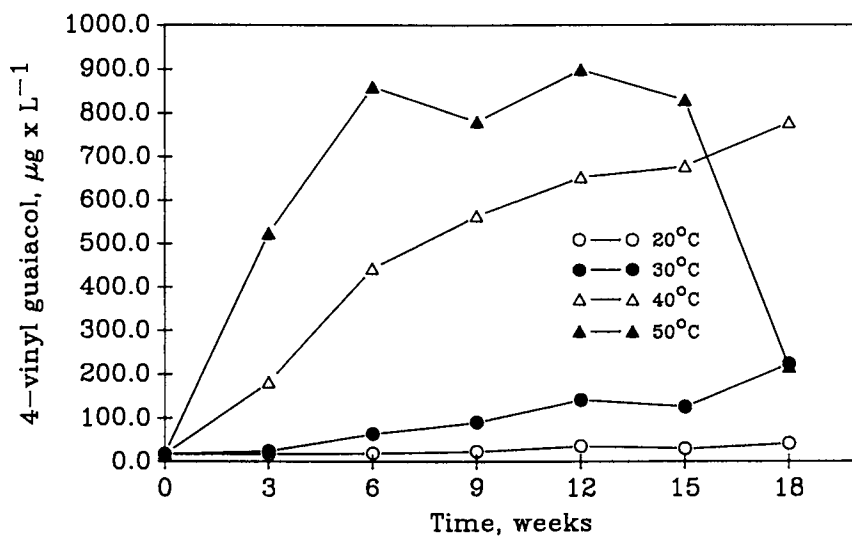


Figure 2. Influence of storage conditions on 4-vinyl guaiacol formation in bottled orange juice.

geranial (all present in citrus juice) produces compounds possessing onion-like aromas. One reactive aldehyde, furfural, is virtually absent in freshly processed juice but increases to significant amounts during high-temperature storage. Since hydrogen sulfide and furfural form during storage, it has been suggested that these two components spontaneously interact to form thiofurfural which imparts a skunky-like aroma (2).

By-Products of Nonenzymic Browning. Volatile products of nonenzymic browning reactions have been classified into three groups (30): (a) simple sugar dehydration/fragmentation products, (b) simple amino acid degradation products, and (c) volatiles produced by further interactions. Table III lists compounds formed during nonenzymic browning of citrus products and from model systems simulating orange juices. A large number of these compounds arise through deteriorative reactions involving sugars, amino acids and ascorbic acid which is present in citrus. Heat-induced degradation leads to formation of furans, pyrones, cyclopentanones, carbonyls, pyrroles and acids which possess objectionable flavor qualities at low concentrations. Most have odors typically described as caramel-like and burnt sugar-like.

Furans appear to largely determine the overall odor of processed citrus products. Twelve furan derivatives have been identified in citrus products and model systems (Table III). From a sensory point, furan derivatives are considered very important aroma constituents. These generally impart sweet, fruity, nutty or caramel-like odor impressions (31). Hydroxymethyl furfural (HMF) was the main furan compound observed in browned citrus juice powder (32), and was also the main carbonyl compound that had increased during storage of grapefruit juices at high temperatures (33). Sensory properties of HMF were described as hay-like and caramel (31); however, it has limited use in correlating flavor change in stored orange juices because of its high aroma threshold value (200 ppm). In stored citrus juice, this level is seldom attained (34).

Another predominant furan, namely furfural, is described as sweet and bread-like caramellic. This furan can interact with hydrogen sulfide of juice to produce thiofurfural, a compound with a skunky odor (35). Furfural has an important role in the monitoring of citrus juice quality (36), and has an especially significant relationship to browning (34). The main source of furfural in aged citrus products is by oxidative degradation of ascorbic acid. Furans, such as deoxyfuroin, furoin and furil are probably formed by self-condensation of furfural (37).

The odor of isomaltol is described as sweet, but with a burnt sugar and fruity character (31). 2-Acetylfuran and 2-hydroxyacetylfuran impart burnt sweetish flavors. Furfuryl alcohol and 5-methyl-2-furaldehyde have sweet caramellic flavors (31). The major formation route to most furans in heated-abused citrus products has been postulated as acid-catalyzed enolizations and dehydrations of sugars (38). Furans, such as 2-hydroxyacetylfuran and isomaltol

Table III. Browning Degradation Products Identified in Stored Citrus Products and Model Systems

ACIDS

Acetic acid
Formic acid
Levulinic acid

CYCLOPENTANONES

Methylcyclopentenolone

FURANS

2-Acetyl furan
Furfural
Furfuryl alcohol
2-Hydroxyacetyl furan
5-Hydroxymethyl furfural
Isomaltol
5-Methyl-2-furaldehyde
2-Furoic acid
2,2-Difurylmethane
Deoxyfuroin
Furoin
Furil

FURANONES

α -Angelica lactone
 β -Angelica lactone
2,5-Dimethyl-4-hydroxy-3(2H)-furanone
 γ -Butyrolactone

KETONES

Acetylformoin

PYRANONES

2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one
3-Hydroxy-2-pyrone

PYRROLES

2-Acetylpyrrole
N-Ethylpyrrole-2-carboxyaldehyde
5-Methylpyrrole-2-carboxyaldehyde

Adapted from Shaw et al. (36,37) and Tatum et al. (4,45,48).

(3-hydroxy-2-acetylfuran) are probably derived via a less favorable 2,3-enolization reaction (39).

Furanones and pyranones are oxygen-containing heterocyclic compounds associated with caramelized flavor notes. Most common flavors of these compounds are caramel-like, sweet, fruity and burnt (40). 2,5-Dimethyl-4-hydroxy-3(2H)-furanone (DMHF) was one

of the malodorous compounds detected in aged orange juice (4). The aroma of DMHF is pineapple-like, and is not considered too detrimental. However, it can mask the fresh orange-like aroma at levels above 0.05 ppm. Shu et al. (41) noted that DMHF is a precursor of various flavor materials when reacted with amino acids at high temperatures. Many acyclic carbonyl and 3(2H)-furanone derivatives can be formed as primary and secondary degradation products during thermal degradation of DMHF (42). Thus, the potential role of DMHF as a flavor impact compound and as an intermediate to form additional flavor compounds in heat-abused citrus products should be carefully considered. 2,3-Dihydro-3,5-dihydroxy-6-methyl-4-H-pyran-4-one was also isolated from dehydrated orange juice (37) and is known as a novel nonenzymic browning reaction product. This pyranone has a high threshold value, over 200 ppm (38), and appears to possess negligible odor character.

Furanones and pyranones constitute a family of compounds that derive from a common α -dicarbonyl intermediate, namely, $\text{CH}_3\text{-CO-CO-(CHOH)}_n\text{-CH}_2\text{R}$ (where R=H or OH). Acetylformoin, which has a fruity-caramel² aroma, has been isolated from browned instant orange juice (43) and from a sugar model system containing a catalyst (38). It can be formed by dehydration and cyclization of methyl dihydroxy-ethyl reductone (enediol of the methyl dicarbonyl intermediate), but it decomposes easily in air to pungent compounds (44).

Methylcyclopentenolone has a strong caramel-like, maple-like or licorice-like aroma (31), and was found in browned, dehydrated orange juice at the 1 ppm level (43). Its synergistic flavor effect with other compounds, such as 5-methyl-2-furfural and N-ethylpyrrole-2-carboxyaldehyde, was reported (43). Although it is present at levels five times below its threshold value, it still impacts on the heat-abused flavor of dehydrated orange juice. This ketone probably results from amine-assisted sugar degradation (45).

Pyrroles are nitrogen-containing heterocyclic compounds, and three pyrrole derivatives have been identified in stored instant orange juice (46). There are less of these compounds than other heterocyclic compounds, possibly due to their stability on heating (47). Pyrroles would be formed by thermal reaction of sugars with amino acids and by thermal reaction of furan derivatives with α -amino acids (48). Shaw and Berry (45) proposed that 2-acetylpyrrole might be formed from a 3-deoxy hexosulose derivative of fructose through Strecker degradation and subsequent enolization and dehydration reactions. Similarly, 5-methylpyrrole-2-carboxyaldehyde could be formed through enolization, Strecker degradation, cyclization and dehydration of hexosulose-3-ene from fructose. N-ethylpyrrole-2-carboxyaldehyde is probably formed by some mechanism other than that involving Strecker degradation.

Off-flavors From Nonidentified Compounds

Cardboard (Oxidized) Flavor or COF. This thermally initiated off-flavor, first noted in the early 1950s, was described by early

investigators as a castor oil (aroma), tallowy (mouth feel) and/or cardboard flavor that occurred from time to time in juices prepared from concentrate. It was observed in concentrated orange and grapefruit juices after two days to several weeks frozen storage. It occurred most often with early season fruit. Extreme container to container variation was observed, i.e., the intensity of this off-flavor varied considerably in samples from the same evaporator run (concentrating process). Moreover, the off-flavor tended to disappear after prolonged frozen storage. Finally, this flavor note was encountered most frequently in products which were relatively bland in aroma and was not easily perceived when the full aromatic components of citrus were present in relatively high concentrations. Orange or grapefruit cold-pressed oil is commercially added to the respective concentrate to "mask" this off-flavor. Chemical interactions between components in the oil and the component(s) responsible for this off-flavor might explain the disappearance of this flavor.

Two related hypotheses have been suggested to explain the occurrence of this off-flavor. Blair and co-workers (50) suggested that tallowiness and malodor are of biochemical origin and represent stages in the biosynthesis of lipid from sugar. They suggested that the specific causative agents were products from dehydration rather than oxidation and were α , β -unsaturated aldehydes.

Olsen et al. (51) assumed that cardboard off-flavor was due to oxidation of components in the concentrated juice. They proposed calling this "citrus oxidized flavor" or COF and concluded that COF could be avoided by maintaining the peel oil content above a certain level, based on the observation that 10 of 16 commercial samples which exhibited COF had very low peel oil levels. It was also recommended that care be taken to avoid the incorporation of air during the manufacture of concentrate. The exact nature of the substances oxidized and the mechanism of oxidation remain to be determined.

Heated Off-Flavor. Heated or cooked off-flavor is attributed to the thermal abuse of citrus juices. Citrus juices are very susceptible to this flavor defect and are accordingly heated only enough to thermally inactivate most of the pectinesterase enzymes rather than undergo complete pasteurization. This flavor defect is observed most commonly in canned citrus juices which are hot-filled and cooled slowly or in single-strength juices reconstituted from concentrate. Reconstituted juices have been heated twice, once during the concentration process and again after being diluted with water before filling.

Very limited information has been published on this subject. Using a sensory panel which employed quantitative descriptive analysis, Rouseff (21) was able to demonstrate that heated off-flavor was the major quality factor in determining the perceived quality of the juice. Some flavor descriptors used in this study are shown in Figure 3. Panelists evaluated each flavor descriptor using a 10-cm line anchored with weak and strong on the ends, and overall quality, previously defined using a 100-point

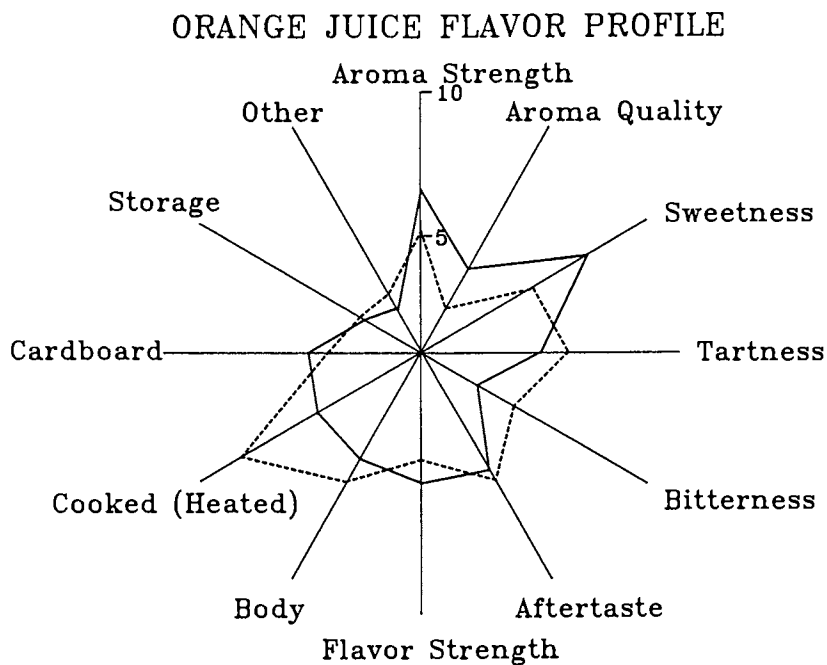


Figure 3. Flavor profiles of commercial single-strength orange juices: canned (-----), chilled (——).

scale. The juice with the dashed line (Figure 3) was a commercial canned single-strength orange juice and that with the solid line was a sample of commercial chilled orange juice. The canned juice was perceived as being less sweet, more bitter and tart, and having a more intense heated flavor. The overall quality rating for the chilled juice was 35, whereas that for the canned juice was 22. Using multivariate statistics to evaluate the data from all the juices, Rouseff (52) found that the heated flavor accounted for about 65% of the total variance with respect to overall quality. Thus, heated off-flavor is extremely important to the perceived quality of citrus juices. Unfortunately, the specific causative agent(s) for this off-flavor have yet to be identified.

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Chapter 32

Aroma Composition of Canned Black Truffles

T. Talou, M. Delmas, and A. Gaset

Laboratoire de Chimie des Agroressources, Ecole Nationale Supérieure
de Chimie, Institut National Polytechnique de Toulouse, route de Narbonne
31077 Toulouse Cédex, France

The volatile constituents of canned Black Perigord Truffles (Tuber Melanosporum) were analysed by dynamic headspace concentration gas chromatography - mass spectrometry. A total of 36 compounds were identified and described for the first time as canned black truffle aroma constituents. The modification of flavor and the possible formation of the compounds due to the heating treatment are discussed.

Black Perigord truffles (Tuber Melanosporum) are underground mushrooms that grow in symbiosis with certain trees, especially oaks. One finds them in Europe, particularly in France where their aroma is very much appreciated by gourmets. Due to their limited harvesting season (mainly during winter), truffles must be thermally processed for culinary use through the year.

We have previously carried out studies on fresh black truffle aroma by strip and trap / dynamic headspace analysis. Experiments were carried out with truffle flesh (1,2), entire truffles (Talou, T. et al., Proc. 19th International Symposium on Essential Oils and other Natural Substrates, in press; Talou, T. et al, J. Sci. Food Agric., in press) and by atmospheric capture of stored truffles aroma (Talou, T. et al, Proc. 3rd Chemical Congress of North America, in press).

Since cooks report that processed black truffles have a particular aroma which is different and stronger than fresh truffle aroma, we decided to investigate the volatiles of canned black truffles.

For this purpose, we developed a modified dynamic headspace technique for the analysis of truffle flesh and the aromatic liquid released during cooking.

The aim of this study was to identify the volatiles of canned black truffles by capillary gas chromatography - mass spectrometry in order to assess the modification of aroma due to the thermal processing.

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Experimental.

Material. Fresh Black Perigord Truffles (Tuber Melanosporum) were purchased by Pebevre Ltd. (Cahors, France), a company specialized in truffle marketing. These truffles were collected mainly in the South East of France. They were fully ripe (3) and released their characteristic aroma. The truffles were received the day after gathering in wicker-baskets, and then stored in cold storage.

After brushing and additional hand-sorting, truffles were placed in 1/8 cans (100 mL) and then industrially processed by heat treatment at 115°C for 90 min. The weights of analysed canned truffles ranged from 30 to 50 g.

Sample Preparation. During the thermal processing, truffles released large amount of water (25% of their weight). The highly aromatic juice obtained, called truffle juice, was characteristically flavoured.

Sampling was carried out immediately after the can was opened on both truffle flesh and juice.

Headspace Sampling Technique. The method used a new gas chromatographic desorption - concentration - GC introduction device (D.C.I.) based on dynamic headspace analysis and available from Delsi Instruments (Paris, France). This apparatus made it possible to isolate volatiles from both solid and liquid samples (4).

In the case of the truffle juice, a 1 mL sample was put in a washing bottle topped by a condenser which was kept at 6°C by circulation of cold water. Above this was located a fixed Tenax trap (0,2 g Tenax GC, 60-80 mesh) packed into a 7 cm by 2 mm i.d. stainless steel tube. The system was held at a controlled temperature (75°C) and low pressure (0.05 psi), while the washing bottle was purged by a 25 mL/min flow of helium for 12 min. The apparatus is shown in Figure 1. The truffle volatiles were then concentrated and trapped in the Tenax trap cooled at -40°C by circulation of liquid nitrogen. By switching a rotary valve the carrier gas was backflushed through the trap and into the GC column. Using thermal desorption at 240°C aroma volatiles were directly transferred onto the GC column. A diagram of the D.C.I. device has been reported previously (1).

In the case of the canned truffle flesh, the washing bottle was replaced by a desorption oven and the sample was put into a vessel which was placed in the oven. The method was the same as we described previously for the study of fresh truffle flesh and conditions of analysis are the same also (2).

Combined Capillary Gas Chromatography-Mass Spectrometry. A GIRDEL 31 gas chromatograph equipped with a D.C.I. system (Delsi Instruments, Paris, France) was coupled by means of a glass lined interface to a NERMAG R10-10B quadrupole mass filter spectrometer (Nermag Ltd., Paris, France). The system was connected on-line to a dedicated data processing system consisting in a Digital Equipment Co. PDP8 Computer, using SIDAR software incorporating the NIH/EPA library of mass spectral data (5).

The capillary column used was 50 m x 0.32 mm (i.d.) fused silica UCON 75H 90000 wall coated column (Delsi Instruments, Paris,

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In Thermal Analysis; Adams; 1989; Vol. 1, p. 1-10; ACS Symposium Series; American Chemical Society: Washington, DC, 1989.

ACS Symposium Series; American Chemical Society: Washington, DC, 1989.

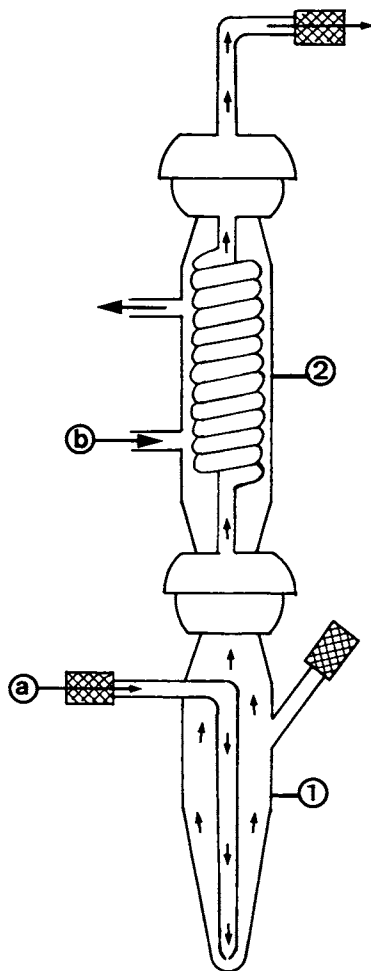


Figure 1. Schematic diagram of the desorption flask
a) purge gas; b) cold water
1) washing bottle; 2) condenser (water-jacket).

France) . The column oven temperature was held 10 min then programmed from 30 to 180°C at a rate of 3°C/min with a final 10-min hold. Column inlet pressure of helium was 7 psi and the splitter flow 30 mL/min.

Significant Mass Spectrometry operational parameters were as follows : ionization voltage, 70 eV; ionization current, 200 μ A; source temperature, 200°C; electron multiplier voltage, 1.9 kV; integration time, 1 ms/a.m.u.. For optimum sample transfer, a transfer temperature of 200°C was employed.

Reagents. Helium was purified by passage through charcoal, molecular sieve, and an Oxisorb trap. Tenax adsorbent was obtained from Alltech Assoc. Inc (Deerfield, Ill, USA). Chemical compounds for comparisons of mass spectra and GC retention times were obtained from commercial sources (Aldrich, Milwaukee, Wis, USA).

Sensory Validation of Sampling and GC Techniques. The sensory evaluation was carried out by a panel of three judges (employees of Pebevre Ltd.). For this study, an external odor port was attached to the gas vent of the D.C.I. system. After the thermal desorption of the volatiles from the trap, the rotary valve was positioned so that the unresolved aroma isolate went to our sniffing port. The response was mesured as similarity or dissimilarity to canned black truffle aroma.

Results and Discussion.

Optimization of the sampling and analytical techniques. Although Tenax adsorbent was hydrophobic, large amount of water could condense on a cold Tenax trap (6) and then interfere with the analysis in two ways: i) by physically blocking a significant portion of the available trapping surface reduced trapping efficiency, degrading both sensitivity and reproducibility; ii) by degrading the column used and interfering into the detection process by extinguishing the FID. This problem was particularly important in the case of analysis of aqueous samples.

So in the case of the analysis of the truffle juice, experimental factors which influenced desorption of volatile compounds from the sample and their adsorption on the trap, that is to say trap cooling temperature, amount of sample, washing bottle heating temperature, purge gas flow, purging time and trap reheating temperature, had to be optimized in order to avoid both water trapping and breakthrough from the Tenax, i.e. elution of volatiles partially through the trap during the adsorption phase.

The traditionnal "factor by factor" methodology consisting in treating each factor separately (by changing just the level of one parameter, maintaining other ones constant) was very time consuming. Furthermore, if several factors played a role, their interaction could not be discernable even if they were dominant because factors were often interdependent (7-9). On the opposite, the "factorial methodology" also called Experimental Designs method, makes it possible to obtain the maximum information with minimum tests (for each factor, different levels were fixed and combined with one another according to a factorial experimental design). This methodology was successfully applied in other fields of analytical chemistry e.g., atomic and plasma emission spectrometry (10-14).

A fractional factorial design was selected for the study of the five experimental factors retained. The responses measured were the total peaks area and the number of peaks, obtained with an ENICA 10 integrator (Delsi Instruments, Paris, France). The computerized treatment of the results (15) allowed us to quantify the main effect and interaction effects of the five factors under study and subsequently to determine the optimum conditions of analysis (Talou, T. Chromatographia, in press.).

Under our optimized sampling conditions, no blocking of the trap (visually verified at the manometer of the scavenging gas circuit) and no losses of volatiles during the adsorption phase (sensorially verified at the odor port) were observed.

The volatile isolate desorbed and sensorially assessed at our odor port was described as typical of processed black truffle, showing that the Tenax had adsorbed and desorbed volatile components responsible for canned black truffle aroma.

Identification of volatiles compounds. Separate gas chromatographic - mass spectrometric analysis were made on five different canned truffles processed during the January - March 1987 period.

The chromatographic profiles of headspace volatiles from canned flesh and juice were similar, but more intense in the case of the juice.

A typical total ion current chromatogram of the Tenax trapped canned black truffle (Tuber Melanosporum) juice volatiles is shown in Figure 2. The compounds identified by GC-MS are listed in Table I in the order of elution from the GC column with their characteristic mass spectral data. The identification of these compounds was based on comparison of the mass spectra obtained with those stored in the NIH/EPA library and also with those of authentic compounds. Moreover, an additional search of published standard mass spectra to confirm the identity of unknowns was undertaken (16).

The major volatile components identified in canned black truffles were alcohols and carbonyls, including acetaldehyde, acetone, 2-butanone, 2-methyl-1-propanol and the two methyl-1-butanols. Also in relatively large amount was dimethyl sulfide.

Found in processed truffles but absent in fresh truffles were two furans (compound 4 and 7) and a pyrazine (compound 24). Furans are well known to result from the thermal degradation of carbohydrates while alkyl pyrazines result from the reaction of amino acids and alpha-diketones. A number of methyl ketones were also observed in the canned product which were absent in the fresh product. Methyl ketones are known to result from the thermal decomposition of beta-keto acids (17-19).

Modification of Flavor Due to Thermal Processing. Despite the similarity of the chromatographic profiles of headspace volatiles of canned and fresh truffles, a sensory analysis carried out by a panel of experts reported a marked difference between the two aromas.

Aroma isolates of processed and unprocessed truffles which were assessed at our odor port were described as typical of the respective aromas.

Since the most striking difference between canned and fresh truffle aroma was the presence of some minor constituents, this

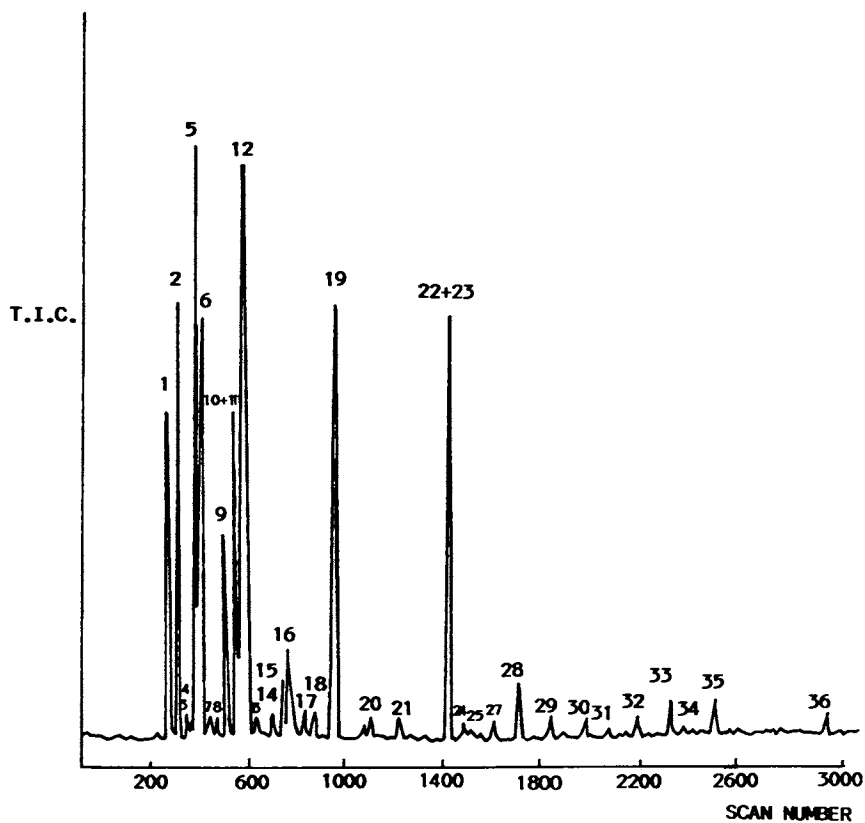


Figure 2. Reconstructed capillary GC-total ion current chromatogram of headspace volatiles of canned black truffle (*Tuber Melanosporum*) juice.

Table I. Volatile compounds identified in canned Black Truffle (Tuber Melanosporum)

Peak Number	a Compound	Mass Spectral Data ^b	Ident. GC,MS	Present in ^c		Rel. % ^d in canned
				canned	fresh	
1	acetaldehyde	29,44,43,42	GC,MS	+	+	3
2	dimethyl sulfide	42,62,45,46	GC,MS	+	+	6
3	propanal	29,28,27,58	GC,MS	+	+	0.2
4	furan	39,68,29,38	MS	+	-	0.3
5	acetone	43,58,42,39	GC,MS	+	+	10
6	2-methylpropanal	43,41,72,27	GC,MS	+	+	6
7	2-methyl furan	82,53,39,81	MS	+	-	tr
8	ethyl acetate	43,29,61,45	MS	+	+	0.2
9	2-butanone	43,72,29,27	GC,MS	+	+	5
10	2-methylbutanal	41,57,29,58	GC,MS	+	+	5
11	3-methylbutanal	44,41,43,29	GC,MS	+	+	tr
12	ethanol	31,45,46,29	GC,MS	+	+	30
13	2-pentanone	43,86,27,29	MS	+	-	tr
14	chloroform	83,85,47,48	MS	+	+	tr
15	2-butanol	45,31,59,29	GC,MS	+	+	0.5
16	1-propanol	31,29,42,27	GC,MS	+	+	1
17	dimethyl disulfide	94,45,79,46	MS	+	+	tr
18	2-methyl-2-butenal	55,84,29,27	MS	+	-	0,1
19	2-methyl-1-propanol	43,33,42,41	GC,MS	+	+	17
20	1-butanol	31,56,41,43	MS	+	-	tr
21	2-heptanone	43,58,41,71	MS	+	-	tr
22	2-methyl-1-butanol	41,29,57,56	GC,MS	+	+	15
23	3-methyl-1-butanol	41,29,42,55	GC,MS	+	+	tr
24	2-methyl pyrazine	94,28,67,40	MS	+	-	tr
25	1-pentanol	42,55,31,70	MS	+	-	tr
26	3-octanone	43,57,29,99	MS	+	-	tr
27	2-octanone	43,58,71,41	MS	+	-	tr
28	anisole	108,78,65,39	GC,MS	+	+	0.5
29	1-hexanol	56,43,55,42	MS	+	-	tr
30	3-octanol	59,55,41,83	MS	+	-	tr
31	methyl anisole	122,91,107,77	MS	+	-	0.1
32	1-octen-3-ol	57,43,29,72	MS	+	-	tr
33	acetic acid	45,43,60,28	GC,MS	+	+	tr
34	2-ethyl-1-hexanol	57,43,55,56	MS	+	-	tr
35	benzaldehyde	105,106,77,51	GC,MS	+	+	0.1
36	2-formyl thiophene	111,112,39,45	MS	+	+	tr

a) The peak numbers correspond to numbers in Figure 2; b) The four most intense peaks are reported; c) key: + = present; - = not detected; d) key : tr = < 0.1%

could be responsible for the difference in the overall aroma. In addition to the volatile identified compounds which are generated by thermal processing, other non-volatile compounds are also created and could be participate to the flavor impression of the canned product. For example, amino acids and peptides may be liberated and non-enzymatic browning products can be formed. But they would not be identified by the analytical methodology developed.

Conclusion.

The headspace technique developed in the present study to isolate volatiles from canned black truffles performed satisfactorily. The aroma isolate obtained was described as typical, and 36 compounds were identified for the first time as canned black truffle constituents. The formation of the major part of them could be correlated to the thermal treatment applied.

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Chapter 33

Flavor Constituents of Roasted Cashew Nuts

A. Jayalekshmy and C. S. Narayanan

Regional Research Laboratory, Commonwealth Scientific and Industrial
Research Organisation, Trivandrum-695019, India

The flavor constituents of plain and roasted cashew nuts have not been previously reported in the literature. In the present study, aroma compounds have been isolated from plain, oven-roasted and oil-roasted cashew nuts by simultaneous distillation extraction and by steam distillation followed by selective extraction, after pH adjustment. Compound identification was carried out by GC and GC-MS analyses. Esters and lactones are present in plain cashews whereas roasted samples also contain pyrazines.

The cashew tree (Anacardium occidentale, Linn.) was introduced into India from Brazil by the Portugese some 400 years ago and became established on the west coast. The tree is very much valued for the tasty and nutritious cashew nut kernels and, the cashew apple finds limited use in making fermented juice products like 'Feni'.

India has been one of the foremost exporters of cashew nuts and during 1987 nearly 12,942 tons were exported to United States (1). In the traditional way of processing, the fully mature nuts in shells are kept on wire trays and roasted for 80 to 90 seconds in a bath of cashew nut shell liquid (CNSL) maintained at a temperature of 180°C. Due to the high temperature and presence of moisture inside the kernel, the nuts are roasted to a desired degree and can be easily broken and manually shelled. After this, skilled labourers remove the brown testa without damaging the kernel and the nuts are graded according to market standard. Usually, export kernels are packed in vacuum with or without carbon dioxide.

¹Cashew nut shell liquid (CNSL) is the exudate from the cashew nut shell. It is mainly phenolic in nature. Industrially used for making polymeric resins etc. A bath of the CNSL is traditionally used for heating the whole nuts with shells.

Cashew kernels possess pleasant taste and flavor and are eaten either raw or roasted with salt. The raw nuts, which are packed in flexible packages, are usually marketed as 'plain' cashews. The 'roasted' nuts are usually fried in vegetable oil to light brown colour, salted and packed in cans. The flavor characteristics of plain cashew nuts are enhanced as a result of oil roasting. A literature review revealed that the flavor constituents of plain or roasted cashew nuts have not been investigated previously. In the present study, the authors have attempted to isolate the flavor compounds by steam distillation and extraction and to identify them by GC and GC-MS techniques. Thirty six compounds have been identified for the first time.

Experimental

Commercial cashew nuts available in the market were purchased. Proximate analysis of the plain cashews was carried out in triplicate according to AOAC methods (2).

Flavor studies

Commercial samples used in the experiment were both plain and oil-roasted cashew nuts. In order to determine the volatiles formed from cashew nuts without the interference of the vegetable oil medium, plain cashew nuts in the form of small bits (1 cm length) were roasted in an air oven at 150°C for 10 min. The flavor extracts of plain, oven-roasted and oil-roasted nuts were isolated by two different methods: (a) simultaneous distillation and extraction in a simple SDE apparatus (3) and (b) by steam distillation and selective extraction of basic, neutral and acidic components following classical pH adjustment procedure. Distilled methylene chloride (DCM) was used as the extraction solvent in the first method and the distillation was carried out at atmospheric pressure. Two hundred grams of powdered cashew nuts were used for extraction and the methylene chloride removed at low temperature ($\leq 50^\circ\text{C}$) over a water-bath using a Vigreux column of 30 cm length and 5.0 cm od. The concentrated extract (0.5 ml) was sealed and kept frozen (-10°C).

In the case of selective extraction of compounds from steam distillate after pH adjustment, about 1.5 L distillate were collected by co-distilling 200 g of powdered nuts with 2.5 L of water. The basic, neutral and acidic compounds were extracted using either methylene chloride or ether and the solvent was removed by distillation. In the case of the acidic fraction, the ether extract was methylated by refluxing with methanol-sulphuric acid (50:1) reagent for 2 hours. The methylated samples were washed free of acid and extracted with redistilled hexane, dried and stored frozen.

Analytical methods

GC analysis: Preliminary GC analysis of total extracts (SDE method) of all the three samples were carried out in a Hewlett Packard

5840A gas chromatograph equipped with a flame ionization detector. Nitrogen was used as the carrier gas at a flow rate of 20 ml/min. A packed s.s. column of 3 percent OV-17 (1.83 m x 3 mm id) was used under the conditions, initial temperature 80°C, then programmed at 5°/min rate to 225°C and held at that temperature for 15 min. The experiments were repeated in the same column under same conditions using TCD detector and helium as carrier gas. The effluent was 'sniffed' by a panel of judges and the consensus of their independent description taken.

GC Retention time indices (I_E): The I_E values were calculated by linear interpolation of the unknown between retention times of a series of methyl esters of normal carboxylic acids under the GC conditions cited for OV-17 column and nitrogen as carrier gas. The method was similar to the one suggested by Van den Dool and Kratz (4). The I_E value of each standard was arbitrarily given the carbon number of the acid of the ester; thus hexanoate was given a value of 6.0. The I_E values of the various peaks in the total extracts (SDE method) of all the three samples and of various authentic compounds were determined under corresponding GC conditions using the same column.

Capillary GC and GC-MS analysis: The flavor extracts isolated by SDE and selective extraction methods of plain, oven-roasted and oil-roasted cashew nuts were analyzed using a cross-linked methyl silicon column (60 m x 0.2 mm id) made of fused silica coupled to a Hewlett Packard 5995B model quadrupole mass spectrometer. The ionisation voltage was 70 eV and electron multiplier voltage was 1600V. The analysis was done in split mode with a ratio of 1:75 and the carrier gas was helium. Following GC conditions were found to be reasonably good for the various flavor extracts; isothermal at 90° for 5 min, then temperature programmed to 225° at the rate of 5°/min with a final temperature hold for 25 min. The basic and neutral compounds were analyzed in this column under the same conditions. The acidic fractions were analyzed, as methyl esters, on a methyl silicon column (12 m x 0.2 mm id) under the above said program conditions. In all the experiments, the instrument was tuned and calibrated with PFTBA (Per Fluoro Tri Butyl Amine). Methyl heptanoate was used as the internal standard. The mass spectral data of the various fractions were matched with the NBS library of Flavor and Fragrances in the data base system provided with the instrument. Mass spectra of compounds which were not covered under this library were compared with published spectral data (5), (6).

RESULTS

The proximate composition of plain cashew kernels is given in Table I.

Table I. Proximate composition (%) of plain cashews

Moisture	5.00
Fat	45.40
Protein	22.58
Soluble sugars	9.79
Total carbohydrates (by difference)	25.83
Ash	2.19

The pH of the steam distillate was 6.2 in all cases. The total flavor isolate (by SDE) of plain cashews had, on the whole, a strong pungent and green aroma, reminiscent of the cashew nut testa and cashew shell, whereas the flavor isolates from roasted samples had the characteristic mildly nutty aroma also. The flavor fractions obtained by selective extraction method gave some information about the chemical nature of compounds responsible for the characteristic flavor notes. Accordingly, the basic fraction of roasted nuts, (both oven- and oil-roasted), had the typical nutty aroma associated with pyrazine compounds. The basic fraction of plain cashews did not have any characteristic flavor in particular.

The neutral fraction of plain cashews had a mildly estery, fragrant, green flavor, typically reminiscent of freshly plucked cashew nuts. The neutral fraction of roasted nuts had an estery, nutty and slightly pungent, heavy aroma. The acidic fractions of plain and roasted samples had the typical, short chain fatty acid note. The quantitative distribution of various fractions is shown in Table II.

Table II. Quantitative distribution of flavor extract

Nature of extraction	Quantity of aroma from cashew nuts (mg/kg)		
	Plain	Oven roasted	Oil roasted
Simultaneous distillation & extraction (SDE) (Total extract)	100	250	273
Steam distillation and fractional extraction by pH adjustment (selective extraction)	105	300	309
Basic compounds	3	100	112
Neutral	84	115	107
Acidic	18	85	90

The authenticity of the peaks in GC and GC-MS analyses were tested by the relative retention time of the standards with reference to methyl heptanoate. In addition to this, the I_E values were calculated on OV-17 column, for the standards and sample peaks. Compounds for which standards were not there, but had very good mass spectral agreement (correlation ratio ≥ 0.95) were also considered as present positively. A few peaks for which

spectral agreement was less, have been described as 'tentative'. Relative concentrations of compounds of the different extracts and flavor fractions were calculated from the electronically integrated peak areas of the respective peaks as against the total area of all the peaks in that sample. From these values and the quantitative distribution data, the actual concentration of each compound was calculated. The results are shown in Table III.

Preliminary GC analysis revealed that there are more number of peaks in roasted samples compared to plain cashews. Also selective extraction method was found to be slightly superior to the SDE method under the conditions of the experiment adopted in this study. However, the compounds in oven-roasted and oil-roasted samples did not differ much, qualitatively and quantitatively. In total, 26 compounds have been identified in plain cashews and 36 compounds in roasted samples. The identified peaks constituted 70 percent of the total peaks registered in GC analysis of the individual samples. The descriptive flavor profile of the eluting peaks of the plain and roasted samples were studied. Since the flavor isolate from oven roasted cashew nuts contained the flavor components of plain cashews also and since analysis showed that there was not much difference between the flavor constituents of oven-roasted and oil-roasted samples, the aromagram of the oven-roasted cashew nuts (SDE) was taken as representative. Fig.1 gives the GC profile of oven roasted cashew nuts and the sensory properties of the numbered peaks are included in Table III.

Discussion

Of the 26 compounds identified in plain cashews, 5-heptene-2-one was found to be most abundant (30 percent). The eluting peak had an intense green aroma. Another important compound was found to be 1,3-propanediol diacetate, the actual nasal impact of the corresponding peak being fresh green, cashew nut-like aroma. Butyl acetate, methyl and ethyl esters of higher fatty acids and the lactones may add to the estery, oily and nutty flavor of plain cashews. The green, mango-like aroma, identical with peak No.5, could be possibly due to myrcene. The phenolic compound (peak No.36) had the typical aroma of cashew nut shell. The acidic fraction contained fatty acids (C_6 to C_{14}).

The analysis of roasted samples (both oven- and oil-roasted) reveal the presence of 7 pyrazines, on an average, contributing 30% of total aroma. Among the pyrazines, 2,6-dimethyl pyrazine occurs in large concentration and has a green, nutty odour. The peaks corresponding to methyl pyrazine, 2-ethyl-6-methyl pyrazine and methyl-pyrrolo (1,2- α) pyrazine registered a mild roasted, sensory impact. The last one, a bicyclic pyrazine, has been reported in model system studies, related to coffee roasting (6). As Maga reviewed, the pyrazines are mostly responsible for the roasted flavors of many food systems (7).

Analysis of the neutral fraction of flavor isolates revealed that most of the neutral compounds like esters, lactones and carbonyls present in plain cashews were found in roasted samples also. In addition, three furans have been detected in the roasted

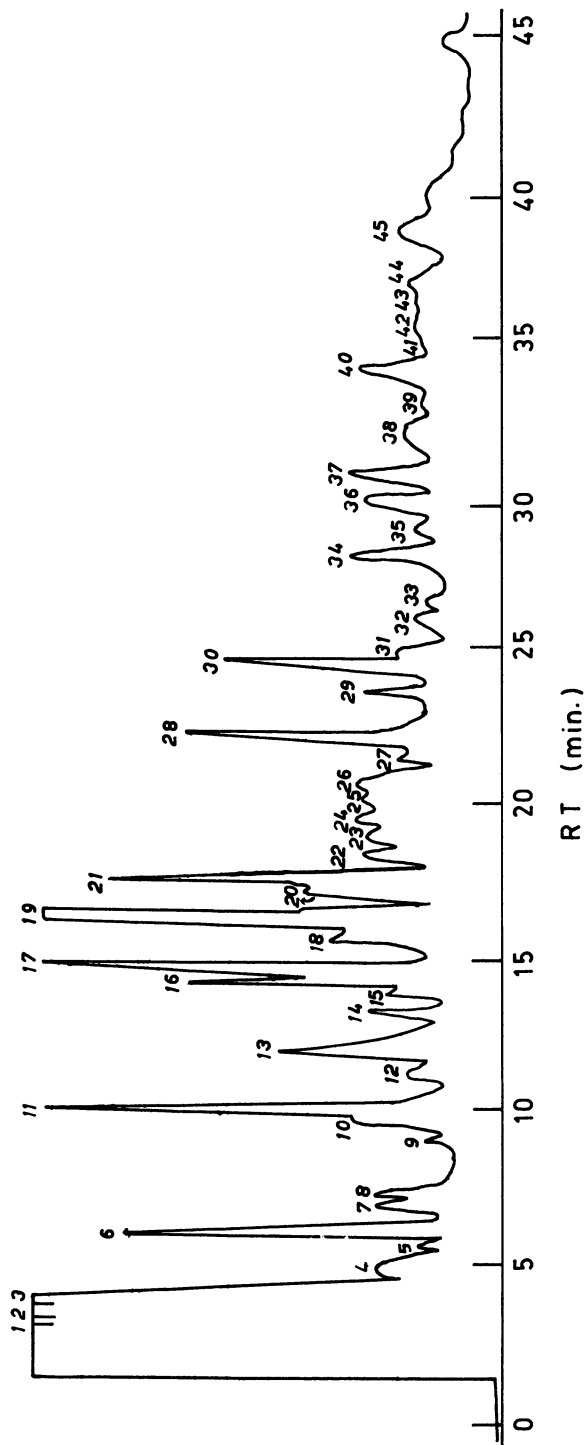


Fig.1 : GC profile of total flavor extract (SDE method) of oven-roasted cashew nuts

Table III. Flavor compounds of plain and roasted cashew nuts identified by GC and GC-MS

Peak no. Corr. to Fig 1	Compounds identified	I _E (OV-17)	R _T in capillary methyl silicone (4)	MS fragments	Sensory properties	Concentration of flavor compounds (mg/kg)			
						Plain	Oven-roasted	Oil-roasted	Total extd. of oven-roasted
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
	Basic								
5	Methyl pyrazine	5.95	0.34	94,67,54,43,53	raw green, mildly nutty	1.0	10.0	12.2	5.6
8	2,6-dimethyl pyrazine	6.20	0.40	108,42,47,41,100,81	green, nutty		26.5	28.3	20.9
13	2-ethyl-6-methyl pyrazine	7.80	0.52	121,122,39,94,56,44	mild roasted		13.5	11.0	7.0
11	2-ethyl-3-methyl pyrazine	7.30	0.54	121,122,67,81,39,43	green, raw, nut-like		8.5	8.1	6.4
11	2-ethyl-5-methyl pyrazine	7.30	0.56	121,122,39,56,96,42	raw green smell		8.0	8.8	6.9
17	2,6-diethyl pyrazine	8.80	0.71	135,136,39,108,121,53	steam cooked smell		9.9	9.3	13.2
	Methyl pyrrolo (1,2- α)pyrazine resolved	not resolved	0.90	Tentative	cashew nut-like		8.2	10.0	2.8
2	Neutral 5-heptene-2-one	5.00	0.27	43,69,41,57	intense, fresh green aroma	30.2	24.0	25.0	27.0
3	butyl acetate	5.02	0.29	43,56,73,41,61	ester like fruity	5.1	4.1	4.2	2.0
1	butoxy-methyl benzene	4.80	0.30	91,92,65,39,41,51	mild hydro carbon-like	1.9	2.3	2.0	3.5

Continued on next page

Table III. Continued

Peak no. corr. to Fig. 1	Compounds identified	I _E (OV-17)	R _T in capillary methyl silicone	MS fragments	Sensory properties	Concentration of flavor compounds (mg/kg)			
						Plain	Oven-roasted	Oil-roasted	Total extd. (SDE) of oven-roasted
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
3	furfural	5.03	0.34	96,95,39,42,41	fermented	0.1	0.8	6.2	7.0
11	2-methyl-3-butyn-2-ol	not resolved	0.36	69,43	slightly pungent	0.5	1.7	2.0	0.5
4	1,3-propane diol diacetate	5.35	0.39	43,44,61	fresh, green	3.0	1.1	0.8	0.5
5	myrcene	5.70	0.40	tentative	cashew nut-like green, mango-like	1.2	0.8	0.5	0.8
20	γ-butyrolactone	9.70	0.42	85,56,41,70	pleasant nutty	3.0	2.0	2.3	2.0
10	benzaldehyde	7.00	0.50	106,105,77,51,78,50	smell of bitter almonds	1.1	1.5	2.0	1.0
9	2-pentyl furan	6.90	0.56	81,82,138,53,67	green, raw	-	0.9	0.5	0.6
13	2-thiophene carboxaldehyde	7.60	0.61	111,112,45,39,83,113	flavor note	1.0	0.9	1.0	0.7
24	δ-hexalactone	10.40	0.72	99,55,42,114	nutty smell	4.3	2.1	3.0	4.0
16	phenethyl alcohol	8.70	0.79	91,92,122,65,78,77	flowery, ester-like	4.0	5.0	4.8	4.3
19	naphthalene	not resolved	0.92	128,51,129,64,127,63	aromatic	-	0.7	0.5	0.9
	5-methyl-[2-(furan-2-yl)methyl]furanone	9.60	0.97	81,43	steam cooked, toasted cereal-like	-	15.4	13.3	23.0
23	ethyl octanoate	10.30	1.02	88,101,57,41,55,127	oily, nutty	1.0	1.1	1.8	1.5
22	2,4-undecadienal	10.10	1.20	81,41,67,152,95,77	raw, green	0.5	1.5	2.0	1.6

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
29	methyl tetra- decanoate	11.85	1.25	74,87,43,55,41	mildly nutty, oily	4.3	4.0	3.7	4.5
34	methyl hexa- decanoate	13.30	1.34	74,87,75,43,41	mildly nutty, oily	2.4	7.0	6.0	7.6
26	5-ethyl-2- methyl oxazole	10.80	1.40	111,96,82,54, 55,43	mild, roasted aroma	trace	1.7	0.9	2.3
36	bis(1,1-dimethyl ethyl)-4-methyl phenol	13.85	1.51	205,161,220, 41,208	phenolic smell typical of cashew shell	4.9	6.0	6.3	7.0
30	2-butyl benzo- thiazole	12.20	2.01	149,41,56, 57,150	slightly pungent	7.7	8.0	7.6	8.0
38	tetradecane	14.65	2.10	57,71,85,99, 43,55	hydrocarbon- like	2.1	2.0	3.0	2.0
28	Acidic (analysed as methyl esters) hexanoic acid	11.25	0.49	74,84,51,47, 41,88	rancid oil- like	3.1	12.0	10.0	9.6
32	octanoic	12.75	1.59	74,87,55,41, 69,158	unpleasant, rancid	3.4	6.0	6.7	4.8
33	decanoic	13.00	3.75	74,87,143, 43,55,106.	stored oil-like	1.8	5.2	6.1	2.6
40	dodecanoic	14.20	6.47	74,87,41,55,43	rancid-slightly fruity	1.0	6.3	4.0	4.3
37	dodecenoic	14.15	6.61	55,74,87,55	slightly pungent	1.1	8.0	9.1	9.3
45	tetradecanoic	16.40	8.65	74,87,41,43,55	rancid oil- like slightly fruity	1.0	9.2	14.0	11.1

samples. Of these, furfural and 2-pentyl furan have been reported in most systems (8). The latter was identified to have a green, grassy flavor note. The third furan derivative is (2-furanyl methyl)-5-methyl-2-(5H) furanone which is noticed to have a strong, toasted cereal-like aroma. This is formed in considerable amount during roasting. Furans are formed by the thermal degradation of sugars (8). The compound 2,4-undecadienal found only in roasted cashew nuts must be derived from lipids of cashew nuts. The corresponding peak had a raw green aroma. The formation of benzaldehyde during thermal degradation of lipids is suggested; however, in cashew nuts benzaldehyde could be detected in plain cashews also, which had not undergone much of heat treatment.

