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Thermally Generated Flavors

Maillard, Microwave, and Extrusion Processes

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Foreword

THE ACS SYMPOSIUM SERIES was first published in 1974 to provide a mechanism for publishing symposia quickly in book form. The purpose of this series is to publish comprehensive books developed from symposia, which are usually "snapshots in time" of the current research being done on a topic, plus some review material on the topic. For this reason, it is necessary that the papers be published as quickly as possible.

Before a symposium-based book is put under contract, the proposed table of contents is reviewed for appropriateness to the topic and for comprehensiveness of the collection. Some papers are excluded at this point, and others are added to round out the scope of the volume. In addition, a draft of each paper is peer-reviewed prior to final acceptance or rejection. This anonymous review process is supervised by the organizer(s) of the symposium, who become the editor(s) of the book. The authors then revise their papers according to the recommendations of both the reviewers and the editors, prepare camera-ready copy, and submit the final papers to the editors, who check that all necessary revisions have been made.

As a rule, only original research papers and original review papers are included in the volumes. Verbatim reproductions of previously published papers are not accepted.

M. Joan Comstock
Series Editor

Preface

AROMA IS A KEY ATTRIBUTE that influences our enjoyment of food. The chemistry of food aromas has been studied intensely for more than 50 years. During that time many researchers have focused on how aroma chemistry changes as a function of thermal processing or cooking. Numerous books, technical papers, and symposia proceedings have been published covering the aroma chemistry of conventionally cooked foods.

Two relatively new methods of cooking, microwave and extrusion, are becoming more prevalent, especially in the United States. Both methods reduce overall cooking time relative to more conventional heating processes, and cooking time ultimately influences the aromas we associate with many foods. Because microwave and extruder cooking are relatively new, the aroma chemistry of these processes has not been comprehensively reviewed. Microwave oven use has grown significantly in the past decade; however, one of the shortcomings is the lack of heat-generated aromas. Although extrusion cooking is of great commercial importance, little research has been reported on optimizing desirable flavors. In both cases, it is primarily the Maillard reaction we are striving to control.

The purpose of this book is to bring together aspects of aroma research covering topics of microwave, extrusion, and Maillard generated aromas. We intend this book to complement and expand on other flavor-related books such as the ACS Symposium Series' *The Maillard Reaction in Foods and Nutrition* (No. 215), *Biogeneration of Aromas* (No. 319), *Thermal Generation of Aromas* (No. 409), and *Flavor Precursors* (No. 490).

The symposium on which this book is based brought together researchers from diverse backgrounds in academia, government, and industry, and was international in scope, with contributions from Canada, Germany, Great Britain, Italy, and the Netherlands.

The first section of this book provides an overview along with discussions of regulatory and legal perspectives. The second section presents several applications of newer analytical methodologies to aroma research. The remaining sections focus on Maillard chemistry, extrusion-generated aromas, and microwave-generated aromas. This book is intended to serve as a reference to researchers in the food and flavor industry.

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Chapter 1

Maillard, Microwave, and Extrusion Cooking

Generation of Aromas

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This symposium is very timely and important since extrusion and microwave food processing are rapidly being commercialized and applied by the food and consumer products' industries. These processes give food manufacturers the opportunity to process foods more efficiently and develop new innovative food products for their worldwide consumers. A common problem in both of these processes is that the heat induced Maillard aroma development which occurs during extrusion or microwave cooking is less acceptable than the aroma developed during conventional cooking. Nevertheless, rapid industrial and consumer acceptance of extrusion and microwave cooking will require both understanding and application of the fundamental chemistry associated with Maillard browning to overcome the aroma deficiency. Other shortcomings associated with microwave and extrusion processing will be minimized as the technologies further develop, but the fundamental aroma deficiency will remain, and the solution is a challenge to food chemists. The intention of this symposium is to focus on this important and relevant problem.

At first glance it might seem unusual to hold a single symposium on what appear to be three different subjects. A person not familiar with the food industry, or new to food chemistry, might even wonder as to the intent, or lack of intent of the organizers. For those familiar with the processes of microwave and extrusion cooking and the chemistry of Maillard browning, the relationship between the three processes is clear. Furthermore, if one is cognizant of the consumer driven changes that are occurring at the retail level of the food business, the importance of this symposium becomes even more apparent.

A simplistic comparison of extrusion and microwave cooking leads the

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author to the conclusion that these are different methods of cooking foods with certain common advantages, and most important, a common deficiency. The deficiency is a lack of flavor development primarily caused by insufficient Maillard browning during the heating or cooking process. Obviously there are other deficiencies, such as color and textural problems that cannot be simply dismissed. Nevertheless, the extreme importance of flavor development in cooked foods make it imperative to review the latest technical developments in Maillard flavor development since this chemistry is responsible for flavor development during microwave and extrusion cooking.

Microwave Cooking

Although microwave cooking has some industrial applications, this chapter and the chapters to follow primarily deal with food products prepared for home microwave cooking. Every year numerous food surveys summarize the reasons for food selection and purchase. Five reasons consistently appear on the top of the list. They are:

LOW CALORIE (Health)

CONVENIENCE

VARIETY

ENJOYMENT

COST

Twenty years ago low calorie was considered part of the small dietetic market and would not have appeared on this list. At that time there was an expression in the food industry which said "You cannot sell health". Today, 3 out of 4 Americans consume low calorie and/or low fat foods and beverages. At first glance this phenomenon does not appear to have anything to do with microwave and extrusion cooking, but the removal of calories usually results in a reduction in desirable flavor and texture. This relationship will be discussed later. Convenience is undoubtedly the single most important reason for the commercial success of the microwave oven. The lack of enjoyment caused by insufficient flavor development and methods to overcome it is the reason for this symposium. Cost is always a factor and this will be discussed later in the chapter.

Microwave oven sales began to grow in the early 60's and as the price rapidly declined they became common household items. Although there are many reasons for changing from conventional cooking to microwave cooking, the major ones are apparent. The primary reasons are the time it saves and the convenience it affords. The microwave oven fits the family lifestyles we have developed, and there is no reason to assume major changes in this lifestyle will occur in the immediate future.

In this book there are numerous chapters that deal with the differences in the sensory properties of microwave and conventionally cooked foods. Lower hedonic ratings are readily assigned to flavor, texture and color of many micro-

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wave cooked foods by the consumer. The major reason for this is that the temperature rise of the food in microwave cooking tends to be uniform throughout the food, independent of the thickness. Also, the cool oven temperature eliminates surface browning and crust flavor development which is necessary for most baked products. In addition, since the absorption of microwave energy is related to the dielectric constant of the food, hot spots can develop. In conventional oven cooking, heating occurs on the outside and is slowly transferred to the interior of the food resulting in a temperature gradient from the exterior to the interior. The flavor chemicals produced because of the different degrees of Maillard browning are quantitatively and possibly qualitatively different from those produced during the more uniform microwave cooking. Food cooked in a conventional oven has become the "gold standard". It is interesting to speculate if we invented the microwave oven before the conventional oven, would we be trying to decrease flavor production and eliminate exterior browning?

An interesting observation is that in spite of the obvious sensory deficiencies of microwave cooked foods, microwave oven use continues to increase. In other words, the American consumer appears to be willing to "trade down" and sacrifice some sensory enjoyment for the convenience afforded by microwave cooking. Obviously there are limits to "trading down", but it is an interesting observation with implications for the food industry that will be covered at the conclusion.

A problem with microwave ovens that is not being discussed at this symposium but is worthy of mention is that microwave ovens are designed, manufactured and marketed by companies which are not in the US food business and have little or no appreciation for the problems associated with food formulations. At the same time, American food companies probably do not fully appreciate the problems of the microwave oven industry. The result is a wide range of oven wattages and even different wattages among what appear to be identical models. This makes it very difficult, if not impossible, for the food manufacturer to give the consumer proper cooking instructions. Obviously, some form of oven standardization is needed and is long overdue. Since it will greatly benefit both industries, some form of standardization will occur regardless of the problems which arise when two different industries, including competitors, must agree to a single standard.

Extrusion Cooking

Extrusion cooking is to the food industry what microwave cooking is to the American household. The processes may be different but the advantages they offer, mainly speed and convenience, are similar.

Cooking extrusion consists of heating and working moistened food and food components until they reach a viscous, plastic-like state and then extruding them through a die to obtain the desired configuration. It is a continuous process and the material is moved through the barrel by a rotating flighted screw. Heat for the cooking process is obtained by direct steam injection, heating the

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extruder barrel, and by the conversion of mechanical energy to heat through the shearing that occurs. The cooking extruder was developed in the 1940's and the first major food application was extruded pet foods. Since then, the technology and equipment have developed to the point whereby a wide variety of food products are now manufactured by this process. Some examples are cookies, snacks, ready to eat cereals and textured vegetable protein. As a process it offers many advantages, but three are most important:

CONTINUOUS

VERSATILE

HIGH PRODUCT QUALITY

Almost by definition, continuous processes result in high productivity and low manufacturing costs. It is a versatile process since the equipment does not have to be dedicated to a single product. A wide variety of foods using different ingredients can be produced on the same machine. The nature of the process results in high quality products with excellent microbiological and physical properties. However, the flavor quality of these products is a problem and is one of the reasons for this symposium.

If there is a flavor deficiency problem in extrusion cooked products then two parts of the process bear investigation. This is a high temperature, short time process with the following typical parameters:

TEMPERATURE	180 to 200° C
TIME	5 to 10 seconds
PRESSURE	800 to 1,200 psi

Are these temperatures and time sufficient to develop fully the desired Maillard browning flavor? If not, what are the qualitative and quantitative differences between the developed flavor and the standard? The second problem is caused by the rapid drop in pressure that occurs when the product exits the extruder barrel. The rapidly escaping gasses cause the desirable puffing that is seen in many products, but from a flavor viewpoint, there is a rapid and significant loss of moisture which results in loss of desirable volatile flavor components. These technical aspects will be discussed during this symposium.

At the beginning of this overview it was noted that 3 out of 4 Americans consume low calorie and/or low fat foods and that this trend has implications for microwave and extrusion cooking. Edible fats and oils are major contributors to the mouthfeel and flavor of foods. Poor flavor development and flavor losses are inherent in the microwave and extrusion cooking processes. Adding to this problem is a marketplace that is demanding low fat foods. The industry is faced with a dilemma. To meet the nutritional demands of the marketplace the food industry is removing important flavor contributors from food formulations which are going to be subjected to cooking processes where flavor development is a problem.

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In spite of the technical challenges that microwave and extrusion cooking present, they also present opportunities for the American food industry. Earlier in this chapter the concept of "trading down" was noted with the fact that the American consumer appears to be willing to "trade down" to obtain the desired convenience and nutritional characteristics. However, if we could partially or completely eliminate the sensory deficits in these products, then growth would be rapid and product failures would be minimal. Remember, it was not lower labor costs that allowed foreign automobile manufacturers to capture 40% of the U.S. market; they simply made a better product and the customer did not have to "trade down". A major objective of this symposium is to provide the critical chemical information that will help the American food industry make the best microwaveable and extruded food products in the world.

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Chapter 2

Regulatory Status of Maillard Reaction Flavors

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Maillard reaction flavors have been introduced into the food supply based on manufacturers' conclusions that the scientific community has considered them to be GRAS. At the present time, FDA does not have enough information to disagree with such conclusions. An analytical method is being developed to determine the amounts and identities of heterocyclic amines which may be present in those flavors. This information is needed to clarify the GRAS status of Maillard reaction flavors.

When a food ingredient (new or old) is used as a component of food, a regulatory burden is associated with its use. The first question is whether the ingredient is a food additive under the definition provided in the Federal Food, Drug, and Cosmetic Act (referred to hereafter as the statute). If it is a food additive, it must obtain pre-market approval from the Food and Drug Administration (FDA) before it can be marketed for use in food. On the other hand, if use of the ingredient is generally recognized as safe (GRAS) or has been sanctioned by FDA or the U.S. Department of Agriculture (USDA) before 1958, the ingredient is not a food additive. The latter two categories of substances, GRAS and prior-sanctioned, are exempt from the pre-market approval required of a food additive.

Maillard reaction flavors are referred to here as processed flavors. They are produced from complex mixtures that are converted to flavors (e.g., meat or chicken flavor) by a heating

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process (*1-3*). The recipes for the precursor ingredients vary from company to company but invariably include, as essential ingredients, specific amino acids, reducing sugars, and animal or vegetable fats or oils. Optional ingredients may include hydrolyzed vegetable protein, onion extract, thiamine, inositol monophosphate, meat extract, and other substances (*4*). All of these precursor materials are common food and flavoring ingredients. However, the safety of processed flavors centers around the heating process which allows these ingredients to develop a desirable flavor in the reaction mixture. Because of this reaction, the review of the safety of processed flavors must be focused on the reaction products rather than simply on the precursor materials. There is no question that processed flavors so produced are flavoring ingredients and thus their use must be in compliance with the statute. The first question we will ask about processed flavors is: Is such a flavor GRAS, prior-sanctioned, or a food additive?

Regulations Pertaining to Processed Flavors

Before we try to determine the categories to which processed flavors may belong, let us discuss the legal definitions for food additives, GRAS substances, and prior-sanctioned substances, as well as some historical background related to the FDA's applications of the GRAS concept.

According to section 201(s) of the statute, food additives include any substance, the intended use of which results, or may reasonably be expected to result, directly or indirectly, in its becoming a component of, or otherwise affecting the characteristics of, any food. Food additives must be approved by FDA, based on a fair evaluation of the entire record available to the agency in accordance with section 409 of the statute. Food additives are deemed to be safe when there is a reasonable certainty that the additive is not harmful under the intended conditions of use.

Section 201(s) also gives a brief definition of GRAS substances. GRAS substances include any substance, which is generally recognized among experts qualified by scientific training and experience to evaluate its safety, as having been adequately shown through experience based on common use in food before January 1, 1958, to be safe under the conditions of its intended use. GRAS substances also include any substance, which is generally recognized by the same experts as having been adequately shown through scientific procedures to be safe under the conditions of its intended use. The statute did not appoint FDA as the sole authority to determine which substances are GRAS and which are not. The statute, in fact, explicitly recognizes the scientific

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community's expert opinions on safety of food substances as a basis for determining whether they are GRAS. Because of this statutory definition requiring general recognition, it is quite obvious that any substance that is secret or not well-known to the scientific community cannot be GRAS.

Prior-sanctioned substances include those approved by FDA under the statute or by USDA under the Federal Meat Inspection Act or the Federal Poultry Products Inspection Act before September 6, 1958, as described in section 201(s)(4) of the statute. The purpose of the exemption was to make it unnecessary to seek approval of those substances that had been sanctioned by these two government agencies before the effective date of the 1958 Food Additives Amendment.

GRAS substances are an open group of substances tied to the current state of scientific information and expert opinions. In contrast, prior-sanctioned substances are a closed group of substances determined by pre-1958 FDA or USDA decisions.

The concept of "GRAS" is unique; no country other than the U.S. has such a category for food ingredients. The creation of the GRAS concept in 1958 by Congress was practical. The lawmakers at that time recognized that to subject a large number of GRAS substances to safety testing would not only disrupt the food supply, but would also place an unnecessary financial burden on industry. However, there was considerable debate over the usefulness of the GRAS concept during the hearings held in Congress before it was finally adopted. Many people expressed concern that the term "GRAS" was imprecise and vague, and did not specify how general the recognition of safety must be in order for a substance to be GRAS. Others envisioned that the GRAS provision may present a problem to food manufacturers who must decide if a new food ingredient would be GRAS. Nevertheless, Congress adopted the GRAS concept with the support of FDA officials, who claimed that the GRAS provision had been working well with new drugs and with pesticide chemicals.

There was little activity up to 1971 on the part of FDA to define what "GRAS" meant. From 1959 to 1971, 21 CFR 121.3, which described GRAS criteria, stated that "... any substance added to food which has no history of common use as a food ingredient should be regarded as a substance that is not generally recognized as safe...unless it has been scientifically tested and shown to be safe."

On June 25, 1971, 21 CFR 121.3 (based on a proposal of December 8, 1970) was revised to be more specific about "GRAS." The revised version declared that any substance of natural biological origin (including those modified by conventional processing)

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consumed primarily for nutrient properties before January 1, 1958, without detrimental effect, would be considered GRAS and there was no need for a promulgation in the Federal Register for this type of substance. It also listed five categories of substances which were considered eligible for GRAS classification, provided that convincing evidence of their safety (including comments from qualified experts) was obtained by the agency.

> (1) Substances defined in subparagraph (1)(i) of this paragraph that have been modified by processes proposed for introduction into commercial use after January 1, 1958, where such processes may reasonably be expected to significantly alter the composition of the substance.
>
> (2) Substances that have had significant alteration of composition by breeding or selection and the change may reasonably be expected to alter to a significant degree the nutritive value or the concentration of toxic constituents therein.
>
> (3) Distillates, isolates, extracts, concentrates of extracts, or reaction products of substances considered as GRAS.
>
> (4) Substances not of natural biological origin including those for which evidence is offered that they are identical with a GRAS counterpart of natural biological origin.
>
> (5) Substances of natural biological origin intended for consumption for other than their nutrient properties.

Four of these five categories were substances of natural biological origin and the remaining one was substances not of natural biological origin but identical to their natural counterparts that were GRAS. 21 CFR 121.3 also stated that substances that were neither of natural biological origin nor identical to GRAS substances of natural biological origin would not be eligible for GRAS status if they had no history of food use. The 1971 version of 21 CFR 121.3 marked the agency's first attempt to define a boundary for eligible GRAS substances.

A more significant change in the FDA's interpretation of the GRAS concept occurred when the agency proposed to further revise 21 CFR 121.3 on September 23, 1974. The proposal, which was

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finalized with little change on December 7, 1976, represented a refined and restricted view of what the GRAS criteria were supposed to be from the agency's point of view. The new 21 CFR 121.3 (recodified in 1977 as 21 CFR 170.30) defined "GRAS" as:

> (1) General recognition of safety through scientific procedures must ordinarily be based on published literature, and requires the same quality and quantity of scientific evidence that would be required for approval of a food additive regulation.
>
> (2) General recognition of safety based on history of common use in food does not require the same quality and quantity of scientific evidence required of a food additive, but shall ordinarily be based on generally available data and information.

This change was particularly significant. It declared that any GRAS substance, based on scientific procedures, should be required to pass the same rigid safety standards set for approval of a food additive, i.e., the same quality and quantity of scientific evidence.

Current Regulatory Status of Processed Flavors

Are processed flavors GRAS, prior-sanctioned, or food additives? We know that processed flavors have not been regulated by FDA as food additives, and we are also certain that processed flavors were not sanctioned by USDA or FDA before September 6, 1958. Therefore, processed flavors must have been marketed for use in food on the basis of their manufacturers' determination that such flavors are GRAS. As stated earlier, the statute authorizes the scientific community, not FDA, to be the arbiter in deciding which substances are GRAS and which are not. Thus, a manufacturer may consider that a particular food ingredient is GRAS, based on its judgment that the scientific community has considered that particular ingredient to be GRAS. This type of determination is often referred to as an independent GRAS determination made by individuals outside FDA. However, such a unilateral determination is subject to the risk that FDA may object to it and may challenge its use in food on the ground that the ingredient is an unapproved food additive. An example is the recent court cases on evening primrose oil, in which FDA challenged independent GRAS determinations made for the oil by its promoters (*5,6*). Once FDA

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and the companies contest the GRAS status of a substance in court, the ultimate decision as to whether the substance is truly GRAS lies with the presiding judge.

Processed flavors may be considered GRAS for the following reasons: (1) The manufacturing of processed flavors mimics high-temperature cooking such as barbecuing. The major difference between the flavors in cooked meat and processed flavors is that the latter are a mixture of selected food ingredients rather than a raw agricultural commodity. Also, most processed flavors are produced at temperatures below 150°C (*4*), much milder than those used in barbecuing. (2) When preparing a gravy, a chef mixes selected food ingredients and cooks at a certain temperature for a specified period of time. Such a gravy is in fact similar to a processed flavor, although the temperature used for the gravy is lower. (3) The use level of processed flavors is low, as is true with other flavoring ingredients.

Safety of Processed Flavors

To date, we are not aware of any significant adverse effects associated with processed flavors used as food ingredients. However, many studies reported that meats cooked at high temperatures contain heterocyclic amines (*7-11*). Some processed flavors may also contain small amounts of heterocyclic amines, which are present as by-products or impurities. The following nine such heterocyclic amines have been isolated from cooked meat or fish.

(1) 2-amino-3-methylimidazo[4,5-f]quinoline (IQ),

(2) 2-amino-3,4-dimethylimidazo[4,5-f]quinoline (MeIQ),

(3) 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx),

(4) 2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline (DiMeIQx),

(5) 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP),

(6) 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole (Trp-P-1),

(7) 3-amino-1-methyl-5H-pyrido[4,3-b]indole (Trp-P-2),

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(8) 2-amino-6-methyldipyrido[1,2-a:3',2'-d]imidazole (Glu-P-1),

(9) 2-aminodipyrido[1,2-a:3',2'-d]imidazole (Glu-P-2).

These compounds were found to be mutagenic by the Ames test. Moreover, dietary exposure to all of these compounds, except DiMeIQx, increased the incidence of tumors in mice and rats (Carrington, C. D., FDA, personal communication, 1992). The principal site for tumor induction was the liver. Malignant lesions following exposure to these compounds were also noted in the forestomach, intestine, blood vessels, and the zymbal and clitoral glands of rats. Furthermore, in an ongoing study conducted by the National Cancer Institute, IQ caused liver tumors in monkeys. After 5 years, 8 out of 20 monkeys with an intake of 10 mg IQ/day and 16 out of 20 monkeys receiving 20 mg IQ/day developed liver tumors, beginning from 21 months to 40 months. Studies in monkeys, sponsored by National Cancer Institute, with MeIQx and PhIP, are also under way. After 3.5 years, no tumors have been observed in the study with MeIQx. The same is true with the study using PhIP, which has been ongoing for 2 years.

At this time, most of the information on heterocyclic amines in foods relates to cooked meat products. We do not have comparable information on processed flavors. Therefore, new information must be generated to show whether processed flavors contain heterocyclic amines in amounts sufficient to affect their safe use in food. An analytical method working group, started by FDA and the Flavor and Extract Manufacturers' Association (FEMA) in 1990, involving government and industry laboratories is developing a protocol for quantification of heterocyclic amines in processed flavors. FEMA has also conducted a survey of the industry to estimate the types and amounts of processed flavors made by the industry (*4*). The analytical method developed will then be used to determine the amounts and identities of heterocyclic amines present in randomly selected processed flavors. We hope that this information will make it possible to see the patterns in which heterocyclic amines are formed in the manufacturing of processed flavors. If so, manufacturers will be able to control their manufacturing conditions to minimize the presence of heterocyclic amines in processed flavors.

Reliable information on the identities and amounts of heterocyclic amines in processed flavors will also make it possible to use risk assessment procedures to estimate the upper-bound limit

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of risk presented by any of these compounds present in processed flavors. By using risk assessment procedures, the scientific community will be in a better position to render a more informed judgment on the safe use of processed flavors.

Summary

1. The industry basis for considering processed flavors to be GRAS is presumably due to the similarity in processing conditions of these flavors to some home-cooking operations, e.g., barbecue sauce and gravy preparations.

2. Safety of processed flavors should be based on reaction products and not on initial ingredients.

3. The FDA does not have any data to challenge the industry's assertion that processed flavors are safe.

4. More needs to be known about the composition of reaction flavor products, particularly the heterocyclic amine content.

5. Some heterocyclic amines are toxic. Using the information from point 4, risk assessment can be performed.

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Chapter 3

Process Flavors and Precursor Systems

Commercial Preparation and Use

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Flavors developed by using thermal treatment of selected precursor materials have become standard commercial flavors sold around the world. More than thirty years ago the first prototype based on the modification of simple hydrolyzed vegetable proteins (HVP) with various amino acids and sugar led the way to the current practice of the industry. Today, the degree of our current knowledge, as reinforced by the chapters in this book, has allowed precise selection of the precursors and processing conditions needed to create high quality flavors.

European regulators are establishing a special class of flavors, designated as "process flavors," and are currently establishing regulatory guidelines for the class. The class of flavors represents a material that is closer to food stuffs in their composition than traditional flavors and, therefore, present a unique problem in establishing regulatory standards.

Man has long used thermal processing or cooking of food as a means to develop a desirable flavor in his food stuff.

In the last 25 years the scientific community has discovered the chemical mechanisms and identified the precursor chemicals that selectively give rise to aromas and taste similar to those of cooked foods. The thermal generation of aroma has been the subject of many scientific papers and symposia and has enabled flavor industry to create a line of flavors known as process flavors (1, 2).

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Process flavors are flavor creations which mimic cooked food flavors, but are used at a low dosage because of the high concentration of the flavor components. The flavor and aroma profile may be reminiscent of a roasted chicken, a Dutch chocolate or any food product which has been altered or created by heat. The sophistication and demands for these flavors have steadily increased over the years as flavor science, food technology and the development of new food products have expanded. This product class currently represents significant business growth and creative development opportunity for the Flavor Industry. It also represents a challenge; like foodstuffs themselves, problems exist in identifying and defining the product and evaluating its safety in use.

Flavor Manufacturing Practices

For the last ten years the Flavor and Extract Manufacturers' Association (FEMA) has had a working committee which has dealt with many basic issues related to process flavors. This committee was instrumental, by working through the International Organization of Flavor Industries (IOFI), in establishing a guideline for the manufacturer of process flavors. That guideline is part of IOFI's Code of Practices and the basis of the current European Industry/Government discussions related to the regulation of process flavors in Europe (3). These flavors have been designated as a separate class of flavor known as "process flavors." Unlike the basic term "flavor" which represents the extracts of botanical or food materials or pure aromatic chemicals approved for flavor use, the "process flavors" are identified as complex mixtures which have been converted to flavors by heat processing step(s).

In the United States, the focus on the definition and regulation of process flavors has not been as clear. They are considered flavors, and their use in food is based upon the assumed safety of the flavor and its relationship to foods. Because of the complexity of process flavors, it is more related to foods and, therefore, is in contrast to safety reviews and evaluations given to pure chemicals used to flavor foods. These traditional flavor materials are the so-called "GRAS" or generally recognized as safe materials used by the USA manufacturers of artificial flavors.

To give the reader a better perspective of what a process flavor is and how it functions, let us draw analogies of prior art and the practice of the chef and Grandma. The chemical bases of flavor created by thermal treatment will be the focus of many chapters in this book and has been covered in many previous symposia. The commercial reasons for the use of

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preparing fine sauces and gravies goes back to the beginning of the great culinary schools of Europe and the Orient. It was the chef who experimented with many ingredients to produce a flavor profile which fit the needs of the entree he was serving. The culinary art to chose the proper ingredient and cooking conditions has led to the great gustatory delights such as Bearnaise sauce and other great sauces of Germany, France and China. They are all based on the development of flavors by the careful selection and processing of ingredient. So too are the sauces and gravies created by Grandma or today's homecooks and the Food Technologists of the world's food industry. All this creativity was based upon intuition- a true art form. This symposium, and others like it, will discuss the scientific knowledge which identifies the key ingredients and processing temperature and time needed to develop that aromatic blend of volatile substances that we call flavor.

Figure 1 shows that a chef may use ingredients I to I10 to create the gustatory delight while the food product developer or Grandma at home has a simpler approach and knows that selected ingredients I, I4, I5 and I10 are key to creating a useful, although not award-winning, sauce. They use those ingredients and process, or "cook" them at a given temperature for a given time.

The scientist now looks at what has been created and isolates the key aromatic components which make up the flavor. He or she also studies the chemical mechanisms and precursor chemicals which give rise to the volatile aromatic components. By legal definition, flavors are ingredients added to food at low quantities for flavor effect and make little or no significant contribution to the nutritive content of the food. Therefore, the flavor chemist will focus on recreating the flavor profile by the use of very specific chemical precursors or food components. The flavor chemist knows that certain key ingredients or isolates for these ingredients used by the chef or Grandma will create a "flavor" which may be used by the food industry to enhance the quality of the prepared food. He or she, therefore, chooses I, a component of I3, I4 and I5 and I10 to make the flavor. Such a flavor may look like this qualitative formulation:

+ Meat extract
+ Onion extract
+ Cystein
+ Thiamine
+ Glucose
+ Beef Fat

*** Heated at 120°C for 60 minutes at a concentration of 70% solids.**

*** The product is a strong meat-like aroma.**

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This is the evolution path from a purely empirical approach (the art of cooking) to the scientific approach (the science of creating a flavor).

The classical approach for making a "flavor" requires the use of natural extracts or distillates and/or synthetic aromatic chemicals (typically those found in nature). These materials are blended together to create the flavor profile. However, it is nearly impossible to create the correct flavor profile of a cooked, roasted food without subjecting ingredients or precursors to a thermal treatment. The classical approach allows the flavor chemist to use materials which are pure (synthetic chemicals) or are natural extracts or distillates which have been used for some time and are considered safe for use. These materials are considered by the 1958 Act as " generally recognized as safe" or GRAS. Dr. Lawrence Lin's chapter in this book and his previous publication have indicated the principals of the GRAS concept (4). Our process flavor is different from these well known substances. Our process flavor becomes unique, as a modified sauce or gravy. It is a complex mixture with substantially different characterization from its individual components, and as prepared foods, it would be difficult to define the product except its recipe or formulation prior to processing.

The GRAS Concept

In 1958 the Food Additives Amendment to the Federal Food, Drug and Cosmetic Act of 1938 created a concept of evaluating the safety of substances added to foods. The Act defines a food additive as any substance whose intended use results in its becoming a component of food or otherwise affecting the characteristics of food if the substance is not "generally recognized, among experts qualified by scientific training and experience to evaluate its safety, as having been adequately shown through scientific procedures to be safe under the condition of its intended use." This provision is the concept exception that played a major role in the safety evaluation of flavoring substances used to make flavors (5).

Classical flavoring components are a unique set of substances that fit easily into the GRAS concept. They are typically used at levels below 10 ppm in food and in many cases less than 1 ppm. This is an order of magnitude less than traditional food additives (6). Typical threshold levels for various components known to occur in process flavors is shown in Table I. The level of the use of flavors and flavoring components are usually self-limiting because of the intensity of the product. Most of the materials are extracts of foods,

CHEF... I = INGREDIENTS

= I-1 + I-2 + I-3... I-n (COOK) ⟶ SAUCES

GRANDMA OR FOOD TECHNOLOGIST...

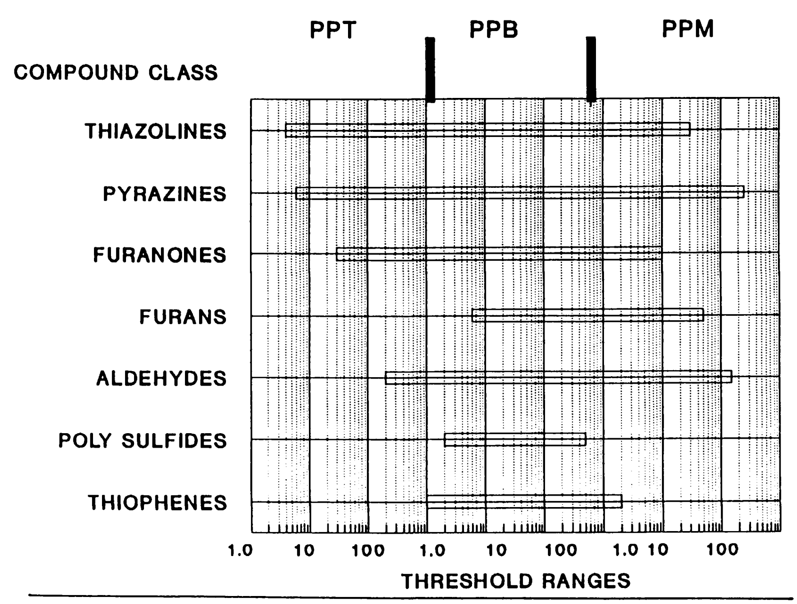
= I-1 + I-4 + I-6 + I-12 (PROCESS) ⟶ CONCENTRATED SAUCE (PREPARED FOOD)

FLAVOR CHEMIST...

= I-1 + I-4 AND COMPONENTS OF I-2, I-3, I-5 AND I-12 (HIGH TEMP) ⟶ FLAVOR

Figure 1. Use of ingredients to produce flavors.

Table I. Threshold values of some flavor components found in cooked meats



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In Thermally Generated Flavors; Parliment, T., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1993.

herbs or spices or are chemicals which are found in those foods, herbs or spices. These factors put flavors into a special class of ingredients used in foods and suggest a much lower exposure and/or risk to consumers than the functional food additives. The GRAS concept as practiced by the flavor industry requires that a panel of experts review certain toxicology data on chemical components and determine by knowledge of the additives' structure, available toxicology data and estimated human exposure if there is reasonable concern for the safety of the consumer at the intended use of the substance. While this procedure does not guarantee complete safety, it does establish a presumption of no harm (7).

Congress, when it established the 1958 Food Additives Amendment to the 1938 Food Drug and Cosmetic Act, 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.

There exists a list of GRAS materials in the Food and Drug Administration regulation under Title 21 of the Code of Federal Regulation (CFR) (8). The flavor industry also maintains a list of substances approved by the Flavor and Extract Manufacturers' Association (FEMA) Expert Panel (9). Both lists are used by the industry to select materials for use in creating flavor. Both lists are considered to contain legal substances for use in the American food system. As noted, the classic flavors are compounds/mixtures of these materials. Just as foods are complex mixtures of ingredients so, too, are flavors. The final flavor is considered to be safe and legal because it is composed of materials which are considered to produce no harm at the intended use.

The Definition of a Process Flavor

In the USA all flavors are considered and titled "flavors" with no regard on how they are produced. However, in Europe, the European Community (EC) has identified and titled a number of types of flavors based on processing, including "process flavors" and "smoked flavors". The EC definition of process flavors identifies the types of materials which may be used as precursors and the time/temperature treatment that the mixture may be given. Table II presents the key features of the International Organization of Flavor Industries' (IOFI) guideline for the manufacture of process flavors. It is interesting to note that the United State Department of Agriculture in a final regulation issued March 1991 identified certain materials used to make process flavors as component which need to be identified in the label statement (10). Note, however, that the USDA did not set a guideline for

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manufacture, but establishes labeling criteria for materials used to produce the process flavor. Table III gives the key features of that regulation.

Therefore, a process flavor is reasonably unique as a flavor since it has its flavor profile developed after it has been compounded and subjected to cooking or roasting conditions. Process flavors are sold to the food industry as the flavor, whereas microwave flavor may be sold as precursor mixture prior to the development of flavor. The heat/energy effects of the microwave oven would produce and release the final flavor. Microwave flavor "precursors" systems are needed in certain prepared food to create the aroma profile which would not be developed because of the lower temperature and shorter time of the microwave treatment. Other chapters in the book will review the chemical basis and indicate the chemistry for producing good flavor profiles from precursors.

These flavors exist to give prepared foods a cooked or roasted profile, for example, in dry mix instant soups. Add hot water and enjoy the flavor. Or start from scratch where the precursors (ingredients) are added and then cooked to develop the flavor. The rapid pace of our modern lives has mandated the former method as the food of convenience. This is the basis of our "ready-to-eat" society- instant prepared foods. Process flavors and microwave products were invented to satisfy that need.

Like the concept of food safety accepts the belief that, if the individual components are safe, then the final prepared food should also be safe. Then the safety concept also fits the development of process flavors.

Modern Safety Evaluation

The requirements of the food law is that the manufacturer does not introduce a potential hazardous material into the food system. This problem has been demonstrated a number of times where certain constituents of a food have been found to be a hazard. For example, aflatoxins in peanuts, nitrosamins in malted grain for beer production, and solanine in potatoes, to mention only a few prominent cases. Upon finding a substance or substances in a food that possess a hazard to man, an effort must be made to remove the hazard or lower to a level which experts would agree that it would possess no general threat to the consumer. In the cases above, processing changes have been made so that the foods do not represent a problem. Regulatory groups around the world will set levels for these types of constituents found in foods. This is also true of food additives, even when the trace contaminants are recognized as carcinogens while the additive has not been shown to have these properties.

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Table II. Process flavor manufacture guidelines

• WITH THE FOLLOWING EXCEPTIONS, AND UNDER THE CONDITIONS DESCRIBED BELOW, INGREDIENTS CONSUMED IN THE REACTION MAY BE LISTED COLLECTIVELY AS REACTION (PROCESS) FLAVORS

✓ EXCEPTIONS:

- ALL INGREDIENTS OF ANIMAL ORIGIN
- ALL NON-ANIMAL PROTEINACEOUS SUBSTANCES (INCLUDING MSG HVP AND AYE)
- THIAMINE HYDROCHLORIDE, SALT AND COMPLEX CARBOHYDRATES
- ANY OTHER INGREDIENT THAT IS NOT CONSUMED IN THE REACTION

✓ CONDITIONS:

- REACTION CONTAINS AMINO ACIDS(S), REDUCING SUGAR(S), AND PROTEIN SUBSTRATES
- TREATED WITH HEAT 100 C OR GREATER FOR A MINIMUM OF 15 MINUTES

Table III. USDA regulation for ingredient labeling

• INTERNATIONAL ORGANIZATION OF FLAVOR INDUSTRIES (IOFI) CODE OF PRACTICE GUIDELINES- PROCESS FLAVORS:

• ARE PRODUCTS OF A MIXTURE PREPARED FOR ITS FLAVORING PROPERTIES

• ARE PRODUCED FROM INGREDIENTS OR MIXTURES OF INGREDIENTS WHICH ARE THEMSELVES PERMITTED FOR USE IN FOODSTUFFS

• OR ARE NATURALLY IN FOODSTUFFS

• OR ARE PERMITTED FOR USE IN PROCESS FLAVORS

• AND ARE GENERATED BY A PROCESS USED FOR THE PREPARATION OF FOODS FOR HUMAN CONSUMPTION

• THE DEFINITION DOES NOT APPLY TO EXTRACTS, PROCESSED NATURAL FOOD SUBSTANCES OR MIXTURES OF FLAVORING SUBSTANCES

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Recently certain chlorinated glycerides have been found hydrolyzed vegetable proteins (HVP). HVP's have been used for more than 100 years to add meat-like flavor to prepared foods. They represent one of the earliest forms of process flavors, and they are composed of amino acids generated by the hydrolysis of vegetable protein with HCl. The heat used to hydrolyze the protein also produces a roast-like flavor character (11). During the reaction the HCl chlorinates glycerine which results from trace amounts of fats found in the vegetable protein. Changes in the method of processing will lower the amount of the chlorinated glycerine to a safe level. Some European countries have set 50 ppm as a safe level.

A number of researchers have recently reported that certain amino acids, specifically creatine and creatinine, form a number of heterocyclic amines when heated in the presence of other amino acids. These substituted imidazo quinolines have been found to be genotoxic and carcinogenic (12). Creatine and creatinine are found in muscle protein and may permit the development of the heterocyclic amines when meat is cooked or process flavors, based on meat extracts or these specific amino acids, are prepared. The flavor industry reported, at the last Toxicology Forum held in Aspen, Colorado, that a joint Flavor Industry/Food Drug Administration Task Force was studying the levels of heterocyclic amines in process flavors by establishing a standard analytical method via HPLC for the quantification of the heterocyclic amines (13). Such a method will allow the FDA to determine whether unsafe levels of heterocyclic amines exist in process flavor and if so, to establish guidelines as has been done in the past.

Conclusions

Process flavors and precursor systems represent new scientific advances on the old culinary art of preparing fine sauces, gravies and other similar foods. Just as a food represents a complex mixture of chemical substances so, too, do process flavors. They represent a new class of flavors and are being designated also in Europe. Guidelines exist for their manufacture and the EC will be adopting them as official standard procedures for this class of flavors.

As another example of regulating a "new" food, the FDA Commissioner, Dr. David Kessler, has recently proposed that foods created by food biotechnology need not undergo a premarket review unless the new food represents some type of possible hazard. The FDA has indicated these new foods should be regulated under its postmarket authority which leave the premarket evaluation of safety and nutritional concerns to industry with guidelines established by the administration (14).

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Regulation of foods, new and old, and flavors based on food concepts such as process flavors are reasonably regulated by the same policy. Safety concerns, as noted, should be focused on the identification and evaluation of containment which may be considered hazardous to the consumer.

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Chapter 4

Basic Principles for Protecting New Developments

A Guide to Patent and Trade Secret Protection

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This chapter presents a discussion of the basic principles for protecting new ideas in the field of flavor and aroma chemistry. The basic categories for which patent protection is available, maintenance of adequate records, avoiding statutory bars to patentability and alternatives to patent protection are reviewed and evaluated.

This paper will highlight certain concepts and legal principles that have far reaching effects in the protection of new inventions and discoveries, particularly in the field of chemistry. By keeping these principles in mind, scientists can enhance the chances for obtaining maximum protection for new developments and minimize the risks of inadvertent loss of valuable intellectual property rights.

Forms of Protection for New Developments in Chemistry and Food Science

Two forms of protection are available for inventions and developments in the field of chemistry (and indeed most other scientific endeavors). One approach is to keep the invention or development as a trade secret. The other approach for securing protection is via the patent laws.

Trade Secret Protection

Trade secret protection is available under state law (not federal law) and the definition of what comprises a trade secret varies from state to state.

One widely accepted definition of trade secrets is in Comment b. in §757, of the Restatement, Torts (1939).

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> A trade secret may consist of any formula, pattern, device or compilation of information which is used in one's business, and which gives him an opportunity to obtain an advantage of competitors who do not know or use it. It may be a formula for a chemical compound, a process of manufacturing, treating or preserving materials, a pattern for a machine or other device, or a list of customers....
>
> Secrecy - The subject matter of a trade secret must be secret. Matters of public knowledge or of general knowledge in an industry cannot be appropriated by one as a secret. Matters which are completely disclosed by the goods which one markets cannot be a secret....

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In simple terms, a trade secret comprises information (which may be a plan, process, tool, mechanism, or compound) known only to the owner, Information that is known to the trade or otherwise in the public domain cannot be a trade secret.

Often, trade secrets become the subject of a legal dispute when an employee terminates employment with one company and accepts employment with a competitor. Although the employee may lawfully use general knowledge, experience, memory and skill gained over the course of his employment, he or she may not lawfully use or reveal any trade secrets of the former employer (1).

In ascertaining whether there is a trade secret, several factors are considered by the courts. These include: 1. The extent to which the alleged "secret information" is known outside of the employer's business; 2. The extent to which the trade secret information is known by the employee; 3. The measures taken by the trade secret owner to maintain secrecy; 4. The value of the trade secret information; 5. The amount of effort or money expended by the employer in developing the information; and 6. The degree of difficulty involved for others to acquire the information on their own (2).

Of these factors, the measures taken by an employer to maintain secrecy are probably most critical (3). This is often the reason for (a) limiting access to laboratory and production facilities and to secret information, (b) non-competition clauses in employment agreements, and (c) implementing routine security procedures to guard against unprotected disclosure.

In general, no protection is available against a person who learns the trade secret honestly and upon whom no duty of confidence has been imposed (4). On the other hand, a trade secret owner is entitled to protection against the *unlawful* appropriation of his trade secrets, even if the trade secret could have been learned lawfully (5). Thus, a court is likely to enjoin the use of trade secret information sold to a competitor by a dishonest employee.

Benefits of Trade Secret Protection. The protection afforded to trade secret information may last indefinitely, although the information must be kept secret.

Thus the United States Supreme Court found that an inventor "may keep his invention secret and reap its fruits indefinitely" (6).

There is no cost, or fee to obtain trade secret protection. However the cost of maintaining the information in secrecy may be considerable.

The level of inventiveness required is much less than for patents.

Worldwide protection commences immediately upon recognition or development of the trade secret. It is not necessary to wait for a grant by a government (as with patents) before the protection afforded by the law comes into force.

Various categories of information and discoveries that cannot be patented can be protected as trade secrets. Thus, a manufacturer of boron fiber composites for jet aircraft wings seeking to prevent delamination of the wing under jet flight conditions discovered that one particular brand, from among hundreds of others, of epoxy cement (sold in hardware stores) worked well in this application. This discovery was immediately protectable as a trade secret despite the fact that patent protection was not available for the use of an epoxy cement to adhere the fiber components together.

Disadvantages of Trade Secret Protection. Trade secret rights are lost if the subject matter of the secret is disclosed to the public, discovered independently or reverse engineered. Trade secret rights can also be lost by failure or inability to protect the secret. For example, if the trade secret is embodied in a commercial product that can be purchased by competitors in the open market, it may be possible to identify it using standard analytical techniques. Thus, the discovery that a laminate containing polyvinylidene chloride (PVdC) is an excellent flavor barrier for use in food packaging materials is not suitable for trade secret protection. Detecting the presence of Pvdc in a food container would require little ingenuity or effort. Trade secret protection is of little value for this discovery. On the other hand, a process that is to be practiced solely in a plant or factory is generally well suited for trade secret protection.

In summary, although the benefits of trade secret protection can be obtained at virtually no cost, the expenses involved in maintaining the trade secret can be high. Also, it is not possible to prevent the loss of trade secret protection to competitors who independently discover or reverse engineer the information. One court has said that reliance on trade secret laws subjects the trade secret information to "a high probability that it will be soon independently developed" (7). Thus, trade secret protection is usually better suited to inventions and discoveries that cannot be reverse engineered and/or will be practiced entirely "in-house" and away from public access.

Patent Protection

A patent is a grant by a government agency, to an inventor, of the right to exclude others from practicing the patented invention for a limited period of time. In contrast to trade secrets, the grant and enforcement of patents is the subject of federal (not state) law. A patent grant is made only in return for complete public

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disclosure of the invention. Inventions in the field of chemistry are the subject of Utility patents. Patents are also granted for Designs and for certain Plants.

Certain inventions, even though they are new, are not statutory subject matter for utility patents. Laws of nature, physical phenomena, and mere ideas are not patentable. A patent cannot be obtained for a system of doing business, an arrangement of printed matter, (8), a mental process, (9), a naturally occurring item, (10), or a scientific principle. Perpetual motion machines are deemed impossible, and are therefore unpatentable.

Mathematical algorithms are also not statutory subject matter (11). Therefore, a patent will not be granted for a computer program encompassing an algorithm (12). It is possible, however, to obtain a patent for a machine that includes a programmed computer or for a process that performs a function using a programmed computer (13). Therefore, although ideas are not eligible for patent protection, the application of an idea is. The bare idea, once known, lies outside the scope of patent protection, and cannot be withheld from the public.

A so-called utility patent may be obtained for "any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof." (See The Patent Act, Title 35 U.S.C. § 101). For the most part these terms are interpreted by the law to have their everyday meaning.

Process. A "process" may be thought of as a means to an end. The Supreme Court defined process as "a mode of treatment of certain materials to produce a given result. It is an act, or series of acts, performed upon the subject matter to be transformed and reduced to a different state or thing" (14).

Processes are intangible. They are a way of getting somewhere or something else from a starting point. A process may be either a way of getting to something that is a new invention or a new method of getting to something known. Typically, a patentable chemical process begins with some raw materials and produces a particular compound (often a patentable composition of matter) through a series of steps (a patentable process). The steps may, but need not, be embodied in a (patentable) machine.

Product. Product is a broad term that encompasses machine, manufacture and composition of matter. Products are physical entities, or fabricated items.

Machine. A Machine is an instrument that consists of parts or elements that are organized to cooperate, when set in motion, to produce a definite, predetermined result (15). A machine is usually a mechanical device. The terms "apparatus," "mechanism," "device," or "engine" are all synonymous (16).

Manufacture. A Manufacture is loosely thought of as non-decorative "goods" or "products." This is a residual category, encompassing most patentable inventions that are not easily characterized by the other categories and includes toys, gadgets, and novelties.

Composition of Matter. A Composition of Matter describes what most people imagine to be the goal of the typical laboratory inventor, since it is usually a new chemical compound or a mixture of ingredients. However, a composition of matter may be any composition of materials, not limited solely to chemicals (17).

Improvement. An improvement is an addition to, simplification of, or change in a process, machine, manufacture, or composition of matter. An improvement over an earlier invention can be patented. In the field of chemistry an improvement invention often comprises a more active form of a compound, or a more efficient way of carrying out a process.

Benefits of Patent Protection. Under U.S. patent law the patent owner can prevent independent discoverers from making, using or selling the subject matter of the patent. See 35 U.S.C. § 154. In contrast, a trade secret owner cannot prevent an independent discoverer from using the subject matter of his trade secret. Trade secret law gives no protection against "honest" discoverers; the patent law does.

The limit (exact boundaries) of protection is clearly defined in the patent claims. This is in contrast to trade secret protection in which the specific identity of the trade secret is open to interpretation.

Disadvantages of Patent Protection. Patent protection is more expensive to obtain. Thus, government fees for filing a U.S. patent application and keeping the resulting patent in force can amount to several thousand dollars. This is exclusive of the expense involved in compiling the information required for patenting and preparation of the application documents.

The duration of U.S. patent protection is currently limited to 17 years from the date of grant. (35 U.S.C. §154). Overseas patent protection is generally for a period of 20 years from the filing date of the patent application.

U.S. courts have recognized that obtaining the grant of a patent is relatively difficult (18).

In contrast to trade secrets which require a relatively low level of invention, a successful patent application requires a relatively high level of invention, i.e., in order to obtain a patent the invention must be new (35 U.S.C. §102), useful (35 U.S.C. §101) and something that would not have been obvious to a person of ordinary skill in the art at the time the invention was made. (35 U.S.C. §103). U.S. courts have recognized that information that is not patentable may be protected as a trade secret (19).

Patents are subject to attack on the grounds of invalidity over the prior art or for failure to comply with formal Patent and Trademark Office (PTO) procedures. Additionally, potential infringers can raise prior art not before the PTO to show that the patent is invalid for lack of novelty, or for obviousness.

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Guidelines for Deciding on Trade Secret or Patent Protection

Because trade secret protection can be lost if the invention is independently discovered or reversed engineered, it is in general not suitable for discoveries that will be revealed to the public. Thus, inventions comprising compositions of matter that are sold to the public (and that can be readily reversed engineered) are best protected via the patent system. On the other hand, inventions comprising methods and processes that are practiced "in-house" are good subjects for trade secret protection provided the trade secret proprietor is prepared to make the necessary commitment to maintain them in secret. Also, trade secret protection should be considered for inventions to be included in products that will be sold without restriction to the public, but only if the trade secret cannot be readily reversed engineered, e.g., aroma chemicals present in trace quantities and whose identity is masked by the presence of many other of the typically hundreds of other volatiles present in a flavor.

Although U.S. patents provide protection for a limited term (17 years) while trade secret protection can last indefinitely, this disadvantage is offset by the fact that the patent protects against independent discovery and reverse engineering. Once a patent has been granted on a product, process, or method, the patentee may assert the patent to prevent anyone else (even those who make the same discovery independently) from making, using or selling the invention. (See Table I.)

Table I. Comparison of Patent and Trade Secret

	Type of Protection	
	Patent	Trade Secret
Maximum Duration:	17 years from the date of issue (U.S.). 20 years from the filing date (Europe).	Indefinite, but must be kept secret.
Cost to Obtain Protection:	Relatively high cost to obtain and enforce.	Relatively low. But the cost of maintenance may be high.
Protection vs. Reverse Engineering:	Yes.	No.
Protection vs. Independent Discovery:	Yes, even against a "good faith" inventor.	No.
Enforceability:	Patent Infringement Suit. Automatic jurisdiction in Federal Courts.	Suit for Specific Performance (Contract) or Injunction (no automatic federal court jurisdiction).

Scientists should keep in mind that trade secret protection is available for the subject matter of a pending U.S. patent application. Under U.S. law patent applications are maintained in confidence by the PTO until the patent is granted. During the period a patent application is pending in the PTO (and assuming the subject matter of the invention has not been freely disclosed to the public) trade secret protection is available. Hence, unauthorized use or disclosure of trade secret information (which will be disclosed later on in the granted patent) often can be halted by initiating legal action under trade secret theories prior to the patent grant.

Priority Rules

Perhaps the most significant differences between the U.S. patent system and the system in other countries are the rules for granting patents to competing patent applications. The rule in the U.S. is that a patent is granted to the first to invent. The last to file a patent application in the U.S. may still be awarded the patent.

Outside the U.S. the patent is granted to the first party to file a patent application. In these countries, there is a so-called "race to the Patent Office." This has the practical effect of encouraging the filing of a patent application at the earliest possible time in order to avoid having a patent granted to another party (who might actually have made the invention at a later date).

In terms of obtaining a U.S. patent, an early filing date is less important. This is because the first to invent (the first to conceive the invention and diligently reduce it to practice) is entitled to receive the patent for the invention even though another inventor may have been the first to file a patent application. See 35 U.S.C. § 102(g). However the first inventor must file a patent application before his patent rights are barred, e.g., by a printed reference (35 U.S.C. §102(b)).

Who is the Inventor (the U.S. Rule)

Under U.S. law, the act of invention requires two steps; conception and reduction to practice. The party who establishes that he is the first to conceive and diligently reduce an invention to practice is deemed the inventor and is entitled to receive the patent (provided the other requirements for patentability are met). The second party to conceive and reduce the same invention to practice will be awarded priority of invention (i.e., the patent) if he can show that the first inventor abandoned, suppressed or concealed the invention (20).

Interference Practice; Resolution of Conflicting Claims to Inventorship in the U.S. Under U.S. law, when two inventors have each filed a patent application claiming the same invention, the PTO initiates an interference proceeding. The interference is an adversarial proceeding (a form of mini-trial in the PTO). The objective of the proceeding is to determine which applicant was the first to invent and is therefore entitled to grant of a patent for the invention (21).

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch004

Conception. "Conception" is the mental part of the inventive act and involves "formation in the mind of the inventor, of a definite and permanent idea of the complete and operative invention as it is to be applied in practice" (22).

To establish conception, a party must show possession of every feature of the invention and prove by corroborating evidence that he disclosed to others a completed thought in such clear terms as to enable those skilled in the art to make the invention (23). Note, a valid invention disclosure as described below is almost always sufficient.

Reduction to Practice. "Reduction to practice" is the physical part of the inventive act. To establish reduction to practice of a chemical composition, it is sufficient to prove that the inventor actually prepared the composition and knew it would work (24).

Two procedures exist for reducing an invention to practice; actual reduction to practice and constructive reduction to practice. Proof of actual reduction to practice requires a demonstration that the embodiment relied upon as evidence of priority actually worked for its intended purpose (25).

The filing of a patent application is a constructive reduction to practice (26).

An invention is not made until there has been a conception and a reduction to practice. Conception is established by showing formation in the inventor's mind of a definite and permanent idea of the complete and operative invention as it is thereafter to be applied in practice. In cartoon drawings the act of conception is often depicted as a light bulb flashing "on" over the inventor's head. In order to establish conception of a complete and operative invention as it is to be reduced to practice, evidence is required that the inventor was in possession of the idea and a way to reduce it to practice. Additionally, a corroborating witness must know and understand what the invention is. It is not a legally adequate conception where an inventor merely has an idea of what he intends to achieve.

Actual reduction to practice comprises (a) actually constructing the invention and (b) testing the physical embodiment of invention to ascertain that it performs in the manner contemplated by the invention. At the time of testing, the inventor must recognize that the test is successful. Actual reduction to practice need not be performed by the inventor, but should be done under his supervision or on his behalf.

In some cases, particularly in the field of complex chemical compounds, conception and reduction to practice occur simultaneously. That is to say, the invention is conceived at the same time that it is reduced to practice. (27).

Diligence. The inventor must work diligently between the time of conception and reduction to practice. As used in the patent statute, the term "diligence" can be defined simply as continued work by the inventor (or his designees) on the invention with the goal of reducing the invention to practice. Although full time effort is not required in order to fulfill the requirement of diligence, an inventor must account for the entire period between conception and reduction to practice by

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establishing activity directed toward reduction to practice and/or an excuse for inactivity (28).

Some Rules of Priority Applicable to New Chemical Compounds

In general, the first inventor to conceive one species of a generic class is the first to conceive the generic invention (29). On the other hand, merely making a chemical compound without any understanding of its activity does not constitute a conception (30). Also, conception of an entire genus is not conception of any specific species within the genus (31).

Given the complex rules for establishing conception and reduction to practice, the message to laboratory scientists is clear. Laboratory work should be documented clearly, in a step-by-step fashion, and in writing. When laboratory work leads to an unexpected result or a new product, the result or product should be recorded and reported to patent counsel or the party responsible for patent matters. An appropriate disclosure should provide sufficient information to enable a person skilled in the field to recreate the unexpected results or new product, i.e. to practice the invention.

Contents of an Invention Disclosure

An adequate invention disclosure should contain at least the following elements:

(a) A broad description of the invention
(b) A synopsis of the prior art and its shortcomings
(c) A description of the manner in which the invention overcomes the shortcomings of the prior art (and why the solution would not have been obvious to a person of ordinary skill in the field of the invention).
(d) A detailed description of the invention (including illustrations - if appropriate).
(e) A description of the preferred embodiment of the invention.
(f) Pertinent references to notebooks or other experimental data.
(g) If more than one person worked on the invention a summary of the contribution made by each inventor.
(h) Identification of witnesses to conception and/or reduction to practice.

This information can be submitted on an invention disclosure form (often provided by corporate patent departments or patent counsel), or in the form of a memorandum to the person responsible for patent matters. All of the above-noted information need not be provided at once, but the date of conception will be found to be that date when enough information is contained in the disclosure to allow one skilled in the art to reduce the invention to practice.

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch004

The invention disclosure form should be dated and signed by the inventor(s) and witnessed by at least two other individuals (who are capable of understanding, and did not make any contribution to, the invention).

Although the invention disclosure can help to establish a date of conception, it will not, standing alone, serve to establish reduction to practice or diligence.

Maintenance of Adequate Notebook Records

In order to assist in establishing conception, diligence and reduction to practice, maintenance of adequate and accurate records of the inventive process, from conception through reduction to practice, is important. To assist in establishing diligence, it is best to maintain a periodic record of work on the invention. This is usually in the form of a laboratory notebook in which the inventor records experimental methods, procedures, observations and results.

Notebook records should clearly show when work was started on a particular project, what was intended to be done, what was actually done (in detail), and what the results were. Observations should be recorded carefully and any equipment or procedures identified. It is best to err on the side of having more (rather than less) detailed notes.

In larger companies it is often customary to have numbered notebooks assigned to each investigator. This helps to provide a notebook record and to establish the dates on which entries were first made. The individual responsible for notebook assignment is informed when the book is completely filled (at which time it is usually returned for safe-keeping) and keeps a record of the dates on which the notebooks are forwarded to patent counsel for evaluation.

Bound hardcover notebooks (not loose leaf or spiral bound), with numbered pages should be used. Preferably, each page should bear the notebook and page number to facilitate identification on photocopies. Entries should be written in ink, or with an indelible marker. Blank spaces and skipped (blank) pages should be avoided; if blank pages are inevitable, a line should be drawn through the unused space and the date entered adjacent to the line. It is best not to erase entries. Rather, unwanted entries should be marked through with a single line, but not so as to destroy legibility. Additional recorded data (e.g. from analytical instruments) can be taped onto the relevant notebook pages in a manner that does not cover up any other information.

On a periodic basis, the notebook should be reviewed by an individual who understands (but is not contributing to) the work being carried out. This individual should sign and date each notebook page and should also be asked to observe critical activities, i.e. first and last steps in synthesizing a new compound, initial analytical activities and recording of results.

In summary, a thorough contemporaneous record of laboratory work should be maintained. The record should enable the researcher and at least one non-inventor to identify with some precision — many years after the event — what was done, and when.

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch004

The need for properly maintaining notebook records that will ultimately serve as corroborating evidence of a reduction to practice has been made clear by the courts. One court (32), noted that:

> The purpose of the rule requiring corroboration is to prevent fraud. . . . Berry's notebook is a contemporaneous record of his thoughts and actions during the performance of an organized research endeavor. . . . Moreover, Berry's laboratory partner, Simmons, testified to having observed the experimental work being performed, understanding the recorded results,and witnessing the notebook records. This establishes that the notebook was in existence and properly kept at the time alleged. To require more corroboration than that set forth above would not, in our view, serve to dispel the possibility of fraud to any significantly greater extent than has already been done in this instance, and would rather appear only to lead to an unjust result.

Avoiding Loss of Intellectual Property Rights

Activities of the inventor or his associates can result in the loss of trade secret and/or patent protection.

Trade Secrets. Trade secret protection is lost if the subject matter of the secret enters the public domain. I.e., is disclosed in a non-confidential situation. Thus, publication of the trade secret information in a journal article, a newspaper, or unrestricted disclosure in a public forum (i.e. in a speech given at a trade meeting or an informational seminar for customers) can result in the loss of trade secret protection. The same applies if a product embodying the trade secret is lawfully acquired and the substance of the secret obtained through reverse engineering.

Hence, it is important to maintain trade secret information in confidence and to avoid unrestricted disclosure. Courts will usually look at the precautions taken by the trade secret proprietor to prevent disclosure, as part of their consideration as to whether information should be accorded trade secret protection.

Patents. Patent protection for an invention can also be barred if certain acts take place prior to filing a patent application. For the most part, protection is barred by disclosing or revealing the invention to the public in some fashion. E.g. displaying the invention at a trade show, publishing a journal article, or offering the invention for sale. However, under U.S. patent law (35 U.S.C. §102) there is a one-year grace period in which to file a patent application after an invention has been disclosed to the public. Thus, under U.S. law an inventor can safely publish a paper describing his invention or place the invention on sale without first having applied for patent protection, provided a U.S. patent application is filed within one year from the date of public disclosure or sale.

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch004

The Absolute Novelty Rule. In Europe and elsewhere, the one year grace period allowed under U.S. law is not available. Most countries outside the United States follow the so-called "absolute novelty" rule. Under this rule, virtually any public disclosure of the invention prior to the filing of a patent application destroys "novelty" and eliminates the possibility of obtaining patent protection. Both the Strasbourg convention on the harmonization of European national patent laws and the European patent convention define prior art to include "everything made available to the public by means of a written or oral description" before the effective filing date, which includes a pre-filing disclosure by the inventor/applicant himself.

Although inventors are generally aware of the one-year grace period under U.S. law (and therefore feel safe in publishing and/or selling their inventions before applying for patent), these feelings of safety are ill-founded if foreign patents are desired for the invention. A sale or other public disclosure (even if the sale or publication is in the United States) of the invention prior to filing a patent application (in the U.S. or elsewhere) can result in the loss of foreign patent rights. The U.S. sale or publication is a novelty defeating act under foreign patent law if it takes place prior to patent filing.

Publications in Scientific Journals. Publication (prior to patent filing) in scientific journals can be a special problem. Journals are often published months after an article has been submitted and without prior notification. Inventors are often surprised to find that their foreign patent rights have been lost because a journal has unexpectedly published their article some time prior to the expected date of publication. Often, more than foreign patent rights are lost through publication. Pre-filing publication of information that is related to the invention for which U.S. patent protection is being sought can form the basis for a rejection of the U.S. patent application (33). Public disclosure or sale of an invention for which patent protection is to be sought should be deferred until after the first patent application has been filed for the invention. Adhering to this rule can avoid the inadvertent loss of patent rights.