The short chain fatty acids (C_6 to C_{14}) detected in plain cashews could be detected in roasted samples also, in higher amounts. Dodecenoic acid was the only unsaturated fatty acid among them. These fatty acids are formed by the degradation of glycerides by mild enzymatic action or hydrolysis.

To sum up, the flavor constituents of plain and roasted cashew nuts are reported here for the first time. The mild flavor of cashew nuts can be attributed to the carbonyls, esters and lactones, especially to 5-heptene-2-one and 1,3-propanediol diacetate. Upon roasting, 2,6-dimethyl pyrazine, 2,6-diethyl pyrazine and the furanone are formed in larger amounts and from flavor profile also these compounds are likely to play a significant role in the characteristic aroma of roasted cashew nuts.

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Chapter 34

Volatile Compounds in Ginger Oil Generated by Thermal Treatment

Chu-Chin Chen¹ and Chi-Tang Ho

Department of Food Science, New Jersey Agricultural Experiment Station,
Cook College, Rutgers, The State University of New Jersey,
New Brunswick, NJ 08903

Ginger oils from steam distillation and liquid carbon dioxide extraction (600 - 700 psi) were fractionated into hydrocarbons and oxygenated hydrocarbons by silica gel column chromatography. Volatile hydrocarbons and oxygenated hydrocarbons were analyzed by capillary GC and GC-MS. Monoterpenes, sesquiterpenes, aliphatic aldehydes, 2-alkanones, citral, monoterpene alcohols and sesquiterpene alcohols were major categories of ginger components which were affected or generated by thermal induced degradation during steam distillation.

Steam distillation is the most common process for the extraction of essential oils from plants (1-3). It provides a fast and simple way to obtain aromatic components which bear the characteristic odor of that species. However, "still notes" or "burnt notes" are frequently found in freshly distilled oil. The off-flavor results in most cases from thermally induced hydrolytic or degradative reactions (4).

Volatile ginger oil obtained from steam distillation has been the subject of many research studies (5-12). However, the thermal degradative effects of steam distillation upon volatile and nonvolatile components of ginger were seldom discussed. Recently, Moyler (1) compared the advantages of liquid carbon dioxide extraction over conventional methods such as solvent extraction or steam distillation by showing reconstructed GC chromatograms which clearly displayed the differences. However, quantitative results showing the differences were not mentioned.

The present study compares the capillary GC analysis of volatile compounds derived from steam distillation of ginger with those extracted by liquid carbon dioxide. Volatile components affected by thermal treatment during preparation were of major concern.

¹Current address: Food Industry Research and Development Institute, Hsinchu, Taiwan, 30099, Republic of China

Materials and Methods

Reagents. The solvents (n-pentane, diethyl ether) were reagent grade and glass distilled. n-Aldehydes (C6-C14) were obtained from Alltech Associates (Deerfield, IL). 2-Heptanone, 2-nonanone and 2-undecanone were obtained from Aldrich (Milwaukee, WI); 2-tridecanone was obtained from Caro (Tokyo, Japan). Unless otherwise stated, all other chemicals were of reagent grade. Zingerone was obtained from ICN Biochemical (Plainview, NY).

Liquid Carbon Dioxide Extraction. Mature ginger (*Z. officinale* Roscoe) rhizomes were obtained from a supplier in Hsinchu (Taiwan). The rhizomes were washed, sliced, freeze-dried, ground and sieved (200 mesh). About 110 g of freeze-dried ginger powder was placed in a glass Soxhlet extractor installed in a stainless steel liquid carbon dioxide extractor. Operating procedures were described previously (13). A golden brown oil material (3.44%) was obtained by the liquid carbon dioxide extraction.

Fractionation of Volatile Compounds by Column Chromatography. Steam-distilled ginger oil (0.96 g) and liquid carbon dioxide-extracted ginger oil (1.71 g) were applied separately to a glass column (40 cm x 2.0 cm) packed with silica gel (50 g, 60/200 mesh; Mallinckrodt). The hydrocarbon fraction was eluted with pentane (1 L, Aldrich) and the oxygenated hydrocarbon fraction was eluted with ethyl ether (1L, E. Merck). The pungent gingerol compounds were not eluted under these conditions. Tetradecane (Matheson, 7.60 mg) and ethyl decanoate (Lachat Chemical, 3.8 mg) were added to the hydrocarbon and oxygenated hydrocarbon fractions, respectively, as internal standards.

GC and GC-MS Analysis of Volatile Components. The two fractions of ginger oil extracted by liquid carbon dioxide were subjected to gas chromatographic analyses on a Varian 3400 gas chromatograph. A fused silica column with a stationary phase equivalent to Carbowax 20M (DB-WAX+, 60 m x 0.32 mm; J&W Scientific) was used. The oven temperature was programmed linearly from 50 to 225°C at 1.5 C/min and was held at 225°C for 80 min. Other operating conditions were as follows: injector and detector temperatures, 250°C; makeup helium flow, 30 mL/min; detector hydrogen flow, 30 mL/min; detector air flow, 30 mL/min. The samples were injected in the split mode with a split ratio of 1/100. The linear velocity of the helium carrier flow was 22 cm/s. Quantitative determinations were made with a Varian 4270 integrator. Linear retention indices for the volatile components were calculated by using n-paraffins (C8-C25; Alltech Associates) as references (14). Capillary gas chromatography-mass spectrometry was carried out on a Hewlett-Packard 5985 B system equipped with a Hewlett-Packard 5840A gas chromatograph. A fused silica capillary column (Carbowax 20M) was used. Analytical conditions were as follows: temperature program, 50 - 200°C, 1.5 C/min, isothermal at 200°C, 50 min; injector temperature, 250°C; helium carrier velocity, 30 cm/s; ion source temperature, 200°C; ionization voltage, 70 eV; electron multiplier voltage, 2600 V.

Results and Discussion

In order to investigate the thermal degradative reaction during the preparation of volatile ginger oil, freeze-dried ginger powder extracted by conventional steam distillation was compared with low temperature extraction using liquid carbon dioxide. Both extracts were further fractionated into hydrocarbon and oxygenated hydrocarbon fractions. GC and GC-MS identifications of volatile compounds were accomplished by comparing with previous report (15). Tetradecane and ethyl dodecanoate, respectively, were added as internal standards.

Figures 1 and 2 show the capillary gas chromatographic analyses of the hydrocarbon and the oxygenated hydrocarbon fractions. In the hydrocarbon fraction, 13 selected monoterpene compounds and sesquiterpene compounds were compared. In the oxygenated hydrocarbon fraction, 22 volatile components, which include aliphatic aldehydes, 2-alkanones, citral, monoterpene alcohols and sesquiterpene alcohols, were compared.

Table I. Comparison of Selected Hydrocarbon Compounds in Steam Distilled and LCO₂ Extracted Ginger Oil

Peak ^a No.	Compound	% (w/w)	
		Dist.	LCO ₂
Monoterpenes			
1	α-pinene	0.16	0.98
2	camphene	0.40	3.08
3	β-pinene	0.02	0.16
4	myrcene	0.08	0.90
5	β-phellandrene	0.09	0.89
6	limonene	0.31	2.75
7	terpinolene	0.11	0.11
Sesquiterpenes			
8	β-elemene	0.29	0.31
9	Zingiberene	14.19	24.15
10	γ-bisabolene	3.47	8.40
11	β-bisabolene	6.37	5.60
12	β-sesquiphellandrene	10.62	10.14
13	ar-curcumene	16.30	7.96

^a Number refers to Fig. 1

Table I shows the quantitative comparison of selective compounds in the hydrocarbon fraction. The total amount of monoterpenes in the steam distilled sample was less than that in the liquid carbon dioxide extract. These compounds could have been lost during distillation. The significant decrease in zingiberene and the concomitant increase in ar-curcumene confirmed that zingiberene was converted to ar-curcumene (16). β-Sesquiphellandrene, another compound which could be converted to ar-curcumene, decreased slightly. The scheme of oxidative conversion of zingiberene and β-sesquiphel-

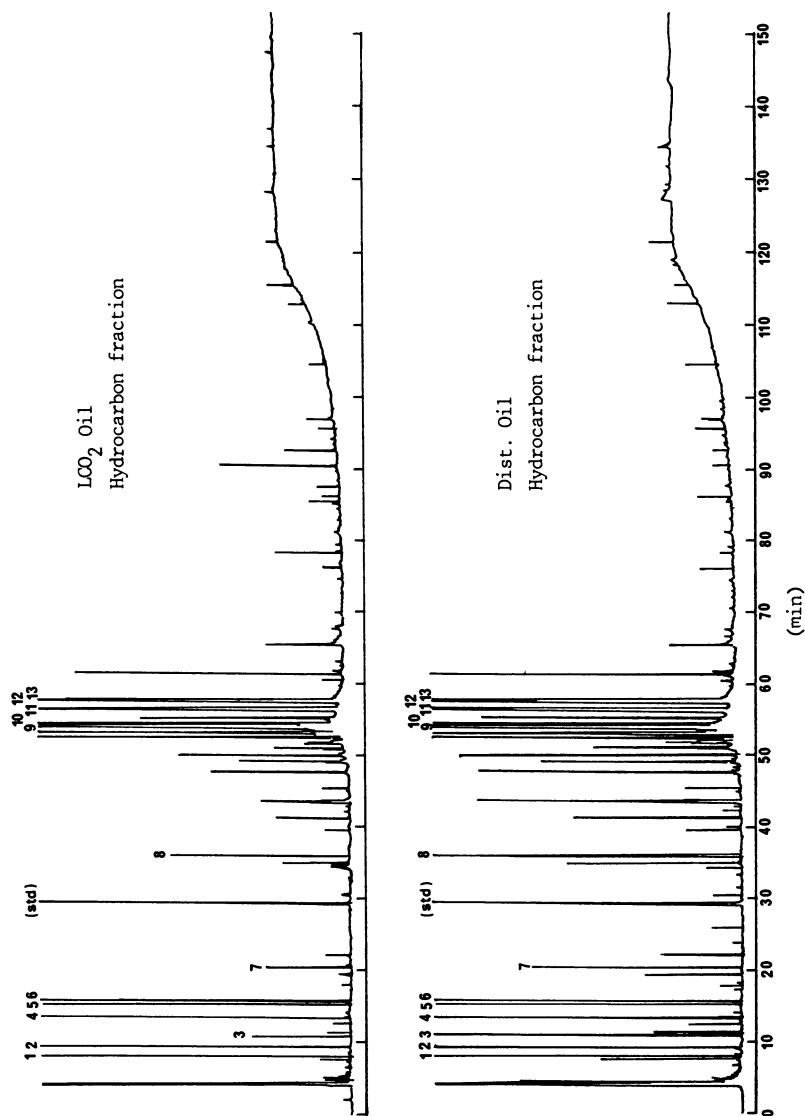


Figure 1. Capillary gas chromatographic analyses of hydrocarbon fractions of liquid carbon dioxide extracted and steam distilled oils.

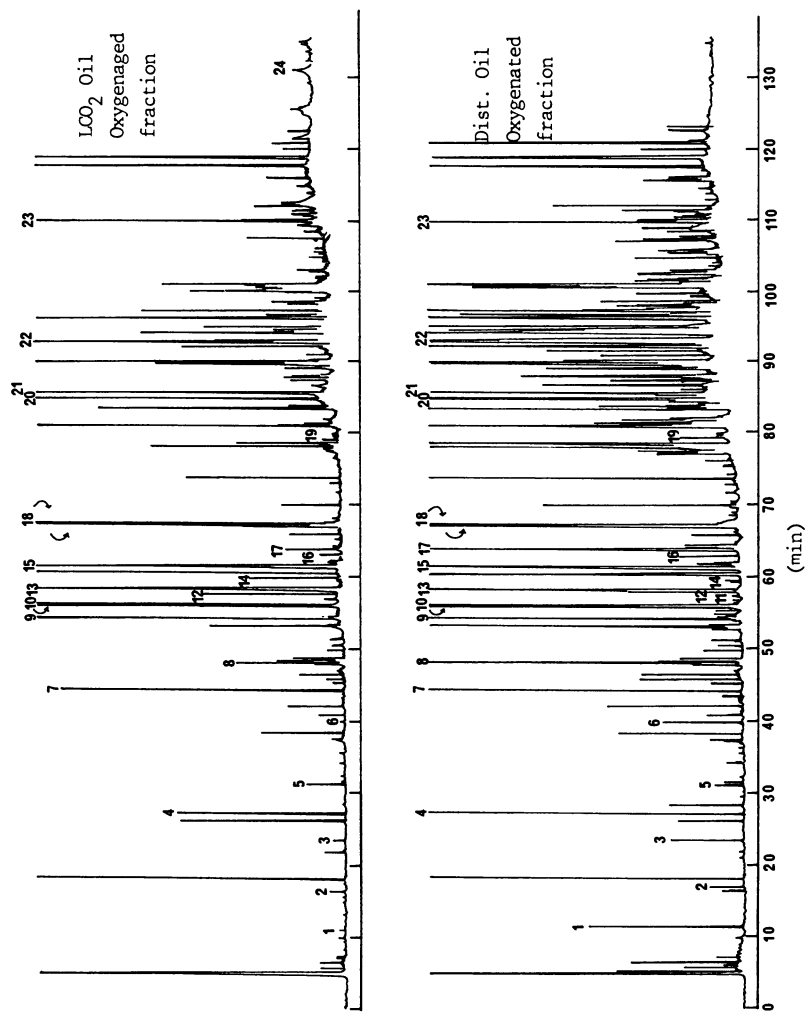


Figure 2. Capillary gas chromatographic analyses of oxygenated hydrocarbon fractions of liquid carbon dioxide extracted and steam distilled oils.

landrene to ar-curcumene is shown in Fig. 3. Such changes will lead to diminution of fresh flavor of ginger oil.

Table II shows the quantitative comparison of selected compounds in the oxygenated hydrocarbon fraction.

Table II. Comparison of Selected Oxygenated Hydrocarbon Compounds in Steam Distilled and LCO₂ Extracted Ginger Oil

Peak ^a No.	Compound	% (w/w)	
		Dist.	LCO ₂
Aldehydes/Alkanones			
1	hexanal	0.05	+
2	2-heptanone	0.01	0.01
3	octanal	0.03	0.01
5	2-nonanone	0.01	0.04
6	decanal	0.04	+
8	2-undecanone	0.29	0.13
11	dodecanal	0.01	-
16	2-tridecanone	0.04	0.02
24	zinggerone	-	+
Citral			
4	2-methyl-2-heptene-6-one	0.24	0.17
9	neral	1.54	2.32
13	geranial	2.58	4.62
Monoterpene Alcohols			
7	linalool	0.26	0.35
15	citronellol	1.25	0.79
17	nerol	0.22	0.08
18	geraniol	1.74	1.02
Sesquiterpene Alcohols			
19	nerolidol	0.03	+
20	β -sesquisabinenhydrate	0.90	0.45
21	zingiberenol	1.42	0.93
22	β -eudesmol	0.90	0.57
23	t- β -sesquiphellandrol	0.21	0.48

^a Number refers to Fig. 2.

Gingerol compounds, the dominant pungent principles of ginger, are thermally labile due to the presence of a β -hydroxy keto moiety in their structure (17, 18). In our previous report (19, 20), two homologous series of gingerol compounds, i.e., 6-, 8-, 10-, 12-, 14-gingerols and methyl-6-, methyl-8-, methyl-10-, methyl-12-gingerols have been identified (Fig. 4). It was found that upon injection of gingerol compounds into a gas chromatograph, straight chain aldehydes (C₆, C₈, C₁₀, C₁₂ and C₁₄) and 2-alkanones (2-heptanone, 2-nonanone, 2-undecanone and 2-tridecanone) were generated due to thermal degradative reactions (retro-aldol and retro-Claisen Schmidt reaction) (20, 21). It is also interesting to note that the presence of hexanal, octanal, decanal, 2-heptanone, 2-nonanone and 2-undecanone in steam distilled oil of ginger has been well documented (7-9, 11-12). It is not known whether these compounds are related to the nonvolatile gingerol compounds.

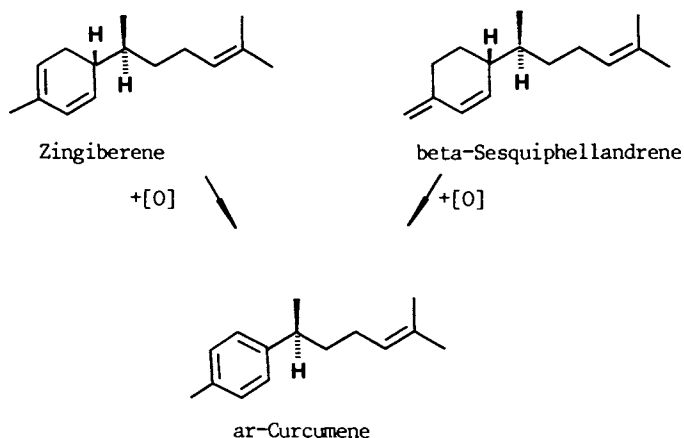


Figure 3. Oxidative conversion of zingiberene and β -sesquiphellandrene into ar-curcumene.

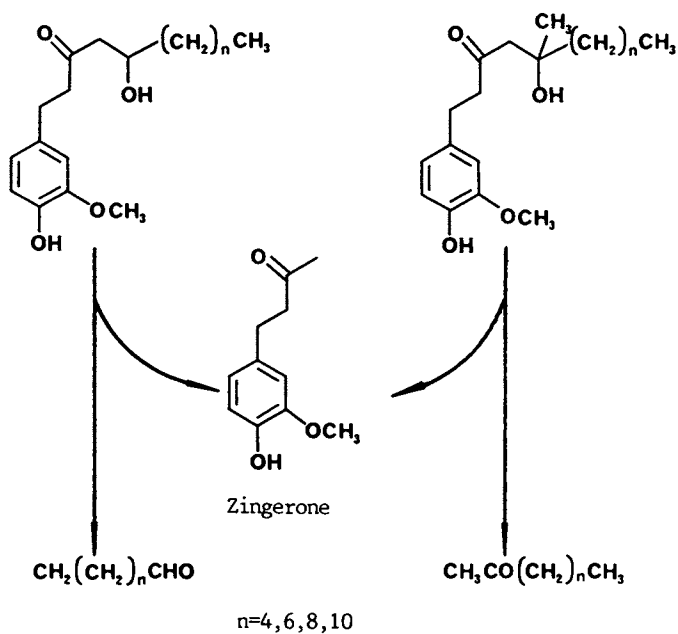


Figure 4. Structure of gingerol compounds and their thermal degradation products.

In Table II, the significantly higher concentration of aliphatic aldehydes (C6, C8, C10, C12) and 2-alkanones, (2-heptanone, 2-undecanone and 2-tridecanone) in the steam distilled oil suggested that thermal degradation of gingerol compounds during steam distillation may cause such changes. With the exception of 2-nonanone, aliphatic aldehydes and 2-alkanones were in lower concentration in the liquid carbon dioxide extract than in steam distilled oil. This is a good indication of less severe thermal treatment during the liquid carbon dioxide extraction. Also, the finding of a trace amount of zingerone (peak 24) in the liquid carbon dioxide extract shows that it is possible that trace amounts of gingerol compounds may appear in various fractions. These trace amounts of gingerol compounds may explain the detection of aliphatic aldehydes and 2-alkanones during gas chromatographic analysis.

Figure 5 shows the thermally induced hydrolytic degradation of citral (geranial and neral) into 2-methyl-2-hepten-6-one and acetaldehyde. It is similar to the reaction mechanism as proposed by Josephson and Lindsay (22, 23). The relatively higher amount of 2-methyl-2-hepten-6-one and the concomitant lower amount of geranial and neral in steam distilled oil confirmed again a previous report (24).

In vegetables, fruits and plants, it has become well-known that some of the terpenoid alcohols originated from nonvolatile terpenoid glycosides through the action of enzymes, acid and/or heat. Vege-

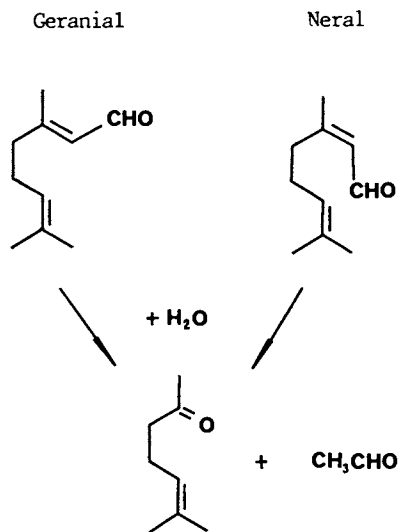


Figure 5. Thermally induced hydrolytic degradation of citral (geranial and citral) into 2-methyl-2-hepten-6-one and acetaldehyde.

tables, fruits and plants reported in such categories include tomato (25, 26), tea shoot (27), grape (28-30), passion fruit (31) and papaya (32).

Recently, Sakamura (33) reported that the amounts of monoterpene alcohols in young rhizomes were less than those in mature rhizomes. Whether enzymic activity is involved during the growth period was not mentioned.

In the present report, the amount of most monoterpene alcohols and sesquiterpene alcohols in distilled oil was higher than those extracted by liquid carbon dioxide. It is possible that the differences were due to the thermal degradative effect of steam distillation upon the nonvolatile glycosides of monoterpene alcohols and/or sesquiterpene alcohol, as in the case of tomato.

Conclusion

Thermal treatment, such as steam distillation during sample preparation, will cause considerable degradative reaction to both volatile and nonvolatile compounds of ginger. To the contrary, however, preparation under low temperature, such as liquid carbon dioxide extraction, can effectively eliminate thermally induced degradative reactions.

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Chapter 35

Identification and Formation of Characteristic Volatile Compounds from Cooked Shrimp

Kikue Kubota, Chinatsu Uchida, Keiko Kurosawa, Ayako Komuro, and Akio Kobayashi

Ochanomizu University, Laboratory of Food Chemistry, 2-1-1, Ohtsuka, Bunkyo-ku, Tokyo 112, Japan

An investigation into the aroma of cooked small shrimp and krill revealed more than forty sulfur- and/or nitrogen-containing heterocyclic substances. Among these eight were newly identified as contributors to food flavor. The main ring structures encountered were pyrazine, trithiolane, dithiin and dithiazine. It was a characteristic of small shrimp flavor that many varieties of cyclic polysulfides were formed during cooking. Some distinction in the composition of these heterocyclic substances were also observed among the shrimp species or from the treatment before heating, and led to the following conclusion: the content of free amino acids should give the initiative for the formation pathway of the heterocyclic substances in small shrimps.

(All-Z)- and (5E,8Z,11Z)-5,8,11-tetradecatrien-2-ones were determined as other important thermally generated constituents in shrimp. These two isomers had the most acceptable seafood aroma among the eight possible geometrical isomers that were synthesized.

Seafood is one of the world's main protein sources. Among seafood, shrimp is consumed in large quantity throughout the world because of its pleasant flavor.

Raw shrimp has little odor, but develops a pleasant characteristic roast aroma upon cooking. Antarctic krill is becoming important protein source because of its abundance. Although taxonomically different, krill is much like shrimp in appearance and will be treated as small shrimp in this report. Cooked krill, however, possesses a less acceptable aroma compared to shrimp.

From our aroma research on boiled small shrimps, almost one hundred volatile components were identified. Among them, more than forty components were determined as sulfur- and/or nitrogen-containing heterocyclic substances, together with various kinds of volatiles that are well known to be thermally generated such as hydrocarbons, carbonyl compounds, alcohols and phenols. The shrimp

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samples evaluated include Sergia lucens (1-3), Acetas japonicus (4), Pleoticus muelleri (5), Euphausia superba (Antarctic krill) (6-8) and Euphausia pacifica (9,10). It seems worthwhile to summarize the main characteristic heterocycles in the volatiles from cooked shrimps, because of the attention payed to heteroatomic substances as food flavors (11).

In addition, two unknown polyunsaturated methylketones were isolated in the volatiles of boiled small shrimp. They drew our particular interest because their structure contained a linolenic acid type component and because of their seafood-like odor.

In the present paper, we describe the identification and formation of the previously-described heterocyclic compounds and polyunsaturated methylketones in the volatiles of boiled small shrimp.

Identification of Sulfur- and/or Nitrogen-Containing Heterocyclic Components

The volatiles from cooked small shrimps were obtained using a simultaneous distillation and extraction apparatus. These volatiles were separated into neutral and basic fractions. The fractions were then analyzed by gas chromatography using a polar column and a FPD detector. Sulfur-containing compounds were found in a segment of the high-boiling fraction of S. lucens volatiles. The high boiling fraction contained many interesting polysulfur-containing heterocyclic compounds which were determined spectroscopically. On the other hand, the basic fraction, which had a strong roasted odor, contained the largest portion of the volatiles from S. lucens. Many nitrogen-containing heterocyclic substances were found here. This pattern was also observed in other shrimp samples. The structures of forty-four sulfur- and/or nitrogen-containing heterocyclic compounds were conclusively determined. The identified compounds and their occurrence in small shrimps are listed in Table I.

Most classes of heterocycles found are also commonly observed in other cooked food flavors. Pyridines, pyrroles, thiophenes and alkylthiazoles were minor components. As main constituents, ten methyl- and ethyl-substituted alkylpyrazines were identified. The pyrazines occurred markedly in all samples and seemed to be one of the main contributors to the roasted-nut like flavor of cooked small shrimp.

Six kinds of alkyltrithiolanes were found, and among them, dimethyl homologs were principal in the shrimps. 3,5-Dimethyl-1,2,4-trithiolanes have been found in the volatiles from cooked beef (12), soy bean (13) and some shell fish (14) and 3-ethyl-5-methyl- and 3,5-diethyl-1,2,4-trithiolanes were identified in a commercial beef extract (15) and Allium plant (16), respectively. They have a strong Allium plant-like odor. 2,4,6-Trimethyl-1,3,5-dihydrodithiazine (thialdine), which had been identified as the flavor constituent of cooked meats (12, 17-21) and beans (13,22), was also found in all samples and it was the main constituent in both S. lucens and A. japonicus. The odor of thialdine at pH 8.2 has been evaluated as that of medium-roast shrimp by Kawai et al. (23); therefore, it is thought that thialdine influenced the flavor of our shrimp samples. On the other hand, dimethyl and methyl ethyl-1,3-dithiins, and ethyl-, propyl- and butyl-substituted homologs of dihydrodithiazines

Table I. Heterocyclic Compounds Identified in the Volatiles from Boiled Small Shrimps

Compound	Rem *	Compound	Rem *
<u>Pyridine</u>		<u>Thiophene</u>	
Pyridine	S, A, Ea, Ep	2-Formyl-	S, Ea
2-Methyl-	Ea	2-Formyl-6-methyl-	Ea
3-Methyl-	Ea	2-Acetyl-	Ea
3-Ethyl-	Ea	2-Acetyl-6-methyl-	Ea
3-Ethyl-5-methyl-	A		
<u>Pyrazine</u>		<u>1,2,4-Trithiolane (cis & trans)</u>	
Pyrazine	A, Ea, Ep	3,5-Dimethyl-	S, A, P, Ea
2-Methyl-	S, A, Ea, Ep	3-Ethyl-5-methyl-	S, P, Ea
2,3-Dimethyl-	S, A, Ea, Ep	3,5-Diethyl-	Ea
2,5-Dimethyl-	S, A, Ea, Ep		
2,6-Dimethyl-	S, A, Ea, Ep	<u>1,3-Dithiin</u>	
Trimethyl-	S, A, Ea, Ep	2,6-Dimethyl-	S, P, Ea
2-Ethyl-5-methyl-	Ea, Ep	6-Ethyl-2-methyl-	Ea
2-Ethyl-3,5-dimethyl-	S, A, Ea	<u>1,3,5-Dihydrodithiazine</u>	
2-Ethyl-3,6-dimethyl-	A, Ea	2,4,6-Trimethyl-	
Tetramethyl-	S, A, Ea	(Thialdine)	S, A, P, Ea, Ep
		2-Ethyl-4,6-dimethyl-	S, A, Ea
		4-Ethyl-2,6-dimethyl-	S, A, Ea
<u>Pyrrole</u>		2,4,6-Triethyl-	P
Pyrrole-	Ea	4,6-Dimethyl-	P
1-Methyl-	Ea	4,6-Dimethyl-	
		2-propyl-	S, Ea
<u>Indole</u>		4-Butyl-2,6-dimethyl-	S, Ea
	S, A, P, Ea		
<u>Thiazole</u>		<u>Bicyclo-1,3,5-dithiazine</u>	
Acetyl-	S, A, Ea, Ep	Pyrrolidino[1,2-e]	
2,4-Dimethyl-	Ea	4H-2,4-dimethyl-	S, Ea
4,5-Dimethyl-	Ea		
2,4,5-Trimethyl-	Ea		
2-Ethyl-4,5-dimethyl-	Ea		
2-Isopropyl-4,5-dimethyl-	Ea		

* Remark shows in which samples each compound occurred.

S: S. lucens (1,3), A: A. japonicus (4), P: P. muelleri (5),
Ea: Antarctic krill (1,3,7,8), Ep: Pacific krill (9).

were newly identified in the volatiles from shrimp, which have only previously been reported as products of the model or synthetic reactions from corresponding aldehydes, hydrogen sulfide and ammonia or aldehydes and ammonium sulfide (10, 23-26). To date, no report concerning these as products from foodstuffs has been available. The concentration of these compounds were not as high as that of thialdine; however, as each compound had a characteristic odor like fuel gas or Allium plant, and the threshold values were low, they must also have had a significant effect on shrimp flavor. It is concluded that the formation of various cyclic polysulfides is important to obtain an characteristic cooked shrimp flavor.

Recently, we identified a new type of bicyclic derivative of a dithiazine compound, i.e. pyrrolidino[1,2-e]4H-2,4-dimethyl-1,3,5-dithiazine (10) in the cooked volatiles from S. lucens and precooked E. superba. Such a bicyclic compound is not common in the volatile of food, and no bicyclic compound has previously been found under the formation system for thialdine homologs; in this case, a nitrogen source other than NH_3 should be considered. Further details on the precursor are under investigation. The odor of this bicyclic dithiazine was similar to that of thialdine and the concentration was almost as large as that of thialdine among the dithiazine contents in S. lucens and E. superba.

Composition of the Main Heterocyclic Compounds

As previously mentioned, the main components of the volatiles from small boiled shrimp were pyrazines and cyclic polysulfides. The composition of the volatiles was roughly divided into two types, those with a higher content of pyrazines or with a higher content of cyclic polysulfides. The compositions of pyrazines, trithiolanes and dithiazine derivatives in the volatiles from boiled shrimps were calculated from the GC peak areas (1,4,7,27) and are shown in Figure 1.

The contents of trithiolanes and dithiazines were larger than that of pyrazines in raw S. lucens and A. japonicus, both of which belong to the same taxonomical group. Conversely, in raw Antarctic krill, alkylpyrazines comprised the predominant part of the volatiles with few cyclic polysulfides also present. The composition of precooked krill corresponded to that of S. lucens, and that of fermented A. japonicus to raw krill.

Some reports (23-25) proved that trithiolanes, dithiins and dithiazines are easily produced from aldehydes, ammonia and hydrogen sulfide, which are universally generated from the thermal cleavage of sugar, lipid and amino acid in the cooked foodstuffs. As shown in Table II, it seems that the main reaction was due to the content of total free amino acids. As the protease activity of Antarctic krill is high (28), raw krill in the market contains more free amino acids than S. lucens or A. japonicus. Precooked krill was produced on a commercial basis by boiling on board for 5 minutes in 90°C sea water just after harvest to deactivate protease (28), the elution of free amino acids into sea water lowering the amino acid content in precooked krill. On the other hand, the content of free amino acids are greatly increased during the formation of raw fermented A. japonicus by its own protease.

In conclusion, the concentration of free amino acids as the

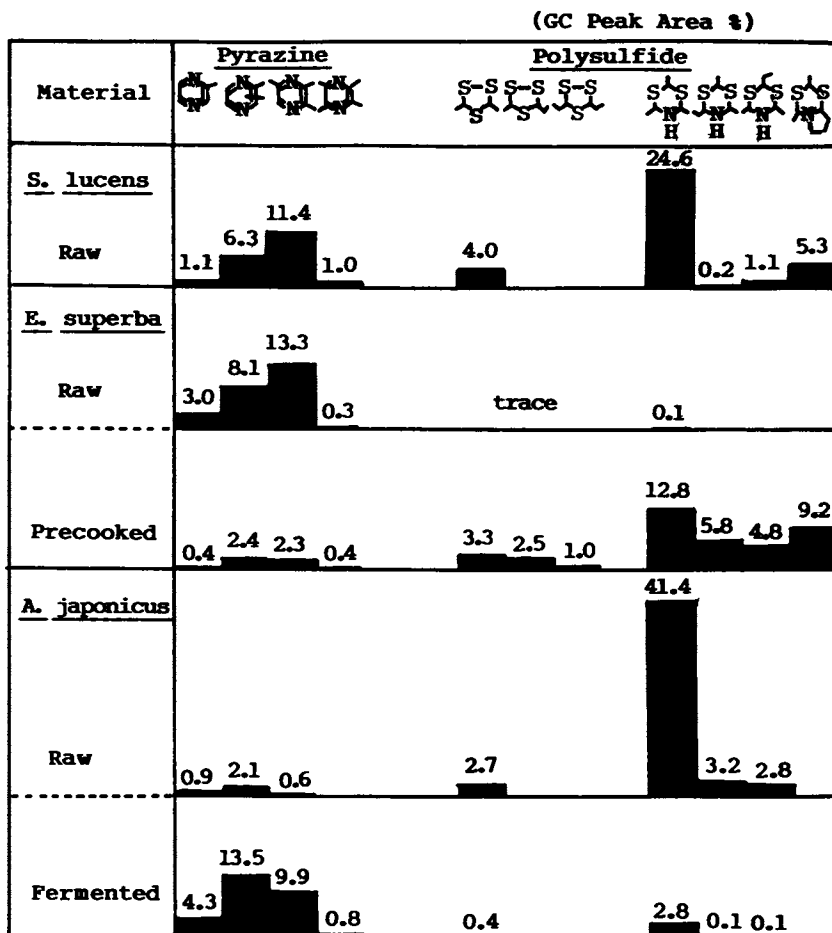


Figure 1. Compositions of Main Heterocycles in the Volatiles from Cooked Shrimps.

precursor should give the initiative for the formation pathway of the cooked volatile in small shrimps; i.e. a larger quantity of free amino acids would result in the predominant formation of pyrazines and, on the contrary, the formation of such cyclic polysulfides as trithiolanes and dithiazines would precede the pyrazine formation when the concentration of free amino acids is less in shrimps.

Table II. pH, Ammonia N and Free Amino Acid Content of *S. lucens*, *E. superba* (7) and *A. japonicus* (4)

Sample	pH*	Ammonia N	Amino acid	
			Cys	Total
		mol/g		mol/g**
<i>S. lucens</i> (raw)	8.0-8.8	41.3	5.8	701.7
<i>E. superba</i> (raw)	7.7-8.4	50.1	11.9	1113.4
(precooked)	8.0-8.7	10.4	1.8	479.6
<i>A. japonicus</i>				mol/N 100g**
(raw)	7.4		12.9	4794.5
(fermented)	6.9		39.4	8190.4

* pH shows before cooking-after cooking

** in dry weight of sample

Identification of 5,8,11-Tetradecatrien-2-one

The cooked aroma of *E. pacifica* (Pacific krill) is fishy and relatively unacceptable. Its GC profile is quite different than that of other samples, because N,N-dimethyl-2-phenylethyl amine was a principal volatile component while only small quantity of pyrazine and thialdine were observed (see Table I). This amine was not produced by cooking, it occurs in vivo (27). Volatiles from both raw and boiled (85°C, 90 min) *E. pacifica* were prepared by steam distillation under reduced pressure (20 Torr).

Gas chromatograms of these volatiles are shown in Figure 2. The GC profiles show that two compounds (peaks A and B) were newly produced by cooking. By sniffing the effluents from a gas chromatograph, it was determined that these two components have a characteristic seafood-like aroma, although not intense.

The spectroscopic and synthetic investigations suggested that these two compounds A and B were novel unsaturated methylketones determined as (5Z,8Z,11Z)- and (5E,8Z,11Z)-5,8,11-tetradecatrien-2-one, respectively (10). They are stereoisomers to each other, compound A having the same partial structure and configuration as that of a ω -3 fatty acid.

Both these methylketones were detected together in the volatiles from all cooked samples of *S. lucens*, *A. japonicus*, *P. muelleri* and *E. superba*. It appears that these novel methylketones are important constituents in addition to the above-described heteroatomic substances of the flavors of cooked shrimps.

Sensory Evaluation and Occurrence of Eight Isomers

As these methylketones have three double bonds in their structures, there should exist eight geometrical isomers. We synthesized all eight isomers (29) and checked the occurrence of the isomers in the volatiles of shrimps by mass chromatography. Only the (all-Z)- and (5E,8Z,11Z)-isomers could be found in shrimp volatiles.

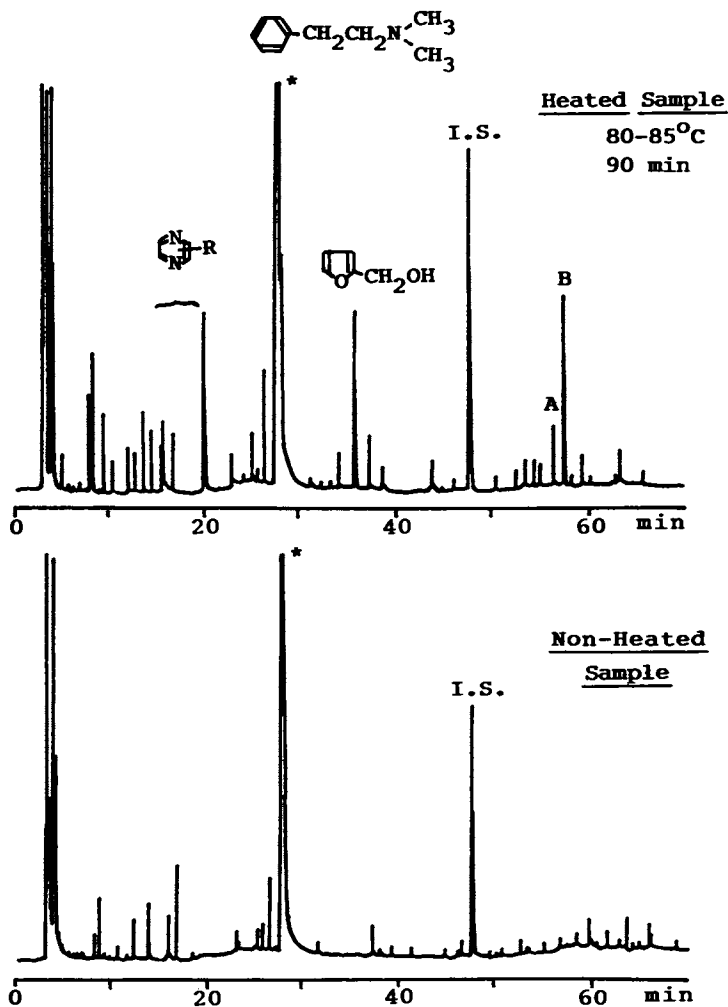


Figure 2. Gas Chromatograms of the Volatiles from *E. pacifica* Prepared by Steam Distillation under the Reduced Pressure.

Conditions; Column, PEG 20M 0.25mm i.d. X 50m FS-WCOT,
Oven Temp., 60 \rightarrow 180°C, 2 °C/min

I.S.: Internal standard, Undecanol.

Peaks A and B: 5,8,11-Tetradecatrien-2-one

In addition, their aroma characteristics were evaluated by a sensory test, the results being summarized in Table III (29). It is interesting that two ketones found in *E. pacifica* (I and II) had a typical seafood aroma reminiscent of cooked small shrimps and shell fish. Since compounds III and V also had an aroma like seafood products, the presence of the C-11 (Z) double bond seems to be a key factor in the aroma of seafood products.

Table III. Kovat's Index Data and Aroma Characteristics of Isomers of 5,8,11-Tetradecatrien-2-one

Isomer	KI Value*	Aroma** Characteristics
I (5 <u>Z</u> ,8 <u>Z</u> ,11 <u>Z</u>)***	2014	shrimp, crab, shell fish
II (5 <u>E</u> ,8 <u>Z</u> ,11 <u>Z</u>)	2033	shrimp, crab, sea cucumber
III (5 <u>Z</u> ,8 <u>E</u> ,11 <u>Z</u>)	2037	fruity, oily, seafood
IV (5 <u>Z</u> ,8 <u>Z</u> ,11 <u>E</u>)	2011	fruity, oily, milk
V (5 <u>E</u> ,8 <u>E</u> ,11 <u>Z</u>)	2041	short-legged clam, seafood
VI (5 <u>E</u> ,8 <u>Z</u> ,11 <u>E</u>)	2029	short-legged clam, oily
VII (5 <u>Z</u> ,8 <u>E</u> ,11 <u>E</u>)	2030	fruity, cucumber, white-meat fish
VIII (5 <u>E</u> ,8 <u>E</u> ,11 <u>E</u>)	2033	oily, fishly, dry bonito

* KI value was measured by using a PEG 20M FS-WCOT column.

** Sensory test was done by smelling paper dipped into a 3-4% ethanol solution of each sample.

*** Numbers are the carbon numbers, and Z and E are the arrangements of their atoms in space.

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One of the naturally occurring methylketones had an E double bond in its structure; however, it retained two Z configurations, and no other E isomers could be found through our study. We assume that both partial structures of tridouble bonds were originally present in raw shrimps, and were not isomerized into each other during the heating and extracting process.

Formation of 5,8,11-Tetradecatrien-2-one

Considering that unconjugated (Z)-double bonds separated by methylene is a typical partial structure of a natural unsaturated fatty acid, it was investigated whether the lipid fraction of shrimp are involved in the formation of these methylketones or not. Neither a lipid extract with chloroform-methanol (2:1) nor that with isopropanol from small shrimp produced any isomers of 5,8,11-tetradecatrien-2-one on heating. In contrast, both the (all-Z)- and (5E,8Z,11Z)-isomers of the methylketone were detected in volatiles from the defatted residue heated with water. Furthermore, the quantity formed was influenced by the heating temperature and time. As shown in Figure 3, when the heating temperature was raised to c. 80°C, the amount of methylketones formed was increased markedly; and the longer the heating time, the greater the quantity of ketones produced. In both cases, the increase of (E,Z,Z) isomers was

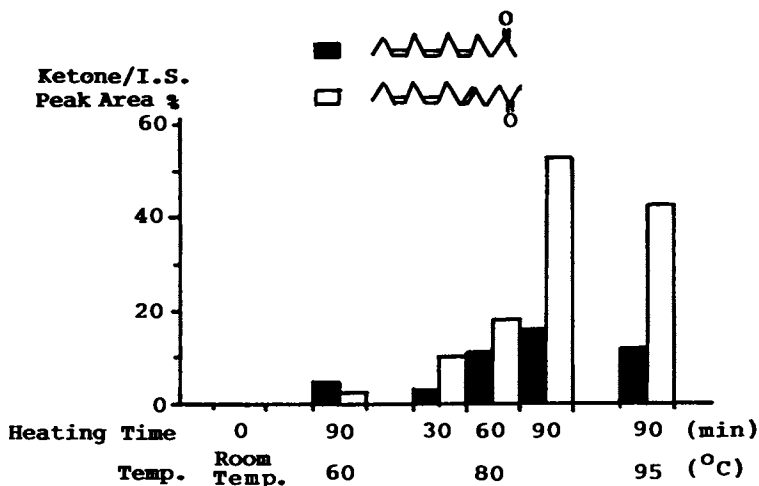


Figure 3. Effect of Heating Temperature and Heating Time on the Formation of 5,8,11-Tetradecatrien-2-ones in the Volatile of *E. pacifica*.

greater. Denaturation of the protein in the sample was observed at c. 80°C. These results show that the precursor existed in the defatted fraction and that a temperature higher than 80°C was needed for the formation of methylketones. We are now assuming that the protein fraction should have some relationship to the methylketone formation.

Conclusion

Various kinds of heterocycles and two unsaturated methylketones were identified as characteristic components in the volatiles from cooked small shrimps. Without exception, they were all thermally generated compounds. Some volatile components from cooked small shrimps were in common with those of other animal protein foodstuffs like meat; however, various types of compounds found in another foodstuffs were composed of the volatiles from specific shrimp species. Both the precursors and the formation pathways for the typical aroma compounds have already been elucidated, even though it is difficult to explain the different constituents of the volatile components among shrimp species. In future, it will be necessary to investigate the key factors which define the possible pathway to form characteristic volatiles in each foodstuff.

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Chapter 36

Volatile Flavor Components in Thermally Processed Louisiana Red Swamp Crayfish and Blue Crab

T. C.-Y. Hsieh, W. Vejaphan¹, S. S. Williams², and J. E. Matiella

Department of Food Science, Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, Baton Rouge, LA 70803 and College of Agriculture and Louisiana Sea Grant College Program, Louisiana State University, Baton Rouge, LA 70803-4200

Louisiana crayfish (*Procambarus clarkii*) and blue crab (*Callinectes sapidus*) were analyzed for volatile flavor components. Dynamic headspace sampling, capillary column gas chromatography, mass spectrometry and chromatography-coupled aroma perception were used for characterization. Over 100 volatile components were identified in boiled crayfish tail meat, boiled crayfish hepatopancreas and/or pasteurized crabmeat.

Crayfish and crabs are two important crustacean seafoods in Louisiana, one of the most important seafood-producing states. Crayfish are about the size of a shrimp and look like small lobsters. Crayfish, also known as "crawfish" in Louisiana, have been regarded as one of the most important elements in Cajun cuisine, which is famous for its unique flavors. Louisiana's crayfish industry comprises the largest commercial crustacean aquaculture industry in the United States with an annual harvest exceeding 45 million kg and is increasing (1). The red swamp crayfish is the major commercial species. While the tail meat has been the major edible portion of crayfish, its hepatopancreas, also known as crayfish "fat" in Louisiana, is traditionally considered a rich source of natural crayfish flavors. Kinlin et al. (2) used steam distillation as a method of isolation and analyzed the volatile components of hepatopancreas from a boiled crayfish sample after the sample had been stored frozen for 3-4 months. Information on the flavor components in freshly boiled crayfish is needed for crayfish product quality improvement.

¹Current address: Lever Brothers Thailand, Inc., Bangkok, Thailand

²Current address: T. J. Lipton, Inc., Englewood Cliffs, NJ 07632

Besides the Atlantic coast, Louisiana and the Gulf coast states are also famous for their abundant supply of blue crabs. The meat from cooked blue crabs, with its delightful aroma and taste, has long been appreciated by people worldwide. The highly perishable character of this delicacy limited its market to coastal regions. Since the development of pasteurization processes for crabmeat, shelf life under refrigeration was extended from 7-10 days to about 9 months, enabling market expansion to distant regions. The presence of certain objectionable flavors in pasteurized crabmeat has been a periodic complaint. Unfortunately, very little information is available on the composition of volatile flavors of this product.

This paper discusses initial results obtained from analysis of the volatile components of the tail meat and the hepatopancreas of boiled crayfish and the volatile components from pasteurized crabmeat samples using a procedure of combined dynamic headspace sampling, capillary column gas chromatography and mass spectrometry.

MATERIALS & METHODS

Materials

Live red swamp crayfish (*Procambarus clarkii*) and pasteurized blue crab (*Callinectes sapidus*) meat samples were purchased from local seafood retailers.

Sample Preparation

Live crayfish were processed and the tail meat prepared as described by Vejaphan et al. (3). The hepatopancreas samples from boiled crayfish were briefly ground in a mortar and pestle and were analyzed immediately. The pasteurized crabmeat samples were refrigerated in the original sealed cans for 290 days and were analyzed immediately upon opening.

Dynamic Headspace Sampling/Gas Chromatography/Mass spectrometry

Procedures used have been described elsewhere (3).

RESULTS & DISCUSSION

Fifty-eight volatile compounds were identified in the dynamic headspace of the tail meat from boiled crayfish. Forty-nine volatile compounds were identified in the dynamic headspace of boiled crayfish hepatopancreatic tissue, while forty-five volatile components were identified in the dynamic headspace of pasteurized crabmeat. These compounds are listed in Table I with the exception of n-alkanes.

Table I. Volatile components identified in the dynamic headspace of tail meat (T) and hepatopancreas (H) from boiled crayfish and from pasteurized crabmeat (C)

Compound ^C	Retention Index	Area %		
		T	H	C
3-methylbutanal	910	0.2		6.0
2-propanol	926			2.2
2-ethylfuran	945			0.4
pentanal	975			23.9
2-pentanone	980			0.3
2,3-butanedione	982	2.3		
alpha-pinene	1017		0.3	
toluene	1040			5.6
3-hexanone	1052			1.4
2,3-pentanedione	1070	0.1	0.1	
dimethyl disulfide	1079	0.1 ^d	0.1	3.0
2-hexanone	1088	0.1 ^d		0.5
hexanal	1090	0.6 ^d	0.7	5.9
2-methylthiophene	1100	0.1 ^d		1.1
2-methyl-2-butenal	1103		0.1	
p-xylene	1132	0.1	0.4	1.1
ethylbenzene	1132	0.1	1.6	0.7
t-2-pentenal	1138		1.5	
m-xylene	1144	0.2	1.0	1.8
1-butanol	1156	0.1		
o-xylene	1188		0.4	1.0
2-heptanone	1190	0.3	0.8	0.4
pyridine	1189		0.2	
heptanal	1193	0.4	1.4	0.4
limonene	1197	0.2	0.3	0.4
propylbenzene	1214	0.1 ^d	0.1	0.2
3-methyl-1-butanol	1218	0.1		
3-hexanola	1220			0.5
4-ethyltoluene	1227			0.6
t-2-hexenal	1228		0.3	
3-ethyltoluene	1230			0.6
2-pentylfuran ^a	1235	0.1 ^d	0.2	0.7
2-hexanola	1245			0.3
1,3,5-trimethylbenzene	1251	0.1	0.4	0.4
thiazole	1258		0.1	
1-pentanola	1266	0.4		0.7
styrene	1267	0.1 ^d		
ethyltoluene	1269			0.2
methylpyrazine	1273	0.1	0.1	
p-cymene	1275	0.1		
1,2,4-trimethylbenzene	1288	0.4		0.7
C4-alkylbenzene	1288		1.1	
C4-alkylbenzene	1291		5.4	

Continued on next page

Table I. Continued

Compound ^c	Retention Index	Area %		
		T	H	C
2-octanone	1293	0.1 ^d	0.1	0.1
octanal	1297	0.3	0.7	0.1
cyclohexanone	1296	0.1 ^d		
1,4-diethylbenzene	1305	0.1 ^d		
cyclohexanone ^a	1307			0.1
a C4-alkylbenzene ^a	1308			0.1
a C4-alkylbenzene ^a	1310			0.1
2-penten-1-ol	1324		0.1	
2,5-dimethylpyrazine	1325	0.1		
2,6-dimethylpyrazine	1331	0.1	0.1	
t-2-heptenal	1334		0.4	
a C4-alkylbenzene ^a	1334			0.1
2-ethylpyrazine	1339		0.1	
1,2,3-trimethylbenzene	1344	0.2	0.6	
a C4-alkylbenzene ^a	1347			0.2
1-hexanol	1359	0.2 ^d		
3-nonanone	1362	0.1 ^d		
a C4-alkylbenzene ^a	1363			0.1
1-hexanol ^a	1370			0.1
cyclopropylbenzene ^a	1377	0.1		
a C4-alkylbenzene ^a	1378			0.1
dimethyl trisulfide ^a	1389	0.1		
3-ethyl-2-methyl- pyrazine	1390		0.2	
2-nonanone	1396	0.5	2.3	
nonanal	1401	0.2	0.4 ^d	
trimethylpyrazine	1407		0.1 ^d	
2-butoxyethanol	1410	0.2		
a C4-alkylbenzene	1433		0.4	
a C4-alkylbenzene	1435		0.5	
5-nonen-2-one ^a	1437	0.3		
t-2-octenal	1438		1.0	
a C4-alkylbenzene ^a	1446			0.1
an ethyl-dimethyl- pyrazine	1448		0.1 ^d	
1,3-dichlorobenzene	1453	0.8		
1,4-dichlorobenzene	1454		0.3	0.3
octenol ^a	1456		1.7	
1-octen-3-ol	1456	0.1		
1,4-dichlorobenzene	1456			0.3
1-heptanol	1463	0.2		
3-methylthiopropional	1469		0.1 ^d	
2,4-heptadienal ^{a,b}	1475		0.9	
furfural	1481			0.1
a C4-alkylbenzene ^a	1495		0.3	
1,2-dichlorobenzene	1497	0.2		
decanal	1498			0.2

Continued on next page

Table I. Continued

Compound ^c	Retention Index	Area %		
		T	H	C
2-decanone	1500	0.2		
<u>t,t</u> -2,4-heptadienal	1503		0.9	
n-decanal	1506	0.6		
1H-pyrrole	1526	0.3		
benzaldehyde	1535	0.7	0.5	0.1
<u>t</u> -2-nonenal	1544	0.1	0.3	
1-octanol	1565	0.2		
3-undecanone ^a	1571	0.1		
2,6-nonadienal ^{a,b}	1594		0.2	
2-undecanone	1603	0.4	0.8	
acetophenone	1660	0.2		
1-nonanol	1665	0.2		
naphthalene	1748	0.3		
1-decanol	1765	0.1		
<u>t,t</u> -2,4-decadienal	1821		0.8	
2-methylnaphthalene	1863	0.2		
1-undecanol	1876	0.1		
1-methylnaphthalene	1898	0.1		
phenol	2014	0.1	0.3	0.2

^a : tentatively identified by matching the sample spectrum with literature reference spectra without confirmation of the retention index with that of an authentic standard.

^b : configuration of geometric isomers not determined.

^c : n-alkanes not included.

^d : area% smaller than the number indicated.

Three alkylpyrazines, including methylpyrazine, 2,5-dimethylpyrazine and 2,6-dimethylpyrazine, were identified as nutty components in boiled crayfish tail meat. Six alkylpyrazines (methylpyrazine, 2,6-dimethylpyrazine, ethylpyrazine, 3-ethyl-2-methylpyrazine, trimethylpyrazine and an ethyl-dimethylpyrazine) were identified in the headspace of crayfish hepatopancreas. Pyrazines are generally considered as key flavor components in many heat processed foods because they contribute nutty, roasted or toasted aromas. Since the formation of alkylpyrazines has been found to increase when food samples were heated at higher temperatures (4-5), cooking crayfish at higher temperatures such as frying may enhance the formation of these and other nutty flavors. Interestingly, no pyrazines were found in the pasteurized crabmeat.

1H-pyrrole was identified in the boiled tail meat. Although it did not give a strong nutty aroma comparable to those of alkylpyrazines, 1H-pyrrole could still be a desirable flavor component of boiled crayfish tail meat due to its slightly burnt and sweet character.

Dimethyl disulfide and dimethyl trisulfide were identified in crayfish tail meat with the former also identified in the boiled crayfish hepatopancreas and the pasteurized crabmeat. These two straight chain sulfur-containing compounds exhibit a cooked cabbage and spoilage odor. Although not present as major components in crayfish, these compounds generally are considered undesirable. Dimethyl disulfide and dimethyl trisulfide have been found previously in thermally processed seafoods and meat products (6-11).

A heterocyclic sulfur-containing compound, 2-methylthiophene, was identified in boiled crayfish tail meat and pasteurized crabmeat. Thiazole and 3-methylthiopropional were identified in the crayfish hepatopancreas. Heterocyclic sulfur-containing compounds play important roles in generating meaty aromas in a variety of meat products and are considered important volatile aroma components of marine crustaceans (12-14). The 2-methylthiophene could be an important flavor component in boiled crayfish tail meat. Both thiazole and 3-methylthiopropional were important contributors to the desirable meaty aroma associated with crayfish hepatopancreas. The 3-methylthiopropional, identified in boiled crayfish hepatopancreas, is derived from Strecker degradation of methionine (15), and has been considered to be an important component in basic meat flavor (16). Pyridine was detected in the headspace of the hepatopancreas from freshly boiled crayfish. Pyridine and 2-ethylpyridine have been previously reported as components in the atmospheric distillate from a sample of crayfish hepatopancreas frozen for three months (2).

As shown in Table I, many volatile flavor components were identified in the samples analyzed. Lipid composition generally affects storage stability of various foods. Omega-3 polyunsaturated fatty acids (PUFA) have been identified in the edible portions of pond-raised and wild crayfish. Crayfish were reported to have higher levels of omega-3 PUFA than

either brown shrimp or fresh water prawns (17). The high susceptibility to quality deterioration and the formation of lipid degradation products in crayfish are probably directly related to its high omega-3 PUFA concentrations.

Eight volatile aldehydes containing 5 to 10 carbons were identified in the crayfish tail meat. Fifteen aldehydes were detected in the dynamic headspace of crayfish hepatopancreatic tissue and included 4 saturated aldehydes, 6 alkenals, 4 dienals and 1 aromatic aldehyde. Five saturated aldehydes were identified in pasteurized crabmeat. All of these compounds have been previously identified in the steam distillate of the crayfish hepatopancreatic tissue; however, only pentanal and hexanal have been detected in the headspace fraction (2). In our study, the saturated and mono-unsaturated aldehydes gave greasy green plant-like odors and probably were not desirable crayfish flavor components. The compound 3-methylbutanal was identified in the crayfish tail meat and the pasteurized crabmeat, giving a green plant-like aroma. This compound was reported as one of the major constituents in sheep liver volatiles (18). Benzaldehyde gave a pleasant almond nutty and fruity aroma and was identified in pasteurized crabmeat and both of the crayfish samples. Furfural, mainly a carbohydrate degradation product, also was identified in the pasteurized crabmeat sample with an interesting slightly sweet and cooked flavor. This compound might be an important component consistent with the overall desirable aroma of pasteurized crabmeat.

The alkadienals could be formed from the autoxidation of PUFA and may contribute desirable aromas to freshly prepared foods (19). Further degradation of alkadienals often increased undesirable flavors. Josephson and Lindsay demonstrated that 2,4-decadienal could produce 2-octenal and ethanal (20); and 2,6-nonadienal could produce 4-heptenal and ethanal (21) via retro-aldol condensation mechanisms. Hsieh et al. (22) reported that isomers of various alkadienals and alkatrienals gave green, greasy and oxidized fish oil odors in crude menhaden fish oil.

Ketones were found to be the major volatile components in boiled crayfish tail meats. A total of seven saturated ketones (C6 - C11), one unsaturated ketone, one cyclic ketone, one aromatic ketone and two alkanediones were identified in the boiled crayfish tail meat. The two alkanediones, 2,3-butanedione and 2,3-pentanedione, gave an intense buttery and desirable aroma. Acetophenone imparted sweet rose floral odor. Four methyl ketones (C7 - C11) and 2,3-pentanedione were identified in boiled crayfish hepatopancreas. Four methyl ketones (C5 - C8) were identified in pasteurized crabmeat. These methyl ketones were usually associated with green, fruity aromas and gave more floral aromas as chain length increased. Several ketones (C4 - C8) also have been reported as volatile flavors of shrimp (13). The diketones might be important aroma components for crayfish tail meat and hepatopancreas products in providing desirable balance of the meaty and buttery notes.

A series of alcohols (C4 - C11) were identified in the tail meat. Odor threshold concentrations were generally higher for alcohols than the aldehyde counterparts. Except for 1-pentanol, the remainder of alcohol peaks were very small and might not be significant in overall aroma of boiled crayfish tail meat. Josephson et al. (23-25) found 1-octen-3-ol, an enzymatic reaction product derived from lipids, to be one of the volatile components widely distributed in fresh and saltwater fish. The compound 2-butoxyethanol identified in crayfish tail meat (3) has been reported in beef products (26-27). GC aroma perception of standard 2-butoxyethanol gave a spicy and woody note, hence this compound could be an important flavor component of the boiled crayfish tail meat.

Ethylfuran was identified in pasteurized crabmeat. 2-Pentylfuran was also identified in all three samples and contributed negatively to the flavor quality of both crayfish and blue crab. Limonene was identified in all three samples and possibly entered these crustaceans via food ingestion. Limonene has been found as a volatile component in fish (24), krill (28) and shrimp (13).

Eleven, thirteen and twenty alkylbenzenes were identified among the volatiles in the boiled crayfish tail meat, hepatopancreas and the pasteurized crabmeat, respectively. The alkylbenzenes and the naphthalenes might have come from environmental pollutants. Lee et al. (30) reported rapid uptake of naphthalene in marine fish. Several chlorobenzenes identified in the crayfish and crabmeat samples possibly were degradation products of various pesticides. Neff et al. (29) reported that aromatic hydrocarbons accumulated in fish to a greater extent and were retained longer than the alkanes. Phenol was also identified in all three samples. The medicinal odor of phenol contributed negatively to these products. Kubota et al. (28) identified xylenes and phenol as volatile components that contributed undesirable odors to cooked krill.

Besides those discussed above, many areas in the GC aromagrams of the volatiles from the three samples contained interesting aroma characteristics, including strong nutty, musty nutty, nutty meaty, good meaty, salty-meaty, mushroom-like and cooked egg-yolk like aromas.

A substantial amount of information on volatiles can be obtained with less than 30 g of each of these samples in a direct DHS/GC/MS analysis. DHS operation sweeps volatile flavors from the surface of food samples in a similar way as we sniff for the volatile flavors of a food. DHS does not require high sampling temperature or solvent for extraction and may be considered as a low-artifact aroma sampling technique. The concentrating effect of DHS provides better sensitivity than static headspace sampling. Techniques such as GC-coupled aroma perception and GC/MS identification can be used to complement other approaches in improvement of flavor quality of a variety of products.

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Chapter 37

Thermally Generated Volatile Compounds in Packaging Materials

H. Kim and S. G. Gilbert

Department of Food Science, New Jersey Agricultural Experiment Station,
Cook College, Rutgers, The State University of New Jersey,
New Brunswick, NJ 08903

The increasing application of complex natural and/or synthetic polymers to food packaging has required definitive information on the characteristics of the finished products. High temperature encountered during the manufacturing process may induce thermal decomposition products which can migrate into the packaged product and cause undesirable flavor. A general methodology for testing polymer odor and odor contributors is discussed in this paper with examples representing the odor of PVC film, ionomer laminate and gelatin. The precursors and the mechanisms of the major volatile components of each packaging material are presented, including the effects of processing conditions on the odor quality of polymers.

Food packaging has been dominated in the last few decades by innovations in plastic packages. These include the developments of different types of polymers, copolymers, the laminates and, especially, packaging materials suitable for microwave heating. Considerable improvement in design effectiveness has been produced with the introduction of new materials and processes, making this area one of the most active in food product development. This growth has been paralleled by an increasing attention to the interaction between plastic packaging materials and foods.

One of the practical concerns in polymeric package is the presence of compounds in toxicologically insignificant amounts, but at levels affecting aroma and/or taste of the packaged food (1-2). There has been an extensive history of odor and taste problems experienced in the development of new packages (3-7). A package can directly disrupt the flavor balance of a food in three ways; (a) subtraction, (b) reaction, and (c) addition. Subtraction occurs when components contributing to the desired flavor of the product are absorbed by the package. Reaction takes place when package components chemically interact with the food product to

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produce flavor artifacts. Addition occurs when the package releases compounds which alter the flavor balance of the food. Addition is by far the most common (3).

The manufacture of packaging materials is often conducted under conditions of high temperatures. A burnt polyethylene odor has been experienced in the paper/foil/ polyethylene laminate field. These conditions can easily induce thermal degradation with the formation of volatile compounds in packaging materials (8-13).

This article summarizes volatile compounds derived during manufacturing packaging materials as well as the precursors of the major volatile components.

Methodology

Sensory Evaluation. The odor quality of test plastic materials such as resins, films or laminates can be directly estimated by panelists using rating scale evaluation of intensity or forced choice methods such as triangle test as the test forms (14-15). The extent of migration of residual volatile compounds in packaging materials to the contacting phase can be evaluated after exposing water or actual food to packaging materials at accelerated conditions or at ambient temperature (3,8). Food can be exposed to packaging materials by direct contact or by vapor phase transfer to determine if the adverse odor/flavor problem is caused by any of the more volatile package constituents or caused by constituents that can only be transferred by direct contact solvation. Glass containers are generally used as control packages.

Sensory evaluation can be paralleled with objective instrumental analyses and the correlation between the sensory and instrumental results can be obtained (16).

Instrumental Analysis. Direct headspace analysis has been generally used to analyze the residual solvents in packaging materials (1,4,5,7,17-19). Vapor samples can be prepared by drawing the headspace of sealed packages through tubes containing sorbents such as Tenax GC. Both filled and empty packages can be used in these analyses. A gentle vacuum was applied to one end of the trap using an aspirator with a needle attached at the other end of the trap to puncture the bag (20). The concentration of volatiles in the headspace is necessary to obtain a more complete picture of the volatile composition as well as to obtain a positive identification of volatile compounds. Dynamic headspace/gas chromatography (DH/GC) has gained popularity as an effective and sensitive technique for the analysis of volatile compounds (21). Residual volatile compounds in the plastic materials were investigated using similar procedures (8,11,12,14,22). Kinetic studies on the effect of oxygen concentration, temperature and additives, such as antioxidants, on the generation of volatiles under simulated processing conditions were conducted with resins (Paik, S. W. and Gilbert, S. G., Rutgers University, unpublished data). A total of 15 mg of resins were ground to about 40 mesh by a Freezer/mill (Spex Industries

Inc., Metuchen, NJ) at liquid nitrogen temperature, mixed with 3 g of DMCS treated 60/80 mesh glass beads (Alltech Assoc., Deerfield, IL) and packed inside a 1/4" ID x 6' L stainless steel tube. The sample-containing tube was placed inside an oven and connected to heated oxygen and nitrogen and the reaction proceeded under a gas flow rate of 15 ml/min at an increased temperature for one hour. Oxygen concentration in the carrier gas was varied by diluting the oxygen with nitrogen gas and monitored by injecting 0.5 ml into a gas chromatograph equipped with a CTRI-8700 column (Alltech Assoc., Deerfield, IL) and a thermal conductivity detector (TCD). Degradation products were collected during the reaction at from 160 to 220° C directly in the analytical column kept at a subambient temperature using dry ice. The reaction was terminated by switching to helium gas at room temperature and turning off the reaction oven. After removing the dry ice from the analytical oven, the collected compounds were analyzed for total peak area for kinetic studies.

The rate of reaction (dA/dT : area/min) at different oxygen concentrations (%) in the carrier gas provides the effect of oxygen concentration on the generation of volatile compounds. Likewise, the effect of temperature was obtained from the rate of reaction against the reaction temperature.

Volatile Compounds Identified in Specific Packaging Materials

Polyethylene Terephthalate (PET). The major significant volatile compound in the polyethylene terephthalate (PET) was characterized as acetaldehyde, also known to be a major cause for the color change of PET during aging and of concern in odor quality. The mechanism for the thermal degradation of PET is considered to be similar to that for simple ester pyrolysis proceeding via a cyclic six-member transition state (23-24). PET decomposes by a molecular mechanism with random chain scission at the ester links (25). Thermal degradation products derived from polyester have been studied using model compounds such as ethylene dibenzoate, 2-hydroxyethyl benzoate and diethylene glycol dibenzoate (26-27). The vinyl compounds formed during these reactions can undergo further reactions to give a complex mixture of end products.

Rubber Articles. Rubber articles have a characteristic objectionable odor formed by a thermal degradation of polyunsaturated compounds. Thermal oxidation products of rubber articles have been extensively reviewed (28-29). Major volatile compounds generated from oxidation of synthetic cis-polyisoprene contained a (CH₃CO) terminal group including acetaldehyde, methyl formate, propionaldehyde, acetone, methyl acetate, methanol, methacrolein, ethanol, butanone, butenone, pyruvaldehyde, acetic acid, formic acid, and levulinolaldehyde, most of them having a pungent odor. The yield of volatile products increased as the temperature was raised, paralleling increased scission efficiency. As in purely thermal degradation, thermal oxidation of rubber is accompanied by formation of low-molecular-weight products in yields too high to be accounted for by random attack on the

hydrocarbon. Unlike thermal degradation, in which, "unzipping" can yield many small molecules from each primary scission, oxidation involves formation of a single low-molecular-weight compound or small group of compounds at each scission event and is detectable down to room temperature (30). Oxidation without scission has not been experimentally observed, although possibly photooxidation at low temperature may involve peroxidation without scission (31).

Polyethylene (PE). In an unpublished study, pouches were made from paper/foil/PE laminates, and headspace gas was taken from the bag after incubation at 60° C for 20 minutes and analyzed by a gas chromatograph. Three major components were identified as acetaldehyde, allyl alcohol and acrolein. When odorous bags were compared with non-odorous bags, there showed a direct correlation between odor, acetaldehyde and allyl alcohol levels. Those compounds were considered to be thermal oxidative decomposition products of polyethylene (Baxter, J. A., W. Grayson and Assoc., Ltd., unpublished data).

Usually LDPE (Low Density Polyethylene) tends to degrade to shorter chain hydrocarbons at temperatures greater than 300° C and further undergoes oxidation to form a large number of oxidation products such as aldehydes and alcohols (9). The products of thermo-oxidative reactions of polyolefins can develop adverse flavors that can be easily detected in degraded samples. A free radical chain mechanism is generally accepted to explain the thermal oxidation of polyethylene (9). Structure, morphology, presence of stabilizers, and type of environment are the most relevant factors in the degradation of polyethylene. Interactions of the polymer with very small amounts of oxygen can cause oxidative degradation, and exposure to elevated temperatures in the absence of oxygen can also promote degradation (10).

Ionomers. Ionomers are defined as linear organic polymers which are copolymerized with a minor portion of an acid function which is neutralized to varying degrees by a metal or quaternary ammonium ion (32) and the unique properties of this class of compounds are useful in flexible food packaging materials.

Extrusion laminates are produced from ionomers eliminating the need for adhesives with solvent problems, simultaneously reducing cost. However, the process of ionomer resins as coating for structures involves two high temperature steps that can promote some thermo-oxidative reactions in the material. The first step occurs during the pelletization of the resin, when temperatures of 230° C are used and oxygen is present, the second during the extrusion coating process, when the temperatures are raised above 300° C for relatively short periods of time. Degradation can be initiated during the first step and accelerated during the second step. Fernandes et al (8) studied the formation of volatile compounds during extrusion lamination of ethylene ionomer. A large number of hydrocarbons, alcohols, aldehydes, ketones and etc. were identified from ethylene ionomer resins and foil/ionomer laminates and many of those compounds have been identified in the degradation products of polyethylene (9,13,33). Therefore, the

major mechanism for the formation of those compounds is considered to be similar to that for the degradation of polyethylene.

The total arbitrary peak area of each GC/FID profile obtained from three different ionomer laminates processed at different processing conditions was correlated to the sensory evaluation data as shown in Fig. 1. Likewise, the total peak area of each GC/FID profile obtained from three different ionomer laminates which contained different levels of an antioxidant was plotted versus the antioxidant level and shown in Fig. 2. The data showed a significant contribution of an antioxidant to the inhibition of thermal degradation reaction (Paik, S. W. and Gilbert, S. G., Rutgers University, unpublished data). These data demonstrate the dependence of degradation on the processing condition as well as a high correlation of sensory data with the formation of volatile compounds during processing.

Polyvinylchloride (PVC). Kim et al. (11) analyzed the volatiles of polyvinylchloride (PVC), one of the most versatile packaging materials. The major precursors for the formation of the volatile compounds were considered to be PVC polymer, bis-(2-ethylhexyl) adipate (DOA), bis-2-(ethylhexyl) phthalate (DOP) and tris-nonylphenyl phosphite and epoxidized soybean oil.

The most predominant volatile compound identified was dibutyl adipate which is a decomposition product of DOA. The second predominant volatile compound was 4-nonyl phenol which is again a decomposition product of the antioxidant tris-nonylphenyl phosphite. Most short-chain phthalates were considered to be either decomposition or transesterification products of DOP, which indicated that the type and the amount of additives could be a major cause of off-odor problem.

The composition of thermal degradation products was previously shown to be greatly affected by the processing temperature (34). PVC is inherently unstable because of the presence of allylic chlorine atoms throughout the polymer. These chlorine atoms are easily removed by exposure to minimal heat and/or light which results in the well-known unzipping reaction (35).

Gelatin. Gelatin is used as an edible packaging material in food and particularly in pharmaceutical applications (36). Volatile compounds were isolated and identified from commercial granular gelatin and were considered to be formed through the thermal decomposition of amino acids (12). Phenylethyl amine identified in the study had a characteristic fishy odor and was considered a major contributor to the objectionable fishy odor of the granular gelatin sample. It was presumed to be derived from phenylalanine.

Paperboard. Vaccaro identified several volatiles released from PET coated paperboard during heating (37). When the paperboard and PET films were analyzed separately to establish the origin of the released volatiles, no volatiles were detected from the PET film used for the coating during analysis. All of the volatiles detected were released from the paperboard. Seven compounds were identified: acetone, 2,3-butanedione, chloroform, furan, furfural,

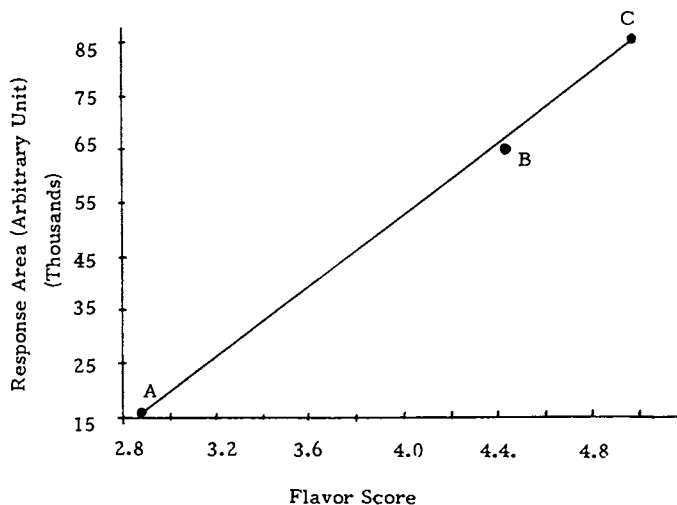


Figure 1. Total peak area vs sensory evaluation data of ionomer laminates extruded at different temperatures. (A: Ionomer Processed at 282° C; B: Ionomer Processed at 304° C; C: Ionomer Processed at 324° C)

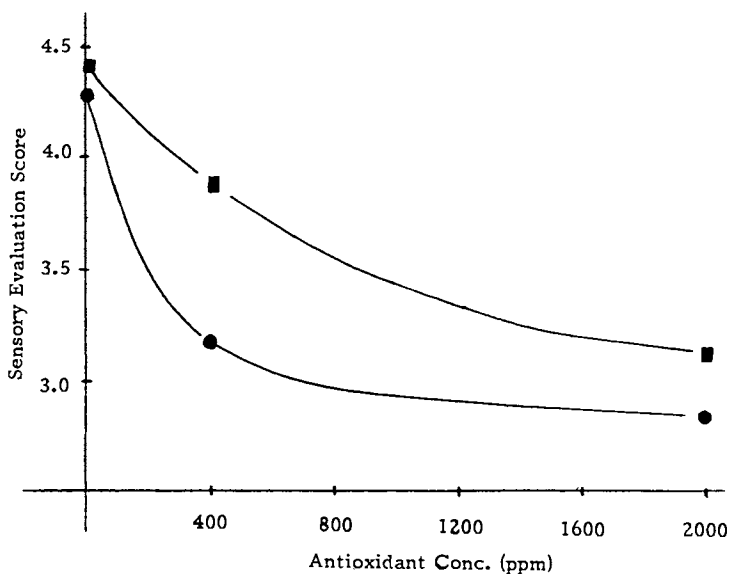


Figure 2. The effect of the antioxidant level on the generation of volatile compounds. (●; Ionomer Resin 1, ■; Ionomer Resin 2)

and methylene chloride, carbon disulfide, and acetaldehyde. The chlorinated compounds may be present as a result of the board bleaching process and the CS₂ as a result of sulfate pulp process to remove lignin. The other compounds are commonly associated with the decomposition of wood through browning reaction (38). Among them, furfural which is the primary pyrolysis product of paperboard can contribute an undesirable flavor to contained foods.

The absorption of identified volatiles by food simulants such as oil saturated cotton balls and gelatinized corn starch was also studied. The actual quantities of three compounds (methylene chloride, carbon disulfide, and chloroform) released from the ovenable board during heating ranged from 0.3 to 3.3 x 10⁻⁶ g compound/g board.

The transfer to food simulants of the three target compounds released from paperboard during heating was evaluated by two methods: partition studies and oven test studies. In closed systems, the partition coefficients for methylene chloride, carbon disulfide and chloroform between the headspace and food simulants (oil and corn starch gel) were determined at 80, 100, and 120° C. Although sorption of the three target compounds into oil was detected at 120° C, none of the three compounds tested were transferred at the ppb level of detection from an unlidded ovenable tray to the food simulants during cooking in an actual oven. This result was attributed to the high temperature of the foods during cooking which may actually favor desorption.

In contrast to the conventional oven, the surface temperature of food in the microwave generally does not exceed 100° C. Therefore, partition coefficient of the volatiles would be different from that in the conventional oven, which indicates the probability that the volatiles emitted from an ovenable tray during cooking may be transferred to the food contents.

In summary, headspace concentration method is the usual way of analyzing the odor quality of packaging materials and has been shown to give reliable data which can be correlated with sensory evaluation data. Oven-heated microwavable packaging materials may pose a special flavor problem in the food consumed.

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Chapter 38

Maillard Technology as Applied to Meat and Savory Flavors

Lawrence L. Buckholz, Jr.

International Flavors and Fragrances, Dayton, NJ 08810

The purpose of pyrolytically treating certain foods is to promote flavor changes that increase their overall palatability. A knowledge of the composition of flavor volatiles produced by heating foods would therefore be most desirable. The Maillard reaction is responsible for these changes and it is involved with food processing in three quite different ways: 1) The development of flavors in traditional roasting, baking and cooking processes. 2) The rational use of "reaction flavor" technology to manufacture engineered foods and processed flavors. 3) Efforts to inhibit undesirable browning, such as that of whey in cheese powders. The development of cooked meat flavors for new "fast" food, frozen entree, and microwave applications is strongly impacted by the Maillard reaction. This review discusses the use of Maillard technology to generate meat and savory flavors, with emphasis on flavor chemistry, precursors, volatiles, and recent flavor improvements.

Man has been involved with Maillard reaction technology from the dawn of time when he first used fire to cook his food. The changes that occurred during the cooking of meat were the result of non enzymatic browning. The flavor produced was the result of sugar-amino acid reactions, Strecker degradation, and fat oxidation; thus, without his awareness, man was using the browning reaction to render meat tender, flavorful and palatable.

Many bland and even unpleasant tasting food substances are transformed into some of the most desirable flavors by roasting (1). Heat-treated foods comprising such different tastes and aromas as chocolate, bread, coffee, roasted meats, cereals, and toasted nuts undergo Maillard browning reactions. Enormous flavor variety is generated from the large number of possible permutations among relatively few primary reactants (amino acids and sugars). While its flavor applications have been well studied, much remains to be understood about basic principles governing Maillard chemistry (2).

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Historical Perspective

The earliest reported scientific studies of the Maillard reaction were initiated by Louis Camille Maillard (1). In a misguided attempt to determine the biological synthesis of proteins, he heated concentrated solutions of D-glucose and amino acids. What he observed was a gradual darkening and frothing, with the development of odors reminiscent of the baking of bread or the roasting of animal or vegetable products. This work attracted sufficient attention to persuade many others to continue the study of what has come to be called the Maillard reaction.

On November 27, 1911 Maillard first reported a condensation of amino acids by the use of glycerol. In a later report he cited a paper on malting by Ling (3), who noted the effects of kilning or heat drying at 120°C to 150°C. Others performed further experiments following Maillard's methods; in 1912 Lintner reported obtaining dark reaction products which were responsible for flavor and aroma (4). Four years later, Pictet described the formation of pyrazine and isoquinoline bases from the acid hydrolysis of casein in the presence of formaldehyde (5). Additional historical details of the Maillard reaction are available (6).

Both the volume of the published work and the recognition of its potential importance to the food industry led to the first general review of the subject in 1951 by Danehy (7). Only two years later a second review, limited largely to a consideration of model systems and mechanistic interpretations was published by Hodge (8). In it, he concluded that "...the control of browning reactions to produce only wanted flavors and odors is an intriguing possibility, ...but progress toward this goal can be made only as the reaction mechanisms are better understood." More recently, three international symposia have been devoted to the Maillard reaction (9-11).

Chemistry of the Maillard Reaction

The Maillard reaction is actually a complex group of hundreds of possible reactions. The initial reaction sequence is similar to that for caramelization, except that the sugars thermally condense with amino acids, peptides, and proteins. Condensation is followed by enolization and dehydration. Two of the several branches of the Maillard reaction are shown in Figure 1 (12). The major pathway leads from the 1,2-eneaminol of the Amadori compound to 5-hydroxymethyl-1,2-furaldehyde. The amino acid may be retained in some molecules of this pathway. The minor branch begins with the 2,3-enediol of the Amadori compound; the amino compounds can then condense with the acyclic carbonyl forms of sugars. Amino acids, peptides, and proteins offer basic amino groups when the pH of the adjacent medium rises above the isoelectric point of the amino compound. The initial rate of browning of a reducing sugar with a given amino compound is directly related to the rate at which the sugar's ring opens to a reducible form. Pentoses and 2-deoxy-D-ribose react faster than 2-deoxy hexoses, which in turn react faster than hexoses. Among the hexoses, browning rates decrease in the order: D-galactose > D-mannose > D-glucose. The general properties and characteristics of the Maillard reaction are summarized in Table I (13).

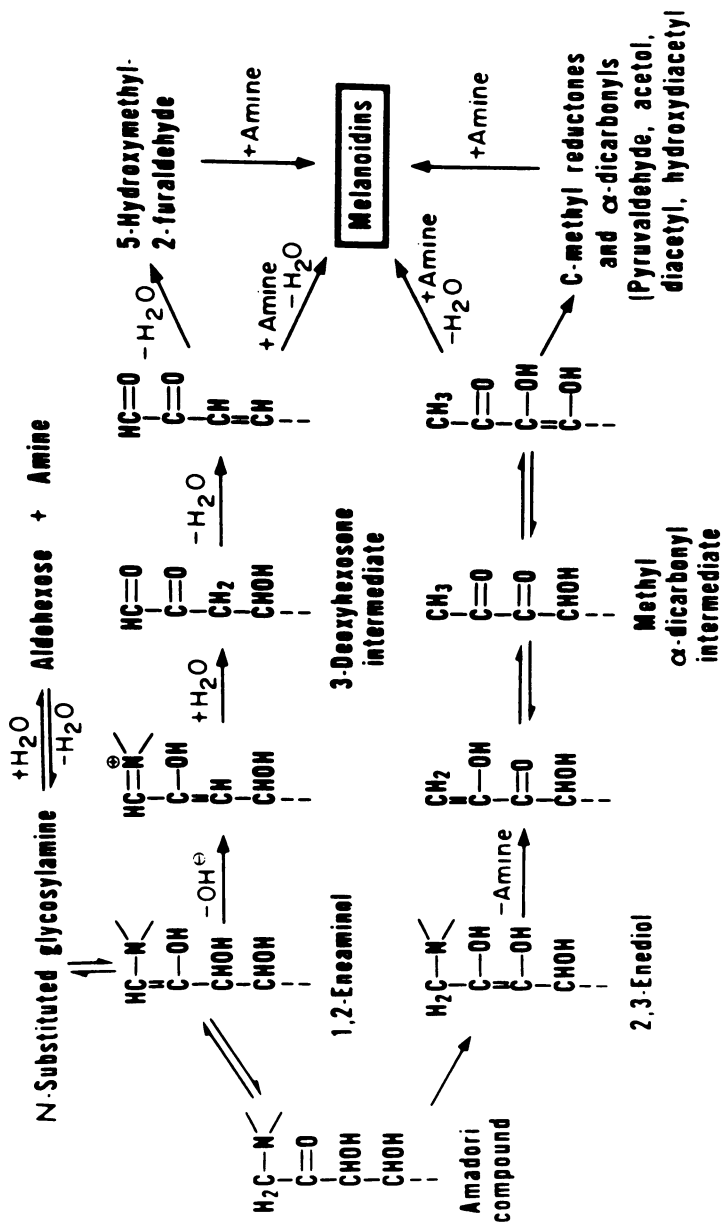


Figure 1. Sugar-amine Maillard browning reactions: Two pathways to melanoidins and by-products. (Reprinted with permission from ref. 12. Copyright 1967 Avi Publishing.)

Table I. Characteristics of Maillard-type Browning Reactions

Stage	Color	Reactions	Properties
<u>INITIAL</u>	Colorless	Condensation Enolization Amadori rearr.	1:1 Glucose:protein-NH ₂ (browns with time) Reducing pwr. in alkalis No absorption in near-UV
<u>INTERIM</u>	Buff yellow	Sugar dehydr.: 3-Deoxyglucosone 5-(MeOH)furfural 2-(AcOH)furan Sugar frag.: α -Dicarbonyls Reductones Pigments	Sulfites decolorize Reducing pwr. in acids pH Decreases Sugars react > amino acids Positive Elson-Morgan test for Amadori cmpds.
<u>FINAL</u>	Red-brown Dark brown	Aldol condens. Polymerization Strecker degrad. CO ₂ evolution	Sulfites do not decolorize Acidic; fluorescence Reducing pwr. in acids Caramel, roasted aromas Colloids, melanins form

Source: Adapted with permission from Ref. 13. Copyright 1976 Marcel Dekker Inc.

In the Maillard sequence, the initial reaction between a carbonyl group and a trivalent nitrogen atom is perhaps the most thoroughly investigated and best understood. As early as 1963, Reynolds (14) published a review with 140 references limited largely to the studies of reactions of aldoses with amines, the determination of the structures and properties of the first products of reaction (a glycosylamine), and the rearrangement of the latter to a more stable ketoseamine.

Typically an aldose reacts simultaneously and reversibly with an amine to form an aldosylamine (an N-alkyl glycoside). Much of the earlier work was done with aromatic amines, but a wide variety of primary and secondary aliphatic amines have also been used. The esters of amino acids readily yield crystalline glucosylamines. In neutral or alkaline solution these glucosylamines exhibit no reducing properties, since they are the nitrogen analogs of the O-glycosides. The glucosylamines are readily hydrolyzed by acids to the parent aldose and amine.

When the carbonyl compound is a ketose (e.g. fructose) rather than an aldose, the fructosylamine precursor (an N-alkyl fructoside) undergoes a Heyns rearrangement to form a 2-alkylamino-2-deoxy-D-glucose (15). These Heyns compounds are precursors of the browning phenomenon.

Another important aspect of the Maillard reaction involves the Strecker degradation of α -amino acids. At elevated temperatures α -dicarbonyl compounds, such as 3-deoxy glucosone, pyruvaldehyde, glyoxal, and dihydroascorbic acid will cause the degradation of an

α -amino acid to the next lower aldehyde. This reaction is illustrated in Figure 2 for glyoxal and glycine.

Pyrazines are formed from transamination reactions, in addition to carbon dioxide and formaldehyde. A requirement is that the carbonyl compound contains a dione and the amino group is alpha to the carboxyl group (16). If the hydrogen on the α -carbon of the amino acid is substituted, a ketone is produced. Newell (17) initially proposed a pyrazine formation mechanism between sugar and amino acid precursors. (See Figure 3). The Schiff base cation is formed by addition of the amino acid to the anomeric portion of the aldo-hexose, with subsequent losses of water and a hydroxyl ion. Decarboxylation forms an imine which can hydrolyze to an aldehyde and a dieneamine. Enolization yields a ketoamine, which dissociates to amino acetone and glyceraldehyde. 2,5-Dimethylpyrazine is formed by the condensation of the two molecules of amino acetone.

More recently, a new Maillard reaction pathway involving sugar fragmentation and free radical formation prior to the Amadori rearrangement has been proposed (18).

Precursors and Model Systems

Bailey (19), did an extensive study of the Maillard reaction and meat flavor. Many desirable meat flavor volatiles are synthesized by heating water-soluble precursors such as amino acids and carbohydrates. These latter constituents interact to form intermediates which are converted to meat flavor compounds by oxidation, decarboxylation, condensation, and cyclization. O-, N-, and S-heterocyclics including furans, furanones, pyrazines, thiophenes, thiazoles, thiazolines, and cyclic polysulfides contribute significantly to the overall desirable aroma impression of meat. The Maillard reaction is important to the formation of Strecker aldehydes, hydrogen sulfide and ammonia.

There are two approaches to meat flavor analysis: one is concerned with the isolation and identification of volatile flavor components, and the other involves identification of non volatile flavor precursors.