Patent Harmonization

The patent defeating inconsistencies resulting from the availability of a one-year grace period after publication in the U.S. and the lack of such a grace period outside the U.S. may soon be resolved. Legislation has been introduced in congress (S2605 and HR4978) that would harmonize U.S. and foreign patent laws. Under the proposed legislation the U.S. would switch over to a "first to file" system thereby eliminating patent interferences and priority contests. At the same time, the term of an issued U.S. patent would be 20 years from the date of filing (instead of 17 years from the date of patent grant). The latter provision is expected to be an incentive for expedited prosecution of pending patent applications.

Many commentators believe the first to file system is fairer because it does away with the expense and uncertainty of priority contests (interference proceedings) under existing U.S. law. Others, including academic institutions, believe it

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puts them at an unfair advantage by requiring an early decision on expensive patent filings.

Conclusion

Once they have been delegalized, the principles underlying the protection of inventions and discoveries are relatively straightforward. Scientists and engineers can, and should, familiarize themselves with, and practice, these principles in order to become more effective advocates for protection of their creations. Observation of the principles and concepts in this article is a step along point on the road to this objective.

Legend of References (for Chemists Non-Lawyers). Most of the references in this article are to law reports. These texts can be found in law libraries (which may be in corporate law departments, court-houses and often in public libraries). The reference reporters referred to in this article are identified below.

C.C.P.A.	=	Court of Customs and Patent Appeals Reports
Fed.	=	Federal Reporter
Fed. 2d	=	Federal Reporter 2nd Series
F. Supp.	=	Wests Federal Supplement
L.Ed.	=	Lawyers Edition (Supreme Court cases)
U.S.	=	U.S. Supreme Court Reports
U.S.L.W.	=	United States Law Week
U.S.P.Q.	=	United States Patent Quarterly

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Chapter 5

Detection of Amadori Compounds in Heated Foods

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Amadori compounds represent precursors of flavor and off-flavor components formed during heat processing of foods. They can be detected in dried vegetables, malt, and cocoa.
For separation and determination of Amadori compounds we used amino acid analysis and HPLC followed by a post-column reaction with triphenyltetrazoliumchloride.
For gas chromatographic analysis, the Amadori compounds are oximized and converted into the trimethylsilyl derivatives. This method also allows the determination of amino acids, reducing sugars, 3-deoxyglucosone, hydroxymethylfurfural and organic acids.
By these methods Amadori compounds formed during vegetable processing, kiln-drying of malt and during roasting of cocoa beans can be detected.

If thermal treatments are employed in food processing, the non-enzymatic browning reaction (Maillard reaction) becomes predominant (*1-4*). It may create desirable aroma components like in baking and roasting processes (*5-7*), on the other side it often causes detrimental nutritional and sensory changes (*8-10*).

In Figure 1 the main pathways of the early Maillard reaction are shown. The first intermediate products of the reaction between aldoses and amino acids are N-substituted 1-amino-1-deoxy-2-ketoses (ketose-amino acids, "Amadori compounds") formed by Amadori rearrangement of the primary aldosyl-amino acids. As Figure 1 shows, Amadori compounds decompose via 1,2- or 2,3-enolization to yield 3-deoxyosones or 1-deoxyosones, respectively. 3-Deoxyosones cyclize to form hydroxymethylfurfural (HMF). 1-Deoxyosones go on to form maltol and isomaltol.

Furthermore, the deoxyosones may participate in the Strecker degradation of amino acids, thus creating volatile flavor components (*11,12*). Deoxyosones may, also, undergo polycondensation to form brown products.

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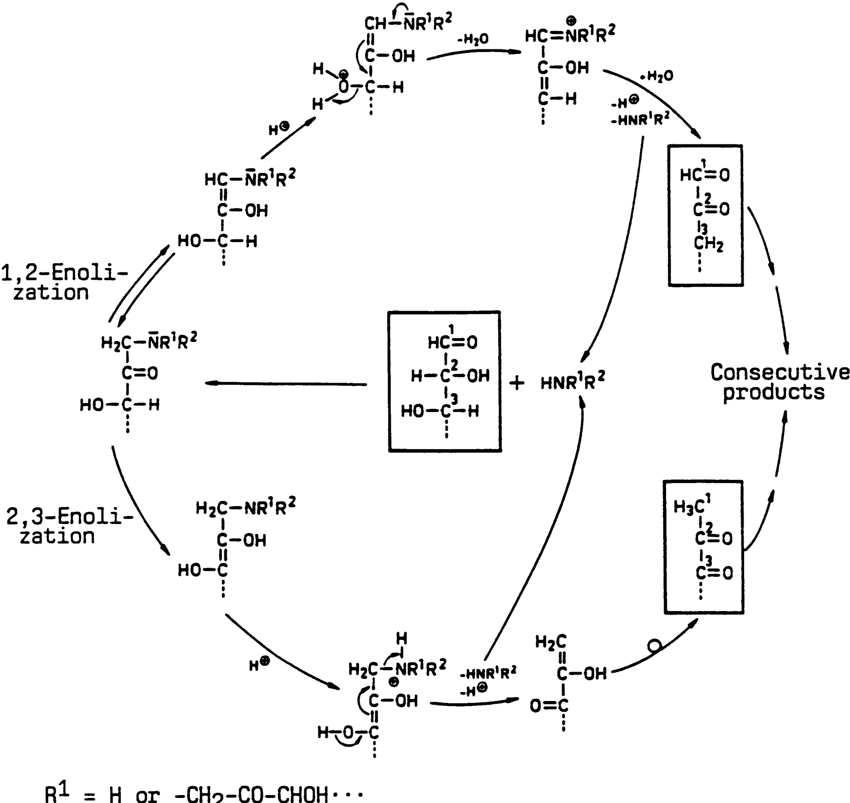

Figure 1. Decomposition of Amadori compounds (ketose-amino acids) to 3- and 1-deoxyosones.

In Thermally Generated Flavors; Parliment, T., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1993.

Since browning and formation of flavor compounds develop later in the Maillard reaction, it seems meaningful to use the Amadori compounds as indicators for early recognition of the Maillard reaction. Amadori compounds can be detected by amino acid analysis, high pressure liquid chromatography (HPLC) and gas chromatography (GC).

Amino Acid Analysis

Several authors (*13-15*) have applied amino acid analysis to separate Amadori compounds. These compounds can be detected in dried vegetables, which contain high amounts of reducing sugars and amino acids. To prepare samples for this analysis, dried products were extracted with cold water, and then the extract was centrifuged. The supernatent was analyzed directly for amino acids and Amadori compounds.

Figure 2 shows a shortened amino acid chromatogram of dried carrots (*16*). Peak C consists of several Amadori compounds which generally are eluted earlier from the cation exchange column than the respective amino acids. The extent of reaction can be expressed as the mol% Amadori compounds, which is calculated based on the initial molar concentration of the pertinent amino acids (*15*). We used the relative concentration of Amadori compounds to control and optimize the drying of foods of plant origin (*16*).

To determine how the concentration of Amadori compounds influence off-flavor development, the concentrations of 2-methylbutanal and 3-methylbutanal (isovaleraldehyde) were determined in samples of freeze-dried, cold-spray-dried and heat-spray-dried tomato powder. The samples contained 7, 15, and 25 mol% Amadori compounds (peak C in Figure 2), respectively. Prior to analysis the samples were reconstituted with water and heated to 70 C. Results, shown in Figure 3, indicate that the concentrations of 3-methylbutanal and 2-methylbutanal, which are formed by Strekker degradation of leucine and isoleucine, increase in proportion to the degree of thermal impact during drying (*17*). From these results it becomes evident that Amadori compounds may act as precursors for off-flavor development caused by Strecker aldehydes.

High Pressure Liquid Chromatography

In view of the drawbacks described for amino acid analysis, a more efficient separation system combined with a more specific method for detecting Amadori compounds needed to be developed.

To date, few methods have been described for the separation of Amadori compounds by HPLC (*14,18-20*). For the separation experiments described below, N,N-diethylaminoethyl-modified silica gel (DEAE-Si 100, 3μm, Serva) was the stationary phase and an acetonitrile-phosphate buffer (70:30, v/v; 0.05 mol phosphate/L; pH 5.8) was the mobile phase (*21*). To achieve a specific detection, an alkaline solution of triphenyl-tetrazolium-chloride (TTC) (3.5g TTC/L 0.05 m NaOH) was added to the column eluate. In the post column reaction coil (85 C, retention time 40 sec), the Amadori compounds reduce the TTC to red 1,3,5-triphenyl-formazane, which is then detected at 480 nm (*21*). Reducing sugars, such as fructose and glucose, react similarly.

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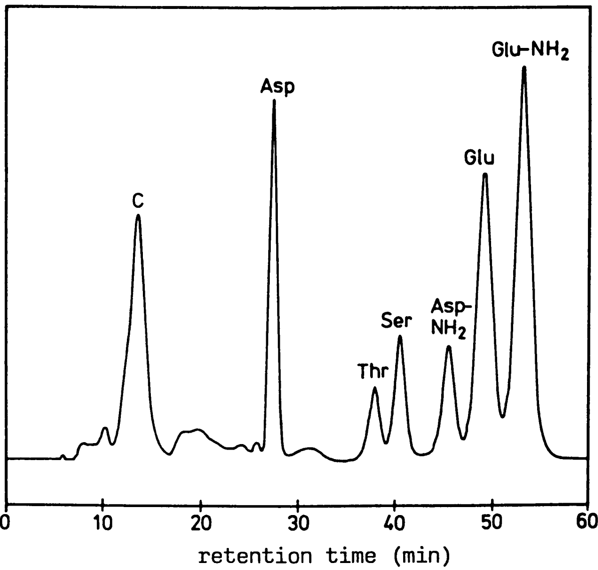

Figure 2. Shortened chromatogram of amino acids in air-dried carrots. Peak C represents Amadori compounds formed by reaction between glucose and the amino acids threonine, serine, asparagine, glutamic acid, and glutamine.

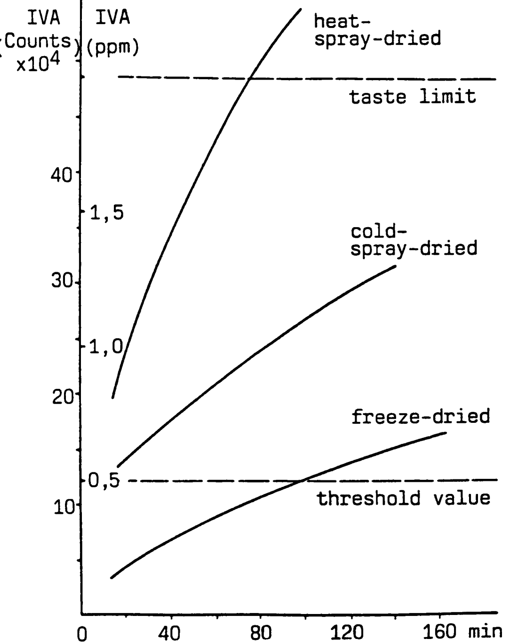

Figure 3. Increase of the concentration of isovaleraldehyde (IVA, sum of 2-methylbutanal and 3-methylbutanal) in freeze-dried and industrially spray-dried tomato powder reconstituted with water (3 ml/g tomato powder) at 70 C.

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Figure 4 shows the chromatogram of a standard mixture of Amadori compounds containing glucose and fructose. In contrast to the amino acid analysis, this separation method does not allow detection of free amino acids; therefore, the amino acids cannot interfere with the separation of Amadori compounds.

By evaluating the chromatograms, it can be concluded that both ion exchange and chromatographic distribution effect the elution sequence of individual components. As an example, Figure 5 shows an HPLC-chromatogram of an aqueous extract of drum-dried tomato powder.

The HPLC method allows detection of fructose-pyrrolidone-carboxylic acid, which is the cyclization product of fructose-glutamic acid or fructose-glutamine (peak 12 in Figures 4 and 5). This compound, which is not detected when amino acid analysis is used, is an indicator of more extensive thermal impact. Therefore, the sum of the indicators fructose-glutamic acid, fructose-glutamine, and fructose-pyrrolidone-carboxylic acid must be taken into consideration when evaluating the course of Amadori compound formation due to thermal impact (*22*). Depending on the extent of heat treatment during drying, relatively high amounts of Amadori compounds could be detected in various dried vegetables like tomato powder, pepper, asparagus, cauliflower, carrot, and celery, as shown in Table I (*21*).

Table I: Occurrence of Amadori Compounds in Various Dried Vegetables (Concentrations in mg/100 g dry matter)

Amadori compounds	Tomato-powder I	Tomato-powder II	Bell pepper	Red pepper	Asparagus	Cauliflower	Carrot	Celery
Fru-Leu+ Fru-Ile	158	41	78	191	59	25	28	50
Fru-Phe	250	108	37	nd	33	15	20	32
Fru-Val	42	48	167	33	50	39	34	47
Fru-Ala+ Fru-Gln	494	516	1599	302	1053	397	418	413
Fru-Asn	945	513	1073	783	1458	127	115	285
Fru-Thr	368	189	213	77	27	18	21	nd
Fru-γ-Abu	1462	1044	317	257	33	105	76	42
Fru-Ser	127	69	338	46	120	75	17	35
Fru-Pyr	594	2148	238	326	60	44	30	27
Fru-Arg	150	nd	288	80	nd	111	82	nd
Fru-Glu	3788	676	62	nd	32	89	52	265
Fru-Asp	948	659	155	62	64	55	34	70

(Fru-γ-Abu = Fructose-γ-aminobutyric acid; Fru-Pyr = Fructose-pyrrolidone-carboxylic acid) (nd = non detectable) (Adapted from ref.*21*).

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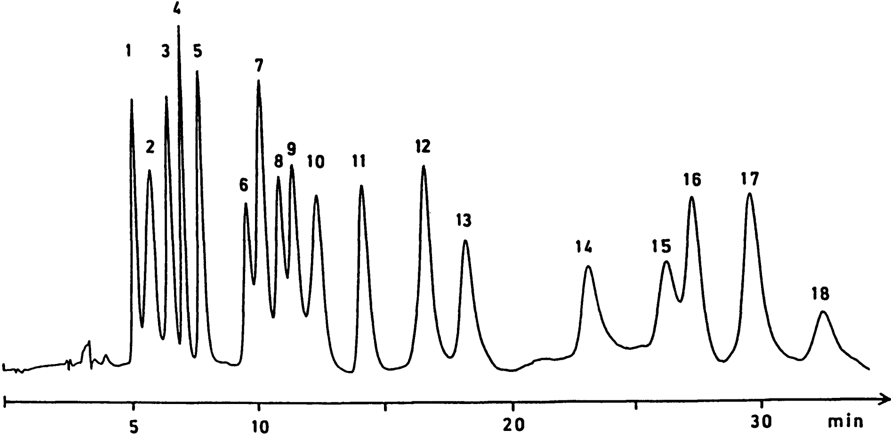

Figure 4. HPLC-chromatogram of a standard mixture of Amadori compounds (post-column reaction with TTC)
1 Fru; 2 Glc; 3 Fru-Leu; 4 Fru-Phe; 5 Fru-Val; 6 Fru-Hyp; 7 Fru-Ala + Fru-Gln; 8 Fru-Asn; 9 Fru-Thr; 10 Fru-γ-aminobutyric acid; 11 Fru-Ser; 12 Fru-pyrrolidone-carboxylic acid; 13 Fru-Arg; 14 α-Fru-Lys; 15 ε-Fru-Lys; 16 Fru-Glu; 17 Fru-Asp; 18 α-,ε-Difru-Lys. (Reproduced with permission from ref.*21*. Copyright 1989 Springer Verlag, Heidelberg).

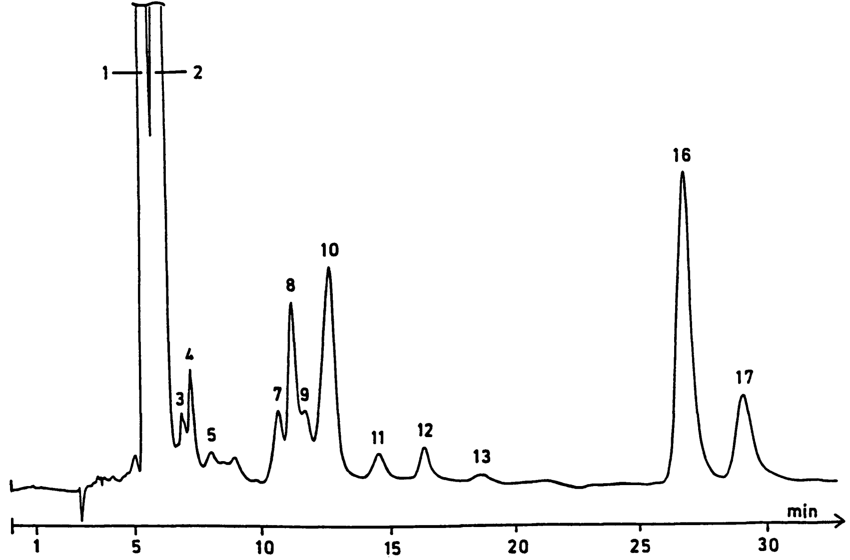

Figure 5. HPLC-chromatogram of an extract of drum-dried tomato powder (peak numbering: cf. Figure 4). (Reproduced with permission from ref.*21*. Copyright 1989 Springer Verlag, Heidelberg).

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Gas Chromatography

Methodology. Prior to gas chromatographic separation, the Amadori compounds and other Maillard reaction intermediates were converted into the corresponding oximes (syn- and anti-form) with hydroxylammonium chloride in pyridine (30 min, 70 C) followed immediately by treatment with N,O-bis-(trimethylsilyl)-acetamide and trimethylchlorsilane leading to the trimethylsilyl derivatives (30 min, 70 C) (*23*). The gas chromatographic separation was carried out on a glass capillary (Duran 35 m x 0.3 mm), covered with methyl silicone (OV-101). Temperature program: 120 - 280 C with a heating rate of 4 C/min. Fig. 6 shows a standard chromatogram of Amadori compounds and different sugars (*23*).

Each compound appears as a characteristic double peak, corresponding to the syn- and anti-form of the oximes; the first peak represents the syn-form, the second one the anti-form.

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Detection of Amadori Compounds in Malts. During malting the Maillard reaction produces the desired color and aroma. Depending on the degree of thermal impact, Amadori compounds are expected to be formed during this process. The Amadori compounds, sugars and free amino acids were extracted from the malts with ethanol/ water (70 ml/100 ml) and bound on a cation exchange resin. The sugars were washed from the resin with water and the Amadori compounds were eluted with 0,5 m trichloroacetic acid (TCA). The derivatization was performed after extracting TCA with ether, evaporating the aqueous solution under vacuum, and freeze-drying (*23*).

Figure 7 shows a capillary gas chromatogram of Amadori compounds in a light colored malt.

The TCA eluate also contains amino acids, which do not interfere with the gas chromatographic separation because they appear before the fructose-amino acids in the chromatogram (provided they form stable derivatives).

The fructose-pyrrolidone-carboxylic acid formed by cyclization of fructose-glutamine and fructose-glutamic acid is not bound on the cation exchange resin, hence, it is washed out together with the sugars. Furthermore, most of the isolated fructose-glutamic acid cyclizes under the derivatization conditions as can be seen in Figure 7. Therefore, the sum of fructose-glutamic acid and fructose-pyrrolidone-carboxylic acid from the TCA fraction corresponds to the original content of fructose-glutamic acid in malt.

Darker malts contain higher concentrations of Amadori compounds than light-colored malts due to the stronger thermal treatment (temperature above 100 C) (*22,23*). Furthermore, light and dark malts show characteristic patterns of Amadori compounds (*22,23*).

Beers produced from those malts again show typical patterns of Amadori compounds (*22,23*). The patterns are characterized by a significant decrease of fructose-valine. There is considerable degradation during the mashing and worting process, but no changes in the concentration of Amadori compounds could be observed during fermentation.

On the other hand, in very dark colored malts, produced at 200 C, no Amadori compounds can be detected , because they degrade under those conditions.

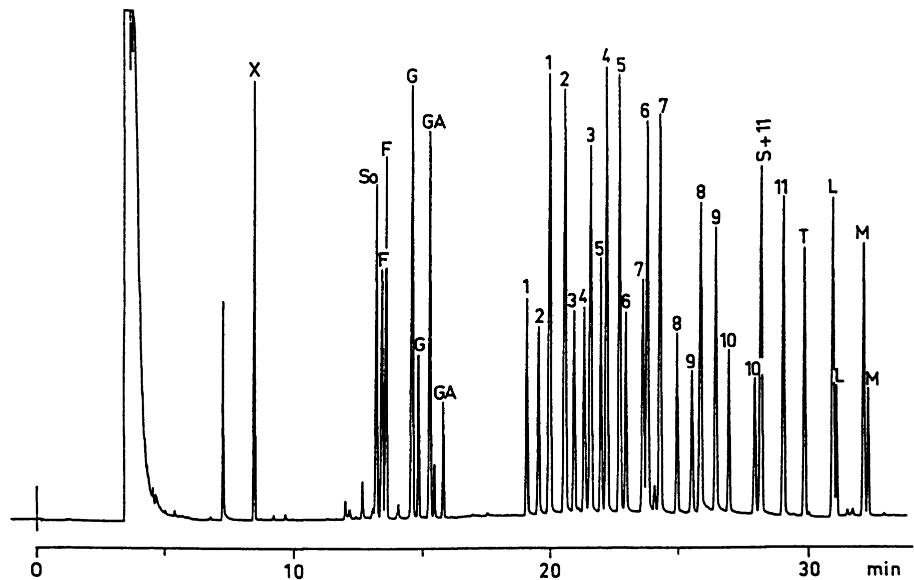

Figure 6. Separation of Amadori compounds and sugars by capillary gas chromatography (standard chromatogram) 1 Fru-Ala; 2 Fru-Gly; 3 Fru-Val; 4 Fru-Leu; 5 Fru-Ile; 6 Fru-Ser; 7 Fru-Thr; 8 Fru-Pyr; 9 Fru-Asp; 10 Fru-Glu; 11 Fru-Phe; So = sorbitol; G = glucose; GA = galacturonic acid; S = saccharose; L = lactose; M = maltose.
Internal standards: X = xylitol; T = trehalose; Pyr = pyrrolidone-carboxylic acid. (Reproduced with permission from ref.*23*. Copyright 1989 Springer Verlag, Heidelberg).

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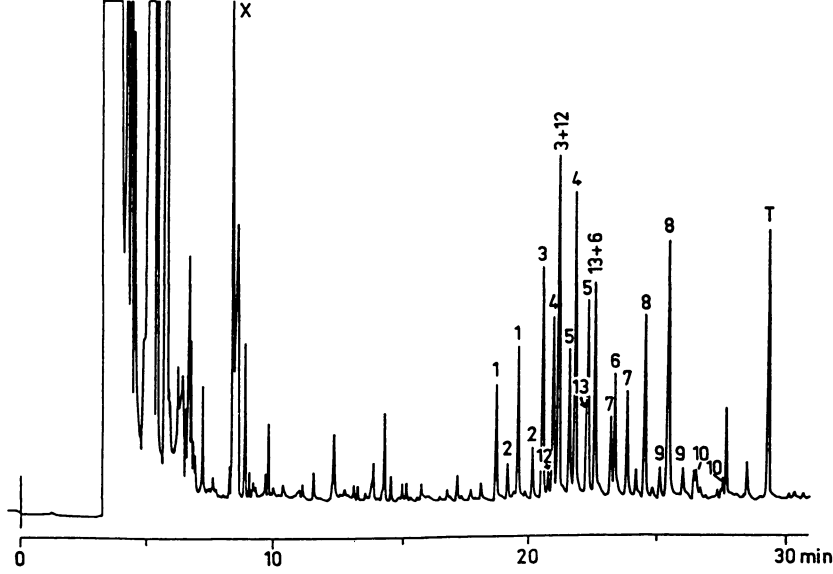

Figure 7. Separation of Amadori compounds from light malt (GC conditions: cf. Fig. 6)
1 Fru-Ala; 2 Fru-Gly; 3 Fru-Val; 4 Fru-Leu; 5 Fru-Ile; 6 Fru-Ser; 7 Fru-Thr; 8 Fru-Pyr; 9 Fru-Asp; 10 Fru-Glu; 12 Fru-γ-aminobutyric acid; 13 Fru-Pro. Internal standards: X = xylitol; T = trehalose. (Reproduced with permission from ref. *23*. Copyright 1989 Springer Verlag, Heidelberg).

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Detection of Amadori Compounds in Cocoa Beans. The gas chromatographic method described above was also applied to cocoa beans. These investigations were performed in connection with the question about the role of Amadori compounds as aroma precursors (*24,25*).

In Figure 8 a capillary gas chromatogram of Amadori compounds isolated from unroasted cocoa is presented (*24*). Again, each compound appears as the characteristic double peak, which corresponds to the syn- and anti-form of the oximes. The first peak (syn-form) is always smaller than the second (anti-form).

From Figure 8 it becomes clear that Amadori compounds are formed during the fermentation and drying process. Our investigations revealed that they act as real precursors of the cocoa aroma (*25*).

In Table II the concentrations of different Amadori compounds in unroasted cocoa beans are presented (*24*).

Table II. Concentrations of Amadori Compounds in Unroasted Cocoa Beans

Amadori compound	Concentration (mg/100g[a])
Fru-Ala	11.9
Fru-Gly	3.0
Fru-Val	14.8
Fru-Leu	25.4
Fru-Ile	6.3
Fru-Gaba[b]	16.9
Fru-Ser	3.6
Fru-Thr	5.7

a: related to fat-free cocoa
b: Gaba = γ-aminobutyric acid (Adapted from ref.*24*).

Determination of Amadori Compounds and Maillard Intermediates in Model Systems. In order to trace the course of the Maillard reaction as a function of reaction conditions, the most important reaction intermediates should be easy to detect. Therefore, we tried to apply the gas chromatographic separation method described above to detection of additional Maillard reaction intermediates. Fructose-glycine and its decomposition products were used as a model system. The model was prepared by heating fructose-glycine to 90 C in a citrate buffer (pH 3.0) for 24 hrs. Figure 9 shows that fructose-glycine (Fru-Gly); its decomposition product, 3-deoxyglucosone (3-DG); its cyclization product, hydroxymethylfurfural (HMF); and glycine, set free from Fru-Gly, can be analyzed in one chromatogram .

At pH 3.0 the main decomposition pathway proceeds via 3-DG (formed via 1,2-enolization of fructose-amino acids, cf. Figure 1) to HMF. During decomposition of Fru-Gly at 90 C, pH 3, the proportion of 3-DG reaches a maximum after about 15 hrs., above which no more accumulates (formation = decomposition). In contrast, formation of HMF increases continuously throughout the reaction (*22*).

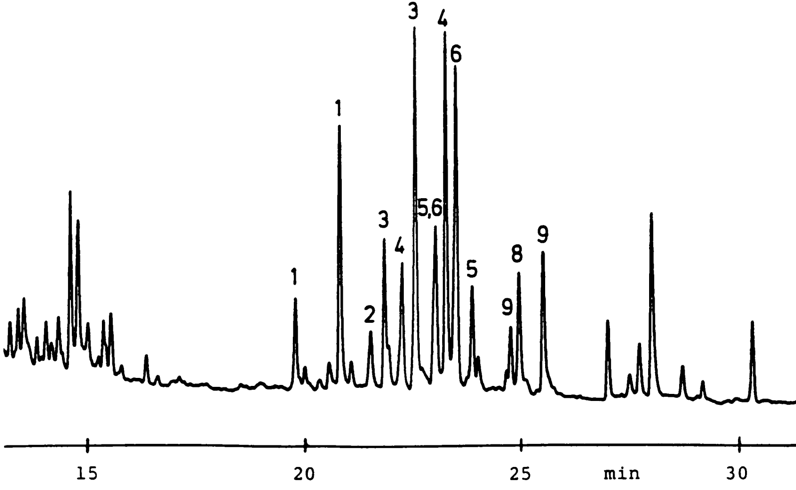

Figure 8. Capillary gas chromatogram (essential section) of oximized and trimethyl-silylized Amadori compounds isolated from unroasted cocoa beans (*24*). Column: DB-5, 32 m; ID 0,252 mm; film thickness 0,1 µm; 21,1 cm/sec N_2. 140 - 300 C with 4 C/min.
1 Fru-Ala; 2 Fru-Gly; 3 Fru-Val; 4 Fru-Leu; 5 Fru-Ileu; 6 Fru-γ-aminobutyric acid; 8 Fru-Ser; 9 Fru-Thr. (Reproduced with permission from ref.*24*. Copyright 1991 Springer Verlag, Heidelberg).

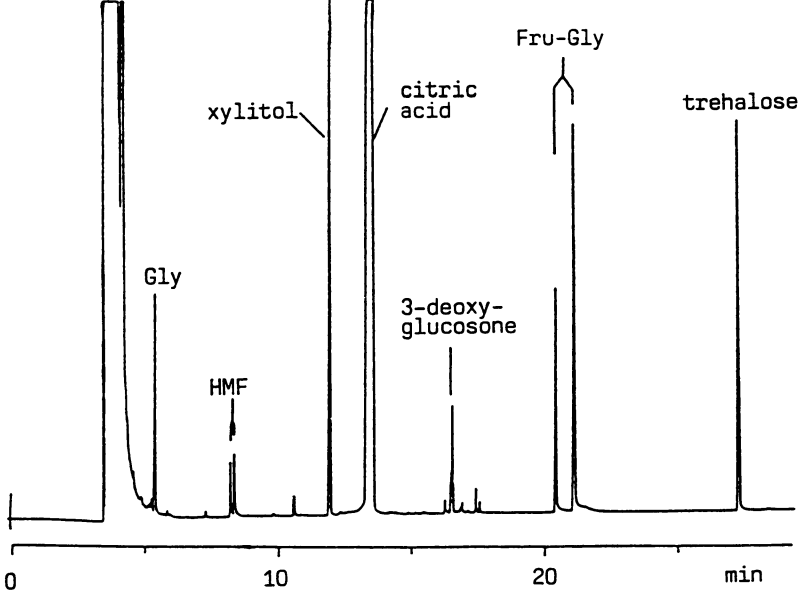

Figure 9. Decomposition of fructose-glycine (Fru-Gly) in a citrate buffer pH 3 at 90 C; reaction time: 24 h. Gas chromatographic conditions: cf. Figure 6. Internal standards: xylitol, trehalose.

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Conclusion

Analytical determination of Maillard reaction intermediates (Amadori compounds) is suitable for the objective evaluation of chemical changes which occur during thermal processes, such as drying, kilning or roasting. Amadori compounds can be detected by amino acid analysis, HPLC or capillary gas chromatography. They are precursors of flavor and off-flavor development. Therefore, Amadori compounds can be used to recognize early Maillard reactions, and (in combination with characteristic decomposition products) for tracing the course of this reaction, which leads to browning and off-flavour compounds.

Furthermore, thermal processing steps can be controlled so that the tolerable or desirable amounts of those compounds are not exceeded. Examples for such applications are dried vegetables, malts kilned in different ways, as well as roasted and unroasted cocoa beans.

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Chapter 6

Maillard Reaction Products from Microwave Heating of Model Systems

Exploiting Gas Chromatographic Variables

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Model systems containing different amino acids (e.g. glycine, alanine, cysteine and tryptophan) and sugars (e.g. glucose and rhamnose) were subjected to microwave heating to produce a variety of Maillard reaction products. The volatile reaction products were analyzed by gas chromatography, using a variety of standard and special fused silica capillary columns. The effects on separation due to various changes in stationary phase composition, film thickness and column length were investigated. Furthermore, the utilization of several detection systems (FID, NPD and FPD) and mass spectrometry aided in the identification of heterocyclic compounds such as pyrazines, thiazoles, oxazoles, thiophenes, pyrroles, furans and cyclic polysulfides. A newly developed high temperature 50% phenyl methylsilicone column proved useful in the isolation and separation of several high boiling point compounds. The analytical results provide excellent examples of the application of gas chromatographic theory to the solution of practical problems.

Analysis of aroma and flavor compounds from foods and model systems remains an important, yet difficult task. The Maillard reaction, in particular, produces literally hundreds of compounds that are of high significance to food and flavor chemists. The Maillard reaction products (MRPs) themselves are extremely complex and range from highly volatile aroma compounds to non-volatile colored pigments known as "melanoidins". Furthermore, the polarity of such compounds range from non-polar insoluble products to extremely polar water soluble products. These combined factors present a considerable chromatographic challenge to the flavor and aroma chemist.

Maillard reaction products have been shown to possess some very interesting chemical properties such as antioxidative activity, both mutagenic and antimutagenic activity, nutritional effects, and of course complex aroma formation (1). For this reason, separation and identification of individual components in the mixture is of utmost importance. Furthermore, the increasing use of microwave ovens for cooking foods has inspired researchers to investigate the lack of browning and preferable flavors (2). Comparison of the volatiles produced from thermal and microwave heating requires optimal gas chromatographic conditions. To complete successfully

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such a task, one must understand the importance of gas chromatographic theory. Optimizing chromatographic parameters such as efficiency (theoretical plates, n), retention (partition ratio, k) and selectivity (relative retention, α) enables the analyst to resolve the aroma components to a high degree.

However, separation of the aroma components from food systems is normally only part of the overall goal. Specific detection systems play an integral role in component identification. The use of element specific detectors such as the nitrogen/phosphorus detector (NPD) and the flame photometric detector (FPD) are two such devices used extensively in flavor analysis. Improved selectivity and sensitivity to nitrogen and sulfur containing compounds, as compared to the more universal flame ionization detector (FID), is critically important in trace analysis of volatile compounds in food systems. Most importantly, gas chromatographic analysis interfaced to mass spectrometry has proved to be a remarkable and dependable detection system for flavor and aroma compounds. This paper illustrates how optimizing critical gas chromatographic parameters, coupled with the utilization of specific and sensitive detection systems, can be extremely important in solving practical problems in food and flavor analysis.

EXPERIMENTAL PROCEDURES

Materials. D-Glucose, L-tryptophan and L-cysteine were purchased from Aldrich Chemical Co. (Milwaukee, WI); L-rhamnose, glycine and DL-alanine were purchased from Sigma Chemical Co. (St. Louis, MO); dichloromethane was purchased from J.T. Baker Chemical Co. (Phillipsburg, NJ). All authentic chemicals were purchased from reliable commercial sources.

Sample Preparation. The method of sample preparation was adapted from Yeo and Shibamoto (3). An amino acid (0.05 mol) and a sugar (0.05 mol) were dissolved in 30 mL of deionized water, and the pH of the solution was adjusted to 9 with 6N NaOH. The solutions were then brought to a final volume of 50 mL with deionized water and covered with Saran brand plastic wrap. Four solutions at a time were irradiated at the high setting of a 700-W microwave oven for 15 min. At 4-min intervals, the irradiation was interrupted and the samples rotated 90° to ensure uniform irradiation.

After microwave irradiation, each brown mass was dissolved in approximately 100 mL of deionized water and allowed to cool to room temperature. The resulting solutions were adjusted to pH 8 with 6N NaOH to enhance the extraction efficiency of nitrogen-containing heterocyclic compounds. The aqueous solution was extracted with 50 mL of dichloromethane using a liquid-liquid continuous extractor for 6 h and the extracts dried over anhydrous sodium sulfate for 12 h. After removal of sodium sulfate, the dichloromethane extract was concentrated to 1 mL by fractional distillation.

Instrumentation. A Quasar Model MQ 7796 AW, 700-W "Easy-Matic Cooking" microwave oven was used for sample irradiation.

A Hewlett-Packard (HP) Model 5890 gas chromatograph equipped with either a flame ioninzation detector (FID), a nitrogen/phosphorus detector (NPD) or a flame photometric detector (FPD) was utilized for sample analysis. The instrument was equipped with a variety of fused silica open tubular capillary columns including DB-5 (5% phenyl methylsilicone), DB-17 (50% phenyl methylsilicone), DB-210 (50% trifluoropropyl methylsilicone), DB-23 (50% cyanopropyl methylsilicone) and DB-WAX (polyethylene glycol) columns supplied by J&W Scientific (Folsom, CA). A newly developed high temperature column (DB-17ht) was also used to separate higher boiling point compounds. Specific temperature programs and gas chromatographic conditions varied, and are shown in each chromatogram.

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A HP Model 5890 GC interfaced to a VG Trio II mass spectrometer was used for MS identification of the GC components using a 30 m x 0.25 mm i.d. DB-WAX bonded-phase fused silica capillary column. The oven temperature was held at 35°C for 2 min and then programmed to 250°C at 3°C/min. Mass spectra were obtained by electron impact ionization at 70 eV and a source temperature of 250°C. The spectral data was recorded on a VG 11-250 computer data system.

RESULTS AND DISCUSSION

Practically speaking, the separation efficiency of a column is a measure of its ability to resolve the components of a mixture into individual compounds. From the equation depicting fundamental resolution given in Equation 1, it is apparent that several chromatographic parameters can be manipulated to provide optimal separation.

$$R = \frac{\sqrt{n}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k}{k+1}\right) \qquad (1)$$

Efficiency (n), retention (k) and selectivity (α) each play roles in the separation process, but to differing degrees. Both column selection and operating conditions should be optimized to ensure the best possible separation of these complex Maillard reaction mixtures.

Selectivity

Frequently, the type and compostion of the column stationary phase may be the most important factor in overall separation of flavor and aroma compounds. In terms of resolution, this factor is a measure of the selectivity of the stationary phase, where the α term is commonly referred to as relative retention. Relative retention is simply the ratio of the adjusted retention times of a mixture of two components and is expressed below in Equation 2, where $t_{R(A)}$ and $t_{R(B)}$ are the retention times of solutes A and B respectively, and t_m represents the "gas hold up time."

$$\alpha = \frac{t_{R(B)} - t_m}{t_{R(A)} - t_m} \qquad (2)$$

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 compounds to be separated and the composition of the stationary phase coated on the inside of the column. The five interactions are dispersion, induced dipole, dipole-dipole, high dipole and hydrogen bonding (4). It is the interactions between the functional groups of the solutes and those of the stationary phase that account for the differences in stationary phase selectivity.

Stationary phase selection is often based upon a previously successful separation completed by another researcher. Although knowledge of separations that have been achieved in the past can provide some useful information, it should be considered as a point of reference from which improvements can be made. Alternatively, an initial point of reference can take the form of a preliminary separation on a short apolar column taken through its temperature limits. Improvements to the separation can be deduced from these preliminary results.

Apolar stationary phases such as methylsilicone (DB-1) and phenyl methylsilicone (DB-5) are usually low selectivity (low α) phases. High quality

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columns prepared with apolar phases are capable of performing a large percentage of all analyses and should be used whenever possible. These lower-α phases offer several advantages such as higher thermal resistances, longer lifetimes, lower bleed rates, and shorter analysis times (5). Gas chromatography is essentially a volatility phenomenon, and solutes elute in a sequence based on their vapor pressures. Apolar phases are limited, however, to dispersive interactions with solutes, and solutes of similar boiling points are prone to coelute on that phase (6).

An L-rhamnose/L-cysteine dichloromethane extract was initially chromatographed on a DB-5 (5% phenyl methylsilicone) fused silica open tubular capillary column and is shown in Figure 1A. This phase was chosen to "learn" about the nature of the sample components and to identify the selectivity required for optimal separation. The chromatogram reveals that the range of volatility of the individual components is quite large. Furthermore, one can observe various coelutions of component peaks and baseline separation is rarely achieved. An extremely large peak located at approximately 20 minutes in the chromatogram is indicative of the coelution of a number of compounds with similar boiling points. Table I lists the boiling points of some characteristic compounds that are generated from this model system. It is thought that these compounds may be responsible for the very wide peak mentioned above. The results of this initial experiment indicate that a more selective and retentive column is required for separation of the L-rhamnose/L-cysteine reaction products.

Table I. Boiling Point Data of Some Common Volatile Maillard Reaction Products

Compound	Molecular Weight	Boiling Point (°C)
2,5-Dimethylpyrazine	108	154-155
2,6-Dimethylpyrazine	108	153-154
2-Ethylpyrazine	108	152-153
2,3-Dimethylpyrazine	108	154-156
4,5-Dimethylthiazole	113	157-158

The substitution of a stationary phase that is capable of interacting (e.g. by dipole interaction or hydrogen bonding) to a greater degree with the solutes of interest will permit their separation. Knowing exactly what substitution to make may be far from simplistic, and usually entails some degree of trial and error. Burns and Hawkes (7) attempted to relate quantitatively modes of interaction with specific stationary phases through interaction indices. These types of generalizations can aid the chromatographer in appropriately selecting a stationary phase.

Figure 1B shows a chromatogram of the same L-rhamnose/L-cysteine extract on a DB-210 (50% trifluoropropyl methylsilicone) capillary column. The DB-210 stationary phase separates solutes primarily on the basis of high dipole interactions, an interaction the DB-5 column does not possess. The DB-210 column appears to have greater selectivity and retains the later eluting components for a longer time resulting in enhanced separation. However, the cluster of peaks previously coeluting still remains, indicating that the phase is not yet selective enough for separation of the earlier eluting peaks.

When both the stationary phase and the solute possess permanent dipole moments, the alignment of the two dipoles can result in a strong interaction. In some cases the close proximity of a strong dipole in the stationary phase can even generate an induced dipole in the solute. The DB-23 (50% cyanopropyl methylsilicone) is one such phase that generates extremely strong dipole-dipole interactions. Figure 1C displays the chromatogram of the identical L-rhamnose/L-cysteine extract on a DB-23 column. This particular column significantly improves the separation of the volatile

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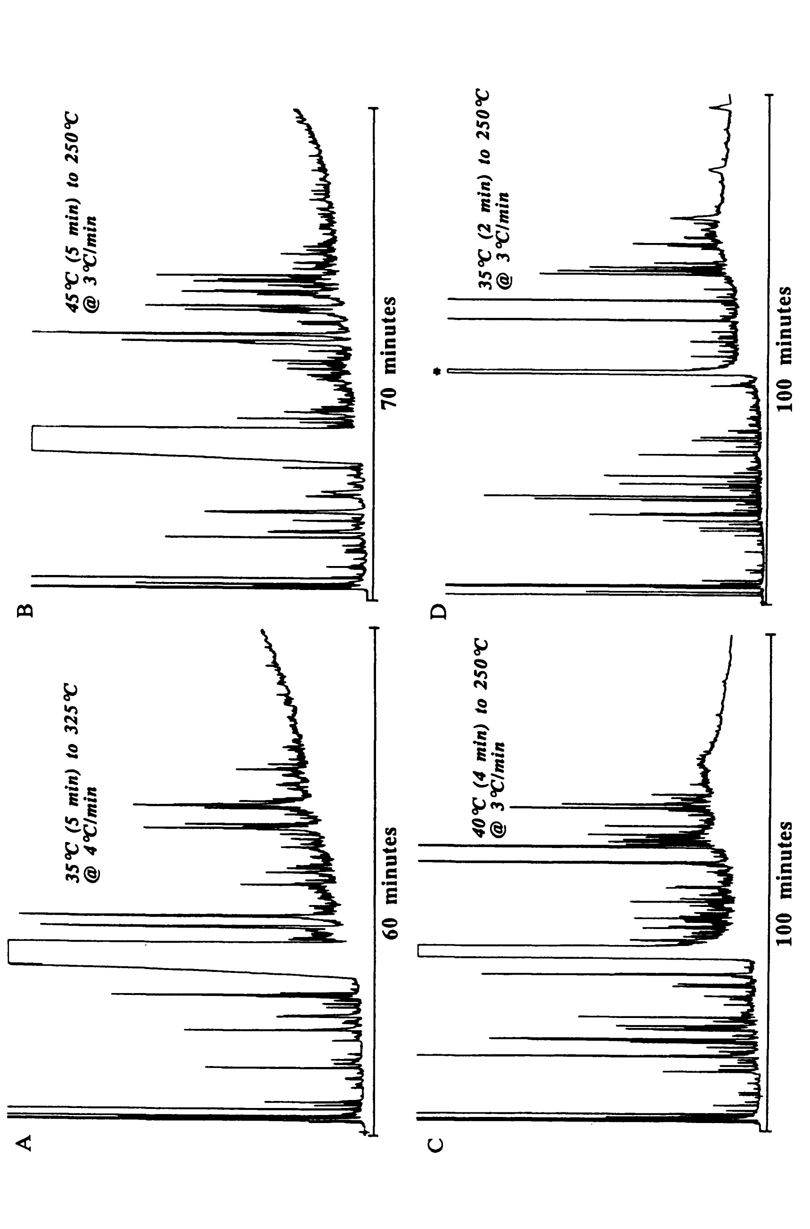

Figure 1. Chromatogram of an L-rhamnose–L-cysteine extract obtained on a 30 m × 0.25 mm i.d. (d_f = 0.25 μm) (A) DB-5, (B) DB-210, (C) DB-23, and (D) DB-WAX capillary columns. Helium carrier gas flow was set to 33 cm/sec with a split injection of 1:40. Injector and detector temperatures were 250 and 275 °C, respectively.

components compared to either of the other two columns mentioned previously, however, at the expense of a longer analysis time.

Thusfar, we have discussed only the application of polysiloxane stationary phases for solute separation. Another type of extremely useful material for separating polar volatile compounds is the polyethylene glycol stationary phases. These phases can also participate in a high degree of hydrogen bonding, and are considered highly selective.

Figure 1D shows a chromatogram of the L-rhamnose/L-cysteine reaction extract on a DB-WAX (polyethylene glycol) capillary column. The increased selectivity due to the hydrogen bonding interactions, coupled with moderate dispersive and dipole forces, resulted in the best separation of all the phases tried. However, because of the increased selectivity and "retentiveness" of this phase, analysis time was increased. Even though more of the peaks in the mixture were resolved, a large peak marked with an asterisk shows backside tailing and is indicative of extreme activity with the stationary phase. Following GC/MS and retention data analysis, this compound was identified as 2,5-dimethyl-4-hydroxy-3(2H)-furanone (DMHF or Furaneol).

The hydroxyl and ketone moieties on this compound make it susceptible to intense hydrogen bonding interactions with the polyethylene glycol stationary phase. Furthermore, DMHF has been found in high concentration as a sugar decomposition product in this and other studies of the Maillard reaction. Its high concentration may therefore also be contributing to the intense activity by overloading the capillary column.

A major disadvantage of polyethylene glycol stationary phases is oxygen sensitivity, especially at higher temperatures. This results in significantly shorter column lifetimes and higher bleed rate during analysis. Among the decomposition products of these phases are acetaldehyde and acetic acid (8), of which both are found in food systems. These problems can be minimized by the removal of oxygen from the carrier gas.

A final example of column stationary phases is a newly developed high temperature 50% phenyl methylsilicone (DB-17ht) capillary column. A chromatogram of a L-rhamnose/L-tryptophan reaction extract is shown in Figure 2. The early part of the chromatogram is again indicative of a low selective stationary phase for this type of analysis. However, several compounds were separated at elevated temperatures to 365°C. No compound identifications were performed in this analysis, but the compounds were shown to contain nitrogen following analysis by a nitrogen/phosphorus detector, also shown in Figure 2. This example shows the great potential for the DB-17ht to aid in the separation and identification of novel high molecular weight Maillard reaction products.

Retention

Now that we have identified the selectivity of the stationary phase (the α term in the fundamental equation of resolution) required to separate the Maillard reaction products, the other variables can be adjusted to fine tune the chromatographic separation.

The solute partition ratio (k) is defined as the amount of solute in the stationary phase compared to that in the mobile phase. The value for k is calculated for each component from the corrected retention time (t'_R) and the gas hold up time (t_m) shown in Equation 3.

$$k = \frac{t_R - t_m}{t_m} = \frac{t'_R}{t_m} \qquad (3)$$

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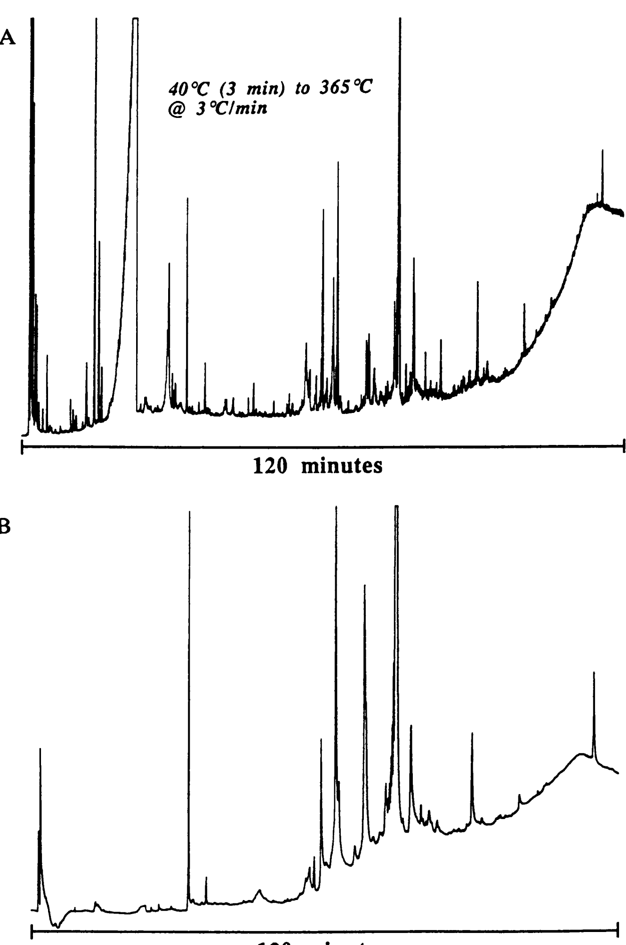

Figure 2. Chromatograms of an L-rhamnose/L-tryptophan extract on a 30m x 0.25 mm i.d. DB-17ht (50% phenyl methylsilicone) high temperature capillary column obtained by FID (A) and NPD (B). Injector and detector temperatures were both 300°C. All other conditions were as described in Figure 1.

Optimizing the value of k is extremely important for the analysis of volatile compounds such as Maillard reaction products. k is strongly dependent upon temperature and the phase ratio (β) of the column. The relationship between k, β, and K_D is expressed below in Equation 4.

$$K_D = \beta k \qquad \text{where } \beta = \left(r/2d_f \right) \text{ and } k = \left(\frac{C_s}{C_m} \right) \left(\frac{2d_f}{r} \right) \quad (4)$$

Since the value of k is strongly dependent upon temperature, column temperature is clearly the most convenient way to affect k. However, 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. Another route to influencing k is through the column phase ratio (β), which is a function of column radius (r) and stationary phase film thickness (d_f). β can then be optimized by either selecting a column with a smaller radius or by using a column with a greater film thickness. Since there are limitations in decreasing column radius, a standard 0.25 mm i.d. column was used throughout this study. Grob and Grob (9) were among the first to explore the practical significance of varying d_f, particularly with thicker film columns.

Figure 3 compares the separation of the L-rhamnose/L-cysteine reaction extract on a DB-WAX column using both 0.15 and 0.50 µm film thickness. A clear observation is noted in the significant increase in column bleed in the thicker film column. It is interesting to note that the amount of normal column bleed will increase with increasing film thickness, column diameter and length. It is evident from these chromatograms that increasing the film thickness directly decreases β and subsequently increases k. This increase in k allows for observably improved separation of the more volatile components at the front of the chromatogram. For the higher molecular weight solutes eluting late in the chromatogram we endeavor to reduce the solute partition ratio (k). Therefore, increasing film thickness directly increases k and leads to peak broadening, loss of resolution, and longer analysis time. Decreasing the film thickness leads to the opposite effect on high boiling point compounds: sharper peaks and shorter analysis time. For most applications, however, a standard 0.25 µm film thickness will provide the required separation for low and higher boiling point compounds alike.

Efficiency

Gas chromatographic separation using open tubular capillary columns is often referred to as high resolution gas chromatography because it is possible to generate very large theoretical plate numbers. Equations by van Deempter et al. (10) and Golay (11) make it obvious that column efficiency is inversely proportional to column diameter. However, as stated previously there are practical limitations to decreasing column diameter and for most separations, a 0.25 mm i.d. column will be suitable. Column diameters equal to or less than 0.10 mm lead to complications in sample injection, sample capacity, overloading, detection, and/or data acquisition and processing.

Another method of increasing column efficiency is to increase column length. However, the advantages here are limited since analysis time is proportional to column length, and the resolution increases only with the square root of column efficiency. Also, because optimum gas velocities vary inversely with column length, solutes must not only traverse a greater column length, but do so at a lower velocity per unit length. In addition, column pressure drop varies directly with column length. High pressure drops not only complicate the injection process, but also generate steep van Deempter curves (5).

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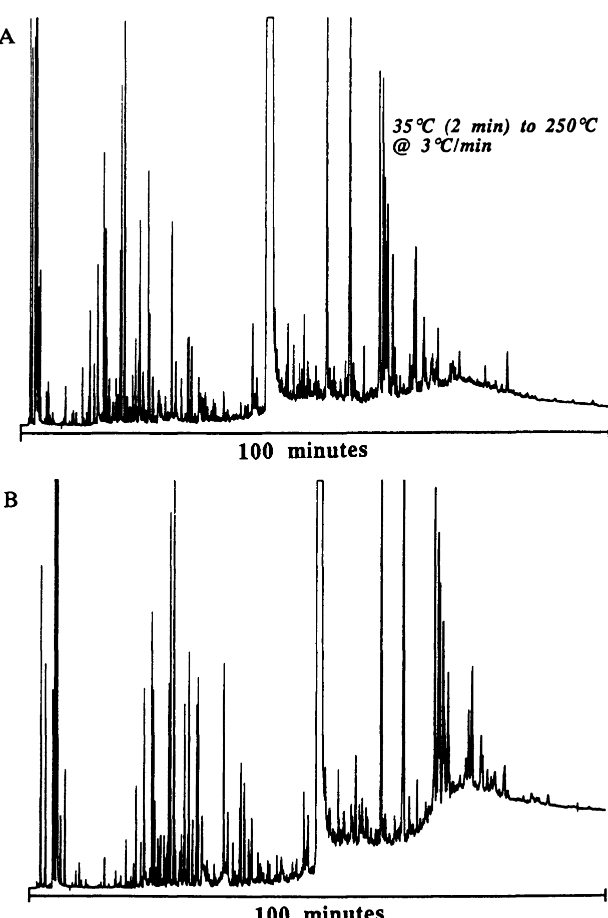

Figure 3. Chromatograms of an L-rhamnose/L-cysteine extract on a DB-WAX capillary column with a film thickness of 0.15 μm (A) and 0.5 μm (B). GC conditions were as described in Figure 1.

Figure 4 compares the chromatographic separations of the L-rhamnose/L-cysteine reaction extract on a DB-WAX column using both a 30 and a 60 meter column. As expected the advantages of using a longer column to generate more theoretical plates are shadowed by minute differences in separation and longer analysis time. Only with the very volatile compounds early on in the chromatogram is separation enhanced to any extent. Ideally, the column length should be just sufficient to provide the number of theoretical plates required for the separation, and no longer. In this case, a 30 meter column provided the theoretical plates necessary to yield adequate separation of the Maillard reaction products.

Figures 5 and 6 show examples of extracts obtained from different sugar/amino acid mixtures chromatographed on a 30 m DB-WAX column. These examples are practical results of exploiting several GC variables to optimize separation of a complex matrix.

Specific Detection Systems

Optimization of the proper chromatographic parameters to reach a suitable separation is normally only part of the overall analytical goal. Specific detection systems can play a crucial role in component identification. For most applications an initial screening of the components with a flame ionization detector (FID) is preferred and recommended. However, the use of specific detection systems such as the nitrogen/phosphorus detector (NPD) and the flame photometric detector (FPD) increase the selectivity and sensitivity to nitrogen- and sulfur-containing compounds, respectively. The sensitivity of such detectors can be several orders of magnitude greater than the FID.

Figure 7 shows chromatograms of the L-rhamnose/L-cysteine reaction extract on a 30 m DB-WAX column generated from an FID, NPD, and FPD, respectively. Assuming that the carrier gas flow rates and the temperature program are the same, one can compare the chromatograms to identifiy which components contain carbon, nitrogen, sulfur, or a combination of these elements. It is interesting to note that some component peaks not observed in the FID scan are very abundant in the element specific scans. Figure 8 compares the NPD chromatograms of L-rhamnose/L-cysteine and L-rhamnose/DL-alanine extracts. The distribution of nitrogen-containing compounds is quite different in the two extracts and may be useful in characterizing chemical activities and aromas.

Lastly, interfacing gas chromatography to mass spectrometry provides the analyst with the ultimate detection system. The relatively unique spectrum obtained from the fragmentation of each compound, coupled with gas chromatographic retention data and element specific detection allow the analyst to identify nearly all components of a complex mixture.

CONCLUSIONS

The volatile compounds produced by microwave heating of Maillard model systems are extremely complex and pose a significant challenge to the chromatographer. By sequentially optimizing specific chromatographic parameters (n, k, and α) we were able to separate the Maillard reaction products to a high degree. Each of these parameters play a role in the separation process, however, to differing degrees. The results obtained in this study are simply one approach to solving such a chromatographic problem, and separation of other types of volatile aroma compounds may require quite different parameters.

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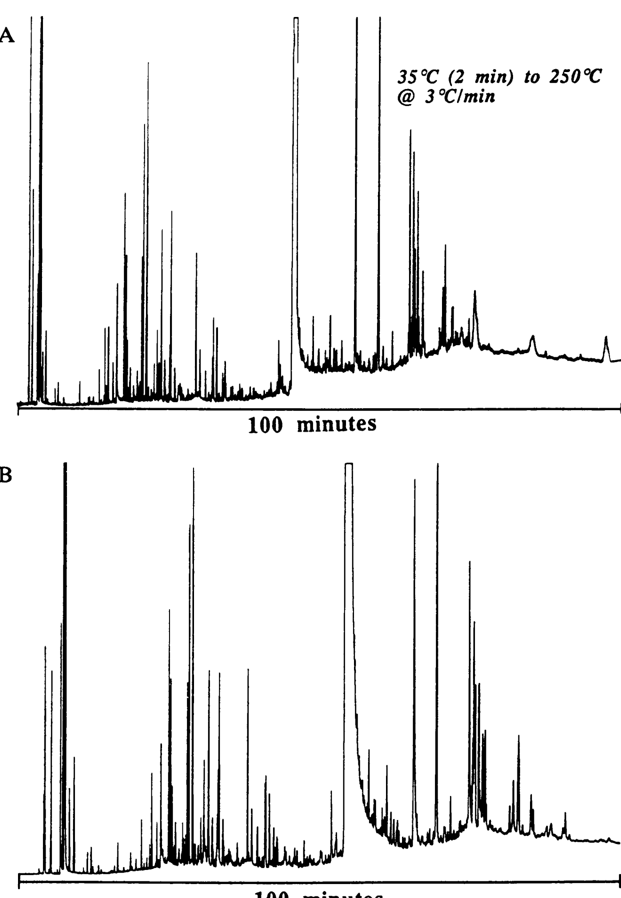

Figure 4. Chromatograms of an L-rhamnose/L-cysteine extract on a 30 m (A) and 60 m (B) DB-WAX capillary column. GC conditions were as described in Figure 1.

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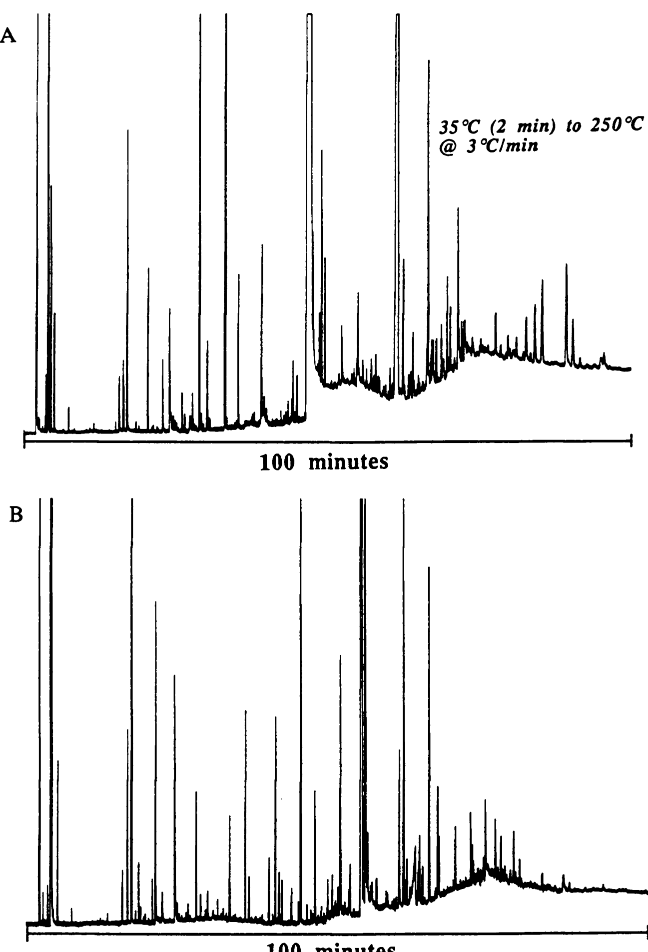

Figure 5. Comparison of the chromatograms of L-rhamnose/glycine (A) and D-glucose/glycine (B) extracts obtained on a DB-WAX capillary column. GC conditions were as described in Figure 1.

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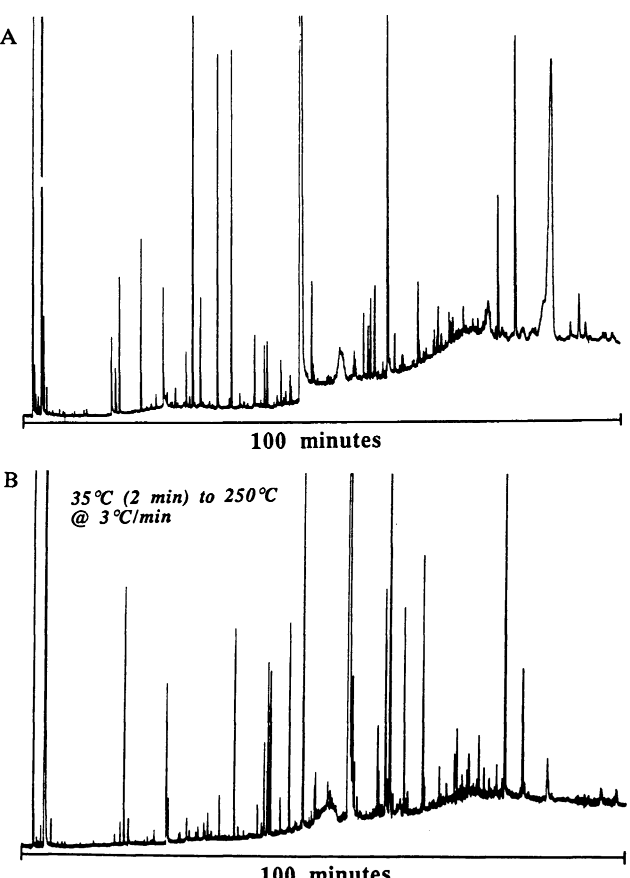

Figure 6. Comparison of the chromatograms of L-rhamnose/L-tryptophan (A) and D-glucose/L-tryptophan (B) extracts obtained on a DB-WAX capillary column. GC conditions were as described in Figure 1.