Non-volatile Precursors. Water-soluble meat flavor precursors encompass a number of different organic classes of compounds including nucleic acids, nucleotides, nucleosides, peptides, amino acids, free sugars, sugar amines, glycogen, and amines (20-30). The influence of heating on the dialyzable low molecular weight constituents (amino acids, carbohydrates, nucleotides, nucleosides) in beef, pork and lamb was studied (25-29). The predominant amines in the dialyzable diffusate were alanine, anserine-carnosine, and taurine, and these decreased considerably during heating. Other amino acids decreasing during heating included methylhistidine, isoleucine, leucine, methionine, cystine, serine, lysine, glycine and glutamic acid. Glucose, fructose, and especially ribose and ribose-5-phosphate readily decomposed during heating at 100°C for one hour (30).

Volatile Precursors. Investigations involving model systems for meat aromas have been reviewed (31-34). More than 600 volatiles have been identified from meat or simulated meat precursors.

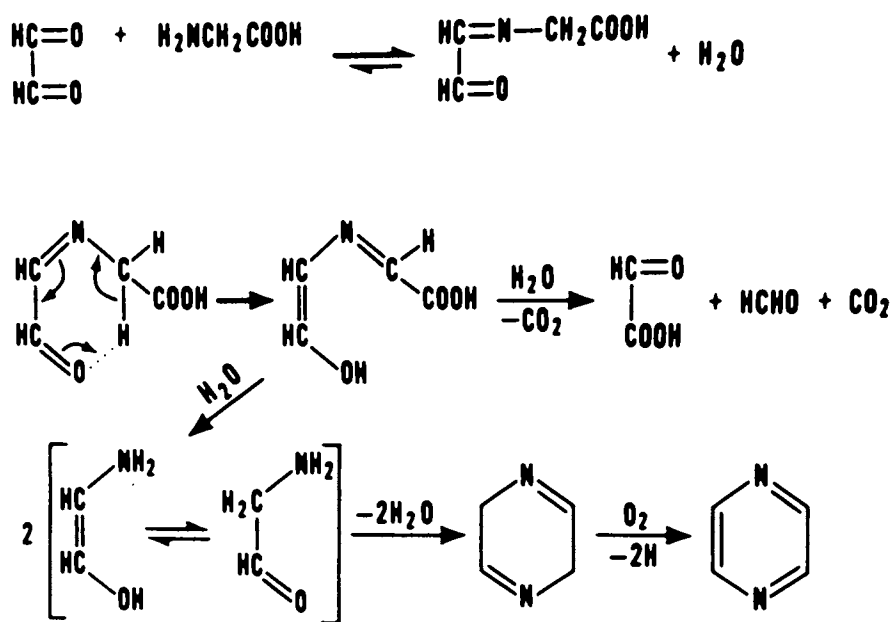


Figure 2. Strecker degradation reactions and products. (Reprinted with permission from ref. 13. Copyright 1976 Marcel Dekker.)

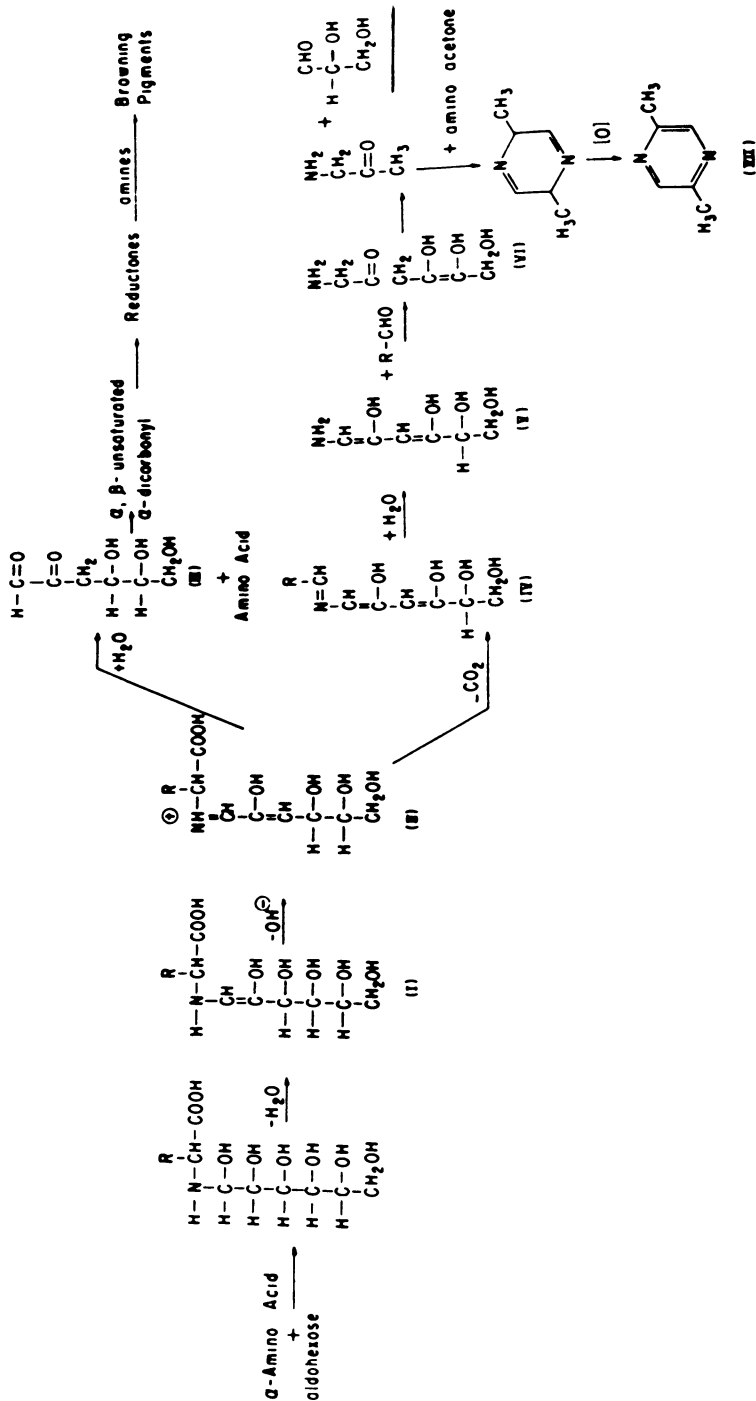


Figure 3. Mechanism for formation of pyrazines. (Reprinted from ref. 17. Copyright 1967 American Chemical Society.)

Further evidence of the importance of the Maillard reaction in the formation of volatile flavorants from meat precursors is gleaned by examining ingredients in reaction mixtures patented as synthetic meat constituents. Ching (31), examined 128 patents of meat flavor and found that 55 specified use of both amino acids and sugars. Cysteine, cystine, and glutamic acid were used in 39 such mixtures. Over 80 patents describe meat flavor "reaction products" (32).

Meat aroma is not the result of one chemical constituent but the sum of the sensory effects of many of these volatiles. Over 90% of the volume of volatile constituents from freshly roasted beef is from lipid, but 40% of the volatiles from the aqueous fraction are thought to be heterocyclic compounds. Heterocyclic compounds contribute significantly to the overall aroma impression of meat.

Although some investigators believe that dihydrofuranone is derived from ribose-5-phosphate through a dephosphorylation-dehydration mechanism, others believe it can be formed by a typical Maillard reaction between amines and sugars, in which the Amadori products dehydrate and eliminate to amines. Both routes are likely to occur.

Furans and Furanones

Eleven furans and seven furanones have been identified from reaction mixtures containing precursors responsible for beef flavor (31). Furfural derivatives were obtained by heating water-extractable components from beef and simple amino acid-sugar mixtures. Furanones can be formed by Amadori compound pathways: Hexoses produce 5-methylfurfurals and 4-hydroxy-2,5-dimethyl-3-(2H)-furanone, while pentoses yield furfural and 4-hydroxy-5-methyl-3-(2H)-furanone. The latter compound was synthesized by heating amines with xylose, ribose, ribose-5-phosphate, or gluconic acid (35,36). Furans that do not contain sulfur are usually nutty, fruity, and caramel-like in odor. The furanones described above have burnt pineapple and sweet roasted notes.

Sulfur Compounds

Thiophenes and Furanthiols. Thiophenes are extremely important contributors to cooked meat flavor and are responsible for mild sulfurous aromas. The sulfur in thiophenes may be derived from amino acids (cysteine, cystine, methionine) or from thiamine. Over 36 thiophene derivatives have been found during various investigations of meat or meat constituents (32).

Probably the most important reactant in the formation of volatile meat flavor compounds is hydrogen sulfide. It can be formed as a Strecker degradation product of cysteine in the presence of a diketone (37).

Several S-substituted furans have been identified from Maillard reaction mixtures which possess meaty aromas including 3-mercapto-2-methylfuran and 3-mercapto-2,5-dimethyl furan (38).

Thiazoles and Thiazolines. Thiazoles and thiazolines provide nutty, roasted notes to meat flavors. These compounds can be formed by combining a diketone, such as 2,3-butanedione (diacetyl), with acetaldehyde, hydrogen sulfide, and ammonia (39). 2-Acetyl-2-

thiazoline is postulated to be formed by Strecker degradation of cysteine followed by oxidative cyclization (40). Other thiazoles have been isolated from meat or meat constituents which have undergone Maillard-type reactions (41).

Polysulfide Heterocyclics. Polysulfur heterocyclics, including thialdine (5,6-dihydro-2,4,5-trimethyl-1,3,5-dithiazine) and trithioacetone (2,2,4,4,6,6-hexamethyl-1,3,5-trithiane), have been identified in meat flavor extracts (41). The formation of these compounds is shown in Figure 4.

1-(Methylthio)ethanethiol. This component was identified in the headspace volatiles of beef broth as a result of Strecker degradation (42). Although it has a roasted onion-like odor, this compound is a significant contributor to beef flavor.

Pyrazines

The identification of 49 pyrazines in heated beef and other meats has been extensively reviewed (32, 43). Several mechanisms have been proposed for pyrazine formation by the Maillard reaction. Dicarbonyl compounds can initiate Strecker degradation of amino acids to yield α -amino ketones, which in turn can undergo condensations and oxidizations to form substituted pyrazines (13).

Sugar-Amino Acid Model Systems

A thorough study of the aromas produced from over 400 model Maillard reaction systems was performed (44). Combinations of 21 amino acids and 8 sugars were evaluated under different conditions of temperature and humidity. Table II lists beefy or meaty aromas produced from thermal interactions between glucose and eight amino acids.

Table II. Generation of "Meaty, Beefy" Aromas in Heated Mixtures of Amino Acids with Glucose

Amino Acid	With Glucose				Alone			
	Temperature (°C)				Temperature (°C)			
	100	140	180	220	100	140	180	220
Asn					•		•	
Cys	•	•	•		•		•	
Cys ₂		•	•					
Glu							•	
Gly					•	•		
Ile					•			
Ser					•	•	•	•
Thr	•				•	•	•	•
Total Meaty Aromas:	3				7			

Source: Adapted from Ref. 44.

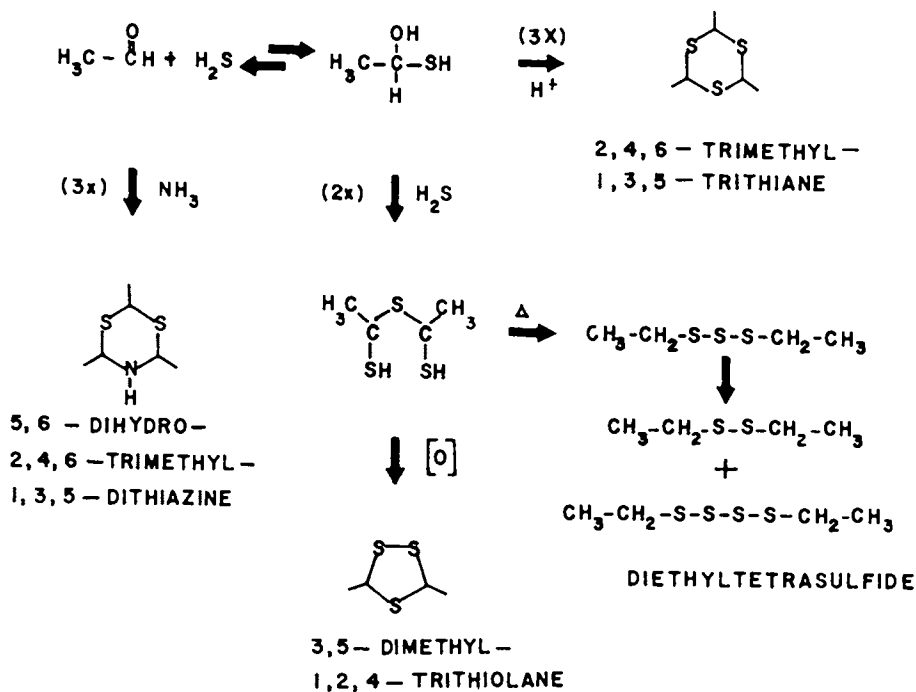


Figure 4. Formation of important meat aroma polysulfide heterocyclics by heating acetaldehyde and hydrogen sulfide. (Reprinted from ref. 39. Copyright 1976 American Chemical Society.)

The sensory properties of nearly 450 volatile Maillard reaction products and related compounds have been compiled (45). The review includes quantitative aroma and flavor descriptions, as well as sensory threshold values for different media, classified according to chemical structure.

Patent Review

While the majority of Maillard technology patents deal with the production of meat-like flavors (1), it is appropriate to comment on the significance of "reaction flavor" patents. During the past 30 years, several hundred patents have been granted worldwide for processes and reaction products based on non-enzymatic browning technology. Less than 100 of these are included in Chemical Abstracts, since subsequent patents are listed in patent concordance.

There appears to be about 45 "standard patents", i.e. patents which specify mixing one or more amino acids with one or more carbonyl compounds, followed by processing over a range of temperatures. No single patent provides a rigid specification. These patents have little instructive or commercial value because the extreme complexity of the reaction product composition would make it impossible to determine by examination how they were made. Secondly, in view of the redundancy of the patents, it would be overwhelmingly difficult to determine patent infringement.

Nevertheless, the patent literature can provide insights to the use and importance of Maillard technology. The key involvement of organic sulfur compounds with the development of meat-like flavors was announced simultaneously in 1960 by several investigators (46). The earliest paper to describe attempts to produce aromas useful in foods via Maillard reactions noted that both cysteine and cystine gave meaty aromas when heated with reducing sugars (47). Heating cysteine or cystine with furan, substituted furans, pentoses or glyceraldehyde was claimed to give a meat-like flavor (48-52).

Most of the original patents referring to meat flavors utilizing Maillard technology were claimed by Unilever (48-52; 56,57). More recent patents are involved with the production of meat-like flavors. While a majority of patents are concerned with cysteine, cystine, or methionine as the sulfur source, others claim alternatives such as mercaptoacetaldehyde, mercaptoalkamines, etc. Several patents (53,54), declare the contribution to meat-like flavors produced from thiamine in the Maillard reaction. Alternately, a technical report describes the volatile flavor compounds produced by the thermal degradation of thiamine alone (55).

Maillard reaction flavors have been manufactured for years by various food and flavor companies. Principal companies utilizing Maillard technology to currently develop flavors are Pfizer, Fidco, Alex Fries, Fritzsche Dodge & Olcott, Dragoco, Haarman & Reimer, Quest, Fries & Fries, and International Flavors and Fragrances. Givaudan has pursued new natural raw materials to use for generation of Maillard flavors (58).

Following is a discussion of the more recent patents concerning meat-like aroma and flavoring agents. Meaty flavors are developed by heating reducing sugars with cysteine or cystine (59). Removing cooker juice from fish and processing it by Maillard reactions to

remove fishy odors is claimed to provide meat flavor (60). Meat flavor is reported by heating hydrogen sulfide precursors with an edible proteinaceous material (such as soy protein) in an aqueous medium (61). For microwave cooking applications, a browning agent comprised of a hydrolyzed protein (collagen or gelatin) in the presence of a reducing sugar was produced (62). A glaze for refrigerated dough applications that rapidly browns in the microwave oven has been developed in the author's laboratory (63).

Many individual flavor chemicals which were isolated and identified from Maillard "side reactions" have been reported in the patent literature. It is evident from these patents that much work has been done to glean specific flavor chemicals from the complexities of the Maillard reaction. 3-Furyl alkyl sulfide, disulfide, and β -chalcogenalkyl sulfide derivatives are claimed to provide bloody, meaty, and roasted notes to beef broth and beef products (64-66). 3-Methylcyclopent-2-en-1-one was declared for its flavor enhancement of beef bouillon (67). Firmenich claimed 2,6-dimethyl-2-octenal and its analogs as possessing meat flavor qualities (68). A method to produce disulfides for application to meat and savory flavors was patented (69).

Meaty and sauteed onion-like flavors are imparted by 2-(α -mercaptoalkyl)-3-thiazoline when added to foods (70). A synthesis of 4-hydroxy-5-methyl-2,3-dihydrofuran-3-one was described, which is claimed to provide meaty or beefy flavor (71). 2-(2,6-Dimethyl-1,5-heptadienyl)-1,3-dithiolanes possess hydrolyzed vegetable protein-like or cooked liver aroma and flavor. Methyl (methylthioalkyl)-1,3-dithiolanes produce vegetable-like, sweet meat-like, turkey, chicken, and pork-like aromas and flavors. At the proper concentration, bready notes can also be achieved. Furyl and phenyl mercaptals are used to enhance roasted nut, roasted meat, beef broth, black pepper, onion, fine herbs omelet, and cooked onion omelet foods. 4,5-Dimethyl-2-(2-methylthioethyl)-1,3-oxathiolane provides beef broth, onion, garlic, bloody, and mushroom notes to foods and snacks. Dialkylthioalkenes and derivatives produce vegetable protein-like and beany nuances. Thioalkanoic acid esters of phenylalkanols produce floral, roasted nutty, roasted peanutty, sesame, coconut macaroon, yeasty, and hydrolyzed vegetable protein-like nuances. *o*-Dioxybenzaldehyde dimethyl mercaptals produce beef extract, oniony, cabbage-type aroma and flavor in specified food applications (72-78).

Conclusion

Modern Maillard reaction technology is critical to today's food industry. The development of convenience foods and quickly-prepared meals requires the use of natural flavor enhancements to overcome the sensory gaps when compared with "traditional" cooking methods: the basting of roasts, the simmering of soups, gravies, meats, etc. Maillard technology can be used to provide the flavor, browning, and room aroma that would be lacking in quick, conveniently prepared foods. As more is learned about non-enzymatic browning and specific flavor chemistries, the ability to maintain or generate desired flavors and aromas will increase, regardless of food application.

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Chapter 39

Reaction Flavors of Meat

Milton E. Bailey and Richard G. Einig¹

Food Science and Nutrition Department, University of Missouri,
Columbia, MO 65211

Organoleptically desirable odors and flavors of meat develop by reaction of precursors during heating. Many types of heat-induced reactions are involved in the formation of meat flavor compounds and almost 1000 volatile compounds related to meat flavor have been identified. Reactions include the pyrolysis of amino acids in the presence of sugars, the oxidation, hydrolysis and decarboxylation of lipids and the interaction of degradation products as sulfur compounds, ammonia and carbonyl compounds. The most important precursors of brothy flavor are low molecular weight water-soluble dialyzable components of protein and carbohydrate metabolism. Pyrazines, pyrroles, furans, furanones, acids, lactones and S-compounds are the major components formed when these precursors are heated. Heating meat also results in the evolution of volatiles responsible for undesirable flavor caused by animal feeding and oxidation.

The flavor of raw fresh meat is bland, metallic and slightly salty, whereas desirable meaty flavor is apparent only after heating. The precursors of brothy-desirable meat flavor have been studied extensively. More than 700 volatile components have been identified from meat reaction systems and undoubtedly many others are formed. Despite these efforts, the elucidation of the precise compounds responsible for "meaty" flavor remains an attractive endeavor for food chemical researchers.

The primary reactions occurring during heating include changes in lipids which are degraded hydrolytically and oxidatively and in water-soluble constituents which are degraded during the first stages of the Maillard reaction.

It has long been known that the basic "brothy" flavor of cooked meat is due to the interaction of low molecular weight, water-soluble constituents of muscle. The species specific flavor compounds are

¹Current address: Hoechst-Celanese Corporation, 500 Washington Street, Coventry, RI 02816

essentially associated with the lipid fraction along with other important attributes of flavor, such as "grassy" flavor of forage-fed beef and lamb, and warmed-over flavor of cooked meat. All of these flavor attributes can be considered to result from reactions occurring during or after heating.

The essential precursors of "meaty" flavor in the water-soluble fraction include amino acids, peptides, glycopeptides, proteins, sugars, sugar phosphates, nucleotides, nucleosides, purines and pyrimidines. The major ingredients of the lipid fraction that change during heating are fatty acids, triglycerides and phospholipids. The natural precursors react during heating in primary reactions to produce intermediate products which can further react with other degradation products to form the complex mixture of compounds needed for meat flavor. The total predictable flavor components formed by interactions of these precursors could be over 12,000.

Many of the reactions occurring in lipids are oxidative or hydrolytic and their products can further react with primary reaction products of the Maillard reaction occurring between amino acids and carbohydrates.

Lipid Degradation by Heating

Lipids contribute extensively to the flavor of meat through heat-induced reactions. The steam distillation of freshly cooked roast beef yields volatile lipids consisting largely of high (C_{13} - C_{18}) molecular weight aldehydes, which constitute over 80% of the total volatiles (1). These components decrease in concentration during storage of cooked roast beef at 4°C and probably polymerize over time to form dimers and trimers (2), or they may be degraded to lower molecular weight volatiles which increase in concentration during storage.

Ching (3) found fatty aldehydes to be the major volatile lipid fraction formed during heating of beef phospholipids in water at 155°C for 40 minutes followed by ether-extraction at 100°C in a modified Likens-Nickerson extractor. These aldehydes appeared in all other beef samples which were heated and extracted in this system in the presence of water. The other volatile components formed during the heating of phospholipids included low molecular weight (C_5 - C_9) aldehydes, alcohols (C_4 - C_{10}) and hydrocarbons (C_6 - C_{18}). These compounds, along with 2-pentyl furan, constitute about 8% of the remaining volatiles of heated phospholipids, which appear to be their major precursors (4-6).

Other investigators (7-9) have identified a large number of carbonyls from heated fat. The remaining meat aroma components derived by heating lipids are esters, lactones, alkan-2-ones (methyl ketones), benzenoids and other alkylfurans. Several investigators have analyzed volatile compounds formed during thermal degradation of fatty acids (10-12).

Another class of lipid components formed by heating which contribute to meat flavor is the lactones, which are formed by the lactonization of γ - and δ -hydroxy fatty acids. δ -Long chain (δ -tetradecalactone) lactones predominate. Lactones can also be formed by conversion of low molecular weight saturated fatty acids, aldehydes and alcohols during heating of meat fat (13). Larick et al. (14) found C_{12} - C_{18} δ -lactones associated with more desirable flavor of beef

finished on grain contrasted to finishing on grass. A sulfur substituted lactone has been found in beef volatiles (15) indicative of the apparent reaction between H_2S and lipids during heating. The compound is 3-methylthio- γ -butyrolactone formed by the reaction between methanethiol and 2-butenolide.

Esters formed during heating of lipids are contributors to pork flavor. Raw pork contains only a small number of esters, while cooked pork contains significantly more, and acetates are the most prominent volatiles. Esters of cooked pork are derived from C_1-C_{10} acids, which impart a fruity sweet note to pork meat (16). Beef contains a higher proportion of esters derived from long chain fatty acids which possess a more fatty flavor character (16). The characteristic odor of mutton is believed to be due to the evolution of 4-methyloctanoic acid, 4-methylnonanoic acid and similar compounds during heating (17).

Two other types of oxidative changes occur during heating of lipids which result in undesirable flavor of meat. These are referred to as "warmed-over flavor" (WOF) and "grassy" flavor.

WOF has been adequately reviewed (18-20). It is normally caused by the heating of meat which results in denaturation of myoglobin, activating iron which can in turn catalyze the oxidation of phospholipid fatty acids. It is a rapid developing phenomenon caused by heating, contrasted to the long-term oxidative rancidity occurring in meat during frozen storage.

WOF is characterized sensorially as being "old, stale, rancid, metallic and painty". These flavors are highly related to concentrations of pentanal, hexanal, 2,3-octanedione and total volatiles in chicken, turkey and beef (21) and to twenty-one different oxidative volatiles in pork (22). The compounds were quantitated by the GLC/MS method of Suzuki and Bailey (23) and appear to be excellent markers for WOF.

"Grassy" flavor of beef and lamb is recognized as undesirable in many consumer markets. It is the result of heating fat from animals that have been finished on a forage diet. "Grassy" flavor has been described as "milky-oily", "fishy", "putrid" and "stale" (24), among other terms. Some of these complex characteristics are developed during heating, although other related character is developed in unheated samples from beef and lamb and the flavor has been recognized in butter from animals finished on forage. This flavor characteristic was recognized in beef 40 years ago by Wanderstock and Miller (25) and has been studied in beef and lamb by many workers (14, 26-32).

The former workers (14) used the GLC/MS method of Suzuki and Bailey (23) to study the influence of feeding cattle on three different pasture systems (orchardgrass, tall fescue and bromegrass) on the flavor of beef compared to animals finished in the feed lot on corn. Bailey et al. (33) also concluded that meat from lambs finished on forage had a higher degree of "grassy" flavor than that from animals finished on grain. Among forage treatments, lamb from animals finished on clover had the highest sensory score for "grassy" flavor followed by that of animals on ryegrass, lucerne, lotus, radish tops, fescue-corn and corn grain, respectively.

Prominent volatiles related to "grassy" flavor of lamb according to Suzuki (34) were: hexanal, heptanal, 2,3-octanedione, 2,4-heptadienal, 3-hydroxy-2-octanone, 2-nonenal, 2-decenal, 2,4-decadienal,

2-tridecanone, heptadecane, phyt-1-ene, neophytadiene, phyt-2-ene, 9-octodecanoic acid and phytol. Similar studies have been carried out on beef by Larick et al. (14), who listed 15 terpenoids (including phytol) in fat from forage fed beef.

Phytol is a precursor for several other diterpenoids (phyt-1-ene, phyt-2-ene, phytane, neophytadiene, phytadiene and dihydrophytol) that produce a "grassy" odor when heated at 150°C or higher and heating may contribute directly to this undesirable flavor.

"Meaty" Flavors from Meat Precursors

Wood and Bender (35) and Wood (36) were among the first to realize that the "meaty-brothy" flavor of meat was the result of heating the low molecular weight, water-soluble constituents of meat. They were able to produce commercially desirable flavor and color from non-protein water extracts of ground beef, which contained amino acids, peptides, creatine, purines, organic acid, ammonia and reducing sugars. They later identified amino acids, glucose, glucose-6-phosphate, fructose-6-phosphate, ribose-5-phosphate and diphosphopyridine nucleotide in these extracts. Ribose-5-phosphate reacted most readily and liberated inorganic phosphate during the disappearance of ribose. The phosphate-sugars could react under extremely mild conditions and cause rapid browning with a mixture of amino acids. One mole of reducing sugar reacted with 6 moles of amino acids.

Hornstein and Crowe (37) prepared a water extract of lean beef, concentrated the extract by freeze-drying and dialyzed the solution at 0°C against an equal volume of water. The dialysis procedure was repeated several times and the combined diffusates were lyophilized to yield a white, fluffy powder that rapidly browned on exposure to air to give a "meaty" odor.

Heating the diffusate in water produced an odor reminiscent of roast beef broth. The diffusate could be separated into amino acid and carbohydrate fractions by ion exchange chromatography and neither produced a meaty odor upon heating, but recombining the subfractions produced a mixture with a "beef-like" odor upon heating.

Browning of glucose and simple mixtures of amino acids did not produce authentic meaty aromas, but heating a synthetic mixture of all compounds identified in beef extract in the appropriate concentration, including alanine, glutamic acid, asparagine, taurine, carnosine, anserine, histidine, cystine, creatinine, hypoxanthine, lactic and succinic acid, produced a highly desirable meaty odor.

Macy et al. (38-39) later quantitatively studied the reactivity of water soluble constituents of meat during cooking. Significantly, some sugars and cystine disappeared completely during meat cooking.

Diffusate powder prepared by freeze-drying dialyzable water-soluble solutes from beef is undoubtedly the best precursor mixture for producing "meaty" odor and flavor since it is these ingredients that are largely responsible for the flavor of cooked meat.

Some of the volatiles identified from these precursors are listed in Table I (3, 39-40).

Three compounds appear to be of particular importance in flavor volatiles identified by Ching (3) from beef diffusate or dried beef. These are 2-hydroxy-3-methyl-2-cyclo-penten-1-one, 4-hydroxy-5-methyl-3(2H)-furanone and 4-mercapto-5-methyl-tetrahydro-3-furanone.

Table I. Volatile Compounds Identified in Heated Diffusate from Beef

Compound	Dry ¹	EE ²	PT ³
<u>HYDROCARBONS, ALIPHATIC, ACYCLIC</u>			
2-Methoxy-1-propene			+
Pentane			+
2-Methyl pentane			+
3-Methyl pentane			+
Hexane			+
2-Methyl hexane			+
3-Methyl hexane			+
2,2-Dimethyl hexane			+
Heptane			+
Octane			+
Nonane			+
Undecane			+
Dodecane	+		+
Tetradecane	+		
2,3,4-Trimethyl-pentane			+
3,4-Dimethyl-2-pentene			+
<u>HYDROCARBONS, ALIPHATIC, CYCLIC</u>			
Methyl cyclopentane			+
Terpene			+
7-Ethyl-1,3,5-cycloheptatriene			+
1-Methyl-4-(1-methylethenyl)cyclohexane			+
2,6-Bis(1,1-dimethylethyl)-4-methyl phenol			+
1-Methyl-4-(1-methyl)cyclohexene			+
<u>HYDROCARBONS, AROMATIC</u>			
Benzene	+	+	+
Methyl benzene			+
1,2-Dimethyl benzene			+
1,2,3,5-Tetramethyl benzene			+
Pentamethyl benzene			+
<u>ALDEHYDES, ALIPHATIC</u>			
Ethanal (acetaldehyde)			+
2-Propenal			+
2-Methyl propanal			+
3-Methyl butanal			+
2-Methyl butanal (isovaleraldehyde)	+		+
2-Methyl-2-buten-1-al			+
Pentanal	+		
2-Methyl penten-2-al	+		
2-Methyl-penten-4-al			+
Hexanal			+
Heptanal			+
Octanal			+
Nonanal			+
2-Nonenal			+
Decanal	+		+
Furfural		+	+
2-Methyl furfural		+	+
<u>ALDEHYDES, AROMATIC</u>			
Benzaldehyde			+
Benzacetaldehyde	+		+

Continued on next page

Table I (continued)

Compound	Dry ¹	EE ²	PT ³
<u>ALCOHOLS</u>			
2-Propanol			+
2-Methyl propanol			+
Cyclohexanol			+
2-Ethyl-1-hexanol			+
Nonanol			+
2-Methoxyethanol			+
2-Furfuryl alcohol	+	+	
Butanol		+	
Ethanol	+	+	+
Ionol	+	+	
<u>ACIDS</u>			
Acetic acid		+	
Butyric acid		+	
Isobutyric acid		+	
Capric acid		+	
Caprylic acid		+	
<u>KETONES, ALIPHATIC, ACYCLIC</u>			
1-Hydroxypropanone		+	
2-Propanone (acetone)			+
2-Pentanone		+	+
2,3-Pentanedione		+	+
3-Methyl-2-butanone		+	+
2,3-Butandione			+
3-Hydroxy-2-butanone (acetoin)		+	+
2-Hexanone			+
2-Methyl-3-hexanone			+
6-Methyl-5-hepten-2-one			+
3-Methyl-2-pentanone			+
3-Hydroxy-2-pentanone		+	
4-Methyl-2-pentanone			
<u>KETONES, ALIPHATIC, CYCLIC</u>			
1-Cyclopropylethanone			+
Cyclobutanone			+
Cyclopentanone			+
1-Cyclohexene-1-one			+
2-Hydroxy-3-methyl-2-cyclopentenone		+	
<u>AMINES, AMIDES</u>			
Trimethylamine			+
Butanamide			+
Benzacetamide			+
N,N-Dimethyl formamide		+	+
<u>HALOGENATED COMPOUNDS</u>			
Chloroform	+	+	+
1-Chlorobutane			+
<u>ESTERS</u>			
Ethyl formate		+	
Ethyl acetate		+	+
Propyne-1-ol-acetate			+

Continued on next page

Table I (continued)

Compound	Dry ¹	EE ²	PT ³
PYRIDINES			
Pyridine			+
2-Methyl pyridine			+
PYRONES			
2-Pyrone		+	
2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one			+
3,5-Dihydroxy-2-methyl-4H-pyran-4-one			+
PYRROLES			
1-H-pyrrole			+
2-Methyl pyrrole	+		
3-Methyl pyrrole			+
2-Acetyl pyrrole	+	+	
2-Acetyl-N-ethyl pyrrole	+		
2-Formyl-1-methyl pyrrole		+	
2-Formyl-5-methyl pyrrole		+	
1-Ethyl-2-formyl pyrrole	+		
OTHER OXYGEN COMPOUNDS			
2,6-Dimethyl-1,4-dioxane			+
1,4-Dioxane			+
3,3,6,6-Tetramethyl 1,2,4,5-tetraoxane			+
SULFUR COMPOUNDS			
Dimethyl sulfide			+
Benzylmethyl sulfide		+	
Furfurylmethyl sulfide		+	
Methanethiol			+
Dimethyl disulfide	+		+
Dimethyl trisulfide	+	+	
2-Methyl thiophene		+	
Thiapentane			+
Methional	+	+	+
Benzothiazole			+
Thiazole			+
2-Butoxythiazole		+	
4-Acetyl thiazole		+	
2-Isopropyl thiazole		+	
FURANS			
2,5-Dimethyl furan			+
2-Pentyl furan	+	+	+
2-Acetyl furan	+	+	
2-Methylthio-5-methyl furan		+	
3-Phenyl furan	+		
INDOLES			
Indole		+	
ETHERS			
Ethylidene diethyl ether (acetal)	+	+	
FURANONES			
2-Methyl-4,5-dihydro-3(2H)-furanone	+	+	+
2,5-Dimethyl-4-hydroxy-3(2H)-furanone		+	
4-Hydroxy-5-methyl-3(2H)-furanone		+	

Continued on next page

Table I (continued)

Compound	Dry ¹	EE ²	PT ³
LACTONES			
5-Hydroxy- γ -butyrolactone			+
5-Ethoxy-5-hydroxy- γ -butyrolactone		+	
PYRAZINES			
Pyrazine			+
2-(2'-Furyl)-6-ethyl pyrazine	+		
2-(2'-Furyl)-6-methyl pyrazine	+	+	
2-(2'-Methylbutyl)-3-methyl pyrazine	+		
2-Acetyl-3-ethyl pyrazine	+		
2-Acetyl-3-methyl pyrazine	+		
2-Acetyl-5-ethyl pyrazine	+		
2-Ethyl pyrazine			+
2-Ethyl-3-methyl pyrazine			+
2-Ethyl-6-methyl pyrazine	+	+	
2-Ethyl-6-vinyl pyrazine	+		
2-Ethenyl-6-methyl pyrazine			+
2-Isobutyl-3-methyl pyrazine	+		
2-Isopentyl-3-methyl pyrazine	+		
2-Methyl-3-n-pentyl pyrazine	+		
2-Methyl-5-vinyl pyrazine	+		
2,3-Dimethyl pyrazine	+		+
2,3-Dimethyl-5-isopentyl pyrazine	+		
2,5-Diethyl pyrazine		+	
2,5-Diethyl-3-methyl pyrazine	+		
2,5-Dime 3 ethyl pyrazine			+
2,5-Dimethyl pyrazine	+	+	+
2,5-Dimethyl-3 vinyl pyrazine	+		
2,5-Dimethyl-3-(3-methylbutyl)-pyrazine	+		
2,6-Diethyl pyrazine	+		
2,6-Diethyl-3-methyl pyrazine	+		
2,6-Dimethyl pyrazine	+	+	
2,5-Dimethyl-3-ethyl pyrazine			+
6,7-Dihydro-2-ethyl-5(H)-cyclopentapyrazine		+	
6,7-Dihydro-2-methyl-5(H)-cyclopentapyrazine	+	+	
6,7-Dihydro-2,5-dimethyl-5(H)-cyclopentapyrazine	+	+	
6,7-Dihydro-3,5-dimethyl-5(H)-cyclopentapyrazine		+	
6,7-Dihydro-5-methyl-5(H)-cyclopenta-pyrazine	+	+	
2-Methyl pyrazine	+	+	+
2,3,5,6-Tetramethyl pyrazine	+		
2,3,5-Trimethyl pyrazine	+	+	+
Unsaturated pyrazine (M.W. 134)	+		

¹Dry - Heated at 150°C for 12 minutes and sampled directly. Data from Einig (40).

²Ether Extracted - Heated at 155°C for 40 minutes and extracted with ether. Data from Ching (3).

³Purge and Trap - Heated at 140°C for 2 hours and refluxed to collect volatiles. Data from Shin-Lee (22).

The first volatile is cyclotene (2-hydroxy-3-methyl-2-cyclopenten-1-one), identified by Ching (3) in heated diffusate. Cyclotene is a commercially available food flavorant similar to the furanones and possesses a fragrant, sweet-caramel aroma. It is a thermal degradation product of glucose and is a Maillard reaction product. It has been described by Nishimura et al. (41) as reacting with ammonia and hydrogen sulfide to form meaty odors. Methyl cyclopentanones also produced meaty odors showing the possible importance of the other cyclic ketones found in diffusates. These investigators also found cyclic methylene polysulfides like 1,2,4-trithiolane, S-trithiane and 1,2,4,6-tetrathipane in reaction mixtures between H₂S and cyclotene, indicating that the latter compound might be fragmented into small carbon units that react with H₂S.

Walradt et al. (42) identified some 6,7-dihydro-5H-cyclopentapyrazines in roasted peanuts and presumed that cyclotene was the precursor. Tricyclic pyrazines are also formed by reacting cyclotene with alanine (43).

The alicyclic ketones have been identified as being important flavor precursors by Flament et al. (15) and more recently by MacLeod and Ames (44), who identified a similar compound (3-methyl-cyclopentanone) in heated ground beef. Flament et al. (15) singled out the importance of these compounds, particularly in the presence of alhyppyrzines. Nishimura et al. (41) produced a meaty odor by heating 2-hydroxy-3-methylcyclopent-2-enone with cyclotene and H₂S. Two volatile compounds described as having meaty odor were 2-methylcyclopentanone and 3-methylcyclopentanone. Thus, cyclotene appears to be a key precursor of "roast beef" flavor.

4-Hydroxy-5-methyl-3(2H)-furanone and similar compounds appear to be important in meat flavor and have been identified in many meat samples and in beef diffusate by Ching (3).

Hodge et al. (45) discussed mechanisms for formation of methyl furanones and related substances from Amadori compounds. They have been produced by heating D-ribose and D-ribose phosphate with ammonia (46;47). Hicks and Feather (48) demonstrated that the Amadori compound 1-benzylamino-1-deoxy-D-threo-pentulose dehydrates to 4-hydroxy-5-methyl-3(2H)-furanone and it has also been identified as a degradation product of L-ascorbic acid. This compound is believed to be formed from ribose-5-phosphate, and gained prominence when it was isolated from beef by Tonsbeck et al. (49). It became more apparent as a precursor of meat flavor when Van den Ouweland and Peer (50) reacted it and its thio analog with H₂S to produce a number of sulfur compounds, some of which had meaty odors.

Other compounds produced by this reaction having a meat-like odor were: 4-mercapto-2-methyl-furan, 3-mercapto-2-methyl-4,5-dihydrofuran, 4-mercapto-3-oxotetrahydrofuran, 3-mercapto-2-methyl thiophene and 3-mercapto-2-methyl-2,3-dihydrothiophene (50).

2-Furfural identified in beef diffusate appears to be a prominent meat flavor intermediate. It is a dehydration product of pentoses similar to formation of hydroxy methyl furfural from hexoses. These compounds are formed by dehydration of 1,2-enediols derived from deamination of Amadori compounds (51).

Shibamoto (52) reacted furfural with H₂S and ammonia to produce oxazoles, pyrazines, furans and thiophenes. This is supporting evidence that the sulfur atom from H₂S or the nitrogen atom from ammonia can exchange with the oxygen atom of furan. Furfural may also be

degraded and react with sulfur to form 1,2,4-trithiolane, S-triathiane or 1,2,4,6-tetrathiepane (53).

These compounds identified in meat diffusate (Table I) are only a small percentage of volatile compounds from heated meat or meat systems reviewed and discussed by MacLeod and Seyyedean-Ardibili (54), Bailey (55) and Shahidi et al. (56).

N, S and O heterocyclic compounds, along with noncyclic sulfur compounds and hydrocarbons, are predominant in "meaty" flavor volatiles. The mechanisms of heterocyclic formation by Maillard and pyrolysis reactions have been reviewed by Vernin and Parkanyi (57) and the Maillard reaction itself is a recurring subject of review (58). Since other speakers contributing to this volume will discuss these aspects of meat flavor, they will not be repeated in this presentation.

Heterocyclic compounds related to meat flavor have been produced in the following reacting systems:

- Reactions of reducing sugars and amino acids.
- Thermal degradation of Amadori and Heyns rearrangement compounds.
- Thermal degradation of sugars.
- Pyrolysis of α -amino acids.
- Reaction of α -dicarbonyl compounds and aldehydes with hydrogen sulfide and/or ammonia.

These reactions can lead to the formation of numerous heterocyclics associated with meat flavor as described by Vernin and Parkanyi (57), including furans, thiophenes, pyrroles, oxazoles, imidazoles, thiazoles, pyrans, pyridines, pyrazines, cyclic sulfides and polysulfides.

Furans are formed by heating sugar-amino acid mixtures, sugars, vitamins or Amadori compounds.

Thiophene derivatives arise mainly from thermal degradation of sulfur amino acids in the presence of reducing sugars.

Pyrroles are derived from heating sugars and amino acids or Amadori intermediates or by heating furfural and α -amino acids.

Oxazoles are formed by decarboxylation of hydroxylated amino acids such as serine or threonine followed by condensation with aldehydes and cyclization.

Pyrans and pyrones can be formed by decomposition of 1-deoxy-1-L-proline-D-fructose or by heating 5-hydroxy-5,6-dihydromaltol.

Pyrazines are formed from many amino acid-sugar systems by the condensation of α -diketones arising from sugar fragmentation with amino acids via Strecker degradation and ammonia or amino acids.

Cyclic sulfur compounds are mainly formed by interaction of aldehydes and H_2S with ammonia.

Heterocyclic aroma compounds found in meat primarily arise from interactions between mono- and dicarbonyl compounds, H_2S and ammonia. The carbonyl compounds are derived from the Maillard reaction, including Strecker degradation of amino acids, oxidation of lipids and aldolization reactions. H_2S is produced by thermal degradation of sulfur amino acids and ammonia by amino acid pyrolysis.

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Chapter 40

Process Meat Flavor Development and the Maillard Reaction

G. A. M. van den Ouweland, E. P. Demole, and P. Enggist

Firmenich SA, P.O. Box 239-CH-1211 Geneva, Switzerland

The flavor industry has introduced, over the years, methods of developing meat flavors by processing appropriate precursors under carefully controlled reaction conditions. As a result, meat flavors having a remarkably genuine meat character in the beef, chicken and pork tonalities are available for the food industry. It has repeatedly been stated that the Maillard reaction is particularly important for the formation of meat flavors. However, of the 600 volatile compounds isolated from natural beef aroma, only 12% of them find their origin in sugar/amino acid interactions and of these 70% are pyrazine derivatives.

The precursors used for process meat flavors are reviewed and also discussed will be non-Maillard interactions of ribose-5-phosphate and lipid degradation products with sulfur giving a real meaty odor and meat specie specific odor compounds, respectively.

More than 50 years were needed before the flavor industry started to use Maillard's original work of heating together carbohydrates and amino acids for the development of flavoring materials. Processing hydrolysed vegetable protein with carbohydrates and cysteine giving a savoury meat-like flavor was one of the first examples of using the Maillard reaction on a commercial scale. Since then the development of process meat flavors has evolved in such a way that currently the food industry has a choice of chicken, beef, pork and ham flavors, which have authentic meat tonalities. The creation of these flavors is based on the processing of selected raw materials. For example in the cooking of meat complex flavor systems obtained are from a combination of fat oxidation products, thermal degradation products of sugars, amino acids, thiamine and nucleotides as well as Maillard reaction products. In the early 1980's the flavor industry in Europe, through IOFI, produced guidelines for the composition and manufacture of process flavorings and the principal raw materials to be used.

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In the USA, the expert committee within FEMA has produced similar guidelines for the preparation of such products. In this paper the relative importance of the Maillard reaction is discussed as well as the interaction of this with ribose-5-phosphate and lipid degradation products.

MAILLARD REACTION and MEAT FLAVOURS

It has been repeatedly stated that the Maillard reaction is very important for the formation of meat flavor. The first stage of the Maillard reaction involves the condensation of the amino-group of the amino acid to the carbonyl-group of the sugar followed by a rearrangement. These rearrangements of glucose and fructose are often referred to as the Amadori and Heyns rearrangement products (RP's). In our study investigating the occurrence of RP's in processed foods we looked at roasted meat for such products and could only find the RP derived from glucose and glycine (5). In other processed foods the presence of RP's is more abundant as shown in Table I.