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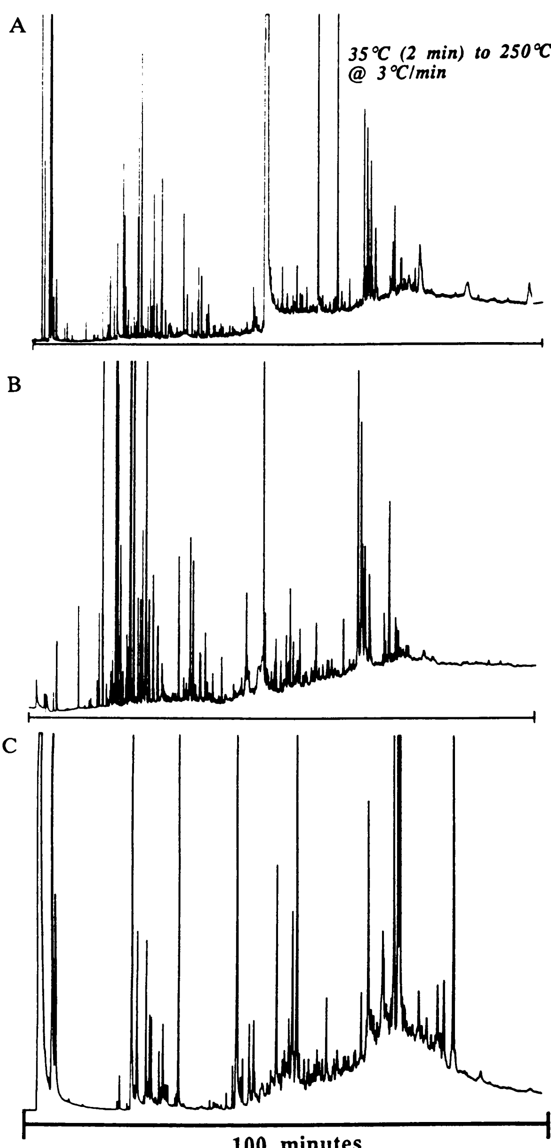

Figure 7. Comparison of chromatograms of an L-rhamnose/L-cysteine extract obtained on a DB-WAX capillary column by FID (A), NPD (B) and FPD (C). GC conditions were as described in Figure 1.

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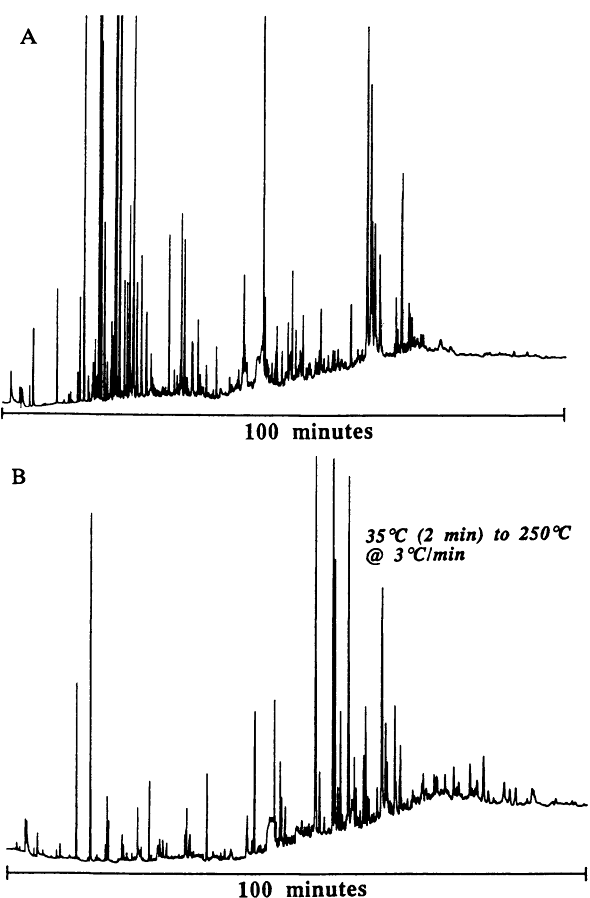

Figure 8. Comparison of the chromatograms of L-rhamnose/L-cysteine (A) and L-rhamnose/DL-alanine (B) extracts obtained on a DB-WAX capillary column by NPD. GC conditions were as described in Figure 1.

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Chapter 7

Gas Chromatography–Olfactometry of Glucose–Proline Maillard Reaction Products

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The aroma produced by the reaction between glucose and proline at 200 °C is primarily due to 5 compounds: diacetyl, 2-acetyl-1,4,5,6-tetrahydropyridine, 2-acetyl-1-pyrroline, 2-acetyl-3,4,5,6-tetrahydropyridine, and furaneol. Gas chromatography-olfactometry using the technique CharmAnalysis ™ produced chromatograms of odor-activity which, with gas chromatography - mass spectroscopy, allowed the identification of the odor-active compounds based on their retention index. At a lower reaction temperature, 180 °C, the minor odor-active compounds were not detected, thus altering the aroma profile. Maltoxazine, a major volatile, produced zero odor potency while the main volatile product of the reaction, 5-acetyl-2,3-1H-pyrrolizine, was found to contribute only 0.3 % to the total aroma.

Maillard browning is one of the most complex and important odor and color producing reactions in food. In order to study the contribution of the Maillard reaction to flavor, the volatile products of a model reaction between a particular amino acid and monosaccharide are often identified. Several comprehensive books and review articles contain a vast amount of Maillard reaction literature, covering both model reactions as well as in actual food systems [1-4].

More than 120 volatiles produced by monosaccharide-proline reactions have been characterized including twenty-two 2,3-dihydro-1H-pyrrolizines and twenty-eight additional pyrrolidines, pyrrolines, piperidines, pyrroles, pyridinones, pyridines, pyrazines, and pentenimines [5-9]. Specific glucose-proline reaction products include furfuryl alcohol, diacetyl, 2-acetyl-1,4,5,6-tetrahydropyridine, furaneol, 2-acetylpyridine, and the most abundant volatile, 5-acetyl-2,3-dihydro-1H-pyrrolizine [7,10,11]. The odors of many of these glucose-proline reaction products are caramel, bready, cracker-like, burnt, and maple. The presence of a volatile with odor, though, is not a measure of it's relative importance to Maillard browning aroma. The gas chromatography-olfactometry (GCO) techniques called CharmAnalysis [12] and aroma extraction dilution analysis (AEDA)[13] are odor bioassays in which the dilution to threshold approach quantitates each odor's potency. In this study, the CharmAnalysis procedure was applied to determine

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which of the many volatiles produced by a glucose-proline reaction are important to the aroma, based on odor potency.

Materials and Methods

Chemicals. Pure samples of the diacetyl, 2-acetyl-pyridine, and furaneol were obtained commercially (Aldrich, Milwaukee, Wis.) while 2-acetyl-1-pyrroline was a gift from Ron Buttery. The synthesis of 2-acetyl-1-pyrroline was reported by Buttery[14]. 2-Acetyl-1,4,5,6-tetrahydropyridine and it's tautomer, 2-acetyl- 3,4,5,6-tetrahydropyridine were synthesized by heating sodium bisulfite, proline, and dihydroxyacetone, dissolving the product in water, adding NaOH, and extracting with pentane[15]. The pentane extract was analyzed by GC/MS employing the conditions described below and found to contain the two tautomers of 2-acetyl-tetrahydropyridine as the major products.

Sample Preparation. *Reaction of L-Proline and D-Glucose.* Equimolar amounts (.02 mol) of D-glucose and L-proline dissolved in 100 mL of water were heated for 1 min at 180 ^{0} C and 200 ^{0} C in a closed reactor as described in "High temperature short time kinetics for the formation of Maillard products in the proline/glucose model system", Stahl, H.D. and Parliment, T.H., *ibid.* The resulting aqueous solutions were extracted sequentially with an equal volume of Freon and an equal volume of ethyl acetate. Both Freon and ethyl acetate phases were dried with anhydrous magnesium sulfate.

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Gas Chromatography-Olfactometry (GCO). The CharmAnalysis bioassay characterizes potent odor-active volatiles when humans sniffing the gas chromatographic effluent note the presence of an odor at a particular retention index (Kovats). In this study, one μL samples were injected onto a GCO system made from a Hewlett Packard 5890 Gas Chromatograph (Datu Inc. Geneva, NY). Two Hewlett Packard fused silica capillary columns were used: Carbowax 20M (12 m x 0.32 mm x 0.3 μm film thickness) and OV101, a cross-linked methyl silicone (12m x 0.32mm x 0.52μm film thickness). The samples were injected at 35 ^{0}C and after 3 min, the oven temperature was raised by 6 ^{0} C/min to 225 ^{0}C (200 ^{0}C for Carbowax). The column was either connected to a flame ionization detector for determination of n-paraffin retention times in order to calculate retention indices or to an olfactometer. The olfactometer mixed the effluent into a stream of humidified air (20L/min). A person who was previously screened for olfactory ability with a set of 6 standard odors sniffed the effluent. Responses were recorded as the presence and quality of odor, and the time recorded on a Macintosh computer using specially designed software (DATU, Geneva, NY).

The samples were diluted by factors of three and the analysis repeated until no more odor was observed (the detection threshold). The combination of these runs produced a charm chromatogram with odor potency defined as the area of the chromatographic peaks. This is in contrast to the dilution value, which is peak height. All charm values reported are the sum of the Freon and ethyl acetate fractions. Theoretically, charm is proportional to the ratio of the amount of odor compound to it's detection threshold[16]. The compounds with high charm values were then identified by matching their mass spectra, retention indices on two substrates, and odor quality to authentic standards. Compounds with no authentic standard were labelled tentatively identified.

Capillary Gas Chromatography / Mass Spectrometry (GC/MS). The characterization of the odor-active compounds was done by concentrating the original extract 270 fold and injecting 1μL on a Hewlett Packard 5890 Gas Chromatograph connected to a 5970 Series Mass Selective Detector, operated in electron impact at 70 eV. Two fused silica Hewlett Packard capillary columns were used: OV101, a methyl silicone, (25m x 0.2mm x 0.33μm film thickness) and Carbowax 20M (25m x 0.32mm x 0.3μm film thickness). The samples were injected at 35 ⁰ C and after 3 mins, the oven temperature was raised by 4 ⁰C/min to 225 ⁰C (200 ⁰ C for Carbowax 20M). In comparison to GC/MS, the rate of temperature increase for GCO was slower and the column was half as long to minimize charm chromatogram acquisition time. The mass spectrum of each odor-active compound was determined by using n-paraffin retention indices (RI) between GCO and GC/MS columns.

Results and Discussion

CharmAnalysis. The predominant odor qualities characterizing glucose-proline reaction products were burnt caramel, popcorn, and cotton candy. Nutty was previously used to describe the main odor in the glucose-proline reaction over a range of reaction temperatures [17]. Over 93% of the aroma potency in the glucose-proline reaction, as measured in charm units, can be explained by 4 compounds, and over 98% by 7 compounds, as seen in Table I.

The two charm chromatograms made using different capillary columns are shown in Figure 1. The 200 ⁰ C samples on Carbowax 20M yielded a charm chromatogram with more narrow retention indices and better separation of the odor active regions than on the OV 101 column. Thus, five main odor-active compounds (No. 1,2,3,5 and 6) were seen.

The largest peak in the OV101 charm chromatogram, RI 1000-1100, was separated into 4 odor-active compounds on Carbowax 20M at RI 1332, 1539, 1619, and 1982. The compound with the largest odor potency was 2-acetyl-3,4,5,6-tetrahydropyridine, which when synthesized, showed a retention index of 1110 on OV 101 and 1613 on Carbowax. A very similar mass spectrum to that of 2-acetyl-3,4,5,6-tetrahydropyridine was seen at RI 1018 on OV101 and RI 1331 on Carbowax, due to the presence of the tautomer, 2-acetyl-1,4,5,6-tetrahydropyridine. These tautomers have very low odor thresholds, 1.6 μg/L in water [18]. It has been reported that the tautomers are formed in concentrations up to 200 μg/g in proline model experiments with reducing sugars, as well as detected in the low ng/g range in beer, malt, and bread[5]. A possible mechanism for their formation involving intermolecular condensation to form 5-substituted hexahydrocyclopenta-(b)-pyridinones has been proposed [5].

2-Acetyl-1-pyrroline is a high charm glucose-proline reaction product which has a very low threshold (0.1 μg/L in water) [19] and a popcorn aroma. In cooked, especially aromatic rice, wheat bread crust, and popcorn, 2-acetyl-1-pyrroline is a major contributor to the odor [19-21]. Model experiments have shown the formation of 2-acetyl-1-pyrroline from an acetylation reaction between 1-pyrroline and 2-oxopropanal [22].

Furaneol (2,5-dimethyl-4-hydroxy-3(2H)-furanone) found in the ethyl acetate fraction has a very low odor threshold, 0.03 μg/L[23], and is a character impact compound in the Maillard reaction. It is also a potent natural product contributing to the odor of fruit, especially the strawberry, pineapple, mango, raspberry, and grape [23-26].

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Table I. Odor-active Compounds in Proline/Glucose Reaction

No	Compound (type of identification)	Odor	% Charm	RI OV101	RI C20M	Mass Spectra m/e (relative intensities)
1	Diacetyl (a,b,c)	Buttery	0.5*	604	900	86(M, 13), 44(4), 43(100), 42(12), 41(3)
2	2-Acetyl-1-pyrroline (a,b,c)	Popcorn	19	898	1257	111(M, 15), 83(29), 69(11), 68(13), 43(100), 42(26), 41(55)
3	2-acetyl-1,4,5,6-tetrahydropyridine (a,b,c)	Burnt Caramel	12	1018	1332	125(M, 49), 95(25), 83(49), 82(56), 55(75) 54(59), 43(100), 41(24)
4	2-acetylpyridine (a,b,c)	Caramel	0.5	1005	1539	121(M,83), 120(20), 106(16), 93(58), 79(100), 78(93), 67(13), 52(33), 51(33), 50(32), 43(47)
5	2-acetyl-3,4,5,6-tetrahydropyridine (a,b,c)	Burnt Caramel	63	1110	1619	125(M,74), 124(23), 93(15), 92(10), 83(9), 82(100), 80(13), 55(24), 54(60), 53(10), 52(11), 43(54)
6	Furaneol (a,b,c)	Cotton Candy	3.9	1032	1982	128(M,60), 85(17), 57(58), 43(100)
7	5-acetyl-2,3-1H-pyrrolizine (a,b)	Medicinal	0.3	1354	2033	149(46), 135(9), 134(100), 106(23), 79(18), 78(11), 77(15), 52(11), 51(15), 43(20)
			99 %			

a = mass spectral match , b = retention index match, c = standard match with odor
* 12% of diacetyl recovered after extraction

2-Acetylpyridine is a minor odor compound in the glucose-proline reaction with a caramel aroma. It has been described as having a bread crust or popcorn odor and was isolated in peanuts, beer, and rye crust [18,27-29]. It's reported threshold is 19 ng/mL in water [18].

The "buttery" smelling compound at RI 604 on OV 101 and 900 on Carbowax 20M is diacetyl (2,3-butanedione). It's reported threshold ranges from 2.6 ng/mL to 2.3 µg/mL [18]. The extraction process used in this study showed a low recovery for diacetyl (12 % yield, Laurent, M.H., Cornell University, unpublished data.). Diacetyl was present at higher levels in the original heated fraction than the extracted sample for CharmAnalysis. The analysis of headspace volatiles recovers more diacetyl than extraction. A study of the headspace volatiles of a glucose-glycine mixture heated at 95 ^{0}C for 2 hours showed diacetyl as the major volatile[30]. The 0.5 % charm shown for diacetyl is an underestimate, and the value is more likely 4.2% charm. It's formation is at the early stage of the browning reaction and is assumed to be formed through fission of the C2 -C3 bond or C4-C5 bond of glucose[30]. It's presence is not limited to Maillard browning products, though, and is ubiquitous in dairy products from lactic acid bacteria fermentation [31].

Temperature Effect. Heating at 200 ^{0}C showed three of the same odor active regions (RI 1070, 937, 600 on OV101) found in the 180 ^{0}C reaction but produced more minor odor-active compounds (Figure 2). The compounds 7 and 8 from the 200 ^{0}C reaction were not detected in the 180 ^{0}C reaction. Slightly different aroma profiles emerge from this 20 ^{0}C difference in temperature. This is consistent with the Shigematsu variation theory of Maillard browning aroma formation where higher temperatures cause the amino acid to be degraded by pathways other than the Strecker degredation. Amines and ammonia are formed by decarboxylation and decarboxylation/deamination, thus providing further reactants to produce compounds such as pyridines [32]. In a study investigating the odors of Maillard browning and their change with reaction conditions, aromas became stronger, more unpleasant, aldehydic, and burnt with increasing temperature and time [17]. Charm values of the 200 ^{0}C samples were indeed larger than for the 180 ^{0}C samples, with 8 and 9 dilutions required to reach threshold for 200 ^{0}C samples as compared to 5 and 7 dilutions for the 180 ^{0}C samples. Several low charm aromas which appeared at 200^{0}C were unpleasant: garbage, sweaty, and medicinal.

Comparison of Odor with Amount. The major volatile product in the glucose-proline reaction is 5-acetyl-2,3-1H-pyrrolizine [7,10]. Figure 3 shows the GC-FID chromatogram for the glucose-proline reaction with the pyrrolizine (No. 7) as the major product. The pyrrolizine's odor, though, makes up less than 1% of the total odor-activity as can be seen by the corresponding charm chromatogram. Maltoxazine (No. 9) is another major volatile, at RI 1622 on OV101 and RI 2428 on Carbowax, which does not exhibit an odor. In fact, most of the major volatile products have very little odor, while trace volatiles account for most of the odor potency.

Conclusion

The key odor-active compounds in the glucose-proline reaction have been identified and are the trace, not the major volatile products of the reaction. Figure 4 shows the main compounds that compose 100% of the charm in the reaction sample. The

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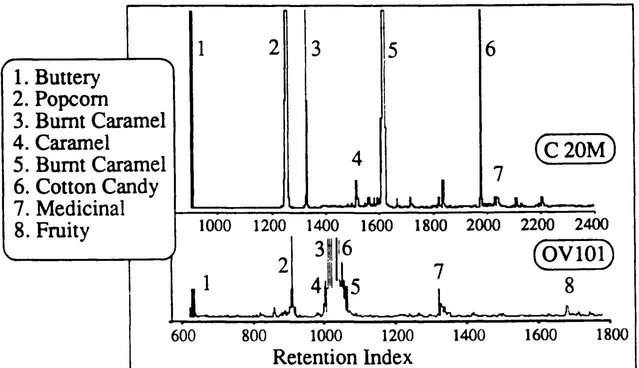

Figure 1. Charm chromatograms of the volatile 200 °C glucose-proline reaction products on OV101 and Carbowax 20M columns. Compound numbers refer to Table I. Y-axis measures dilution value and x-axis is n-paraffin retention index.

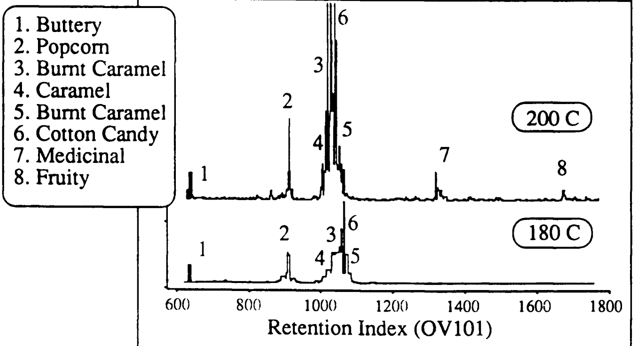

Figure 2. Charm chromatograms of the volatile glucose-proline reaction products under different temperatures, 200 °C and 180 °C. Y-axis measures dilution value and x-axis is n-paraffin retention index.

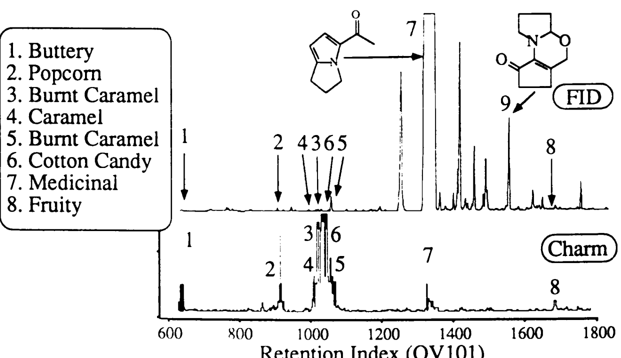

Figure 3. Comparision between a Charm and FID chromatogram of the volatile glucose-proline reaction products at 200 ⁰C. Y-axis measures dilution value and x-axis is n-paraffin retention index.

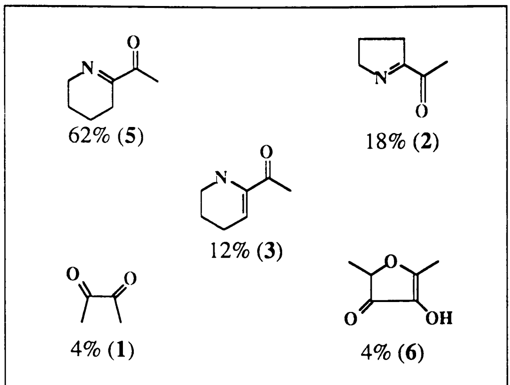

Figure 4. Summary of the major odor-potent compounds in the glucose-proline reaction, with % Charm, 1: diacetyl, 2: 2-acetyl-1-pyrroline, 3: 2-acetyl-1,4,5,6-tetrahydropyridine, 5: 2-acetyl-3,4,5,6-tetrahydropyridine, 6: furaneol.

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relation of percent charm to the odor quality in a mixture has yet to be determined. Based on charm values, one compound dominates the odor, that is 2-acetyl-3,4,5,6-tetrahydropyridine. Two compounds, 2-acetyl-1-pyrroline and 2-acetyl-1,4,5,6-tetrahydropyridine comtribute moderately to the aroma of the glucose-proline reaction. Furaneol and diacetyl contribute minor amounts to the aroma. Quantitating the levels of these potent flavor compounds can be used to optimize the production of food with the desired level of glucose-proline Maillard browning aroma.

Acknowledgments

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Chapter 8

Molasses Flavor Investigations with Sulfur Chemiluminescence Detection

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Sugar cane molasses is a processed food ingredient used as much for its characteristic flavor as its sweetness. Well over eighty volatile compounds have reportedly been found in molasses. Of those, only three contain sulfur. Sulfur volatiles can have a profound effect on the overall flavor of a food and are often present near the limits of detection. The flavor of molasses develops in part as a result of Maillard browning. It is reasonable to expect to find more sulfur compounds based on the precursors available. A fairly new GC detector for sulfur compounds has become available. With its inherent sensitivity, selectivity, and ease of use, the sulfur chemiluminescence detector (SCD) is a powerful tool in flavor research. Its use is demonstrated in detecting sulfur volatiles from molasses flavor isolates.

The term molasses is applied generally to a number of sugary syrups derived from sugar beets, sugar cane, sorghum, citrus, and corn sugar. Although its characteristic flavor makes it a common ingredient in a number of processed foods, it is usually considered a by-product of the sugar industry and is used for its sweetness. Most commercially available molasses comes from the sucrose crystallization process of sugar cane juice.

Molasses is classified according to the number of times it has undergone the reclamation process. There are basically two categories of molasses: imported molasses and New Orleans molasses. Imported molasses has a fine flavor. It is the whole juice of West Indies sugar cane, which has been evaporated to a heavier consistency. It is sweeter than other molasses grades, because it contains all the sugar of the original cane. New Orleans molasses is derived from domestically grown sugar cane. It has a different flavor that results from the shorter maturation time of the Louisiana cane, and the use of sulfur dioxide during clarification. In the production process, first molasses is the resultant residue from the first crystallization of sucrose. Heat is applied during the process and a dark color begins to appear. Second and third molasses are the products after subsequent sucrose extractions. With each extraction, the total sugar content continues to decrease, the flavor becomes stronger, sometimes bitter, and the color gets darker. Blackstrap is the final product from which no more

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sucrose can economically be extracted. It is generally considered inedible and used for animal feed or fermented and distilled to make rum.

For the most part, the flavor of molasses is the result of carbohydrate degradation brought on by acid, thermal, or Strecker degradation, and Maillard browning. Several investigations have been made attempting to characterize the major flavor volatiles present (*1*). A number of isolation, extraction and concentration strategies have been employed. These sometimes elaborate and involved methods combined some form of liquid-liquid extraction of flavor components either before (*2*) or after vacuum distillation (*3*). Fractionation of the resultant isolates was often carried out to either clean up the sample (*4*), focus on the aroma bearing fraction (*3,5*) or simplify the sample for instrumental analysis. Direct analyses of molasses was also accomplished with modified direct inlet systems of a gas chromatograph (*6,7*). Enriquez, Sanz and Cano (*8*) tested a number of strategies for isolating and identifying final molasses volatiles. Along with the more traditional methods of direct liquid-liquid extraction and acid base fractionation, they tested simultaneous distillation-extraction (SDE).

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Flavor Considerations

The number of components identified in molasses flavor (all grades) exceeds eighty compounds. A compounded flavor based on this list lacks total molasses impact. This leads one to suspect that additional key components remain unidentified. It is reasonable to assume that these compounds are present in low concentration, yet impart a significant contribution to the flavor.

Sulfur compounds can be detected by the olfactory system at extremely low levels, and possess some of the lowest threshold values for flavor chemicals. Typical reported threshold values (*9*) are: hydrogen sulfide (0.5 - 10 ppb), methanethiol (0.02 - 2.1 ppb), dimethyl sulfide (0.33 - 12 ppb), methional (0.2 - 10 ppb), 2-isobutylthiazole (2 - 3.5 ppb), and 3,4-dimethylthiophene (1.3 ppb).

Many sulfur compounds are generated by the Maillard reaction (*10-14*); manufacture of molasses provides ample opportunity for these reactions. A more detailed discussion of formation of sulfur compounds via the Maillard reaction appears in another section of this book.

Although a great deal of work has been done isolating and identifying sulfur flavor volatiles, the difficulties involved limit the amount of information we have in respect to identity, quantity, and formation pathways. To date, only three sulfur compounds have been reported in molasses flavor; dimethyl sulfide, carbon disulfide, and sulfur dioxide. Of those, dimethyl sulfide predominates in importance to molasses aroma (*1*). At the appropriate concentration level it has a pleasant molasses aroma. It is thought to arise as a thermal degradation product of S-methyl methionine sulfonium salt.

Sulfur Compound Detection

Selective GC detectors aid in the detection and identification of compounds containing specific groups (halogens with electron capture detector (ECD)) or individual elements

(nitrogen and phosphorus with nitrogen-phosphorus detector (NPD); sulfur and phosphorus with flame photometric detector (FPD); sulfur with sulfur chemiluminescence detector (SCD), electrolytic conductivity detector (ELCD), and ECD). A flavor isolate can contain hundreds of compounds and achieving baseline separation of all components in one GC run is not always possible. With a selective detector, compounds bearing the group or element of interest can be located and quantified even in the presence of co-eluting compounds. Ideally, the specific detector should only respond to the analyte containing the species of interest. In practice, however, this is not always the case. Compounds bearing the same specific element of interest can produce uneven detector response depending upon molecular characteristics such as size, additional functionalities, oxidative state, unsaturation, or other elemental interference.

Selective detection of sulfur compounds is especially important in foods, beverages, pesticides, biological samples, and environmental analyses. This has been predominantly accomplished with the use of the FPD. Based on the original West German patent of Draegerwerk and Draeger (*15*), Brody and Chaney (*16*) produced a gas chromatographic detector specific for phosphorus and sulfur compounds.

In the FPD, column effluent is incinerated in a hydrogen/oxygen flame producing an interrelated product mix of sulfur species from the original sulfur compound. The fuel mix is hydrogen rich and leads to the formation of H_2S, HS, S, S_2, SO and SO_2 when sulfur compounds are added (*17*). The FPD is set up to photometrically detect the chemiluminescence of the excited S_2^* species, with the emission of photons exhibiting a maximum at 394 nm (*16*). When the sulfur compound contains carbon, as in most GC flavor effluents, the complexity of the flame chemistry increases due to CS, CS_2, and OCS species.

Difficulties in its use and non-uniformity of results has led to frustration in the application of the FPD to sulfur compound detection (*17*). Most often cited flaws of the FPD (*16-18*) are its non-linear response, reduced response with co-eluting hydrocarbons (quenching), inconsistent selectivity, sensitivity, and limits of detection (LOD).

Other schemes to selectively detect sulfur compounds from GC effluents have been devised (*19-22*). Generally, these detection techniques suffer from one or all of a number of disadvantages including varying sensitivities, operational complexity, large size, or prohibitive cost. Detection based on chemiluminescence continues to receive a great amount of attention due to its inherent selectivity and sensitivity.

Recently, a new sulfur selective detector has become commercially available (Sievers Research, Inc., Boulder, CO). Similar to the FPD, the detection scheme incorporates combustion of the GC column effluent in a hydrogen rich flame with chemiluminescence detection. However, the sulfur species response is due to an excited state of sulfur dioxide (SO_2^*), not S_2^*. The SO_2^* is produced by the reaction of SO with ozone (O_3) in a separate reaction cell. Even though SO is a free radical it can be sufficiently stabilized in a flow system under reduced pressure (*23,24*) to be sampled and transferred to a vessel to react with O_3. Based on these observations, Benner and Stedman (*25*) concluded that SO produced in a flame could be easily detected. They modified a redox chemiluminescence detector (*26*) to produce a Universal Sulfur Detector (USD). A linear response between 0.4 ppb and 1.5 ppm

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(roughly equal to 3 to 13,000 pg of S/sec) was demonstrated with equal response to the five sulfur compounds tested. This detection scheme has been utilized as the basis for the commercially available GC detector.

Evaluation of the commercially available GC detector, now known as the sulfur chemiluminescence detector (SCD), was detailed by Shearer et al. (*24*). Emphasis was focused on chemical and petrochemical applications, but the fundamental characteristics are relevant to other fields as well. Subsequent work by Benner, Hosick, and Stedman (*27*) confirmed that the sulfur species undergoing reaction with O_3 was indeed SO. The detector can be interfaced with any GC having a flame ionization detector. A ceramic sampling probe is positioned above the tip of the FID flame. Approximately 90-95% of the flame combustion products (*28*) are drawn into the ceramic probe via a transfer line, under reduced pressure, to the reaction chamber. The formation of SO in the flame is optimized by adjusting the air and hydrogen flows to the FID. Oxygen or compressed air is passed through an ozone generator situated adjacent to the reaction cell. The SO from the flame combustion of sulfur compounds reacts with O_3 under reduced pressure to produce SO_2^*, which then emits photons as it returns to its ground state. The light is detected by a photomultiplier tube (PMT) with a UV band pass filter (225 - 450 nm) located between the reaction cell and the PMT. The combination of the flame and UV band pass filter virtually eliminates interferences from non-sulfur containing analytes. The reduced pressure is provided by a rotary vane pump which also serves to sweep the cell of product and unreacted effluent. A Hopcalite trap is used for removal of unreacted ozone and oxides of nitrogen prior to the trap. The signal from the PMT is processed by the electronics of the SCD for output to a recording device. The FID signal and the SCD response can be recorded simultaneously due to the mounting configuration of the SCD probe. However, the flow rate of hydrogen relative to air in the flame for optimum SO production results in a reduction in FID sensitivity by about two orders of magnitude (*24*).

The reported advantages of the SCD (*27,29,30*) over other existing sulfur selective detection systems include linear dynamic range between three and five orders of magnitude, high selectivity for sulfur (10^5 for compounds containing heteroatoms; 10^6 for hydrocarbons (*24*)), absence of quenching due to co-eluting compounds, lower detection limits, and a nearly equimolar response for all sulfur compounds.

The SCD has been optimized for use as a GC detector. Work has also been done investigating the optimum GC capillary column dimensions and stationary phases (*28*) for sulfur compound analyses. A range of column lengths (15 - 100 meters), interior diameters (0.25 - 0.53 mm i.d.), and film thicknesses (0.5 μm - 5.0 μm df.) were explored. Less stable stationary phases tend to foul the ceramic probe and build up inside the reaction cell of the SCD. Fairly thick film coatings of methyl silicone tend to work out best for retaining and separating sulfur compounds with minimal bleed problems.

The aim of this research is to focus on the sulfur compounds to be found in molasses flavor. An integrated analytical approach to isolation, detection, and identification is used which includes sample preparation (SDE, purge and trap), GC separation, SCD detection, and mass spectral identification.

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Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch008

Analyses

Instrumentation. A Sievers sulfur chemiluminescence detector model 350A was interfaced with a GC/FID for the selective detection of sulfur compounds. Liquid isolates were analyzed using a Hewlett-Packard 5890A GC/FID equipped with an on-column injector. Trapped headspace volatiles were thermally desorbed into a Varian 3400 GC/FID. GC/MS analyses of the liquid isolates and thermally desorbed volatiles were performed on a Hewlett-Packard 5890 Series II GC interfaced with a HP 5971A MSD and a Varian 3400 GC interfaced with a Finnigan ITS40, respectively. The GC columns used for the studies were 30m x 0.32mm id. fused silica capillary with a 4μm film stationary phase of methyl polysiloxane (SPB-1).

SCD Performance. The performance characteristics of the SCD were checked by making injections of dimethyl sulfide with either diethyl ether or dichloromethane as the solvent. Sensitivity and linear range tests were made with dimethyl sulfide in diethyl ether, because the analyte elutes separately from the solvent. Evaluating the SCD for quenching by other coeluting species was accomplished by injections of dimethyl sulfide in dichloromethane, since the two coelute under the chromatographic operating conditions.

The detector exhibited a linear response over three orders of magnitude with the limit of detection being 44 picograms of sulfur (pg S as dimethyl sulfide) at a signal to noise ratio of 3:1. No signal quenching was observed. The GC oven conditions for the tests were: 30° - 60°C at 7 °C/min, helium carrier gas at 35 cm/sec, 1μl injection on-column. The SCD integration time was set at 0.03 sec.

Micro-SDE. Molasses flavor isolates were prepared using a micro-steam distillation-extraction apparatus (Chrompack). Two different solvents (diethyl ether and dichloromethane) were used to check qualitative extraction differences. Three different commercially available molasses (Table I) were extracted with both solvents resulting in six different isolates. Three hundred fifty grams of molasses mixed with one liter

Table I. Molasses Flavor Isolates

SAMPLE	MOLASSES	SOLVENT
1A	BRER RABBIT GREEN LABEL	$(CH_3CH_2)_2O$
1B	BRER RABBIT GREEN LABEL	CH_2Cl_2
2A	BRER RABBIT ORANGE LABEL	$(CH_3CH_2)_2O$
2B	BRER RABBIT ORANGE LABEL	CH_2Cl_2
3A	GRANDMA'S	$(CH_3CH_2)_2O$
3B	GRANDMA'S	CH_2Cl_2

of water was distilled for one hour. Volatiles were concurrently extracted into a final solvent volume of between 0.5 and 1 ml. The flavor isolate was then dried over anhydrous sodium sulfate to remove all water. The flavor isolates were immediately injected onto the GC/FID/SCD and the signals simultaneously recorded. Directly afterward a second injection of the same isolate was made on the GC/MS. The ovens were temperature programmed as follows: initial temperature, 30°; initial time, 1 min; 30° to 220° at 2°/min; final time, 20 min. Helium carrier gas at 30 cm/s was used.

Dynamic Headspace Purge and Trap/Thermal Desorption. Headspace volatiles were collected with a Dynatherm Dynamic Thermal Stripper 1000. Purified nitrogen was used to purge the headspace above approximately 5 grams of molasses inside a 60 cc sample vial. The flow rate was 90 ml/min for 30 minutes. Stripper temperatures were: oven, 90 °C; block, 70 °C; tube, 70 °C. Sorbent traps were packed with a layered progression of Tenax TA/Ambersorb XE-340/Charcoal to effectively trap all purged volatiles.

Thermal desorption was accomplished with a Dynatherm ACEM 900 system. Sample tubes were desorbed at 220° for 3 minutes. The focusing trap was packed with Tenax TA and Carboxen 1000 and desorbed for 5 minutes at 220°. The valve box and transfer lines were maintained at 235°. Column flow was maintained at 30 cm/s. The oven program was as described for the liquid samples.

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Results

The selective detection of sulfur compounds from an SDE isolate is shown in Figure 1. The top trace is the FID chromatogram of sample 3A and the bottom is the corresponding SCD trace. The chromatograms were individually recorded from separate 3μl injections. Results from the six different samples indicate at least fifteen sulfur species are present. Of particular interest is the qualitative similarities between the molasses samples (Figures 2a-c). All molasses samples contain the same major sulfur peaks, with the exception of the peak appearing at about 21 minutes. In the B sample chromatograms, the peak appears as a singlet. The A samples show this to be a doublet. Quantitative differences for individual sulfur peaks are noticeable, but in the absence of an internal standard, specific conclusions cannot be drawn. However, some general quantitative effects can be observed. The B samples show a greater overall concentration of peaks in the earlier portions of the chromatograms than do the A traces. Also, the second sulfur peak appearing at about 6.5 minutes is significantly larger for the B samples.

Of the sulfur peaks detected, five have been positively identified by GC/MS, and confirmed with authentic compound injections (Table II). A sixth identification was tentatively made but not confirmed (peak 4). The retention times listed are referenced to the sample 3A SCD chromatogram (Figure 1).

The SCD chromatogram from the thermal desorption of trapped volatiles is shown in Figure 3. At least fourteen sulfur compounds have been detected in sample 2. Both methanethiol and dimethyl sulfide have been positively identified. Other identifications are pending further work.

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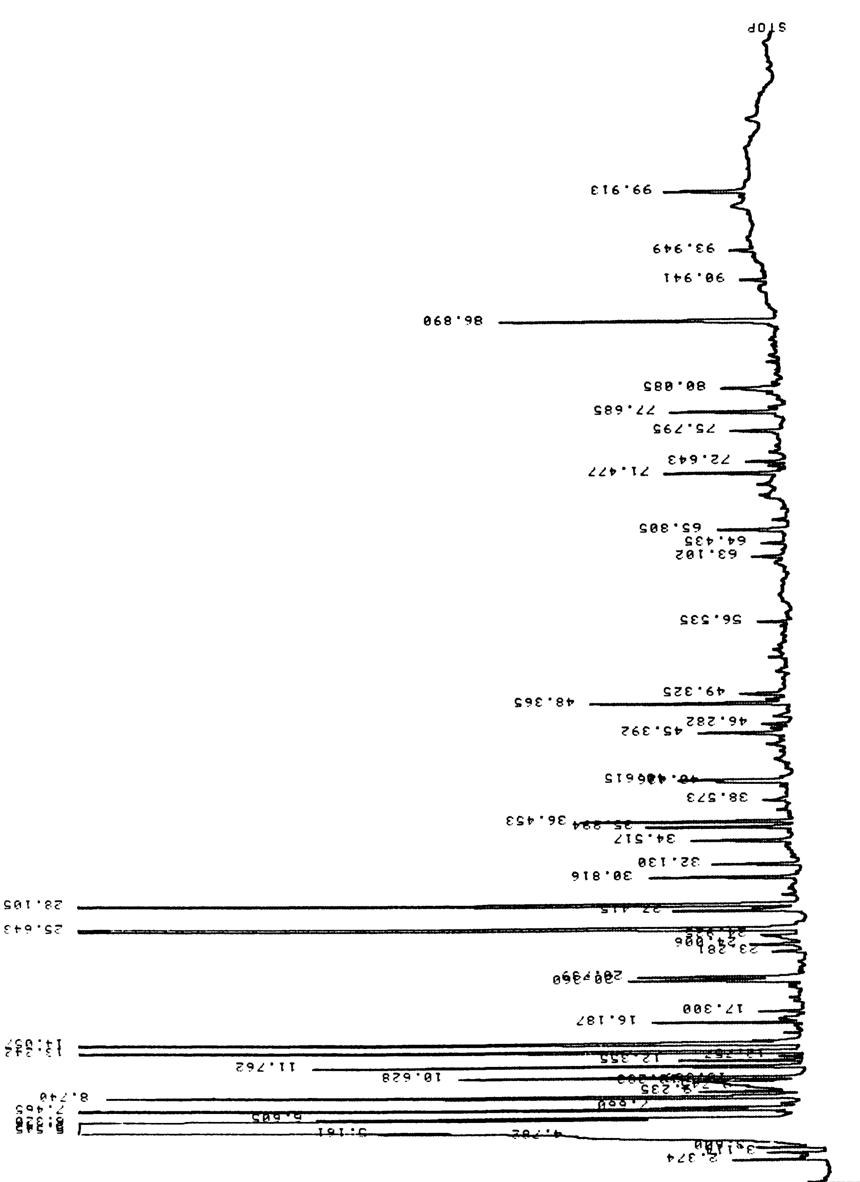

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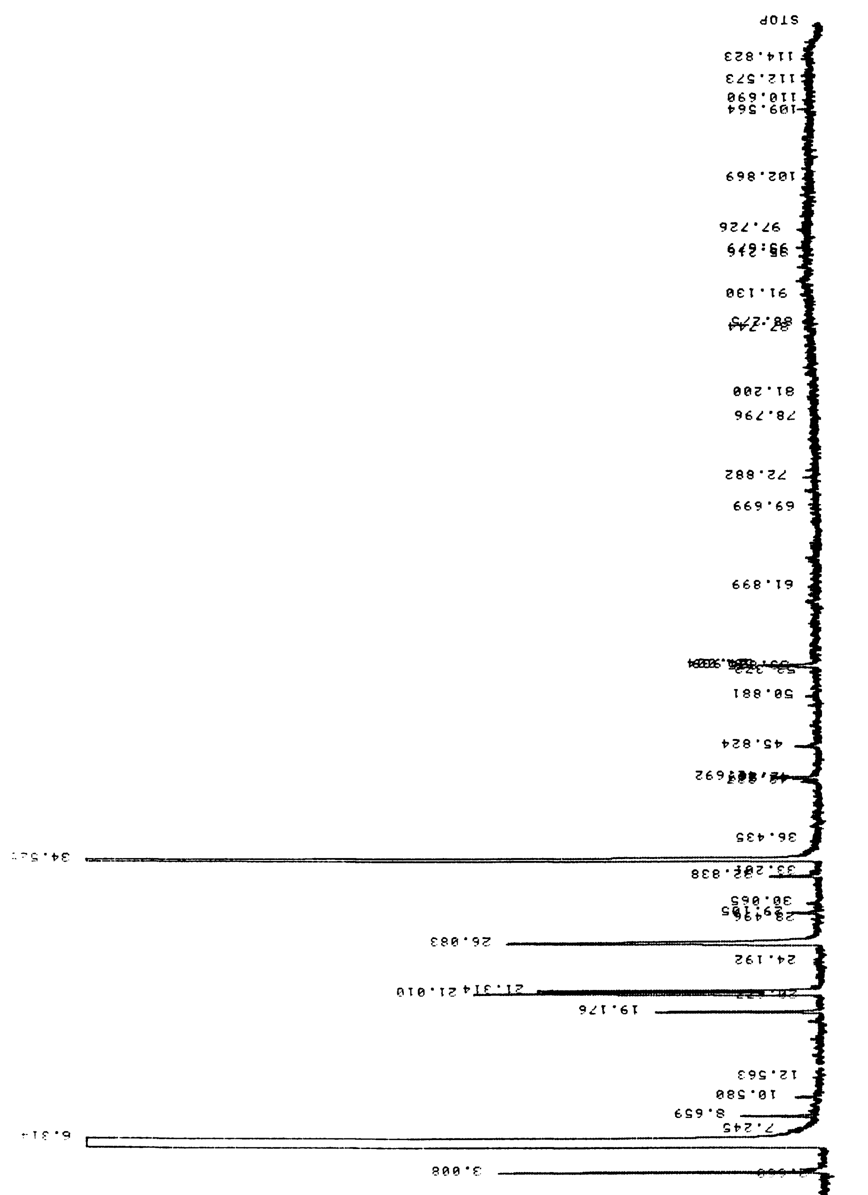
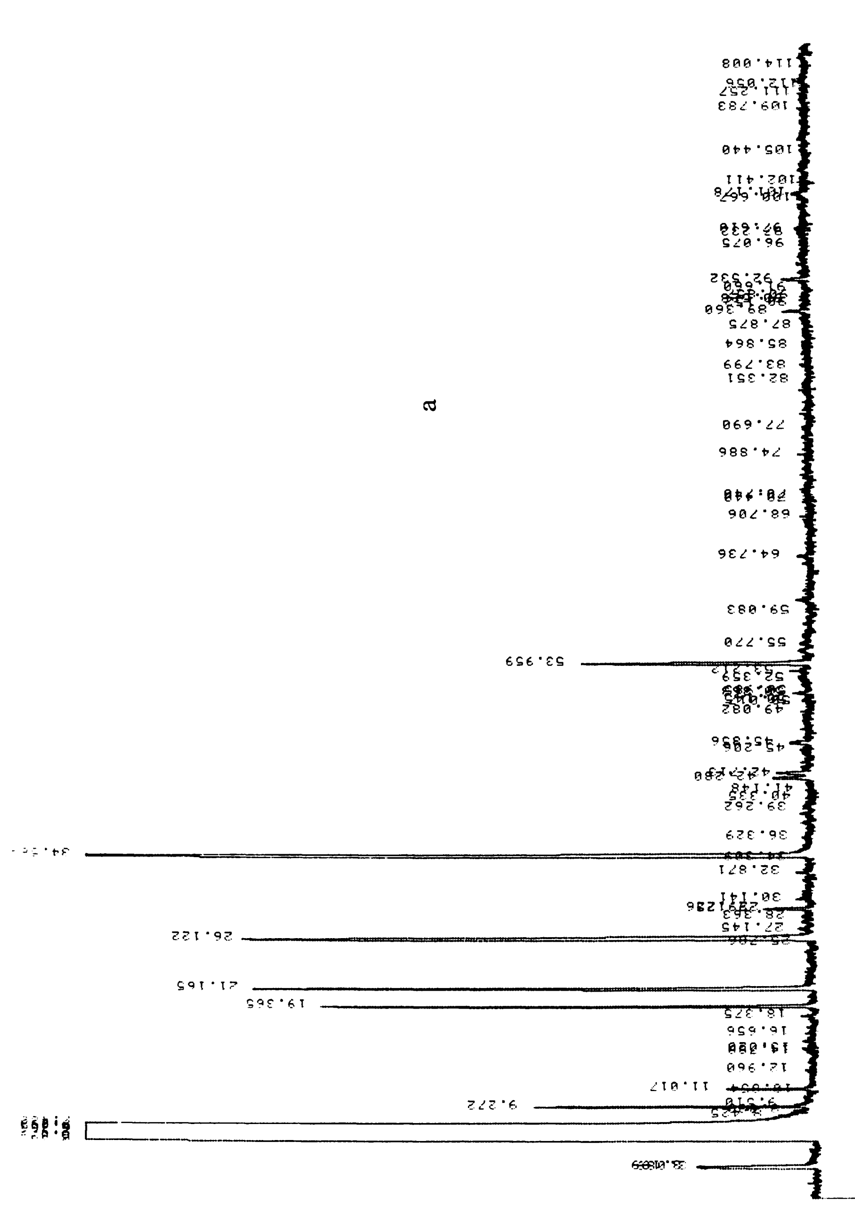


Figure 1. Chromatograms of sample 3A; FID (top) and SCD (bottom).



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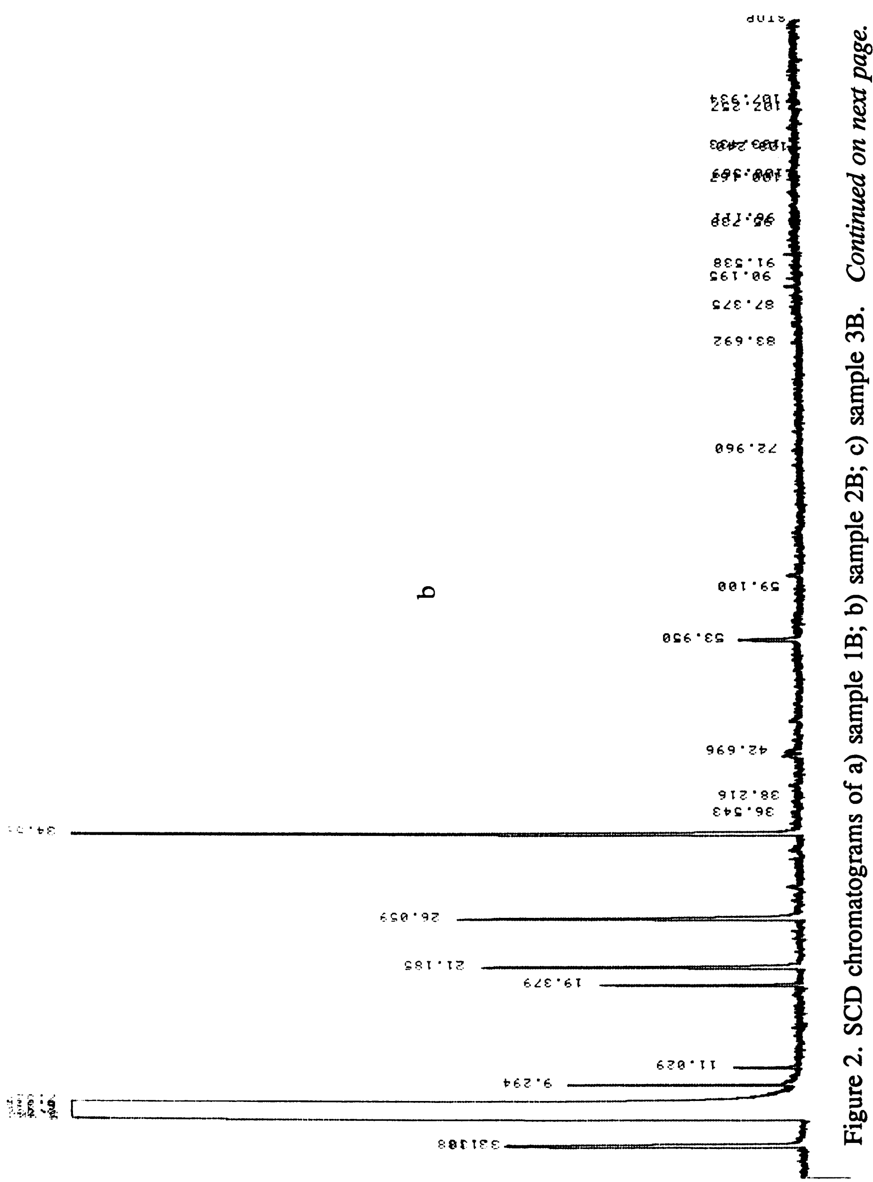

Figure 2. SCD chromatograms of a) sample 1B; b) sample 2B; c) sample 3B. *Continued on next page.*

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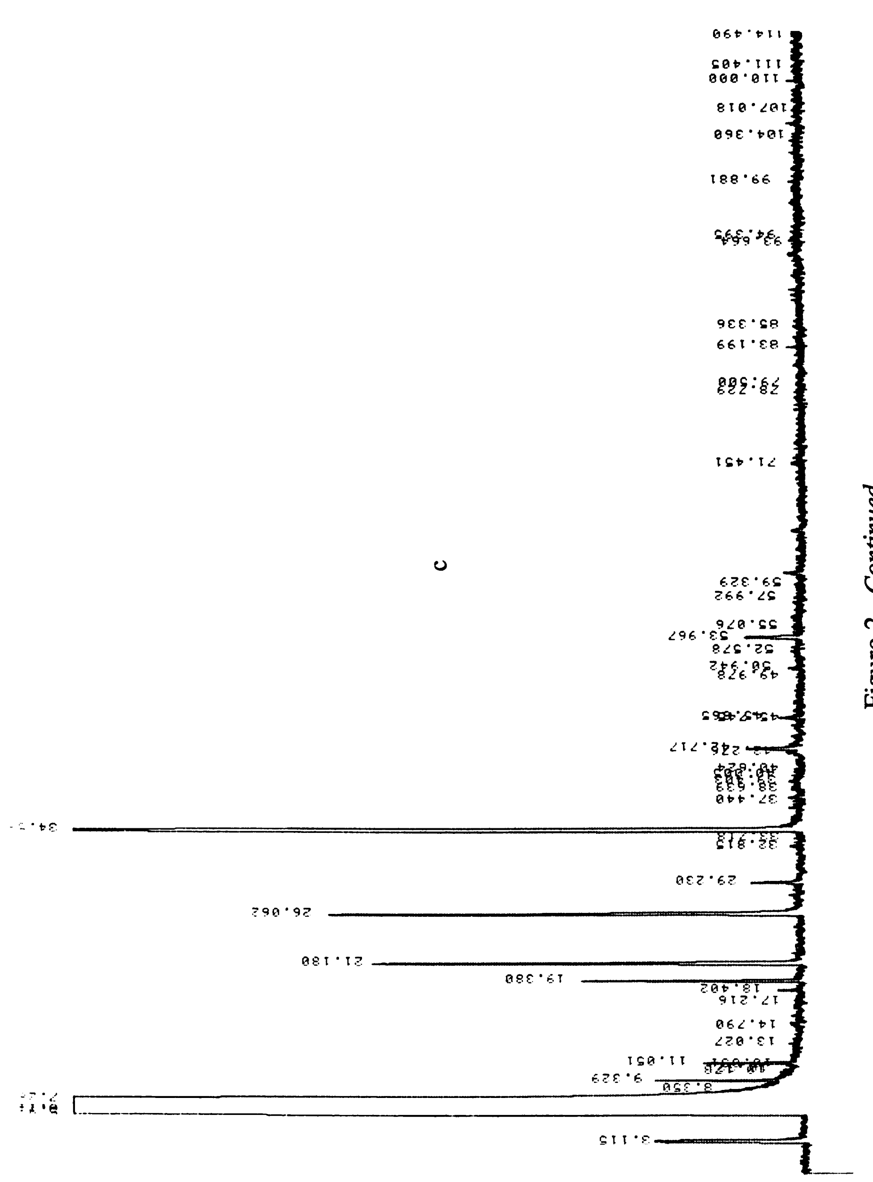

Figure 2. *Continued.*

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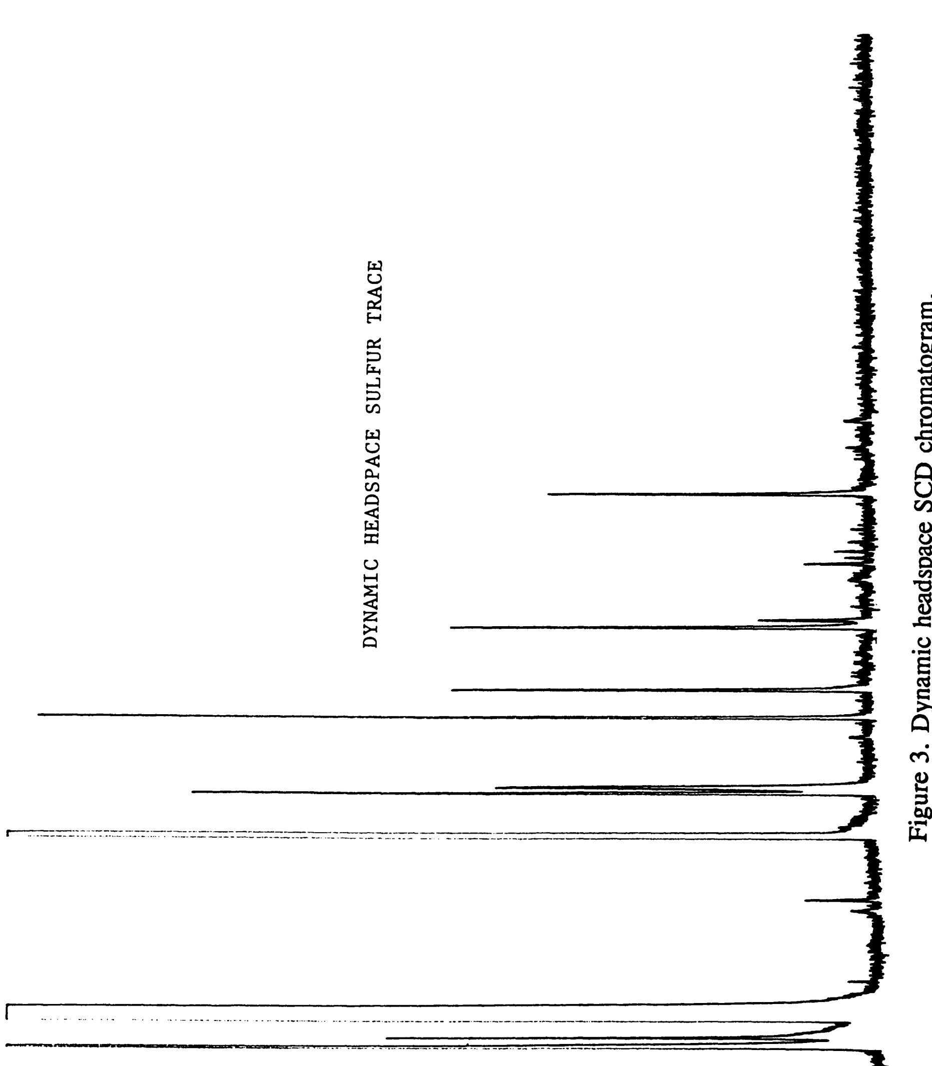

Figure 3. Dynamic headspace SCD chromatogram.

Table II. Sulfur Peak Identifications

PEAK NUMBER	RETENTION TIME	COMPOUND NAME	EMPIRICAL FORMULA
1	3.008	METHANETHIOL	CH_3SH
2	6.314	DIMETHYL SULFIDE	$(CH_3)_2S$
3	19.176	THIAZOLE	C_3H_3NS
4	21.010	1-(METHYLTHIO)-PROPANE	$C_4H_{10}S$
5	21.314	DIMETHYL DISULFIDE	CH_3SSCH_3
6	34.525	METHIONAL	C_4H_8OS

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Discussion

The limit of detection for the SCD was not quite as low as reported in the literature (low ppb or picogram detection). There is some confusion as to how this LOD is calculated and reported. In a recent users survey, users of the SCD reported a wide range of sensitivity (ppm to sub ppb). Most reported the LOD as the detection limit of injected compound. Since the SCD has a near equimolar response, a more accurate specification is detection of sulfur rather than actual compound. A signal to noise ratio of 3:1 is usually specified for instrumental sensitivity, although in older publications S/N criteria for reporting detection limits is 4:1. The SCD manufacturer reports sensitivity by a statistical treatment of the various parameters involved. This takes into account the peak area, the root mean square of the noise, and a ninety-five percent confidence level for the standard deviation of the noise:

$$LOD = \frac{S*n*(3.29/5)}{ph*pw}$$

where S is the mass of sulfur represented by the peak in picograms; n is the noise magnitude measured in mm peak-to-peak; ph is the peak height in mm; pw is the peak width at half height in seconds; the rms noise level is n divided by 5 and multiplied by 3.29 to achieve 95% accuracy in detection limit. Applying this equation to my LOD (44 pg of S as $(CH_3)_2S$) results in 1.6 pg S/sec.

Adequate preparation of flavor isolates is fundamental to successful flavor research. Micro-SDE was selected for several reasons. It is an efficient, effective means of obtaining a flavor isolate without expending a great deal of effort or time. The process by which molasses is made involves the repetitive application of heat to reclaim sucrose from sugar cane. Therefore, there should be a minimal number of reaction products and artifacts from atmospheric distillation. Since micro-SDE does not require additional concentration of the extract, there should be a negligible loss of

the lower molecular weight, highly volatile compounds. This was demonstrated by the detection and identification of methanethiol in the isolates.

The choice of which extraction solvent to use depends on the chemistry of the analytes of interest. Commonly used low boiling solvents cover a range of analyte polarity. The two solvents selected for this work were chosen to determine which was more effective at extracting the sulfur volatiles. While dichloromethane was better at extracting and retaining the lighter sulfur compounds, diethyl ether facilitated the separation of 1-(methylthio)-propane from dimethyl disulfide in the chromatographic runs. Apparently there is a greater difference in the solvent-solute interactions between $(CH_3CH_2)O$ and the two analytes than there is with CH_2Cl_2.

Of the six sulfur compounds identified in this work, five are reported for the first time in molasses (Table II). Only one of the three previously reported sulfur compounds was positively identified; dimethyl sulfide. Sulfur dioxide and carbon disulfide were not found in the liquid isolates. These compounds may have been driven off by the distillation-extraction process. They may be present in the thermally desorbed chromatogram as two of the early eluting peaks yet to be identified.

The power of utilizing a selective detector in concert with high resolution GC and mass spectrometry is clearly demonstrated in this research. However, in order for the overall strategy to yield desired results, there are limitations and requirements that must be satisfied. Selective detection is of limited use in identifying unknown analytes if the compounds are not adequately separated into individual bands on the GC. This is demonstrated in the chromatograms from sample 3A (Figure 1). The peak in the SCD trace appearing at 26.083 minutes should be identifiable with the MS, especially since solid identifications were made on smaller peaks. However, this peak co-elutes with the peak at 25.643 minutes of the FID chromatogram, making it impossible to identify. A similar situation exists with dimethyl sulfide, which co-elutes with dichloromethane. Fortunately, the use of another solvent allowed its identification.

Another major limiting factor is the actual concentration of the analyte. The sensitivity of the SCD is orders of magnitude better than the mass spectrometer. So, as in the case for the smaller sulfur peaks, there is insufficient analyte present to be identified by the MS.

The results from the thermal desorption of headspace volatiles showed about the same number of sulfur peaks on the SCD chromatogram; however, identifications were problematic. While the early region of the chromatogram shows response to five different sulfur compounds, only two were positively identified. Use of the ion trap mass spectrometer requires consideration of sample limitations unique to the trap. One can easily overload the ITS-40 with analyte and the resultant spectra become atypical of electron ionization spectra. Also, certain classes of molecules behave differently in the trap versus a linear quadrupole or sector instrument. This can lead to skewed abundance levels of molecular as well as fragment ions. These problems appear most pronounced with the earlier eluting compounds and certain oxygenated species.

Conclusions

This work has described and demonstrated the utility of the SCD as a sensitive, selective detector of sulfur flavor compounds in molasses. Its sensitivity and selectivity

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make it a valuable tool for detecting trace amounts of sulfur compounds in food. Five sulfur compounds have been identified that have not been previously reported in molasses.

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Chapter 9

Isolation of Aroma Volatiles from an Extruded Oat Ready-To-Eat Cereal

Comparison of Distillation–Extraction and Supercritical Fluid Extraction

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Supercritical carbon dioxide extraction of oat ready-to-eat cereals requires less time than vacuum steam distillation-solvent extraction for the isolation of aroma volatiles. Recoveries of hexanal and methylpyrazine extracted from an oat cereal increase as extraction pressure, extraction time, and modifier concentration increase. However, extraction of lipid also increases as these parameters increase. Co-extraction of lipid is not desired: lipid dilutes the aroma isolate, or makes it necessary to isolate the aroma volatiles form the lipid. Exposing the sample to high pressure supercritical carbon dioxide, then depressurizing rapidly to 1 ATM prior to extraction, improved recovery of methylpyrazine by 10%, and reduced lipid extraction.

Sales of ready-to-eat (RTE) cereals in the United States total approximately 5 billion dollars (*1*). One factor influencing consumer acceptance of RTE cereals and, thereby sales, is flavor. The Quaker Oats Company produces and markets a variety of extruded RTE cereals, which contain oats as their principle ingredient, for example Quaker Oat Squares, Quaker Oat Life, and Quaker Oat Bran. Flavor of these products is primarily generated during the cooking process. To investigate development of desirable flavor in these products distillation-extraction and SFE methodologies were evaluated and compared.

Isolation of volatile chemicals from food systems is the starting point for all flavor investigations. Criteria for selecting an isolation procedure (*2-4*) are that artifacts should not be formed and the sample should not require additional clean-up prior to analysis. For oat products, where flavor is thermally generated, and there is a significant concentration of unsaturated lipids, these criteria translate to isolating volatiles under mild thermal conditions without co-isolation of lipids. One procedure that may best meet these criteria is vacuum steam distillation (VSD) followed by solvent extraction. Risch and Reineccius (4) point out this two step procedure is often easier than trying to control simultaneous distillation-extraction under vacuum.

VSD has proven to be very useful for preparing aroma isolates; however, it is slow. Consequently researchers continue to look for faster sample preparation techniques. One procedure that appears promising for the isolation of flavor volatiles from extruded oat RTE cereals is extraction with supercritical carbon

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dioxide (*5-7*). Supercritical fluid extraction (SFE) allows isolation under mild thermal conditions; thereby, minimizing artifact formation. In addition, the solvating power of the supercritical fluid is dependent on density and can be varied as a function of temperature and pressure. This makes it possible to alter the selectivity of the isolation. There are many examples in the literature of isolation of aroma chemicals by SFE (*9-14*). Much of this work was done with custom built apparatus. Introduction by various vendors of self contained instruments for supercritical fluid extraction made it feasible to compare this technology with vacuum distillation-extraction.

The objective was to determine if SFE could replace VSD for the isolation of aroma compounds from oat RTE cereals. To make this assessment, it was necessary to determine if volatiles could be isolated with SFE without any contaminating lipid, and to compare volatile recoveries from both SFE and VSD.

Experimental

Before SFE could be assessed target recoveries were established for VSD. In a typical VSD procedure, modified from Haydanek and McGorrin (*15*), 200 to 1000 grams of cereal, 6 liters of deionized ultra-filtered water (DIUF) water buffered to pH 7, and an internal standard (2-methoxy-3-methylpyrazine, 0.090 ppm w/w cereal) were combined in a 12 liter round bottom flask. The sample was distilled under vacuum (approximately 70 mm Hg) for 3 hours at a distillation temperature of 45 °C. The distillate was collected in a trap cooled with dry ice. After thawing, NaCl was added to the distillate (10% w/v), the distillate was extracted with dichloromethane, then dried over sodium sulfate, and concentrated to approximately 10 ml in a Kundera-Danish apparatus. The distillate was further concentrated to approximately 0.3 mL under a stream of dry nitrogen prior to GC analysis. Recoveries of volatiles from VSD are based on the internal standard. For these experiments, recoveries were determined as the peak area ratio of the specified compound to that of the internal standard. NPD chromatograms were used to determine pyrazine recoveries. FID chromatograms were used to determine recoveries of other aroma chemicals.

SFE Procedure. The SFE extraction system used was the Dionex SFE-703, since it has the largest extraction cells available in a commercial instrument. In a typical experiment the sample (50 g) was ground, mixed with methanol modifier (5.0 g), and loaded into a 32 mL cell (approximately 20 grams). The cells were equilibrated at the extraction temperature (55 °C), the contents were physically disrupted (decompression), and the samples were extracted. In the "decompression" step the sample was pressurized to 250 ATM for 2 min., followed immediately by decompression to 1 ATM. Extracts were collected into vials containing dichloromethane. Post extraction, standard 1 (2-methoxy-3-methylpyrazine, 0.101 ppm) was added, samples were concentrated under a stream of nitrogen to 0.1 to 0.3 mL, standard 2 (2-methoxy-3-isobutylpyrazine, 0.106 ppm) was added, and the sample was analyzed by GC. Recoveries from the SFE experiments are reported as the percent ratio of the recoveries from VSD.

The first series of extractions were done at 175 ATM and 55 °C for various times. Prior to extraction, the sample was modified with methanol (10% w:w), and was pressurized to 250 ATM for 2 min, then rapidly decompressed to 1 ATM. The flow rate of supercritical carbon dioxide was 0.509 mL/min during extraction.

In the second series of extractions conditions were identical to those described above, except the extraction pressure was 150 ATM. The flow rate of supercritical carbon dioxide was 0.465 mL/min during extraction.

In the third series of extractions decompression pressure was varied: 0, 250, 375, and 500 ATM. Modifier concentration, pre-pressurization time, and

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extraction conditions were held constant at 10% (w:w), 2 min, and 150 ATM, 55 °C, 105 min, respectively.

In the forth series of experiments modifier concentration was varied: 0, 5, 10, and 15% (w:w sample). Decompression was from 250 ATM. Extraction conditions were 150 ATM, 55 °C, 90 min.

Chromatography. The GC used was a Hewlett-Packard 5880A GC with dual capillary injectors (250 °C) and FID and NPD detectors (275 °C). The GC was equipped with J&W Scientific DB-1 capillary columns (60 m, 0.32 mm, film thickness 1.0 μm). Split injection (1:10) was used, and the column temperature program was 3 min isothermal at 40 °C, 40-220 °C at 3 °C/min, then isothermal at 220 °C for 10 min. Helium (4 mL/min) was the carrier gas. A PE Nelson 2600 Chromatography Data System was used to analyze the data.

Results & Discussion

As with any new instrument, the first experiments were exploratory in nature. These experiments indicated that at extraction times of more than 60 min, 175 atmospheres (ATM) appears to be the upper pressure limit for isolating volatiles without extracting the lipid. Addition of 10% methanol to the sample improved volatile recovery. A second extraction of the same sample often provided higher recoveries than the first extraction, which was thought to be due to a decompression, i.e. physical disruption, of the sample. Initial work indicated that quantitation, of the supercritical extracts, based on internal and external standard methods was similar, except when a decompression step was involved. In experiments with a decompression step there was significant loss of internal standard.

Extraction pressure, extraction time, decompression pressure and modifier concentration were investigated. During the course of the experiments there was some difficulty concentrating certain extracts to the desired volume, due to the occurrence of a phase separation while concentrating some samples. Phase separation probably resulted from the lipid isolated with the aroma volatiles. When phase separation occurred, it inhibited concentration to the desired volume and increased variability of aroma recovery. In order to monitor this variability, experiments were modified to include a second standard. Standard 1 was added prior to concentration and standard 2 was added after concentration. The normalized area ratio of Standard 1/Standard 2 provided an estimate of volatile loss during concentration.

Extraction at 175 ATM. Figure 1 shows relative recoveries of hexanal and methylpyrazine as a function of extraction time. Hexanal and methylpyrazine are representative of the two principle paths to thermal aroma generation: lipid oxidation and Maillard reactions. These compounds represent the lower range of volatility of compounds thought to be extracted with supercritical carbon dioxide. Recoveries leveled off after extraction for 60 min (Figure 1). In samples extracted longer than 60 min there was a significant loss of standard 1. This coincided with a phase separation during concentration of the extract. Phase separation occurred earlier in the concentration step as the extraction time was increased. As extraction time increased lipid recovery also increased. At 175 ATM the best volatile recoveries were after extraction for 60 min. However, recoveries were not large enough to make this a reasonable substitute for VSD.

Extraction at 150 ATM. Figure 2 shows that under these conditions there was an increase in hexanal recovery up to 105 min. Methylpyrazine recoveries remain constant from 68 to 105 min, but increased at 120 min. Loss of standard 1 also

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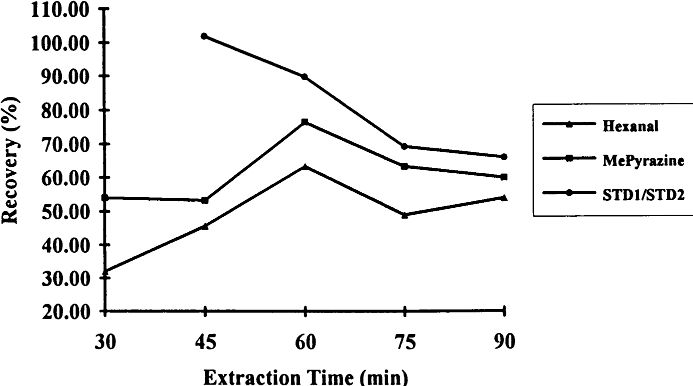

Figure 1. Extraction at 175 ATM and 55 °C. Cereal contained 10 % methanol modifier and was decompressed from 250 ATM prior to extraction. Recoveries of hexanal and methylpyrazine are given as the percentage of the recoveries from the VSD procedure. Standard 1/Standard 2 indicates volatile loss during concentration.

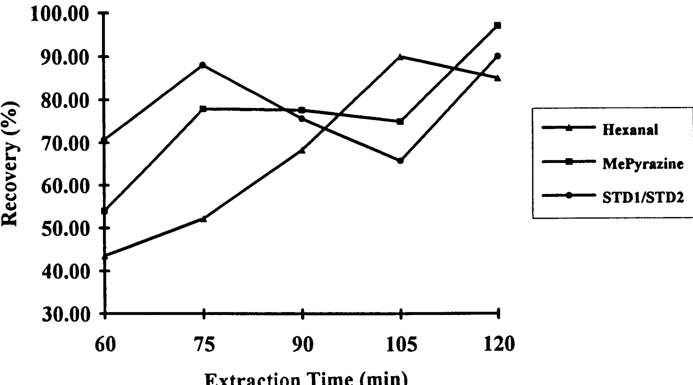

Figure 2. Extraction at 150 ATM and 55 °C. Cereal contained 10 % methanol modifier and was decompressed from 250 ATM prior to extraction. Recoveries of hexanal and methylpyrazine are given as the percentage of the recoveries from the VSD procedure. Standard 1/Standard 2 indicates volatile loss during concentration.

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occurred after sample concentration. Extraction at 150 ATM for 120 min gave results comparable to those obtained by VSD.

Extraction as a Function of Decompression. Figure 3 indicates that at higher decompression pressure there is better recovery of methylpyrazine and standard 1. Improved recovery of standard 1 implies less phase separation, which in turn indicates that there is less lipid recovered. Research on polymer systems has shown that supercritical carbon dioxide can influence swelling, glass transition temperature and plasticization (*16*). Alteration of the physical state of bio-polymers should influence the binding (*17*) and, hence, the extractability of aroma compounds. Research on how the matrix influences extraction of aroma compounds is needed.

Extraction as a Function of Modifier Concentration. Figure 4 shows that the optimal modifier concentration was 10% when methanol was used as the modifier. Below 10 % modifier, recoveries of hexanal and methylpyrazine were poor. When extractions were run with more than 10 % modifier, there was significant phase separation during the concentration step, indicating that modifier concentration greater than 10% increases lipid extraction.

Comparison of SFE and VSD. The comparison of VSD and SFE was based on both recovery of volatiles and time efficiency. Relative recoveries, of the representative aroma volatiles given in Table I, indicate that SFE can be used in place of VSD for volatile extraction.

Table I: Recovery of Volatiles by SFE

	SFE/VSD
Hexanal	91.59
2,4-Decadienal	89.37
Benzaldehyde	46.98
Methylpyrazine	85.05
2,5/6-Dimethylpyrazine	97.54

Decompression: 375 ATM/2 min.
Extraction: 150 ATM/105 min

Comparison of the time it takes to prepare a sample by VSD and SFE, Table II, indicates significant time advantages to sample preparation by SFE. In addition, using the Dionex SFE-703, it is possible to prepare up to 8 samples simultaneously.

Table II. Time Required to Isolate Volatiles

	VSD	SFE
Set-up	1 hr	40 min
Distillation	3 hr	
Thaw	2 hr	
Extraction	1 hr	120 min.
Concentration	2 hr	40 min.
Clean-up	2 hr	10 min.
Total	11 hr/sample	210 min. = 3.5 hr/sample

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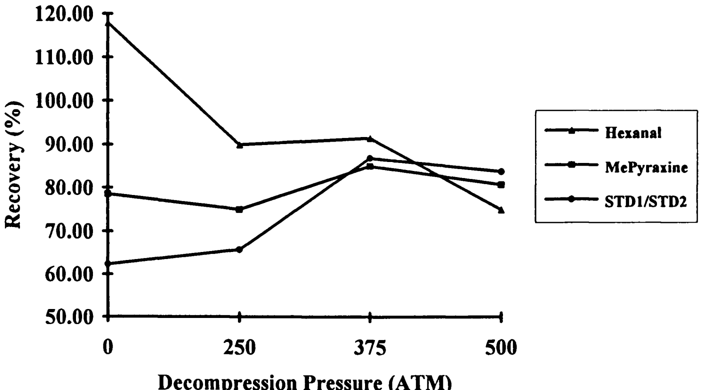

Figure 3. Extraction after decompression from different pressures. Cereal contained 10 % methanol modifier and was extracted at 150 ATM, 55 °C for 105 min. Recoveries of hexanal and methylpyrazine are given as the percentage of the recoveries from the VSD procedure. Standard 1/Standard 2 indicates volatile loss during concentration.

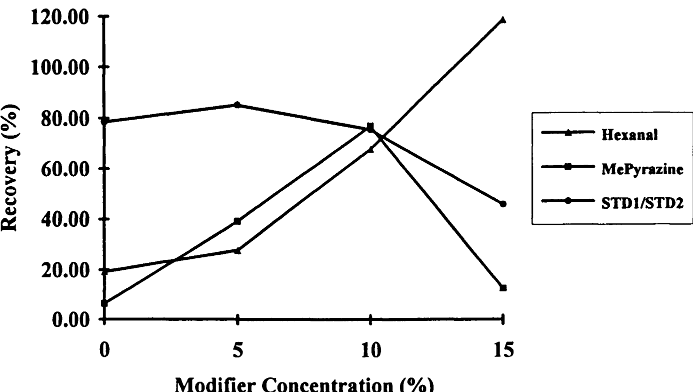

Figure 4. Extraction of cereal containing different concentrations of modifier. Samples were decompressed from 250 ATM and extracted at 150 ATM, 55 °C for 90 min. Recoveries of hexanal and methylpyrazine are given as the percentage of the recoveries from the VSD procedure. Standard 1/Standard 2 indicates volatile loss during concentration.

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Isolation of aroma volatiles from extruded oat RTE cereals by supercritical carbon dioxide is influenced by extraction pressure, extraction time and modifier concentration. A decompression step can enhance volatile extraction. Optimization of SFE parameters allows recovery of most aroma volatiles in proportions similar to those obtained from vacuum steam distillation/solvent extraction. Time expended to optimize the SFE procedure will be recovered due to the overall greater time efficiency of the SFE technique.

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Chapter 10

Flavor Compounds Formed during the Maillard Reaction

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The Maillard reaction is one of the most important routes to flavor compounds in cooked foods. The initial stages of the reaction involve the condensation of the carbonyl group of a reducing sugar with an amino compound, followed by the degradation of the condensation products to give a number of different oxygenated compounds. The subsequent stages of the Maillard reaction involve the interaction of these compounds with other reactive components such as amines, amino acids, aldehydes, hydrogen sulfide and ammonia. These additional reactions lead to many important classes of flavor compounds including furans, pyrazines, pyrroles, oxazoles, thiophenes, thiazoles and other heterocyclic compounds. The large number of different reactive intermediates that can be generated in the Maillard reaction gives rise to an extremely complex array of volatile products. This review discusses some of the reaction pathways by which the important aroma compounds of different cooked foods may be formed.

The term "Maillard Reaction" is used to describe the complex series of chemical reactions between carbonyl and amino components derived from biological systems. In foods it results in the production of flavors and the browning of the food. The reaction is named after the French chemist Louis-Camille Maillard who, in 1912, observed the formation of brown pigments when heating a mixture of glucose and lysine *(1)*. The significance of the Maillard reaction for food is not confined to the production of color and flavor; reduction of nutritional value from the loss of essential amino acids other nutrients, such as ascorbic acid, the possible formation of toxic components, such as imidazoles, and the antioxidant properties of Maillard reaction products are all areas of much interest to the food industry. However, the reaction is one of the main sources of flavor in cooked foods and, with the increased availability of sophisticated analytical techniques, studies of the Maillard reaction as

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the route to characteristic food flavors is an area that has seen considerable activity in recent years. The reaction has been examined in model systems with a range of different reducing sugars and amino acids, and the results used to explain the formation of flavors in heated foods.

It was Hodge in 1953 who first explained the complex series of reactions that comprise the Maillard reaction *(2)*; forty years later, his scheme is still the basis for current understanding of the essential features of the reaction. In relation to flavor formation, the Maillard reaction is best considered in three stages. The initial reaction involves the formation of a glycosylamine and its subsequent rearrangement. This is followed by dehydration to furan derivatives, reductones, and other carbonyl compounds. The final stage involves the conversion of these furan and carbonyl intermediates into aroma compounds usually by reaction with other intermediates such as amino compounds or amino acid degradation products. The mechanism of the initial and intermediate stages of the reaction have been discussed in a number of reviews *(3-7)*; therefore, this paper will concentrate on the later stages of the reaction and discuss the possible routes by which a number of important classes of aroma compounds may be formed.

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Classification of Flavor Compounds from Maillard Reactions

The aroma volatiles produced in the Maillard reaction were classified into three groups by Nursten *(8)*, and this provides a convenient way of viewing the origin of the complex mixture of volatile compounds derived from the Maillard reaction in foods :

1. "Simple" sugar dehydration/fragmentation products:
   - Furans
   - Pyrones
   - Cyclopentenes
   - Carbonyl compounds
   - Acids

2. "Simple" amino acid degradation products:
   - Aldehydes
   - Sulfur compounds (e.g. hydrogen sulfide, methanethiol)
   - Nitrogen compounds (e.g. ammonia, amines)

3. Volatiles produced by further interactions:
   - Pyrroles
   - Pyridines
   - Pyrazines
   - Imidazoles
   - Oxazoles
   - Compounds from aldol condensations
   - Thiazoles
   - Thiophenes
   - Di- and Trithiolanes
   - Di- and Trithianes
   - Furanthiols

Group 1 contains those compounds produced by the breakdown of the glycosylamine in the early stages of the reaction, and includes many of the compounds found in the caramelization of sugars. Many of these compounds possess aromas that could contribute to food flavor, but they are also important intermediates for other compounds. The second group comprises simple aldehydes, hydrogen sulfide or amino compounds that result from the Strecker degradation occurring between amino acids and dicarbonyl compounds.

All these Maillard products are capable of further reaction, and the subsequent stages of the Maillard reaction involve the interaction of furfurals, furanones, and dicarbonyls with other reactive compounds such as amines, amino acids, hydrogen sulfide, thiols, ammonia, acetaldehyde, and other aldehydes. These additional reactions lead to many important classes of flavor compounds that comprise the last group of the classification of Maillard aroma compounds.

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch010

Early stages of the Maillard reaction

The first step of the reaction is the formation of a N-aldosylamine, and the classical mechanism of Hodge *(2)* proposes that this involves an addition reaction between the carbonyl group of the open chain form of an aldosugar and the amino group of an amino acid, peptide or other compound with a primary amino group (Figure 1). It is convenient to view the reaction occurring between the open chain form of the sugar although some authors suggest that the cyclic pyranose or furanose conformation of the sugar is more likely to be involved since it is the most abundant form of sugars in aqueous solution *(9)*. The subsequent elimination of water and molecular rearrangement gives a 1-amino-1-deoxy-2-ketose (Amadori product). A similar sequence of reactions occurs with ketosugars, with the initial formation of a N-ketosylamine, which undergoes the Heynes rearrangement to give the corresponding 2-amino-2-deoxyaldose.

These Amadori and Heynes intermediates themselves do not contribute to flavor, however they are important precursors of flavor compounds. They are thermally unstable and undergo dehydration and deamination to give furfurals, reductones, and fission products such as dicarbonyls. The nature of the products depends on the nature of the amino substituents on the Amadori product, and on the reaction conditions. A low pH tends to favor 1,2-enolization, and the loss of the amino compound from the tautomeric 1,2-eneaminol gives a 3-deoxyosone; elimination of water and cyclization yields furfurals (Figure 2). At higher pH a 2,3-enolization is favored, and here the elimination of the amine gives a 1-deoxyosone. Further dehydration and intermolecular cyclizations can lead to 5-methyl-4-hydroxy-3(2*H*)-furanone from pentose sugars or the 2,5-dimethyl homologue from hexoses. Maltol (3-hydroxy-2-methyl-4*H*-pyran-4-one),5-hydroxy-5,6-dihydromaltol,andisomaltol(1-(3-hydroxy-2-furanyl)-ethanone) are other important dehydration products of hexoses. Fragmentation of the carbohydrate chains of the deoxyosones intermediates can lead to a variety of α-dicarbonyl compounds such as pyruvaldehyde, 2,3-butanedione, hydroxyacetone, and 3-hydroxy-2-butanone.

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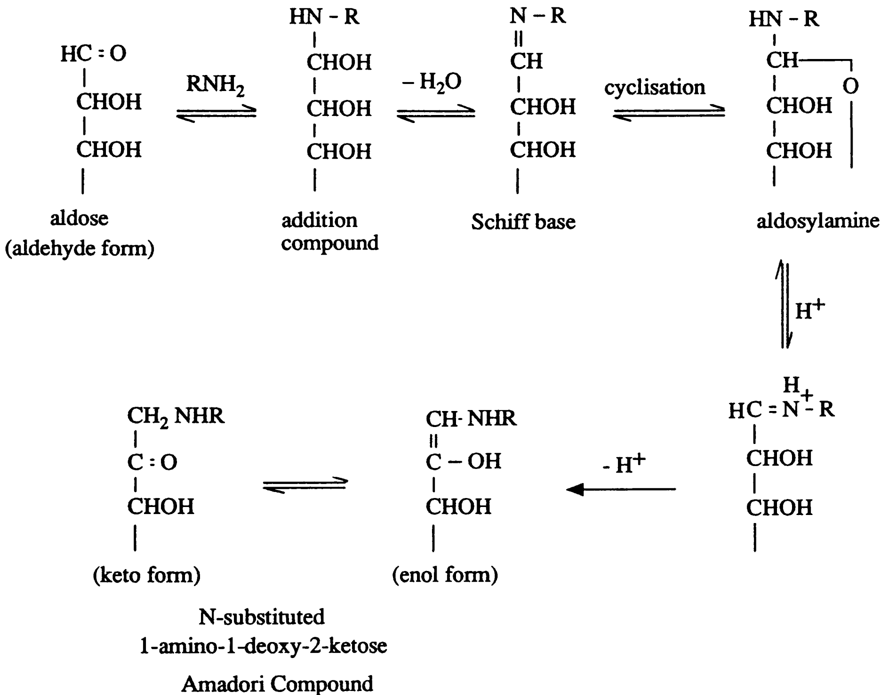

Figure 1. Formation of Amadori compounds in the initial stages of the Maillard reaction. (Adapted from ref. 2).

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch010

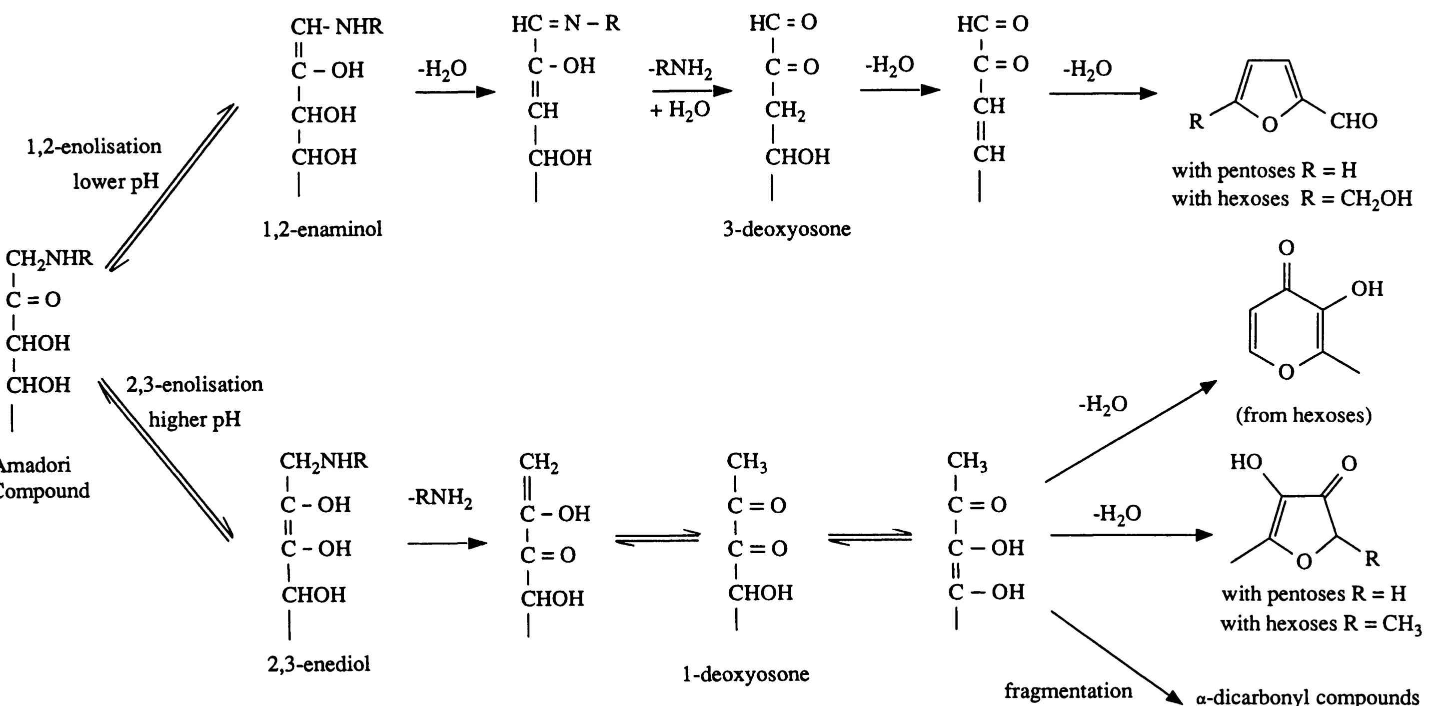

Figure 2. Decomposition of Amadori compounds in the Maillard reaction. (Adapted from ref. 2).

Strecker Degradation

Strecker degradation is one of the most important reactions associated with the Maillard reaction, and involves the oxidative deamination and decarboxylation of an α-amino acid in the presence of a dicarbonyl compound (Figure 3). This leads to the formation of an aldehyde, containing one fewer carbon atoms than the original amino acid, and an α-aminoketone. These aminoketones are important intermediates in the formation of several classes of heterocyclic compounds including pyrazines, oxazoles, and thiazoles *(6)*.

The sulfur-containing amino acids are important sources of S-heterocycles, which contribute to characteristic aromas in many foods. In the Strecker degradation of cysteine, hydrogen sulfide, ammonia, and acetaldehyde are formed, as well as the expected Strecker aldehyde, mercaptoacetaldehyde, and an aminoketone *(10)*. These compounds are important as reactive intermediates for the formation of many S and N compounds with low odor threshold values, which play important roles in the characteristic flavors of a number of foods, meat being a most important example.

Proline and hydroxyproline differ from the other amino acids as they contain the secondary amino group in a pyrrolidine ring; therefore, they do not produce amino-ketones and Strecker aldehydes in the reaction with dicarbonyls. However, nitrogen heterocyclics are produced including 1-pyrroline, pyrrolidine, 1-acetonyl-2-pyrroline, and 2-acetyltetrahydropyridine (Figure 4) *(11)*.

Later Stages of the Maillard Reaction

As discussed above, the initial stages of the Maillard reaction are responsible for the formation of a number of oxygenated sugar degradation products. These are similar to those formed in the caramelization of sugars, containing one or more carbonyl groups, but in the Maillard reaction they are formed at lower temperatures than those required for caramelization. They will readily undergo further reaction, especially at the elevated temperatures associated with the cooking of food. Other reactive compounds are also produced including Strecker aldehydes, ammonia, and hydrogen sulfide. Many of these compounds will possess aroma or taste, but they also provide an essential source of intermediates for the production of the characteristic flavors associated with cooked foods. A number of reviews have examined the pathways by which some of these compounds are formed *(3-8)*]. Another review of particular interest, to the flavor chemist interested in aromas produced by the Maillard reaction, is the paper by Fors *(12)* that lists the reported aromas and odor thresholds of compounds produced in the Maillard reaction.

In the following sections, the possible routes to a number of important classes of aroma compounds found in heated foods are presented, together with some comments about their aroma characteristics, and their role in food flavor.

Oxygen-Containing Aroma Compounds from the Maillard Reaction

Furans with oxygenated substituents (furfurals, furanones) occur in the volatiles of all heated foods, and are among the most abundant products of the Maillard reaction. Oxygenated furans and pyrans, such as furfural, 5-methylfurfural, 2-acetylfuran,

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$-H_2O$

$-CO_2$

$R'-CHO$ $+ H_2O$

[O]

For cysteine
R' = $HS.CH_2$-

$H_2S + NH_3 + CH_3CHO +$

alkylpyrazine

Figure 3. Strecker degradation of α-amino acids and the formation of alkylpyrazines.

Figure 4. Formation of compounds with bread-like aromas in the reaction of proline with pyruvaldehyde. (Adapted from ref. 11).

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maltol, and isomaltol, generally impart caramel-like, sweet, fruity characteristics to foods. 2,5-Dimethyl-4-hydroxy-3(2*H*)-furanone and its 5-methyl homologue, which have been found in a number of foods, have aromas described as caramel-like, burnt pineapple-like, although at low concentrations the dimethyl derivative attains a strawberry-like note. The odor threshold values of furfurals and furanones are generally at the ppm level *(12)*. In their own right, oxygenated furans may contribute to caramel-like, sweet aromas in heated foods; however, they are important intermediates to other flavor compounds, including thiophenes, furanthiols, and other sulfur-containing compounds.

Aliphatic carbonyl compounds, such as diacetyl which has a butter-like odor, also may contribute to the aromas derived from the Maillard reaction, and many of the Strecker aldehydes also have characteristic aromas. For example, 3-methylbutanal, which is derived from leucine, is an important odor impact compound in malt.

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch010

Nitrogen-Compounds

Pyrazines. These important aroma compounds are believed to contribute to the pleasant and desirable flavor of many different foods. Although tetramethylpyrazine was first isolated from the molasses of sugar beet in 1879 and several alkyl pyrazines were found in coffee in 1928, it was not until the mid-1960s that their occurrence in foods was widely reported, and since then this class of aroma compound has received considerable attention *(13)*. The alkylpyrazines generally have nutty, roast aromas with some eliciting earthy or potato-like comments *(12)*. The odor threshold values of the mono-, di-, tri-, and tetramethylpyrazines are all relatively high ($>$ 1 ppm), and these pyrazines probably only play minor roles in food aromas. However, replacing one or more of the methyl groups with ethyl can give a marked decrease in threshold value *(14)*, and some ethyl substituted pyrazines have sufficiently low threshold values for them to be important in the roast aroma of cooked foods. Apart from acetylpyrazine, relatively few pyrazines with oxygen-containing substituents have been reported as products of the Maillard reaction in foods, although methoxypyrazines are important biosynthesized aroma components in a number of vegetables. Acetylpyrazines have some importance as flavorings, having popcorn-like aromas, and odor threshold values in the low ppb range *(12)*.

Pyrazines are one of the major classes of compounds formed in the Maillard reaction, and their formation has been studied in many different model systems involving amino acids and reducing sugars, as well as simpler systems in which ammonia was the source of nitrogen or carbonyls replaced the sugar *(6)*. It has been shown that factors such as time and temperature of reaction, pH, reactant concentration, and water activity are important variables in determining the nature and quantity of the products from such reactions *(15)*. Several mechanisms have been proposed for the formation of pyrazines in food flavors *(6,13)*, and one important route is from the α-aminoketones, which are products of the condensation of dicarbonyl with an amino compound via Strecker degradation (Figure 3). Self-condensation of the aminoketones, or condensation with other aminoketones, affords a dihydropyrazine that is oxidized to the pyrazine.

Oxazoles and Oxazolines. Oxazoles have been found in relatively few cooked foods, although over 30 have been reported in coffee and cocoa, and 9 in cooked meat. Oxazolines have been found in cooked meat and roast peanuts, but not to any extent in other foods. 2,4,5-Trimethyl-3-oxazoline has been regularly detected in cooked meat *(16)*, and when it was first identified in boiled beef *(17)* it was thought that the compound possessed the characteristic meat aroma; however, on synthesis it was shown to have a woody, musty, green flavor with a threshold value of 1 ppm *(18)*. Other 3-oxazolines have nutty, sweet or vegetable-like aromas and the oxazoles also appear to be green and vegetable-like *(18)*. The contribution of these compounds to the overall aroma of heated foods is probably not as important as the closely related thiazoles and thiazolines.

Pyrroles, Pyrrolines and Pyrrolidines. Pyrroles are found in the volatiles of most heated foods *(19)*, although they have received less attention than some other classes of aroma volatiles. Some pyrroles may contribute desirable aromas, e.g. 2-acetylpyrrole has a caramel-like aroma, and pyrrole-2-carboxaldehyde is sweet and corn-like, but alkyl- and acylpyrroles have been reported to have unfavorable odors *(12)*. Many more volatile pyrroles have been found in coffee than in other foods *(20)*, and they are common products of amino acid - sugar model systems. Pyrroles are closely related in structure to the furans, and they are probably formed in a related manner from the reaction of a 3-deoxyketose with ammonia or an amino compound followed by dehydration and ring closure (Figure 5) *(21)*.

The characteristic aroma of wheat bread crust has been attributed to 2-acetyl-1-pyrroline, and its formation depends on the presence of bakers' yeast *(22)*. In model systems it was demonstrated that the acetylpyrroline is formed from the reaction of proline with pyruvaldehyde or dihydroxyacetone. Other compounds with bread-like aromas formed in the reaction of proline with pyruvaldehyde include 1-acetonyl-2-pyrroline and 2-acetyltetrahydropyridine (Figure 4).

Since proline already contains a pyrrolidine ring it provides a potential source of nitrogen heterocyclics in the Maillard reaction, and a number of proline containing model systems have been examined. Tressl identified more than 120 proline-specific compounds in the reaction of proline or hydroxyproline with various sugars *(23,24)*. These include pyrrolines, pyrroles, pyridines, indolines, pyrrolizines, and azepines, but relatively few of the compounds have been identified among food volatiles.

Sulfur-Compounds

Sulfur-containing compounds contribute to the pleasant as well as unpleasant aromas of many foods, and constitute the essential character impact compounds of a significant number of foods. They may be formed by natural metabolic pathways in plants, or be derived from thermally-induced reactions. In meat the importance of sulfur compounds, both aliphatic and heterocyclic, to the characteristic aroma has been recognized for many years, and in the search for meat-like aroma chemicals much attention has been directed towards the organic chemistry of sulfur. In a recent review, MacLeod *(25)* listed 78 chemical compounds that have been reported in the literature as possessing meat-like flavors; seven are aliphatic sulfur compounds, 65 heterocyclic sulfur compounds, and the remaining six non-sulfur heterocyclics. Many

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch010

of these compounds arise from the prolific patent literature on this subject, and only 25 of the compounds have actually been identified in meat.

Hydrogen sulfide is an essential compound in the formation of many sulfur-containing aroma compounds, and it is found in many uncooked and cooked foods, especially meat. It is produced from cysteine by hydrolysis or by Strecker degradation; ammonia, acetaldehyde, and mercaptoacetaldehyde are also formed. All of these are reactive compounds, providing an important source of reactants for a wide range of flavor compounds.

Thiazoles and Thiazolines. These are closely related in structure to the oxazoles and oxazolines, have lower threshold values, and are more prolific in food volatiles than their oxygenated analogues. The first thiazoles were isolated from food volatiles in 1966, and they are now recognized as important constituents of food aromas, especially in roasted or fried products *(26)*. The highest number have been identified in roast or fried meat (41 thiazoles and 5 thiazolines) and coffee (28 thiazoles) *(16,20)*. Nearly all are alkyl-substituted, although some acetyl derivatives have also been reported. In studies on the aroma qualities of synthesized thiazoles it has been reported that in general 2-alkylthiazoles possess green, vegetable-like properties, while increasing the substitution added nutty, roasted, and sometimes meaty characteristics *(27,28)*. The nature and number of alkyl substituents appears to be important in determining the aroma character and certain di- and tri-alkyl derivatives have roast, meaty notes. A particularly interesting compound is 2,4-dimethyl-5-ethylthiazole, which has a nutty, roast, meaty, liver-like flavor, and a low odor threshold value of 2 ppb *(6)*.

Several mechanisms have been proposed for the formation of thiazoles and thiazolines *(6)*. The thermal degradation of thiamin is one source of thiazoles in heated foods *(29,30)*. A possible route in the Maillard reaction involves the action of hydrogen sulfide and ammonia on mixtures of aliphatic aldehydes and 1,2-dicarbonyl compounds (Figure 6), which is closely related to the pathway to oxazoles. In heated foods the main source of the aldehydes for these reactions is via Strecker degradation of amino acids. However, in fat-containing foods an additional source of aldehydes is from lipid oxidation. Trialkylthiazoles containing long-chain 2-alkyl substituents have been reported recently in fried chicken *(31)*, roast beef *(32)*, and fried potatoes *(33)*; these compounds could only result from the participation of lipid-derived aldehydes in the above reactions.

Polysulfur Heterocyclics. A number of heterocyclic compounds with two or three sulfur atoms in five and six membered rings have been reported in the volatiles of heated foods, especially in meat. Some of these have attracted interest as possible contributors to food aromas. The most frequently found is 3,5-dimethyl-1,2,4-trithiolane, which was first isolated from the volatiles of boiled beef by Chang et al. in 1968 *(17)*, and has subsequently been identified in other cooked foods including potatoes, nuts, and toasted cheese. Other sulfur-heterocyclics found in meat include trithioacetaldehyde (2,4,6-trimethyl-1,3,5-trithiane), trithioacetone (hexamethyl-1,3,5-trithiane), and thialdine (5,6-dihydro-2,4,6-trimethyl-1,3,5-dithiazine) *(16)*. The latter compound was the main volatile component obtained from a sample of boiled mutton *(34)*. Thialdine was first described in 1847 as a product from the reaction of hydrogen

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Figure 5. Formation of pyrroles from intermediates of the Maillard reaction.

Figure 6. Formation of thiazoles and thiazolines from intermediates of the Maillard reaction.

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch010

sulfide, ammonia, and acetaldehyde *(35)*, and in recent years has been reported in a number of heated foods. Although the reaction occurs readily, the compound is thermally labile; therefore, larger amounts might be expected when cooking and volatile extraction are carried out at lower temperatures *(36)*. The compound has also been shown to be formed during volatile extraction, and this, together with its thermal lability, may explain why large amounts have been obtained in some systems but not in others. Thialdine has been reported to have a roast beef-like aroma *(37)*, and trithioacetaldehyde and trithioacetone were also reported in the patent literature to have meaty characters *(38)*, although Boelens et al. *(36)* described trithioacetaldehyde as dusty, earthy, nutty.

One of the most important routes to sulfur-containing volatiles involves the reaction of carbonyls and/or dicarbonyls with ammonia, hydrogen sulfide or thiols, which are products of the Strecker degradation of cysteine or methionine. An example of this has already been discussed in relation to the formation of thiazoles. Boelens et al. *(36)* examined the products from the reaction of a number of aldehydes with hydrogen sulfide and thiols. Acetaldehyde reacted with gaseous hydrogen sulfide with the formation of trimethyl-substituted dioxathianes, oxadithianes, and trithianes (Figure 7). When the reaction was carried out with liquid hydrogen sulfide, under pressure, bis-(1-mercaptoethyl) sulfide was formed, which readily oxidized to 3,5-dimethyl-1,2,4-trithiolane. Inclusion of ammonia in the reaction produced thialdine.

Furan- and Thiophenethiols, Sulfides, and Disulfides. The odor properties of furans and thiophenes with thiol and methylthio substituents have been examined along with corresponding disulfides, and some have been found to have distinct meaty characteristics *(39,40)*. Furans (and also thiophenes) with a thiol group in the 2-position appear to have burnt or sulfurous aroma characteristics, while the isomers with 3-thiol substitution have meat-like aromas. A number of furanthiols, thiophene-thiols, and disulfides have been found in the volatiles from heated model systems containing hydrogen sulfide or cysteine and pentoses or other sources of carbonyl compounds, and some are reported to have meaty aromas. The odor threshold values of compounds of this type are very low, and that of bis(2-methyl-3-furyl) disulfide has been reported as 0.00002 ppb; one of the lowest known threshold values *(41)*. Although compounds with these structures had been quoted in patents relating to meat aroma, it is only recently that such compounds have been reported in meat itself *(16,42)*. These compounds and their formation are discussed in more detail in another paper in this book.

Thiol substituted furans have also been implicated in coffee aroma, and 2-furyl-methanethiol was identified as a key component in coffee aroma as early as 1926 *(20)*. It has an odor threshold value of 0.005 ppb, smells like coffee at low concentration, but is more sulfury at higher levels. Sulfide and disulfide oxidation products of these thiols are also thought to contribute to coffee aroma. An interesting bicyclic compound 2-methyl-3-oxa-8-thiabicyclo[3.3.0]-1,4-octadiene(kahweofuran), which is closely related to the 2-methyl-3-furanthio compounds, has also been identified in coffee.

Thiophenes. Many thiophenes have been reported in the volatiles of cooked foods, especially meat and model Maillard systems. The majority of the thiophenes found

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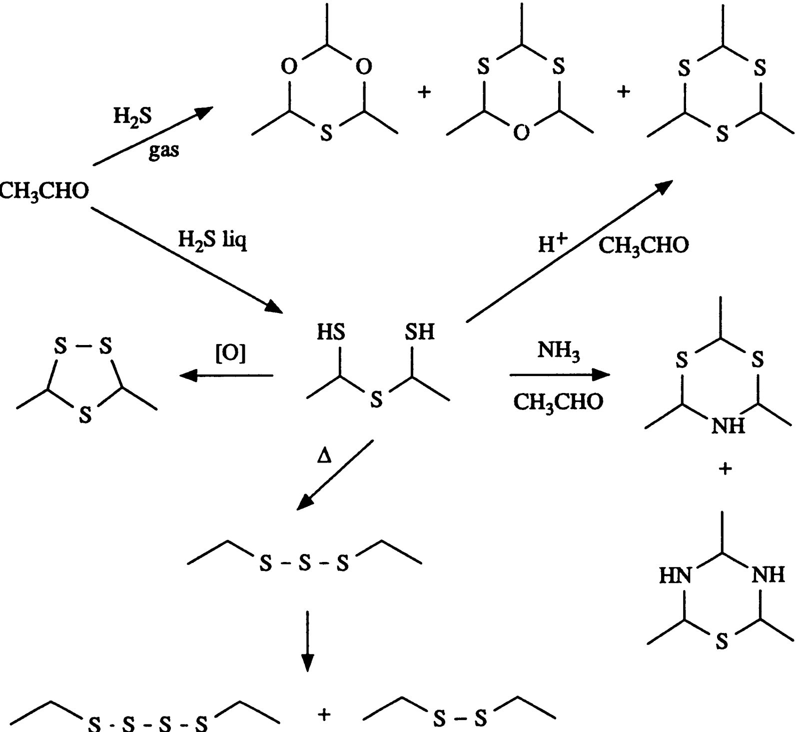

Figure 7. Formation of some sulfur-containing aroma compounds in the reaction of acetaldehyde, hydrogen sulfide, and ammonia. (Adapted from ref. 36).

are substituted in the 2-position with n-alkyl or acyl groups, although 3-(2*H*)-thiophenones are also found. The majority of thiophenes that have been examined for their sensory properties show odor threshold values in the ppb range; therefore, they should be considered as potential contributors to the aroma in foods. The alkyl thiophenes are reported to have aromas reminiscent of roast onions *(43,44)*; 2-formylthiophene is reported as benzaldehyde-like, while 5-methyl-2- formylthiophene gives a cherry-like odor at a level of 0.5 ppm *(45)*. 2-Acetylthiophene has an onion- or mustard-like aroma although in coffee it is reported to develop a malty, roast note. Its odor threshold value in water was found to be 0.1 ppb, which was some 2000 times lower than 2- formylthiophene *(46)*. As discussed above, thiophenes with a thiol group substituted in the 3-position are known to possess meaty characteristics, and a number have been reported in model systems.

There are a number of possible routes to the thiophenes *(6)*, involving the reaction of hydrogen sulfide, or some other sulfur compound also derived from the sulfur amino acids, with intermediate sugar degradation products from the Maillard reaction, such as deoxyosones (Figure 2). The mechanisms are similar to those for the formation of pyrroles. Long-chain 2-alkylthiophenes have recently been shown to be formed when a phospholipid was heated in a Maillard system of cysteine and ribose *(47)*, and a pathway has been proposed involving the reaction of hydrogen sulfide with a 2,4-dienal derived from lipid degradation *(48)*.

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Factors Influencing the Maillard Reaction

Most of the information available on the factors influencing the Maillard reaction in food relates to the formation of color or loss in nutritional value, and there have been few systematic studies on the role of various factors in flavor formation. An excellent summary of those factors that have been studied in relation to flavor formation has been given by Reineccius *(49)*. Factors include time/temperature conditions, water activity/moisture content, and pH, as well as the composition of the system.

Much of the work on the Maillard reaction in flavor formation has been carried out in model systems in which a single amino acid has been reacted with a reducing sugar or a sugar degradation product, and in many cases the reaction has been carried out in aqueous solution. Even in such "simple" systems the number of volatile products is very large, and interpretation of the results has largely been limited to postulating mechanisms of flavor formation and evaluating the sensory properties. There is a lack of kinetic data on the formation of flavor compounds in the Maillard reaction, and little information relating the model studies to real food systems.

Mixtures of amino acids and sugars stored at refrigerated temperatures can show signs of Maillard browning on storage; the reaction increases markedly with temperature, and the desirable flavors and brown colors associated with cooking are formed at the elevated temperatures associated with cooking. It is generally recognized that the reaction proceeds more readily at low moisture levels, and the brown colors and characteristic flavors associated with the outside areas of roasted or baked foods, where dehydration has occurred, are cited as examples of the importance of low moisture content in the Maillard reaction. It has been suggested that the optimum reaction rate for Maillard reactions occurs at a water activity of 0.65 - 0.75. In a kinetic study of pyrazine formation in non-fat dried milk at water

activities ranging from 0.32 to 0.84, Leahy and Reineccius found that pyrazine formation increased to reach a maximum at an a_w of about 0.75 *(50)*. However, other work has shown that for some other classes of volatiles the rates increase or decrease with changes in water activity depending on whether or not water is required for their formation *(30,51,52)*.

It is well known that color formation in the Maillard reaction is much greater at pHs above 7. This may be explained by considering the formation of melanoidin pigments from reactions of amino groups with Maillard intermediates, which will be inhibited at lower pH due to protonation of the amino group. Similarly it has been shown that the rate of pyrazine formation in model systems increases with increasing pH, and pyrazines are not found when the reactions are carried out at pH less than 5.0 *(50)*. Other classes of compounds, such as furfural and some sulfur compounds may be favored by lower pH. The breakdown of the Amadori product during the intermediate stages of the Maillard reaction is pH dependent; a mechanism involving 1,2-enolization of the Amadori compound is favored by a low pH, while 2,3-enolization is more likely to occur at higher pH (see Figure 2). In unbuffered model systems, a pH change of 3 or more pH units may occur during heating, and this may affect both the rate and the pathways by which volatile and colored products are formed *(47,53)*. Thus, in model systems used to study the influence of pH on the Maillard reaction, it is very important to maintain a constant pH during the reaction. Recently, in model systems containing cysteine and ribose it was shown that small changes in pH over the range 4.5 - 6.5 had a marked effect on the nature and concentration of the volatile products *(54,55)*. Furanthiols and disulfides, which are believed to be important in meat aroma were favored by lower pHs, while pyrazines were only formed at pH above 5.0.

Interaction of Lipid with the Maillard Reaction

The other important flavor forming reactions in heated foods, apart from Maillard reactions, involve lipids. These undergo thermal degradations to give a large number of volatile products, especially aldehydes, alcohols, and ketones from the oxidation of the alkyl chains of fatty acids *(56)*. Such compounds are reactive and could be expected to undergo further reactions in the heated foods, and interaction with Maillard intermediates could be anticipated. In an examination of the contribution that lipids make to the development of aroma during the heating of meat, the phospholipids were shown to be particularly important *(57)*. When inter- and intra-muscular triglycerides were removed from lean muscle using hexane, the aroma after cooking could not be differentiated from the untreated material in sensory triangle tests; both preparations being judged to be meaty. However, when a more polar solvent (chloroform - methanol) was used to extract all the lipids - phospholipids as well as triglycerides - a very marked difference in aroma resulted; the meaty aroma was replaced by a roast or biscuit-like aroma. Comparison of the aroma volatiles from these meat preparations showed that the control and the material extracted with hexane had similar profiles, dominated by aliphatic aldehydes and alcohols. Removal of phospholipids as well as triglycerides gave a very different profile of volatiles; the lipid oxidation products were lost, but there was a large increase in the amounts of alkyl pyrazines. This implied that in normal meat, lipids or their degradation products

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch010

inhibit the formation of heterocyclic compounds by participating in the Maillard reaction.

Model Systems Containing Amino Acids, Ribose and Lipid. The results, showing a reduction in amounts of volatile Maillard products in defatted cooked meat, led to investigations of the effect of phospholipids on the volatile products of heated mixtures of amino acids and sugars *(47,58-60)*. Several amino acids were used, including cysteine. Ribose was chosen as the reducing sugar, because of its recognized role in the formation of meat flavor. Concentrations were selected to approximate their relative concentrations in muscle. Reactions were carried out in aqueous solution, buffered at pH 5.6 with phosphate, under pressure in sealed glass tubes at 140 ^{0}C, and the effect of phospholipid on the volatiles from the reaction was examined. In the absence of lipid the reaction mixtures yielded complex mixtures of volatiles including furfurals, furanones, alkylpyrazines, and pyrroles. The volatiles from reactions involving cysteine were dominated by sulfur-containing heterocyclics, particularly thiophenes, thienothiophenes, dithiolanones, dithianones, trithiolanes, and trithianes, together with 2-methyl-3-furanthiol, 2-furanmethanethiol, and 2-methyl-3-thiophenethiol.

In the presence of phospholipids a reduction in the amounts of many of these volatiles was observed, confirming the observations in meat that phospholipids exert a quenching effect on the quantities of heterocyclic compounds formed in the Maillard reaction. As would be expected the inclusion of phospholipids in the reaction mixtures produced many volatiles derived from lipid degradation, such as hydrocarbons, alkylfurans, saturated, and unsaturated alcohols, aldehydes, and ketones. In addition the reaction mixtures contained several compounds derived from the interaction of the lipid or its degradation products with Maillard reaction intermediates. In reaction mixtures containing cysteine, ribose, and phospholipid, the most abundant of these compounds were 2-pentylpyridine, 2-pentylthiophene, 2-hexylthiophene, and 2-pentyl-2*H*-thiapyran. Smaller amounts of other 2-alkylthiophenes with C4 to C8 n-alkyl substituents were found, together with 2-(1-hexenyl)thiophene, 1-heptanethiol, and 1-octanethiol. All these compounds are probably formed by the reaction of lipid breakdown products with hydrogen sulfide or ammonia derived from cysteine. Figure 8 shows a scheme for the formation of 2-pentylpyridine, 2-hexylthiophene, and 2-pentyl-2*H*-thiapyran from 2,4-decadienal, which is one of the major oxidation products of polyunsaturated fatty acids.

The particular role of the phospholipids, compared with triglycerides, was also examined *(60)*. The structural phospholipids contain a high proportion of polyunsaturated fatty acids, particularly those with three or more double bonds such as arachidonic acid (20:4), and these could be expected to breakdown during heating to give products that could react with Maillard products. The triglycerides in meat contain only a very small proportion of polyunsaturated fatty acids, and this may explain the observations on defatted meat that suggested that phospholipids rather than triglycerides were important for meat flavor. Reaction mixtures of cysteine and ribose were prepared with different lipids: triglycerides (BTG) and phospholipids (BPL) extracted from beef, and commercial egg phosphatidylcholine (PC) and phosphatidylethanolamine (PE). The aroma of the reaction mixture without any lipid was described as "sulfurous, rubbery", but there was a distinct underlying meaty aroma.

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Addition of the beef triglyceride did not effect the aroma; however, when beef phospholipids were used the meaty aroma was more intense and the sulfurous notes less pronounced. Similarly, the addition of PC or PE gave mixtures with increased meatiness, with the mixture containing PE exhibiting the most meaty character. The lipid preparations differed in the way they influenced the profile of volatiles from the reaction mixtures. All the phospholipids produced 2-pentyl pyridine, 2-pentylthiapyran, and the 2-alkylthiophenes, but the quantities differed markedly. Only trace amounts of these compounds were found in the triglyceride-containing system. Many Maillard reaction products showed marked reductions on addition of lipid, although not all the volatiles were effected to the same extent, and the different lipids did not all behave in the same way. In general BTG showed some effect on the Maillard volatiles, but this was not as marked as the phospholipid preparations. These results clearly demonstrated the important role lipids, especially phospholipids, play in those Maillard reactions that are the basis of flavor formation in meat.

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Volatiles in Foods from Lipid - Maillard Interaction. In a recent review, Whitfield *(61)* examined the volatiles identified in meat and other foods that could be formed from the interaction of lipid with the Maillard reaction. Such compounds include pyridines, pyrazines, thiophenes, thiazoles, and oxazoles with alkyl substituents. They have been reported in the different meat species and fried or baked potatoes but, as yet, they have not been found to any extent in other fried or fatty foods *(61,62)*. In meat the triglycerides or phospholipids provide fatty acid degradation products for interaction with the Maillard reaction, while in fried food it is the frying fat. In 1977, Buttery et al. reported a number of pyridines with C4, C5 or C6 n-alkyl substituents in roast lamb fat *(63)*; subsequently, 2-pentylpyridine has also been found in all the other main species of meat. As discussed above, the reaction of ammonia or amino acids with 2,4-dienals is a likely route to this type of compound (Figure 8). Related reactions involving hydrogen sulfide instead of ammonia provide pathways to the 2-alkylthiophenes, which have also been found in roast or pressure cooked beef and fried chicken. Several thiazoles with long n-alkyl substituents in the 2 position have been reported in roast beef, fried chicken, and fried bacon by the research group at Rutgers University *(31,32,64)*. Fried potatoes were also found to contain similar n-alkyl substituted compounds *(65,66)*. Oxazoles with similar n-alkyl substituents were also found in fried potato, but in roast beef and fried bacon the oxazoles reported had the long n-alkyl substituents in the 4 position *(32,33)*. Mechanisms for thiazole and oxazole formation in heated foods have been suggested to involve aldehydes, usually acetaldehyde and Strecker aldehydes (Figure 6), and it is reasonable to assume that lipid derived aldehydes can participate in these reactions to yield the long chain 2-alkyl thiazoles and oxazoles, although the formation of the 4-alkyloxazoles requires a modification to this mechanism.

Other heterocyclic compounds with long n-alkyl substituents found in meat and baked potatoes include butyl and pentyl pyrazines, and it has been suggested that these could be formed from the reaction of pentanal or hexanal with a dihydropyrazine, formed from the condensation of two aminoketone molecules *(66-68)*. Pentanal and hexanal also appear to be involved in the formation of 3-butyl-3-methyl-1,2,4-trithiolane and its 3-pentyl homologue, which have both been reported in fried chicken *(66)*. Trithiolanes can be formed from aldehydes and hydrogen sulfide *(36)*,

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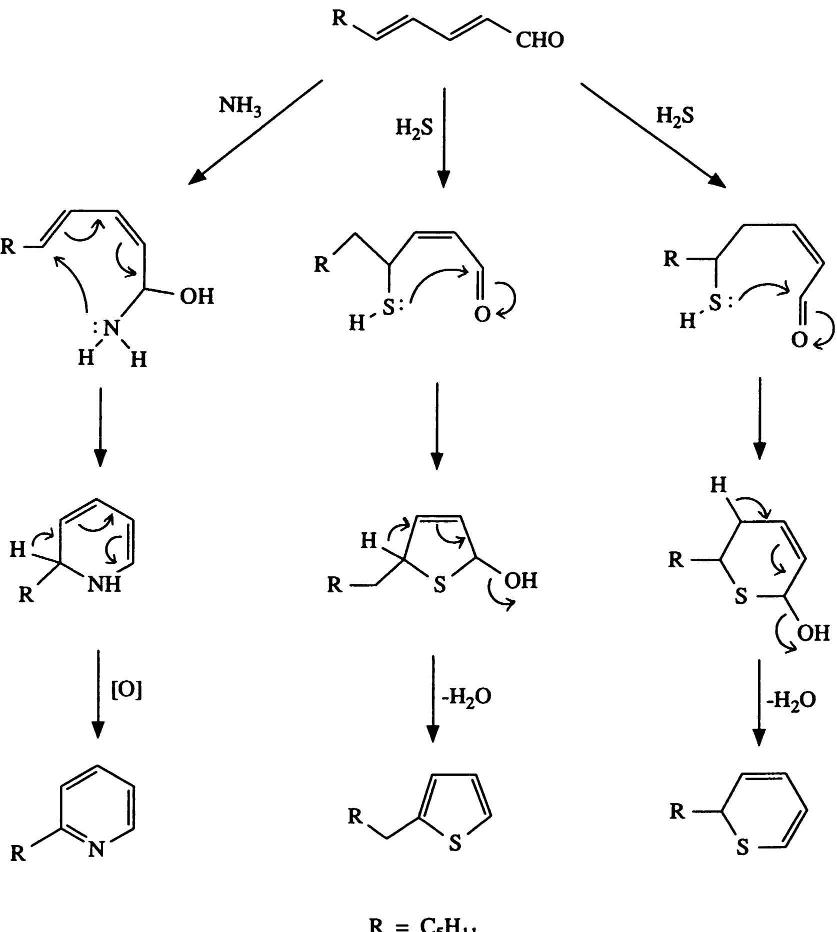

Figure 8. Reaction of 2,4-decadienal with hydrogen sulfide and ammonia. (Reproduced with permission from ref. 60. Copyright 1990 Society of Chemical Industry.)

and the reaction of hydrogen sulfide, acetaldehyde, and pentanal or hexanal has been suggested as the route to these butyl and pentyl trithiolanes *(66,68)*.

The aroma characteristics have only been reported for some of these alkyl substituted heterocyclic compounds, but those which have been examined suggest that they may contribute to fatty, fried aromas *(63,66)*. The reactions leading to these compounds may influence the aroma of heated foods in another way; that is, they provide competing reactions, which modify the extent to which other Maillard reactions can occur; thus affecting the balance of aroma compounds produced during the cooking. The early stages of the Maillard reaction give rise to many reactive intermediates, of which dicarbonyls, furfurals, furanones, Strecker aldehydes, ammonia, and hydrogen sulfide are the most important. These provide the reactants for most of the important classes of aroma volatiles. The relative amounts of the different volatiles produced from these intermediates must depend on the concentrations produced by the early Maillard reaction, and the relative rates of the different reactions. The addition of lipid (especially unsaturated lipids) and lipid degradation products (such as aldehydes, ketones, and alcohols) to this mixture of Maillard intermediates provides competing reactions that produce other volatiles and effect the relative proportions of compounds produced by the other reactions. Hydrogen sulfide and ammonia are extremely important intermediates in so many of the aroma forming reactions, and their interaction with lipid degradation products is a clear example of how lipid will influence the relative proportions of heterocyclic Maillard reaction products.

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Chapter 11

Dicarbonyl Sugar Derivatives and Their Role in the Maillard Reaction

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The Maillard reaction involves the interaction of reducing sugars with protein amino groups to give 1-amino-1-deoxy-2-ketose derivatives (Amadori compounds), followed by their degradation. During this degradation, deoxy-dicarbonyl sugar derivatives are formed, which play an important role in subsequent stages of the reaction. The role of "3-deoxyglucosone", as well as other dicarbonyl intermediates produced from Amadori compounds are discussed herein. Ascorbic acid also undergoes Maillard type reactions with amino acids. This is probably due to the fact that it undergoes degradation to give carbonyl and dicarbonyl intermediates. Among the degradation products detected are L-threose as well as two 5-carbon dicarbonyl compounds. Data are presented with respect to the identification of these compounds as well as how they arise from ascorbate or its oxidation products. Finally, the results of some studies of aminoguanidine (guanylhydrazine) as an inhibitor of the Maillard reaction are presented.

The Maillard reaction represents a complex series of degradation reactions that is initiated by the interaction of a carbonyl compound, usually a reducing sugar, with an amino group, usually a protein or an amino acid. The reaction is a degradative reaction with respect to both the amine and the reducing sugar, both of which eventually disappear from the reaction solution or the food preparation, if the reaction is allowed to proceed sufficiently long. During the Maillard reaction, polymeric pigments are formed (Maillard polymers), which contain carbon atoms derived from both the sugar and the amine; an increase in the ultraviolet absorbance is observed (a UV maximum gradually develops at about 300 nM); food flavor and aroma

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constituents are produced; and an increase in the amount of carbonyl groups is observed. The latter is due, in large part, to partially dehydratedsugar-derived dicarbonyl compounds that are produced as intermediates in the reaction. In addition, proteins undergo functional modifications, since some of the free amino functions on them have reacted with reducing sugars, and extensive protein crosslinking is also observed. The modification of protein significantly alters the nutritional value of food proteins, especially the availability of lysine, which has a free epsilon amino group, that can easily react with reducing sugars. The reaction is of considerable and obvious interest to food chemists and technologists, but has also recently been found to be an *in vivo* reaction, which may have deep seated health related consequences. A number of international symposia have been held on the subject and the proceedings have been published (*1-4*). In addition, the excellent reviews by Ledl (*5*) and by Danehy (*6*) are noteworthy. The latter is of special interest with respect to the generation of food flavors and aromas in foods and the influence of amino acids on this reaction.

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Initial Stages of the Maillard Reaction

From the standpoint of carbohydrate chemistry, the initial stages of the reaction are reasonably well understood, when aldose sugars, which are the most common reactants in food systems, serve as participants. As shown in Figure 1, using glucose as an example, the sugar initially reacts with an amine to give an intermediate glycosyl amine, which rapidly rearranges to a 1-amino-1-deoxy-2-ketose. The conversion of a sugar into an Amadori compound constitutes the Amadori rearrangement, and the product of the reaction is commonly referred to as an Amadori compound. Amadori compounds are conformationally unstable, and in solution, exist in both the pyranose and furanose forms as well as measurable amounts of open chain form. This has been shown to be the case for a number of Amadori compounds that were prepared from a variety of amino acids and was demonstrated by 13C NMR spectroscopy (*7*). Compared to the parent aldose sugar, Amadori compounds are unstable (*8*), and they undergo a variety of degradation and dehydration reactions, which depend on the solution pH, temperature, water content of the reaction mixture, and the duration of heating time. The difference between the rate of decomposition of an Amadori compound and the parent aldose sugar is large. Amadori compounds, for example, when heated at pH 2.0, will completely decompose in a matter of hours, while a sugar, such as D-glucose is stable at these conditions for weeks, or even months. Thus, the formation of an Amadori compound provides a pathway for a relatively stable sugar, such as D-glucose, to undergo a variety of degradation reactions at relatively mild conditions, as would be encountered during the cooking or processing of foods. Amadori compounds may be expected to form any time reducing sugars (or compounds that contain them) are found in the presence of protein or amino acids. They can be expected to be formed in almost all types of foods, particularly when they are heated, a process that increases the rate of reaction between sugars and amino groups.