Table I RP's isolated from processed foods

<u>RP's derived from</u>	<u>Isolated from</u>							
<u>GLUCOSE and</u>								
Glycine	A	B	C	D				G
Alanine	A	B	C		E	F		G
4-aminobutyric acid	A		C					G
Valine					E			
Aspartic acid	A	B	C			F		G
Asparagine					E	F		
Glutamic acid								G
Proline					E	F		
Tyrosine					E			
Phenylalanine					E			
Theanine								H
<u>FRUCTOSE and</u>								
Glycine		B						G
4-aminobutyric acid								G
Aspartic acid						F		

- A= Apricots [1]
 B= Liver [2] [3]
 C= Beet Molasses [4]
 D= Roasted meat [5]
 E= Flue Cured Tobacco [6]
 F= Liquorice [7]
 G= Tomato powder [5]
 H= Black Tea [5]

When we first investigated processed foods for the occurrence of RP's there was no direct method for the determination of their conformation. An RP can exist in the open keto-form or hemiacetal ring structures derived from it (Figure 1)

Generally the presence or absence of a C=O absorption band in the IR spectrum is used to assign the keto structure or the cyclic forms, respectively. As far as the cyclic forms were concerned, no further spectroscopic evidence was then available. The structures of RP's were characterized by chemical methods, which often involved acid hydrolysis followed by identification of the amino acid, which was split off in this decomposition reaction. Examination of the 220 MHz PMR spectrum of the RP isolated from the roasted meat i.e. derived from glucose and glycine (Figure 2) recorded in D₂O at pH 3 shows that the product exists at least as a mixture of two components. The assignment of the proton signals of the major pyranose component (85%) is given and it appears that the coupling constants are identical with those of β (D)-fructose. The AB doublet resonating at 3.33 and 3.38 ppm can be assigned to the methylene protons adjacent to the nitrogen atom. The complete exchangeability of these protons with Deuterium at pH 9.5 provides confirmatory evidence of this assignment and indicates that in D₂O solution an equilibrium exists between the various possible structures, i.e. the pyranose form, furanose form and the open chain structure. The major component in the mixture has the six-membered structure and exists exclusively in the β (D)-²C₅ conformation and the minor component has the five membered ring.

The presence of exclusively the RP from glucose and glycine in roasted meat is remarkable, especially in the light of the quantities of other free amino acids present in beef diffusate.

More than 600 components have been isolated [8] from natural beef aroma. We have estimated that only 94 of these volatiles find their origin in the Maillard reaction and of these 50% are pyrazine derivatives which contribute more to a roasted aroma than to a meat aroma.

REACTION of HYDROGEN SULPHIDE with METHYLFURANOLONE

One reaction system delivering a meaty odour where the Maillard reaction is not involved is the conversion of ribonucleotides via ribose-5-phosphate and methyl-furanolone into meat flavor [9].

Many of the individual reaction products isolated from this reaction have a characteristic meat odour, with thiofuran derivatives the most interesting. The amount of unsaturation and the position of the methyl group play predominant roles for the odour quality as illustrated in Figure 4.

As shown for compounds 1-4, a flat 5-membered ring with at least one double bond and a methyl group adjacent to the thiol group gives the most characteristic meat flavor. Compounds 2 and 3 were also isolated as thiamine degradation products [10] and the occurrence of 3 in cooked meat was recently proven [11]. The importance of the position of the methyl group is also illustrated for some thiophenols (Figure 4). Only the methyl group on the ortho position gives meat-like flavor. Compounds 5,6,7 were isolated from a natural meat aroma. Of the 600 or more substances isolated from natural beef aroma, only a very few possess a meat-like aroma. A 5 or 6 membered more or less planar ring substituted with an enol-thiol and a methyl group adjacent to the thiol seems to be necessary for a meaty aroma.

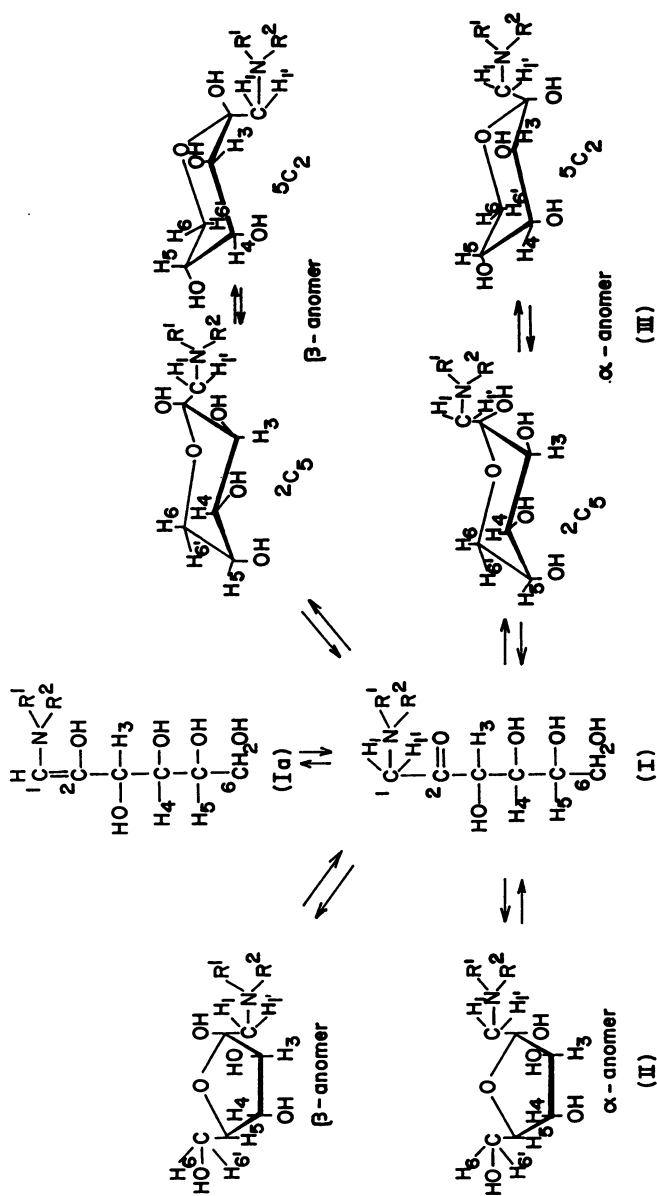


Figure 1. Different possible structures for an Amadori rearrangement product.

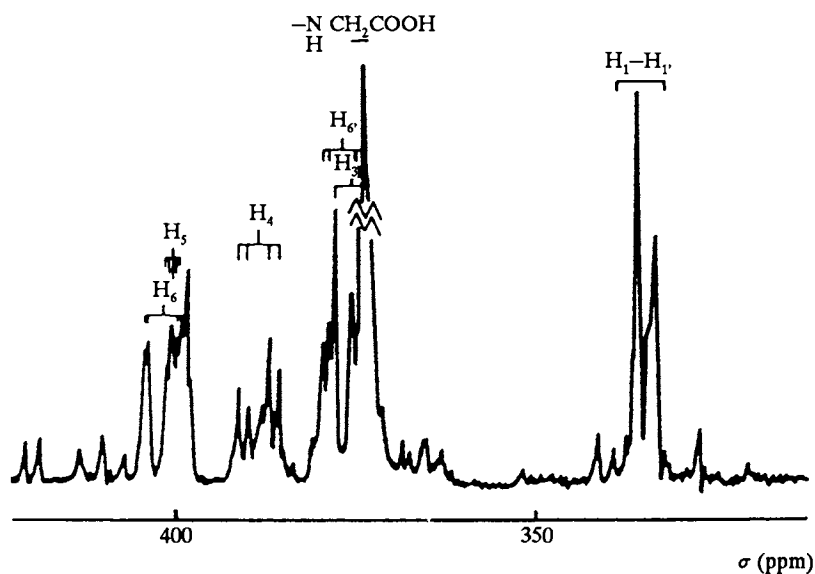


Figure 2. 220 MHz PMR spectrum of the RP from glucose and glycine.

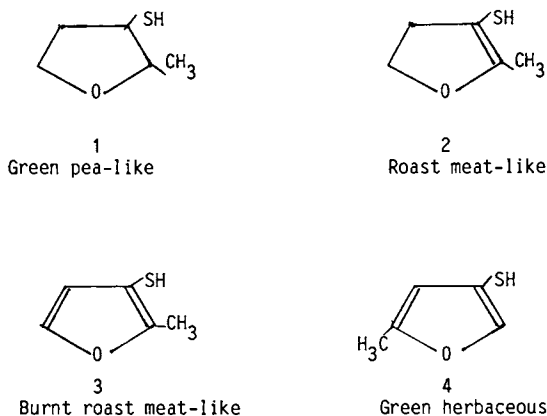


Figure 3. Odor description of some mercapto furan derivatives isolated from methylfuranolone - H₂S reaction.

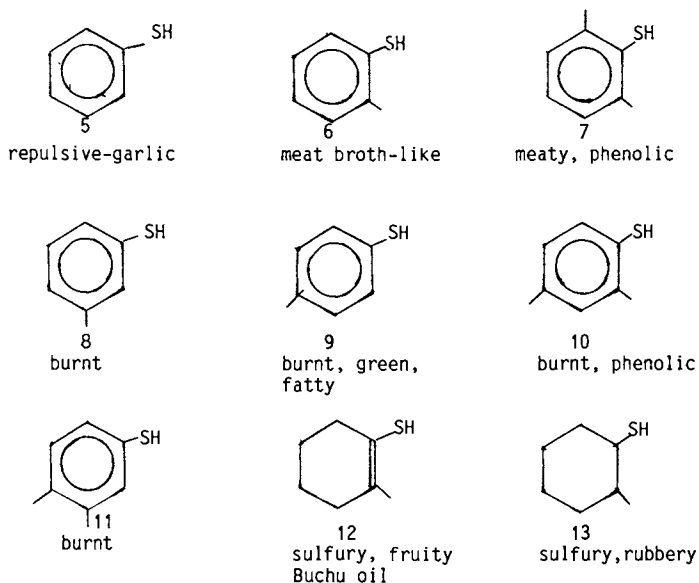


Figure 4. Odor description of some cyclic mercapto derivatives.

REACTION of HYDROGENSULFIDE with unsaturated ALDEHYDES

The lipid fraction of meat is an important group of precursors for meat flavor and a source of flavor differences between the various species. During cooking, thermal and oxidative degradation of lipids proceed simultaneously and a range of flavoring compounds are formed. These degradation reactions of lipids are more pronounced for meat with a relatively high content of polyunsaturated fatty acids, i.e. chicken [13], than is the case for beef [12]. These carbonyl compounds are potent aroma chemicals which not only contribute to cooked meat flavor, but also are involved in the formation of other strongly odorous compounds. An example of this was given by Pippen and Mecchi [14] who observed that when hydrogen sulfide is passed through molten chicken fat, a chicken meat-like aroma is formed.

In heated chicken fat mono-, di-, and triunsaturated aldehydes are present. In our investigation of the reaction of hydrogen sulphide with these aldehydes we chose the C-10 aldehydes. The base catalyzed reaction between 2-decenal and hydrogen sulphide in aqueous solution at $\text{pH} \geq 7$ follows essentially the same routes as described earlier for 2-butenal by Badings et al [15] and outlined in Figure 5.

The simple addition product 3-mercaptodecanal 14 is obtained as the main product. On standing this compound changed into a viscous odorless oil of structure 15. As expected [16] compound 14 reacts in the presence of NH_3 and acetaldehyde to thiazine derivatives 16 and 17 as indicated in Figure 6.

In contrast to the reaction of 2-decenal with H_2S a much more complex reaction mixture is obtained from the reaction of 2,4-decadienal with H_2S in an aqueous medium at $\text{pH} \approx 8$.

In view of the expected instability of the mercapto aldehydes likely to be formed, the reaction mixture was extracted and the concentrated extract treated with LiAlH_4 /acetic anhydride/pyridine. The acetates/thioacetates isolated from this reaction mixture were analyzed with MS/NMR spectroscopy. From the results of these analyses, the reaction routes as indicated in Figure 6 are followed.

Compound 24 is obtained as the major reaction product. The mercaptoaldehydes 19 and 20 obtained via 1,6- and 1,4-addition of H_2S to the starting aldehydes can be considered as the precursors for compounds 22, 24 and 25. The formation of the thiophene derivative 23 can be explained to proceed via mercaptoaldehyde 18, although the latter could not be detected as its acetate/thioacetate in the crude reaction mixture.

In view of the ease with which H_2S reacts with polyunsaturated aldehydes it is tempting to assume that during the cooking of meat interaction of these protein and lipid degradation products occurs. The reactive H_2S -addition products initially formed can then easily react further with aldehydes and NH_3 to give a vast variety of odorous compounds which would have a major contribution to the flavor of cooked meat.

The identification of these types of compounds in meat flavor remains a major challenge to the flavor chemist with sophisticated modern analytical tools.

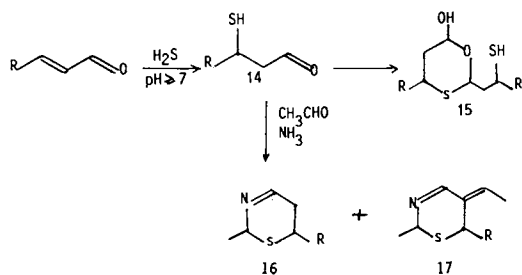


Figure 5. Reaction of 2-decenal in process flavors.

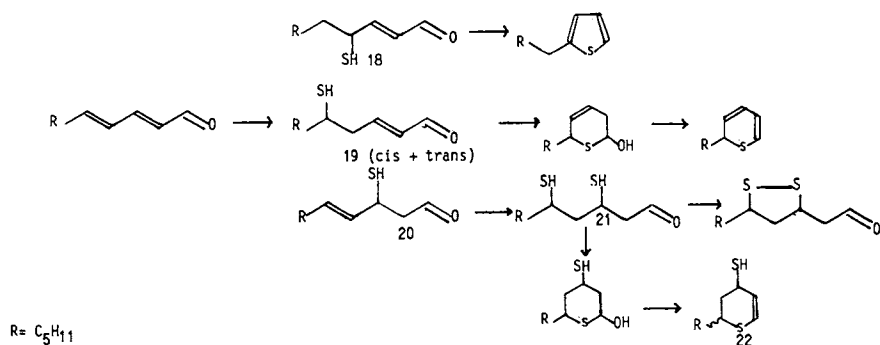


Figure 6. Reaction of 2,4-decadienal with H_2S .

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Chapter 41

Flavor Formation in Meat-Related Maillard Systems Containing Phospholipids

Donald S. Mottram¹ and Linda J. Salter²

¹Department of Food Science and Technology, University of Reading, Whiteknights, Reading RG6 2AP, United Kingdom

²Bristol Laboratory, AFRC Institute of Food Research, Langford, Bristol BS18 7DY, United Kingdom

In heated foods the main reactions by which flavors are formed are the Maillard reaction and the thermal degradation of lipids. These reactions follow complex pathways and produce reactive intermediates, both volatile and non-volatile. It has been demonstrated that lipids, in particular structural phospholipids, are essential for the characteristic flavor development in cooked meat and that the interaction of lipids with products of the Maillard reaction is an important route to flavor. When model systems containing amino acids and ribose were heated in aqueous buffer, the addition of phospholipids had a significant effect on the aroma and on the volatile products. In addition a number of heterocyclic compounds derived from lipid - Maillard interactions were found. The extent of the interaction depends on the lipid structure, with phospholipids reacting much more readily than triglycerides.

The characteristic flavors associated with cooked foods such as meat have proved particularly difficult to characterize, both for the sensory analyst and the flavor chemist. Large numbers of volatiles comprising aliphatic, aromatic and heterocyclic compounds have been identified in cooked foods, and meat, with almost 1000 volatiles, contains the largest number of compounds identified in any food or beverage. Despite this considerable research interest, the key aroma component(s) of meat remain elusive and it is probable that no simple combination of compounds is responsible for meat aroma (1,2). Meat flavor is thermally derived, since uncooked meat has little or no aroma and only a blood-like taste. The major precursors of meat flavor can be divided into two categories: water soluble components (amino acids, peptides, sugars, nucleotides, thiamine, etc) and components of lipids (principally the fatty acids). The two main types of flavor-forming reactions which occur during the cooking of meat are the Maillard reaction between amino compounds and reducing sugars, and the thermal degradation of lipids.

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The Maillard Reaction in Meat Flavor

The importance of the Maillard reaction in meat flavor has long been recognized and it has formed the basis of the large number of patents for "reaction product" simulated meat flavorings. Research on cooked meat in the 1950s had shown that during cooking there were significant depletions in the levels of reducing sugars and amino acids, in particular cysteine (3,4). This led to the classic patent of Morton et al in 1960 which involved the formation of a meat-like flavor by heating a mixture of cysteine and ribose (5). Practically all the subsequent proposals for meat-like flavorings from Maillard systems involved sulfur, usually as cysteine or other sulfur-containing amino acids.

Heterocyclic compounds are dominant among the aroma compounds produced in the Maillard reaction, and sulfur-containing heterocyclics have been shown to be particularly important in meat-like flavors. In a recent review, MacLeod (6) listed 78 compounds which have been reported in the literature as possessing meaty aromas; seven are aliphatic sulfur compounds, the other 71 are heterocyclic of which 65 contain sulfur. The Strecker degradation of cysteine by dicarbonyls is an extremely important route for the formation of many heterocyclic sulfur compounds; hydrogen sulfide and mercaptoacetaldehyde are formed by the decarboxylation and deamination of cysteine and provide reactive intermediates for interaction with other Maillard products.

Lipid-derived Volatiles in Meat Flavor

The volatiles from cooked meat contain large numbers of aliphatic compounds including aldehydes, alcohols, ketones, hydrocarbons and acids. These are derived from lipids by thermal degradation and oxidation (7) and many may contribute to desirable flavor. In addition, the aldehydes, unsaturated alcohols and ketones produced in these reactions, as well as the parent unsaturated fatty acids, are reactive species and under cooking conditions could be expected to interact with intermediates of the Maillard reaction to produce other flavor compounds.

Meat contains neutral triglyceride lipids in the fat depots and in the intra-muscular fat cells. In addition, the polar phospholipids are important structural components of all tissues and, although they comprise less than 1% of muscle, the phospholipids contain a much higher proportion of polyunsaturated fatty acids than triglyceride lipids and therefore provide a significant source of autoxidation products. The role of phospholipids in the development of off-flavors in reheated meats (warmed-over flavor) has long been recognized (8), but until recently their possible role in the development of desirable flavors has been largely unexplored.

Lipids and the Maillard Reaction

In an examination of the contribution which lipids make to the development of aroma during the heating of meat, the phospholipids were shown to be particularly important. Consumer and taste panel studies had failed to show any relationship between the meaty flavor of lean meat and the level of fat on the carcass, apparently

confirming early studies on meat flavor which associated meatiness with water soluble flavor precursors and species characteristics with the fat (9,10). However, the volatiles of cooked meat are dominated by lipid-derived compounds and it is difficult to believe that such compounds play no role in meat flavor. The contribution these volatiles of lipid origin make to meat aroma was investigated by observing the changes in aroma which occurred when lipids were extracted from the meat prior to cooking (11).

The inter- and intra-muscular triglyceride fats were removed from lean muscle by solvent extraction with hexane, and the material was subsequently cooked. The resulting aroma was very similar to that from the untreated material; both preparations were judged to be meaty and could not be differentiated by aroma in sensory triangle tests. However, when a more polar solvent (chloroform - methanol) was used to extract all the lipids - phospholipids and triglycerides - a highly significant difference in aroma resulted; the meaty aroma was replaced by a roasted, biscuit-like aroma. Examination of the aroma volatiles showed that both the control and the material without triglycerides had similar profiles dominated by aliphatic aldehydes and alcohols, while removal of phospholipids as well as triglycerides gave a markedly different profile; the lipid oxidation products were lost, but there was a large increase in the amounts of alkylpyrazines. This increase in alkylpyrazines, volatile products of the Maillard reaction, implied that in normal meat the lipids or their degradation products inhibit the formation of certain heterocyclic compounds by participating in the Maillard reaction.

There is increasing evidence that the interaction of lipids with the Maillard reaction is relevant to the generation of flavor in many cooked foods. For instance, the removal of lipids from coconut has been shown to cause flavor changes in the roast material (12). Uncooked coconut contained significant amounts of lactones as the main aroma components; on roasting pyrazines, pyrroles and furans were also found in the aroma volatiles which added a strong nut-like aroma to the sweet aroma of the unroasted coconut. When ground coconut was defatted and then roasted, the sweet aroma due to lactones disappeared and the product possessed a burnt, nut-like aroma. A marked increase in the number and amount of Maillard reaction products, in particular pyrazines, was found.

It has been known for many years that Maillard Reaction products can behave as antioxidants in food systems (13,14), and they have been shown to inhibit warmed-over flavor development in cooked meat which is caused by the autoxidation of lipids, especially phospholipids. There has been a significant amount of research examining the Maillard reaction products and intermediates from model systems which may have antioxidative properties. However, the formation of volatile aroma compounds from the interaction of Maillard intermediates with lipid-derived compounds has received little attention.

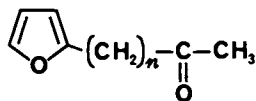
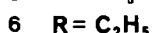
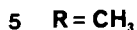
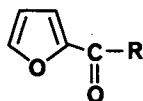
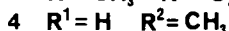
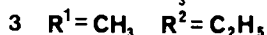
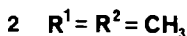
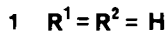
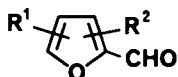
In some recent research on flavor formation during deep-fat frying at Rutgers University, a number of heterocyclic compounds with long-chain alkyl substituents were found the volatiles of fried chicken (15) and fried potato (16). These included pyridines, thiazoles, oxazoles, trithiolanes and a pyrazine. Only the involvement of lipids or lipid degradation products in the formation of

these compounds could account for the long-chain alkyl substitution. In a study of volatiles from a heated mixture of corn components (zein, corn starch and corn oil) two alkyl substituted pyrazines (2,5-dimethyl-3-pentylpyrazine and 2-methyl-3(or 6)-pentylpyrazine) were identified (17); again these compounds must arise from a lipid-protein-carbohydrate interaction. The interaction of simple triglycerides with amino acids at high temperatures (270°C) produced fatty acid amides (18), and when beef fat was heated to 200°C with glycine, butyl- and pentylpyridines were found among the lipid degradation products (19). The conditions prevailing in all these systems were essentially low moisture and high lipid, while in meat much higher water (ca. 75%) and lower lipid (2-3%) levels exist, and this could result in different reaction pathways to volatile compounds.

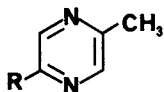
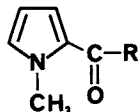
Reaction of Amino Acids, Ribose and Phospholipids.

The reduction in levels of volatile Maillard reaction products observed in defatted cooked meat led to an investigation of the effect of phospholipids on the volatile products from aqueous model systems containing amino acids and sugars (20-22). Several amino acids were used including glycine, the simplest amino acid, and cysteine, a sulfur containing amino acid. Ribose was employed as the reducing sugar because of its recognized role in the production of cooked meat flavor and its high reactivity in Maillard systems. In initial work, L- α -phosphatidylcholine (lecithin) from egg yolk was selected as the phospholipid, and later studies compared other phospholipids and lipid extracts from meat. As the study originated from investigations of cooked meat flavor, the model system reactions were carried out in aqueous solution buffered with phosphate at an initial pH of 5.7 and concentrations of the reactants were selected to approximate their relative compositions in mammalian muscle. The reactions were carried out under pressure at 140°C for 1 hour in sealed glass ampoules. At the end of the reactions volatiles were collected by headspace entrainment on Tenax GC and analysed by capillary gas chromatography - mass spectrometry.

Volatiles from Amino Acid + Ribose alone. All the reaction systems produced complex mixtures of volatiles, containing large numbers of heterocyclic compounds, many of which have not been reported previously in Maillard systems. In the absence of phospholipid 74 compounds were separated and identified from the glycine system (21); furfurals and furanylketones dominated and the three largest peaks in the chromatogram were 2-furfural [1], dimethylfurfural [2] and ethylmethylfurfural [3].



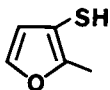
Other furans included methyl- 2-furfural [4], 2-acetylfuran [5], 2-propionylfuran [6], 1-(2-furanyl)-2-propanone [7] and 1-(2-furanyl)-3-butanone [8]. Several alkyldiazines were also found, with the 2,5-dimethyl [9] and 2-methyl-5-ethyl [10] the most abundant derivatives, and some 2-acyl-1-methylpyrroles of which only the formyl [11] and acetyl [12] derivatives were conclusively identified.

9 R = CH₃10 R = C₂H₅

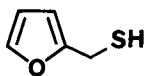
11 R = H

12 R = CH₃

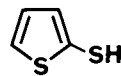
When cysteine was used instead of glycine the reaction volatiles became dominated by sulfur containing heterocyclics (22); the alkyldiazines and furanylketones were reduced to trace amounts and, although a series of alkyldiazines were found, the levels were much lower than in the glycine system. A total of 118 volatile compounds were isolated from the cysteine + ribose reaction; the major components were 2-methyl-3-furanthiol [13], furanmethanethiol [14], 2-thiophenethiol [15], 2-methyl-3-thiophenethiol [16], dihydro-2-methyl-3(2H)-thiophenone [17] and 3-mercapto-2-pentanone [18]. A number of other furanthiols, thiophenethiols and thiophenones were also found including a series of acyl thiophenes analogous to the furfurals and furanylketones found in the glycine reaction. Other heterocyclic compounds identified included several alkyldiazoles, three isomeric thienothiophenes [19-21] and their methyl- and dihydro-derivatives, as well as alkyl-substituted 1,2-dithiolan-4-ones [22-24], 1,2-dithian-4-ones [25-27], 1,2,4-trithiolanes [28] and 1,2,4-trithianes [29].



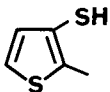
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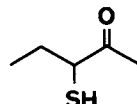
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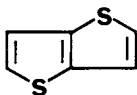
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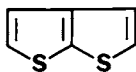
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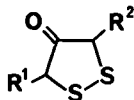
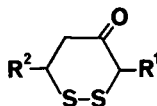
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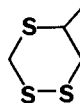
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22 $R^1 = H$ $R^2 = CH_3$,23 $R^1 = R^2 = CH_3$,24 $R^1 = CH_3$ $R^2 = C_2H_5$,25 $R^1 = R^2 = H$ 26 $R^1 = R^2 = CH_3$,27 $R^1 = H$ $R^2 = CH_3$,

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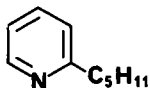
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Effect of Phospholipids on Reaction Volatiles. As would be expected, the inclusion of phospholipids in the reaction mixtures produced many volatiles derived from lipid degradation; these included hydrocarbons, alkylfurans, saturated and unsaturated alcohols, aldehydes and ketones. However, two other important observations were made. First, the concentrations of most of the hetero- cyclics, formed by the amino acid + ribose Maillard reaction, were reduced. For most of the major volatiles this reduction was of the order of 40 - 50%, but in the case of thiophenethiol and methyl- furanthiol the reduction was over 65%. This appears to support the findings that in meat and coconut, lipids exert a quenching effect on the amount of heterocyclic compounds formed in Maillard reactions during heat treatment (11,12). Second, and perhaps more important, the addition of phospholipid to the reaction mixtures resulted in the production of large amounts of compounds derived from the interaction of the lipid or its degradation products with Maillard reaction intermediates.

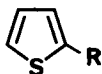
In the glycine reaction, 2-pentylpyridine [30] was formed. This compound has been reported in the volatiles from heated lamb fat (23), fried chicken (15) and french fried potato (16), and was a major product in the thermal interaction of valine and linoleic acid (24). It is postulated to be formed by the reaction of decadienal and ammonia, and large concentrations of 2,4-decadienal were found in the volatiles of lecithin when heated alone or in the Maillard mixtures. The addition of phospholipid to the cysteine - ribose reaction system gave over 10 times more 2-pentylpyridine than the corresponding glycine reaction. In the Strecker degradation of cysteine by dicarbonyl compounds, ammonia is known to accompany the formation of hydrogen sulfide (25,26) and this will provide a ready source of ammonia for reaction with dienals from lipid breakdown. With glycine, ammonia is not formed so readily at the slightly acidic pH employed in the reaction mixture and hence the lower concentrations of 2-pentylpyridine.

Among the most abundant components of the cysteine + ribose + lecithin reaction mixture were the 2-pentylpyridine and two long-chain alkyl substituted thiophenes: 2-pentyl- and 2-hexylthiophenes [31,32], together with a related compound with molecular formula and

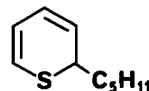
mass spectrum very similar to 2-hexylthiophene but with a later retention time. This hexylthiophene isomer was tentatively identified as 2-pentyl-2H-thiapyran [33]. Closer examination of the GC-MS analysis revealed a series of 2-alkylthiophenes with n-alkyl substituents between C3 and C8 inclusive. Each had an isomeric compound with very similar mass spectrum eluting close to the next higher 2-alkylthiophene. Other lipid derived compounds included 2-hexenylthiophene [34], 1-heptanethiol and 1-octanethiol. All these compounds are probably formed by the reaction of lipid breakdown products, such as dienals, with hydrogen sulfide or mercaptoacetaldehyde obtained in the Strecker degradation of cysteine.



30



31 R = C₅H₁₁
 32 R = C₆H₁₃
 34 R = CH=CH-C₄H₉



33

Hydrogen sulfide is also a key component in the formation of sulfur-containing compounds in the Maillard reaction (27). Thiophenones may be formed by the action of hydrogen sulfide on sugar degradation products such as dehydroreductones, furfural (28) or furanones (29), while thiazoles can be produced by the reaction of hydrogen sulfide with dicarbonyls and ammonia or an amino acid (30). The small amount of furfural in the cysteine mixture compared with that containing glycine may be due to its reaction with hydrogen sulfide to give furanmethanethiol. In the reaction mixtures containing lecithin, the decrease in concentrations of many of the sulfur-containing heterocyclic compounds derived from the basic cysteine + ribose reaction can be explained in terms of competition for hydrogen sulfide by lipid degradation compounds resulting in other sulfur compounds such as the alkylthiophenes and alkanethiols.

Aroma of Reaction Mixtures The aroma of the glycine + ribose reaction mixture after heating was described as "caramel", "sweet", "burnt sugar" while the addition of lecithin added "oily" and "chicken-like" notes. The overall odor of the cysteine containing reaction mixtures was described as "sulfurous, rubbery" but there was a distinct underlying meaty aroma. This "cooked meat", "beef" aroma was much more pronounced in the mixture containing the phospholipid. When the aromas eluting from the GC column were evaluated, the cysteine + ribose + lecithin system gave at least six areas where meaty aromas could be detected. Only one of these was detected in the absence of the phospholipid and this was attributed to 2-methyl-3-thiophenethiol; none of the other meaty odors could be assigned to particular compound identifications. The alkylthiophenes and the other identified products of the lecithin - Maillard interaction did not have meat-like aromas.

Comparison of Different Lipids

The original work on defatted meat suggested that phospholipids rather than triglycerides were important in determining meat flavor and the participation of phospholipids in the Maillard reaction between amino acids and ribose has been clearly demonstrated in the model systems. In mammalian tissue the structural phospholipids contain significant amounts of polyunsaturated fatty acids, in particular arachidonic (20:4), docosapentaenoic (22:5) and docosahexaenoic (22:6). Beef triglyceride fat contains less than 2% polyunsaturated fatty acids, mainly linoleic (18:2) and, although pork contains higher levels of linoleic acid, the triglyceride lipids of the different meat species do not contain significant amounts of C20 or C22 polyunsaturated fatty acids. Differences in fatty acid composition may be one feature of phospholipids which will result in a different behaviour from triglycerides in Maillard systems, while the other feature is the polar nature of the phosphatidyl group.

The observations on the aromas from cysteine + ribose reaction mixtures have been extended to compare the effect of different lipids: triglycerides and phospholipids extracted from beef, and commercial egg lecithin (phosphatidylcholine) and egg cephalin (phosphatidylethanolamine) (L.J. Salter; D.S Mottram, unpublished data). The inclusion of the beef triglycerides (TG) did not appear to have any effect on the aroma of the cysteine + ribose reaction mixture, which was sulfurous with an underlying meatiness. However, when beef phospholipids (PL) were used the meaty aroma increased markedly. Similarly, addition of egg lecithin (LEC) or egg cephalin (CEPH) to the cysteine + ribose reaction mixture gave increased meatiness, with the cephalin-containing mixture being judged to have the most meaty character.

The effect of these different lipids on the formation of selected volatiles has also been evaluated (Table I). Many compounds

Table I. Relative concentrations of some heterocyclic compounds from cysteine + ribose Maillard reaction mixtures containing different lipids

Compound	No Lipid	Beef TG	Beef PL	Egg LEC	Egg CEPH
2-Furanmethanethiol	1	0.67	0.63	0.62	0.72
2-Methyl-3-furanthiol	1	0.40	0.15	0.27	0.24
2-Thiophenethiol	1	0.32	0.03	0.46	0.10
2-Pentylpyridine	0	0.09	1	18.65	1.54
2-Pentylthiophene	0	0	1	23.86	10.86
2-Hexylthiophene	0	0.15	1	6.63	2.38
2-Pentylthiapyran ^a	0	0.01	1	11.02	3.97

^atentative identification

derived from the basic cysteine + ribose system were reduced in the presence of lipids, with the phospholipids in general having more effect than the triglycerides. The alkylthiophenes and other compounds resulting from the interaction of lipids with Maillard intermediates were only found in trace amounts in the presence of triglycerides, while considerably larger amounts were found in all the phospholipid-containing mixtures.

Conclusion

Lipids play an important part in the development of aroma in cooked foods, such as meat, by providing a source of reactive intermediates which participate in the Maillard reaction. Phospholipids appear to be more important than triglycerides. The addition of phospholipid to aqueous amino acid + ribose mixtures leads to reductions in the concentrations of heterocyclic compounds formed in the Maillard reaction. This effect could be due to lipid oxidation products reacting with simple Maillard intermediates, such as hydrogen sulfide and ammonia, to give compounds not normally found in the Maillard reaction. The precise nature of the odoriferous products obtained from lipid - Maillard interactions is dictated by the lipid structure and may depend on the fatty acid composition and the nature of any polar group attached to the lipid.

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Chapter 42

Thermal Generation of Sulfur-Containing Flavor Compounds in Beef

J. R. Vercellotti, J. W. Kuan, A. M. Spanier, and A. J. St. Angelo

Southern Regional Research Center, Agricultural Research Service, U.S.
Department of Agriculture, P.O. Box 19687, 1100 Robert E. Lee Boulevard,
New Orleans, LA 70179

Positive cooked-beef flavor components as perceived by descriptive sensory panelists are lost during free radical catalyzed meat flavor deterioration (MFD) while negative flavor notes with descriptor definitions of cardboard and painty intensify. Although the cardboard and painty off-flavors correlate well with lipid oxidation products and can be measured easily by gas chromatography (1), much less is known about the fate of the positive flavors in this MFD process. Previously a purge and trap gas chromatographic method was reported by the authors for cooked beef heterocyclics (2). A method of extracting and concentrating positive cooked beef flavors has been developed. A flame photometric detector specific for sulfur compounds revealed a mixture of sulfur compounds in the cooked meat extract which quantitatively changed with storage. Three major sulfur compounds were identified as markers for flavor changes, namely methional, methyl sulfone, and benzothiazole.

Although considerable effort has been expended on the identification of sulfur-containing flavor components in beef (as reviewed by MacLeod et al., (3); Shahidi et al., (4)), little effort has been expended upon the dynamic chemistry of these compounds during the cooking and storage phases of the meat. Using a previously developed concentration method, MacLeod employed trapping methods (5-7) to determine the effect of water activity on the production of cooked-beef aroma compounds. These were significant studies because they demonstrated that different compounds develop at 58% or 17% moisture in rehydrated cooked ground beef, contrasting this work to the report of Hartman (8), wherein maximum flavor volatiles production was reported at a water activity of 0.72 (about 64% moisture).

Since several studies identified the aliphatic and heterocyclic sulfur compounds as being important to cooked-beef flavor (9-11), the present report focuses upon a few key sulfur compounds that contribute to flavor of cooked meat as they change with time and temperature. Previous work has been directed to obtaining reliable correlation of sensory panel data with objective instrumental data describing meat flavor deterioration on storage (1). The present study is targeted toward obtaining a reliable, objective assay

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for desirable beef flavor in cooked meat products using sulfur specific detection techniques in gas chromatography.

Beef Preparation. Beef was obtained from a local supermarket as USDA "choice" grade, top round steaks. Excess fat was trimmed and the lean muscle used for these experiments. Beef patties were prepared with minor variation to the methods for sensory analysis as described in Johnsen and Civille (12) and Love (13). Only the *semimembranosus* muscle of the top round steaks was used. The sample was ground twice and then made into 85 g patties containing about 3% fat. The patties were cooked on a Farberware grill for 8 min on each side to a mean internal temperature of $168^{\circ} \pm 3.5^{\circ}\text{F}$ (75°C). Weight loss was 37.1% on cooking. For storage studies the freshly cooked patties were cooled to below 50°C and reground to prepare a more homogeneous mixture of the cooked muscle. Ground meat samples (100 g) were placed in Petri dishes and stored with glass covers at 5°C for zero days, 2 days, 4 days, and 7 days, respectively. Samples were reheated at full power for 1 minute in a microwave oven prior to proceeding with flavor volatile analysis (60°C internal temperature). A reproducibility study was carried out on 5 identical, 100 g samples that had been stored for 3 days after cooking, except that they were not reheated in the microwave prior to analysis. An *ad hoc* panel convened for these experiments consisted of two trained meat flavor panelists who scored the samples for characterization of MFD according to descriptive sensory methods described by Johnsen and Civille (12) and Love (13). The panelists were also active members of a twelve member descriptive sensory panel at the Center. Two duplicate repetitions were carried out for each experiment (4 samples studied).

Gas Chromatography (GC). GC was performed using a Hewlett-Packard 5880 gas chromatograph equipped with a capillary splitter on the injection port. A 50 meter, 0.31 mm fused silica capillary column coated with 0.52 micron film thickness of SE-54 (Hewlett-Packard Ultra 2, 5% phenylmethyl silicone) was used for the separations with detection via flame ionization (FID) and flame photometric (FPD; Hewlett-Packard Model #19256A). Injections were performed manually with the injection port at 200°C , splitter ratio 8:1, and 1 μl sample injected with temperature programming from 50°C (5 minute hold) at a $5^{\circ}\text{C}/\text{minute}$ ramp to 180°C (10 minute hold). Carrier gas flow was 2.5 ml/min at 40°C and gas regulation on the FPD was hydrogen, 70 ml/min, and air, 80 ml/min for operation (310 ml/min total for ignition). FPD temperature was set at 200°C .

Standards of typical meat flavor sulfur aliphatics and heterocyclics were made from 5 ng/ μl to 500 ng/ μl in hexane to determine response factors as well as reproducibility in the flame photometric detector. Background sulfur compounds were checked in concentrated reagent blanks.

GC peaks were identified using the Finnigan Ion Trap Detector (ITD) and its programs for library comparison. To aid in the positive identification of peaks, a library of ITD mass spectra was generated using standards of compounds equivalent to those found in this work. Capillary GC (Perkin-Elmer Sigma-300) for the ITD was carried out similar to the conditions above, i.e., splitter ratio, 20:1, oven temperature, 50°C (5 minute hold) followed by a $5^{\circ}\text{C}/\text{min}$ ramp to 180°C ; the 10 minute hold was followed by a $5^{\circ}\text{C}/\text{min}$ ramp to 200°C (20 minute hold to remove impurities).

Extraction. The sulfur compounds of interest and other meat flavor principles in the cooked meat patties were extracted and concentrated

according to the procedures seen in Figure 1. Prior to passing the final extract through silica gel, the internal standard, benzothiophene (2500 ng in 100 μ l of hexane), was added to the volume of organic solvent. Note that the meat flavor principles of most interest are not hexane soluble but are water soluble, and are finally back-extracted into methylene chloride-methanol (9:1).

Sulfur Compounds of Beef Flavor. Methional, which results from the degradation of methionine, is an important contributor to flavor in meat. Thiolanes, formed during the cooking of beef, have peculiar oniony flavors that also augment the quality of the meaty flavor. Thiophenes and thiofurans are also important to meaty flavors. Sulfides, such as methyl sulfide, are oxidized to methyl sulfoxide and methyl sulfone. Condensation reactions of Maillard browning products also result in thiazoles such as benzothiazole, an important component of meat flavor.

From the literature, 19 commonly reported beef flavor aroma compounds containing sulfur were chosen as representative of the capillary gas chromatographic spectrum of substances active in the FPD and are noted in Table I. The reproducibility of the instrument was demonstrated by repeated injections to identify the range of sulfur volatiles in beef samples. The internal standard chosen, benzothiophene is also listed in Table I.

The FPD detector is quite specific for sulfur compounds and enhances the signal several fold over the FID. The total ion current chromatogram of these same sulfur standards were run with capillary GC on a Finnegan ITD. The mass spectral identification of each compound was quite efficient with the ITD system, even at 25 nanograms sample per peak.

Table I: Sulfur Compounds Chosen for this Study

dimethyl sulfide	2-ethylthiophene	2-acetylthiazole
thiophene	2,5-dimethylthiophene	2-butylthiophene
thiazole	2-methyl-2-thiazole	3-acetylthiophene
dimethyldisulfide	4,5-dimethylthiazole	2-acetylthiophene
2-methylthiophene	2-propylthiophene	BENZOTHIOPHENE*
3-methylthiophene	dimethyltrisulfide	benzothiazole
4-methylthiophene	2,4,5-trimethylthiazole	

* BENZOTHIOPHENE = internal standard injected at 25 ng

Volatile profiles of raw and cooked-beef flavor samples, prepared by the procedures of Figure 1, were obtained after capillary GC and FPD. Although the identification of these sulfur containing compounds is as yet incomplete, the chromatograms demonstrated that there were a number of new sulfur compounds produced on cooking that were not present in the raw beef. Three prominent sulfur compounds were identified as markers in subsequent meat flavor deterioration experiments, namely, methional (13.2 min), methyl sulfone (13.8 min), and benzothiazole (25.3 min). Each compound produced an adequate mass spectrum for spectral library search and positive identification.

A typical cooked-beef flavor deterioration sample was prepared as described in Beef Preparation section to observe changes in key sulfur marker compounds over a period of 0-, 2-, 4-, and 7-day storage. Sulfur markers, methional, methyl sulfone, and benzothiazole were compared with benzothiophene as internal standard to follow the course of free radical reactions taking place in the stored cooked-beef. Results are plotted in

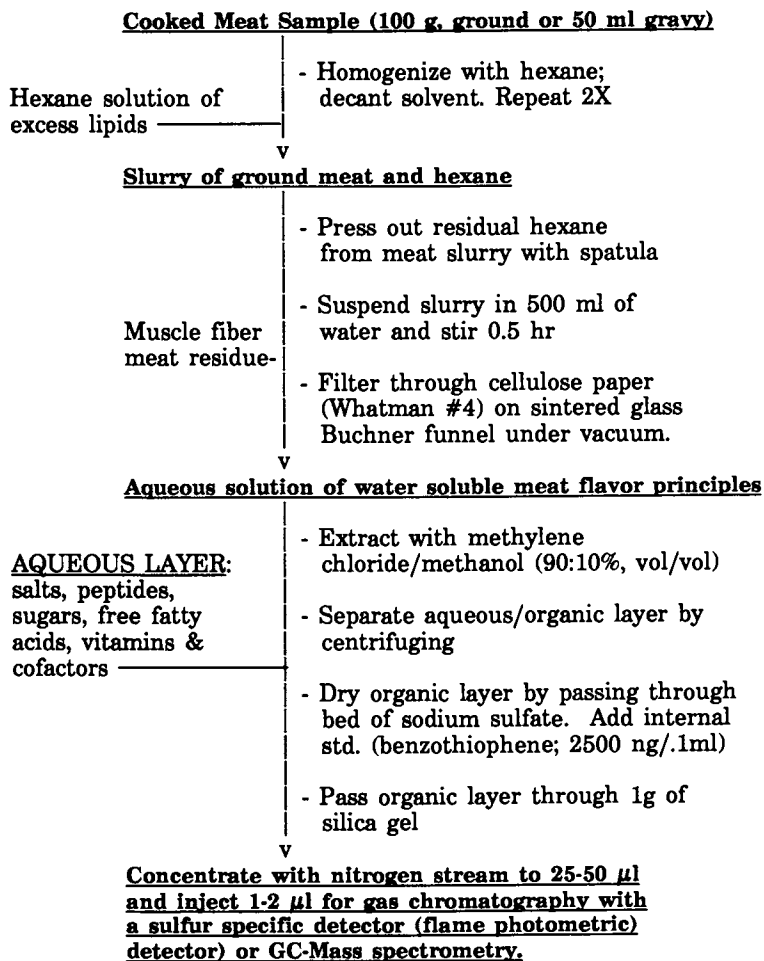
EXTRACTION OF SULFUR HETEROATOMIC COMPOUNDS FROM COOKED MEAT

Figure 1. Extraction/flow diagram of sulfur heteroatomic compounds from cooked meat.

Figure 2 and listed in Table II for the time course of 7 days of storage with standard deviation limits for FPD responses of the three sulfur markers.

Table II: Effect of storage on the content of sulfur heterocyclics in ground-beef

Compound	Daily concentration (ppb) after storage (4 °C)			
	0 day	2 day	4 day	7 day
methional	19.31	52.19	61.04	41.33
methyl sulfone	32.31	27.06	28.18	27.39
benzothiazole	1.83	2.41	2.82	3.04

Figure 3 plots intensity changes perceived by *ad hoc* descriptive sensory panelists in intensity analysis for character notes involved in meat flavor deterioration during storage of the grilled beef samples reported in Figure 2 and Table II (12, 13). The loss of intensity for certain descriptors in Figure 3 is in accord with sensory panel experience that the positive notes such as cooked-beef brothy diminish with formation of new off-flavor compounds represented as cardboard and painty.

Discussion and Interpretation.

The authors have previously reported a general screening method for beef flavor compounds with high impact and low thresholds of human perception (2). These sulfur, nitrogen, and oxygen heteroatomic or heterocyclic compounds are present in meat in the low parts per billion range. Preliminary data on the fate of sulfur compounds during meat flavor deterioration reactions was reported by St. Angelo et al. (14). Of greater importance in the present paper is the question of the dynamics of the sulfur flavor compounds made by thermal generation in meat (10, 11, 15-17) and rapid degradation of these key substances through a variety of free radical mechanisms during storage and reheating of meat.

Positive cooked-beef flavor components as perceived by descriptive sensory panelists are reduced during free radical catalyzed meat flavor deterioration (MFD) while negative flavor notes with descriptor definitions of cardboard and painty intensify, as reviewed recently by Love (13). Although the cardboard and painty off-flavors correlate well with lipid oxidation products and can be measured easily by gas chromatography (1, 14, 18), much less is known about the fate of the positive cooked-beef flavors in this MFD process (13).

A large concentration differential exists between lipid oxidation products to beefy flavors (high ppm for lipid oxidation products vs low ppb for cooked-beef aromatics, respectively). Therefore, a method of extracting and concentrating positive cooked-beef flavors has been developed (Figure 1). The cleanup steps so described removed protein and a good deal of fat soluble materials, but the final residue upon drying and solvent removal remained quite identifiable as a representative roast beef aroma according to Johnsen and Civille (12) and Love (13).

A mass spectral chromatogram for the extract from grilled beef is quite complex with over 100 peaks. In spite of the complexity of this chromatogram, several sulfur-containing compounds are consistently identifiable by ITD from these cooked meat extracts, which correspond to the FPD-active peaks. The three principal marker compounds identified

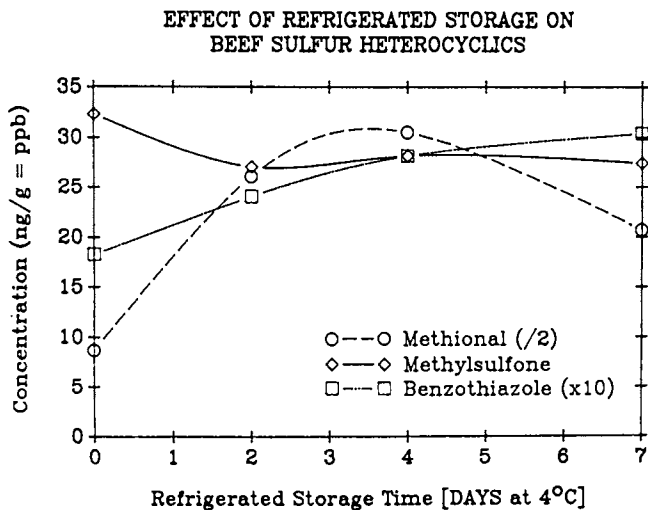


Figure 2. Effect of storage on four FPD active sulfur compounds extracted from grilled beef stored up to 7 days at 4°C.

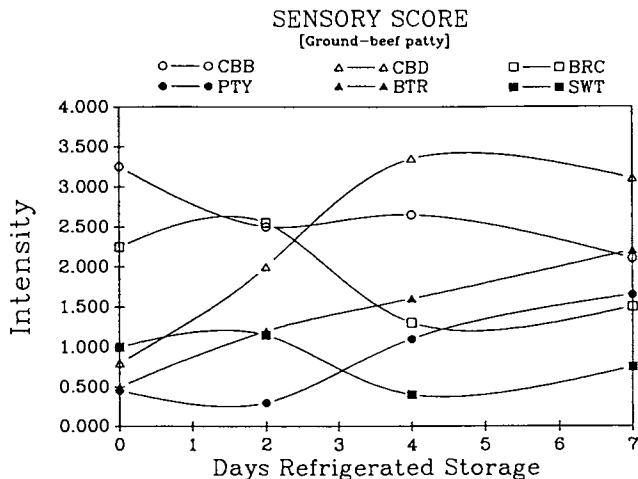


Figure 3. Changes in panelists' (n = 2) perceived intensity of sensory descriptors from grilled ground beef patties during storage at 4°C. Aromatics: CBB: cooked beef/brothy; BRC: browned caramel; CBD: cardboard; PTY: painty. Tastes: BTR: bitter; SWT: sweet.

were methional, methyl sulfone, and benzothiazole, which were later quantitated from their FPD response curves using benzothiophene as an internal standard.

The kinetics of marker sulfur compound turnover in grilled beef patties stored from time of cooking through 7 days as described herein (Table II and Figure 3) indicated that the free radical process is very dynamic for these sulfur compounds. Some sulfur flavor compounds in cooked-beef were destroyed during the storage and rewarming process while others were being produced by free radical reactions that may contribute to off-flavors. Although at low concentration methional can be a positive flavor contributor, when too high or out of balance it forms a boiled sweetcorn-like odor. Methional is formed from the Strecker degradation of methionine, and methional itself undergoes several free radical reactions to form further thiols or sulfides. Methional maximizes between days 2 and 4 of storage and then drops off in concentration. These concentrations reported for the storage study of grilled beef patties (Table II) are far above methional's threshold of human perceptibility (19), which is in the lower parts per billion range. The gradual formation of the stable end product, benzothiazole, during meat flavor deterioration is an interesting study in reactivity. Benzothiazole is a frequent roast beef positive flavor in low concentration. However, benzothiazole is a definite off-flavor (19) in the concentrations built up during the grilled beef patty storage process. Again, the blending and balance of these flavors is probably important to their overall impact. The nearly odorless and tasteless end-product of methionine oxidation, methyl sulfone, is practically unchanged throughout the storage period and, not surprisingly, is quite stable. This work would indicate that in the more severe thermal degradation conditions of roasting the beef methyl sulfone is formed whereas the kinds of free radical processes generated during storage further degrade methional and lead to the formation of the stable aromatic negative flavored end product, benzothiazole.

The intensities of character notes in descriptive sensory analysis of these grilled beef patties are shown in Figure 3. Although these intensities were estimated by experienced meat flavor panelists, they should be viewed as preliminary characteristics of MFD. However, these results largely parallel the long experience of these panels with grilled beef patty flavor deterioration (12, 13). There are increases of the off-flavor character notes in Figure 4 that parallel changes in two of the dominant sulfur derivatives, methional and benzothiazole (Figure 2 and Table II). The desirable flavors, cooked beef brothy (CBB) and browned caramel (BRC), declined noticeably (cf., 12 and 13). Perhaps some of the cardboardy (CBD) off-flavor of MFD is attributable to changes in these methionine-related sulfur compounds. An interesting hypothesis from this work is that the descriptor cardboardy is perceived as wet, Kraft box material; paper mill odor; or sulfite liquors with a definite connotation of sulfury as conveyed by the sulfur-containing marker compounds found here. The painty (PTY) meat off-flavor, on the other hand, which is reminiscent of aged linseed oil, is more easily identifiable with the strong concentration of lipid oxidation products. Sweet (SWT) and bitter (BTR) tastes diverge during beef patty storage with SWT declining and BTR increasing.

An obvious deficiency in the present marker study is that practically none of the other sulfur-containing molecules, known to be positive contributors to cooked-beef flavor, are detectable by this extraction and FPD gas chromatographic method (2-11, 20). The fate of more of these other contributors to the blend of cooked-beef flavor should also be studied to gain a better perspective. However, this extraction method is simple to use and is recommended for its reproducibility and high recovery of useful markers.

As described in MacLeod and Coppock (20) subtleties in flavor do exist even between samples of the same meat cooked in different conventional ways, e.g., boiled versus roasted. The present method is a good route to screening many compounds in these samples also by FID or GC/Mass Spec after various treatments such as the use of preservative antioxidants.

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Chapter 43

Isolation and Characterization of Volatile Sulfur-Containing Meat Flavor Components in Model Systems

P. Werkhoff, R. Emberger, M. Güntert, and M. Köpsel

Haarman & Reimer GmbH, Research Department, D-3450 Holzminden,
Federal Republic of Germany

Reaction of an aqueous solution of cystine with thiamin, glutamate, and ascorbic acid produces a complex mixture of compounds with an overall flavor resembling that of roasted meat. The reaction was carried out at 120°C for 0.5h at pH 5.0 in a closed system. The aroma compounds were isolated by means of the simultaneous steam distillation/solvent extraction method. The flavor concentrate was pre-separated by liquid chromatography on silica gel and subsequently analysed by GC and GC/MS. Unknown flavor components were isolated by preparative capillary gas chromatography and the structures were elucidated on the basis of spectroscopic studies. Various heterocyclic thioethers, disulfides and dithiohemiacetals were identified for the first time in the volatiles of the heated meat flavor model mixture. Sensory properties of newly identified flavor components are discussed. In most cases, identifications were confirmed by organic syntheses.

It has been reported that many types of chemical reactions are responsible for meat flavor due to different water-soluble flavor precursors which generate volatile components on heating. The chemistry of meat flavor formation has been extensively investigated and reviewed by many authors (1-3).

A great number of meat flavor components have been isolated from model systems. Studies of reaction flavors or model systems are extremely helpful in the identification of organoleptically interesting meat flavor components. Many of the compounds present in cooked or roasted

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meat flavor are also formed in the model systems. On the other hand it must be emphasized that many flavor components have not yet been identified in natural meat aroma, although some have been isolated from model systems.

Remarkable progress has been made in meat flavor research over the past ten years. Chemical compounds occurring in natural meat volatiles are listed in a recent review article by F. Shahidi et al. (4).

There is no doubt that sulfur-containing components play a most important role in roasted and cooked meat flavors because only trace amounts of these compounds need be present to be aroma effective.

The investigation of a series of model meat systems has demonstrated the important role of volatile sulfur-containing heterocyclic components substituted with sulfur in the 3-position. One of these 3-substituted sulfur compounds, 2-methyl-3-methylthio-furan was identified recently in the volatiles from cooked beef aroma (5) and from a heated yeast extract composition (6) and is considered a meaty character impact compound.

In this context, it is worth mentioning that in natural meat volatiles mainly 2-substituted derivatives of furan or thiophene have been identified. This does not mean, however, that there are no 3-substituted sulfur-derivatives in natural meat products. It is more likely that these components substituted with sulfur in the 3-position are present in trace amounts in natural meat volatiles.

In addition to analyzing meat, we decided to investigate a relevant model meat flavor system approximating cooked and/or roasted meat that was prepared by heating an aqueous solution of cystine, thiamin, glutamate, and ascorbic acid. The purpose of the present investigation was to study the formation of volatile sulfur-containing components responsible for interesting meaty flavor notes in this model meat system based on naturally occurring precursors.

EXPERIMENTAL SECTION

Preparation of the Reaction Mixture. A mixture of 100g cystine, 100g thiamin-HCL, 100g ascorbic acid, 500g monosodium-glutamate, and 2 l of distilled water was placed in an autoclave equipped with a stirrer arm. The pH of the mixture was measured as 5.0. The reaction mixture was heated to 120°C for 0.5 hr and allowed to cool to room temperature.

Isolation of Volatiles by Simultaneous Distillation/Extraction. The dark brown reaction mixture was placed in a 4 l round bottom flask and continuously extracted for seven hours with 200 ml pentane/diethyl ether (1:1) at atmospheric pressure according to the procedure described by Likens/Nickerson. The pentane/ether extract

was dried over anhydrous sodium sulfate and the organic solvent was removed on a 25 cm x 1 cm Vigreux distillation column. The concentrate was stored under nitrogen.

Pre-separation by Adsorption Chromatography. The components were pre-separated into twenty fractions by medium pressure liquid chromatography on silica gel using a pentane/ether gradient as mobile phase. The aroma concentrate was placed on a cooled column (480 mm x 37 mm I.D.) filled with 240g of silica gel (25 - 40 μ m). The elution rate was 10 ml/min. All eluates were dried over anhydrous sodium sulfate. The individual fractions were concentrated to a volume of 1 ml by using a 25 cm x 1 cm Vigreux column. Further concentration to about 100 μ l was slowly performed by a procedure described by W. Dünge (7).

Capillary Gas Chromatography (HRGC). Analytical separations were performed on a Varian 3700 GC instrument as well as on a Carlo Erba type 5360 Mega Series gas chromatograph. The Varian 3700 GC system was modified with a hot split/splitless injector and additionally equipped with a commercially available inlet splitter (Gerstel, Mülheim/Ruhr) in order to install two capillary columns of different polarity. The Carlo Erba 5360 gas chromatograph was fitted with a so-called "glass-cap-cross" inlet splitting system. This system for double-column GC analysis in combination with the cold on-column injection technique has been developed in our laboratory quite recently (8,9).

Columns used: (1) Polar column A: 60 m x 0.32 mm I.D. fused silica capillary column coated with DB-WAX (0.25 μ m film thickness). (2) Non-polar column B: 60 m x 0.32 mm I.D. fused silica capillary column coated with DB-1 (0.25 μ m film thickness).

A Helium carrier gas flow rate of 2 - 3 ml/min and an oven temperature programmed from 60°C to 220°C at 3°C/min were used. The temperature of the injector (Varian 3700) was 250°C and the detector temperature was 275°C.

Furthermore, GC samples were separated on a Carlo Erba type 4200 gas chromatograph fitted with a normal FID and with a flame photometric detector (FPD) operating in the sulfur-mode at 394 nm. Again, the dead-volume free "glass-cap-cross" was used in order to split the carrier gas flow. By means of these sulfur chromatograms mass spectral evaluation could be focussed on certain compounds in very complex mixtures.

Preparative Capillary Gas Chromatography. A Carlo Erba type 5360 Mega Series gas chromatograph equipped with a flame ionization detector and a split/splitless injector was used to isolate individual components from the eluent of a capillary column.

A "glass-cap-cross" effluent splitting system was installed in this GC instrument and the splitting ratio

between the detector and the trap was adjusted to be 1:5 or 1:10.

All preparative GC separations were performed on wide-bore fused silica capillary columns (30 m x 0.53 mm I.D. / film thickness between 1.0 μm and 3.0 μm) combining the advantage of sufficient separation efficiency with relatively high sample capacity. A high sample capacity is especially desirable to reduce the number of injections.

Prior to spectroscopic investigations the collected samples were re-injected into an analytical capillary GC system in order to determine the purity of the condensed components.

GC - Sniffing. For odor evaluation, a GC instrument was equipped with an all-glass effluent splitter ("glass-cap-cross") with one splitter arm going to an FID and the other splitter arm was connected via a fused silica capillary to a sniffing port. The components separated by GC were evaluated by their smell at the sniffing port. GC conditions were similar to those mentioned above.

Gas Chromatography - Mass Spectrometry (GC-MS). The column and operating conditions employed for the gas chromatograph in GC/MS analysis were similar to those described above.

The system used to obtain the mass spectra was a Finnigan MAT Series 8230 instrument interfaced to a Carlo Erba 5360 Mega Series gas chromatograph (open split coupling via a flexible transfer line).

The operating conditions were as follows: Temperature of the transfer line, 250°C; temperature of the ion source, 220°C; electron energy, 70 eV; cathodic current, 1 mA; accelerating voltage, 3 kV; resolution, 900; scan speed, 1sec / dec.

The compounds were identified by comparison of the GC retention indices and mass spectra with reference data from authentic components and with data of our own MS library. Detailed spectral data will be published separately.

IR- and NMR - Analysis. Infrared spectra were obtained in CCl_4 using a Perkin Elmer 983 G type instrument.

^1H -NMR spectra were measured at 200 MHz in CDCl_3 or C_6D_{12} on a Varian XL-200 instrument with tetramethylsilane as an internal standard. Detailed IR and NMR data will be published separately.

RESULTS AND DISCUSSION

A similar meat flavor model system - comprising also monosodium glutamate, ascorbic acid, thiamin HCl and cystine - was investigated by another research group in 1984. Only eighteen sulfur-containing flavor components were identi-

fied during this earlier study (10,11). Obviously, previous work on the volatile sulfur-containing components has not been very extensive or the formation of sulfur-substituted flavor components proceeded quite differently due to different reaction conditions (e.g. effect of reaction temperature, reaction time, pH, solvent or molar ratio).

By way of contrast, a total of 70 sulfur-containing compounds were identified in the volatile components isolated from our model meat flavor system. A rough survey of the chemical classes represented in the processed meat aroma is shown in Table I.

Table I. Chemical Classes of Volatile Sulfur-Containing Components in a Model Meat Flavor System

Class	Number
Aliphatic Mercaptans	3
Heterocyclic Mercaptans	7
Heterocyclic Thioethers	11
Heterocyclic Disulfides	7
Thiophenes	22
Thiazoles	6
1,2-Dithianes	2
1,2-Dithiolanes	1
1,2,4-Trithiolanes	2
1,2,4,5-Tetrathianes	2
Thia-alkanethiols (Dithiohemiacetals)	2
Thia-heteroarylalkanethiols	3
Miscellaneous	2

Of particular interest is the identification of five different types of heterocyclic sulfur-containing flavor components, the preponderance of which were furans and thiophenes substituted with sulfur in the 2- and 3-position. The bulk of these flavor compounds had not been identified in meat and had not been reported in the literature so far. These sulfur-substituted heterocyclic flavor components will be further discussed below.

Identification of these components was based on GC/MS and retention index information. Novel compounds were isolated by preparative capillary gas chromatography and spectroscopically identified by interpretation of infrared, nuclear magnetic resonance and mass spectra. In

most cases, the structure was ultimately confirmed by chemical synthesis.

FURANS AND THIOPHENES SUBSTITUTED AT THE THREE POSITION WITH SULFUR

Table II lists some furan and thiophene components substituted with sulfur at the three position on both heterocyclic rings generated in one reaction system. Also included are Kovats retention index data and references reporting the occurrence in foods or model systems.

The occurrence of 1 and 3 in a flavor model system has been pointed out by G.J. Hartmann et al. (10,11). Moreover, 2-methyl-3-furanthiol 1 and bis-(2-methyl-3-furyl)-disulfide 3 have already been identified as major constituents in a model meat system that was prepared by refluxing an aqueous solution of cysteine hydrochloride, thiamin hydrochloride, and hydrolyzed vegetable protein (12,13) for four hours.

G.J. Hartmann et al. (14) and G.A. Reineccius and R. Liardon (15) have recently studied the volatile products from thermally degraded thiamin. A number of different decomposition components were identified including 2-methyl-3-furanthiol and bis-(2-methyl-3-furyl)-disulfide. A schematic representation of the formation of these extremely important meat flavor compounds from thiamin is outlined by G. MacLeod (3). The odor threshold of bis-(2-methyl-3-furyl)-disulfide is remarkably low (16). Similarly, comparably low odor thresholds are expected for the related structures shown in Table II.

Furthermore, 2-methyl-3-furanthiol and bis-(2-methyl-3-furyl)-disulfide were recently identified in the volatiles from a simulated meat flavor (17,18) as well as from a heated yeast extract composition (6).

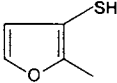
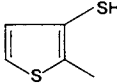
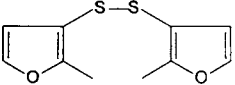
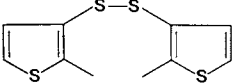
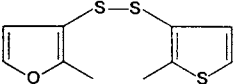
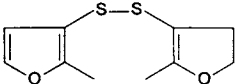
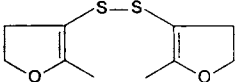
2-methyl-3-thiophenethiol 2 was identified in a heated model system of hydrogen sulfide with 4-hydroxy-5-methyl-3(2H)-furanone (norfuranol) (19).

In contrast to 3, bis-(2-methyl-3-thienyl)-disulfide 4 is cited in the literature only once, in a Russian publication dealing with the synthesis and some transformations of sulfides of the thiophene series (20).

Bis-(2-methyl-3-thienyl)-disulfide was prepared from 2-methyl-3-thienylthiol in our laboratory. This heterocyclic mercaptan was dimerized in an alkaline solution in the presence of H₂O₂. After removing the solvent, the product was purified by fractional distillation. The synthetic compound proved to be identical with the flavor compound isolated from the complex model mixture.

2-methyl-3-(2-methyl-3-thienyldithio)-furan 5 has not been previously described in the literature. This latter component was characterized as a main constituent in our model system. Furthermore, this component has also recently been identified in our laboratory in the headspace of beef (unpublished results). 5 was prepared by

Table II. Volatile sulfur-containing components identified in a model meat flavor system

Chemical structure	Name of component	Occurrence in food or model systems	Kovats-Index DB-1 ³⁾
	<u>1</u> 2-Methyl-3-furanthiol	<u>6, 10, 11, 12, 13, 14, 15, 17, 18</u>	846
	<u>2</u> 2-Methyl-3-thiophenethiol	<u>19</u>	1030
	<u>3</u> Bis-(2-methyl-3-furyl)-disulfide	<u>6, 11, 12, 13, 14, 15, 17, 18</u>	1494
	<u>4</u> Bis-(2-methyl-3-thienyl)-disulfide		1867
	<u>5</u> 2-Methyl-3-(2-methyl-3-thienyldithio)-furan ¹⁾		1681
	<u>6</u> 2,3-Dihydro-5-methyl-4-(2-methyl-3-furyldithio)-furan ²⁾	<u>14</u>	1627
	<u>7</u> Bis-(2-methyl-4,5-dihydro-3-furyl)-disulfide ²⁾	<u>14</u>	1675

¹⁾ Reported for the first time²⁾ Tentatively identified³⁾ 60 m x 0,32 mm I.D. DB-1/film thickness 0,25 μm/60°C-3°C/min - 220°C

oxidizing a mixture of 2-methyl-3-furanthiol and 2-methyl-3-thiophenethiol.

The structure assignment of the reaction products 6 and 7 was solely based on the interpretation of MS and NMR data (micro samples) and was not confirmed by synthesis. Both components have been mentioned in the literature from the thermal degradation of thiamin (14). However, it is worth mentioning that our spectroscopic data for 7 are not in accordance with MS-data published by G.J. Hartmann et al. in 1984.

All three mass spectra of the heterocyclic disulfides 3,4 and 5 are characterized by intensive molecular ions. The main feature of the fragmentation is the cleavage of the disulfide bond system, giving intensive fragments at m/z 113 (2-methyl-3-furylthio cation) and m/z 129 (2-methyl-3-thienylthio cation). It is worth mentioning that the fragment ion m/z 43 (acetyl cation) plays an important role in the fragmentation process of disulfides and thioether compounds. This ion is a clue to the presence of the 2-methyl-3-furylthio structural fragment for this type of sulfur-containing components.

For the sensory evaluation, experienced assessors were used. Prior to sensory evaluation the purity of all flavor components was carefully checked because undesirable trace impurities may severely affect the odor quality of a component and can lead to erroneous conclusions. Different concentrations of each flavor substance in aqueous solution were used (based on their detection thresholds). Furthermore, sensory evaluation was also performed by sniffing the GC-eluate.

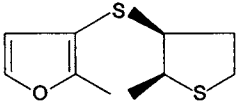
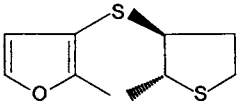
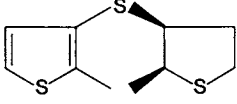
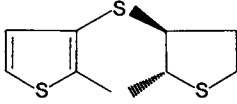
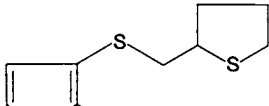
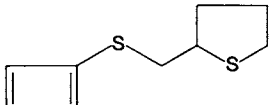
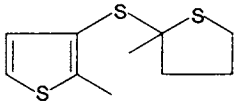
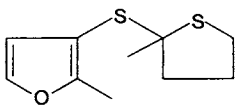
1 and 3 possess characteristic meat flavor notes and are likely to be of prime importance in cooked meat aromas (3). 2 exhibits strong odor and flavor of roasted meat while 4 has less powerful organoleptic properties and is described as sulfurous, metallic and rubbery having only a slight meat character. Compound 5 delivers a meaty aroma character but has additionally an allium-like flavor of onion or garlic with metallic and fatty background notes.

The presence of the heterocyclic disulfides in the aroma mixture is easy to understand and can be generally postulated as oxidative decomposition products of the corresponding monomers. Even air oxidation of the monomers may result in dimerization without effort.

HETEROCYCLIC THIOETHERS

Another important type of sulfur compound identified in the model reaction mixture is represented in Table III. Eight heterocyclic thioethers were identified for the first time. Both heterocyclic ring systems of components 8 - 11 are substituted by sulfur in the 3-position. Due to the connection of a furan or thiophene ring system with a substituted tetrahydrothiophene structure, the

Table III. Newly identified heterocyclic thioethers in a model meat flavor system

Chemical structure		Name of component	Kovats-Index DB-1
 $C_{10}H_{14}OS_2$	<u>8</u>	2-methyl-3-(cis-2-methyl-3-tetrahydrothienylthio)-furan	1561
 $C_{10}H_{14}OS_2$	<u>9</u>	2-methyl-3-(trans-2-methyl-3-tetrahydrothienylthio)-furan	1607
 $C_{10}H_{14}S_3$	<u>10</u>	cis-2-methyl-3-(2-methyl-3-thienylthio)-tetrahydrothiophene	1754
 $C_{10}H_{14}S_3$	<u>11</u>	trans-2-methyl-3-(2-methyl-3-thienylthio)-tetrahydrothiophene	1799
 $C_{10}H_{14}OS_2$	<u>12</u>	2-methyl-3-(2-tetrahydrothienylmethylthio)-furan	1645
 $C_{10}H_{14}S_3$	<u>13</u>	2-methyl-3-(2-tetrahydrothienylmethylthio)-thiophene	1845
 $C_{10}H_{14}S_3$	<u>14</u>	2-methyl-2-(2-methyl-3-thienylthio)-tetrahydrothiophene	1727
 $C_{10}H_{14}OS_2$	<u>15</u>	2-methyl-3-(2-methyl-2-tetrahydrothienylthio)-furan	1537

formation of *cis*- and *trans* stereoisomers is possible. This class of flavor components has not previously been reported in a food system or in a model system. To confirm these structures we have prepared the compounds 8, 9, 10, and 11 by synthesis from 2-methyl-3-furylthiol or 2-methyl-3-thienylthiol and 2-methyl-3-*p*-toluene-sulfonyloxy-tetrahydrothiophene (Scheme 1).

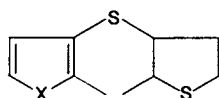
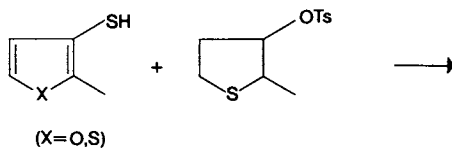
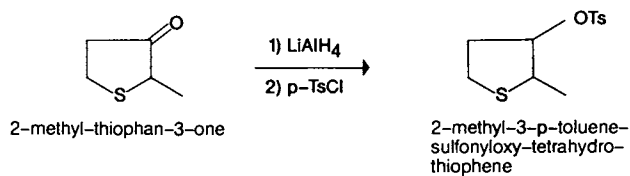
Compounds 8, 9, 10 and 11 are described as being strongly odorous. The compounds 8 and 9 also illustrate the fact that in spite of close similarities in chemical structure, there are differences in sensory quality. On the one hand, 8 was mainly associated with herbaceous, nutty, carrot-like, mushroom-like and bread crust-like odor impressions. On the other hand, this component contributes to the overall odor and taste of boiled and roasted meat. Moreover, 8 possesses a strong odor of freshly grilled liver. Component 9 has a characteristic roasted note, reminiscent of roasted filberts. Additionally, 9 produces carrot-, potato-, vegetable-, asparagus-, and mushroom-like sensory impressions and seems to contribute a desirable odor and taste to cooked meat products. The flavor thresholds in water of 8 and 9 are below 1ppb.

Products 10 and 11 are meaty in aroma and flavor. 10 possesses a characteristic meaty note reminiscent of liver-sausage whilst 11 has a roasted meat odor and a burnt meat taste. In addition, the *cis* stereoisomer has a carrot-like and mushroom-like aroma and a herbaceous and tropical fruit note. The flavor thresholds in water of 10 and 11 are below 100 ppb.

Two new substances 12 and 13 which had not been previously identified as flavor compounds are also depicted in Table III. These components are also heterocyclic thioethers and have the molecular formula $C_{10}H_{14}OS_2$ and $C_{10}H_{14}S_3$, respectively.

Compound 12 was identified as 2-methyl-3-(2-tetrahydrothienylmethylthio)-furan and compound 13 was identified as 2-methyl-3-(2-tetrahydrothienylmethylthio)-thiophene. Accordingly, the furan or thiophene ring system is substituted with sulfur in the 3-position while the tetrahydrothiophene ring is connected via the 2-position. Thus, a characteristic feature of these components is the thiomethylene bridge between the two heterocyclic ring systems. The definite structural proof of these two thioether compounds was given again by synthesis as shown in Scheme 2.

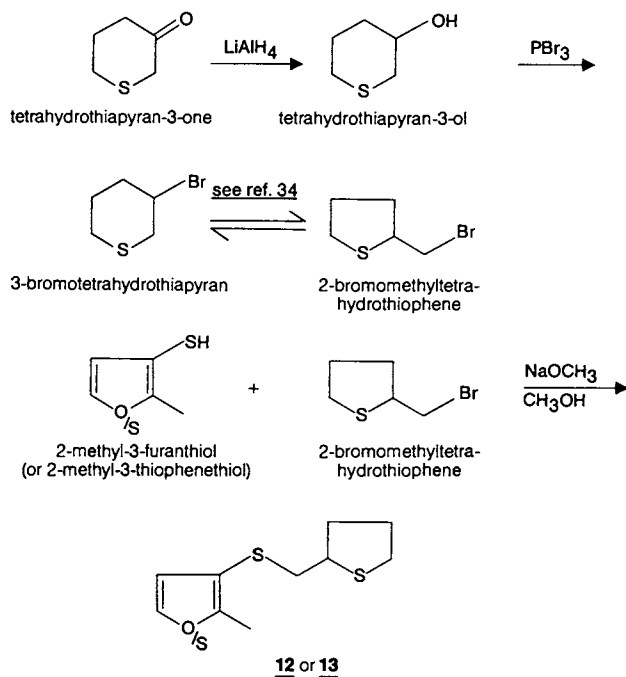
A very important fragment ion is m/z 87 which is significant in the mass spectra of 12 and 13. Obviously, m/z 87 stands for a dihydrothienyl cation and is therefore an essential indicator for the presence of a thiomethylene group between the two ring systems just as the fragment ions at m/z 127 or m/z 143 which are also only present in conjunction with structure 12 or 13.



$(X=O \rightarrow \mathbf{8, 9}$ /mixture of cis/trans stereoisomers)

$(X=S \rightarrow \mathbf{10, 11}$ /mixture of cis/trans stereoisomers)

Scheme 1. Synthesis of cis/trans stereoisomers of 2-methyl-3-(2-methyl-3-tetrahydrothienylthio)-furan and 2-methyl-3-(2-methyl-3-thienylthio)-tetrahydrothiophene



Scheme 2. Synthesis of 2-methyl-3-(2-tetrahydrothienylmethylthio)-furan **12** and 2-methyl-3-(2-tetrahydrothienylmethylthio)-thiophene **13**

The flavor description of 12 and 13 is strong sulfury, having only a slight meat character. In addition, 12 possesses also leek-, chives-, and garlic-like and oniony notes as well as an estragole-like flavor. Compound 13 has an oniony and rubbery character in both aroma and taste and exhibits the odor and flavor of a tropical fruit at very low concentration.

In Table III two additional heterocyclic thioethers are shown which have not been identified as natural products thus far. Their structures were elucidated on the basis of infrared, $^1\text{H-NMR}$ and mass spectrometry to be the 2-methyl-2-(2-methyl-3-thienylthio)-tetrahydrothiophene 14 and the 2-methyl-3-(2-methyl-2-tetrahydrothienylthio)-furan 15, i.e. in this case the thioethers are substituted with sulfur in the furan or thiophene ring systems at the β -position while the sulfur in the tetrahydro-part of the structure is at the α -position.

Contrary to the mass spectra of mercaptans or disulfides, however, the mass spectra of various thioether compounds (e.g. 8, 12 and 15) show no loss of an acetyl cation m/z 43 or the thioacetyl cation m/z 59, respectively. Summing up, it may be said that the mass spectra of the isomeric heterocyclic thioether structures are very similar and mainly differ in the stability (i.e. the relative intensity) of the molecular and few specific ions.

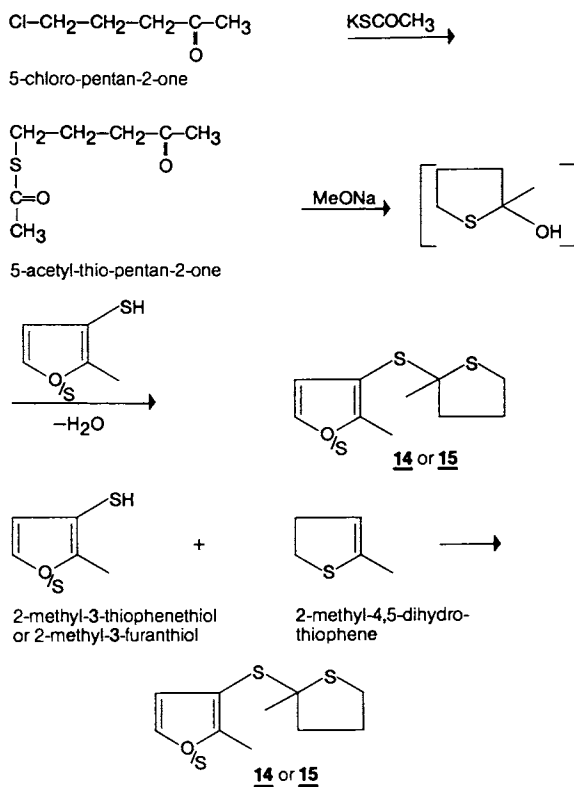
It should be noted that both components have already been described in the patent literature for use in gravies and meat products (21). In this patent, much spectroscopic data was reported. There is a significant discrepancy between our analytical data and the spectroscopic data reported by de Roos et al. (21). We were not able to reproduce MS data summarized in the patent and obtained completely different spectroscopic results.

The routes by which 14 and 15 were synthesized are shown in Scheme 3.

14 and 15 have a very strong odor with a typical meat note. 14 was described as having a "characteristic roast meat aroma". When 14 was added to meat products at the ppb range, the effect was positive, while at higher levels, sensory notes described as metallic, oniony, cabbage and burnt became apparent.

15 has a weaker and rather non specific odor with sulfury, metallic, minty and green flavor notes and is recommended to improve the taste of foodstuffs. 15 imparts a meaty or roasted meat organoleptic impression to foods.

As far as the sensory evaluation of the various types of heterocyclic thioethers is concerned, it is worth noting that most of the heterocyclic thioethers are highly potent flavor components and are mainly associated with a meat-like odor impression at low concentration. At higher concentration, however, they show a terpene-like and/or carrot-like character, while an intense sulfur



Scheme 3. Synthesis of 2-methyl-2-(2-methyl-3-thienylthio)-tetrahydrothiophene 14 and 2-methyl-3-(2-methyl-2-tetrahydrothienylthio)-furan 15

odor is observed in a pure state. Generalizing from these observations it may be said that the odor quality of the newly identified thioether components highly depends on the substance concentration and may change from one concentration to another. Many compounds are meaty only at certain concentrations, usually very low concentrations.

ALIPHATIC AND HETEROCYCLIC DITHIOHEMIACETALS

Finally, we would like to report on four additional important flavor compounds that we have isolated and identified from our model mixture, two of which are, to the best of our knowledge, new to the literature. The components are illustrated in Table IV. Two aliphatic dithiohemiacetals (methylthiomethanethiol 16 and 1-methylthioethanethiol 17) as well as two heterocyclic dithiohemiacetals (1-(2-methyl-3-thienylthio)-ethanethiol 18 and 1-(2-methyl-3-furylthio)-ethanethiol 19) were identified.

The synthesis of methylthiomethanethiol 16 has already been described in the literature (22-25), but the component has never been mentioned in the context with flavor chemistry. Thus, methylthiomethanethiol 16 is reported here for the first time as flavor component. In contrast with 16, 1-methylthioethanethiol 17 was reported in the headspace volatiles of beef broth by Brinkmann et al. (26) and was described as having the odor of fresh onions. This component is formed when ethanal, methanethiol, and H₂S are heated in aqueous solution at pH 6 (27).

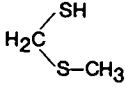
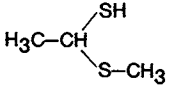
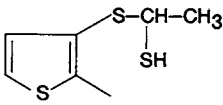
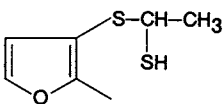
The chemical class of the thiaalkanethiols has been reported by different authors as flavor constituents (17,18,28-30). According to the patent literature, 1-methylthioethanethiol 17 is extremely useful for meat flavors (31,32). A one-step synthesis of dithiohemiacetals was published by L. Schutte in 1971 (33).

The other two heterocyclic dithiohemiacetals shown in Table IV are, to our knowledge, reported here for the first time.

18 possesses sulfurous, carrot-like, leek-like, but also meaty flavor notes. This component imparts pleasant and interesting meaty, yeast-like and onion-like flavors to food products. 18 improves the taste and/or smell of meat products by giving them a boiled meat flavor as well as a typical brothy character.

Organoleptic properties of 19 were reported by several members of the panel. For example, terms like roasted, brothy, spicy, onion, garlic, vegetable, meat and gravy, were frequently used. In particular 19 has a powerful flavor with good meat character in a highly dilute solution. It possesses a typical savoury meat note reminiscent of roast beef. Both heterocyclic components possess more interesting olfactory properties than the alkyl thioether substances. Therefore, it is likely that

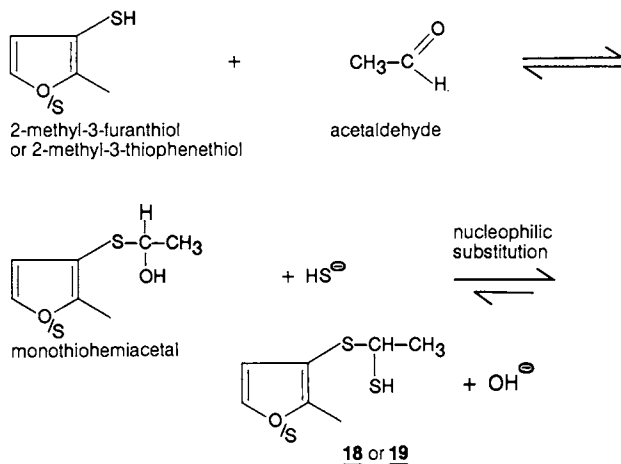
Table IV. Newly identified dithiohemiacetals in a model meat flavor system

Chemical structure		Name of component	Kovats-Index DB-1
	<u>16</u>	methylthiomethanethiol	793
	<u>17</u>	1-methylthioethanethiol	815
	<u>18</u>	1-(2-methyl-3-thienylthio)-ethanethiol	1396
	<u>19</u>	1-(2-methyl-3-furylthio)-ethanethiol	1214

these heterocyclic components are mainly responsible for the interesting sensory properties of the model meat flavor system. Their flavor thresholds in water are below 50 ppt.

18 and 19 were synthesized from hydrogen sulfide, acetaldehyde and 2-methyl-3-thienylthiol (or 2-methyl-3-furylthiol), and compared directly with the dithiohemiacetals isolated by preparative capillary gas chromatography from the model system. The synthetic procedure is shown in Scheme 4. Both synthetic components 18 and 19 proved to be identical with their "natural" counterparts. We believe that these components form in the model system in a manner similar to that by which they are synthesized, because acetaldehyde and hydrogen sulfide are the primary thermal degradation products from cystine.

In summary, model studies are very efficient for the identification and structure elucidation of important flavor components. Most of the compounds reported here have not been identified in meat and have not yet been reported as constituents of food volatiles. Nevertheless, there are good reasons to believe that minute traces of these sulfur-containing components are present in roasted and/or cooked meat volatiles because our model system was based solely on naturally occurring precursors. We believe that only minute trace amounts of these types of components need to be present in natural products to be of prime significance due to their extremely low odor threshold values.



Scheme 4. Synthesis of 1-(2-methyl-3-thienylthio)-ethanethiol 18 and 1-(2-methyl-3-furylthio)-ethanethiol 19

ACKNOWLEDGMENT

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Chapter 44

Soy Proteins and Thermal Generation of Alkylpyrazines in Meat Flavor

Richard G. Einig¹ and Milton E. Bailey²

¹Hoechst-Celanese Corporation, 500 Washington Street, Coventry, RI 02816

²Department of Food Science and Nutrition, University of Missouri, Columbia, MO 65211

Concentrations of thermally generated meat flavor components are diminished by protein adsorption when soy extenders are added to fresh meat products before heating. The amounts of individual alkyl pyrazines, thermally generated by heating beef diffusate, decreased linearly as the amount of whole soy, soy 7S or soy 11S proteins were increased in a model system. Similar recoveries were obtained when pyrazines were mixed with soy either as chemical standards or from diffusate. Stoichiometry and energetics of interaction were determined for methyl pyrazine congeners with soy proteins at 120° and 145°C. Results of this study suggest that flavorants can be added in readily determined amounts to compensate for losses due to adsorption in meat-soy products.

In this age of low cholesterol, low fat foods, replacement of a portion of red meat with vegetable protein in heat-and-eat prepared foods is very attractive to both consumer and supplier. However attempts to add a significant portion of soy protein to fresh ground beef have been less than successful based on consumer acceptance. Perceived changes in the traditional psychophysical attributes of hamburger are the major causes of product rejection (1,2).

When texturized soy protein was used to replace some of the meat in patties, undesirable off-flavors were released during preparation and consumption of the meat-soy mix (3). The source of these were identified as oxidation products of unsaturated fatty acids. High protein content soy ingredients have mitigated this problem (4).

Reduction of desirable meat aroma remains as a serious impediment to addition of soy protein. When highly purified soy protein is added to ground patties, thermally generated meat aroma intensity is decreased. Adsorption of flavor compounds onto vegetable protein is a primary mechanism for this aroma loss (5-7).

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In this study the physical parameters involved in interaction of a major class of meat flavorants, methyl pyrazines, with soy proteins were determined at meat roasting temperatures. Beef diffusate, the water soluble, low molecular weight fraction that constitutes about 1% of beef, was shown to contain the necessary precursors to obtain a desirable, thermally generated meat aroma (8). Diffusate was heated under controlled conditions and generated volatiles were transferred to a gas chromatograph for separation and quantitation. Methyl pyrazines, either from heated diffusate or from standard solutions, were measured in the presence of purified soy proteins and the thermodynamics of binding were determined.

Experimental

Diffusate. Beef diffusate was prepared according to the procedure of Ching (9). One kg of top round was ground and blended with an equal weight of deionized water in a Waring Blender. The mixture was centrifuged at 5000 rpm for 25 minutes and the supernatant was filtered through cheesecloth. The filtrate was poured into cellulose dialysis tubing (12000 m.w. cutoff) and dialyzed against 4 L of deionized water for 90 hr at 4°C. Ten g of diffusate was recovered from the 4 L of solution after freeze-drying.

Soy Proteins. Highly purified soy protein and its two major protein fractions, 7S and 11S, were prepared according to a modified procedure of Thanh and Shibasaki (10). One and a half kg of defatted soybean meal (49% protein) was extracted with 32 L of 0.03M Tris buffer at pH 8.0. The solution was centrifuged at 15000 rpm and the supernatant was recovered. The 11S protein was precipitated by slowly adjusting the solution to pH 6.4. This protein precipitate was washed with deionized water until creamy white in color, and then freeze-dried. The 7S protein was precipitated by further adjusting the solution to pH 4.8. After the supernatant was decanted, the precipitate was redissolved in 4.8 L of 0.03M Tris buffer at pH 7.6. Polymerized protein was removed by readjusting the solution to pH 6.2 and centrifuging it at 5000 rpm for 10 min. The solution was again slowly adjusted to pH 4.8 and the 7S protein was collected, washed and freeze-dried. Whole soy protein was prepared according to the procedure for the 7S without the intervening precipitation of 11S protein at pH 6.4.

Microreactor. The sample reaction chamber was a 7" x 1/4" o.d. (17.8 cm x 0.64 cm) Pyrex glass tube with a 26 guage needle attached onto one end via a 1/4" x 1/16" reducing union. After sample was added to this tube, it was clamped into a Perkin-Elmer #245-0902 on-column injector barrel as illustrated in Figure 1. A thermocouple was attached to the side of the barrel with fiberglass tape to monitor the reactor temperature. The inlet gas line was connected to one of the chromatograph flow controllers via a three port valve (Whitey #B-41x32). Nitrogen gas at about 25 mL/min flowed either through the microreactor or joined with the flow from the other flow controller to give a total of 30mL/min through the gas chromatographic column. The reactor was fitted with a heater blanket (Glas-Col Apparatus) which was controlled with a Variac.

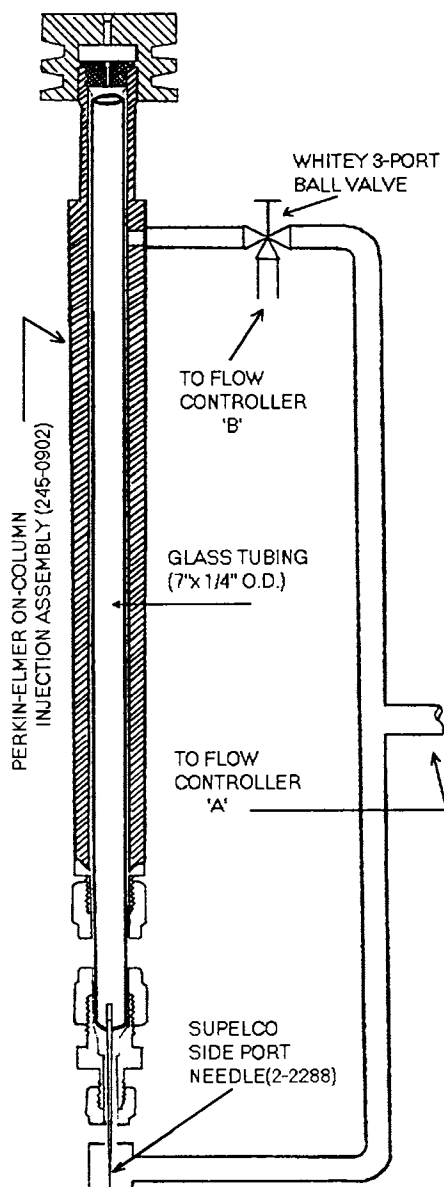


Figure 1. Microreactor for heating Diffusate or Pyrazine Standards in presence of Soy Proteins.

One hundred mg dry diffusate and 0-500 mg soy protein were intimately mixed with 500 mg of 100/140 mesh silanized glass beads and poured into the reactor tube using glass wool plugs on either end. After the tube was installed in the microreactor, the needle was inserted into the gas chromatograph septum. The sample was heated for 13 min at 145°C after which carrier gas was diverted through the microreactor to quantitatively transfer the generated volatiles onto a 3mm x 2mm Pyrex column packed with 5% Carbowax 20M on 100/140 Supelcoport. The column was held at room temperature during the 2 min volatiles purge and then was programmed from 40° to 220°C at 4°C/min. The septum on the microreactor was used in a subsequent experiment to introduce pyrazine standard solutions into the microreactor.