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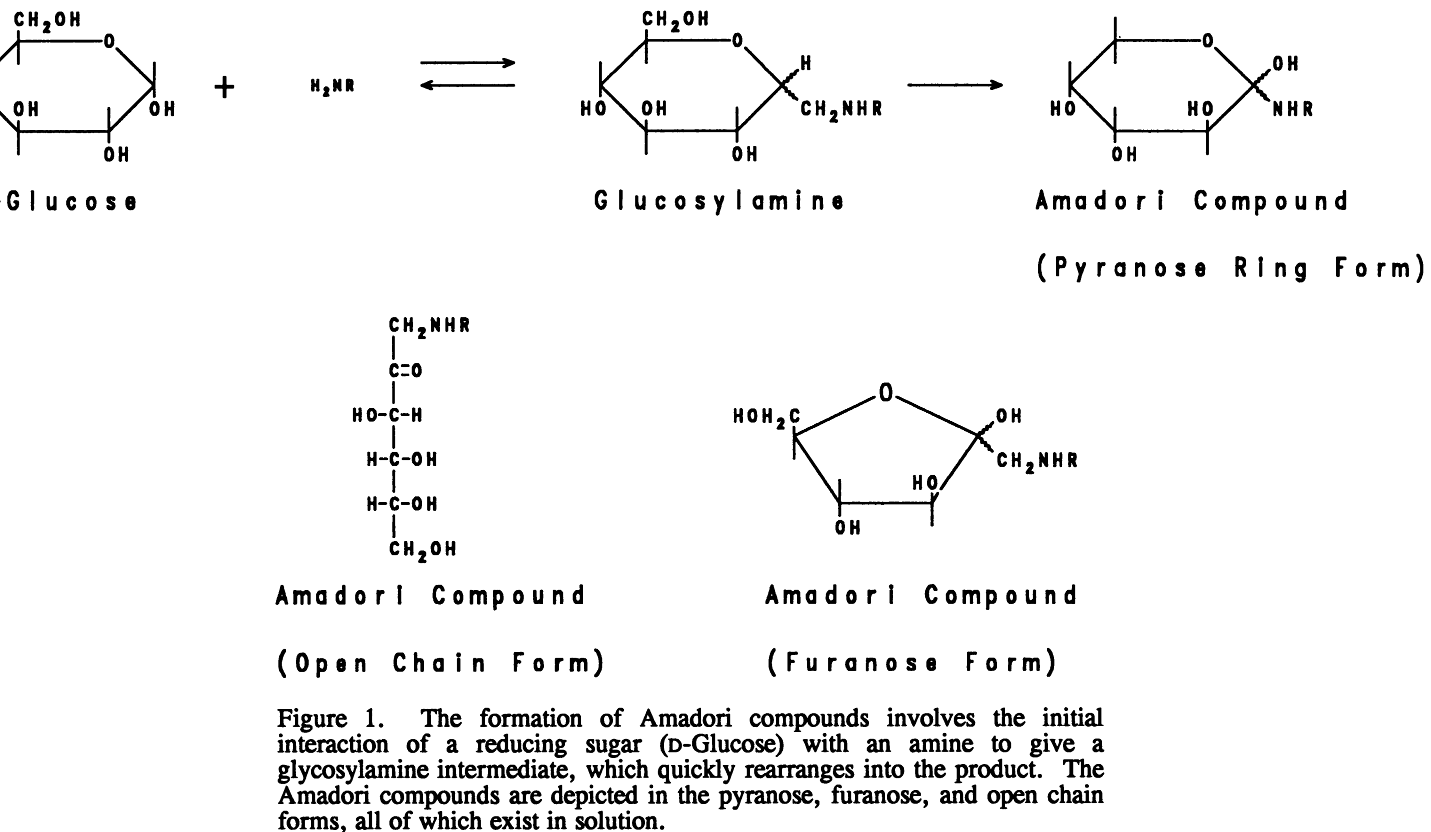

Figure 1. The formation of Amadori compounds involves the initial interaction of a reducing sugar (D-Glucose) with an amine to give a glycosylamine intermediate, which quickly rearranges into the product. The Amadori compounds are depicted in the pyranose, furanose, and open chain forms, all of which exist in solution.

The Degradation of Amadori Compounds

Our knowledge of all the degradative reactions that an Amadori compound can undergo is incomplete at present. Molecular oxygen probably plays a part in some of these degradation reactions, as do solution pH, temperature, and the basicity of the amino group of the Amadori compound that is involved in the reaction.

The present thinking suggests that Amadori compounds initially undergo enolization and dehydration to give partially dehydrated dicarbonyl sugar derivatives. It is these intermediates that serve as the precursors for production of a variety of food flavor and aroma constituents, production of the UV absorbing materials, and possibly as reagents that modify proteins in later stages of the reaction. Interestingly, it is these compounds that we know the least about in terms of their overall participation in the reaction.

Formation of 3-Deoxy-1,2-Dicarbonyl Intermediates. 3-Deoxy-D-*erythro*-hexos-2-ulose (hereafter referred to as 3-deoxyglucosone) represents an important dicarbonyl intermediate and is also the most well studied of the dicarbonyl derivatives. It was originally isolated from a glycine-derived Amadori compound by Anet (*9*), and its preparation, also described by Kato (*10*), results from the reaction of D-glucose with N-butylamine. More recently, we have reported a practical synthesis of it (*11*) starting from the crystalline bis-benzoylhydrazone, which was originally prepared and characterized by El Khadem (*12*) and his co-workers. 3-Deoxyglucosone can now be prepared in reasonably pure, gram sized quantities with relative ease. It is a highly reactive intermediate and represents one of the first formed dicarbonyl compounds from an Amadori compound. It is converted into 5-(hydroxymethyl)-2-furaldehyde (HMF), in acidic solution (*13*). HMF is a strong UV absorber (A=18,000, k=280 nM), which probably accounts for much of the UV absorption normally associated with Maillard reactions. 3-Deoxyglucosone is produced via an initial 1,2 enolization of an Amadori compound, followed by elimination of the amine substituent, as shown in Figure 2. It represents an initial dehydration product derived from a hexose-derived Amadori compound. Experiments using 3-deoxyglucosone as a starting compound indicate that its reactivity is consistent with it being an important Maillard reaction intermediate. When tested with amino acids, it gives brown colors (Maillard Polymers) at a much faster rate than D-glucose itself (*14*), and has been shown to function in Strecker degradation reactions with phenylalanine, converting it to phenylacetaldehyde in high yield (*15*). In *in vitro* experiments, 3-deoxyglucosone has been shown to be involved in protein crosslinking (*16,17*), and has been detected in serum and urine of human subjects (*18*). It is noteworthy that, in these latter experiments, both 3-deoxyglucosone and 3-deoxy-D-fructose were found to be present in both urine and serum, with the latter in the highest yield. It is probable that the fructose derivative is produced from the deoxyosone via enzymatic reduction by aldose reductase.

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Formation of 1-Deoxy-2,3-Dicarbonyl Intermediates. Figure 3 shows a mechanism wherein an Amadori compound undergoes an initial 2,3 enolization, a process that ultimately produces a 1-deoxyglucosone intermediate. For many years, this intermediate was a hypothetical one, having never been isolated or shown to be present during a Maillard reaction. The intermediate was predicted to serve as the precursor for maltol and isomaltol, important pastry flavor and aroma constituents, as shown in Figure 4. Ledl (*19*) and his co-workers have recently confirmed that the 1-deoxyosone is actually produced during Maillard reactions; trapping and identifying it as the quinoxaline derivative. Clearly it is produced in the reaction, but little is known about its reactivity or role in Maillard reactions, other than the fact that it is, as expected, produced and appears to be a precursor of a number of food flavor and aromas. It is noteworthy that the 5 carbon analog, also a hypothetical intermediate, which could be produced from pentoses or hexuronic acids (the latter via decarboxylation) probably serves as the precursor (*20*) of 4-hydroxy-5-methyl-3(2H)-furanone, an important component of cooked meat flavor and aroma, as shown in Figure 5. Indirect evidence (14C tracer studies) supports this hypothes is in the sense that the methyl group of the furanone is derived from C-1 of the reducing sugar and from C-1 of the Amadori compound (*21*).

Formation of 1,4-Dideoxy-2,3-Dicarbonyl Intermediates. Lastly, the 2,3 enolization of an Amadori compound could also give rise to a 1,4 dideoxy-2,3-dicarbonyl derivative that remains attached to the amino group. This type of derivative is also now known to be produced from an Amadori compound, having been isolated by Ledl and his co-workers recently from a synthetically produced Amadori compound (*22*). Some years ago, a substituted lysine derivative was shown to be present when dried milk preparations were subjected to hydrolysis, prior to amino acid analysis. Furosine (*23*), the isolated derivative (Figure 6) would be expected to arise from the 1,4 dideoxyosone derivative, which, in turn, would be expected to be formed from the Amadori compound, produced as a result of reducing sugar reacting with a Lysine residue during the spray drying of milk. This represents a concrete example of how the nutritional availability of lysine in proteins is decreased as a result of the Maillard reaction.

The Role Of Ascorbic Acid In The Maillard Reaction.

Ascorbic acid also serves as a very active participant in Maillard reactions. We have recently initiated some studies relative to the compounds that are produced during the degradation of it and what their role is in the Maillard reaction. At pH 7.0 and at 37 °C in the presence of oxygen, ascorbic acid is very unstable, even in the absence of amino groups. This suggests that it may well decompose to give carbonyl-containing compounds that are capable of interacting with amines to give Maillard reaction products. Some of the compounds produced during the degradation of ascorbic acid are shown in Figure 7, and include threose, glyceraldehyde, as well as xylosone and 3-deoxyxylosone (*24, 25*). The latter would be expected

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch011

CH_2NHR
C=O
HO-C-H
H-C-OH
H-C-OH
CH_2OH

→

CHNHR
C-OH
HO-C-H
H-C-OH
H-C-OH
CH_2OH

$- H_2O$
$- NH_2R$
→

H O (CHO)
C=O
CH_2
H-C-H
H-C-H
CH_2OH

3-Deoxyglucosone

Figure 2. The dicarbonyl intermediate 3-deoxyglucosone (shown above) appears to be produced from an Amadori compound via 1,2 enolization, followed by loss of one molecule of water and hydrolysis of the schiff base substituent.

CH_2NHR
=O
HO—H
H—OH
H—OH
CH_2OH

→

CH_2NHR
C-OH
C-OH
H-C-OH
H-C-OH
CH_2OH

$- H_2O$
$- NHR$
→

CH_3
C=O
C=O
H-C-OH
H-C-OH
CH_2OH

1-Deoxyglucosone

Figure 3. The dicarbonyl intermediate 1-deoxyglucosone (shown above) appears to be produced from an Amadori compound via initial 2,3 enolization, followed by elimination of the amine substituent.

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch011

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch011

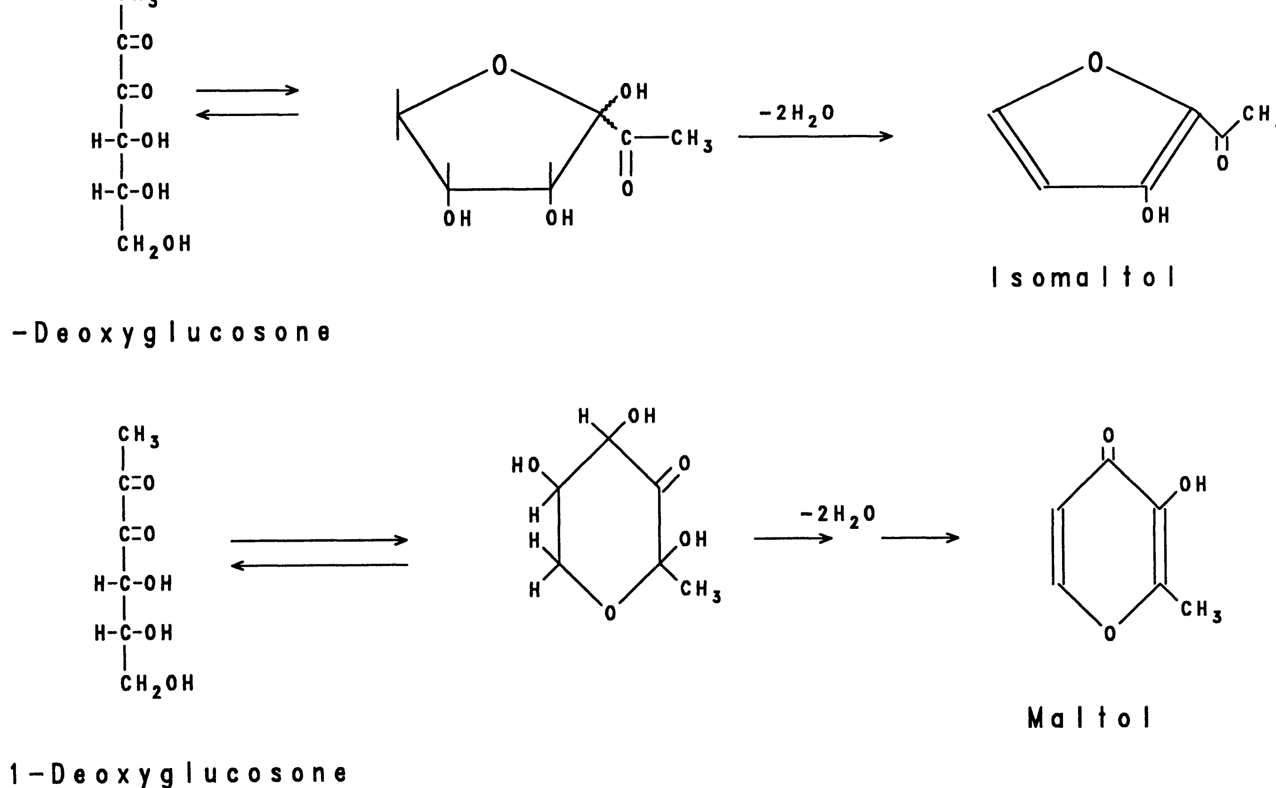

Figure 4. 1-Deoxyglucosone appears to serve as the precursor of isomaltol and maltol, prominent food flavor and aroma constituents. The mechanism for the formation of these compounds are shown above.

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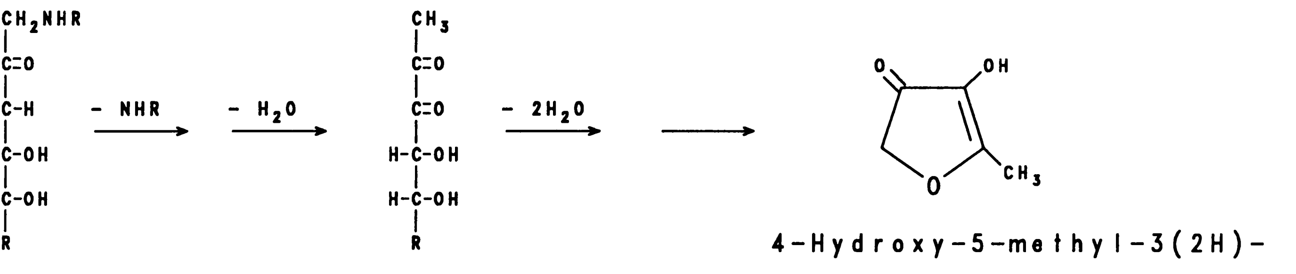

Figure 5. The 1-deoxydiulose derived from either a pentose sugar (R=H) or a hexuronic acid (R=COOH) serves as the source of 4-hydroxy-5-methyl-3(2H)-furanone, a prominent component of the flavor and aroma of cooked beef.

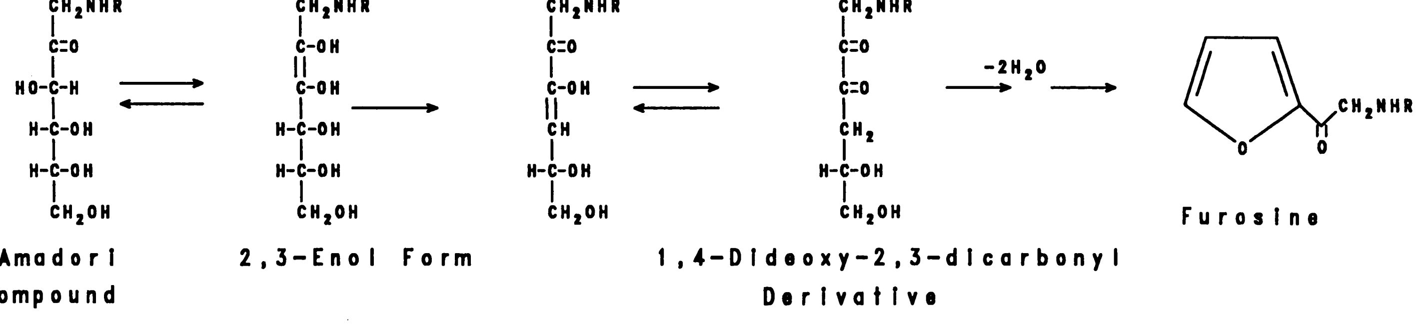

Figure 6. The 1,4-dideoxydicarbonyl derivative produced during Maillard reactions appears to be produced via an initial 2,3 enolization of the Amadori compound. It appears to serve as a source of furosine.

to function as highly reactive intermediates in the same manner as the 6-carbon osones described above. They probably explain the formation of 2-furaldehyde, and related UV absorbing compounds produced during the acid-catalyzed degradation of ascorbic acid. In our initial studies, all of these intermediate compounds were identified by GC (comparason with authentic standards) as well as by GC-MS. It is not altogether clear how these materials arise during the decomposition reactions. At the conditions of the study, ascorbic acid could have been partially oxidized to dehydroascorbic acid, which can also be converted into the open chain form, 2,3-diketo-L-gulonic acid (ascorbic acid is readily converted into dehydro ascorbic acid by molecular oxygen) (Figure 8).

The degradation product produced from ascorbic acid in the highest yield is threose, an aldotetrose (*26*). Threose is a much more highly reactive sugar as regards the Maillard reaction, than the aldohexoses and aldopentoses. Hence, it seems likely that this sugar constitutes the major source of ascorbic acid-derived carbonyl groups in this type of Maillard reaction. In an attempt to better understand how threose is formed during this degradation, we prepared both dehydroascorbic acid and 2,3-diketo-L-gulonic acid and we examined the yield of and the rate of formation of threose from these possible precursors. Figure 9 shows some data on the rates of formation of threose in both the presence and absence of oxygen from all three possible sources. The data supports the following scenario: The source of threose is 2,3-diketo-L-gulonate, which is produced by oxidation of ascorbic acid, followed by hydrolysis of the dehydroascorbate to gulonate. Oxygen is probably not required for threose formation, i.e., the cleavage reaction that gives rise to it is not an oxidative one; oxygen only serves to oxidize ascorbic acid to the diketone, which represents the reactive species. It is also noteworthy that absolute yields of threose, as it is produced in the reaction with 2,3-diketo-L-gulonate, approaches 22 mole%. Threose is also unstable at the conditions at which it is produced and has a half life of about 3.5 days at pH 7.0 and 37 ° C. Therefore, it seems probable, that threose is a major factor in ascorbic acid-catalyzed Maillard reactions. It is highly reactive and is produced constantly during the reaction from the ascorbate in solution; thereby providing a constant source of aldopentose during the lifetime of ascorbate in solution. The other carbonyl and dicarbonyl compounds are produced in much lower yields, but, nevertheless, may well be important in the overall reaction. We rationalize the formation of xylosone as a decarboxylation product arising from dehydroascorbate and 3-deoxyxylosone as arising from ascorbate via similar mechanisms, i.e., a classical ß-keto acid decarboxylation as shown in Figure 10.

Inhibition Of The Maillard Reaction

There is considerable interest in the development of compounds that will inhibit Maillard reactions, particularly with regard to color development. This is of obvious interest to the food processing and related industries where the production of dark colors is undesirable. Bisulfite has been the reagent of choice for this inhibition, because it is effective and cheap. Presumably bisulfite forms addition

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Figure 7. A number of carbonyl and dicarbonyl compounds (shown above) are produced from ascorbic acid during its degradation in solution at pH 7.0 and in the presence of oxygen.

Figure 8. Ascorbic acid undergoes oxidation in the presence of oxygen to give the dehydro form, which can undergo hydrolysis to the open chain 2,3-diketo-L-gulonic acid.

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch011

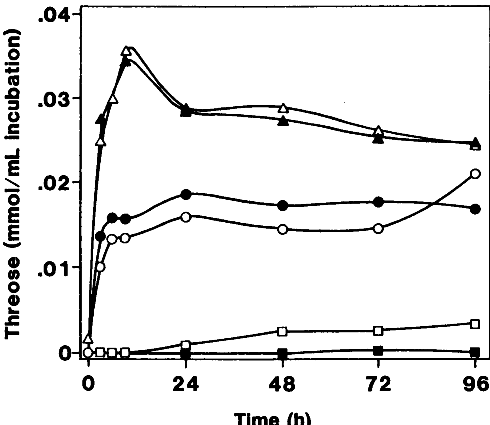

Figure 9. Rates of appearance of threose at pH 7.0 and 37 °C in the presence (open symbols) and absence of oxygen (closed symbols). L-Ascorbic acid (□ ■), dehydro-L-ascorbic acid (○ ●), and 2,3-diketo-L-gulonic acid (△ ▲).

compounds with reducing sugars, possibly Amadori compounds themselves, as well as the dicarbonyl intermediates, preventing them from further reaction. The possibility that bisulfite may be banned as a food additive has stimulated a search for alternatives for this compound. Recently, aminoguanidine (*27*) has been suggested as a possible reagent for this inhibition and has been shown to inhibit Maillard initiated protein crosslinking at physiological conditions, and inhibits brown color formation, when D-glucose is incubated with proteins. Kato (28) and co-workers have also recently shown that aminoguanidine also inhibits crosslinking in model systems using 3-deoxyglucosone and model proteins, suggesting that the site of the inhibition may be at a later stage of the Maillard reaction. We recently investigated the nature of the reaction of aminoguanidine and 3-deoxyglucosone and other dicarbonyl sugar derivatives that might be expected to be produced in Maillard reactions. At pH 7.0 and 37 °C, aminoguanidine rapidly and completely reacts with 3-deoxyglucosone in a matter of minutes, and is converted into a mixture of 5- and 6- substituted 3-aminotriazine derivatives (*29,30*) (Figure 11). By comparison, D-glucose reacts very slowly and incompletely, requiring weeks to give any reaction product, which appears to be a hydrazone. The reaction can be followed by thin layer chromatography or by UV absorbance measurement, since the triazines that are produced in the reaction absorb strongly at about 315 nM, while aminoguanidine itself shows no absorption at this wavelength. The reaction appears to be a general one for any molecules that contain adjacent dicarbonyl functional groups, since the pentose analog of 3-deoxyglucosone (3-deoxyxylosone), as well as glucosone and xylosone also react at about the same rates. The only differences observed were that the 3-deoxy-derivatives give a mixture of triazines, while glucosone and xylosone give exclusively the 5 substituted triazine.

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch011

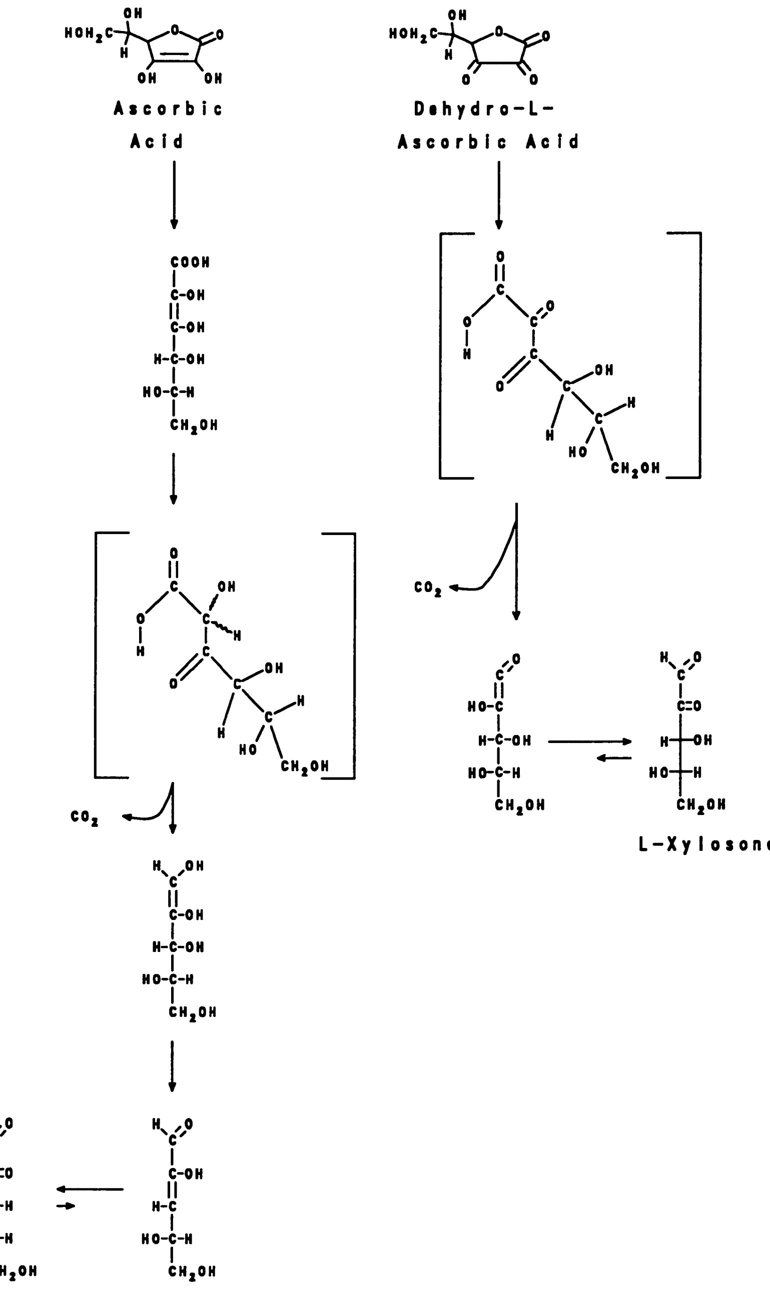

Figure 10. 3-Deoxyxylosone and xylosone are probably produced via ß-keto acid decarboxylation of ascorbic acid and 2,3-diketo-L-gulonic acid, respectively.

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch011

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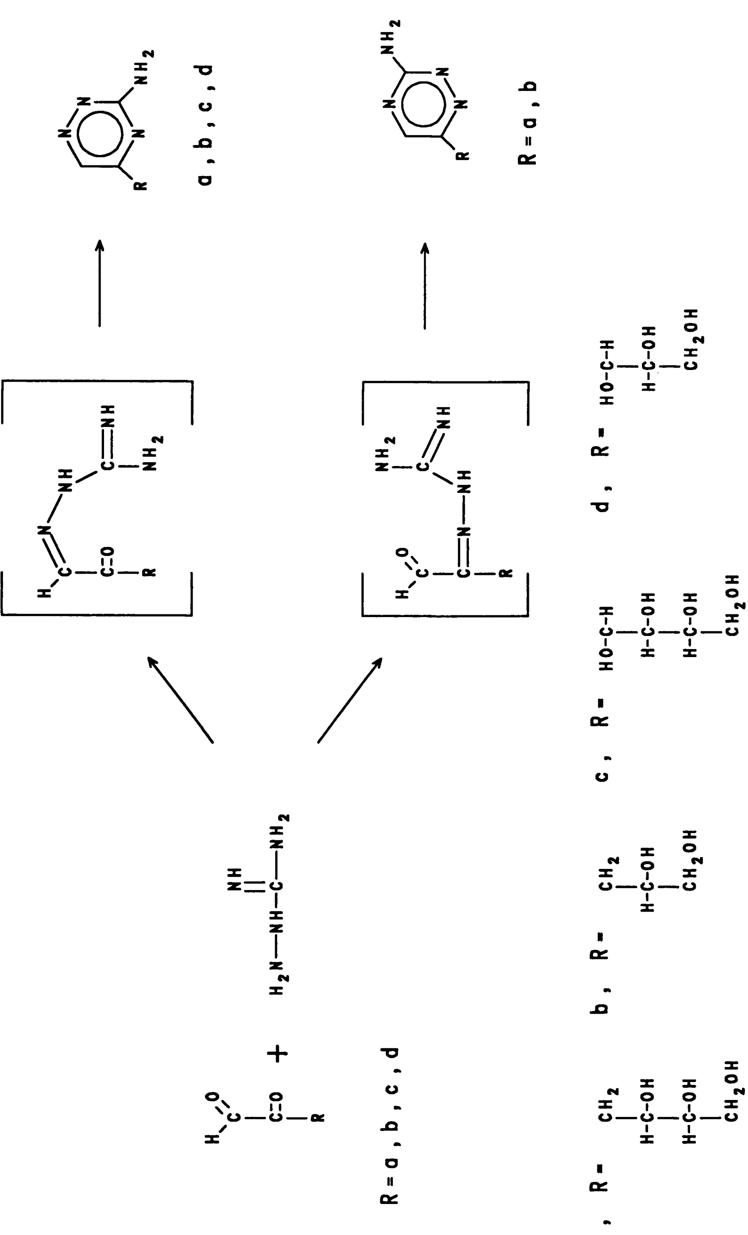

Figure 11. Aminoguanidine appears to exert its effect as an inhibitor of the Maillard reaction by complexing with dicarbonyl intermediates to give the stable 5- and 6- substituted triazine derivatives, which represent irreversibly formed end products.

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Chapter 12

Mechanism of Pyrazine Formation

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Pyrazine formation was studied as a model for carbohydrate fragmentation in the Maillard reaction. Thus 1-^{13}C-glucose, 2-^{13}C-glucose and 1-^{13}C-fructose were reacted with asparagine in 1,2-propanediol, and the volatile products isolated by steam distillation and extraction. The product mixture was analyzed by GC, NMR and GC-MS, and consisted mainly of dimethyl-, monomethyl, and to a lesser extent trimethylpyrazines. Although product yields and ratios for the reaction of asparagine with glucose differed to some extent from the same reaction with fructose, the ^{13}C-distribution of the resulting pyrazines was not much different. The ^{13}C-distribution in pyrazines originating from 2-^{13}C-glucose was quite different from the ^{13}C-distribution in pyrazines which were formed from glucose and fructose labeled at the C-1 position. The ^{13}C-incorporation in the pyrazines obtained from all three labeled hexoses was in agreement with retro-aldolization of the intermediate deoxyglucosones as the main cleavage mechanism (scheme 5). Both 1- and 3-deoxyglucosone appear to play an approximately equally important role in the formation of the methylated pyrazines.

The formation of many flavor substances in the Maillard reaction is initiated by carbohydrate cleavage. The resulting mono- and dicarbonyl products may react further to form pyrazines, thiazoles, carbocyclic compounds, etc., depending on conditions and other reactants (*1-5*). Asparagine appears to be a particularly good amino acid in the formation of pyrazines (*5,6*), and was used here to study pyrazine formation and the underlying carbohydrate cleavage mechanism.

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In the literature three main carbohydrate cleavage mechanisms are described. Hayami (*7*) studied sugar decomposition which was not amine or amino acid catalyzed, but which took place in 40% aqueous phosphate buffer (pH 6.7). Acetol was reported as the main three-carbon fragmentation product. Its formation was explained by hydrolytic cleavage of a β-diketone intermediate, which was generated from 1-deoxyglucosone by isomerization.

In the Maillard reaction the main cleavage mechanism is often considered to be retroaldolization (*8*). The structures of a number of Maillard reaction products permit one to assume that they have been formed by retroaldol cleavage. In a glucose plus β-alanine model reaction, production of C_2 and C_3 sugar fragments was negligible under acidic conditions, but increased with pH, also in agreement with the retro-aldol cleavage mechanism (*9*).

In a glucose plus alkyl amine model system pyruvaldehyde and glyoxal diimines were identified as the main products (*10,12*). It was suggested that C_2 fragments are formed directly from an aldose or the corresponding imine (*10*), and C_3 fragments from deoxyosones (*11*) or Amadori rearrangement products (*12*).

In addition to the retroaldolization mechanism, there seems to be some evidence for an alternative cleavage mechanism in the Maillard reaction: cleavage of α-dicarbonyl species (*8*). This mechanism is supposed to involve immonium ion formation, followed by hydrolytic cleavage, resulting in a carboxylic acid and an immoniumbetaine intermediate. The mechanism is based mainly on products formed from α-dicarbonyl species such as 3-deoxyglucosone.

We recently reported on the reactivity of 3-deoxyglucosone and the formation of pyrazines from 1-^{13}C-glucose (*6*). We now report on the formation of pyrazines from 1-^{13}C-glucose, 2-^{13}C-glucose and 1-^{13}C-fructose, which were analysed by NMR and GC-MS.

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Experimental

General. The reaction conditions for generating the pyrazines were as described before (*6*). Abbreviations in the text are as follows: Pyr: pyrazine; MMP: methylpyrazine; DMP: dimethylpyrazine; TMP: trimethylpyrazine; 1-done: 1-deoxyglucosone; 3-done: 3-deoxyglucosone; 4-done: 4-deoxyglucosone.

NMR. NMR conditions have been described before (*6*). Because samples of the labeled pyrazines were relatively dilute, the chemical shifts of the pyrazine C-atoms varied considerably, making it impossible to assign each peak in the ^{13}C-NMR to a specific pyrazine. For the same reason only some of the methyl signals for 2,5-DMP could be identified individually.

GC and GC-MS. The GC colums used were a 50 m CP-Sil 8CB (Chrompack) and a 60 m Stabilwax (Restek). The CP-Sil column was used in most of our studies, and did not separate 2,5-DMP and 2,6-DMP. The Stabilwax column gave almost complete resolution of 2,5-DMP and 2,6-DMP. The term dimethylpyrazine (DMP) was generally used to indicate a mixture of 2,5-DMP and 2,6-DMP. We used GC-MS data to identify the individual pyrazines, and to quantify the level of ^{13}C-enrichment. The spectra of the labeled pyrazines were compared with the spectra of the unlabeled pyrazines. The $[M]^{+\cdot}$, $[M+1]^{+\cdot}$ and $[M-1]^{+\cdot}$ patterns of the unlabeled pyrazines were used to determine the extent of ^{13}C-enrichment in the labeled pyrazines. MS-MS experiments of unlabeled DMP had indicated this to be an acceptable approach. Using this procedure the ratios of none, once and twice labeled pyrazines were obtained, and were used to calculate total ^{13}C-content. When the same approach was used to determine the ^{13}C-content of the

fragmentation patterns representing $[M\text{-}HCN]^{+\cdot}$ and $[CH_3CNH]^+$, values were obtained which were qualitatively comparable with the NMR results. However the ^{13}C contents of the pyrazines calculated from the ^{13}C contents of the fragments were significantly and consistently different from the ^{13}C contents of the pyrazine molecules based on the molecular ion mass spectrum. We reasoned that the values for the fragments may be effected by isotope effects, and are therefore unreliable.

Results and Discussion

Pyrazines from 1-^{13}C-glucose and 1-^{13}C-fructose. Distribution of ^{13}C in the pyrazine molecules was determined by ^1H-NMR, ^{13}C-NMR and GC-MS (Tables 1–4). ^{13}C-incorporation in pyrazines derived from 1-^{13}C-fructose was found to be slightly higher than in pyrazines derived from 1-^{13}C-glucose. NMR data clearly indicate that C-1 of fructose and glucose is incorporated at the methyl and methine carbon atoms of the methylated pyrazines, but not at the quarternary carbon atom. ^{13}C-incorporation at the methyl position of 2,5-DMP is equal for fructose and glucose, ^{13}C-incorporation at the methine position is slightly higher for 1-^{13}C-fructose derived pyrazines than for 1-^{13}C-glucose derived pyrazines.

All in all there is very little difference in ^{13}C-incorporation into pyrazines from 1-^{13}C-fructose and 1-^{13}C-glucose, which is not necessarily what we expected. Fructose gives pyrazines in a significantly higher yield than glucose (Table 5), and because it is a ketose, it must follow a different route in the formation of the deoxyglucosones (Scheme 1 and 2).

Comparison of the theoretically possible ^{13}C containing retroaldol products from the 1-^{13}C-deoxyglucosones (Scheme 3) suggests that the position of the ^{13}C-label in the methylated pyrazines is indicative for the relative importance of the intermediate deoxyglucosones (*6*). Since 2,3-dimethylpyrazine is formed only to a negligible extent, 4-deoxyglucosone is apparently not an important intermediate. Similarly intermediates **3** and **4** (Scheme 3) can be ruled out as contributing significantly to the product pyrazines. This allows one to assign the ^{13}CH labeled pyrazines as coming from 3-deoxyglucosone, and the $^{13}CH_3$ labeled pyrazines as originating from 1-deoxyglucosone. If we apply this reasoning to the pyrazines derived from 1-^{13}C-glucose and 1-^{13}C-fructose, it can be concluded that 1- and 3-deoxyglucosone are participating approximately equally in the formation of pyrazines from fructose, and that 1-deoxyglucosone is slightly more important in the formation of pyrazines from glucose.

Pyrazines from 2-^{13}C-glucose. Methylated pyrazines that were formed from 2-^{13}C-glucose, were labeled almost exclusively at the quarternary carbon atom, in agreement with the mechanism indicated in Scheme 4. ^{13}C-NMR showed two small signals in the CH region (139.4 and 141.5 ppm) of the spectrum, indicating that there is an additional pathway leading to pyrazines. This can be explained by assuming that retroaldolization of glucose or glucose imine also takes place. This reaction which has been suggested by Hayashi et al. (*10*), results in glycolaldehyde or the corresponding imine, which in turn can lead to methylpyrazine or dimethylpyrazine with a ^{13}C-label at the CH position. Comparison of the integrated signals in the ^{13}C-NMR of 2-^{13}C-glucose derived 2,5-dimethylpyrazine with one of synthetic origin, allowed the quantitation of the methine ^{13}C and the quarternary ^{13}C (Table 4).

Retroaldolization versus other cleavage mechanisms. All our observations are in agreement with retroaldolization of the intermediate 1- and 3-deoxyglucosones

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Table 1. ^{13}C-Incorporation in Pyrazines Based on ^{1}H-NMR [1]

	CH (%)		CH_3 (%)	
hexose	2,5-DMP	all	2,5-DMP	all
1-^{13}C-glucose	22.4	20.4	25.8	29.8
	(21.2-23.6)[2]	(14.6-25.6)	(20.3-29.8)	(23.9-34.5)
1-^{13}C-fructose	26.8	27.0	27.1	30.0
	(25.8-28.0)	(25.9-28.0)	(26.1-28.0)	(29.8-30.1)
2-^{13}C-glucose	<10	<10	0	0

[1] based on 2-4 independent experiments
[2] range

Table 2. ^{13}C-incorporation in pyrazines based on ^{13}C-NMR

hexose	CH	CH_3	quarternary C atom
1-^{13}C-glucose	++	++	-
1-^{13}C-fructose	++	++	-
2-^{13}C-glucose	+	-	++++

Table 3. ^{13}C-enrichment in pyrazines based on GC-MS [1]

hexose	MMP	2,5-DMP	2,6-DMP	TMP
1-^{13}C-glucose	0.81-0.86 [2]	0.87-0.90	0.89-0.90	0.84-0.90
1-^{13}C-fructose	0.94	1.05-1.07	1.10-1.12	1.04-1.05
2-^{13}C-glucose	0.96-1.04	0.91-0.93	0.90	0.78-0.79

[1] average number of ^{13}C-atoms per molecule

[2] range, based on duplicate experiments

Table 4. ^{13}C-incorporation in 2,5-DMP based on NMR and GC-MS [1]

hexose	CH	CH_3	C
1-^{13}C-glucose	0.44	0.52	0
1-^{13}C-fructose	0.54	0.54	0
2-^{13}C-glucose	0.05	0	0.87

[1] number of ^{13}C-atoms per molecule

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch012

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Table 5. Yield (%) of pyrazines from various C-sources and asparagine

C-source	Pyr [1]	MMP	DMP	TMP	n=
glucose	-	1.8±0.3 [2]	3.8±0.3 [3]	0.3±0.0	4
fructose	-	2.0±0.5	6.2±0.5 [4]	0.4±0.1	8
glycolaldehyde	0.1	0.3-0.4	0.2-0.3 [5]	-	2
pyruvaldehyde	-	-	6.2±0.9	1.5±0.1	4
glyceraldehyde	-	-	6.2-6.4	0.5-0.6	2
glycolaldehyde + pyruvaldehyde	-	0.9-1.2	2.2-2.5	0.4-0.5	2
glyceraldehyde + pyruvaldehyde	-	-	7.4-7.6	1.1-1.2	1

[1] for abbreviations : see experimental

[2] theoretical yield ± standard deviation (%)

[3] ratio 2,5-DMP : 2,6-DMP was 4.6 : 1

[4] ratio 2,5-DMP : 2,6-DMP was 4.1 : 1

[5] ratio 2,3-DMP : 2,5/6-DMP was 5 : 1

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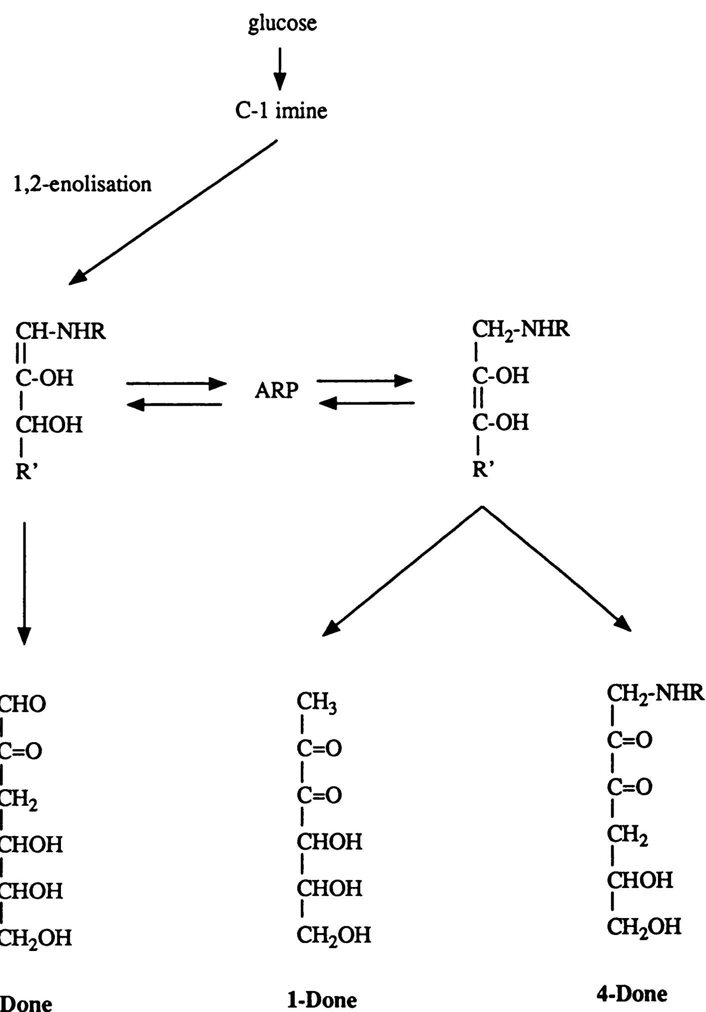

ARP = Amadori rearrangement product

Scheme 1. Proposed formation of deoxyglucosones (Done) from glucose

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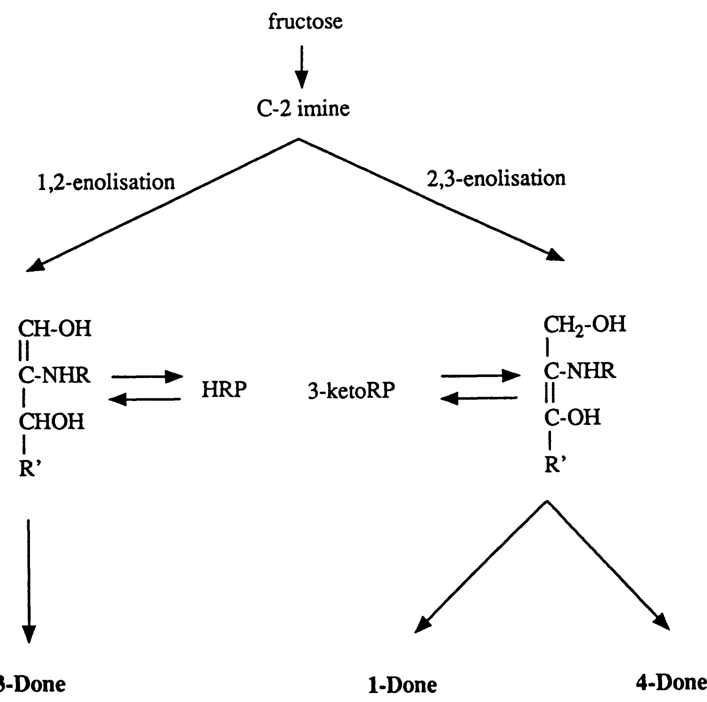

HRP = Heyns rearrangement product

3-ketoRP = 3-keto rearrangement product

Scheme 2. Proposed formation of deoxyglucosones (Done) from fructose

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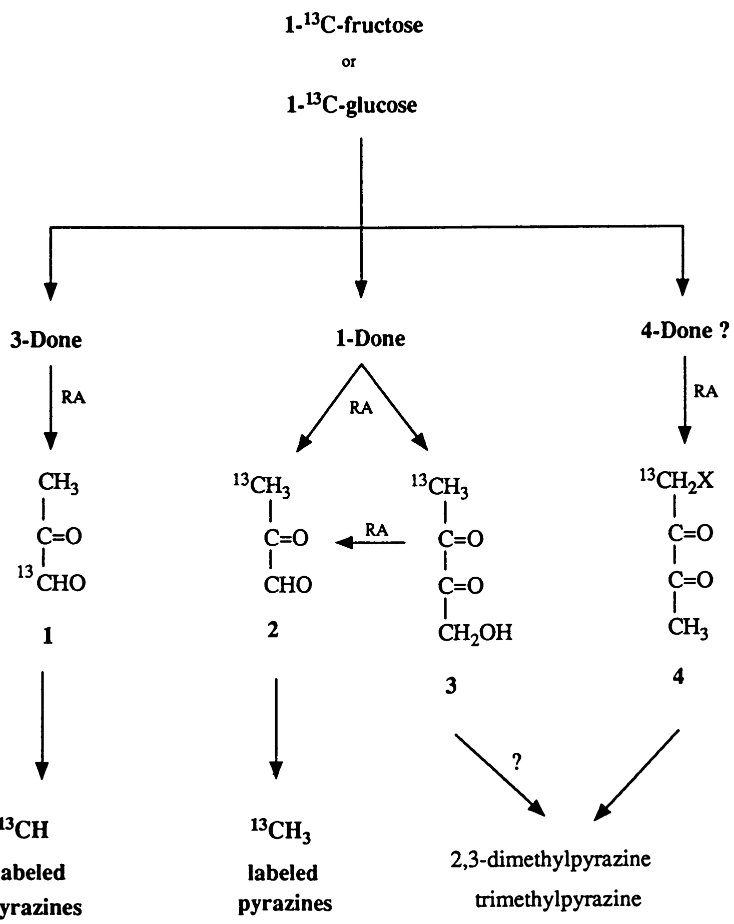

RA: retroaldolization

Scheme 3. Pyrazines from 1-^{13}C-glucose or 1-^{13}C-fructose via deoxyglucosones

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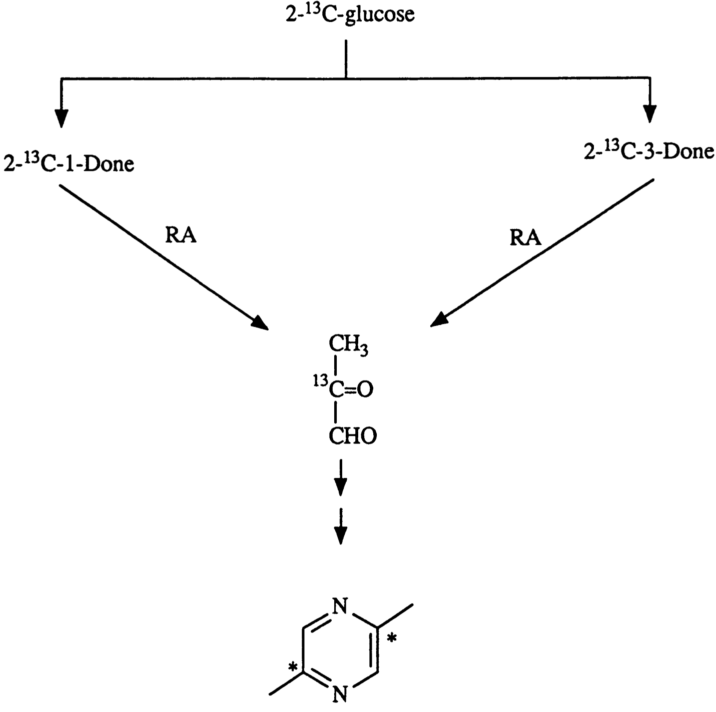

$* = {}^{13}C$-label

RA : retroaldolization

Scheme 4. 2,5-dimethylpyrazine from 2-^{13}C-glucose

as the main cleavage mechanism in the asparagine mediated pyrazine formation from glucose and fructose (Schemes 3-5). Retroaldolization of the starting hexose does not seem important in pyrazine formation, because 2-^{13}C-glucose only generates pyrazines with ^{13}C at the methine positions to a very small extent. We see no reason why fructose would behave differently.

Retroaldolization of the Heyns rearrangement product, and α-cleavage of 1-deoxyglucosone are expected to generate among others acetaldehyde, which should react with the dihydro(di)methylpyrazine intermediates to yield ethyl(di)methylpyrazines. Since we have not observed such pyrazines to a significant extent, we rule out retroaldolization of the rearranged amino acid plus hexose addition products (Amadori and Heyns rearrangement products), as well as α-cleavage, as significant carbohydrate cleavage mechanisms in pyrazine formation.

The mechanism of sugar cleavage proposed by Hayami (*7*), i.e. (hydrolytic) cleavage of β-dicarbonyl intermediates, seems reasonable, also in an amino acid catalysed situation. 1-Deoxyglucosone, a known intermediate in the Maillard reaction, should easily isomerise to the 2,4-diketo isomer, which in turn can be cleaved either by addition-elimination or by retroaldolization (scheme 6). This would result a.o. in the formation of acetol, which reacts with asparagine to form dimethylpyrazines (unpublished observation).

Formation of 2,5-DMP versus 2,6-DMP. Formation of 2,5-DMP from glucose and fructose was about four to fivefold higher than formation of 2,6-DMP. This can be explained with the mechanism outlined in Scheme 7. Formation of **6** will be favoured over **7**, both kinetically and thermodynamically. Similarly **8** will be favored over **9**. Two molecules of **6**, or **6** + **8** lead to 2,5-DMP, which is therefore the main isomer expected.

Pyrazines from C_2 and C_3 precursors. In order to gain more insight into the condensation of the carbohydrate fragments to pyrazines, we reacted the presumed hexose retroaldolization products (Scheme 5) with asparagine. The yields and product distributions are given in Table 5.

Glycolaldehyde should not only lead to 2-methylpyrazine, but also give rise to non-methylated pyrazine. Apparently this reaction is somehow not very productive. When glycolaldehyde was reacted with asparagine, very little pyrazine was formed, but glycolaldehyde together with pyruvaldehyde or glyceraldehyde gave rise to the expected 2-methylpyrazine, dimethylpyrazine and 2,3,5-trimethylpyrazine (Table 5).

As expected pyruvaldehyde alone gave mainly dimethylpyrazines and no 2-methylpyrazine, however it also generated some 2,3,5-trimethylpyrazine. We assume that 2,3,5-trimethylpyrazine can be formed from dihydrodimethylpyrazine and formaldehyde, as explained before (*6*). Formaldehyde can be envisaged to originate from glyceraldehyde by retroaldolization. Formaldehyde formation from pyruvaldehyde can possibly be explained as follows: in the enol form H_2O may add to pyruvaldehyde in a Michael fashion, resulting in glyceraldehyde, which can generate formaldehyde as indicated above.

Interestingly, glyceraldehyde plus pyruvaldehyde gave a slightly higher yield than glyceraldehyde or pyruvaldehyde alone (Table 5). This is in agreement with H_2O elimination as a major pathway to dimethylpyrazines from their hydroxylated dihydrodimethylpyrazine precursors (Scheme 8).

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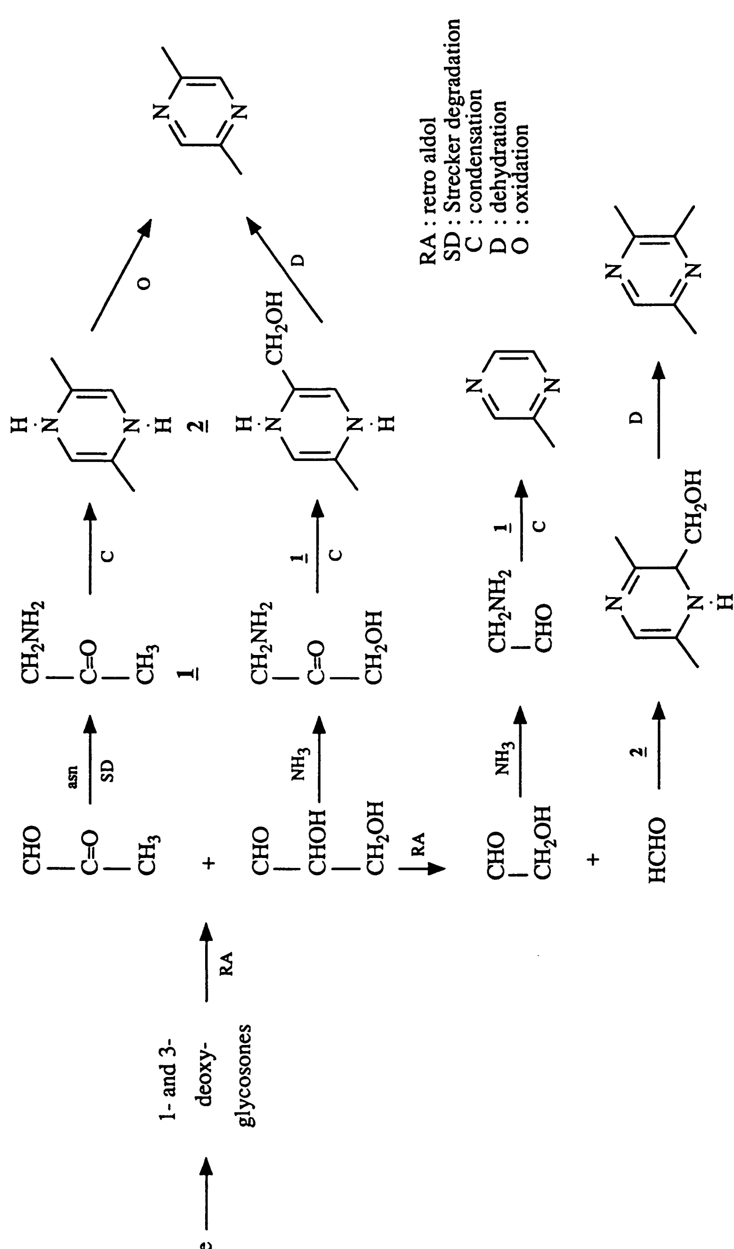

Scheme 5. Formation of pyrazines from hexose and asparagine

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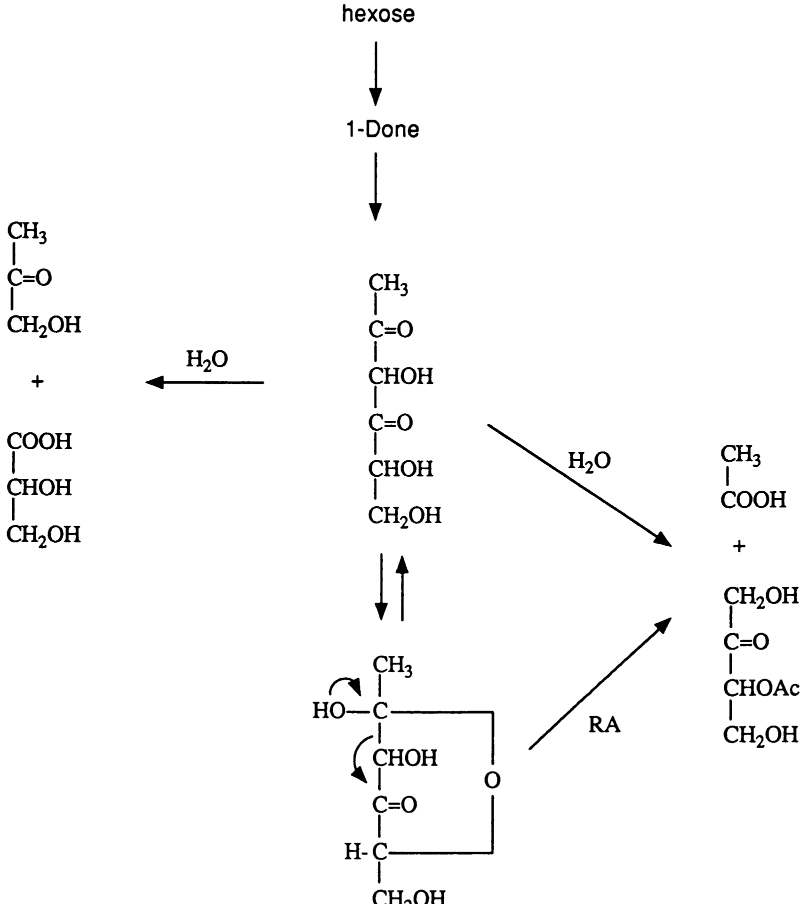

Scheme 6. Carbohydrate cleavage after 1-deoxyglucosone isomerisation

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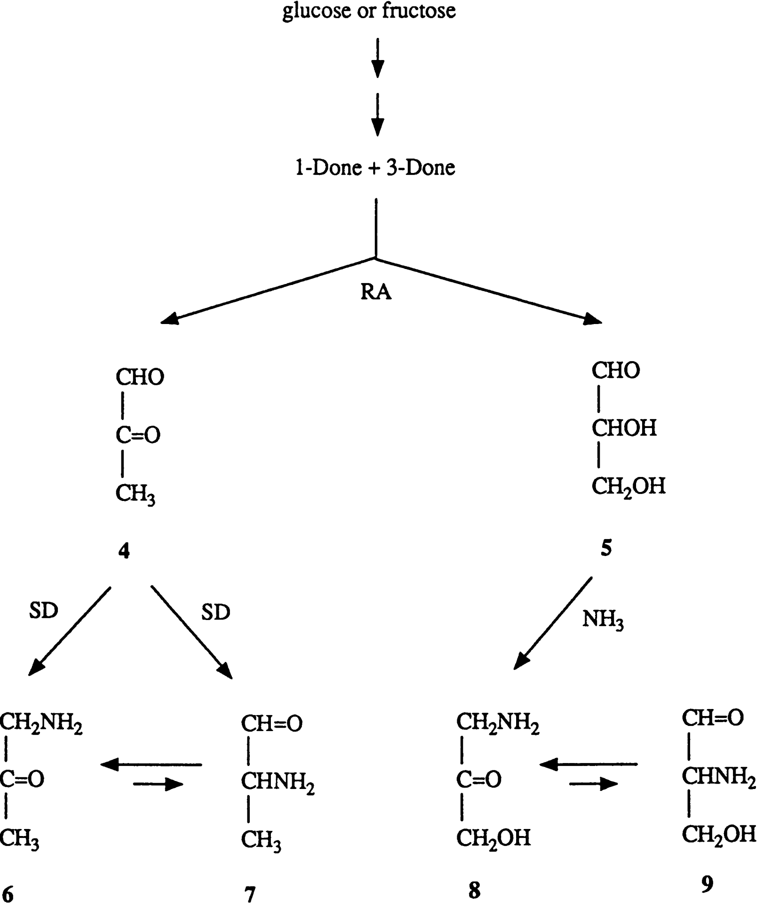

6 + 6 6 + 8 7 + 7 7 + 9	⟶	2,5-DMP
6 + 7 6 + 9 7 + 8	⟶	2,6-DMP

SD = Strecker degradation

Scheme 7. Formation of 2,5-DMP versus 2,6-DMP

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CHO
|
C=O
|
CH_3

4

SD

CH_2NH_2
|
C=O
|
CH_3

6

CHO
|
CHOH
|
CH_2OH

5

NH_3

$CHNH_2$
|
C=O
|
CH_2OH

8

OH
N
N
H

$-H_2O$

N
N

SD = Strecker degradation

Scheme 8. Formation of 2,5-DMP via dehydration

Main conclusions

1. The reaction of asparagine with hexoses, trioses or glycolaldehyde generates methylated pyrazines, with 2,5-dimethylpyrazine as the main volatile compound formed. Pyrazine itself is not formed, or only to a very small extent.

2. All our observations are in agreement with retroaldolization of the intermediate 1- and 3-deoxyglucosone as the main cleavage mechanism in the asparagine mediated pyrazine formation reaction. Retroaldolisation of rearranged isomers of 1-deoxyglucosone may also play a role.

3. In the fructose + asparagine reaction 1- and 3-deoxyglucosone are about equally important, but in the glucose + asparagine reaction 1-deoxyglucosone seems slightly more important than 3-deoxyglucosone. 4-Deoxyglucosone does not seem to play a significant role.

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch012

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Chapter 13

Reactivity of Peptides in the Maillard Reaction

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For one typical monosaccharide (glucose) the reactivity of peptides was studied in relation to chain-length and amino acid type. The reactivity in this case was defined as the rate in which glucose is converted in the early-phase of the Maillard reaction, which was monitored using HPLC. It has been established in model systems containing glucose and glycine homopolymers that the relative reactivity was GlyGly > GlyGlyGly > > Gly. Results obtained from reaction kinetics measurements with a series of dipeptides of the composition GlyX (X=Gly, Val, Thr, Pro, Phe, His, Lys, Asp and Glu) revealed that for all systems, with the exception of GlyGlu, the reactivities were approximately identical. The reaction system GlyGlu/glucose showed a strongly enhanced reaction rate. The same phenomenon ("Glu effect") was observed in the MetX reaction systems (X=Met, Pro, Lys and Glu). However, the "Glu effect" is absent when using dipeptides with a secondary amino terminus. These observations suggest a single reaction mechanism in which carboxylic groups catalyze the early phase of the Maillard reaction. The side-chain carboxylic residue in X-Glu dipeptides is highly effective in catalyzing intramolecular reactions. The mechanism presented also offers an explanation for the observed phosphate-catalysis effect in Maillard-type reactions.

The term "Maillard reaction" is used to characterize a group of chemical reactions involving amino and carboxyl functions in food-stuffs, leading to non-enzymic browning and flavour generation. 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 and lysine. Due to interest by the food industry, the Maillard reaction has been the subject of numerous articles during the past decades.

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Recently some extensive review articles have appeared covering many aspects from a mechanistic or more applied (e.g., nutritional) point of view (*2*).

In a previous publication (*3*), the phosphate catalysis of the so-called "early phase" was studied. It was concluded that phosphate exerts a highly catalytic effect, which can be attributed to a general base-type, exemplified by the observed approximately first-order kinetics of the reaction. In this study, no suggestions referring to the true identity of the catalytic intermediate species were made.

This chapter deals with the reactivity of peptides. Both chain-length as well as composition of simple model peptides are considered. Using high performance liquid chromatography (HPLC) techniques the sugar decomposition is determined for each of the individual reactions.