Binding Study. Concentrations of soy and diffusate were equivalent to ground beef fortified with 5-20% hydrated soy protein. The diffusate/soy mixtures were heated under the same conditions and generated pyrazines were separated and quantitated by gas chromatography with an alkali flame ionization detector (afid). Triplicate results were averaged for each level of vegetable protein and the concentration of pyrazines generated in the presence of soy proteins was determined relative to the control, diffusate heated neat.

In a second experiment designed to determine thermodynamic binding constants of pyrazines with the protein types, only a 400 mg level of protein was used. This was equivalent to a 16% soy substituted ground beef. The protein was preheated in the microreactor prior to injecting 2 microliters of a standard solution of methyl substituted pyrazines in heptane. Six concentrations from 3mM to 0.03mM were tested at 120° and 145°C. The standard solutions were heated under the same conditions as was the diffusate. Replicate values were averaged and binding parameters were determined from Klotz plots of the results.

Results and Discussion

In this model system of soy protein with meat aroma precursors, there was a significant reduction in alkyl substituted pyrazine content with an increase in soy protein content. The percentage loss based on the control for thermally generated pyrazines in the presence of 100 and 500 mg soy protein is shown in Table I.

There was no loss in flavorant at a 100 mg level when either whole soy or soy 7S protein was used, but there was a 14-24 percentage loss when 11S protein was used. Only soy 11S protein affected the substituted pyrazine content of the mixture at all addition levels. The higher the substituted pyrazine congener, the smaller was the percentage loss with any of the protein types. At the 500 mg level, only about 50% of the amount of any of the methyl pyrazines in the control was recovered from either soy 7S or 11S protein while 70% was recovered from whole soy protein. These results greatly extend the initial work reported by Palkert and Fagerson (11) who determined that about 75% of dimethyl thiazole, a sulfur-nitrogen heterocycle, was recovered from dry, textured soy protein.

Table I. Alkyl Pyrazines Thermally Generated
in Matrix with Soy Proteins
(Percentage of Control)

Species	Control	Soy Proteins (mg)					
		7S		11S		Whole	
		100	500	100	500	100	500
Methyl Pyrazine	100	100	52	76	51	100	66
Dimethyl Pyrazine	100	100	54	86	50	100	67
Trimethyl Pyrazine	100	100	54	81	51	100	73
Tetramethyl Pyrazine	100	100	67	84	71	100	74

When standard solutions of methyl pyrazines were used in binding studies with the soy proteins instead of thermally generated pyrazines, the same relationships were observed. Table II is a listing of percentage of the control concentration of the methyl pyrazine congeners from diffusate and standard solutions in the presence of 400 mg soy 11S protein.

Since recoveries were similar for pyrazines from either source, these data suggest that the proteins did not interfere with the production of the individual compounds from their precursors, but rather proteins adsorbed the compounds after they were formed.

The stoichiometry and energetics of alkyl pyrazines binding to soy proteins were determined from the free, [L], and bound, u, ligand concentration and the amount of protein (12). A double reciprocal plot of $1/u$ vs $1/[L]$, a Klotz plot, yielded a straight line with the equation:

$$1/u = 1/nK[L] + 1/n$$

Table II. Alkyl Pyrazines Recovered from Matrix
Containing 400 mg Soy 11S Protein
(Percentage of Control)

Species	From Standard	From Diffusate
Methyl Pyrazine	42	54
Dimethyl Pyrazine	50	56
Trimethyl Pyrazine	52	56
Tetramethyl Pyrazine	60	67

The number of binding sites on the protein, n , and the binding constant, K , were determined from the Y-intercept and the slope. Thermodynamic parameters of free energy of interaction, enthalpy and entropy were found from K .

Measurable loss of pyrazine concentration in the presence of soy proteins was a function of the type of protein. The number of binding sites and intrinsic binding constants on 7S, 11S, and whole soy proteins for the series of pyrazines are given in Table III.

Table III. Stoichiometry and Energetics of Alkyl Pyrazines with Soy Proteins

Species	Soy Proteins					
	7S		11S		Whole	
	n	K	n	K	n	K
Methyl Pyrazine	3	700	3	1000	1	1700
Dimethyl Pyrazine	1	1900	1	1650	1	1500
Trimethyl Pyrazine	1	2900	1	1900	1	2000
Tetramethyl Pyrazine	1	3900	1	1950	1	2400

Most of the pyrazines had only one binding site available. Methyl pyrazine was the exception because, while only one binding site was available on whole soy, the 7S and 11S fractions had multiple sites. Since binding sites for 7S and 11S are on the interior of cylindrical shaped proteins, as the ligand size increased, it became increasingly difficult to fit inside the protein (6).

However binding characteristics of the soy 7S and soy 11S proteins were distinctly different. Binding energies increased for the methyl and dimethyl pyrazines on soy 11S, but were constant for tri- and tetramethyl pyrazines. In contrast, binding energies increased linearly for all the pyrazines with soy 7S protein. An increase of one methyl group on the pyrazine molecule increased the binding constant by an average of 925/M. This difference in binding between the two proteins can be explained by considering steric hinderance around the binding sites for the larger ligands which limits the goodness of fit on the protein. Damodaran and Kinsella (6) proposed that the 7S protein has hydrophobic regions which are accessible for ligand binding compared to the 11S protein in which such hydrophobic regions are buried inside the protein and are not accessible for interaction with the ligand.

Free energies of interaction of the alkyl pyrazines with soy proteins are listed in Table IV.

Table IV. Free Energy of Interaction of Alkyl Pyrazines with Soy Proteins

Species	Soy Proteins		
	<u>7S</u> kcal/M	<u>11S</u> kcal/M	<u>Whole</u> kcal/M
Methyl Pyrazine	-5.4	-5.7	-6.2
Dimethyl Pyrazine	-6.5	-6.2	-6.1
Trimethyl Pyrazine	-6.6	-6.3	-6.3
Tetramethyl Pyrazine	-6.8	-6.3	-6.4

The large negative values for free energy change are evidence that the binding reactions are spontaneous. Since no significant differences were found in the values of K at 120° or 145°C, a plot of $\ln K$ vs $1/T$ had zero slope. Therefore the change in enthalpy was negligible, and the driving force for the binding reaction was primarily the result of an increase in the entropy. This is consistent with both hydrophobic and electrostatic binding. This same effect was described by Damodaran and Kinsella for the binding of 2-nonanone to bovine serum albumin (13) and soy isolate (14).

Proposed explanations of binding data depend on the quaternary structure of the proteins at the time the binding reaction occurs. Differential scanning calorimetry values were obtained on proteins recovered from the microreactor after heating at 120°C and 145°C. The temperature at which thermal transitions occurred were identical to those obtained on proteins which had not been previously heated. Areas under the endotherms were similar confirming that the proteins remained in the native conformation. Transition temperatures in the whole soy protein were 75°C and 93°C compared to those found in the pure soy 7S, 70°C, and soy 11S proteins, 91°C, suggesting chain-chain interaction between these proteins in the whole soy that may have modified the binding affinity of accessible sites. The elevated temperatures and high protein concentrations used in this study promote such interactions (15).

Conclusion

The problem of developing desirable meat flavor in the presence of vegetable protein has been clearly demonstrated in the literature. Physical measurements after heating a meat model system with soy proteins have shown a dramatic reduction in the concentration of alkyl pyrazine compounds due to interaction with the soy proteins. These interactions have been defined in terms of stoichiometry and binding energies from measurements on pure standards of the methyl pyrazines.

Results of these studies demonstrate that flavorants can be added to the protein in readily determined amounts to compensate

for loss of flavor by adsorption. Obviously whole soy protein is the protein of choice because it has the lowest adsorptive capacity at meat roasting temperature. Other psychophysical characteristics of soy supplemented meat products such as mouthfeel and texture are being addressed with extrusion technology.

Acknowledgements

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Chapter 45

Aroma Development in Chinese Fried Pork Bundle

Tzou-Chi Huang, Sen-Far Chang, Chi-Shen Lin, Daniel Y.-C. Shih¹, and Chi-Tang Ho¹

Department of Food Science, National Pingtung Institute of Agriculture, Pingtung, Taiwan, Republic of China

Fried pork bundle is one of the most popular Chinese breakfast foods. Characterized by its sensational flavor, color and texture, fried pork bundle is prepared by (1) boiling prerigor meat from animals not electrically shocked; (2) predrying the cooked and seasoned meat by conductive heat with scraping on the heating surface and (3) finish drying the disintegrated and partially-dried muscle bundle with added lard and wheat flour. In this study, a headspace GC-MS technique was used to analyze the volatile compounds. Pyrazines were found to contribute to the unique flavor of the fried pork bundle. Free reducing sugars and amino acids from pork muscle may be precursors in browning reactions that generate pork bundle aroma.

Fried and disintegrated pork bundle (Zou-Shung) is characterized by its special shape, flavor, color and mouthfeel. Low temperature cooking in the initial stage of production removes moisture, and high temperature in the final stage forms its characteristic flavor. Undesirable flavor frequently occurs if the product is heated too long. Optimum heating is judged currently by the appearance of a brown color. Fried pork bundle usually suffers poor uniformity in color and flavor. This report describes the flavor and color development during the processing of Chinese pork bundle.

Manufacturing Process

Traditionally pork bundle is homemade. In this study, muscle of Rectus femoris was sliced into 20 x 10 x 10 cm³ cubes and boiled in water (100°C) for 1.5 hours to denature the muscle protein. When

¹Current address: Department of Food Science, New Jersey Agricultural Experiment Station, Cook College, Rutgers, The State University of New Jersey, P.O. Box 231, New Brunswick, NJ 08903

most of the water was evaporated, sucrose and salt were added to yield seasoned pork bundle. A stainless steel (diameter 1 m) frying pan, with several scraping knives on the bottom, was used to fry the pork bundles. The scraping knife disintegrated the muscle cubes into bundles with a diameter of 3-10 mm.

The average temperature of the pork in the frying pan was about 80°C. A period of 70-80 minutes was needed to remove nearly 75% water, and the average final water activity was 0.70. The partially dried and disintegrated pork bundle was further fried in the same frying pan to form the desired color, flavor and mouthfeel. In the final frying stage, wheat flour was added to absorb lard and coat the surface.

Sensory Evaluation

Finished fried pork bundles were poured out of the pan when a brown color developed. An eight-member panel was trained in four sections to become familiar with the products, to evaluate the flavor and odor of pork bundles. Panelists scored the aroma attributes by tasting (flavor) or sniffing (odor), marking a 15 cm, 60-point unstructured intensity scale (ex. 0 = no flavor, and 60 = a strong flavor). The scores were collected and analyzed by the Response Surface Method used in the SAS Institute (Cary, North Carolina) to obtain a regression model for plotting a response surface graph.

As shown in Figure 1, a brown color started to develop after 90 minutes of frying, and flavor formed right after the appearance of the brown color.

Model systems composed of the seasoned pork bundle, sucrose and lard were used to investigate the effect of the frying temperature and lard content on flavor and color development in Chinese fried pork bundle. Seasoned pork bundles were prepared as described previously, except superheated steam was used to provide the various heating temperatures following the designs of Box and Behnken (1).

As shown in Figure 2, the raw meaty odor of pork bundles decreased as the heating temperature increased, while increases in lard content had no significant effect. A possible explanation is that the raw meaty odor was covered by the flavor formed during heating.

Frying temperature was found to be the criterial parameter that determined the flavor quality in Chinese pork bundle. Cooked meat aroma increased as the heating temperature varied from 134°C to 172°C., as shown in Figure 3. Below 130°C neither cooked meat aroma nor brown color developed. Slightly higher temperatures have been reported for the optimum flavor formation in fried potato chips at 180°C (2), and roasted beans at 200°C (3).

Amino acid analysis confirmed that Maillard browning reactions occurred during the frying process, since basic amino acids such as lysine and arginine decreased significantly, whereas all others decreased only slightly (Table I).

Pyrazine Formation

Alkylpyrazines have been identified in virtually all roasted foods. The most common descriptions used for alkylpyrazine aroma include

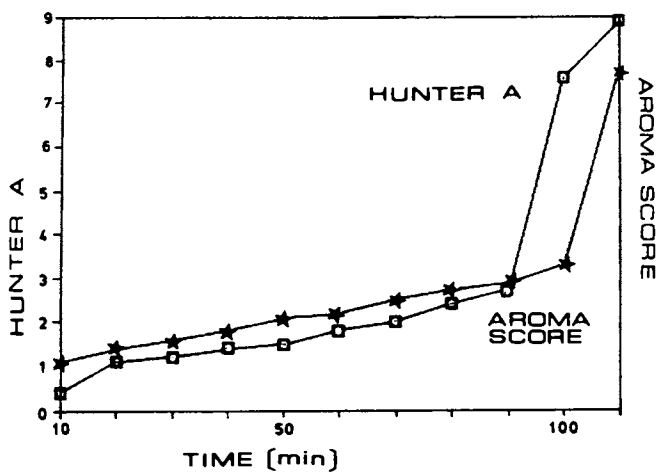


Figure 1. Development of redness and aroma during the frying of pork bundle.

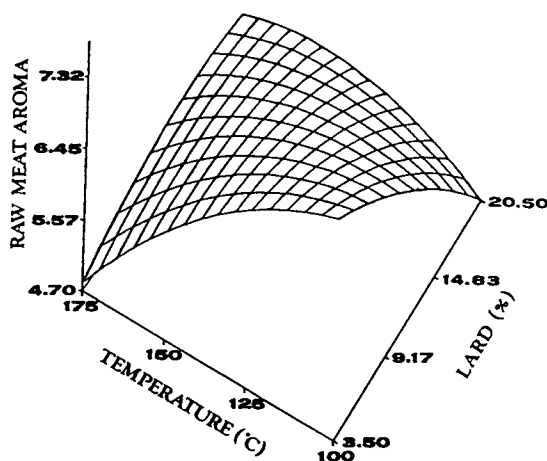


Figure 2. The effect of temperature and lard content on the raw meat aroma of Chinese fried pork bundle.

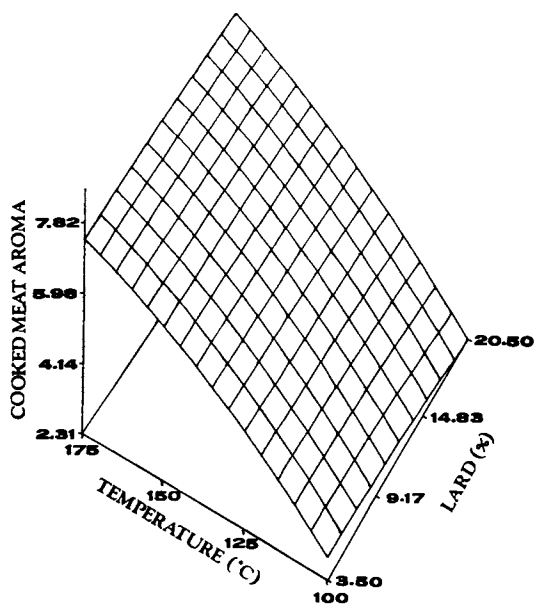


Figure 3. The effect of temperature and lard content on the cooked meat aroma of Chinese fried pork bundle.

Table I. Amino Acid Compositions in Chinese Fried Pork Bundles

Amino Acid	Pork Bundle	
	Seasoned	Fried
Asp	5.3	4.8
Thr	2.7	2.2
Ser	3.2	2.7
Glu	9.5	8.9
Pro	2.6	2.1
Gly	2.7	2.4
Ala	4.1	3.3
Cys	0.6	0.3
Val	3.6	3.1
Met	1.3	0.8
Ile	2.2	1.6
Leu	3.7	3.1
Tyr	0.4	0.2
Phe	2.2	1.9
Lys	6.4	3.9
His	1.3	0.8
Arg	5.4	3.8

nutty, roasted and corny. In the present study, a selective purge-and-trap method was developed to investigate pyrazine formation in the pork bundle samples. Powdered samples were soaked with water in a two-arm round bottom flask. Volatile compounds were purged with nitrogen and trapped with 1 M HCl solution in an Erlenmeyer flask. After titration to pH 13, the basic fraction was purged with nitrogen and trapped on a Tenax TA glass tube; analysis was performed on a Varian 3400 gas chromatograph and a Finnigan MAT 8230 high resolution mass spectrometer.

The basic fraction of the volatiles identified in the fried pork bundle contained 16 alkylpyrazines. Among them, methylpyrazine (nutty, roasted), 2,5-dimethylpyrazine (grilled chicken, roasted peanut), 2,6-dimethylpyrazine (ether-like), 2,3,5-trimethylpyrazine (nutty, roasted) and 2-ethyl-6-methylpyrazine (grassy) were predominant. The combination of these alkylpyrazines may cause the characteristic cooked meat aroma of Chinese fried pork bundle. Quantitative analyses showed that alkylpyrazine formed during the final frying stages, as shown in Table II.

Table II. Alkylpyrazines in Seasoned and Fried Pork Bundle

Alkylpyrazines	Pork Bundle (ppm)	
	Seasoned	Fried
pyrazine	0.2	0.3
methylpyrazine	3.6	7.3
2,5-dimethylpyrazine	12.5	16.4
2,6-dimethylpyrazine	4.2	6.7
2,3-dimethylpyrazine	2.1	4.1
vinylpyrazine	0.6	1.2
5-methyl-2-ethylpyrazine	0.2	0.8
6-methyl-2-ethylpyrazine	3.8	5.3
3-methyl-2-ethylpyrazine	6.4	8.1
propylpyrazine	1	2.5
2-methyl-vinylpyrazine	trace	0.5
3,6-dimethyl-2-ethylpyrazine	9.3	12.5
3,5-dimethyl-2-ethylpyrazine	1.1	2.9
tetramethylpyrazine	0.8	1.6
6-methyl-2,6-diethylpyrazine	0.6	1.2
3-methyl-2,6-diethylpyrazine	0.1	0.5

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Chapter 46

Protein-Generated Extrusion Flavors

Joseph A. Maga and Chin Hong Kim

Department of Food Science and Human Nutrition, Colorado State University, Fort Collins, CO 80523

Varying amounts of defatted soy flour (DSF), soy protein concentrate (SPC) sodium caseinate (SC), whey protein concentrate (WPC), and gluten (G) (0-50%) were added to corn starch and adjusted to either 15 or 25% moisture. The blends were extruded at either 120 or 150°C dough temperature in a Brabender laboratory extruder. Volatiles were recovered from resulting extrudates and analyzed by gas chromatography. Sensory evaluations of blandness were compared with the instrumental results. A greater number of volatiles and/or higher relative concentrations were observed with increasing protein levels. DSF produced the greatest number of detectable peaks while SPC had the least. Low temperature and high moisture extrusion conditions resulted in the most peaks, while high temperature and high moisture yielded the least. Sensory blandness scores magnified with increasing temperature and decreasing moisture.

The extruder is a continuous high-temperature short-time reactor. Ingredients, moisture, temperature, pressure, and shear can interactively produce many Maillard-type flavor compounds. As the extrudate exits the extruder, many of the volatile reaction products may be lost with steam since the extrudate passes from a zone of relatively high pressure within the extruder to atmospheric pressure. By controlling formulation variables, the extruder can serve as a useful tool to thermally produce volatile and nonvolatile compounds which make significant contributions to overall flavor.

Historically, most extrusion processors have relied on post-extrusion flavor application as a means of characteristically

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flavoring their products. This is not ideal in most situations due to cost, flavor usage levels required, and additional steps in processing.

During extrusion, general browning typified by caramelization, Maillard, and oxidative decomposition reactions are paramount in flavor compound formation. Temperature and shear conditions occurring during extrusion can provide the chemical and physical means whereby complex starch and protein can be partially degraded to provide reactants that can then participate in browning.

Browning reactions usually produce product darkening. However, from a flavor generation standpoint, browning is usually a highly desirable reaction during extrusion. Therefore, formulations and operations in most cases should be optimized to take advantage of browning.

In the case of caramelization, a simple sugar such as glucose, can produce a wide variety of flavorful heterocyclic compounds. Glucose can be added or formed during extrusion via starch degradation, enolization, dehydration, and cyclization. It is interesting to note that fructose is generally considered to be more thermally reactive than glucose. However, it has been reported that glucose is more active than fructose during extrusion (1).

By thermal and shear forces during extrusion which cause protein rearrangement/degradation, the Maillard reaction progresses, and a wide variety of potent flavoring compounds can result. Reaction rates in turn are influenced by the types of sugars and amino acids present, temperature, water activity, duration of heating, and pH.

Published studies on extrusion formation/retention of flavor are rather limited (2-8). None have reported the effects of protein sources and concentrations on flavor compound formation in extrudates, the major objective of this study.

Materials and Methods

Ingredients. All ingredients were obtained commercially along with compositional information and consisted of corn starch (National Starch, Bridgewater, NJ), whey protein concentrate (WPC) and sodium caseinate (SC) (Leprino Foods, Denver, CO), defatted soy flour (DSF) (Archer Daniels Midland, Decatur, IL), soy protein concentrate (SPC) (Central Soya Company, Fort Wayne, IN), and gluten (G) (Ogilvie Mills Ltd., Montreal, Canada).

Pre-Extrusion Blending. Based on the moisture contents of the corn starch and various protein sources, blends of 0,5,15,30, and 50% (dry weight) of each protein source were made by first dry mixing for 10 minutes in a Paterson-Kelly Model LB-P-8 twin shell blender followed by the appropriate amount of 20°C tap water to obtain a total moisture content of either 15 or 25% (wet basis). The moistened mixtures were then blended for an additional 20 minutes followed by overnight equilibration at room temperature in air-tight bags.

Extrusion. A Brabender Plasticorder Extruder Model PL-V500 with a 19.05 mm barrel diameter, a 20:1 barrel length to diameter ratio, eight 0.29 x 3.18 mm longitudinal grooves and a die plate equipped with a 4.75 mm diameter by 1.27 cm length die was used. The unit was equipped with a variable speed drive which was set at 120 rpm for all runs. The barrel was equipped with two electrically heated, compressed air cooled collars controlled by thermostats. Nonisothermal temperature conditions were used with increasing temperature toward the die. A thermocouple placed in the dough stream just before the dough exited the die as extrudate was used to measure dough temperatures of 120 and 150°C. A 3/1 compression screw ratio was used for all runs. All formulation and extruder variables/conditions are summarized in Table 1.

Table I. Formulation and Extruder Variables Evaluated

<u>Variable</u>	<u>Level</u>
Protein type WPC, SC, DSF, SPC, G	
Protein level	0, 5, 15, 30, 50%
Feed moisture	15, 25%
Dough temperature	120, 150°C
Screw configuration-speed	Constant (3:1, 120 rpm)
Die size	Constant (4.75 mm)

Samples from each variable were collected and permitted to air dry overnight and then milled to pass through a 1 mm screen.

Extraction and Concentration. Five 5-gram units of each ground extrudate were placed into five micro-Kjeldahl flasks. Fifty ml of 80°C deionized water were added to each flask, the flask connected to the distillation apparatus and 40 ml of distillate collected in a screwcap test tube. Eight ml of freshly redistilled diethyl ether were added to each 40 ml of distillate and shaken vigorously for 30 seconds. The ether layers from the five distillates were combined and cooled in dry ice. Therefore, a total of 25g of each extrudate was extracted. The ether extracts were then concentrated down to approximately 3 ml and transferred to a 5 ml graduated micro vial. The sample was further concentrated to 0.3 ml in a dry ice bath using nitrogen and stored on dry ice until analyzed.

Gas Chromatographic Analysis. A Hewlett Packard Model 5830A gas chromatograph equipped with a Model 18850A data terminal was used. The column was 1/8" by 10' stainless steel packed with 10% Carbowax 20 M on 100/120 mesh Gaschrom P. An initial oven temperature of 100°C was held for 3 minutes after injection and then increased at a rate of 2°C/minute to 207°C and held for another 65 minutes. Injection and detector temperatures were 225°C and nitrogen at a flow rate of 26 cc per minute. Typical separations are shown in Figure 1.

Sensory Evaluation: Two expert sensory evaluation members within the Department were asked to rate the blandness of each extrudate

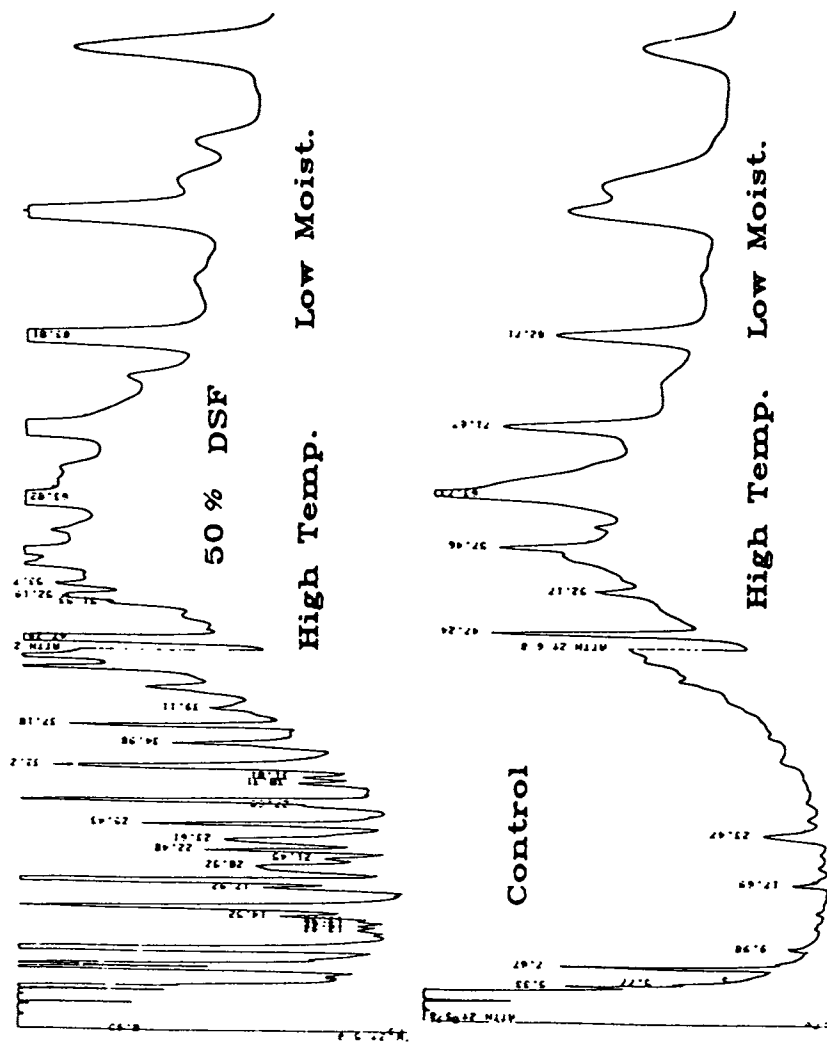


Figure 1. Comparison of gas chromatographic volatile separations for 50% DSF against a control protein source, HTLM conditions.

using a 10-point scale with 1 being bland and 10 strong. Samples were provided for them to taste along with rinse water.

Results and Discussion:

Sensory Blandness. The sensory blandness for all treatments is summarized in Table II.

Table II. Sensory Blandness Scores

<u>% Protein</u>	<u>Conditions</u>	<u>DSF</u>	<u>SPC</u>	<u>WPC</u>	<u>SC</u>	<u>G</u>	<u>Control</u>
5	LTLM	1	2	4	2	2	1
15		3	4	5	6	3	
30		3	6	6	6	3	
50		4	7	7	8	4	
5	LTHM	1	2	2	3	2	1
15		2	3	3	4	2	
30		3	3	4	6	3	
50		5	5	6	7	4	
5	HTLM	4	5	3	4	5	3
15		5	7	4	6	6	
30		6	7	6	8	6	
50		8	9	9	9	8	
5	HTHM	3	3	3	4	3	2
15		4	3	4	5	5	
30		6	6	7	7	6	
50		8	7	8	8	7	

LTLM: Low temperature low moisture; LTHM: Low temperature high moisture; HTLM: High temperature low moisture; HTHM: High temperature high moisture. 1 = Bland; 10 = Strong

As can be seen, as protein concentration increased, blandness decreased. Overall it was concluded that DSF and G were the blandest protein sources evaluated while SC was the least bland. Perhaps the differences in protein contents within the same series (DSF and SPC; WPC and SC) produced these observed differences. It was also apparent that the two high temperature sets of samples were not as bland as the low temperature series. Within each set, high moisture produced blander tasting products than low moisture. More flavorful compounds were produced with high temperature, apparently due to thermal decomposition reactions. Perhaps, high moisture produced starch/protein structures do not permit the rapid and/or complete release of flavor compounds in the mouth.

GC Volatile Comparison. It can be seen from Table III that certain volatiles were only observed in specific protein sources. For example, Peak 2 was only present in milk protein sources (WPC and SC) while Peak 3 was evident in soy protein sources (DSF and

SPC) and the control starch extrudates. Peaks 31, 33 and 35 appeared to be unique to G. On the other hand, some peaks (23, 38, 42-46) were found in all products including the starch control indicating that they were starch derived. It can also be seen that DSF had the most observed peaks while SPC had the least. Perhaps nonprotein components associated with DSF also thermally reacted to form detectable components.

When volatile data are compared to sensory blandness scores as discussed above, it becomes apparent that the measurements do not totally agree. DSF was subjectively evaluated as being rather bland in taste while SPC was stronger. In contrast the objective gas chromatographic data would indicate otherwise. In all probability, certain volatile compounds in SPC at higher levels than DSF (Peaks 7, 18, 20, 23, 24) are responsible for these differences.

With the two milk protein sources (WPC and SC), one would have predicted from a thermal standpoint that WPC should have provided more volatiles than SC because it is a source of highly reactive carbohydrates. From Table III, it can be seen that this was not the case. However, only residual volatiles were evaluated and it is plausible that SC had retained more volatiles than WPC due to its higher protein content.

When extrusion conditions are considered (Table IV), the most severe conditions (HTLM) resulted in the loss or decreased level of certain peaks; however, the remaining components were at relatively high levels. The least severe conditions (LTLM) retained the highest number of volatiles.

Also, certain peaks were associated with high moisture conditions but were not apparent in low moisture conditions, while other peaks were only observed at low temperature conditions. Relative peak concentration for some volatile components increased during severe conditions indicating their thermal formation and retention.

From Table V, which represents increasing levels of SPC, it can be seen that some components were at the same relative concentration throughout, while others magnified. Perhaps variations in water activity and/or shear conditions within the extruder can partially explain some of these differences.

Conclusions

This initial study clearly demonstrated that protein type and amount as well as extrusion conditions can result in a vast array of both sensory and volatile compound differences. Further research, including compound identification, is required to more fully understand the complex interactions observed.

Table III. Gas Chromatographic Volatile Comparison, Extrusion Conditions: HTLM

Peak No.	Protein Sources (50%)					
	DSF	SPC	WPC	SC	G	Control
1	+++	-	-	-	+++	-
2	-	-	+++	+++	-	-
3	+++	+++	-	-	-	++
4	+++	-	++	+++	+++	+
5	-	-	+	++	-	-
6	-	-	-	+	-	-
7	-	+++	-	-	-	++
8	+++	+++	-	-	++	+
9	++	-	++	++	++	-
10	-	-	-	+	-	-
11	+++	++	++	+++	++	-
12	+	-	-	-	+	-
13	+	+	-	++	+	-
14	+	-	-	-	+	-
15	++	++	-	-	++	-
16	-	-	-	+	-	-
17	+++	-	+	++	-	+
18	++	+++	+	++	++	-
19	+++	-	++	++	++	-
20	++	+++	-	+	++	-
21	++	++	-	-	-	-
22	++	-	+	++	-	-
23	++	+++	+	++	++	++
24	++	+++	+	++	++	-
25	++	++	-	-	++	-
26	+++	-	++	++	-	-
27	++	-	-	+	-	-
28	++	-	-	+	-	-
29	+++	++	++	++	++	-
30	++	-	++	++	-	-
31	-	-	-	-	+	-
32	++	-	++	++	-	-
33	-	-	-	-	++	-
34	+	-	-	-	-	-
35	-	-	-	-	+++	-
36	++	-	-	+	-	-
37	++	-	+	+	-	-
38	+++	+++	+++	+++	+++	+++
39	+	-	+	-	-	-
40	+	-	-	-	-	+
41	+	-	-	-	-	-
42	+++	+++	++	+	+	++
43	+++	+++	+++	+++	++	+++
44	+++	+++	+++	++	++	++
45	+++	++	++	++	++	++
46	+++	++	++	++	++	++

+ <10,000 Integrator Peak Area
 ++ 10,000 - 100,000
 +++ >100,000

Table IV. Gas Chromatographic Volatile Comparison

SPC (50%)

<u>Peak No.</u>	Extrusion Conditions:			
	<u>HTLM</u>	<u>HTHM</u>	<u>LTLM</u>	<u>LTHM</u>
1	-	+++	-	+++
2	+++	++	++	++
3	-	-	-	+
4	+++	-	++	++
5	+++	+	+	+
6	-	+	-	-
7	++	+	+++	+++
8	-	-	-	+
9	+	+	-	+
10	++	+	+	++
11	-	-	+	++
12	+++	-	++	++
13	-	-	++	+++
14	-	-	-	++
15	+++	-	-	-
16	++	-	+	+
17	-	-	+	++
18	+++	++	++	++
19	+++	++	++	++
20	++	-	+	+
21	-	-	++	++
22	-	-	-	++
23	-	-	-	+
24	++	-	+	+
25	-	-	-	++
26	-	-	+	++
27	-	-	-	++
28	-	++	+	++
29	-	-	+	++
30	-	-	-	++
31	++	++	+++	+++
32	-	-	+	++
33	-	-	++	+++
34	+	+	++	+++
35	++	+++	++	+++
36	++	++	++	+++
37	++	++	++	+++

Table V. Gas Chromatographic Volatile Comparison,
Extrusion Conditions: HTLM

Peak No.	SPC level (%)				
	<u>0</u>	<u>5</u>	<u>15</u>	<u>30</u>	<u>50</u>
1	-	+++	+++	+++	-
2	++	-	-	+++	+++
3	+	-	-	++	-
4	-	++	++	-	+++
5	++	-	-	-	+++
6	-	-	-	++	++
7	-	++	++	++	-
8	-	+	++	-	-
9	++	++	+	++	++
10	-	-	-	+	-
11	-	+	+	+	++
12	-	+	++	++	++
13	+	++	++	++	+++
14	-	++	-	++	-
15	-	++	++	++	+++
16	-	+	+	++	++
17	++	+	+++	+	+++
18	-	++	++	++	+++
19	-	-	-	+	++
20	-	++	++	++	++
21	-	-	-	+	-
22	-	++	++	++	-
23	+++	+++	+++	+++	+++
24	-	+	+	+	+
25	-	+	-	-	-
26	++	++	++	++	++
27	++	++	+++	+++	+++
28	+++	+++	+++	+++	+++
29	++	++	++	+	+

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Chapter 47

Formation of Volatile Compounds from Extruded Corn-Based Model Systems

Chi-Tang Ho, Linda J. Bruechert, May-Chien Kuo, and Mark T. Izzo

Department of Food Science, New Jersey Agricultural Experiment Station,
Cook College, Rutgers, The State University of New Jersey,
New Brunswick, NJ 08903

Volatile compounds generated by model systems of zein, corn amylopectin and corn oil extruded at barrel temperatures of 120°C and 165°C were analyzed by GC and GC/MS. The largest quantities of lipid oxidation products were detected in systems containing all three components. In each system, the quantity of 2,4-decadienal was low relative to the quantities of hexanal, heptanal and benzaldehyde. Identification of the Maillard reaction products, 2-methyl-3(or 6)-pentylpyrazine, 2-methyl-3(or 6)-hexylpyrazine and 2,5-dimethyl-3-pentylpyrazine, suggested that lipid-derived aldehydes might be involved in the formation of substituted pyrazines. 4-Methylthiazole was identified as a major decomposition product of thiamin when corn meal containing 0.5% thiamin was extruded at a final temperature of 180°C.

Lipid oxidation and the Maillard reaction are significant sources of flavors and off-flavors in processed foods. Thermal processing such as extrusion cooking of foods accelerates lipid oxidation and increases the potential for interactions between lipids, proteins and carbohydrates, and their breakdown products. When foods are extruded, variables such as shear force and pressure, in addition to the expected time and temperature thermal processing variables, also influence these reactions. The flavor of extruded foods is further complicated by the loss of volatiles at the end of the extruder. Although the flavor of many extruded grain products, especially snack products, is highly dependent on the flavor contributed by the base grain (1), only a few papers have been published in this area. Fagerson (2) has reported some qualitative effects of extrusion on the retention of volatiles in textured vegetable protein, and Chen et al. (3) have studied the loss of volatiles during extrusion of a corn-based product.

As a part of a larger cooperative effort to increase the current understanding of extrusion processing, the volatile compounds produced by extruded and baked zein samples were compared. Zein is a

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major storage protein of corn and constitutes at least 50% of the total endosperm protein. It is the most hydrophobic protein known among the prolamines. The volatile compounds generated from the degradation of thiamine during extrusion cooking were also studied in a model system.

Materials and Methods

Extruded Model System. For this investigation, 100 parts zein, 40 parts corn amylopectin and 5 parts corn oil were blended thoroughly, and water was added at 30% of the total dry weight. Volatiles collected from extruded samples of this model system were compared to volatiles from the same system heated in an oven for 30 minutes at 120°C or 180°C and to volatiles from extruded samples of zein with 30% added water. The extruded samples were prepared on a C. W. Brabender single-screw extruder, type 2003, with a barrel diameter of 1.9 cm and an L/D ratio of 20:1. The first heating zone of the barrel was held at 60°C, and the second was set at either 120°C or 165°C. The die diameter was 6.5 cm.

One hundred grams of each sample were ground to a powder and extracted with a total volume of 1000 mL redistilled ethyl ether in two aliquots. Oil, carotenoids and other nonvolatile ether-extractable materials were removed from the extract by a modified Nickerson-Likens procedure (4). The resulting distillate was dried with anhydrous sodium sulfate and concentrated with a spinning band still to a final volume of 100 μ L. The concentrated extracts were stored in a freezer at -40°C to reduce further reactions or decompositions.

A Varian 3400 gas chromatograph equipped with a flame ionization detector and a nonpolar fused silica capillary column (60 m x 0.25 mm i.d.; 0.25 μ m thickness, SPB-1, Supelco, Inc.) was used to analyze the volatile compounds from the model systems. The injector temperature was 250°C, and the detector temperature was 260°C. The flow rate of the helium carrier gas was 1 mL/min and the split ratio was 50:1. The temperature program consisted of a 10 min isothermal period at 35°C, temperature increases of 2°C/min from 35°C to 120°C and of 4°C/min from 120°C to 235°C, and a 40 min. isothermal period at 235°C. The chromatograms were plotted and integrated on a Varian 4270 integrator. Linear retention indices for the volatile compounds were calculated using n-paraffin standards (C6-C25; Alltech Associates) as references according to the method of Majlat and co-workers (5).

The concentrated samples were also analyzed by GC/MS using a Varian 3400 gas chromatograph coupled to a Finnigan MAT 8230 high resolution mass spectrometer. Spectra were recorded on a Finnigan MAT SS 300 Data System. GC conditions were the same as described above.

Extruded Corn Meal with Thiamin Added. Degerminated yellow corn meal with 0.5% thiamin HCl was extruded on a Werner Pfleiderer ZSK-30 co-rotating twin-screw extruder. The five heating zones of the barrel were held at 50°C, 76°C, 100°C, 134°C and 172°C, respectively. The product temperature was 180°C, the torque was 46% at 200 RPM and the pressure was 130-172 psi. The extrudate was allowed to cool to room temperature, then stored in glass vessels under nitrogen at 4°C.

For purge-and-trap analysis, a portion of the extrudate was ground and 15 g were placed in a two-necked sample flask with 30 g of NaCl and 100 ml of distilled water. Headspace components were collected on activated Tenax TA by purging nitrogen gas through the sample suspension at a flow rate of 400 mL/min for 2 hours.

The collected volatiles were desorbed from the Tenax TA at 260°C using a modified GC packed-column injector. A 25 G, 2.5 inch needle at one end of the injector was inserted through the GC septum, and the Tenax sample tube was placed into the injector through a screw cap at the other end. The desorbed volatiles were carried through the injector by a flow of helium and were trapped as a sharp band at the beginning of the GC column by maintaining the GC oven temperature at -40°C with dry ice for the 15 min desorption period. The helium carrier gas supplied by the GC was turned off as long as the needle of the desorption unit was inserted into the GC.

A Varian 3400 gas chromatograph equipped with a flame ionization detector and a fused silica capillary column (60 m X 0.32 mm I.D., $d_f = 1.0 \mu\text{m}$, DB-1, J&W Scientific, Folsom, CA) was used to analyze the trapped volatiles. The injector temperature was 270°C and the detector temperature was 300°C. The flow rate of the helium carrier gas was 1 mL/min, and the injection was splitless. The oven temperature increased from -40°C to 40°C at 10°C/min and from 40°C to 260°C at 2°C/min.

Volatiles isolated by the purge-and-trap method were analyzed by GC-MS using a Varian 3400 gas chromatograph coupled to a Finnigan MAT 8230 high resolution mass spectrometer equipped with an open split interface. Mass spectra were obtained by electron ionization at 70 eV and an ion source temperature of 250°C. The filament emission current was 1 milliampere and spectra were recorded on a Finnigan MAT SS 300 Data system.

Results and Discussion

Comparison of Volatile Compounds in Extruded and Baked Model Systems.

Volatiles from the extruded zine and zein/corn amylopectin/corn oil and the baked zein/corn amylopectin/corn oil samples were separated on a nonpolar SPB-1 capillary column. The quantities of the volatiles from the extruded zein and the extruded and the baked zein/corn amylopectin/corn oil samples are listed in Table I. Larger quantities of volatiles were detected in the baked samples than in the extruded samples, and more volatiles were generated during extrusion at 165°C than 120°C. These data suggest that the actual amount of volatiles generated in the samples extruded at 165°C is much larger than the amount detected. It is expected that reactions to produce flavors during extrusion will increase at higher temperatures, but also that products extruded at higher temperatures will experience more extreme drops of temperature and pressure as they emerge from the die. The rapid expansion and rapid moisture loss that result contribute to an increased loss of volatile compounds. In addition, flavors that are formed in or added to the extruded product should be more susceptible to thermal degradation and sec-

Table I. Quantitation of Volatile Compounds in Extruded and Baked Zein/Corn Amylopectin/Corn Samples

Compounds	Quantitation (ppb)					
	Extruded				Baked	
	Z		Z+O+A		Z+O+A	
	120 (°C)	165 (°C)	120 (°C)	165 (°C)	120 (°C)	180 (°C)
<u>From Lipids</u>						
Hexanal	t*	t	11	42	86	709
2-Heptanone	2	6	4	16	8	91
Benzaldehyde	5	18	17	51	20	351
2-Octanone	t	t	1	6	-	34
3,5-Octadien-2-one	1	3	3	7	-	66
2,4-Decadienal	t	2	6	16	-	185
2-Methyl-3-phenyl- 2-propenal	t	t	6	17	-	103
<u>From Carotenoids</u>						
Toluene	t	t	7	29	10	115
6-Methyl-5-hepten-3-one	t	t	1	4	-	26
Isophorone	8	28	33	94	24	798
α-Ionone	1	3	6	19	-	28
β-Ionone	t	t	3	9	-	18
6,10-Dimethyl-5,9- undecadien-2-one	t	t	1	4	-	-
<u>From Proteins and Carbohydrates</u>						
2,5-Dimethylpyrazine	12	40	33	113	18	255
2-Methyl-5-ethylpyrazine	2	6	6	17	-	59
2,5-Dimethyl-3- ethylpyrazine	t	2	4	10	-	31
<u>From Lipids, Proteins and Carbohydrates</u>						
2-Methyl-3(or 6)- pentylpyrazine	t	1	7	26	-	29
2-Methyl-3(or 6)- hexylpyrazine	t	t	1	4	-	-
2,5-Dimethyl-3- pentylpyrazine	t	t	1	4	-	6

*Trace Amount

A = Amylopectin; O = Oil; Z = Zein

ondary reactions within the extruder at higher temperatures. Therefore the amount of volatiles generated at 165°C must be high in order to compensate for these losses (5).

Linoleic acid is the most abundant fatty acid in fresh corn oil (6), and its primary oxidation products, hexanal and 2,4-decadienal, were identified in both the extruded and the baked samples. However, the total number of aldehydes identified in the extruded samples is considerably smaller than the number identified by Snyder et al. (6) in the headspace volatiles of corn oil oxidized at 60°C for eight days. It is possible that aldehydes produced by lipid oxidation will form Schiff base complexes with primary amino groups in zein. The concentration of a specific aldehyde in a given system should then depend on the rate at which it is produced by lipid oxidation and the rate at which it is bound in the model system. For example, lipid oxidation favors the production of specific aldehydes such as hexanal and 2,4-decadienal, but the rate at which aldehydes are bound to the zein should be fairly uniform for any straight-chain aldehydes present in the system.

The quantity of 2,4-decadienal in the extruded samples was low relative to the quantity of hexanal. It is also much smaller than the quantities of 2,4-decadienal from zein/corn amylopectin/corn oil model systems heated at a constant temperature of 180°C. Kimoto and Gaddis (7) report that conditions of stress, including heat, alkali and copper ion decomposition, increase the quantity of 2,4-decadienal produced from trilinolein. Because extrusion exposes samples to greater stress than constant temperature conditions in an oven, it is reasonable to assume that the amount of 2,4-decadienal produced during extrusion is actually quite large. The quantity of 2,4-decadienal may be reduced in extruded samples compared to baked samples because it is volatilized more easily, because it is degraded more quickly or, because it participates in secondary reactions to a greater extent.

One possible mechanism for the degradation of 2,4-decadienal is the retro-aldol mechanism proposed by Josephson and Lindsay (8) for the oxidation of α,β -unsaturated fatty aldehydes. In their investigation of the degradation products of 2,4-decadienal, Josephson and Lindsay (9) identified 2-octenal and hexanal as primary products. Both 2-octenal and hexanal were identified in the present systems.

Ketones are generally less reactive and less volatile than aldehydes. These characteristics are reflected by the somewhat less extreme differences in the quantities of 2-heptanone, 2-octanone and 3,5-octadien-2-one between the extruded and the baked samples. Two of these ketones, 2-heptanone and 2-octanone, have been identified among the thermal decomposition products of corn oil, and their quantities were found to increase when corn oil was oxidized in the presence of zein (4). The effect of an increased surface area for reaction between zein and corn oil during extrusion is hard to determine from these data.

Significant amounts of several carotenoid decomposition products were also identified in this study. Toluene, α -ionone and β -ionone are well-known decomposition products of carotenoids. In corn grain, the two most abundant carotenoids are lutein and phytoene (10). The formation of isophorone from lutein by a free radical mechanism was reported in an earlier publication (4), and phytoene

is a likely source of 6-methyl-5-hepten-2-one. Marty and Berset (11) reported the formation of 5,6- and 5,8-epoxides in both extruded and baked trans- β -carotene. Epoxide formation at the second double bond from the end in phytoene, followed by cleavage of both the epoxide and the isoprene chain leaving the oxygen on the smaller fragment would produce the 6-methyl-5-hepten-2-one identified in the present study. Similar epoxide formation and cleavage at the third double bond from the end would produce 6,10-dimethyl-5,9-undecadien-2-one, a compound identified by GC/MS in the extruded samples but not in the baked samples.

Isopherone, α -ionone and β -ionone are present in comparable amounts in the extruded and the baked samples at 120°C, but their quantities are smaller in the sample extruded at 165°C than in the sample baked at 180°C. When trans- β -carotene was either extruded or baked at 180°C, the amount of nonvolatile degradation products was higher in the extruded samples (11). It is possible that the formation of nonvolatile degradation products is favored over the formation of volatile products during extrusion. On the other hand, the nonvolatile degradation products might be an indication of the overall degree of degradation that has taken place so that when more nonvolatile products are produced, more volatile products are also produced. At this point of the research, it is not known whether more volatiles will be produced during extrusion at 165°C or during baking at 180°C.

Pyrazines are the most widespread Maillard reaction products found in processed foods. The quantity of each pyrazine identified is greater in the samples extruded at 165°C than in those at 120°C. Extrusion conditions have been noted to favor the production of pyrazines at high temperatures (12), but as extrusion temperatures continue to increase, the rate of volatilization may become greater than the rate of production.

Three pyrazines identified by their mass spectra in the extruded samples, 2-methyl-3(or 6)-pentylpyrazine, 2-methyl-3(or 6)-hexylpyrazine and 2,5-dimethyl-3-pentylpyrazine, appeared to be interaction products between proteins, carbohydrates and lipids. A mechanism for the formation of pyrazines with long-chain substitutions has been proposed by Huang et al. (4).

Volatile Compounds Derived from the Degradation of Thiamin During Extrusion

Volatile products produced during the thermal degradation of thiamin have been of great interest in terms of understanding the mechanisms of nutrient loss as well as aroma production. Dwivedi and Arnold (13) reviewed the chemistry of thiamin degradation in food products and model systems. The heating of thiamin in neutral solutions results in cleavage of its methylene bridge, yielding pyrimidine and thiazole fragments. van der Linde et al. (14) reported that 4-methyl-5-(2-hydroxyethyl)-thiazole and 3-mercapto-5-hydroxy-2-pentanone were prominent primary products of thiamin degradation resulting in secondary volatile compounds identified (furans, thiophenes, thiazoles). The importance of water in the production of volatiles during thermal degradation of thiamin has been reported (15). Most recently, Reineccius and Liardon (16) reported that in

the headspace profile from a heated thiamin solution, 2-methyl-3-thiofuran predominates at pH 7, along with the thiophenes and bis-(2-methyl-3-furyl)-disulfide. At pH 9, the 2-methyl-3-thiofuran and bis-(2-methyl-3-furyl)-disulfide are not significant and the thiophenes are predominant in the profile.

When 0.5% thiamin hydrochloride in degerminated yellow corn meal was extruded on a Werner Pfleiderer ZSK-30 co-rotating twin-screw extruder at the final product temperature of 180°C, numerous volatile components were generated. Table II lists the volatile compounds isolated and identified by purge-and-trap GC/MS method.

Among the volatile compounds listed in Table II, only thiazole compounds are derived from the thermal degradation of thiamin. 5-(2-hydroxyethyl)-4-methylthiazole and 4-methyl-5-vinylthiazole are well-known thermal degradation products of thiamin. 5-(2-Chloroethyl)-4-methylthiazole may form through the interaction of 5-(2-hydroxyethyl)-4-methylthiazole with hydrogen chloride. However, the most abundant product, 4-methylthiazole, has never been identified as a decomposition product of thiamin. The mechanism for its formation is not clear.

Table II. Volatile Compounds Identified in Extruded Corn Meal with 0.5% Thiamin Added

Compound	Relative GC Area (%)
Toluene	1.42
Hexanal	22.71
4-Methylthiazole	47.70
Furfural	3.18
Hexanol	0.40
2-Heptanone	0.58
2-Butylfuran	0.02
Heptanal	0.78
Benzaldehyde	1.38
<i>trans</i> -2-Heptenal	0.92
6-Methyl-5-hepten-2-one	0.68
2-Pentylfuran	1.27
4-Methyl-5-vinylthiazole	0.21
5-(2-Chloroethyl)-4-methylthiazole	0.61
5-(2-Hydroxyethyl)-4-methylthiazole	0.02

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Chapter 48

Design of Flavors for the Microwave Oven

The Delta T Theory

Nadim A. Shaath and Nehla R. Azzo

Research and Development Laboratory, Felton Worldwide Inc., 599 Johnson Avenue, Brooklyn, NY 11237

Over 500 raw materials used to create flavors were analyzed through a series of experiments designed to characterize their heat absorption in the microwave oven. From the data gathered, we have proposed the Delta T ($\Delta T'$) theory to describe the behavior of flavors in the microwave environment. The $\Delta T'$ values calculated for these raw materials, which comprise a range of functional groups, allow for the extrapolation of our data to the thousands of raw materials currently used in creating food flavors. This ultimately will enable the design of flavors which are customized for microwave food applications.

By the year 1990, at least one microwave oven (MWO) will be found in 85-90% of U.S. homes (1). This increasing number of microwave units has created the demand for the introduction of many MWO-related food products. Early consumer trials of cooking in the MWO were from scratch, resulting in food products that were non-palatable and unappetizing. The next trials were of packaged foods originally meant for the conventional oven which were labeled as "microwave-able". The results of these trials were also unfavorable. Consequently, the challenge recognized by food, packaging and flavor companies was to design products exclusively for the MWO that delivered traditionally accepted tastes.

In contrast to conventional ovens that generate and transmit heat through conduction and convection, the MWO produces electromagnetic waves that penetrate food and cause friction among its components, generating heat (2). Microwaves radiate at a frequency of 3×10^2 to 1×10^6 MHz with a corresponding wavelength of 3×10^{-2} to 1×10^7 cm (3). In the U.S., the most common frequency used in household units is 2,450 MHz (2,450 million cycles/second) (2). This radiation, with its alternating electromagnetic fields, causes increased movement of polar and ionic molecules. Microwaves are absorbed by foods and oils yet are reflected by metal; glass, paper and ceramics are transparent (2). It is this inconsistent behavior of objects with microwaves that must be considered in the packaging

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and oven design as well as in the food and flavor development. The uniqueness and advantageous qualities of the MW0 as opposed to the conventional oven may become more prominent with the proper harnessing of different energies and interactions. The possibilities for new and revolutionary methods of preparing meals are therefore many and significant.

Problems Associated with Microwave Heating

The problems associated with the behavior of food products in the microwave oven are either inherent to the properties of microwaves or are a function of the food, packaging and oven design. The microwave oven is designed with metal walls as a reflector to contain the microwaves inside the cavity. However, this creates problems such as hot and cold spots generated from the phenomenon of standing waves (4). Hot spots are areas within the oven cavity that experience excessive amounts of energy and cold spots are areas within the cavity that receive negligible energy (5). Also, the frequency of 2,450 MHz has a wavelength of 12.2 cm which is of a similar magnitude to that of most food products. This creates an increased percentage of microwave rays reflected off the surface of foods (6). When cooking a product in the MW0, the air inside the oven cavity remains cool (air is transparent to microwaves). This produces a food that is heated from the inside but whose surface temperature is lower due to contact with the cooler oven air (2). Because of this, it should also be noted that microbes and bacteria could survive at food surfaces due to lower temperatures (7).

Lack of crisping and browning are two drawbacks of the MW0 (8). Crispy texture and brown color normally achieved in conventional cooking are lacking in microwave cooking. In conventional ovens, high temperatures dehydrate a product's surface, producing a crispy crust on the exterior which also helps to protect the interior from moisture and flavor loss. In the MW0 crisping does not occur, and sogginess may result as well. Sogginess develops in microwaved food products since moisture (and volatile constituents) are driven to the food surface (9). The surface moisture persists because of the cool oven air surrounding the food. The uncrusted product in the MW0 remains prone to the steaming-off of moisture, flavor and other volatile food components. The "flashing-off" and "modification" of flavors in microwaveable foods are often common and predictable occurrences. Because of the dynamic effects of electromagnetic energy on the flavors themselves, the partial or total volatilization of flavor is often the cause for bland taste and the development of off-flavor in foods. Microwave energy can cause physico-chemical changes in specific flavor components, resulting in serious distortion of the final taste profile.

The shortened cooking times common to the MW0 do not allow for pyrolysis, caramelization or flavor-generating reactions such as Maillard, Strecker degradation and Amadori rearrangements to occur (10). This commonly leaves microwave foods (especially baked goods) with a raw, uncooked and underdeveloped taste. These problems are currently being approached through various oven modifications, packaging devices such as susceptors and heating elements, as well as the proper design of flavors and food products for the MW0.

The Delta T (ΔT) Theory (11,12)

Our approach was to understand the nature of the interaction of flavors with microwave energy. The factors that affect microwave heating of foods include the specific heat (s), the dielectric constant (ϵ'), the loss tangent and the dielectric loss factor (13). However, in the MWO, inherent properties of the oven and individual foods become more prominent, such as the density, shape, size, moisture content, quantity and consistency of the food, as well as the starting temperature and power setting of the oven (14). It is thus clearly evident that many complex factors are responsible for the behavior of foods in the MWO. Therefore we decided to develop a simplified approach that can assist our flavor chemists in designing new flavors for the MWO, one which combines the majority of the factors that affect microwave heating. With this goal in mind we conducted a series of experiments that tested the effects of microwaves on individual flavor ingredients.

Since specific heats, dielectric constants and dielectric loss factors are not available in the literature for most of the commonly used flavor ingredients, we searched for alternate criteria to evaluate flavor raw materials. Thus, early in 1985 we embarked on a research project to analyze over 500 chemicals, solvents, essential oils and natural flavoring components that were carefully selected to represent the thousands of raw materials used in the flavor industry. All were analyzed individually in the MWO to determine their characteristic behavior within the electromagnetic environment.

The basic design of the experiment consisted of placing a preweighed sample of material adjacent to an equivalent preweighed sample of water as a standard in the center of a 700 Watt MWO. Each sample was tested under various combinations of power settings (10, 30, 50, 80 and 100%) and heating times (20, 60, 150, 300 sec.). Temperatures were measured at each power and time setting. Eleven combinations of power vs. time settings were conducted for each chemical and their average temperature value was computed. Data was collected and a pattern emerged which reflected the tested material's unique heat absorption in the microwave oven. Each component was given a representative value, Delta T' ($\Delta T'$), which corresponds to the ratio of the temperature increase of the sample to the temperature increase of the standard.

Thus,

$$\Delta T' = \frac{\Delta T (\text{sample})}{\Delta T (\text{standard})}$$

where

$$\Delta T = T_{\text{final}} - T_{\text{initial}}$$

The experimental design included $\Delta T'$ calculations for a number of commonly-used flavor chemicals including a homologous series of alcohols, aldehydes, ketones, esters, lactones, amines, thio-compounds and hydrocarbons.

Table I contains the $\Delta T'$ data on two homologous series,

aliphatic alcohols and aldehydes, compared against s (15) and ϵ' (16) data. The predominant effect of increasing chain length is a

Table I. $\Delta T'$ Values of Two Homologous Series, with Specific Heat and Dielectric Constants

Chemical	$\Delta T'$ Value	s (15)	ϵ' (16)
<u>Aliphatic Alcohols</u>			
Alcohol C-2	1.73	0.59	24
Alcohol C-6	1.13	0.59	13
Alcohol C-8	0.97	0.59	10
Alcohol C-10	0.75	0.54	8
<u>Aliphatic Aldehydes</u>			
Aldehyde C-2	1.28	0.57	27
Aldehyde C-3	1.18	0.56	18
Aldehyde C-5	0.98	0.52	12
Aldehyde C-6	0.93	0.52	11
Aldehyde C-10	0.71	0.50	7

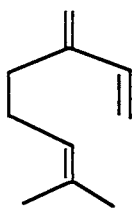
decrease in relative polarity as evidenced by the decreasing $\Delta T'$ values and ϵ' constants. Note that a correlation between the $\Delta T'$ value and the ϵ' constant can be made only if s remains relatively uniform throughout the homologous series. Interfunctional group comparisons also reveal, as expected, that the more polar alcohols generally have a higher $\Delta T'$ value than the corresponding aldehydes.

Table II lists 13 of the most commonly-used chemicals in the flavor industry. For example, cinnamic aldehyde is used for the generation of cassia or cinnamon flavors, carvone for spearmint flavors, citral for citrus flavors, and benzaldehyde for cherry flavors. As can be seen, the known s values for these compounds are

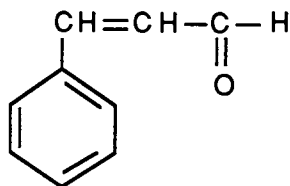
Table II. $\Delta T'$ Values of Common Flavoring Raw Materials with Specific Heat and Dielectric Constants

Chemical	$\Delta T'$ Value	s (15)	ϵ' (16)
Dihydro coumarin	2.65	0.37	25
gamma-Undecalactone	2.64	0.38	23
Cinnamic aldehyde	2.58	0.37	17
Carvone	2.38	0.40	15
Glycerin	2.30	0.57	42
Citral	2.29	0.39	19
Benzaldehyde	2.19	0.40	19
Benzyl alcohol	1.81	0.44	13
Ethanol	1.73	0.59	24
Ethyl benzoate	1.13	0.39	6
Water	1.00	1.00	78
Alcohol C-8	0.97	0.59	10
Aldehyde C-10	0.71	0.50	7
Myrcene	0.09	0.50	3

not fairly constant, hence the correlation of ϵ' constants with the degree of chemical polarity ceases to exist. The $\Delta T'$ value, on the other hand, affords an excellent indicator of a chemical's unique heat absorption in the MWO. A non-polar hydrocarbon such as myrcene (1) has an extremely low $\Delta T'$ value of 0.09, whereas an aldehyde such as cinnamic aldehyde (2) exhibits a very high $\Delta T'$ value of 2.58. These values indicate that, under the same heating conditions in the MWO (for example, 30% power setting and 150 seconds), myrcene reached a maximum temperature of 35°C, versus 175°C for cinnamic aldehyde. Thus, the differences in the $\Delta T'$ values indicate a dramatic difference in the heat absorption capacity of flavor chemicals in the MWO.

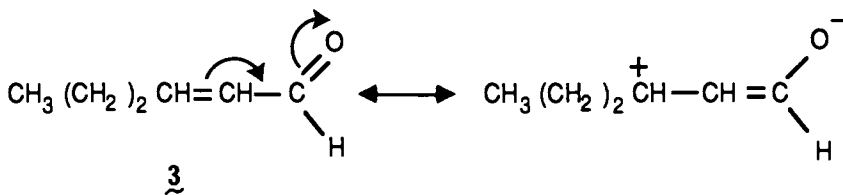


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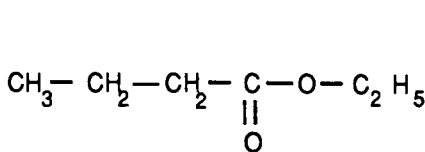
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Numerous additional tests to determine the effect of the carbon chain on the functional group were conducted using a homologous series. The effect of unsaturation is pronounced only in cases where the double bond is conjugated with the aldehydic functional group. For example, trans-2-hexenal (3) had a $\Delta T'$ value of 1.50 as compared to n-hexanal with a value of 0.93. The ease of electron delocalization in trans-2-hexenal, as shown below, increases the polarity of the molecule, hence its higher $\Delta T'$ value.

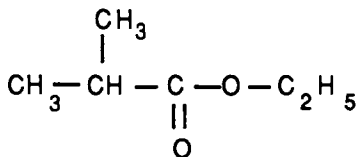


3

Branching has a moderate effect on increasing the $\Delta T'$ values of molecules. For example, ethyl n-butyrate (4) has a $\Delta T'$ value of 0.20, whereas ethyl isobutyrate (5) has a $\Delta T'$ value of 0.37.

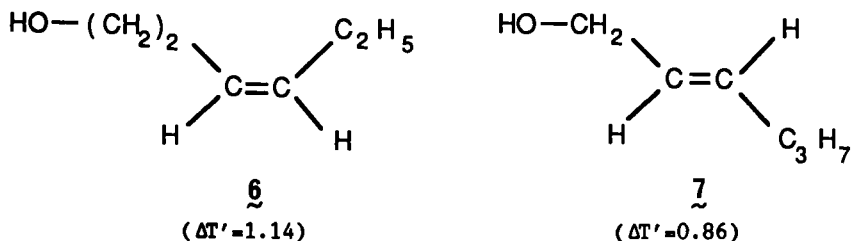


4



5

Geometric isomerism has an obvious effect on the polarity of organic molecules as evidenced by the higher $\Delta T'$ value for cis-3-hexenol (**6**) when compared to that of trans-2-hexenol (**7**) as shown below. Note that the position of the double bond is not relevant for the enol **6** since the alcohol group is unconjugated.



$\Delta T'$ values are thus divided into two major groups: flavor components which were found to possess a high $\Delta T'$ (greater than 1.0, the value for water), and those with low $\Delta T'$ (less than 1.0). High $\Delta T'$ components tend to get hotter in the microwave oven and therefore can be used most effectively in "reaction"-type flavors where browning and caramelization is desirable. Conversely, low $\Delta T'$ values reflect the reduced heat absorbance of flavor components within the microwave oven. They are less prone to microwave-related "modifications" or "flashing-off" and are therefore likely to have superior flavor retention. Experimental data for chemical combinations, essential oils, and flavor systems will appear in a future publication.

Conclusion

The knowledge gained from the testing and evaluation of the dozen functional groups, their homologous series and the effect of chain length, geometry, unsaturation, conjugation and branching on their $\Delta T'$ values has enabled us in our current research to assess the heat absorption characteristics of hundreds of aroma chemicals and their combinations. This, in turn, allows us to design and develop numerous flavor systems tailor-made for many microwave food applications.

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Chapter 49

Influence of Microwave Heating on Flavor

**James A. Steinke, Christine M. Frick, Jo A. Gallagher, and
Kenneth J. Strassburger**

Fries & Fries, 110 East 70th Street, Cincinnati, OH 45216

Flavor systems which perform well in conventionally prepared food are frequently unacceptable when incorporated in microwave heated food products. A frequent problem associated with flavor systems during microwave heating is the loss of flavor attributed to disproportionate distillation or degradation of selected flavor components during microwave heating. High pressure liquid chromatography and gas chromatographic techniques were used to quantitate these flavor components in microwave heated systems. Formation of Strecker aldehydes and loss of flavor components were much greater during microwave heating than conventional heating to comparable temperatures. The loss of a homologous series of volatile acids varied widely depending on the composition of the microwave medium. Factors which affect the dielectric property of the microwave medium such as water and salt concentration had a significant impact on the loss of volatile components.

Consumer interest in microwave products is at an all time high. However, quality of microwave products is frequently marginal. Numerous microwaveable products on the market are the result of a label change rather than a change in product formulation or packaging materials. Additionally, products are introduced as dual microwave and conventional heated products with flavor systems which were not optimized for either process. Quality is marginal. Consumers were willing to sacrifice product quality for convenience. However, this attitude is changing as a result of the large number of new microwave products being introduced. Microwave-only products require a basic understanding of the problems associated with microwave heating.

Conventional heating and microwave heating of food products result in significantly different end products. Foods heated conventionally are subjected to relatively high surface temperatures, 350-450 degrees F., which results in product surface dehydration.

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The low water activity at the product surface favors hundreds of flavor reactions through classical Maillard Browning mechanisms. Product surface also develops a brown color and crisp texture. Microwave heating, however, produces a very different product because the mechanism of microwave heating is so different. Generation of heat within food products in the microwave process is caused by molecular friction attributed to the breaking of hydrogen bonds associated with free water molecules and ionic migration of free salts in an electrical field of rapidly changing polarity. Highest temperatures are typically 190-212 degrees F. Water vapor migrates to the product surface causing evaporative cooling and moisture condensation at the surface. Low product temperatures and the high water activity minimize flavor, color, and texture development during microwave heating of food systems.

Flavors added to microwave food systems have a greatly expanded role compared to flavors added to products prepared by conventional heating. The flavors must provide not only the characterizing flavor (i.e., lemon, butter, vanilla, etc.), but also the typical roasted, toasted, and baked flavors which do not develop in microwave heated products. New flavors designed for use in microwave products must mask the raw uncooked flavor characteristics and other undesirable flavor notes frequently found in many microwave bases. Microwave flavors must also deliver pleasant aromas into the room during the microwave process. Development of these flavors for microwave application is dependent upon a fundamental understanding of microwave heating on flavor performance in food systems.

There is little available literature on the interaction of flavor components with food systems during microwave heating. However, numerous authors have reported on the dielectric properties of nonflavor food ingredients during microwave processing (1,2,3,4).

Individual flavor components are subjected to losses through distillation, flavor binding by starches and proteins, and chemical degradation during the microwave process. Specific data on flavor loss by distillation as affected by the various media and chemical modification of flavor precursors is presented in this paper. Data on flavor binding during microwave processing is addressed in a subsequent paper.

MATERIALS AND METHODS

Preparation of Strecker Aldehyde Samples. Twenty grams of an aqueous solution containing 0.5% amino acid and 1.0% diacetyl were sealed in a 30 ml vial prior to heating. Samples were heated either 4 minutes in a G.E. Space Maker 600 Watt Microwave Oven or held 60 minutes in a 190 degree F. water bath. Microwave samples were heated in 20 second intervals and subsequently cooled until a total microwave heating time of 4 minutes was achieved. Maximum temperature of the microwave sample never exceeded 190 degrees F. The amino acids evaluated were glycine, alanine, and valine. Samples were sealed to prevent the loss of formaldehyde, acetaldehyde, and isobutaldehyde which were formed by Strecker degradation of glycine, alanine, and valine, respectively, when heated in combination with the diacetyl. Samples were analyzed by gas chromatographic headspace analysis.

Preparation of Volatile Acids Solutions in Various Media. Samples of 150 g 90/10 oil/water blend containing 500 ppm concentrations of acetic, propionic, butyric, valeric, and caproic acids were microwaved 0, 1, 2, and 3 minutes in a 600 Watt G.E. Microwave Oven. Temperatures were recorded and a duplicate sample was heated to the same temperatures in a conventional oven. Samples were heated in open containers to permit the loss of acids during heating.

Samples containing 150 g of 500 ppm acids were also prepared using 3% added sodium chloride in the 90/10 oil/water blend, water, and vegetable oil as the microwave medium. These samples were heated 0, 1, 2, and 3 minutes in the microwave. Changes in the acid concentration were determined by high pressure liquid chromatography with an organic acid column and an aqueous mobile phase.

Preparation of Microwave and Conventional Cakes. Diacetyl and acetoin were added at 200 ppm to a commercially available cake mix. The conventional cake was baked 35 minutes at 250 degrees F. in a standard General Electric electric oven. The microwave cake was baked 6.5 minutes in a 600 Watt G.E. Space Maker Microwave Oven. Diacetyl and acetoin concentrations were determined by gas chromatographic headspace analysis as previously described for quantitation of the Strecker aldehydes.

Microwave and Conventional Heating Systems. A General Electric Space Maker 500 Watt Microwave Oven at 2450 MHZ was used in the preparation of all microwave samples. A standard General Electric Hotpoint electric range or a hot water bath was used to prepare conventional heated samples.

Gas Chromatography. A Varian 3700 gas chromatograph equipped with a flame ionization detector, a Hewlett-Packard Model 19395A Headspace Sampler with direct injection, and a Hewlett-Packard 3357 Laboratory Automation System were used. A 60 m x 0.32 mm DB-5 fused silica capillary column was installed in the gas chromatograph. Helium at 25 cm/sec was employed as the carrier gas. Column was equipped with a 50:1 splitter system. Temperature of the injection port was 250 degrees C, temperature of detector was 250 degrees C. Column was maintained at 140 degrees C throughout the analysis. All samples were equilibrated 30 minutes at 50 degrees C in the Hewlett-Packard headspace sampler prior to injection on the column.

High Pressure Liquid Chromatography. The high pressure liquid chromatography system used consisted of a Varian L.C. Model 5000 with a column heater, 50 μ l injector loop, Varian Autosampler and a Varian U.V.-50 variable wavelength detector. All solvents used were HPLC grade from Mallinckrodt Chemicals. Analyses were performed on a Bio Rad Organic Acid Column HPX-87H (250 x 7.6 mm), without the use of guard columns. Flow rate was 0.7 ml/min. An injection volume of 50 μ l was used. Detection parameters were 210 nm at 0.1 AUFS. Column was maintained at 55 degrees C. Mobile phase was 0.016 M H₂SO₄. Concentration of each analyte (acetic, propionic, butyric, valeric, or caproic) in each sample was calculated as follows:
analyte concentration = analyte sample peak area/analyte standard peak area x analyte standard concentration x dilution factor. The

percentage of acid lost was determined by comparison with original acid concentration.

RESULTS & DISCUSSION

Strecker Aldehyde Formation. Formaldehyde, acetaldehyde and isobutyraldehyde were formed by Strecker degradation of glycine, alanine and valine, respectively. Relative concentrations of aldehydes produced by microwave and conventional heating to comparable temperature is shown in Table I. Significantly higher concentrations were observed for microwave heated samples.

Table I. Strecker-Aldehydes Produced by Microwave and Conventional Heating of Amino Acids and Diacetyl

<u>Aldehydes</u>	<u>Conventional (ppm)</u>	<u>Microwave (ppm)</u>
Formaldehyde	10	20
Acetaldehyde	10	30
Isobutyraldehyde	30	130

Other reactions, such as the formation and degradation of 1-amino-1-deoxyketoses, were previously reported to proceed to a much greater extent in microwave products than in those prepared by conventional heating systems (5).

Chemical reactions occur during microwave processing of food systems, however, their contribution to flavor appears to be minimal. The volatile aldehydes quickly flash off during subsequent heating. The desirable baked, toasted, and roasted flavors typical of Maillard Browning do not develop in microwave heated food products.

Loss of Volatile Acids During Microwave and Conventional Heating. The effect of microwave and conventional heating on the loss of volatile acids in an oil/water (90/10) mixture is shown in Table II. Acid losses, regardless of the carbon chain length, were much greater in the microwave heated systems. Significant losses of acetic (33%), propionic (20%), and butyric (9%) were observed during microwave heating to 120 degrees F. Losses observed in conventional heating to this temperature were negligible. Since the temperature of the bulk liquid was the same in both the microwave and conventional heated samples, the observed losses of volatile acids was not attributed to temperature.

Mechanisms of heating, however, were significantly different. The dielectric properties of water and oil differ radically. A high water concentration in food systems greatly increases its dielectric properties. Oil, however, contributes relatively little to the dielectric behavior of a food system (1). Consequently, in the 90/10 oil/water mixture, the microwave energy was directed primarily at the 10% aqueous phase. Acids added to this 90/10 mixture will partition into this aqueous phase to the extent of their relative solubility in the two phases. Greatest losses were observed for acetic acid which exhibits the greatest solubility in water and was concentrated in the aqueous phase. Losses of the more nonpolar acids, i.e. caproic, were also much greater in microwave samples. Losses of the relatively

nonpolar acids were attributed to their orientation at the oil/water interface and proximity to the preferentially heated aqueous phase during microwave processing. Loss of the acids during microwave heating was favored because of the large water losses associated with this process and the corresponding loss of water soluble volatile acids through steam distillation. Conventional heating of these systems to the same temperature demonstrated much lower losses of volatile acids. Conventional heating was typical convective heat transfer and, therefore, dependent upon the specific heat of the oil/water mixture. There was no preferential heating of the aqueous phase.

Table II. The Effect of Microwave vs. Conventional Heating on the Loss of Acids in an Oil/Water (90/10) Mixture

Type of Heating	Acids (% Loss)				
	Acetic	Propionic	Butyric	Valeric	Caproic
120 Deg. F.					
Microwave	33	20	9	0	0
Conventional	1	0	0	0	0
140 Deg. F.					
Microwave	66	44	22	8	1
Conventional	12	10	3	1	0
150 Deg. F.					
Microwave	80	62	44	26	17
Conventional	20	12	5	2	0

Salt Addition to the Microwave Medium. Addition of 3% sodium chloride to the 90/10 oil/water mixture had a significant impact on the loss of volatile acids. Data is summarized in Table III. Loss of acids was much greater in systems with added salt. The increased loss is attributed to the change in dielectric properties because of dissolved salt in the water.

Table III. Effect of Salt on Acid Losses During Microwave Heating

Added Salt	Acid (% Loss)				
	Acetic	Propionic	Butyric	Valeric	Caproic
No Salt	66	44	22	8	1
3% Salt	85	72	54	32	5

The Loss of Volatile Acids in Water. The loss of volatile acids in water during microwave heating is summarized in Table IV. Greatest losses were observed for the more nonpolar although less volatile acids. This was expected and is consistent with vapor pressure data generated for volatile flavor compounds in dilute water systems in a non-microwave environment (6,7). The more polar acids have a greater affinity for the aqueous medium and, therefore, exhibit less loss

on heating. The extent of losses for all acids during microwave heating, however was much greater than expected.

Table IV. The Loss of Acids in Water

Time (minutes)	Acids (% Loss)				
	Acetic	Propionic	Butyric	Valeric	Caproic
1.0	9	10	11	12	14
2.0	16	19	22	26	30
3.0	20	29	37	45	73

Loss of Volatile Acids in Vegetable Oil. Polarity of the microwave medium had a significant effect not only on the type of acid lost but also the extent of that loss. Acetic acid, the most polar acid, exhibited the smallest loss during microwave heating in an aqueous medium. Losses of acetic acid in a nonpolar medium, however, were the greatest as shown in Table V. Losses of volatile acids in vegetable oil were minimal compared to losses in aqueous systems. Loss of volatile acids can be minimized by changing or modifying the microwave medium.

Table V. Loss of Acids Microwaved in Vegetable Oil

Time (minutes)	Acids (% Loss)				
	Acetic	Propionic	Butyric	Valeric	Caproic
1.0	1	0	0	0	0
2.0	15	2	0	0	0
3.0	23	11	0	0	0

Table VI summarizes the effect of heating medium on the loss of acids after 3 minutes of microwave heating. Loss of volatile acids varied widely dependent on the microwave medium. Acetic and caproic acids had losses ranging from 20-80% and 0-73%, respectively, depending on medium composition. The dielectric property, specific heat, or other physical/chemical properties of individual flavor compounds can provide valuable insight into the potential behavior of these compounds during the microwave process. The dielectric property of the total food system and the affinity of the flavor compound for the microwave medium, however, were primarily responsible for the behavior of these flavor compounds during microwave heating.

Table VI. The Effect of Medium Composition on the Loss of Acids During Microwave Heating

Medium Composition	Acids (% Loss)				
	Acetic	Propionic	Butyric	Valeric	Caproic
Oil	23	11	0	0	0
90/10 Oil/Water	80	62	44	26	17
Water	20	29	37	45	73

Microwave Cake and Conventional Cakes. Losses of diacetyl and acetoin (Table VII) were much greater in the microwave cake than in the cake prepared by conventional heating. Losses in the microwave were attributed to volatilization, although flavor binding by starches and proteins is also a factor.

Table VII. The Effect of Microwave vs. Conventional Heating on the Loss of Diacetyl and Acetoin

Type of Cook	Concentration (ppm)	
	Diacetyl	Acetoin
Conventional Cook	80	80
Microwave Cook	50	65

Microwave food products are rarely as simple as the water and oil systems discussed above and caution must be exercised in predicting the reaction of individual flavor components in complex food systems containing salt, proteins, sugars, starches, and other food ingredients. Liquid products quickly dissipate the microwave energy and result in a more uniform product. Solid food products, multiphase systems, or frozen products develop hot spots during heating which further complicate flavor delivery in these systems. Performance of the flavor in the microwave is dependent not only on the physical/chemical properties of individual flavor components, but more importantly, on the interaction of these components with complex food systems.

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Chapter 50

Flavor Development in a Microwaved Versus a Conventionally Baked Cake

C. Whorton and Gary A. Reineccius

Department of Food Science and Nutrition, University of Minnesota,
St. Paul, MN 55108

Many problems associated with successfully identifying and simulating the flavors characteristic of conventionally baked foods have yet to be overcome in the development of new microwave products. This study addresses these problems by identifying compounds most important to the characteristic flavors of white cake batter, microwave and conventionally baked cake. Gas chromatography, mass spectrometry, and odor analysis by sniffing indicated that compounds such as diacetyl, C4-C10 aldehydes, C4-C10 alcohols, C8-C11 dienals, 3-octen-2-one, and 7-octen-4-ol were common to all three flavor systems. Conventional cake was found to contain higher levels of isopentenal and furfural than microwave cake. In addition, compounds such as methyl pyrazine, furan methanol, acetyl furan, and several unidentified compounds with a buttery, caramel character were present only in the conventionally baked cake.

Over the past few years, many advances have been made in the area of microwave product development with items such as popcorn, pizza, and frozen entrees. However, products such as baked goods have met with limited success. To date, many of the texture-related problems have been solved, but the majority of flavor-related problems (particularly flavor development during baking) have not.

One of the major problems associated with the flavor development of microwave products results from the fact that the reduced time/temperature relationship during baking is not conducive to the formation of a crisp, outer crust or many of the Maillard compounds associated with a conventionally baked flavor. These microwave baked products thus have a different flavor character from conventionally baked products and are typically judged "inferior" by consumers.

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To date, there has been virtually no published research specifically addressing the source of these differences in flavor character. Therefore, the purpose of this study was to determine how the flavor system of a microwave baked cake differs from that of a conventionally baked cake. Raw batter (without heat treatment) was also analyzed as a control.

Methodology

A basic white cake was chosen as a model system for the study because of its relative simplicity. Ethyl vanillin was added as a marker compound which was traced throughout the analyses. The formulation (1) was as follows:

Flour	145.0 g
Sugar	150.0 g
Baking Powder	5.5 g
Salt	2.5 g
Shortening	45.0 g
Skim Milk	110.0 g
Egg White	60.0 g
Ethyl Vanillin	0.3 g
Total	518.3 g

Ingredients were mixed and transferred to a glass baking pan. The cakes were then either baked for 35 minutes in a conventional oven preheated to 350°F or microwaved in a Litton II microwave for 5.5 minutes at full power. After baking, cakes were immediately prepared for analysis. Batter was analyzed directly after mixing.

Sample Preparation. For both microwave and conventional cake analyses, a 250 g sample of the top surface and the crumb was removed from the center of the cake, crumbled, and transferred to a 5 L double-necked boiling flask. A 325 g sample of batter was analyzed as a control. To the flask, 2.5 L of preheated Glenwood distilled spring water (Glenwood Company, Minneapolis, MN) was added, and the temperature was allowed to equilibrate to 57-60°C for approximately 3 minutes.

Flavor Extraction and Concentration. The apparatus used for the steam vacuum stripping consisted of a Nickerson-Likens extractor as modified by Schultz et al. (2). The sample/water slurry was maintained at a boil of 57-60°C (600-610 mm gauge pressure) for one hour. During this time, approximately 200 mL of water vapor and flavor volatiles vaporized, condensed, and were collected in the 250 mL flask. A needle-valve was attached to a glass tube in the second neck of the sample flask to admit a controlled stream of charcoal filtered air through the sample for even boiling under vacuum.

Once collected, the aroma quality of the distillate was evaluated, transferred to a 1 L separatory funnel, and extracted with methylene chloride as described by Leahy and Reineccius (3).

Three extractions were completed and pooled per sample of batter, microwave, or conventionally baked cake. The pooled extracts were concentrated to approximately 3 mL in a 600 mL Kuderna-Danish apparatus heated over a gentle steam bath. The extract was then transferred to a glass vial and further concentrated to 0.03 mL. One microliter of the concentrate was placed on a blotter, and the aroma quality evaluated.

Gas Chromatography-Mass Spectrometry. A Hewlett-Packard Model 5890 Gas Chromatograph equipped with a hydrogen flame ionization detector was used in the study. A bonded phase DB-5 fused silica capillary column (30 m x 0.25 mm, 1 μ m film thickness) was used in all analyses (J & W Scientific, Rancho Cordoba, CA). Operating parameters were as follows:

Carrier Gas:	Helium
Head Pressure:	15 psig
Split:	20:1
Injection Volume:	2 μ L
Initial Temperature:	40 C
Initial Time:	2.5 minutes
Rate 1:	5 C/minute
Final Temperature 1:	250°C
Rate 2:	10 C/minute
Final Temperature 2:	275°C
Final Time:	15 minutes

A Hewlett-Packard Model 5970 Mass Spectrometer interfaced with a Hewlett-Packard Model 5890 Gas Chromatograph was used for sample analysis and identification. Chromatographic conditions were the same as above. All mass spectra were obtained at 70 eV EI.

Sniffing. Aroma analysis of separated compounds from each extract was performed on a Hewlett-Packard Model 5880 Gas Chromatograph modified for sniffing off the end of the column. A 3 μ L injection was evaluated. All chromatographic parameters were the same as listed above. Two sniffing trials per extract (two judges each) were completed and recorded for comparison with the mass spectrometry data.

Results and Discussion

A general comparison of the first 32 minutes of the total ion chromatograms (TIC) for batter, microwave, and conventionally baked cakes are shown in Figure 1. Preliminary identifications and the retention times of some significant volatiles shown in the TIC's of the three sample extracts are listed in Table 1. Upon examination, similarities were observed between the three sample chromatograms. In many instances, differences in appearance between the batter and cake samples were due to variation in the relative abundance of a compound alone or in relationship to other peaks. Examples of some compounds found to be in the batter control and the two cake samples include diacetyl, C4-C10 aldehydes, C4-C10 alcohols, C8-C11 dienals,

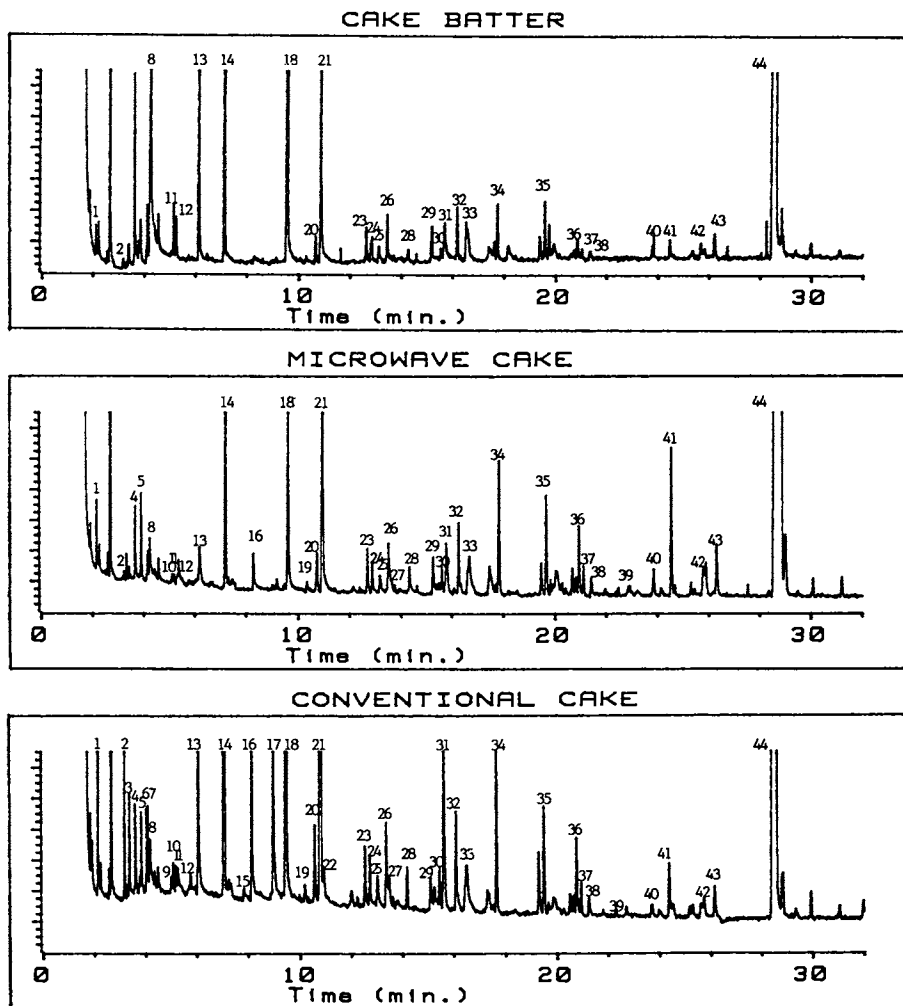


Figure 1. Total Ion Chromatogram Comparisons of White Cake Batter, Microwave, and Conventionally Baked Cake (0-32 min.)

Table 1. Compounds Formed in Batter, Microwave, and Conventionally Baked White Cake (Corresponds with Figure 1)

Peak No.	Compound Name	Retention Time (min.)	Batter	MW Cake	Conv. Cake
1	Diacetyl	2.1	X	X	X
2	Isopentanal	3.2	X	X	X
3	Unknown (caramel)	3.3			X
4	1-Methyl Propanol	3.6		X	X
5	Unknown	3.8		X	X
6	2,3-Pentanedione	4.0			X
7	Unknown (green)	4.1			X
8	Ethylene Glycol	4.2	X	X	X
9	Unknown (caramel)	5.0			X
10	3-Hydroxy-3-Methyl 2-Butanone	5.1		X	X
11	Isopentanal	5.2	X	X	X
12	2,3-Butanediol	5.3	X	X	X
13	n-Pentanal	6.1	X	X	X
14	Hexanal	7.1	X	X	X
15	Methyl Pyrazine	7.8			X
16	Furfural	8.1		X	X
17	Furan Methanol	9.0			X
18	1-Hexanol	9.5	X	X	X
19	2-Heptanone	10.2		X	X
20	Heptanal	10.6	X	X	X
21	2-Butoxy Ethanol	10.8	X	X	X
22	Acetyl Furan	10.9			X
23	t-2-Heptenal	12.5	X	X	X
24	Benzaldehyde	12.7	X	X	X
25	Heptanol	13.0	X	X	X
26	7-Octen-4-ol	13.4	X	X	X
27	2,5-Hexanedione	13.5		X	X
28	Octanal	14.2	X	X	X
29	Ethyl Hexanol	15.1	X	X	X
30	3-Octen-2-one	15.4	X	X	X
31	t-2-Octenal	16.1	X	X	X
32	Octenol	16.5	X	X	X
33	Nonanal	17.7	X	X	X
34	2-Nonenal	19.5	X	X	X
35	Nonanal	19.9	X	X	X
35	Methyl Salicylate	20.8	X	X	X
37	Decanal	20.9	X	X	X
38	2,4-Decadienal	21.3	X	X	X
39	9-Methyl-3-Undecene	22.7		X	X
40	2,4-Undecadienal	23.7	X	X	X
41	Dodecadienal	24.4	X	X	X
42	Unknown	25.7	X	X	X
43	Unknown	26.1	X	X	X
44	Ethyl Vanillin	28.7	X	X	X

3-octen-2-one, and 7-octen-4-ol. Corresponding aroma descriptions such as butter; green, floral, fatty, penetrating; sharp, green, floral; green, vegetable, cucumber; sweet, fatty; and green, mushroom, respectively match cited literature descriptions (4-5). Many of these compounds and their associated aromas have also been previously reported in baked breads (6-7).

Many of the differences between batter, microwave, and conventionally baked cake are also observed in Figure 1. As might be expected, several compounds typically associated with browning notes were present in the conventional cake. Significantly fewer were observed in the microwave cake and batter samples. Peak 2 at 3.2 minutes, identified as isopentenal, was observed in all three samples and noted to have a caramel, tootsie roll-like aroma. However, the relative abundance of isopentenal appeared to vary dramatically between the three samples with batter having the least and conventional cake the most.

Likewise, furfural (peak 16, 8.1 minutes) was observed in both microwave and conventionally baked cake, but at a significantly higher level in the latter. Methyl pyrazine (peak 15, 7.8 minutes), furan methanol (peak 17, 9.0 minutes), and acetyl furan (peak 22, 10.9 minutes), were present in the conventional cake samples as were two unidentified compounds (peaks 3 and 9, 3.3 and 5.0 minutes) observed to have buttery, caramel-like aromas. Several other minor peaks were also observed only in the conventional cake. It should be noted that a few nutty, brown, and potato type smells were detected in areas of the conventional cake chromatogram where no peaks were integrated. These aromas suggest the presence of other Maillard compounds in the extract at levels too low for instrumental detection.

In general, microwave cake appeared to lack many of the nutty, brown, and caramel-type aromas observed in the conventional cake and was in fact more similar to the batter. Table 2 summarizes the predominant aromas noted from each extract in decreasing order. The predominant aromas in both batter and microwave cake were green vegetable notes. Brown, caramel, and potato notes were observed less frequently. The conventional cake profile contained more brown, caramel notes followed by butter, cucumber, potato, and finally, green vegetable aromas.

Table 2. Predominant Aromas Associated With Batter, Microwave, and Conventionally Baked Cake

Batter	MW Cake	Conv. Cake
Green	Green	Caramel/Tootsie Roll
Cucumber	Caramel	Butter
Vegetable	Cucumber	Cucumber
Brown/Caramel	Vegetable	Potato
Floral	Old, Oxidized	Nutty
Potato	Brown	Green
	Potato	Vegetable

Conclusion

Comparison of flavor extracts from white cake batter, microwave, and conventionally baked cakes have provided insight as to the types of flavor compounds initially present before baking and as to the types of compounds which form (or do not form) during the baking process. Ultimately, this type of information will aid in the formulation of conventionally baked flavors to be added to microwave products. When used in conjunction with microwave accessories which promote crust formation, these flavors can benefit the food industry in the development of quality microwave baked products.

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