As a result of the determination of the reactivity of a series of model dipeptides, a mechanistic interpretation is proposed, which can also rather elegantly account for the degree of phosphate catalysis.

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Mechanistic Aspects of the Early-phase Maillard Reaction. Maillard-type chemical processes are quite extensively described in the literature (*2*). Previous work covers both empirical studies, dealing with the Maillard processes in a "black-box" type of approach, which define reagents, processing conditions and reaction output (including patent literature), as well as studies characterized by a more mechanistic organic chemistry approach of dealing with these complex chemical pathways. Investigations which can be considered to belong to the former category clearly underscore the importance of peptides in Maillard-type reactions. It is well established that the omission of peptides in Maillard reactions directly influences organoleptic perception, a deficiency which can not be compensated by adding amino acids (*4*). Studies belonging to the latter category most commonly try to understand and explore the "black box" of Maillard chemistry, which was also the objective of this study.

The Maillard reaction, from a mechanistic point of view, has been extensively reviewed in the literature (*2*). Most of the work presented can be divided in two categories; on one hand are studies concerned with examining relatively simple model systems which attempt to establish individual reaction routes in the Maillard cascade of reactions; alternatively are investigations focused on the formation and identification of many volatiles. From both types of studies it is well established that the Maillard reaction can be subdivided into three different stages (*5*):

1. Maillard early stage, in which the carbohydrate and nitrogen source initially interact, resulting in the formation of a glycosyl-amine. These thermally labile compounds which are subjected to what is called the Amadori rearrangement, are converted into the corresponding Amadori rearrangement product (ARP), the 1-amino acid-1-deoxyfructose. In this stage the reaction route is rather straight-forward, which accounts for in the fact that this portion of the reaction mechanism is well documented.
2. In the advanced stage, sugar fragmentation, condensation, elimination, dehydration and cyclization reactions take place simultaneously, resulting in the formation of a huge diversity of heterocyclic volatiles. Among the numerous important flavour precursor systems being generated are very reactive dicarbonyl sugar derivatives.
3. In the final stage, many of the functional groups generated in the advanced stage interact, yielding highly polymeric, brown coloured materials soluble in water called melanoidins (characteristic of the non-enzymatic browning process).

Since the Amadori rearrangement is considered to be the rate determining step in the overall Maillard reaction (*6*), attention was focused on its early phases. In order to understand the mechanism involved in phosphate catalysis and reactivity-dependency with respect to various peptides, some mechanisms which are involved in the early Maillard phase should be exemplified using the glucose/glycine model system.

As previously mentioned, the conversion of glucose (Scheme 1, compound (**I**)) and glycine (**II**) to the corresponding ARP (**IV**) is a two-step process, the glycosyl-amine (**III**) being the key intermediate. In more detail, the carbonyl group of the linear sugar isomer is nucleophilically attacked by the amino terminus of the amino acid/peptide (Scheme 2, amino acid = glycine). After dehydration, a so-called Schiff-base adduct (Scheme 2, compound (**V**)) is formed. This species can upon nucleophilic attack of the sugar C-5-OH oxygen atom on the C=N bond cyclize to yield the cyclic glycosyl amine. As Potman pointed out correctly, the very low concentration of linear sugar molecules can not account for the relatively high reactivity of the Maillard reaction (*3*). It is therefore very likely that the ring opening reaction is initiated by a direct result of the amino acid nucleophilic attack. It should be noted, however, that the differences between these mechanisms only relate to differences in timing of the individual steps.

The Amadori rearrangement, the conversion of the glycosyl-amine (Scheme 3, compound (**III**)) to the ARP (**IV**), is initiated by protonation of the ring oxygen atom, resulting in a ring opening reaction yielding a protonated iminium cation (**VI**). Deprotonation of the NH^+ results in the open-form glycosyl-amine and is therefore a non-productive reaction. Alternatively, the C-2-H(OH) carbon atom can be deprotonated, resulting in the formation of the enamine adduct (**VII**). Keto-enol tautomerism generates a C=O moiety (open-chain ARP), which upon nucleophilic attack of the C-6-OH oxygen atom gives rise to the cyclic ARP (**IV**).
It should be noted that since the Amadori Rearrangement is the rate determining step in the Maillard reaction (vide supra), effective iminium cation (**VI**) formation and C-2-H deprotonation are very important factors affecting the overall Maillard reaction rate.

Flavour Formation in the Maillard Advanced Phase. The Amadori rearrangement reaction is in itself not responsible for the formation of heterocyclic volatiles. The actual flavour compounds are generated by thermal decomposition reactions of the ARP. Commonly accepted is the conversion of ARP to highly reactive dicarbonyl sugar derivatives such as deoxyglucosones (*7*). Cyclisation and dehydration reactions are considered to produce various O-containing heterocyclic compounds, such as hydroxymethyl-furfural, acetyl-furfural, (dihydro-hydroxy)maltol, etc. In addition, these dicarbonyl species are highly effective in generating Strecker-degradation reactions.

The Reactivity of Peptides. Within the literature, several articles have appeared stating that peptides play a very important role in the flavour generation process during Maillard type reactions (*8*). It has been established that during roasting of cocoa, peptides and amino acids do exhibit synergetic effects; neither the removal of peptides nor of the amino acid can be carried out with impunity (*4*). It is quite striking,

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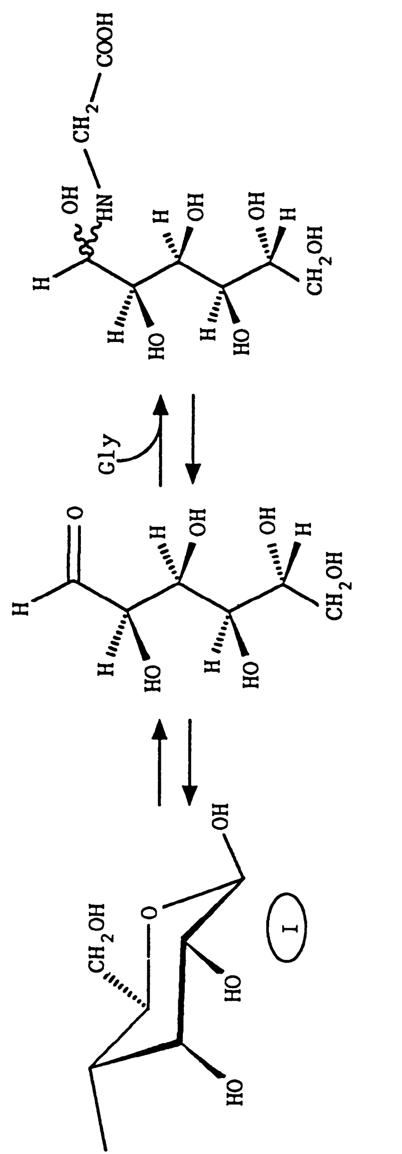
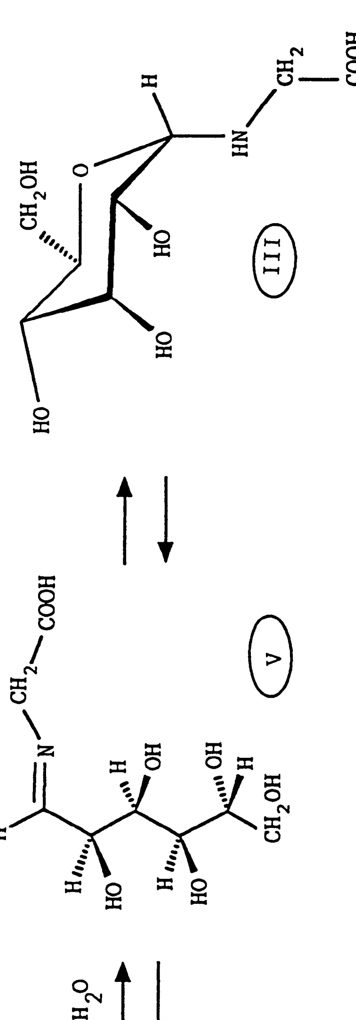
Scheme 1. Overall reaction of the early phase Maillard reaction.

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch013





Scheme 2. Formation of the glycosylamine of glucose and glycine.

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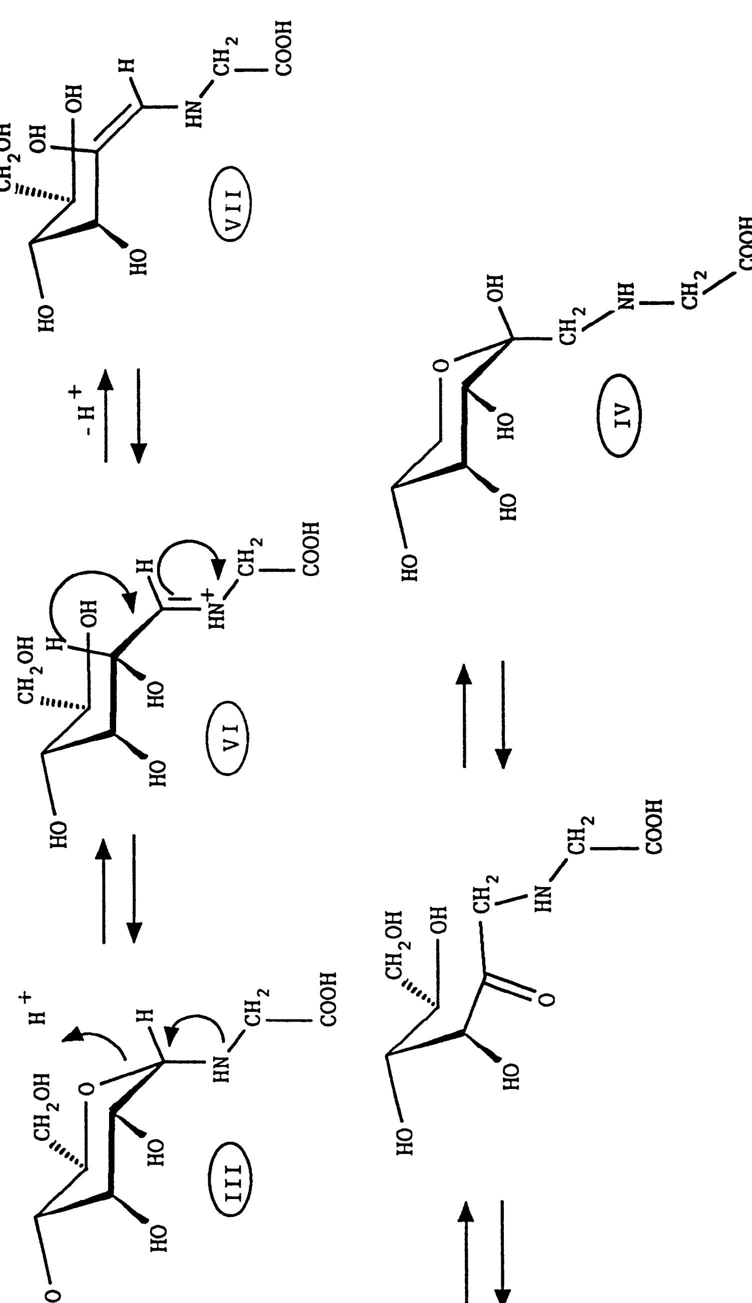

Scheme 3. The Amadori rearrangement reaction.

however, that the subject of reactivity of model peptides in Maillard type processes is barely covered. The few publications which are available are too diverse and non-uniform (different reactants, reaction parameters etc.) to provide a clear view of typical Maillard parameters. Moreover many of the authors determine the rates of browning in an attempt to quantify the peptide Maillard reaction rates.

Fujimaki, already in 1973 (*9*), pointed out that the rate of browning of glycine homopolymers increases with increasing chain length. Experiments in which glyoxal was reacted with these model peptides at 80°C, clearly showed that the browning order is tetraglycine > triglycine > diglycine > dialanine > glycine > alanine. Buera et al. in 1987 (*10*), confirmed these findings by repeating these experiments using glucose and adjusting the water activity to 0.90 with sodium chloride. The latter authors suggested that the amount of browning increased with increasing concentrations of the negative deprotonated species of the peptides and therefore is related to the pKa of the peptide at issue. In an attempt to prove this correlation, they again re-conducted these series of experiments, using equal deprotonated negatively charged peptide concentrations. Browning decreased with increasing chain length. It should be noted however that in this series of experiments at pH 5.0, the overall concentration of glycine was approximately 100-fold that of triglycine. This implies that whenever the rate of reaction is not totally determined by the concentration of the anionic species, the very high concentration of glycine can easily account for its high reactivity relative to the browning rate obtained using triglycine.

It is known, however, that the amount of browning is not per sé proportional to the conversion of amino acids/peptides and/or sugars. It is commonly accepted that the degree of browning depends on the type of melanoidins formed during the Maillard reaction and that the colour of melanoidins derived from different starting materials can substantially deviate, in addition to being highly dependent on the exact reaction parameters. As early as 1973, Motai (*11*) concluded that melanoidins obtained from peptide Maillard model reactions exhibited darker degrees of colour compared with those produced using amino acids. Although browning in itself is a very important parameter, the relation of reaction and generation of volatiles (flavour formation) to the rate is ambiguous. Alternatively, it seems more reasonable to use the rate of starting material-conversion to quantify the Maillard reaction.

Very recently Mori and Manning, after several earlier publications (*12*)(*13*), presented what may be considered the first, profound article in the field of peptide-Maillard kinetics (*14*). They studied the Maillard reaction between peptides (1 mM) and glyceraldehyde (50 mM) at 50 °C pH 7.0, buffering the solution with potassium phosphate. Quantifying the decreasing peptide concentrations, these authors revealed that the reactivity of these reaction systems increases in the order: amino acids < dipeptides < tripeptides. Despite the fact that during these investigations a phosphate buffer was used, their study revealed only a portion of the general concept of the peptide-Maillard kinetics. It should be noted, however, that several of the results presented in their study fit rather nicely with the mechanistic concept presented in this chapter.

It can be advocated, however, that disappearance of the peptide fraction is not the best parameter to describe the rate of the Maillard reaction. On one hand, the Hodge-scheme clearly shows that amino acids/peptides are re-liberated during the course of the advanced Maillard phase, whereas Hashiba (*15*)(*16*) alternatively indicated

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch013

that within the peptide-glucose Amadori rearrangement product, the peptide bond is much more susceptible to hydrolysis compared to that in the free peptide. The authors suggested an intramolecularly catalyzed peptide hydrolysis. Both phenomena do influence the rate of peptide conversion, which directly depends on the peptide composition as well as peptide chain length. Alternatively, the rate of glucose conversion can serve as a parameter to quantify the rate of reaction.

Experimental Methods

Prior to the experiments in which the reactivities of various peptides were determined, it was checked whether the rate of the non-Maillard sugar degradation (caramelization) is of any relevance to the overall reaction rate. This has already been done for the reaction couple glucose/glycine (*3*). Indeed, the sugar degradation in absence of peptide was negligible, as was the hydrolysis of the peptide in absence of glucose.

In order to ensure the uniformity, i.e. the translatability to earlier studies performed within this project (*3*), all reactions were carried out at 100°C in water at pH 5.60. Due to the relative high costs of the peptide material, it was necessary to miniaturize the reaction system. In these studies all the reactions were performed on a 1.4 mL scale within a completely sealed septum flask to prevent water evaporation. Using a micro-reflux condenser was not feasible since the amount of water which appeared to develop on the inside wall of the condenser strongly diminished the reproducibility.

Concentrations of both the reactants were chosen at an equal molar level of approximately 0.4 M. At this concentration the conversion was suitable to determine the reaction rate accurately. In some reaction systems (MetX model peptides), the concentration was lowered to 0.22 M in order to prevent solubility problems.

At this point, it should be noted that the exact reaction parameters are known only at t=0. As a result of the Maillard reaction, the amino termini of peptides are converted whereas the bulk of the COOH moieties remain unchanged. This results in a severe pH drop of the reaction system. Buffering is not feasible since the anionic species of the buffer can exert a severe catalytic effect as has been extensively pointed out for the phosphate ion (*3*)(*17*). Adjusting the pH by adding NaOH is unfeasible since it requires pH measurements at 100°C and due to the addition, dilutes the reaction mixture. Besides this acidification problem, the production of highly reactive species during the Maillard reaction may well influence the observed glucose concentration. This is a direct result of the fact that the stages of the Maillard reaction are analytically indistinguishable from each other during the reaction. In order to cope with these problems a curve fitting procedure is applied. This enables the use of all measurements, even though the reaction parameters at each individual point are not exactly known, to obtain the reaction rate (first derivative) at t=0. As in the previous publication (*3*) an iterative SAS procedure using equation 1 is adopted.

$$[glucose]_t = [glucose]_\infty + ([glucose]_0 - [glucose]_\infty)e^{-k_{obs}t} \quad (1)$$

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch013

The appearance of the term [glucose]$_\infty$ may well be the result of the acidification of the reaction mixture, which will induce an increasing inhibition of the Maillard reaction. As several references indicate (E.g. (*12*) and (*18*)), lowering the pH decreases the reaction rate as a result of the protonation of the amino terminus of the amino acids or peptides, blocking the nucleophilic attack on the sugar carbonyl moiety.

Lastly, all of the reaction rates obtained are corrected for the concentrations measured at t=0. Due to the adjustment of the pH prior to the heat treatment, exact concentrations of the reactants were observed to deviate from their initially intended value. In order to enable comparison of the reaction rates of several Maillard reaction pairs all concentrations were normalized. Doing this, it was assumed that all orders were approximately equal to unity for all of the reaction components (phosphate included, if appropriate). From a mechanistic point of view, as well as from determination of the orders of several compounds (*3*), this assumption is reasonable although only justified within very small concentration ranges.

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch013

Materials. Acetonitrile (Uvasol grade, Cat. No. 16) and $NaH_2PO_4 \cdot H_2O$ (Cat. No. 6346) were obtained from Merck, D-(+)-glucose (Cat. No.17.008.33), glycine (Cat. No. 12.007.76) and GlyGly (Cat. No. 12.014.83) were acquired from Janssen Chimica, whereas the peptides were supplied by Sigma (GlyVal (Cat. No. G4127), GlyThr (Cat. No. G1129), GlyPro (Cat. No. G3002), GlyPhe (Cat. No. G2752) and GlyHis (Cat. No. G1627)) or Serva (GlyGlyGly (Cat. No. 51480), GlyGlu (Cat. No. 51450), GlyLys (Cat. No. 51590), ProGly (Cat. No. 52215), ProPro (Cat. No. 52300), ProLys (Cat. No. 52271), ProGlu (Cat. No. 52210), MetMet (Cat. No. 52085), MetGly (Cat. No. 52065), MetLys (Cat. No. 52083), MetGlu (Cat. No. 52055) and MetPro (Cat. No. 52100))

Reaction conditions. Glucose, the model peptide and $NaH_2PO_4 \cdot H_2O$, if appropriate, were mixed in amounts equivalent to approximately 0.45 mmol (for Met-X dipeptides all amounts were lowered to approximately 0.30 mmol as dictated by the poorer solubility of these peptides). To this mixture 1.4 mL of distilled water was added. The pH of the solution was adjusted to a value ranging between 5.58 and 5.62 using either hydrochloric acid or sodium hydroxide. One mL of this batch was transferred in to a 1 mL vial equipped with a micro magnetic stirring bar, which was subsequently sealed hermetically with a septum cap. Through this cap a calibrated thermocouple was immersed into the solution, enabling the determination of the temperature at each instant. The vial containing the reaction mixture was placed in a preheated thermostrated oil bath of 100-104°C. The instant at which the internal temperature reached 90°C was defined to be t=0. It has already pointed out ((*3*) and additionally checked prior to this study) that at this point no glucose and peptide conversion had taken place. At t=0, 30, 60, 90, 120, 180, 240, 300 and 360 minutes, samples of approximately 100 μL were taken from the reaction mixture directly through the septum cap, using a syringe. These quantities of reaction mixture were rapidly transferred to a weighted quantity (approximately 9.5 mL) of HPLC eluent in a 10.00 mL measuring flask. This procedure assured a fast shut-down of the Maillard reaction as a result of simultaneous rapid temperature decrease and dilution of the sample. Measuring the weight of the resulting diluted sample enable the accurate determination of the sample weight. The total volume was brought to 10.00 mL using

HPLC eluent. The resulting solutions were microfiltered (Chrompack Sample Filter ϕ13 mm, 0.2 μm) then submitted to HPLC analysis in order to determine the reagent concentrations and, if applicable, concentrations of reaction products.

Analytical method. In order to quantify the glucose and dipeptide concentrations, a HPLC technique was adapted (*3*). The eluent consisted of acetonitrile and an aqueous phosphate buffer of which the compositions are listed below.

Reaction systems	vol% Acetonitrile/ vol% Phosphate buffer	Phosphate buffer Conc (M)
GlyGlu/Glc	75/25	0.005
MetMet/Glc	80/20	0.005
MetGly/Glc	85/15	0.005
MetLys/Glc	75/25	0.005
MetGlu/Glc	85/15	0.005
MetPro/Glc	85/15	0.005
ProPro/Glc	80/20	0.005
ProGly/Glc	70/30	0.1
Other systems	70/30	0.005

HPLC system:

Pump:	Waters 510, flow 1 mL/min
Guard column:	Chrompack HPLC Guard Column, reverse phase, 75.2x1 mm
Column:	Nucleosil 100- 5 NH_2, particle size 5 μm, 200x4 mm, ex. Macherey-Nagel (Cat. no. 720008) thermostrated at 35°C
Eluent:	Acetonitrile/phosphate buffer (pH 2.75) as specified below
Detectors:	Waters 410 Differential refractometer Waters 490 Programmable Multiwavelength (220 nm)
Data processing:	Nelson Analytical Interfaces Model 760 (1 sample/sec) Nelson Analytical (Model 2600) Chromatography Software (Version 5.0) run on a Hyundai PC-AT
Injection volume:	20 μL

Quantification was established using calibration curves of all pure compounds which were determined separately.

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Results and Discussion

The Reactivity of Glycine Homopolymers with Glucose. In an attempt to determine the dependency of chain length upon the reactivity of the reaction pairs, glucose/glycine, glucose/diglycine and glucose/triglycine were subjected to a Maillard-type reaction at 100°C. Due to the very poor solubility of higher homopolymers, extension of this series was not feasible (e.g. the pentamer's solubility is only 0.04 M). Low solubility produces a very slow Maillard reaction, which results in the fact that the caramelization reaction predominates the glucose conversion. Simultaneously decreasing the glucose concentration to overcome this problem decreases the Maillard reaction rate approximately 100-fold. The reaction time needed to obtain a substantial glucose conversion increases dramatically, rendering investigation of these reaction pairs unfeasible.

Glycine, diglycine and triglycine (± 0.4 M) were subjected to interactions with glucose (± 0.4 M) at 100°C and pH 5.60 in duplicate; once without phosphate present and the second time when approximately one equivalent (± 0.4 M) of sodium dihydrogen phosphate mono-hydrate was added prior to pH adjustment. As an example, Figure 1 depicts the HPLC concentration profiles of glucose in both the non-phosphate and the phosphate catalyzed reactions of the glucose reactions with Gly, GlyGly and GlyGlyGly respectively. From these glucose concentration profiles, with help of an iterative SAS statistical regression procedure, the reaction rates at $t=0$ were determined. Subsequently, the corrected reaction rates were determined by normalizing the concentrations to 0.35 M for all reagents using a first-order approximation. The values thus obtained are graphically depicted in Figure 2.

The results depicted in Figure 2 clearly show that the reactivity increases from glycine to diglycine for both the phosphate-free and phosphate catalyzed reactions. When the homopolymer chain length was increased by one additional glycine residue, a reversed effect was noticed.

From these results, it is evident that within the series glycine, diglycine and triglycine, diglycine is the most reactive species. This is quite remarkable since the pK_2's of these compounds decrease with increasing chain length (9.77, 8.25 and 7.91, respectively (*19*)). As the basicity can be considered to be a measure for the nucleophilicity of the NH_2 terminus, it was expected that the reaction rates would incline from glycine to triglycine. As a matter of fact, Hashiba et al. (*16*) did find this relation using the conversion of the peptide to determine the reaction rate. It has been advocated (vide supra) that the use of this parameter is not advisable since several other reaction routes lead to the conversion and/or regeneration of the peptide. Mori and Manning (*14*) emphasized that the reactivity of peptides is not solely determined by the NH_2 terminus. They postulated that the proximity of other substituents can effectively catalyze the reaction and they proposed that a positively charged amino group or protonated histidine-imidazole moiety can induce such an effect. They also deny the relevance of a carboxylic residue as a moderator of any type of catalysis. It is argued that at pH 7.0, at which all their experiments were performed, the carboxylic group is fully charged. In addition ethanolamine, in which the COOH group is absent, and the dipeptide AlaAsp, having an extra COOH group, exhibit approximately the same reactivity towards glyceraldehyde as any of the other dipeptides at pH 7.0.

At pH 5.60, which was used for all our experiments, the role of the carboxylic

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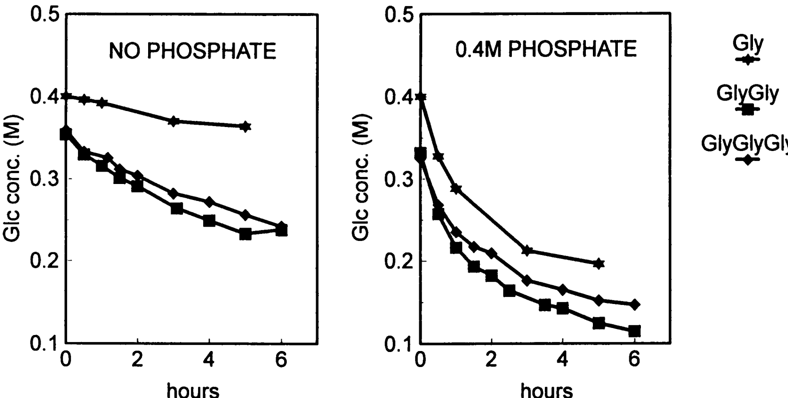

Figure 1. HPLC concentration profiles of glucose in its reaction with $(Gly)_x$ (x=1,2,3).

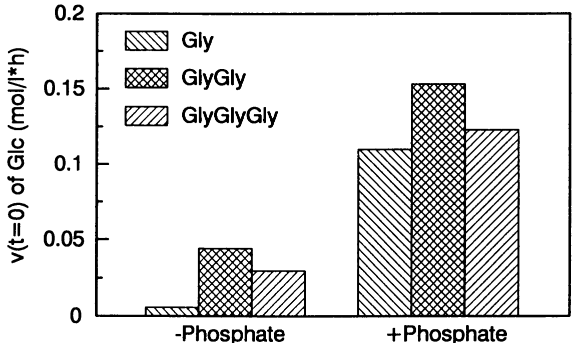

Figure 2. Rate of conversion of glucose at t=0 in its reaction with $(Gly)_x$ (x=1,2,3).

group is very distinct. At neutral pH (7.0) the NH_4^+ group can behave as an acidic moiety. The catalytic role of the carboxylic group in itself is not new. Several references have been made attributing a catalytic effect to the COOH group in the Amadori rearrangement reaction (*20*). These observations only refer to an *inter*molecular type of catalysis due to the use of amino acids. The specific role of the peptide's COOH group has not been referred to, however in an *intra*molecular process the carboxyl group is responsible for the dramatic reaction rate increase from glycine to diglycine in the non-phosphate reaction. It should be noted that the efficacy of the *intra*molecular catalytic process is concentration-independent and is determined only by the ratio of the number of effective conformations and the total of possible conformations. Its *inter*molecular variant, however, is very ineffective at very low concentrations due to the fact that it is at least a bi-molecular reaction.

In order to be able to explain the role of the carboxylic group, it is necessary to present the mechanism of the Amadori rearrangement in some detail (Scheme 4). Although often the formation of the glycosylamine **IX** and the Amadori rearrangement product are presented to be two subsequent reaction pathways (Schemes 2 and 3), they actually proceed via a mutual cation intermediate **VIII**. This cation can be deprotonated in two ways; (i) the abstraction of the nitrogen bonded proton giving rise to the formation of the neutral open-form glycosylamine (**IX**) (Scheme 4, route B) or (ii) the much slower and therefore rate limiting deprotonation of the sugar C-2 atom (*21*) resulting in the formation of the ARP (Scheme 4, route A). From this it is evident that the concentration of the reactive intermediate benefits from an effective protonation of the neutral nitrogen atom of the glycosylamine (**IX**).

Scheme 5 shows the structure of the catalytic conformation of the dipeptide/glucose adduct. Due to the COOH terminus, the imine nitrogen atom of **IX** (Scheme 4) can be protonated *intra*molecularily. Substituting the dipeptide for glycine, however, drastically reduces the number of main chain atoms, thereby prohibiting the formation of a structure with the glycine COOH group in the proximity of its N terminus. The contribution of an *intra*molecular type of catalysis to the overall rate of the reaction is therefore negligible. Increasing the chain length, by substituting the diglycine with triglycine introduces a great amount of new degrees of freedom, i.e. a large number of possible effective conformations. From a statistical point of view, the possibility of the appearance of direct interaction of the COOH group with the amino terminus will decrease with further increasing chain length. It is therefore expected, although testing was not feasible due to experimental problems, that the reactivity will continue to decrease when the main chain is further extended, eventually reaching the minimum reactivity level which equals that observed for the glycine/glucose system (total absence of *intra*molecular catalysis).

The Reactivity of Gly-X Dipeptides with Glucose. Besides chain length, the peptide composition is expected to be a very important parameter in the rate description of the Maillard reaction. Mori and Manning (*14*) stated that at pH 7.0 in the reaction between peptides and glyceraldehyde the proximity of an NH_4^+ group catalyses the ARP formation. In the proceeding it will be established that it is the presence of the COOH group that has a severe impact on the peptide reactivity at pH 5.6.

In order to test the reactivity of peptides differing in composition but having the

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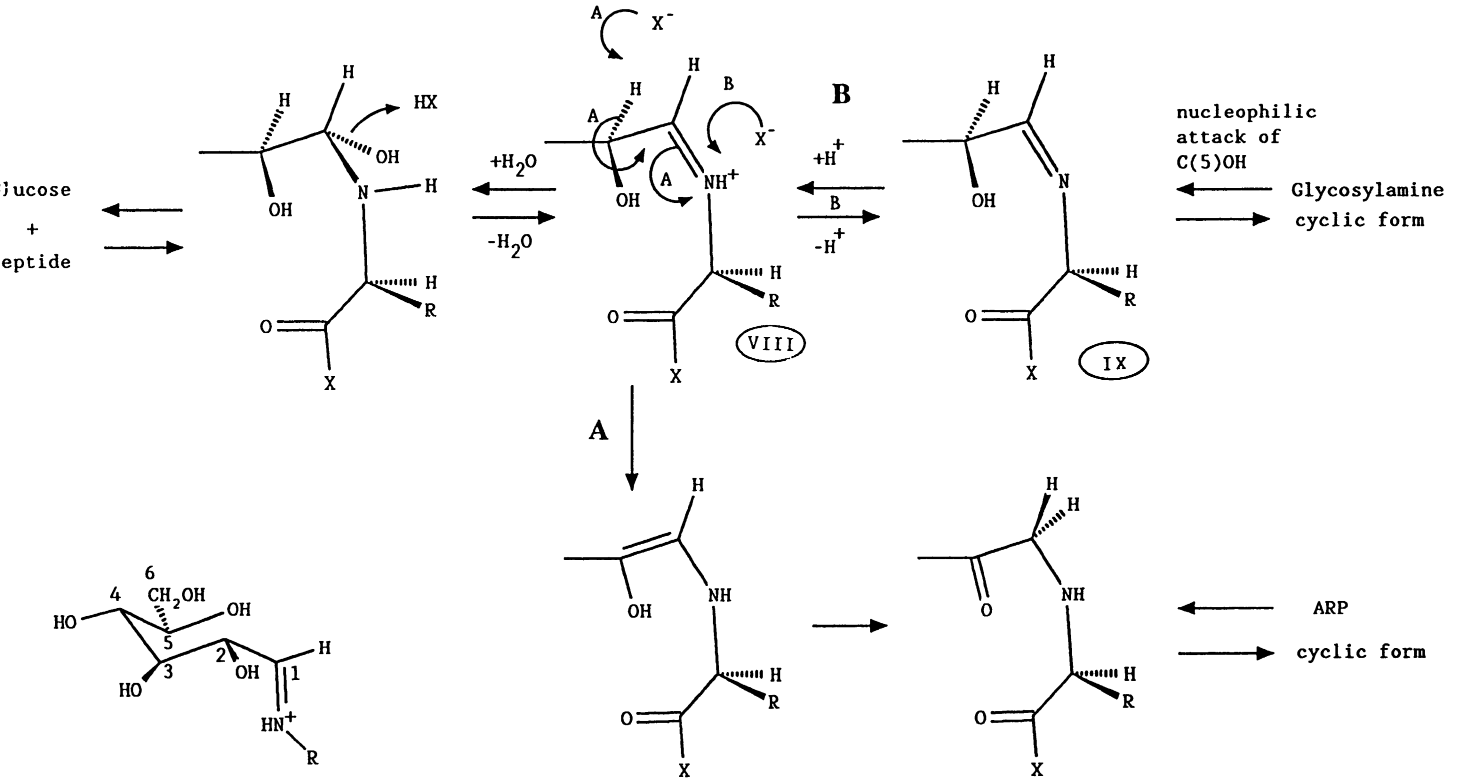


Scheme 4. Detailed mechanism of the ARP formation.

same amino acid at the N-terminus, several Gly-X dipeptides were reacted with glucose at 100°C at pH 5.60. Figure 3 summarizes the concentration-corrected reaction rates for both the non-phosphate as well as the phosphate catalyzed reactions. Of interest is the fact that all dipeptides in the non-phosphate reactions exhibit more or less the same reactivity towards glucose with one exception: the dipeptide GlyGlu is much more reactive. In the phosphate-catalyzed reaction, the reactivity is moderately elevated for dipeptides in which the carboxylic residue contains a basic group (X=His,Lys).

The dramatic reaction acceleration of GlyGlu with respect to the reaction between glucose and any other dipeptide clearly results from the introduction of the extra carboxylic group. Apart from the *intra*molecular catalysis, which results from the interaction between the carboxylic terminus of the main chain of the dipeptide, a very effective *intra*molecular assisted protonation of the intermediate imine group results from the introduction of the carbon-terminus side chain carboxylic group. The latter COOH group is introduced in a very flexible way into the system. This enables the strain-free formation of a cyclic catalytic transition state in which the acid proton is transferred to the imine nitrogen atom. By reducing the C-terminus amino acid side chain with one carbon atom (GlyAsp), the catalytic effect of the side chain COOH group is still present, although less pronounced. The reactivity of GlyAsp is approximately twice the value obtained for GlyGly as is the net COOH group concentration. It is evident that especially in GlyGlu the effect of the extra COOH group is optimal.

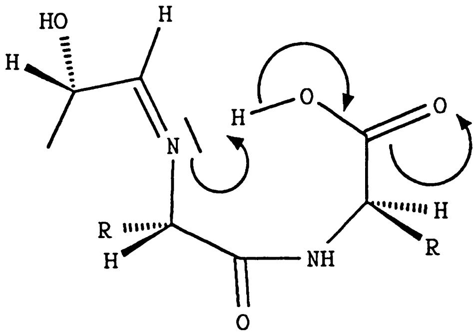
The investigations of Manning and Mori (*14*) revealed an enhanced reactivity of the ValHis-, AlaHis- and GlyHisGly- glyceraldehyde reaction systems. Since these authors performed their reaction in the presence of a phosphate buffer, their findings are in complete accordance with the results of the study presented here. The GlyHis reactivity in the phosphate catalyzed reaction is high with respect to the reaction rates observed for the bulk of the peptides, approximately equal to the reactivity of GlyLys with a basic NH_2 incorporated in the side chain, but relatively low in comparison to the rate obtained for the GlyGlu systems.

The Reactivity of Met-X Dipeptides with Glucose. In order to check whether the "Glu effect" is only observed in the Gly-X system, Met-X dipeptides (X= Met, Gly, Glu, Lys or Pro) were subjected to the same procedure.

Due to the fact that Met-X dipeptides are somewhat less soluble in water at pH 5.60 compared to Gly-X dipeptides, the concentrations of both reactants, i.e. glucose and the peptide under investigation, were lowered to a value of approximately 0.22 M. In the case of MetGlu, the peptide was not completely soluble at room temperature, but the solution clarified at a temperature below 90°C, i.e. before the reference time t=0 was set.

Figure 4 depicts the graphical representation of the normalized reaction rates (0.22 M) at t=0 for the dipeptides MetMet, MetGlu, MetLys, MetGly and MetPro. The reaction rates further substantiate the proposed "Glu effect". In both the non-phosphate as well as in the phosphate catalyzed reaction, MetGlu is significantly more reactive than the other peptides. It is therefore concluded that the "Glu effect" is surely not limited to the Gly-X system. On the contrary, the results presented here strongly suggest that it is a phenomenon which all the primary N-terminal residue containing dipeptides have in common.

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch013



Scheme 5. Catalytic conformation of the dipeptide/glucose adduct.

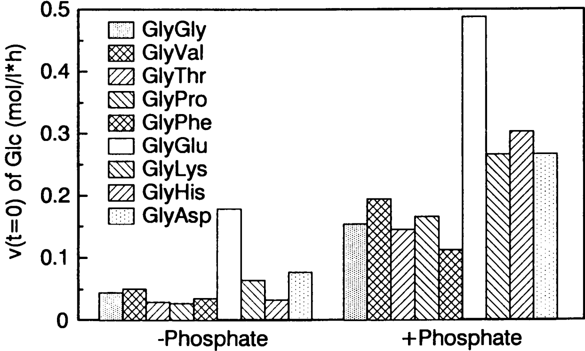


Figure 3. Rate of conversion of glucose at t=0 in its reaction with Gly-X dipeptides.

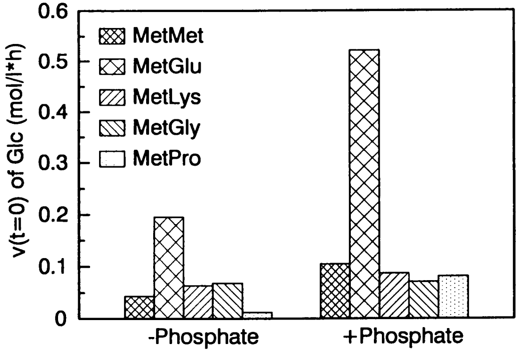


Figure 4. Rate of conversion of glucose at t=0 in its reaction with Met-X dipeptides.

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch013

The relative high reaction rate increase due to the addition of phosphate in the MetHis system will be discussed separately.

The Reactivity of Pro-X Dipeptides with Glucose. Proline and its derivatives, such as hydroxy-proline, are the only amino acids containing a secondary amino terminus. The fact that the main chain nitrogen atom is di-substituted has an impact on its nucleophilicity. Although the extra alkyl group (side chain of C_α) gives rise to a somewhat higher electron density, thereby rendering the N-terminus more nucleophilic, the steric hindrance of the alkyl substituent strongly decreases its reactivity. As a matter of fact, it will be established that dialkyl substitution has a dramatic impact on the mechanism of the catalysis as observed in dipeptide/glucose interactions.

In this part of the investigations, the role of ProGly, ProGlu, ProLys and ProPro in the Maillard reaction with glucose was studied. The peptides tested were soluble in water up to a concentration of 0.4 M at 90°C. This enabled the determination of the rate of reaction at approximately 0.35 M for both of the reactants. The results can be directly compared with the kinetics obtained for the Gly-X system. Only in case of ProLys, the resulting mixture remained turbid when adding the peptide to water at a 0.4 M concentration. It clarified however, upon heating before a temperature of 90°C was reached.

The results obtained from the reaction kinetics measurements are depicted in Figure 5. Strikingly, the "Glu effect", i.e. the catalytic activity of the glutamic acid residue in X-Glu dipeptides, is absent. ProLys, on the other hand, exhibits a markedly high reactivity, in both the non-phosphate as well as in the phosphate catalyzed reaction. This reactivity can not be attributed to a glucose reaction with the extra side chain NH_2 group of lysine, since the Lys-catalytic activity was not observed in the Gly-X and Met-X systems. Taking into account the mechanism depicted in Scheme 4, the key intermediate **(VIII)** in the Pro-X case will have a structure as shown in Scheme 6. It is evident that this iminium cation can not be deprotonated to yield its neutral glycosyl amine derivative. Only two reaction paths remain available; (i) the nucleophilic attack of the C-5-OH group on the iminium C=N carbon atom, resulting in the formation of the cyclic glycosyl amine and (ii) the ARP formation. In consequence, the reaction mechanism for the ProLys system does not comprise a COOH-catalyzed protonation of the neutral imine analogue **(IX)**. The rate determining step in the reaction is therefore shifted to the base induced deprotonation of the C-2 atom of the structure in Scheme 6. It is this step which is not rate determining in the Gly-X and Met-X systems due to the slow imine protonation, that is effectively catalyzed by the *intra*molecular deprotonation exerted by the basic ϵ-NH_2 group of lysine. It should be emphasized, however, that the catalytic effect of a basic group only appears when the protonation step is absent (Pro-X) or very effectively catalyzed (phosphate catalysis).

The Nature of Phosphate Catalysis. All the reactions of glucose with Gly-X, Met-X and Pro-X dipeptides have been performed twice, i.e. once without phosphate added to the reaction and the second time after phosphate was introduced into the reaction mixture at an equimolar concentration prior to adjustment of the pH to 5.60. The

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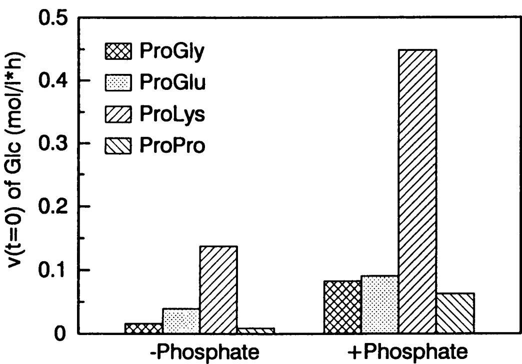
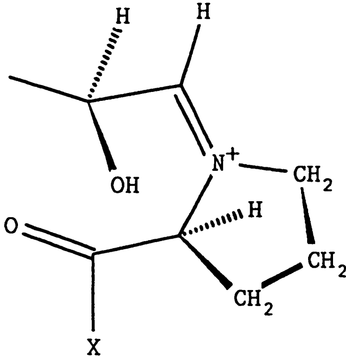


Figure 5. Rate of conversion of glucose at t=0 in its reaction with Pro-X dipeptides.



Scheme 6. Schiff-base intermediate in the reaction of Pro-X and Glc.

results of the kinetic investigation are already incorporated into the Figures 2 to 5. In order to quantify the degree of phosphate catalysis (DPC), the ratios of the reaction rates of the phosphate catalyzed reaction and the non-catalyzed reaction (equation 2) were calculated.

$$\text{DPC} = \frac{\text{Reaction rate of the phosphate catalyzed reaction}}{\text{Reaction rate of the non-phosphate reaction}} \qquad (2)$$

Of special interest is the mechanism of the phosphate catalysis. This study clearly shows that the DPC differs between the various dipeptides, which is not expected when phosphate is only involved in the de-protonation (*3*) of an intermediate species.

Figure 6 suggests that within each series of peptides, the degree of phosphate catalysis (DPC) is negatively correlated to the reactivity of the non-phosphate reaction (relative intrinsic reactivity defined as the normalized reaction rates of the non-phosphate reactions, defining the reaction rate of Y-Gly 1.00 for each of the systems Gly-X, Met-X and Pro-X). As suggested previously, the effectiveness of the *intra*molecular COOH induced protonation of the key-intermediate imine group is related to the overall reactivity of the reaction couple. Furthermore, the phosphate ion, acting as a proton-transfer mediator between the dipeptide COOH group and the reactive centre, can increase the rate of reaction, i.e. the effectiveness of the *intra*molecular protonation (Scheme 7). The amphophilic character is a prerequisite to enable this concept. Indeed, Potman (*3*) argued that the $H_2PO_4^-$ ion is the most effective catalytic species of the series PO_4^{3-}, HPO_4^{2-}, $H_2PO_4^-$ and H_3PO_4. This can surely be attributed to the fact that this ion has two sites available, for both proton accepting as well as proton donation. If this is the actual role of the phosphate ion, the decreasing degree of phosphate catalysis with increasing intrinsic reactivity of the non-phosphate system is easily explained (Figure 6). This phenomenon results from the fact that the reaction rate enhancement originates from an increased effectiveness of direct protonation of the reactive centre by the C-terminus. It is evident that when the direct catalytic process is highly efficient the role of the phosphate ion acting as a bridge between the reactive site and the catalytic site subsides, thereby lowering the DPC.

A careful examination of all the results presented in Figure 6 reveals that, although the correlation is in a qualitative sense valid, the model presented needs some fine-tuning, since some exceptions to this rule are being observed. Most obviously deviating from the correlation is the system GlyHis. Although at first thought its exceptional high DPC is surprising, its nature seems to be the result of the pH 5.60 at which the experiments have been performed (the imidazole group of the histidine residue is approximately 50% protonated). For this reason its efficacy in the catalytic process would be envisioned to be high since at this pH the proton is easily transferred to the catalytic site. The presumed high reactivity is repressed, however, in the non-phosphate reaction by conformation restrictions within the GlyHis dipeptide. The imidazole group especially does not have the conformational degree of freedom. Upon addition of phosphate, however, one, indeed, should experience a dramatic increase in the number of acceptable conformation in which the imidazole group, via the phosphate ion, is able to exert a catalytic effect. In addition, some minor increased

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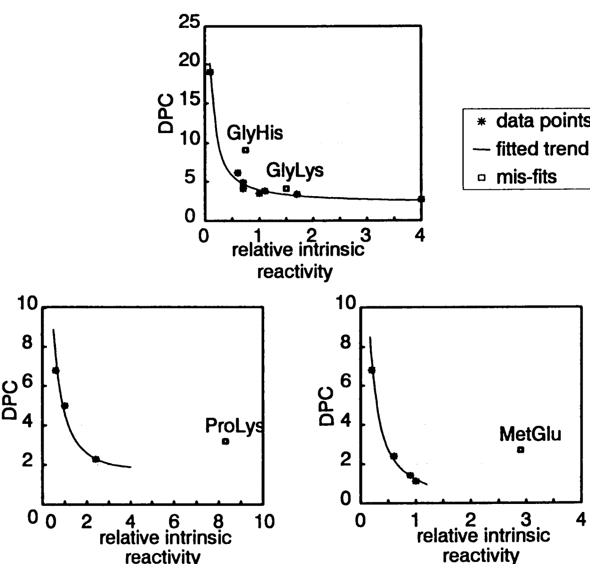

Figure 6. Degree of phosphate catalysis (DPC) plotted versus the intrinsic reactivities of the reaction systems of glucose with Gly-X, Pro-X and Met-X dipeptides, respectively.

Scheme 7. Phosphate as an *intra*molecular proton transfer agent.

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch013

DPC numbers are observed for the systems GlyLys and ProLys. It was already anticipated that the increasing effectiveness of *intra*molecular acid catalysis increases the catalytic effect of additional basic residues. The rate determining step is gradually shifted to the base-induced deprotonation of the sugar residue.

Extending this model even further, one could speculate that the absence of this minor "Lys effect" in MetLys is a result of a shielding effect of the $(CH_2)_2SCH_3$ group, interfering with the COOH-imine interaction, thereby lowering the effectiveness of *intra*molecular catalysis. The MetGlu system suffers from the same conformational hinderance of the methionine side chain, although addition of phosphate slightly reduces this problem.

Finally, it should be noted that the catalysis by amphophiles is not restricted to phosphate. Acetate and citrate have also been put forward to induce catalytic effects within the Maillard reaction, the phosphate effect being much more pronounced (*17*). The mechanism proposed in this paper is directly applicable to catalysis by other amphophilic species.

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Conclusions

The study presented here established that small peptides are more reactive than amino acids in the Maillard-type reaction with glucose at pH 5.6. The reactivity increases going from an amino acid to a dipeptide, decreasing again with increasing peptide chain-length.

In addition, the peptide reactivity is highly dependent on the peptide composition. It appeared that dipeptides having a glutamic acid C-terminal residue show a substantially elevated reactivity with glucose. It is argued that the extra COOH group can effectively catalyze the rate determining step in the Amadori rearrangement reaction due to *intra*molecular protonation. The reactivity is therefore determined by the N-terminal residue and the probability of *intra*molecular proton-transferring transition state formation.

In the case of peptides having a proline N-terminal residue, the "Glu catalytic mechanism" is absent. Alternatively, a C-terminal lysine is a major catalytic factor. This is the consequence of the mechanism proposed and Pro's secondary N-terminus.

The role of phosphate as a catalyst fits with the mechanistic concept presented here. The phosphate ion passes the proton from the COOH group onto the reactive site of the glucose/dipeptide adduct. Increasing effectiveness of the *intra*molecular protonation diminishes the catalytic effect of phosphate, however.

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Chapter 14

Aroma Volatiles from Meatlike Maillard Systems

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Furans and thiophenes with a sulfur-containing group in the 3-position are important in determining meaty aromas. A number of compounds with such structures have been found in meat and model systems. Heating a mixture of 4-hydroxy-5-methyl-3-(2*H*)-furanone and cysteine gave several mercaptoketones and other thiols as well as a number of novel disulfides formed from these thiols. The pH of the reaction mixture had a major effect on the profile of aroma volatiles, especially the sulfur compounds.

Meat flavor develops during cooking from the complex interaction of precursors derived from both the fat and lean components of meat. A number of different flavor characteristics are clearly recognizable in cooked meat; all meats possess fatty flavors, and the different species (beef, pork, lamb, chicken, etc.) have their own distinct flavors. Grilled or roast meats have characteristic roast aroma notes, which differ from the aromas associated with boiled or stewed meat. In addition all meat, regardless of species or cooking method, has a characteristic "meaty" aroma which is a major component of the eating quality. Over 1000 volatiles have been isolated in cooked meat, and those compounds that provide the characteristic "meaty" aroma have attracted the most interest, but have proved to be the most elusive *(1)*.

Recently a number of sulfur containing compounds, which play an important role in meaty flavor, have been isolated from cooked meat and from meat-like model systems. This paper discusses some of the work leading to the isolation of these compounds and presents data on some novel volatiles from a model system containing cysteine and 4-hydroxy-5-methyl-3-(2*H*)-furanone. The influence of pH on the formation of aroma volatiles is discussed.

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Compounds with Meaty Aroma Characteristics

Research during the 1950s and early 1960s demonstrated that the low molecular weight water-soluble fraction of meat contained meat flavor precursors, which included free amino acids and peptides, reducing sugars and sugar phosphates, nucleotides and other nitrogenous compounds such as thiamine. In studies of the effect of heat on these water soluble compounds, depletions in the quantities of carbohydrates and amino acids were observed, the most significant losses occurring for cysteine and ribose. Subsequent studies of the aromas produced on heating mixtures of amino acids and sugars, confirmed the important role played by cysteine in meat flavor formation. This led to the classic patent of Morton et al. in 1960 *(2)*, which involved the formation of a meat-like flavor by heating a mixture of cysteine and ribose. Most subsequent patent proposals for "reaction product" meat flavorings have involved sulfur, usually as cysteine or other sulfur containing amino acids or hydrogen sulfide *(3)*.

In a recent review, MacLeod *(4)* listed 78 chemical compounds that have been reported in the literature as possessing meat-like flavors; seven are aliphatic sulfur compounds, 65 heterocyclic sulfur compounds, and the remaining six non-sulfur heterocyclics. Many of these compounds arise from the prolific patent literature on this subject, and only 25 of the compounds have actually been identified in meat.

In 1976, Evers et al. reported that furans and thiophenes with a thiol group in the 3-position had meat-like aromas *(5)*. The corresponding disulfides formed by oxidation of furan and thiophene thiols were also found to have meat-like characteristics, and exceptionally low odor threshold values. A number of patents were given for the use of such compounds as meat flavorings. Despite the acknowledged importance of furan- and thiophenethiols and their disulfides in meat-like flavors, it is only recently that compounds of this type have been reported in meat itself (Figure 1). MacLeod and Ames *(6)* identified 2-methyl-3-(methylthio)furan as a character impact compound in cooked beef. It has been reported to have a low odor threshold value (0.05 μg/kg) and a meaty aroma at levels below 1 μg/kg. Gasser and Grosch *(7)* identified 2-methyl-3-furanthiol and the corresponding disulfide, bis-(2-methyl-3-furyl) disulfide, as major contributors to the meaty aroma of cooked beef; the compounds were later found in cooked chicken *(8)*. The odor threshold value of the disulfide has been reported as 0.00002 μg/kg, one of the lowest known threshold values *(9)*. More recently, several other disulfides with furyl and thienyl groups have been reported in the volatiles of heated beef muscle, and evaluation of their aroma on elution from the GC column indicated meaty characteristics *(10)*.

Furanthiols and Related Compounds in Model Systems

In meat 2-methyl-3-furanthiol and related compounds could arise from Maillard reactions involving cysteine and ribose; the source of the pentose sugar being ribonucleotides such as inosine monophosphate. Thiamine is another precursor of such compounds. Thiols and disulfides from model systems based on both of these routes have been investigated.

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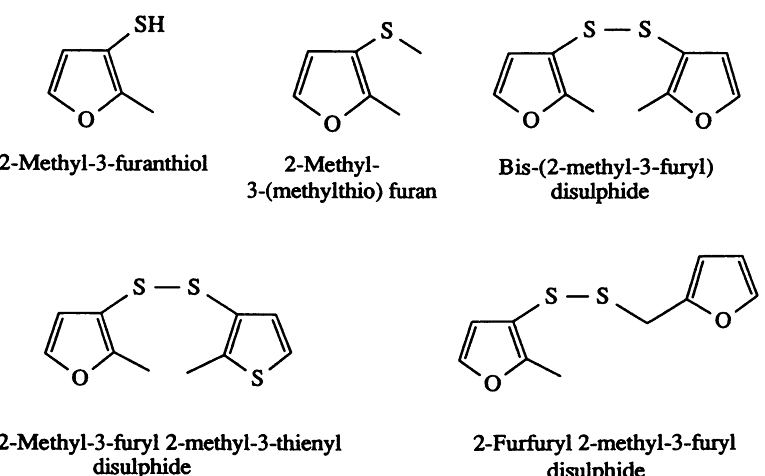

Figure 1. Some thiols, sulfides and disulfides isolated from meat which contribute to meaty aromas.

Thiamine Degradation. The thermal degradation of thiamine produces a number of odoriferous compounds including thiazoles, furanones, thiophenes and furanthiols. van der Linde et al. *(11)* reported that the primary products obtained from heating thiamine in aqueous buffer at 130 °C included 5-(2-hydroxyethyl)-4-methylthiazole and 5-hydroxy-3-mercapto-2-pentanone (Figure 2). Dehydration of the hydroxyethylthiazole gave other thiazoles, while the mercapto compound was an intermediate for the formation of 2-methyl-3-furanthiol, and other furans and thiophenes. The odor associated with thiamine degraded by UV-irradiation was shown to be due to bis-(2-methyl-3-furyl) disulfide *(9)*. In addition to 2-methyl-3-furanthiol, the dihydro- and tetrahydro-2-methyl-3-furanthiols have been found in the thermal degradation products of thiamine, together with disulfides arising from the oxidation of these compounds *(12)*. In investigations of heated model systems containing cysteine, thiamine, glutamate and ascorbic acid, Werkhoff et al. have isolated a number of novel furans and thiophenes containing thiol, sulfide or disulfide groups, many of which had meaty aromas *(13-15)*.

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Cysteine - Ribose Model Systems. The meaty characteristics of heated model systems containing hydrogen sulfide or cysteine and pentoses or other sources of carbonyl compounds have been recognized since the early work on meat flavor precursors. Recent studies on aqueous mixtures of cysteine and ribose heated at 140 °C showed that the volatiles were dominated by sulfur-containing heterocyclic compounds including thiophenones, thienothiophenes, dithiolanones, trithiolanes, dithianones, and trithianes, but the major components were 2-methyl-3-furanthiol, 2-furylmethanethiol, 2-thiophenethiol, 2-methyl-3-thiophenethiol, and 3-mercapto-2-pentanone *(16,17)*. A number of disulfides were also found among the volatiles, albeit at relatively low concentrations. These included bis-(2-methyl-3-furyl) disulfide, bis-(2-methyl-3-thienyl) disulfide, and mixed disulfides derived from 2-methyl-3-furanthiol, 2-methyl-3-thiophenethiol, 2-furylmethanethiol and 2-thiophenethiol *(18)*. Evaluation of the aromas of the GC effluent indicated that some of these compounds had meaty characteristics.

Recent Studies on the Reaction of 4-Hydroxy-5-methyl-3-(2*H*)-furanone with Cysteine

4-Hydroxy-5-methyl-3(2H)-furanone (HMF) is formed in Maillard reactions involving pentose sugars from the dehydration of 1-deoxypentosones. In 1975, van den Ouweland & Peer *(19)* found 2-methyl-3-furanthiol, 2-methyl-3-thiophenethiol, and the corresponding dihydro and tetrahydro derivatives as the main volatile products from the reaction of hydrogen sulfide with HMF. The meaty characteristics of the reaction mixture led to patents dealing with a number of related compounds with potential as meat flavorings *(4)*. In other studies on the reaction between 2,5-dimethyl-4-hydroxy-3(2H)-furanone and cysteine, neither furan nor thiophene thiols were reported. The volatiles were dominated by the dimethyl derivatives of furan-3-one, thiophen-3-one, and trithiolane, although when the pH was increased to 7.1, pyrazines were major products *(20,21)*.

Recently, we have examined the volatile products obtained from the reaction of HMF with cysteine or hydrogen sulfide *(22)*. Reactions were carried out in dilute

Figure 2. Some volatile products from the thermal degradation of thiamine. (Adapted from ref. 11)

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aqueous solution (50 mM HMF) in sealed tubes at 140 °C for one hour and volatiles were analysed by headspace concentration on Tenax followed by GC-MS, as described previously *(17,23)*. The solutions were buffered at pH 4.5, 5.5, or 6.5 using sodium pyrophosphate (0.2M).

Over 80 compounds were identified in the headspace volatiles of reaction mixtures, and included aliphatic ketones (4), furans (10), thiophenes (10), dithiolanones (6), dithianones (3), thienothiophenes (10), thiols (9), sulfides (2) and disulfides (26). Many of these compounds had been identified previously in cysteine - ribose reaction mixtures, which would be expected since HMF is a major product from the degradation of pentose sugars in both Maillard systems and caramelization reactions. However, the majority of the disulfides had not been found in the cysteine - ribose system, and 18 of the compounds had not been reported previously in any food or model flavor system.

The thiols and disulfides were particularly interesting because of the association of this type of compound with meat-like aromas. Three thiol substituted ketones (3-mercapto-2-butanone, 3-mercapto-2-pentanone, 2-mercapto-3-pentanone) were major components of the reaction mixtures, and smaller amounts of three other mercaptoketones were also found. 2-Methyl-3-furanthiol was also present in large amounts as well as the corresponding thiophene derivative. Both of these compounds were found by van den Ouweland and Peer *(19)* when hydrogen sulfide was reacted with HMF in aqueous solution at 100 °C, but the mercaptoketones were not reported. In our reactions involving cysteine a large amount of 2-thiophenethiol was also found, but it could not be detected when hydrogen sulfide was used. The disulfides comprised the oxidation products of these thiols and mercaptoketones. Both symmetrical disulfides, formed from two molecules of the same thiol, and unsymmetrical compounds, formed from two different thiols, were identified (Figure 3).

Both 2,3-butanedione and 2,3-pentanedione were found in the reaction mixtures and in HMF heated alone in buffer. These diones are probably produced from the HMF by reteroaldol reactions. The reaction of the carbonyl groups with hydrogen sulfide, either added to the system or from degradation of cysteine, provides a route to the mercaptoketones. Previous reports have discussed the formation of furan- and thiophenethiols from HMF and hydrogen sulfide. The pathways involved are summarized in Figure 4. The oxidation of these thiol compounds will readily produce the range of disulfides found in the reaction mixtures. The meat-like aroma of many of these compounds suggests that they may be important in the aroma of freshly cooked meat.

Effect of pH on Volatile Formation

The Maillard reaction is known to be affected by pH; as the pH increases the quantities of colored and polymeric compounds increase *(24)*. Alkaline conditions tend to favor the formation of nitrogen-containing volatiles, such as pyrazines, but other volatiles are only formed under acid conditions *(25,26)*. Studies on the effect of pH on the products of the Maillard reaction in model systems have usually involved relatively large pH changes (2 - 5 pH units or more), often without the use of buffers. However, meat has a pH in the range 5.5 - 6.0 and has a high buffering capacity, which results in little pH change during cooking. Recently the effect of pHs

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Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch014

X = O or S

$R_1 = CH_3$ & $R_2 = C_2H_5$ or $R_1 = C_2H_5$ & $R_2 = CH_3$ or $R_1 = R_2 = CH_3$

Figure 3. Thiols and disulfides obtained from the reaction of 4-hydroxy-5-methyl-3(2*H*)-furanone with cysteine.

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Figure 4. Routes for the formation of thiols in the reaction of 4-hydroxy-5-methyl-3(2*H*)-furanone with cysteine.

between 4.5 and 6.5 on the volatiles from model systems containing cysteine and ribose was examined *(18,27)*. Small changes in pH had a major effect on certain classes of volatiles. Nitrogen heterocyclics such as pyrazines were only produced at pHs above 5.5, while the formation of 2-methyl-3-furanthiol and 2-furylmethanethiol were favored by lower pH. The novel disulfides formed in the reaction (see above) were only found at lower pH (Figure 5).

In our studies on the reaction between HMF and cysteine, pH has been shown to be extremely important in determining the nature and quantities of the products. At pH 4.5, the major volatile products were mercaptoketones, furan- and thiophenethiols, 2-methyltetrahydrothiophenone, and 3,5-dimethyl-1,2-dithiolan-4-one (Table I). However, when the reaction was carried out at pH 6.5, the mercaptoketones and other thiols were not detected or were reduced to trace levels. In this reaction mixture pyrazines, thiazoles, oxazoles, and pyrroles became important components. At pH 4.5 the disulfides were present in smaller concentrations than the corresponding thiols, but at pH 6.5 none of the 26 disulfides isolated at pH 4.5 were found, except for a trace amount of one (2-methyl-3-furyl 1-methyl-2-oxopropyl disulfide). When the reaction was carried out using hydrogen sulfide instead of cysteine, a similar effect of pH on the thiol and disulfide compounds was observed. This indicates, the reaction steps that are influenced by pH involve the addition of

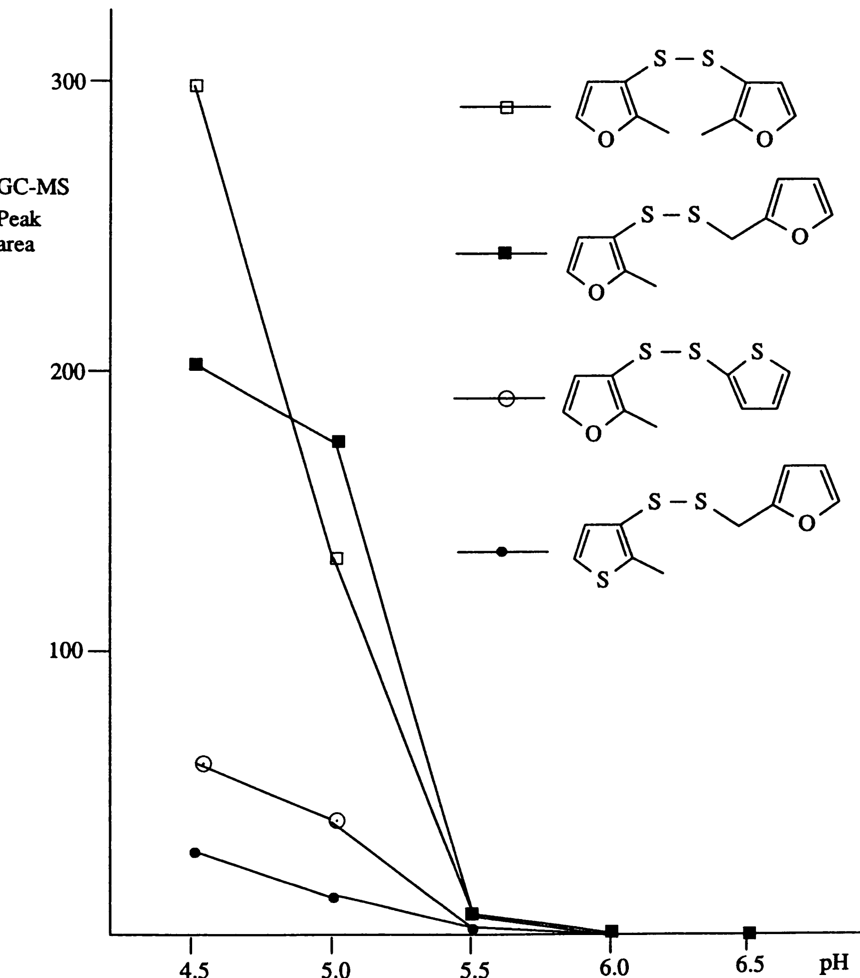

Figure 5. Effect of pH on the amounts of disulfides produced in the reaction of cysteine and ribose. (Adapted from ref. 18).

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Table I. Effect of pH on Major Volatiles from the Reaction of 4-Hydroxy-5-methyl-3(2*H*)-furanone and Cysteine

Compound	*Quantity (μg per 10mg HMF)*		
	pH 4.5	*pH 5.5*	*pH 6.5*
2,3-pentanedione	7	10	8
3-mercapto-2-butanone	70	118	tr
3-mercapto-2-pentanone	57	42	tr
2-mercapto-3-pentanone	62	48	-
2-methyl-3-furanthiol	12	10	tr
2-methyl-3-thiophenethiol	6	6	-
2-thiophenethiol	16	6	-
2-methyltetrahydrothiophenone	65	25	47
3,5-dimethyl-1,2-dithiolan-4-one	18	6	tr
methylthienothiophene	8	1	2
2-methyltetrahydrofuranone	tr	tr	8
trimethyloxazole	-	-	10
2,5-dimethylthiazole	-	-	18
trimethylthiazole	-	-	12
2,5-dimethylpyrazine	-	-	14
trimethylpyrazine	-	-	12
a pyrrole (MW 137)	-	-	29
a pyrrole (MW 151)	-	-	28

tr = trace amount (< 0.5 μg/10mg HMF)
\- = not detected (limit of detection approx 0.1 μg/10mg HMF)

hydrogen sulfide to the carbonyl compounds, rather than the formation of hydrogen sulfide from cysteine.

These results clearly demonstrate the importance of pH on Maillard reactions, and show that small changes in pH can have marked effects on the profile of volatile aroma products. There have been very few reports on the effect of pH on flavor in meat or meat products; however, the work which has been reported shows that as the final pH in the meat increases above the normal range of 5.6 - 5.8, there is a decrease in the amount of meat flavor as perceived by a sensory panel *(28,29)*.

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Chapter 15

New Aroma Compounds in Wheat Bread

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Over 375 aroma compounds generated in the dough, crust and crumb of wheat bread during the baking process were identified and quantitated using dynamic and static headspace techniques in conjunction with gas chromatography - mass spectrometry. Components generated during baking included 114 evolved volatiles, and 86 aroma products in the crumb and 372 in the crust. The evolved volatiles and those isolated from the crumb fraction contained predominantly fat degradation products (alcohols, aldehydes, ketones), while those in the crust were mostly Maillard reaction products (furans, pyrazines, sulfur compounds). For the first time, 217 compounds were identified as bread aroma components. Most of these were previously found in other food systems, including three kahweofurans (coffee aroma) and nine sulfur-containing furans. Newly identified food aroma components are 5-methyl-4,5-dihydro-3-[*H*]-thiophenone and 1-[*H*]-pyrrolo-[2,1-*c*]-1,4-thiazine. Quantitative analyses indicate that aroma components are first formed from fat degradation. After three days storage, their concentration is diminished by vaporization, whereas some of the Maillard products in the crust migrate to the crumb.

The complexity of wheat bread aroma is well established. Initial investigations of the composition of bread aroma were described by Mulders (*1-4*) who identified 102 compounds, followed by Folkes and Gramshaw (*5,6*) who reported 97 new compounds. Subsequent studies were performed by Schieberle and Grosch (*7-10*) and Hiromaka (*11*).

The aim of our research was the identification of aroma compounds generated not only in wheat bread but also those vaporized into the oven during the baking process. This research was combined with attempts to elucidate the rate and mechanism of aroma formation. The main steps of bread production are shown by the following graphic:

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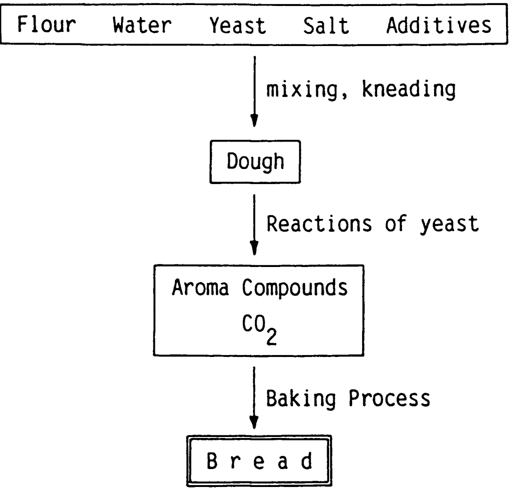

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Experimental Procedure

Bread Preparation. Bread dough was prepared by combining 500 g of wheat flour, 25 g of yeast, 10 g of sodium chloride, and 290 mL of water, then mechanically kneading the mixture for eight minutes. The dough was fermented at 32°C in a special proofing oven for 30 min., and was subsequently left at room temperature for five min. A 600-g portion of dough was formed into a bread loaf shape and proofed in the same oven for 45 min. to prepare it for subsequent baking. The dough surface was then moistened and, after humidifying the oven, the bread was baked in a hermetically-sealed borosilicate glass "baking tin" in a professional-type oven for 30 min. at an average temperature of 230°C. The baking parameters and the experimental design are shown in Figure 1, including the oven, crust and bread temperatures and the oven humidity.

Analytical Methodology. Bread volatiles were purged by a nitrogen stream through a water condenser, which enabled trapping of excess water vapor. (Figure 2) Aroma volatiles were then collected on either a Tenax adsorbent trap or condensed in a liquid nitrogen cold trap. By changing the traps at defined times, it was possible to distinguish the compositions of volatiles which were being formed. In every case, relatively large headspace volumes were injected onto a gas chromatograph, where the aroma compounds were cryofocused onto a retention gap of deactivated fused silica, using liquid nitrogen. The influence of sampling device volume, analyte concentration, and sample matrix have been previously reported (*12*).

Independently of analyzing the volatiles during the baking process, samples of crust and crumb from the freshly baked bread and raw dough were separated, pulverized and analyzed by means of static and dynamic headspace analysis. By this technique, the concentrations of aroma compounds could also be determined. The standard deviations of eight selected compounds were between 6 and 26%, which were higher than those usually observed for purge-and-trap experiments, because of oven temperature deviations (±20°C).

Static Headspace Analysis. A 10-g sample was transferred into a 250-mL Erlenmeyer flask (Figure 3) and held at a suitable temperature (e.g. 20°C) for 1 hr. For volatile sampling, a gas-tight syringe (1 or 10 mL) equipped with a deactivated fused silica capillary (25 cm x 0.17 mm i.d.) was directly introduced a few millimeters above the sample. The volatiles were withdrawn at a rate of 0.5 mL/min, then transferred to the gas chromatography column at the same rate. The gas chromatograph was equipped with a retention gap of deactivated fused silica, which was cooled by liquid nitrogen (Figure 4). By injecting large headspace volumes (up to 20 mL) and cryofocusing them onto the GC column, the sensitivity of the technique was increased relative to conventional static headspace analyses.

Dynamic Headspace Analysis. A 10-g sample was introduced into a pear-shaped two-necked flask as shown in Figure 5 and swept with helium at 50 mL/min. Water was condensed in the first trap, which was cooled with ice water, and the volatile aroma compounds were collected in the second trap, which was cooled with liquid nitrogen. After warming of the second trap at 20°C for 1 hr, 5 mL of the gas phase was injected onto a gas chromatography column as shown in Figure 4.

Gas Chromatography. Separations were performed on fused silica capillary columns using one of the following chromatographs:

1) Carlo Erba Model 6000 Vega, Series 2.

2) Carlo Erba HRGC Model 5160 Mega Series with a Model MFC 500 programming unit.

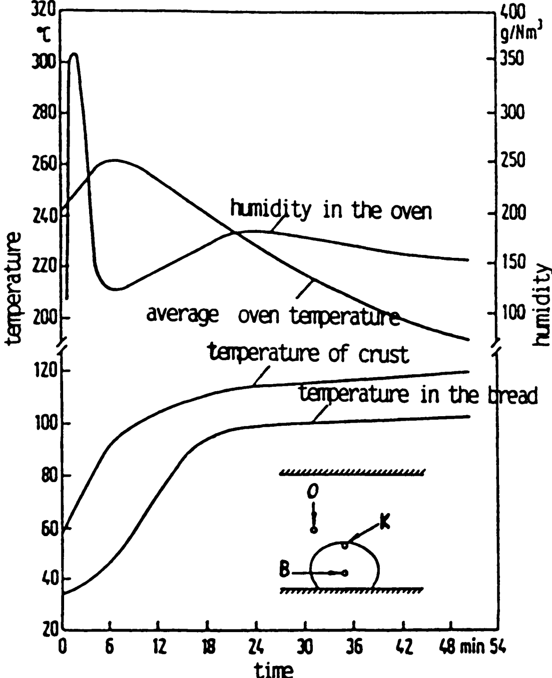

Figure 1. Baking humidities and temperatures as a function of time.

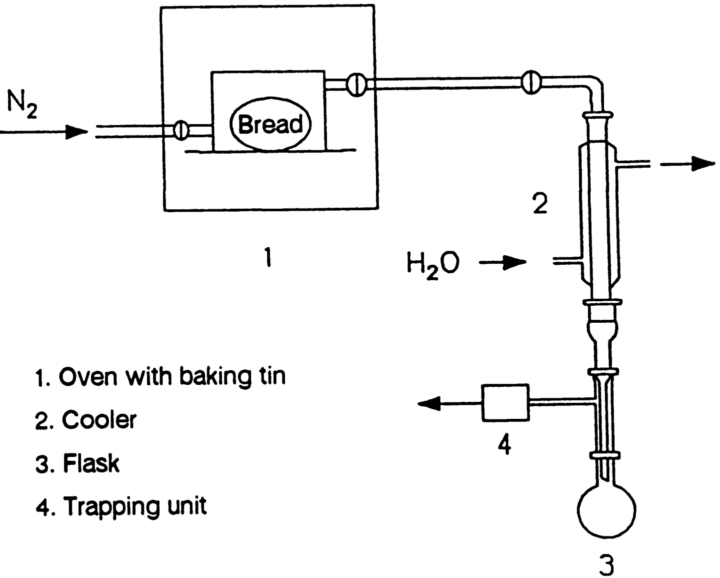

Figure 2. Apparatus for headspace volatile isolation during the baking process.

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch015

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch015

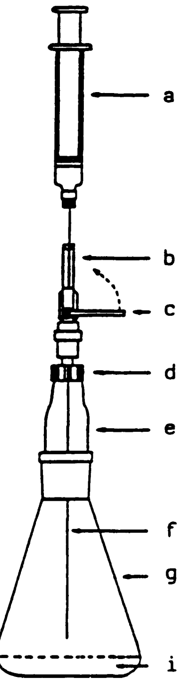

Figure 3. Static headspace sampling device for the collection of large headspace volumes: (a) gas-tight syringe, (b) glass restrictor tube, (c) valve, (d) screw-cap with Teflon ferrule, (e) reducer, (f) fused silica syringe needle, (g) 250-mL Erlenmeyer flask, (i) sample.

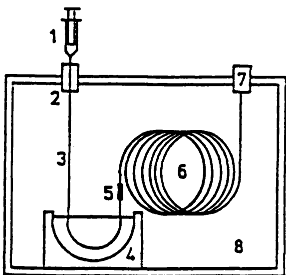

Figure 4. GC interface for the injection of large headspace volumes by cryofocusing: (1) gas-tight syringe, (2) on-column injector, (3) retention gap, (4) Dewar flask containing liquid nitrogen, (5) capillary union, (6) GC column, (7) flame ionization detector, (8) GC oven.

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch015

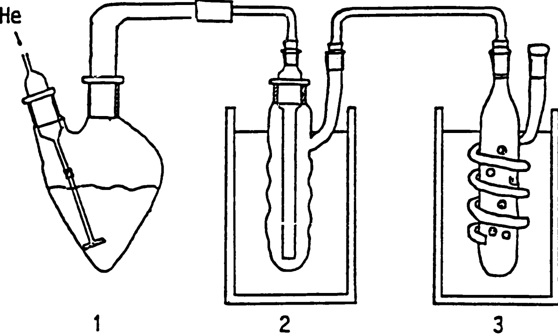

Figure 5. Dynamic headspace sampling device for purge and cold-trapping of volatiles: (1) flask containing sample, (2) cold trap (ice/water), (3) cold trap (liquid nitrogen); this trapping unit was also connected at position (4) in Figure 2.

3) Carlo Erba Model 4130 equipped a Model 180 electrometer. Injections were made in the on-column or split/splitless mode at 280°C. All gas chromatographs contained a flame ionization detector, which was operated at 280°C. The carrier gas was helium at 2 mL/min.

Column 1: 60 m x 0.25 mm i.d., 1.0 µm film thickness DB-1 (J&W Scientific, Folsom, CA) fused silica capillary column; initial temperature 30°C, 5 min hold, then programmed at 3°C/min to 260°C, followed by a 60 min hold. (Used in gas chromatographs 1 and 2.)

Column 2: 60 m x 0.25 mm i.d., 0.25 µm film thickness DB-WAX (J&W Scientific, Folsom, CA) fused silica capillary column; initial temperature 40°C, 5 min hold, then programmed at 2°C/min to 210°C, followed by a 60 min hold. (Used in chromatograph 3.)

Gas Chromatography-Mass Spectrometry (GC/MS). A Finnigan MAT Model 4500 mass spectrometer equipped with a Model 2010 interface and an INCOS 2100 data system was used. Transfer line temperature: 240°C (direct coupling); ion source temperature: 120°C; ionization energy: 70 eV.

Electron impact: Source pressure: approx. 1 x 10^{-6} torr.; mass range 35-350 amu, 0.8 s scan rate.

Chemical ionization: Methane reactant gas; source pressure: 0.7 torr.; mass range 80-350 amu, 0.8 s scan rate.

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch015

Results and Discussion

Volatiles which were evolved during baking consisted primarily of aldehydes, ketones, esters and some hydrocarbons. A majority of these compounds may have been formed from amino acids in the course of the dough fermentation process. Indeed, the research group of Benedito de Barber (*13*) recently demonstrated that during fermentation, most free amino acids are considerably degraded. As was also shown by this group, the fraction of free amino acids in the dough, especially serine, threonine, alanine, leucine, phenylalanine, and lysine, was increased by 64% after yeast addition. For example, the amino acid ornithine, present only as a trace in flour, grew to more than 1% of the total amino acid composition. After fermentation, the free amino acid content dropped significantly, and in baked bread it was reduced to about 30% relative to the original concentration in flour.

The influence of yeast on bread aroma can be considered in several ways:

1) Yeast is an ingredient of dough, which subsequently can generate aroma compounds.
2) Yeast is capable of producing aroma precursors.
3) Yeast possesses its own aromatics, which can enrich the aroma of baked goods, such as cookies.

Figure 6 shows the rates of formation of 14 selected aroma compounds during baking. It was observed that aroma compounds which were formed by yeast such as 1-propanol and 2,3-butanedione were distilled-off very early. Alternately, 3-methylbutanal (formed by Strecker degradation of leucine), pyrrole, and the four furan compounds (presumably formed by the Maillard reaction) were evolved towards the end of the baking time.

Aroma Compounds Identified in Wheat Bread. By means of static and dynamic headspace analysis, we identified 372 compounds in the crust and 86 in the crumb of white bread. Only three compounds (2,4-heptadienal, 2-dodecenal and 2,3-octandione) were formed exclusively in the crumb. The other 83 compounds were also present in the crust.

In the crumb fraction, aldehydes (24), alcohols (17) and ketones (13) predominate, while Maillard reaction products such as pyrazines (11) and furans (6)

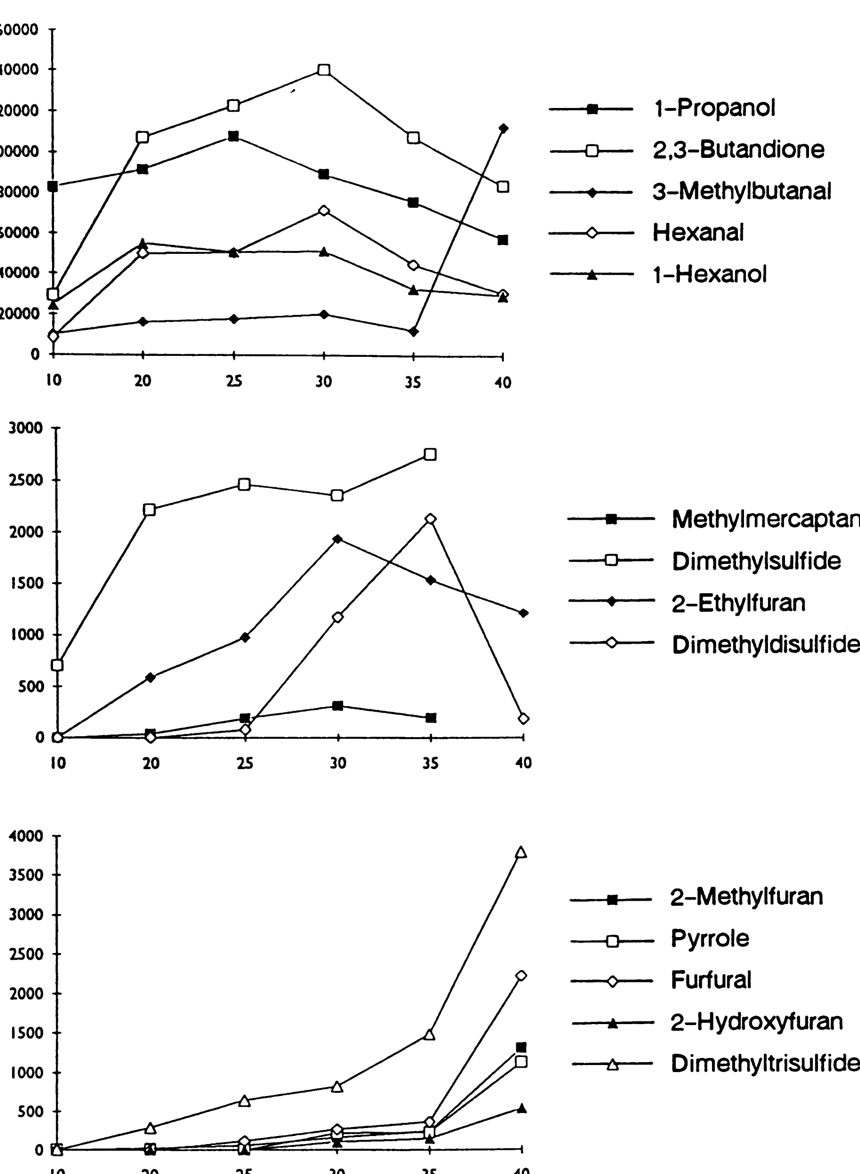

Figure 6. Formation rates for 14 selected aroma compounds in wheat bread during baking as a function of time.

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch015

are less represented. Methional was the only sulfur-containing compound identified. While much fewer volatiles were produced from the crumb during the baking process, their compositions are very similar to those identified in the crust fraction. They are predominantly compounds which have been formed in the dough during the fermentation process or which may have been degraded from precursors in this step. This was verified by identification of similar volatile compounds in the dough.

On the other hand, a large spectrum of aroma compounds was identified in the crust which were predominantly formed by Maillard reactions: 68 pyrazines, 37 pyrroles, 38 ketones, 28 aldehydes, 21 hydrocarbons, 18 alcohols, 15 esters and 12 pyridines. The predominant result of these investigations were, in our opinion, the identification of 57 sulfur-containing compounds, 44 of which were not described to be present in bread aroma until now.

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch015

Aliphatic Hydrocarbons and Alcohols. A variety of hydrocarbons were identified in the crust volatiles. Most important from a toxicological viewpoint may be benzene, toluene, xylene, styrene and ethylbenzene, while some aliphatic hydrocarbons such as *n*-octane, *n*-decane and *n*-dodecane which are derived from fatty acids are not as significant. The aromatic compounds are presumably formed as by-products of sugar degradation. We have identified them following the roasting of sugar/amino acid mixtures (220°C) as well as after pyrolyzing sugars at 700°C (*14*). The threshold values of hydrocarbons are high, which suggests that they are not important as aroma compounds. In addition, alcohols possess high threshold values, therefore they are not important aroma compounds. We have identified 19 alcohols, six of which were not described in bread aroma up to now. Among them, we have identified *(Z)* and *(E)* isomers for both 2-nonen-1-ol and 2-decen-1-ol. The only compound of this class having low threshold values was 1-octen-3-ol, which is well-known to be a character impact compound of mushrooms.

Carbonyl Compounds. Of the 29 aldehydes identified in bread, 3-methylbutanal and 2-nonenal seem to be important for crust aroma, while 2-nonenal and 2,4-decadienal dominate as aroma compounds in the crumb. 2,4-Decadienal possesses a green, fatty aroma and has a threshold value of 0.07 ppb. It was previously identified in potato chips, rice and cooked beef. *(E)*-2-Nonenal (0.08 ppb threshold) is responsible for a cucumber-like aroma. At concentrations between 0.4 and 2 ppb, it possesses a wooden note which gives a fresh aroma character to roasted coffee. The structures of these alcohols and aldehydes, some of which are shown in Figure 7, indicate that they have probably been formed by fat oxidation. Among the 38 ketones which were identified in crust and crumb, 17 are described in white bread for the first time. Most of them can be generated by yeast fermentation as well as by Maillard reactions. The same is valid for some hydroxy ketones such as acetoin and hydroxypropanone. Among them were seven cyclic ketones belonging to a group of pentanones and hexanones. Some of these are important aroma compounds in caramel which are formed through Maillard reactions.

Oxygen Heterocycles. A group of 63 furans and furanones was identified, 43 which are described to be contributors to bread aroma for the first time. This group contains some derivatives with a long alkyl substituent, such as hexyl furan, which are clearly fat oxidation products. The most abundant compound is furfural (14.9% of all volatiles), and it is the principal component of the volatiles from crust. Furan ketones and diketones are described as prominent aroma compounds in bread and coffee which have caramel-like, burnt aroma notes. Of interest is the identification of some condensed furans, some which were also identified in coffee.

Nitrogen Heterocycles. Pyrroles are well-known aroma compounds in foods such as cocoa, coffee, tea and nuts which are formed by Maillard reactions. We

identified 27 of them in the crust, 19 which were found in bread for the first time. Of interest is the identification of some pyrrolizines. The aroma impression of pyrroles are pyridine-like, while acylpyrroles possess mostly a bread-like aroma note. 2-Acetylpyrroline has been described to be a character-impact compound of wheat bread crust. It is formed by the reaction of the amino acids proline and ornithine with sugars in the course of the Maillard reaction. 2-Acetylpyrroline was initially identifed in rice and is responsible for popcorn-like aroma notes. Its threshold value is in the range of 1 ppb in water.

Twelve pyridines were identified, seven of which were found in bread for the first time. One of these is 2-pentylpyridine, which is formed starting with the reaction of 2,4-decadienal and ammonia (Figure 8, R=C_5H_{11}). It possess a fatty, tallow-like aroma note and a threshold value of 0.6 ppb in water. The corresponding thiophene is formed by reaction with H_2S. 2-Acetyltetrahydropyridine was identified by Hunter (*15*) in white bread. It possesses a cracker-like note and a low threshold value (1.6 ppb), and is formed by reaction of proline with pyruvic aldehyde. Through aromatization, 2-acetylpyridine is formed; it possesses a higher odor threshold but similar sensory character as 2-acetyltetrahydropyridine.

Pyrazines are not discussed in this chapter, even though 38 new pyrazines were identified in bread for the first time. However, a huge literature is available concerning pyrazines in foods. For instance, we have previously described 123 pyrazines obtained through the heating of serine and threonine with sucrose. (*16*)

Sulfur Compounds. Of special significance is the identification of 44 sulfur-containing compounds which are described as bread aroma products for the first time. Methionine and cysteine are precursors to a variety of aliphatic sulfur compounds, some of which are shown in Figure 9. Heterocyclic sulfur compounds such as thiophenes, are generated by reactions of the corresponding aldehydes with H_2S or with mercaptoacetic aldehyde (the Strecker degradation product of cysteine.) Examples of these compounds identified in bread aroma include the previously unknown 5-methyl-4,5-dihydro-3-[2*H*]-thiophenone (**26**) and the thiophenones **24** and **25** which are already known from coffee aroma (Figure 10). Thiophenes with alkyl substituents in the 2-position may be derived from 2,4-dienals through fat degradation.

Thiazoles represent another chemical class, the precursors of which appear to be cysteamine (formed by reaction of suitable carbonyl compounds with H_2S and amino compounds). Strecker degradation plays a dominant role in the formation of thiazoles. 2-Acetyl-2-thiazoline, which possesses a strong fresh bread aroma character and was designated as a bread aroma compound by Folkes and Gramshaw (*5*), was not identified in our experiments.

Of special interest are furans, which are easily substituted with sulfur groups. A variety of these types of compounds can be formed by reactions of furfural or pentoses with H_2S. Ten sulfur-substituted furans were identified in bread, nine for the first time (Figure 11). Furfuryl methyl disulfide (**29**) has previously been identified by Mulders (*4*) as a character impact compound of bread. Its threshold is approximately 0.04 ppb in water. Furfuryl mercaptan is important in coffee aroma. Difurfuryl sulfide and disulfide are described to possess a white bread crust-like aroma.

In our opinion, the identification of kahweofuran derivatives in bread aroma is a distinguishing result. Compound **34** was first identified by Stoll in coffee. (Figure 8) In suitable dilution, it possesses a smokey-like odor. Compounds **35** and **36** were also recently identified in coffee. Their aroma qualities are reminiscent of sulfur-like, meaty, or mushroom-like notes. They were formed in a model system reaction between mercaptopropanone and vinyl hydroxymethyl ketone in 50% yield (*17*).

Other sulfur-containing compounds identified include methional, methionol, oxidation products of methyl mercaptan, and 1,2,3,4-tetrathiane. A new compound

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch015

Figure 7. Some selected aldehydes and alcohols identified in wheat bread aroma.

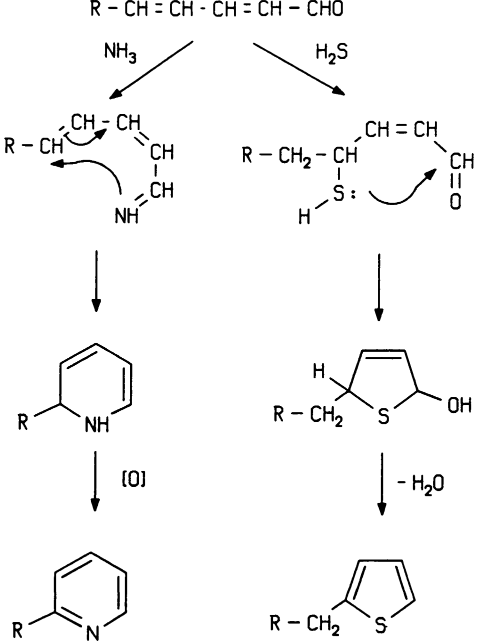

Figure 8. Formation of pyridines and thiophenes with long-chain alkyl substituents *via* reaction of 2,4-dienals.

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch015

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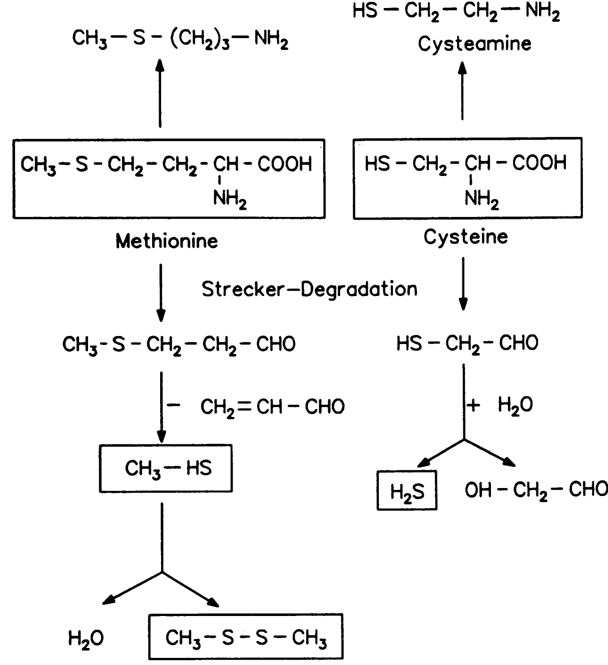

Figure 9. Formation of typical degradation products from methionine and cysteine.

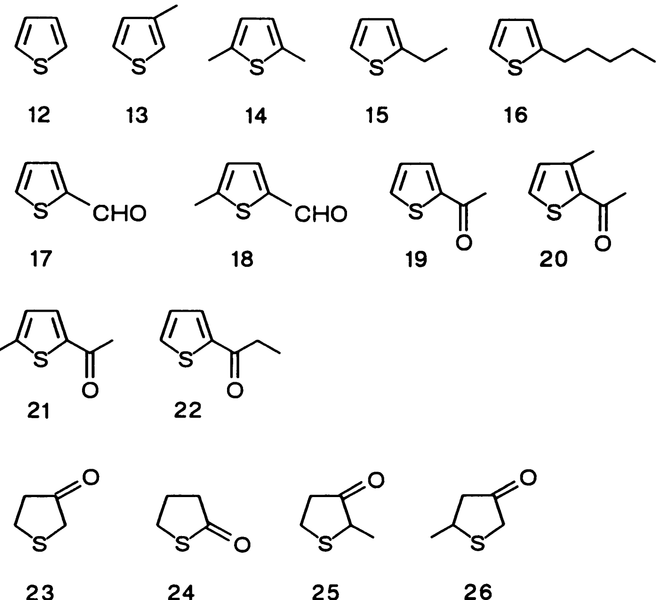

Figure 10. Selected thiophenes and thiophenones formed in wheat bread aroma.

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch015

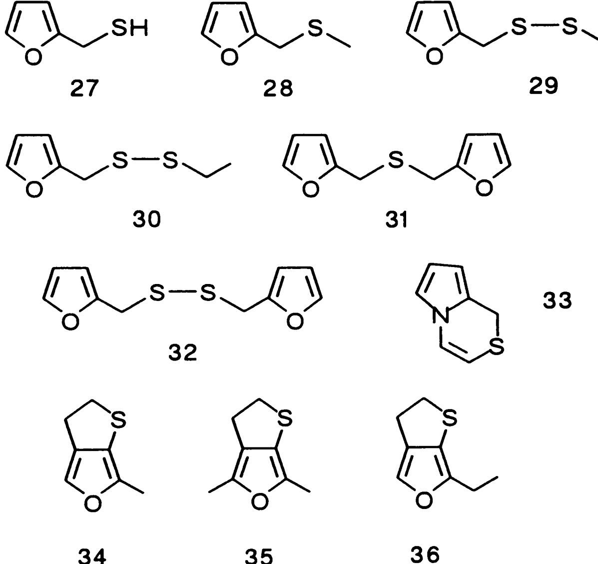

Figure 11. Some selected furans containing sulfur substituents.

which was not described in food systems until now is 1-[*H*]-pyrrolo-[2,1*c*]-1,4-thiazine (**33**) which we identified in the crust volatiles (Figure 11). It was initially described by Werkhoff in a model system consisting of cysteine and ribose (*18*). Its formation is presumed to proceed by reaction of an aminoketone with 3,4-dideoxypentosene, or by reaction of furfural with mercaptoacetic aldehyde. Because of the high concentration of furfural in the bread volatiles, we favor the latter mechanism.

Conclusions

The majority of volatiles identified in wheat bread were found in the crust portion. While the crust was found to contain predominantly Maillard reaction products such as furans, pyrroles, pyrazines and sulfur compounds, the aroma products in the crumb were generated through yeast fermentation processes and included alcohols, aldehydes and ketones. Overall, the most abundant volatiles in bread were alcohols, furans and pyrazines.

During storage of bread for 1, 2 and 3 days, the spectrum of aroma compounds changed. During this time, the concentrations of aroma compounds formed by fermentation such as 2,3-butanedione, 2-butanone, ethyl acetate, 3-methylbutanol and 2,3-pentanedione decreased in the crust. A possible explanation may be diffusion out of the bread together with evaporation of water. Alternatively, the concentrations of pyrazines and furans reached a maximum on the second day and subsequently dropped.

The opposite was observed in the crumb. While the concentrations of fermentation products remained constant, the levels of Maillard reaction products increased. It became apparent that Maillard reaction products in the crust are able to migrate into the crumb, as was determined by measurement of the concentrations of selected aroma compounds at a 5-millimeter distance from the crust.

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Chapter 16

Formation Pathways for Primary Roasted Coffee Aroma Compounds

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The majority of the more than 800 volatiles which have been identified in roasted coffee, are formed by Maillard reactions. However, recent sensory specific investigations showed that only a small number of these contribute to the flavor complex of roasted coffee and that many odorants with a strong flavor impact are generated by formation pathways besides Maillard reaction. This chapter gives an overview of aroma compounds of roasted coffee and their corresponding formation pathways during roasting. Apparently certain pathways are specific for roasted coffee flavor formation.

Generally speaking, the composition of flavors generated by thermal treatment or fermentation is extremely complex eg beer, meat, tobacco or coffee. Recent compilations of volatile compounds in roasted coffee list more than 800 compounds of all chemical types (1, 2). We expect a large number could still be found. This is one reason why the knowledge of coffee aroma chemistry is still incomplete after more than 70 years of systematic research in this field. Identification of unknown coffee volatiles without studying their sensory impact makes sense only for academic purposes, as it does not fit the current requirement of aroma research which focuses more and more on the character impact compounds. The first attempts to investigate the aroma relevance of roasted coffee volatiles have been recently made by application of semi-quantitative GC/sniffing assessments carried out as aroma dilution analyses (3-5). The results indicated that only a comparatively small number of components, between 60 and 80, contribute to the aroma character of roasted coffee. Many of these chemicals arise from Maillard reactions directly or indirectly. However, a number of other aroma impact compounds are generated by different kinds of formation pathways.

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Experimental

Isolation of volatiles of medium roasted Colombian coffee and identification of the character impact compounds was carried out as described in detail earlier (3, 5, 6): Volatiles were collected by means of high vacuum distillation and simultaneous distillation/extraction with a mixture of diethylether and n-pentane. After preseparation by means of column chromatography on silica gel, preparative HPLC and GC, the aroma concentrates were investigated by capillary GC, GC-MS, and simultaneous GC/sniffing. Identification was carried out by GC retention and spectroscopic data compared with authentic reference compounds.

Results and Discussion

Over the last decade, GC-effluent sniffing has proved to be a powerful tool for the investigation of relevant aroma compounds in various kinds of foods and therefore was applied in the characterization of roasted coffee odorants. Table I lists the character impact compounds identified in roasted Colombian coffee by means of combined GC-MS/sniffing. Sotolone and abhexone (see below) were identified by Blank et al. (4). A further 17 aroma notes detectable at the sniffing port have not yet been identified but appear to be of lower sensory importance. The overall number of aroma relevant compounds is therefore few considering that more than 1000 volatiles may occur in roasted coffee. Furthermore, the results indicate that not a single compound exhibits the typical roasted coffee-like aroma impression but the aroma consists of a complex mixture of compounds possessing many different odor qualities. Table I also gives an overview of their predominant formation pathway and precursors during the roasting of the green coffee beans.

Breakdown Products of Lipid Oxidation. Sniffing indicated that compounds such as hexanal, 1-octen-3-one, 2(cis)-nonenal, 2(tr)-nonenal, 2,6(tr,cis)-nonadienal, 2,4(tr,tr)-nonadienal or 2,4(tr,tr)-decadienal contribute to roasted coffee flavor to a limited extent, although the odor notes were weak. Their presence is not surprising considering that green coffee beans which are a seed contain lipids and proteins to support germination. Total lipids amount to about 13 % in Arabica coffee (7), and of this over half is linoleic acid. The formation of various aldehydes and ketones by autoxidation of unsaturated fatty acids via breakdown of hydroperoxide intermediates is well established in the literature (8). 2(tr)-Nonenal is related to "woody" or "cardboard-like" off flavor notes in coffee beverages (9). 2(tr)-Nonenal exhibits synergistic effects and may influence the sour taste perception without changing the pH. The carbonyl compounds mentioned above were identified in the staling of roasted coffee in oxygen-containing atmospheres. Stale notes in roasted coffee could be correlated with the generation of hexanal after 7 weeks storage in air (10, 19). Whether hexanal arises from autoxidation of the lipid complex or from volatile roasting products is not yet clear.

Pyrazines. 2-Methoxy-3-isopropyl pyrazine (III, Figure 1) and 2-methoxy-3-isobutyl pyrazine which possess strong vegetable-like odors are present in green coffee (11) and contribute to the final coffee aroma impression after roasting. As

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Table I. Odor description, intensity and predominant formation pathway of aroma relevant volatiles in roasted Colombian coffee

Compound	Odor Description	Intensity	Pathway/ Precursor
Methanethiol	putrid	+	M,S
Methylsulfide	sulfury	+	M,S
2-Methylpropanal	pungent, fruity	+	M,S
2-Methylbutanal	fermented, fruity	+	M,S
3-Methylbutanal	pungent, fruity	+	M,S
2,3-Butanedione	buttery	++	M
2,3-Pentanedione	buttery	+	M
n-Hexanal	nutty, green	+	L
3-Methyl-2-buten-1-thiol	foxy, skunky	++	P
1-Octen-3-one	mushroom-like	+	L
2-Methyl-3-furanthiol	meat-like	+++	T
2-Ethyl pyrazine	roasty	+	M
2-Ethyl-3-methyl pyrazine	roasty	+	M
2,3,5-Trimethyl pyrazine	roasty, musty	++	M
2-Furanmethanethiol	roasty, coffee-like	+++	M
2-Methoxy-3-isopropyl pyrazine	peasy	+	G
Acetic acid	vinegar-like	+	M
Methional	cooked-potato-like	+++	M,S
2-Ethyl-3,5-dimethyl pyrazine	roasty, musty	++	M
2-Furfurylmethylsulfide	garlic-like	+	M
2(cis)-Nonenal	metallic, tallowy	+	L
3-Mercapto-3-methylbutyl formate	catty	+++	P
2-Methoxy-3-isobutyl pyrazine	paprika-like	+++	G
2(tr)-Nonenal	tallowy, fatty	+	L
Linalool	flowery	+	G
2,6(tr,cis)-Nonadienal	cucumber-like	+	L
5-Methyl-6,7-dihydro-5H- -cyclopenta(b)pyrazine	peanutty	+	M,Ca
2-Phenylacetaldehyde	honey-like	+	M,S
3-Mercapto-3-methyl butanol	broth-like	+	P
2-Methyl butyric acid	fermented, sweaty	+	M,S
3-Methyl butyric acid	footsweat-like	+++	M,S
2,4(tr,tr)-Nonadienal	geranium-like	+	L
2,4(tr,tr)-Decadienal	fried, oily	+	L
ß-Damascenone	fruity, tea-like	+++	C
Cyclotene	spicy	+	Ca
Guaiacol	sweet, phenolic	++	PA
2,6-Dimethylphenol	phenolic	+	PA
2-Phenylethanol	honey, beer-like	+	M,S

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Table I. (continued)

Compound	**Odor Description**	**Intensity**	**Pathway/ Precursor**
Phenol	phenolic	+	PA
4-Ethylguaiacol	clove-like	+	PA
2,5-Dimethyl-4-hydroxy-3- -(2H)-furanone	caramel-like	+++	M
m-Cresol	phenolic	+	PA
3-Ethylphenol	phenolic	+	PA
4-Vinylguaiacol	clove-like	++	PA
Sotolone[a]	spicy	+++	M
3,4-Dimethylphenol	phenolic	+	PA
Abhexone[a]	spicy	++	M
3-Methylindole	faecal	+	M

Compounds listed according to their retention on DB-WAX; **a:** according to reference (4); **+:** weak; **++:** intense; **+++:** very intense; **M:** Maillard Products; **S:** Strecker Degradation; **L:** Lipid Oxidation; **G:** Green Coffee Volatiles; **T:** Thiamine Degradation; **P:** Prenylalcohol; **C:** Carotinoid Degradation; **Ca:** Caramelization; **PA:** Phenolic Acid Degradation.

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch016

opposed to the more than 80 pyrazines identified as roasted coffee constituents, the methoxy alkyl pyrazines are not formed by Maillard reaction but are generated within the coffee plant tissue. One speculative mechanism was proposed by Murray et al. (12) and is depicted in Figure 1. Amidation of valine or leucine, in the case of the isobutyl derivative, gives the intermediate product I, which undergoes a condensation with glyoxal to form compound II. Finally the methoxy alkyl pyrazines are generated via methylation of the hydroxy group. This special pathway may be the reason why III and 2-methoxy-3-isobutyl pyrazine are detectable in other raw plant material such as peas and paprika. Excessive amounts of III were the reason for a "peasy" off flavor note in certain batches of Ruandian coffee (13).

Numerous pyrazines derived from Maillard reactions occur in roasted coffee in remarkable amounts and form about 14 % of the overall volatile content (14). Their formation pathway is shown in Figure 2. α-Aminoketones (IV) with various alkyl groups (generated by Strecker degradation, see Figure 3) can undergo condensation to form pyrazines. However, the majority show only negligible sensory potency. According to the results of semi-quantitative sniffing assessment, only 2,3,5-trimethyl pyrazine (V), 2-ethyl-3,5-dimethyl pyrazine (VI) and some pyrazine-like aroma notes of unknown chemical structure seem to have an influence on coffee aroma. The aroma note "roasty/musty/flowery" typically formed by pyrazines, occurred several times during sniffing and the effect of pyrazines on the overall aroma presumably must be evaluated additively.

Figure 1. Hypothetical biogenesis of 2-methoxy-3-isopropyl pyrazine (12).

Figure 2. Formation of aroma relevant alkyl pyrazines via α-aminoketones.

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Strecker-Aldehydes. Various volatiles derived from Strecker-degradation are important odorants in roasted coffee (Figure 3). α-Dicarbonyls (VII) which are generated by a side pathway of the Maillard reaction are highly reactive and condense with free amino-groups of amino acids. α-Aminoketones (IV) and Strecker-aldehydes (VIII) of various chemical structures are then formed via transamination and decarboxylation, eg 2-methylpropanal (IX), 2-phenylacetaldehyde (X), 3-methyl- (XI) and 2-methylbutanal (XII) or methional (XIII), depending on the precursor amino acid. In addition to methanethiol (XIV, see below), short chain Strecker-aldehydes are key compounds for the pleasant smell arising from freshly roasted and ground coffee (15).

Methional and Methanethiol. Methional, the Strecker-aldehyde of methionine (XIII, Figure 3), is the key compound of cooked potatoes. Methional is essential for roasted coffee flavor, even though its characteristic cooked-potato-like aroma note can be detected in most thermally treated foods (17). Methional can undergo degradation to form the more volatile methanethiol (XIV). Although it exhibits a putrid smell in the concentrated state, in lower levels methanethiol is a key compound for the pleasant aroma arising from freshly roasted and ground coffee (15). Disappearance of methanethiol by oxidative and non-oxidative events correlates to the storage related loss of aroma freshness in roasted whole beans to a significant degree. Quantification of methanethiol has been suggested as an analytical monitoring process for roasted whole bean freshness (18).

Volatile Acids. Several Strecker-aldehydes serve as precursors for low chain fatty acids, eg 3-methyl butyric acid and 2-methyl butyric acid, respectively (XV, XVI, Figure 3). Both isomers show slightly different sensory qualities. The 3-methyl-isomer possesses a well known unpleasant footsweat-like smell, whereas, 2-methyl butyric acid smells fermented and less sweaty. These odorants gave very intense aroma notes during sniffing evaluation. Although the selectivity of the GC column used for GC/sniffing was inadequate for a separation of both isomers, it can be assumed that the 3-methyl isomer is sensorily more important. This is the case because the total amount detected in roasted coffee is about 7 fold higher (19), while the threshold values are 3 fold lower compared to 2-methyl butyric acid (20).

Sotolone and Abhexone. Two "spicy, lovage-like" aroma notes with high sensory potency were identified by Blank et al. (4), recently, as sotolone (4,5-dimethyl-3-hydroxy-2(5H)-furanone, XX in Figure 4) and abhexone (5-ethyl-4-methyl-3-hydroxy-2(5H)-furanone, XXI). The planar enol-oxo function is their common structural element (see also Figure 8). Sotolone was found in considerably higher amounts in Coffea Arabica and correlated to its milder taste compared to Robusta (4). The formation of sotolone is possible under mild fermentation as well as roasting conditions. Kobayashi (21) identified sotolone and abhexone in roast model systems containing α-oxo butyric acid (XVII) and pyruvate (XVIII). Both compounds are presumably formed via intermediate XIX. Abhexone may be formed via self-condensation of α-oxo butyric acid which occurs as an intermediate during acidic degradation of threonine (22).

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch016

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch016

Figure 3. Aroma intense odorants in roasted coffee generated by Strecker-degradation (16), Ox.: oxidation.

Figure 4. Formation of sotolone and abhexone via α-oxo acids (21).

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch016

2-Furanmethanethiol. One of the few odorants which can be described as slightly roasted coffee-like is 2-furanmethanethiol (XXIII, Figure 5) that gave a very intense odor note during sniffing assessment. 2-Furanmethanethiol has been regarded as a significant roasted coffee compound for many years (16, 23, 24). It may be formed by a reaction between hydrogen sulfide and 2-furanaldehyde (XXII) or 2-furfurylalcohol, arising from thermal degradation of pentoses and sulfur containing amino acids during roasting (Figure 5). Recently, 2-furanmethanethiol was identified in roast model systems containing rhamnose or 2-furanaldehyde and cysteine or methionine (25). Thermal breakdown of methionine produces methanethiol which may react in a similar manner to form 2-furfurylmethylsulfide (XXIV). This compound exhibits a strong garlic-like aroma note at the sniffing port and seems to be of lower importance for the overall roasted coffee flavor.

Odorants Derived From Prenylalcohol. Several sulfur containing odorants were identified recentyl (6) and are depicted in Figure 6: 3-Mercapto-3-methyl butanol (XXVI), 3-mercapto-3-methylbutyl formate (XXVII) and 3-methyl-2-buten-1-thiol (XXVIII) contribute to roasted coffee flavor remarkably. They can be regarded as derivatives of the common precursor prenylalcohol (XXV) and gave "skunky", "broth-like" or "catty" aroma notes at the sniffing port. Free prenylalcohol is a well known constituent of roasted coffee (26) and also occurs in green coffee in the ppm range (19), probably arising as an intermediate in the isoprenoid biosynthesis cascade. According to a specific formation pathway first introduced by Tressl et al. (27), prenylalcohol reacts with hydrogen sulfide to form compounds XXVI and XXVIII by a reaction across the double bond and a substitution of the hydroxy group, respectively. Esterification of XXVI yields compound XXVII. The thiol and the alcohol were detected in roast model systems containing prenylalcohol and sulfur containing amino acids (6). 3-Methyl-2-buten-1-thiol is known as an agent causing off flavors in beer (28). Compounds XXVI, XXVII and XXVIII have not been identified in other volatile aromas yet, therefore they may be considered to be roasted-coffee specific.

Degradation Products of Carbohydrates. Among those aroma notes which remained unidentified were several described as "spicy, lovage-like". Many of these aroma notes can be related to cyclopentenolones, a class of compounds which have been identified recently in roasted coffee and roast model systems with sucrose (29). One of the well known compounds is cyclotene (XXX, Figure 7), although its sensory impact is quite low. Cyclopentenolones are formed by condensation of degradation products (XXIX) thermally generated from reducing sugars according to the scheme in Figure 7. Furthermore, some cyclopentenolones can act as precursor compounds and can undergo condensation with α-aminoketones and ammonia to form bicyclic pyrazines such as the "peanutty" smelling 5-methyl-6,7-dihydro-5H-cyclopenta(b)pyrazine (XXXI).

Caramel-like smelling and tasting compounds such as 2,5-dimethyl-4-hydroxy-3-(2H)-furanone (XXXII, Figure 8) are important odorants not only of roasted coffee but also of many kinds of thermally treated foods (30). This compound probably arises from rhamnose under roasting conditions. The intense caramel-like aroma note is due to the planar enol-oxo group (Figure 8).

Figure 5. Generation of odorants from the precursor 2-furanaldehyde (14).

Figure 6. Odorants derived from prenylalcohol.

Figure 7. Formation of cyclotene and bicyclic pyrazines (16).

Figure 8. Planar enol-oxo group in 2,5-dimethyl-4-hydroxy-3-(2H)-furanone (Furaneol).

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch016

Phenols. During sniffing assessment of roasted coffee aroma concentrates several phenolic and clove-like odor notes were perceivable. Most of these compounds are formed by a thermal degradation cascade of phenolic acids (Figure 9). Phenolic acids such as ferulic acid (XXXIII) are major constituents of green coffee (31). Among numerous phenolic compounds only 4-vinylguaiacol (XXXIV) and guaiacol (XXXVI) show a considerable impact on roasted coffee flavor. One of the intermediate breakdown products, vanillin (XXXV), seems to be of lower sensory importance.

Miscellaneous Pathways. Carotinoids like carotene (XXXVII, Figure 10) are widely found in various kinds of foods. Degradation via oxidation and isomerization can lead to the formation of several odorants with low odor thresholds (32). One of these breakdown products is ß-damascenone (XXXVIII) which contributes to the final roasted coffee flavor remarkably. This odorant is present in green coffee, as is true for linalool (XXXIX).

3-Methyl indole (XL), better known as skatole, possesses a very strong and unpleasant "fecal" smell, however there is a contribution to roasted coffee flavor. 3-Methyl indole is formed by an oxidative degradation of tryptophane.

2-Methyl-3-furanthiol (XLIII, Figure 11) exhibits an intense meat-like aroma and was characterized as a key compound of boiled meat previously (17). It contributes to roasted coffee flavor, as Table I shows. Since 2-methyl-3-furanthiol is present at only trace levels, its identification required the work up of 5 kg of roasted coffee beans (5). The formation of 2-methyl-3-furanthiol has been reported in numerous roast model reactions (33). Thiamine (XLI) is considered to be the precursor. Thermal degradation yields 5-hydroxy-3-mercapto-pentane-2-one (XLII) as the major intermediate product. The most aroma intense compound formed in the reaction sequence is 2-methyl-3-furanthiol (XLIII). Its disulfide (XLIV), one of the most potent odorants known so far (23), has been identified in roasted coffee (4).

OH (XXXIII) OH (XXXIV) OH (XXXV)

COOH $-CO_2$ Ox. Ox. H O

Ox. OH COOH $-CO_2$ OH (XXXVI)

Figure 9. Thermal degradation of ferulic acid (16).

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Figure 10. Miscellaneous roasted coffee odorants.

Figure 11. Formation of odorants from thiamine degradation (34).

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Chapter 17

Indicator Compounds and Precursors for Cocoa Aroma Formation

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During cocoa roasting several hundred volatile aroma compounds are formed. Therefore we looked for compounds that would indicate the degree of roasting. It turned out, that within the range of technical roasting processes, the concentration of 2,3-dihydro-3-hydroxymaltol has a linear correlation with respect to the extent of roasting, and the concentration of dimethylpyrazines strongly increases at the beginning of overroasting.
Amadori compounds formed by reaction of aldoses and amino acids are aroma precursors. Their concentrations increase during pre-drying prior to roasting. In model roasting experiments with fructosealanine, a typical aroma precursor detected in cocoa, different classes of aroma compounds (pyrazines, pyrroles, pyridines, furans), which have already been described for the aroma of cocoa, were formed. Their formation depends on reaction conditions.

Cocoa aroma is formed by moderate roasting of fermented cocoa beans at temperatures between 110 and 130 C. (*1*). During cocoa roasting several hundred volatile aroma compounds are formed (*2*), which mainly arise from the Maillard reaction (*3-5*). Aroma precursors; especially reducing sugars, amino acids, and peptides (*4,6*); are formed during the fermentation process (*7*). Reducing sugars react almost completely as roasting times increase, but only about 40% of the free amino acids react.

Historically cocoa is roasted to varying degrees to obtain specific aroma characteristics; therefore, sensory evaluation will always determine the quality of a cocoa product. An analytical determination of the degree of roasting, which uses defined tracer substances, could serve to verify the sensory evaluation. This would provide an objective value independent of the performance of the sensory panel. A linear correlation between the degree of roasting and the tracer substance would be desirable.

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Furthermore, the extent that Maillard reaction intermediates serve as aroma precursors should be investigated in both roasted and unroasted cocoa.

Indicator Compounds for Evaluation of the Degree of Cocoa Roasting

Figure 1 shows the HPLC chromatograms (UV detector) of steam distillates of underroasted and overroasted cocoa. 2,3-dihydro-3-hydroxymaltol (peak 1) increased due to roasting. Different furan and pyrazine derivatives were, also, seen to increase (*10,11*). The concentration of tetramethylpyrazine (peak 7), which is present in unroasted cocoa, stayed almost constant. Concentrations of the remaining compounds, shown in Figure 1, increased characteristically as a function of time.

For cocoa types Elfenbein, Lagos, and Ariba, the concentration of 2,3-dihydro-3-hydroxymaltol increases linearly over the range of medium roasting conditions (between approximately 5 and 15 minutes at 130 C). In the region of overroasting the concentration of 2,3-dihydro-3-hydroxymaltol levels off (Figure 2).

In contrast, compounds contained in peak 2 through peak 6 in Figure 1 increase significantly only after longer roasting times. This is very clear for the dimethylpyrazines. Concentrations of the dimethylpyrazines do not change in the lower roasting range, but they show a sharp increase at overroasting conditions (Figure 3).

From this work we can conclude: 2,3-dihydro-3-hydroxymaltol is a suitable tracer substance for evaluating the degree of roasting in cocoa beans that have been roasted within the normal range. A large increase in dimethylpyrazines can indicate the beginning of overroasting.

These results correspond with investigations of G. Ziegleder (*12*). In model roasting experiments using Ghana cocoa, he showed that formation of 2,3- and 2,5-dimethylpyrazine increases sharply at temperatures above 130 C (roasting time: 15 min) as demonstrated in Figure 4. The methylpyrazines, which generally occur in roasted foods (*4*), may be formed by condensation of α-aminoketones arising from the Strecker degradation of amino acids (*13,14*). Higher concentrations of dimethylpyrazines are formed at temperatures greater than 140 C (cf. Figure 4), and are correlated with a burnt taste. The burnt taste indicates overroasting. In contrast to methyl- and dimethylpyrazines, the increase in concentration of trimethylpyrazine appears to be constant over the temperature range studied. The concentration of tetramethylpyrazine increases during fermentation (*15*); it is possibly formed by reaction of acetoin and ammonia, which arise from bacterial activity during fermentation. The concentration of tetramethylpyrazine also increases at moderate drying temperatures (110 C), apparently because the precursors (e.g., diacetyl and acetoin) are already present. The concentration of tetramethylpyrazine decreases at higher roasting temperatures, possibly due to volatilization.

Ziegleder has also shown (*12*): fermented cocoa with a high concentration of tetramethylpyrazine has higher concentrations of the lower molecular weight methylpyrazines. This is presumed to be due to formation of aroma precursors during fermentation. Furthermore, model roasting experiments showed, that independent of absolute concentrations, the ratios between 2,5-dimethylpyrazine and trimethyl- or tetramethylpyrazine concentrations are related to the degree of roasting as determined by sensory evaluation (*12*).

Analysis of the different pyrazine derivatives can be done by either GLC or HPLC (*12,16,17*)

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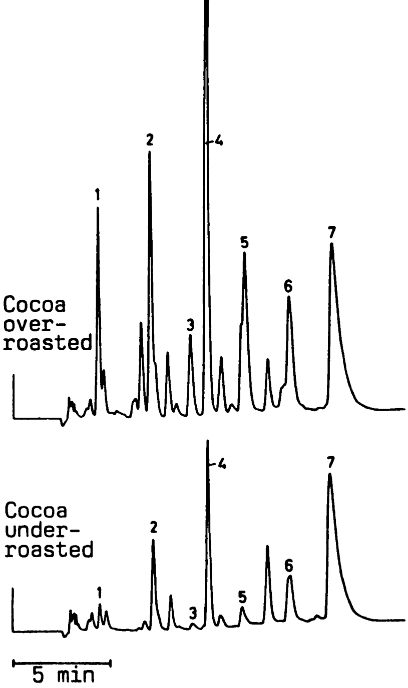

Figure 1. HPLC-chromatogram of the steam distillates of underroasted and overroasted cocoa (variety Elfenbein).
(Lichrosorb RP 18 (5 µm, Merck), gradient of methanol/water (10/90; 80/20 ; 0.09 M acetic acid, 0.005 M KH_2PO_4 (*10,11*); detection at 280 nm).
1) Dihydrohydroxymaltol, 2) Furfural, 3) 2-Methylpyrazine, 2-Acetylfuran, 4) 2-Acetylpyrrole, 5-Methylfurfural, 5) Dimethylpyrazines, 6) Trimethylpyrazine, 7) Tetramethylpyrazine. (Adapted from ref. *10*).

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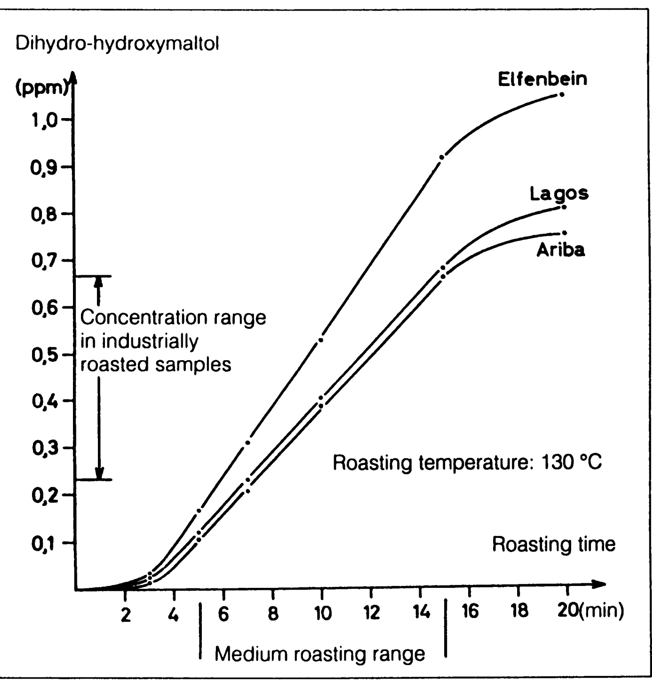

Figure 2. Increase of 2,3-dihydro-3-hydroxymaltol concentration in relation to the degree of roasting of cocoa varieties Elfenbein, Lagos and Ariba (increasing roasting time at constant roasting temperature; amounts in steam distillates in relation to total cocoa). (Adapted from ref. *10*).

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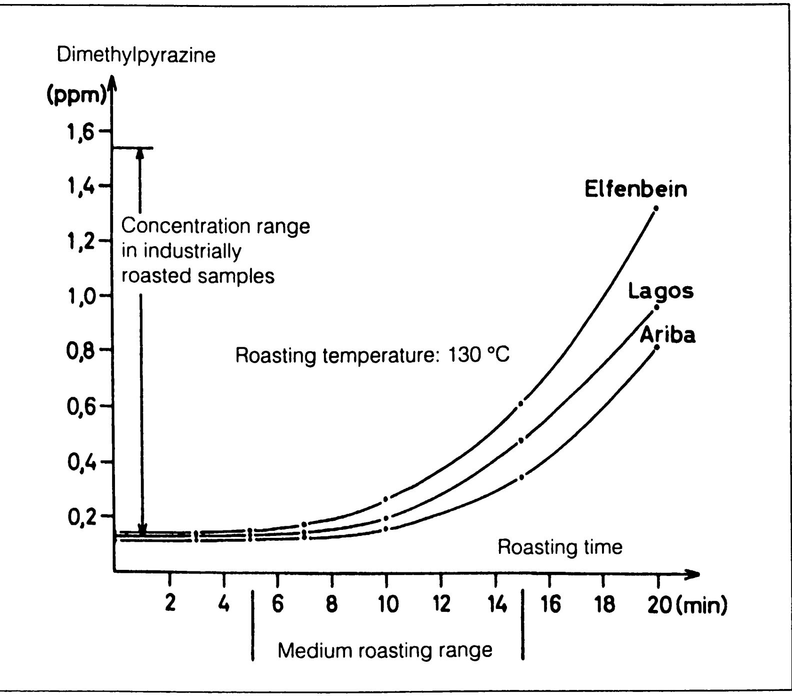

Figure 3. Increase of dimethylpyrazine concentration in relation to the degree of roasting of cocoa varieties Elfenbein, Lagos and Ariba (increasing roasting time at constant roasting temperature; amounts in steam distillates in relation to total cocoa). (Adapted from ref. *10*).

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch017

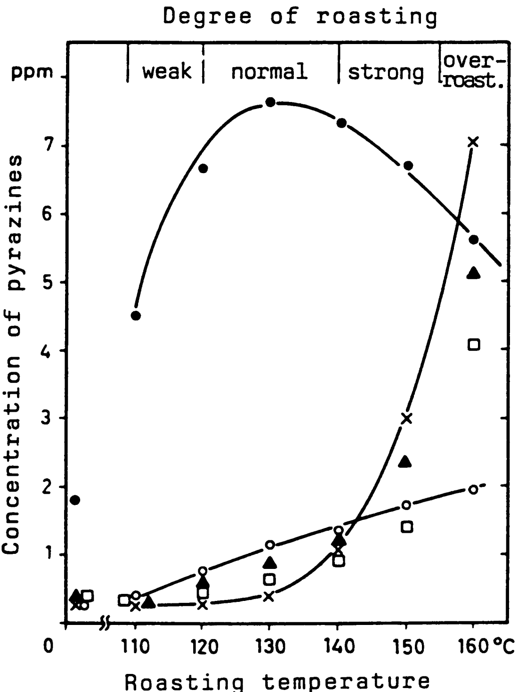

Figure 4. Increase of the concentrations of pyrazine derivatives in comminuted Ghana cocoa beans (∅ 1 mm) dependent on roasting temperature (roasting time: 15 min). □ Methyl-, ▲ 2,3-Dimethyl-, × 2,5-Dimethyl-, O Trimethyl-, ● Tetramethylpyrazine. (Reproduced with permission from ref.*12*. Copyright 1982 Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart).

Amadori Compounds as Aroma Precursors

As already mentioned, the precursors for cocoa aroma formation consist mainly of reducing sugars, amino acids, and peptides (*1,3*). In this connection, the question was raised as to what extent Amadori compounds play a role as aroma precursors. To investigate this question, we studied the formation of these compounds in comminuted cocoa beans under drying (98 C) and roasting (130 and 150 C) conditions (*18*).

The concentrations of Amadori compounds slowly increase during drying (see Figure 5). According to empirical results from the cocoa manufacturing industry, the yield of aroma compounds increases during roasting when cocoa beans have been pre-dried at temperatures around 100 C (*3,9,20*). Furthermore, at roasting temperatures there is an initial increase in the concentration of Amadori compounds, which is followed by a sharp decrease. From these findings it can be inferred that Amadori compounds are aroma precursors.

In order to clarify the role of Amadori compounds as aroma precursors, we performed model roasting experiments with fructose-alanine (Fru-Ala), which is one of the most important Amadori compounds in cocoa (*18*).

Figure 6 shows the decomposition of Fru-Ala at different roasting temperatures. It turned out that Amadori compounds contained in the cocoa matrix (cf. Figure 5) are much less stable than pure Amadori compounds.

Figure 7 shows a gas chromatogram of the aroma components formed when Fru-Ala was heated for 30 minutes at 160 C. All of the aroma compounds listed in Figure 7 are constituents of natural cocoa aroma (*19*). Pyrazines, pyridines, pyrroles, and furan derivatives are generally major components of roasted cocoa aroma (*21*).

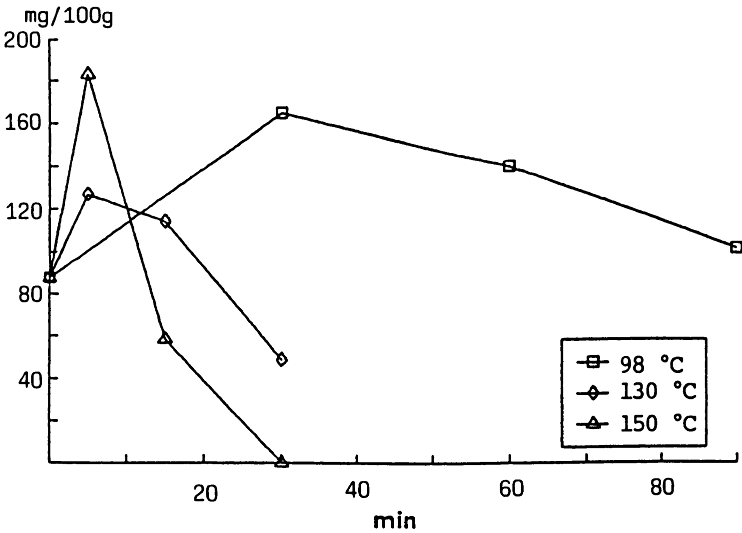

Figure 5. Changes in the concentrations of Amadori compounds during pre-drying, roasting and overroasting of cocoa beans. (Reproduced with permission from ref.*18*. Copyright 1991 Springer Verlag, Heidelberg).

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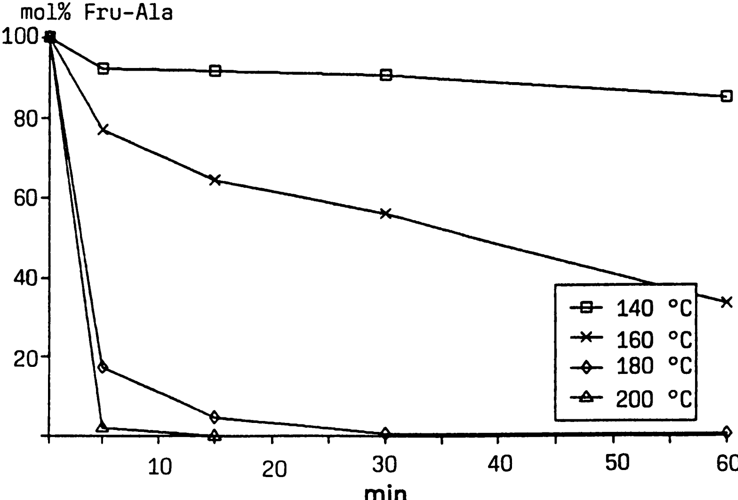

Figure 6. Decomposition of fructose-alanine (Fru-Ala) at different temperatures. (Reproduced with permission from ref.*19*. Copyright 1991 Springer Verlag, Heidelberg).

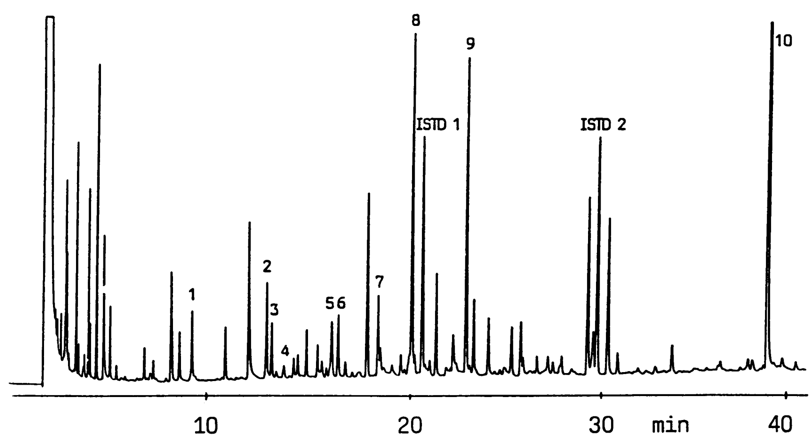

Figure 7. Capillary gas chromatogram of volatile aroma compounds formed by model roasting of fructose-alanine (30 min/160 C).
Column: 35 m, ID: 0.32 mm; Carbowax 20M-TPA; 50-200 C (3 C/min); 1.3 ml/min N_2; FID.
1 = 2-Methylpyridine, 2 = 2,5-Dimethylpyrazine, 3 = 2,6-Dimethylpyrazine, 4 = 2,3-Dimethylpyrazine, 5 = Trimethylpyrazine/2-Ethyl-3-methylpyrazine, 6 = 5-Ethyl-2-methylpyridine, 7 = Furfural, 8 = 2-Acetylfuran, 9 = 5-Methylfurfural, 10 = 2-Acetylpyrrole. (Reproduced with permission from ref.*19*. Copyrright 1991 Springer Verlag, Heidelberg).

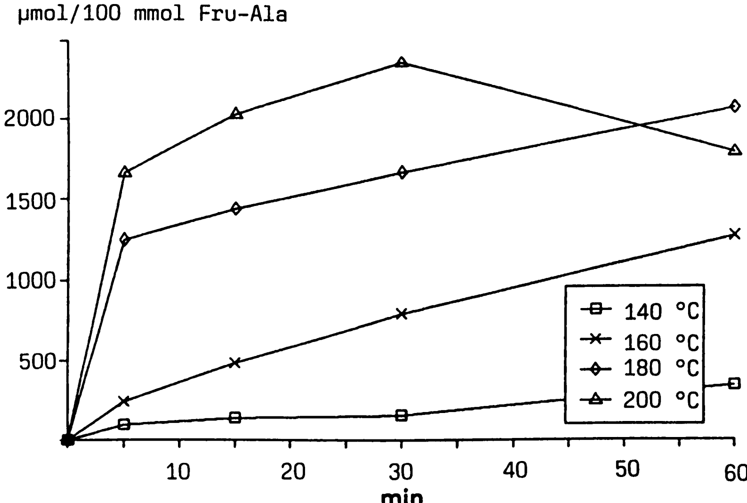

Figure 8. Increase of the sum of identified aroma components (cf. Figure 7) during heating of Fru-Ala at different temperatures. (Reproduced with permission from ref.*19*. Copyright 1991 Springer Verlag, Heidelberg).

Figure 8 shows the increase in total concentration of aroma compounds (the sum of all compounds listed in Figure 7) as a function of time at different temperatures. Figure 8 is the mirror image of Figure 6, and indicates Amadori compounds are real precursors of an important group of cocoa aroma components.

Results from these experiments were compared to those from the reaction of glucose and alanine (glucose and alanine are the reactants which go on to form Fru-Ala.). Furan derivatives were the main products of this reaction. Formation of N-heterocyclic products was delayed. Therefore, pre-drying of the fermented cocoa beans is an important method of generating Amadori compounds, which function as precursors to the desired aroma compounds.

Based on these results, it seems reasonable to divide cocoa roasting plants into pre-drying and roasting sections.

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Chapter 18

Effect of pH on the Volatile Compounds Formed in a Xylose–Lysine Model System

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Analysis of isolates of volatile components formed on heating aqueous 1m solutions of xylose and lysine (initial pH 4.9) either with control of the pH at 5 or without pH control (final pH 2.6), resulted in the identification of 54 and 28 compounds, respectively, from the 2 systems. 2-Furfural was the dominant volatile reaction product in both systems, and constituted 52.2% and 99.9% (m/m) of the volatiles, respectively, from the systems heated with and without pH control. While yields of furans were greater without pH control, more representatives were identified with pH control. Total yields and numbers of nitrogen-containing compounds were greater in the system with pH control, and monocyclic pyrroles, pyridines and 2,3-dihydro-1*H*-pyrrolizines were identified only in that system. This study is the first reporting 2,3-dihydro-1*H*-pyrrolizines in a model system containing lysine as the amino compound.

The Maillard reaction is responsible for the development of aroma and color in many foods on heating. In model systems, careful selection of the reaction precursors, coupled with manipulation of the reaction conditions, e.g. temperature and time of heating, water activity (a_w) and pH, allows some control over the final aroma and color of the system (*1,2*). Of all the variables which affect the reaction, temperature has the greatest influence on the extent of the reaction, an increase in temperature resulting in an increased reaction rate. The Maillard reaction occurs most rapidly at intermediate (0.5-0.8) a_w values (*3*), although the presence of components such as humectants influence the a_w value at which maximum browning occurs (*4*). The pH of the system influences the extent to which the Amadori

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Rearrangement Products (ARPs) degrade by 1,2-enolisation and 2,3-enolisation (which are favoured by lower and higher pH, respectively). The aroma formed in Maillard systems is influenced by temperature-time combinations (*1,2,5*), moisture content (*1,6*) and by pH (*1,7*), changes in each of these variables affecting the profile of reaction products formed.

The main objectives of our study were to investigate the low molecular weight compounds formed in a xylose-lysine model system, in order to gain a better understanding of the routes leading to the formation of both the volatile and non-volatile reaction products relevant to a wide range of foods. Lysine (in the form of lysine monohydrochloride) was chosen as the amino acid. Of all the amino acids commonly found in foods, lysine possesses the highest browning potential. Xylose, being a pentose, is more reactive towards amino acids than hexoses. It is also present in a number of foods in relatively small amounts, including cereals (*8*) and soy sauce (*9*). The study also monitored the effects of time of heating and of pH on the reaction products profile (*10*). This paper deals with the effect of pH on selected volatile reaction products. The effect of time on the profile of volatile reaction products (*10*) and the results of the study of the non-volatile reaction products have been reported (*10*; Ames, J.M.; Apriyantono, A.; Arnoldi, A. *Food Chem.*, in press).

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Experimental

Solutions of xylose (0.5 mol) and lysine monohydrochloride (0.5 mol) in degassed distilled water (500 mL) were refluxed for 1 h in a modified Likens and Nickerson apparatus, with diethyl ether (15 mL) as the extraction solvent. The initial pH was 4.9 and it was either uncontrolled during heating or was adjusted to and maintained at pH 5 throughout heating by the addition of 3M sodium hydroxide solution at approx. 10 min intervals. Each solvent extract was concentrated to a volume of 0.5-1.0 mL using a Vigreux column under reduced pressure. Final concentration (to a volume of 0.05 mL) was achieved using a gentle stream of nitrogen. Volatile isolates were analysed by gas chromatography (GC) and GC-mass spectrometry using a fused silica capillary column (25 m x 0.32 mm i.d.) coated with SE 52/54 (1 μm film thickness; Thames Chromatography, Maidenhead, Berks, UK) and using a fused silica column (50 m x 0.32 mm i.d.) coated with CP-WAX 52 CB (0.2 μm film thickness; Chrompak UK Ltd., London, UK). Individual components were quantified by the use of an internal standard (tetradecane) and linear retention indices (LRI) were obtained with reference to a standard solution of n-alkanes. Full details are given in Apriyantono (*10*).

Results and Discussion

The heated model systems, the solvent extracts and the concentrates all possessed an aroma described as caramel, buttery, slightly cereal and slightly sweet. Maintaining the pH of the model system at 5 during heating resulted in samples which also possessed roasted and nutty notes. The pH of the model system heated without pH control was 4.9 before heating and 2.6 after heating. Fifty-eight and 28

compounds, respectively, were identified from the isolates prepared from the model systems heated with and without pH control. Unidentified compounds represented 0.2% and a trace of the total reaction products from the systems heated with and without pH control, respectively. A complete list of the reaction products identified from the 2 systems has been reported (*10*). The yields and numbers of reaction products by chemical class in each isolate are given in Table I. The yields quoted in all the tables are the means obtained from 2 GC runs. The precision is $\pm 18\%$. Yields of less than 2 nmol/mol xylose are quoted as tr (trace) and "-" indicates that the component was not detected in that isolate. This chapter focuses on the nitrogen-containing compounds, especially the 2,3-dihydro-1*H*-pyrrolizines, and on the monocyclic and dimeric furans formed in the 2 systems.

The main effect of heating without pH control was an increase in the total yield of reaction products by a factor of about one hundred, compared to the system

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Table I. Yields and numbers of volatile compounds identified from a xylose-lysine model system heated either without pH control or with control of the pH at 5, by chemical class

	Without pH control		With pH control	
Chemical class	Yield (nmol/mol xylose)	No. of representatives	Yield (nmol/mol xylose)	No. of representatives
Aliphatic compounds	tr	2	27.6	5
Alicyclic compounds	42.8	2	tr	1
Benzene derivatives	-	0	98.6	1
Monocyclic furans	35.3×10^5	15	14.7×10^3	25
Dimeric furans	29.0 x 10	5	17.4 x 10	4
Benzofurans	tr	1	73.6	2
Monocyclic pyrroles	-	0	24.8	4
1-(2-Furfuryl)-pyrroles	10.5 x 10	2	30.5 x 10	2
Pyridines	-	0	66.2	2
Pyrazines	21.6	1	89.4×10^2	5
2,3-Dihydro-1*H*-pyrrolizines	-	0	12.2 x 10	3
Unknowns	52.2	3	52.2	12
Total	35.3×10^5	31	24.7×10^3	66

heated with pH control, due mainly to an increased yield of 2-furfural. This result was expected since degradation of the ARP via 1,2-enolisation to give 2-furfural as a major product (when the sugar is a pentose) is favoured by lower pH values. Monocyclic furans dominated in both model systems, and constituted 60% and nearly 100% of the total volatiles, respectively, on heating with and without pH control. 2-Furfural was the reaction product identified in the greatest amounts in both model systems and comprised 52.2% and more than 99.9% of the total volatiles, respectively, on heating with and without pH control. However, although total yields of furans were greater without pH control, more representatives were identified when the pH was controlled. In contrast, nitrogen-containing heterocyclic compounds, i.e. monocyclic pyrroles, 1-(2-furfuryl)pyrroles, pyridines, pyrazines and 2,3-dihydro-1*H*-pyrrolizines were present in larger amounts in the system heated with pH control. Monocyclic pyrroles, pyridines and 2,3-dihydro-1*H*-pyrrolizines were only identified in that system. It is well-known that the yields of many classes of volatile compounds from Maillard systems vary with pH (e.g. *11,12*), and it has been established that some nitrogen-containing compounds can only be detected when the pH of the system is above a certain value (e.g. *13,14*).

2,3-Dihydro-1*H*-pyrrolizines. The 2,3-dihydro-1*H*-pyrrolizines were the most interesting compounds identified in this study and 3 representatives, i.e., the 5-formyl, the 5-formyl-6-methyl and a 5-acetylmethyl were tentatively identified from the isolate obtained on heating with pH control (see Table II). (Tentative identifications in all the tables are preceded by "*"). Identifications were primarily based on matching of the experimental EI mass spectral data with published data and comparisons were made using the mass spectral data system. In addition, the CI mass spectral data support the identification of the 5-formyl-6-methyl and the 5-acetylmethyl derivatives. The experimental and literature mass spectral data are given in Table III, and the literature data were obtained from Tressl et al. (*15*). Based on the mass spectral data, the methyl substituent of the 5-acetylmethyl compound could be in either the 6 or the 7 position. Table III shows that there are only small differences between the 2 reference compounds and between the reference compounds and the components from the model system. All the mass spectra possess a base peak at m/z 148 (M-15, corresponding to the loss of a methyl group) and a molecular ion at m/z 163. Another significant ion occurs at m/z 120 (M-43), corresponding to the loss of a CH_3CO group and suggesting the presence of an acetyl group. The LRI obtained for the derivative isolated from the system heated with pH control using the CP-WAX 52 CB was 2144. This value is closer to the literature LRI (*15*) for the 5-acetyl-7-methyl compound (2126) than to that for the 5-acetyl-6-methyl derivative (2187). Mass spectra for the 2 other 2,3-dihydro-1*H*-pyrrolizines tentatively identified in this study were only obtained using the SE52/54 column, and no reference LRI values were available for this stationary phase.

2,3-Dihydro-1*H*-pyrrolizines were first reported from a glucose-proline system heated at 200°C and the 5-acetyl, 5-acetyl-6-methyl and 5-formyl-6-methyl derivatives were identified (*16*). Several years later, in 1981, 8 representatives, including the 3 compounds previously identified, were reported from aqueous

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Table II. Yields of nitrogen-containing compounds identified from a xylose-lysine model system heated either without pH control or with control of the pH at 5.0

	Yield (nmol/mol xylose)	
Compound	Without pH control	With pH control
2,3-Dihydro-1*H*-pyrrolizines		
*5-Formyl-2,3-dihydro-1*H*-pyrrolizine	-	3.0
*5-Formyl-6-methyl-2,3-dihydro-1*H*-pyrrolizine	-	6.8
*5-Acetyl-7-methyl-2,3-dihydro-1*H*-pyrrolizine	-	11.2 x 10
Total 2,3-dihydro-1*H*-pyrrolizines	-	12.2 x 10
Pyrazines		
Pyrazine	-	79.1 x 10^2
2-Methylpyrazine	-	10.2 x 10^2
2,5(or 2,6)-Dimethylpyrazine	-	tr
A propenylpyrazine	21.6	10.0
An ethyl-2-vinylpyrazine	-	tr
Total pyrazines	21.6	89.4 x 10^2
1-(2-Furfuryl)pyrroles		
*1-(2-Furfuryl)pyrrole	13.6	19.0
1-(2-Furfuryl)-2-pyrrolaldehyde	91.4	28.6 x 10
Total 1-(2-furfuryl)pyrroles	10.5 x 10	30.5 x 10
Monocyclic pyrroles		
2-Pyrrolaldehyde	-	14.8
2-Acetylpyrrole	-	tr
A pyrrole, M 137	-	10.0
A pyrrole, M 151	-	tr
Total monocyclic pyrroles	-	24.8
Pyridines		
*An ethylmethylpyridine	-	66.2
*An ethyldimethylpyridine	-	tr
Total pyridines	-	66.2
Total nitrogen-containing compounds	12.7 x 10	94.6 x 10^2

glucose (or maltose)-proline systems (pH 5-6) refluxed for 2h by Tressl and co-workers (*17*), and a total of 22 derivatives were subsequently identified by the same group on heating various monosaccharides with proline at 150°C for 1.5h (*17*). It has been claimed that 2,3-dihydro-1*H*-pyrrolizines are proline-specific Maillard

Table III. Mass spectral data for the 2,3-dihydro-1*H*-pyrrolizines tentatively identified from a xylose-lysine model system heated with control of the pH at 5

Compound	Experimental data	Literature data (13)
5-Formyl-2,3-dihydro-1*H*-pyrrolizine	*135*(100), 106(56), 134(48), 120(15)	*135*(100), 134(67), 106(62), 79(22), 77(14), 120(14), 52(9), 51(8), 85(8)
5-Formyl-6-methyl-2,3-dihydro-1*H*-pyrrolizine	*149*(100), 120(52), 148(48), 108(38), 93(18), 134(18), 150(11), 106(10), 65(9)	*149*(100), 148(78), 120(57), 134(33), 65(16), 39(13), 77(13) 93(12), 41(10), 106(8), 150(8)
A 5-acetylmethyl-2,3-dihydro-1*H*-pyrrolizine	148(100), *163*(87), 120(32), 149(16), 135(14), 65(14)	
5-Acetyl-6-methyl-2,3-dihydro-1*H*-pyrrolizine		148(100), *163*(38), 120(13), 149(10), 65(8), 77(7), 91(7), 43(6), 39(5), 93(5)
5-Acetyl-7-methyl-2,3-dihydro-1*H*-pyrrolizine		148(100), *163*(44), 120(16), 149(9), 43(7), 91(6), 77(6), 162(5), 93(5), 65(4), 164(3), 39(2), 41(2), 79(2), 92(2), 118(2)

reaction products (*15*), but it has been demonstrated that the 5-acetyl and 5-formyl derivatives can be formed on heating sucrose with the hydroxyamino acids, threonine or serine, respectively (*18*). The study reported here is the first reporting the formation of 2,3-dihydro-1*H*-pyrrolizines from a sugar-amino acid model system with an amino acid other than proline, threonine or serine as the amino compound.

Tressl et al. (*15,19*) proposed a mechanism to explain the formation of 2,3-dihydro-1*H*-pyrrolizines involving the reaction of proline with α-dicarbonyls and α-hydroxycarbonyls produced during sugar fragmentation. In order to explain the formation of these compounds from threonine or serine-containing model systems, Baltes and Bochmann (*18*) assumed a mechanism involving reaction of a 6 carbon sugar fragment with α-aminocarbonyl compounds derived from the pyrolytic degradation of hydroxy amino acids. In the current study, it is proposed that 2,3-dihydro-1*H*-pyrrolizines could form by a mechanism similar to that put forward by Tressl et al. (*15*). Such a mechanism would require the formation of a key

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intermediate such as pyrrolidine. It has been suggested that pyrrolidine is formed from the ARP of lysine at high temperatures by an intramolecular substitution reaction (*20,21*), and it is possible that small amounts of this compound are also formed upon refluxing aqueous model systems. Pyrrolidine has also been identified from the reaction of glyoxal or dihydroxyacetone (both of which are sugar degradation products) with lysine at room temperature (*22*). It is suggested that attack by dicarbonyl compounds at position 1 of pyrrolidine would produce reactive iminium ions, which would subsequently react with hydroxycarbonyl compounds to form intermediates, which would then be transformed by Michael addition or aldol condensation, followed by dehydration to 2,3-dihydro-1*H*-pyrrolizines (Figure 1).

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Other nitrogen-containing reaction products. Thirteen nitrogen-containing compounds, other than the 2,3-dihydro-1*H*-pyrrolizines already discussed, were identified and they are listed in Table II. All were identified in the system with pH control whereas only 3 representatives were present when the pH was uncontrolled. This is in agreement with previous studies which have shown that yields of various nitrogen-containing compounds increase with the pH of the model system (e.g., *13,14*). Of all the classes of nitrogen-containing compounds, pyrazines were present in the greatest amounts in the system with pH control and accounted for 36% of the total isolate. Pyrazine was the representative detected in the highest concentration and constituted 88% of the total pyrazines, with 2-methylpyrazine being the second most abundant derivative at 11.4%. Only one pyrazine, a propenyl derivative, was identified in the system without pH control, but the yield of pyrazines formed in Maillard model systems is known to increase with pH over the pH range 5-9 (*7,14*).

Two 1-(2-furfuryl)pyrroles were identified and both were formed in greater amounts with pH control. 1-(2-Furfuryl)pyrrole has previously been reported among the reaction products of a ribose-lysine system (*23*). Monocyclic pyrroles and pyridines were only identified in the isolate prepared from the system with pH control. The 2-pyrrolaldehyde and 2-acetylpyrrole are likely to form by exchange of the ring oxygen of the corresponding furan (both of which were also identified) for the nitrogen atom of ammonia. As far as the pyridines are concerned, the results obtained agree with those reported by Mottram and Leseigneur (*14*), who showed that an ethylmethylpyridine and a dimethylethylpyridine were formed in increasing amounts in a ribose-lysine system on increasing the pH over the range 4.5-6.5.

Monocyclic furans. The monocyclic furans can be categorized according to the number of carbon atoms they possess, relative to the number of carbon atoms in the reactant sugar. In this study, Type I, II and III furans are defined as those possessing less than, the same number as, and more than 5 carbon atoms, respectively. The monocyclic furans identified in each system, together with their yields, are listed in Table IV. (In Table IV, "NQ" indicates that the compound could not be quantified in the isolate concerned since it eluted very close to other reaction products.)

Only one Type I furan was identified, i.e., 2(5*H*)-furanone, and this compound may formed by a mechanism involving sugar fragmentation. Apart from 2-furfural,

Figure 1. Proposed mechanism of formation of 5-formyl-2,3-dihydro-1*H*-pyrrolizine from the lysine ARP. (Adapted with permission from *15,20,21.*)

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Table IV. Yields of monocyclic furans identified from a xylose-lysine model system heated either without pH control or with control of the pH at 5

	Yield (nmol/mol xylose)	
Type of Furan and Compound	Without pH control	With pH control
<u>Type I furans</u>		
2(5*H*)-furanone	12.1 x 10	50.0
<u>Type II furans</u>		
2-Methylfuran	tr	NQ
2-Furanmethanol	-	49.2 x 10
2-Furfural	35.3 x 10^5	12.9 x 10^3
4-Hydroxy-5-methyl-3(2*H*)-furanone	-	tr
Total Type II furans	35.3 x 10^5	13.4 x 10^3
<u>Type III furans</u>		
3(or 4)-Methyl-2-furanmethanol	-	9.0
5-Methyl-2-furanmethanol	tr	7.2
5-Methyl-2-furfural	14.1 x 10^2	8.2
A dimethyl-2-furfural	80.6	28.6 x 10
2-Acetylfuran	86.5 x 10	11.1 x 10
2-Propionylfuran	19.4	53.2
1-(2-Furyl)-2-propanone	11.1 x 10	14.6
1-(2-Furyl)-3-butanone	35.0 x 10	8.0
**cis*-4-(2-Furyl)-3-buten-2-one	37.2 x 10	8.0
trans-4-(2-Furyl)-3-buten-2-one	19.4 x 10	44.0
1-(2-Furyl)-1,2-propanedione	14.3 x 10	28.0
*1-(2-Furyl)-1,2-butanedione	5.2	14.0
2-Furfuryl acetate	-	80.0
A furan M 124	-	tr
A furan M 124	-	tr
A furan M 124	-	tr
A furan M 152	-	tr
A furan M 152	tr	20.0 x 10
A furan M 166	-	tr
A furan M 166	-	4.0
Total Type III furans	35.5 x 10^2	12.2 x 10^2
Total monocyclic furans	35.3 X 10^5	14.7 X 10^3

only one Type II furan was identified in the model system without pH control, i.e., 2-methylfuran. This compound, as well as 2-furanmethanol and 4-hydroxy-5-methyl-3(2*H*)-furanone, were also identified in the model system with pH control.

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Monocyclic furans possessing more than 5 carbon atoms (Type III furans) were present in greater numbers than the other categories of furans in both model systems. Twenty and 12 representatives, respectively, being identified with and without pH control. Nine compounds are reported only in the model system with pH control. Two representatives, i.e., 2-propionylfuran and a dimethyl-2-furfural were identified in greater amounts with pH control, but total yields of Type III furans were about 3 times higher without pH control. There are 3 ways in which furans with a greater number of carbon atoms than the reactant sugar could form in a simple sugar-amino acid model system. Sugar fragmentation products could react with each other, furans formed directly from the sugar could react with sugar fragmentation products (e.g., furan aldehydes and furan ketones can form from the reaction of 2-furfural with aliphatic carbonyl compounds) or Type III furans could result from the degradation of melanoidins (*24,25*). In theory, if furans of Type III are formed from reactions involving sugar fragmentation, the yields from the model system with pH control would be expected to be higher than those in the system heated without pH control. This is because reactions involving fragmentation of the sugar molecule are favoured by neutral or slightly alkaline pH (*26*). Since this was not the case, it seems possible that large amounts of Type III furans were indeed formed in the system heated with pH control, but that a proportion of them participated in further reactions with the production of non-volatile compounds, including some colored components. This is supported by the fact that a higher absorbance (at 420 nm) was obtained for the model system heated with pH control. Alternatively, additional pathways may operate for the formation of Type III furans. For instance, it is feasible that 2-furfural (which was formed in much greater amounts in the system without pH control) could act as a precursor for these compounds. Two of the Type III furans identified have been reported in model systems using 2-furfural rather than a sugar as an initial reactant. 2-Acetylfuran was identified in a 2-furfural-hydrogen sulphide-ammonia system (*27*) and this compound, together with 5-methyl-2-furfural, has also been reported in a heated mixture of 2-furfural, cysteine and methionine (*28*).

Dimeric furans. The dimeric furans identified are listed in Table V and total yields were greater in the isolate from the system without pH control. However, 2 compounds were identified in greater amounts from the system with pH control, and *trans*-2,2'-difurfurylethylene was only identified in that system. *cis*-2,2'-Difurylethylene was by far the most abundant representative in both isolates, with yields being about twice as great without pH control. It has been suggested that certain dimeric furans are formed by polymerization of furan precursors (*29,30*), and all the representatives identified in the current study, with the exception of 5-(2-furfuryl)-2-furfural, have been reported from model systems containing 2-furfural as an initial reactant (*27,28*). Therefore, it seems likely that the greater yields of dimeric furans in the system without pH control are due to the abundant production of 2-furfural.

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Table V. Yields of dimeric furans identified from a xylose-lysine model system heated either without pH control or with control of the pH at 5

	Yield (nmol/mol xylose)	
Compound	Without pH control	With pH control
2,2'-Bifuran	38.8	-
2,2-Difurylmethane	9.4	13.6
2,2'-Difurylethane	13.6	37.0
cis-2,2'-Difurylethylene	22.6 x 10	11.1 x 10
trans-2,2'-Difurylethylene	-	12.6
*5-(2-Furfuryl)-2-furfural	2.2	-
Total	29.0 x 10	17.4 x 10

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Chapter 19

Flavors from the Reaction of Lysine and Cysteine with Glucose in the Presence of Lipids

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The volatiles generated in model systems containing glucose, an amino acid (cysteine or lysine), water, and lipid (corn oil or extra virgin olive oil) were compared with systems containing methyl stearate or lacking any lipophilic phase. This research was done to help determine the effect of vegetable oils on the Maillard reaction. Differences were observed between the various model systems, due in part to lipophilicity and in part to the different features of the lipid. In particular minor components of the lipids were of greater importance than the percent acidic composition. The consequences on flavor are discussed in terms of aromagrams, which take into consideration the threshold values of components.

Flavor of processed foods depends in part on the aroma compounds present in the raw material and in part on how the food was prepared. Cooking is certainly one of the most important preparation. It catalyzes the reaction between amino acids and sugars (Maillard reaction), which produces many volatile heterocyclic compounds. The structures and concentrations of these compounds influence the aroma of a particular food. Fats are, also, present in many foods. In addition to triglycerides, fats contain minor components, which can in part be oxidized to aldehydes and ketones by a radical chain mechanism (1) during storage. These lipid degradation products (aldehyde and ketones) can influence the flavor of food by their presence and through interaction in the Maillard reaction.

Few studies have focused on the interaction of lipids in the Maillard Reaction, of these, many relate to the formation of meat flavor. Defatting meat before cooking strongly modifies the aroma (2), and phospholipids were shown to reduce the formation of heterocycles (3). Scattered information is available on how these interactions influence vegetable flavors. Defatting coconuts before roasting entirely modifies the pleasant aroma, which becomes similar to that of hazelnut (4). The addition of corn oil to zein and corn starch enhances the formation of pyrazines (5).

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Some of them have long alkyl chains and their mechanism of formation has been recently explained (6).

In order to get a better insight on the effect of vegetable oils on the Maillard reaction, we have studied models systems containing glucose, an amino acid (cysteine or lysine), water, and a lipid (corn oil, or extra virgin olive oil); and have compared them to systems containing methyl stearate or lacking any lipophilic phase.

Experimental.

Model systems; containing 6.9 g of glucose, the stoichiometric amount of amino acid (L-lysine or L-cysteine), 30 mL of water, and 50 g of lipid [extra virgin olive oil extra, refined corn oil (both purchased in a local market), or methyl stearate]; were prepared by heating for 3 h at 100°C with vigorous stirring. The mixtures were combined with 200 mL 0.1 M NaOH and 1 mg of quinoxaline (internal standard) and extracted for 8 h with dichloromethane in a Likens-Nickerson apparatus. After drying and careful concentration of the solvent to 1 mL, the mixtures were analyzed by GC-MS.

Model systems without lipid and extracts of the oils were prepared and analyzed in the same way.

GC-MS analyses were performed on a Finnigan TSQ70 instrument. The column was a 30 m x 0.25 mm Supelcowax-10. The temperature program was 70°C for 8 min, 5°C/min, 180°C isothermal. Ions were generated by EI at 150°C. The compound identification was done by comparison with authentic samples, whenever possible, and with NBS mass spectra library.

Results and Discussion

Before analyzing the model systems, the recovery of pyrazines and furans from the oils and methyl stearate was estimated to be on the order of 80 %. The different oils and methyl stearate gave very comparable results. Analysis of the corn oil extract revealed the presence of ketones (2-heptanone, cyclohexanone), alcohols (hex-2-en-1-ol) and aldehydes [(E)-2-decenal, (E,Z)-2,4-decadienal, (E,E)-2,4-decadienal]. Extra virgin olive oil was richer in volatile compounds than corn oil: many alcohols, ketones and aldehydes were detected, and are typical flavor compounds of this oil (7).

The pH of the solution was not controlled during heating: ending pHs were 3.4 and 3.45 for lysine and cysteine, respectively. Amounts of compound were expressed in μg per model system and were calculated based on the amount of the internal standard added. A correction factor of 1 was used for all the compounds.

Figure 1 shows a comparison of the volatiles formed, by the reaction of lysine and glucose in the presence of methyl stearate, corn oil and extra virgin olive oil. The volatile compounds identified from the two oils were, also, detected here, but were not included in the figures.

In the case of lysine, pyrazines are the most important Maillard compounds formed, some in concentrations of milligrams per model system. There are no relevant differences in the structure of the pyrazines produced under the different

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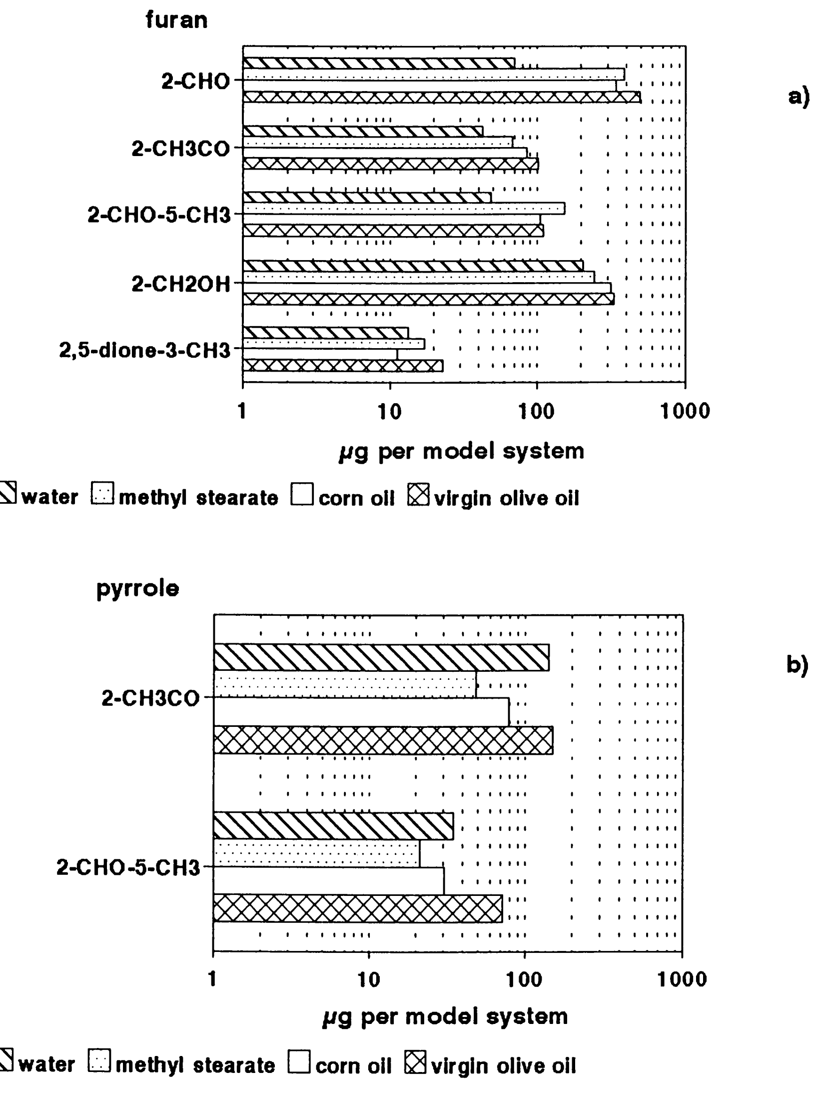

Figure 1. Comparison of the Maillard reaction compounds formed in lysine-glucose model systems heated for 3 h at 100 °C: a) furans, b) pyrroles, c) and d) pyrazines.

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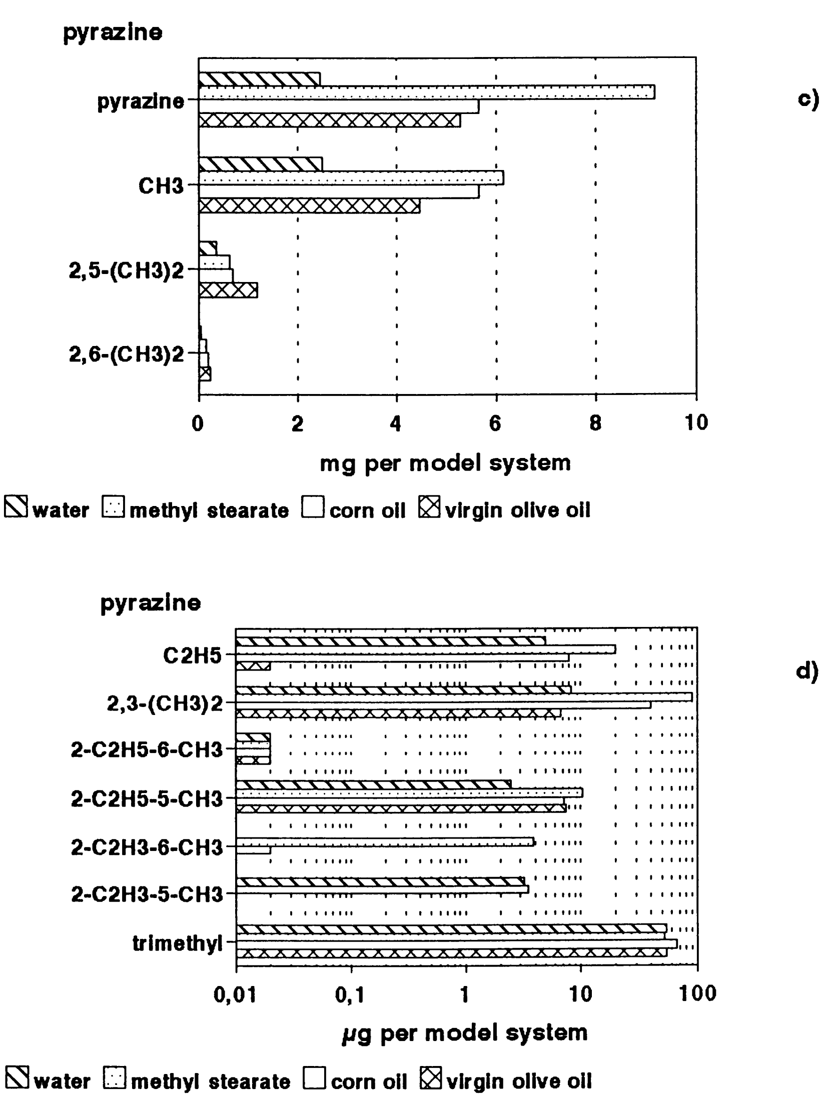

Figure 1. Continued.

conditions, and at 100 °C no pyrazines with long alkyl chains (6) were detected. Only five furans and two pyrroles were observed in these systems. A comparison of the volatiles formed in the absence of any oil, and in the presence of methyl stearate allows us to understand the effect of a lipophilic phase on the Maillard reaction products. In general methyl stearate enhances the formation of pyrazines, in particular the pyrazine and methylpyrazine. Fomation of furans, also, increases, while surprisingly pyrroles, which are considered to be formed from furans, decrease. A possible explanation of this fact is that methyl stearate, dissolving the furans, subtracts them from subsequent reactions. The addition of corn oil decreases this effect, in particular some pyrazines like pyrazine and methyl pyrazine are produced in lower amounts. With furans the situation is less clear, some decrease, and some increase. Pyrroles increase. The decrease of pyrazine concentration is even more clear in the case of extra virgin olive oil, while furan concentration increases both in respect to water and methyl stearate. On the whole, as methyl stearate enhances the formation of both furans and pyrazines, it seems that a lipophilic phase increases the rate of the Maillard reaction: the increased formation of colored compounds, which are soluble in water and not in the lipophilic phase, supported this hypothesis. Corn oil and extra virgin olive oil decrease the rate of formation of pyrazines.

In the case of the cysteine model systems (Figure 2), furans and sulfur heterocycles are the most abundant compounds formed. The latter are typical of this model system. The structures were assigned by comparison with literature spectra [for 1,2,4-trithiolanes and 1,3,5-trithianes see reference (8), for substituted 1,3,5-dithiazines see reference (9)]. 2,4,6-Trimethyl- and 4,6-dimethyl-1,3,5-dithiazin are the most relevant. The addition of methyl stearate enhances the formation of furans, 1,2,4-trithiolan and 1,3,5-trithian. In this system 4-methyl-2-thiazoline (10) was, also, detected. On the contrary pyrroles and pyrazine decrease. Corn oil especially enhances sulfur compound formation. Extra virgin olive oil suppresses formation of furans, increases formation of 2,5-dimethyl and 2,6-dimethylpyrazines and strongly suppresses formation of sulfur compounds, many of which could not be detected. Also in this case a lipophilic phase increases the Maillard reaction rate, the effect is less pronounced in oils. With the two amino acids studied in this work, corn oil resembles the behavior of methyl stearate more than extra virgin olive oil. Taking into consideration the lower degree of unsaturation of olive oil, we can explain this effect by the higher amount of minor components in extra virgin olive oil extra. Olive oil can, therefore, be useful in decreasing the formation of compounds with undesired roasted notes like pyrazines.

Aromagrams. A general problem in the study of aroma is that it is relatively easy to obtain the chromatogram of complex mixtures, while it is more difficult to relate chemical to the sensory differences. Some years ago Teranishi and Buttery (11) proposed aromagrams as a possible solution to this problem. In this technique quantitative results, from analytical chemistry, are "corrected" by taking into account the compound aroma threshold values. This methodology has two major limitations: the limited number of threshold values available and neglect of possible synergism between two or more compounds. The most important advantage of this technique is that very minor compounds, with low odor thresholds gain the correct consideration.

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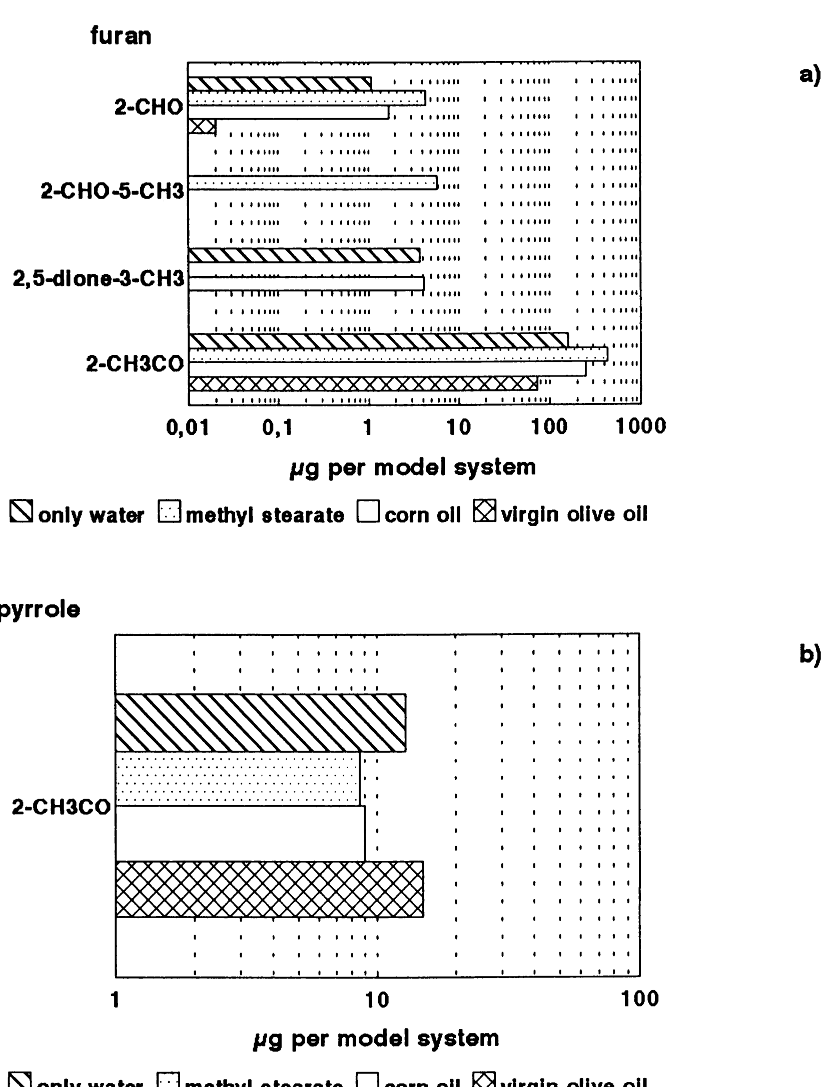

Figure 2. Comparison of the Maillard reaction compounds formed in model systems cysteine–glucose heated for 3 h at 100 °C: a)furans and b) pyrroles. *Continued on next page.*

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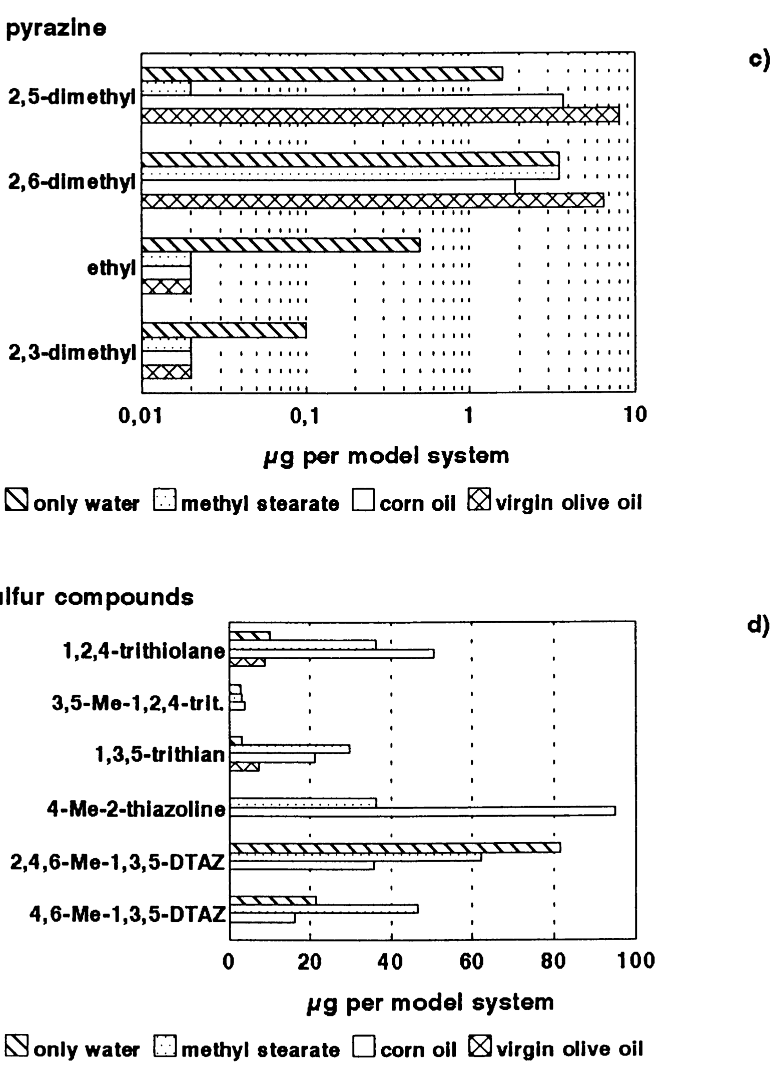

Figure 2. *Continued.* Comparison of the Maillard reaction compounds formed in model systems cysteine–glucose heated for 3 h at 100 °C: c)pyrazines and d) sulfur compounds.

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To our knowledge, aromagrams have never before been applied to Maillard model systems to rationalize the flavor obtained under different conditions. The application of this technique to our model systems has some additional limitations. The threshold values reported in literature are expressed as the concentration in the liquid phase, while the concentration in the vapor phase, which would be more relevant, are only seldom obtained. The amounts of the compounds formed were calculated based on the area of the internal standard using a response factor of 1 for all compounds. The model systems in water and those with lipids had a different volume. We decided to take into account only the volume of the water solution, and kept this constant in all the systems. The threshold values of some of the compounds were not known, while in some cases different Authors give different values for the same compound: in the latter case the average was used. We decided to use odor threshold values in water for Maillard compounds (12), and values in oil for the compounds coming from autoxidation of lipids (13) (in this case the oil volume was used to calculate the concentration). The problem must be considered carefully because the volatility of flavors is much lower in oil than in water and the threshold values are as a consequence rather dissimilar (14).

The application of this methodology greatly changes the results. In the case of lysine (Figure 3, only compounds above the threshold values are reported) the most abundant compounds, pyrazine and methylpyrazine, are of little importance in determining the flavor, while 2,5-dimethylpyrazine and 2,6-methylpyrazine play the most important role. Unsaturated aldehydes are very important but only in the case of virgin olive oil extra. These aldehydes are present in low concentration in fresh oil (7) even before autoxidation, because they are present in the olives. On the contrary, they are under the threshold value in corn oil. These results are confirmed by the aroma, which has a fat note especially in the case of olive oil. In the system without lipids only a few Maillard compounds are above their threshold value.

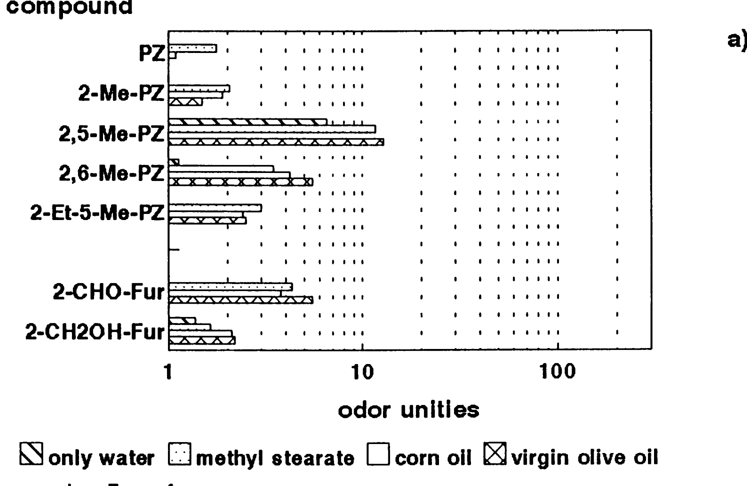

Figure 3. Comparison of aromagrams for the lysine-glucose model systems: a) Maillard compounds, b) aldehydes. *Continued on next page.*

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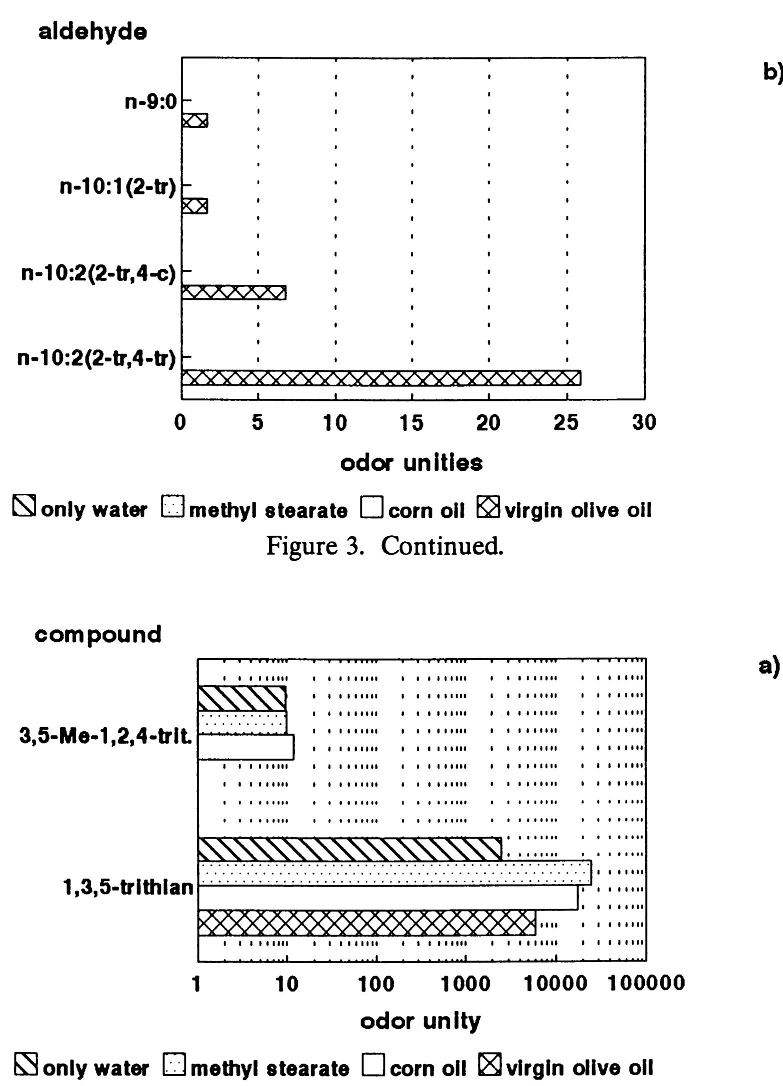

Figure 3. Continued.

Figure 4. Comparison of aromagrams of the cysteine-glucose model systems: a) Maillard compounds, b) aldehydes.

In the case of cysteine the analysis is limited by the fact that only a few threshold values for sulphur derivatives are known. 1,3,5-Trithiane has a very low value and completely conditions the aroma. Pyrazines and furans are completely irrelevant; they are below their thresholds and the models lack roasted aroma.

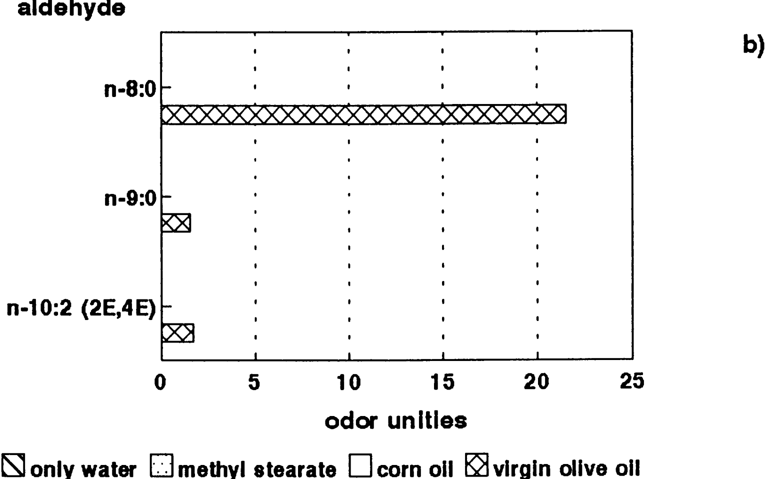

Figure 4. Continued.

In conclusion, aromagrams seem a powerful tool for the comparison of different systems. The determination of the threshold values of more Maillard reaction products would be very useful for a more complete picture of the effects of the various components.

Acknowledgment

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Chapter 20

Formation of Maillard Products in the Proline–Glucose Model System

High-Temperature Short-Time Kinetics

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Equimolar amounts of a proline/glucose aqueous solution were reacted in a continuous flow through reactor under controlled time and temperature conditions in order to derive activation energies and rate constants for the reactants and products. Extent of reaction was monitored by increase in optical density and decrease in glucose and proline concentration. The products were extracted with ether and the organic compounds analyzed by GC/MS. The major compound, 5-acetyl-2,3-dihydro-1H-pyrrolizine, was found to increase according to pseudo zero order kinetics. A number of other organic compounds were also identified and found to correlate with reaction conditions.

Maillard reactions are known to be responsible for a wide variety of desirable food aromas such as those of bread, cereal, coffee, chocolate and meat. Numerous works have been published covering the chemistry and aromas of the products (1-3).

One mixture known to produce particularly interesting and useful aromas is the proline/carbohydrate system. Kobayasi and Fujimaki (4) heated proline with glucose and developed a cracker aroma. Hunter et al. (5) pursued this line of research and proposed that 2-acetyl-1,4,5,6-tetrahydro pyridine was responsible for the desirable fresh-bread odor of the reaction mixture. The structure was confirmed by synthesis by Buchi and Wuest (6) two years later.

Subsequently Shigematsu et al. (7) pursued an indepth analysis of the proline/glucose model system. They heated the reactants in a dry state for 6 min at 200°C and identified eleven compounds including heterocyclic dihydro-1H-pyrrolizines and tetrahydroindolizin-8-ones. Aroma descriptors of these compounds were provided.

In the 1980's Tressl et al. studied the reaction of aqueous solutions of proline and monosaccharides which were heated in an

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autoclave for 1.5 hours at 150°C. Among the classes of compounds identified were dihydro 1-(H) pyrrolizines (8), 2-(1-pyrrolidinyl)-2-cyclo-pentenones, and cyclopent (b) azepin-8(1H)-ones (9), pyrrolidines, and piperidines (10), furylpyrrolidines and -piperidines (11) and 7H-cyclopenta(b)pyridin-7-ones (12). In addition to the above, Tressl and Helak (13) also characterized the tricyclic compound maltoxazine in the glucose/proline reaction system.

Data on the kinetics of the Maillard reaction are not extensive. It is well known that cooking food under different conditions can result in a variety of aromas. Lane and Nursten (14) examined more than 400 model amino acid/sugar systems and demonstrated that different flavors can be generated within the same reaction system by varying time and temperature.

Baltes et al. investigated heating fructose or glucose with serine or phenylalanine at different temperatures. Buffered solutions of the mixtures were heated at 100°C and in an autoclave at 120°, 150° and 180°C (15). Roasting conditions were simulated by heating the sugar/amino acid mixtures on an inert material at 220°C in the presence of 10% buffer. The study demonstrated that many compounds produced upon autoclaving and roasting simulation were identical. The relative quantities of the various classes of product were seen to vary with temperature. They concluded that "reaction temperature is the most decisive factor for formation of thermal aroma."

Reineccius (16) related these differences to reaction kinetics and specifically the activation energy for a specific reaction in a series of reactions. The activation energy determines how temperature affects the rate of formation of a product. Reactions with low activation energies are generally favored at lower temperatures, while those with higher activation energies are favored at higher temperatures. Leahy and Reineccius in two earlier studies examined the kinetics of formation of some alkyl pyrazines. They examined the effect of pH and water activity (17) and the effect of the type of amino acid and type of sugar on the kinetics of alkyl pyrazine formation (18). A high correlation was found between the formation of pyrazines and reaction time indicating pseudo zero order kinetics. Activation energies for the formation of various pyrazines were found to range from 27 to 45 kcal per mole (18).

These results were consistent with the earlier work of Warbier et al (19) and Labuza et al (20) who found that formation of browning products in the Maillard reaction generally followed pseudo zero order kinetics when reactant concentrations were in excess. The loss of reactants (20) and formation of Amadori compounds (21) typically exhibit first order kinetics.

The activation energies for formation of pigment in glycine/glucose and glucose/aspartame systems were determined to be 15 kcal/mole and 22 kcal/mole, respectively (22). Activation energies for the reaction (lysine and glucose) were measured at 25 kcal/mole for both reactants (23).

Leahy and Reineccius believe that the higher activation energy for pyrazine formation suggests a different rate controlling step (18).

It is the purpose of this study to investigate the kinetics of

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the proline/glucose reaction under carefully controlled high temperature short time conditions. The conditions studied range from 160°C to 220°C and from 0.25 min to 5 min and simulate those that occur in many food processes including steam puffing of grains, popping of popcorn, extrusion of cereals, roasting of coffee and toasting in a toaster.

EXPERIMENTAL SECTION

Reaction Conditions.

For the thermal reactions, 2.3 gm proline and 3.6 gm glucose were combined in 100 mL of deionized water (pH= 5.7). This system was pumped through a 1.0 ml sample loop made of 1/16 in stainless steel tubing which was immersed in a silicone oil bath. Pressure was maintained in this system by using a high pressure HPLC valve at the exit of the system. The silicone oil bath was heated by an external heater and controlled to +/- 0.5°C. After passing through the heated loop the product was immediately cooled by immersing the connecting tubing in a cooled water bath. The pump used was a Waters HPLC pump (Model M 6000A) which permitted various flow rates ranging from 0.1 to 9.9 mL/min and thus residence or reaction times ranging from 10 min to about 0.1 min. Connecting tubing was 1/16 in 316 stainless steel tubing (0.031 in i.d.). Two sets of experiments were conducted. In the first, the oil bath was held at 200°C and flow rates were varied to give reaction times ranging from 0.25 min to 5 min. In the second set, the reaction time was adjusted to 1.0 min and the oil bath temperature was varied from 160°C to 220°C.

Analysis.

Eight mL of aqueous reaction product was transfered to a 10 mL Mixxor. One gram sodium chloride was added and the system was extracted with 0.9 gm diethyl ether (containing 1 mg ethyl nonanoate per 10 mL diethyl ether as internal standard). This procedure has been previously described by Parliment (24).

Etheral extracts were analyzed via GC/MS. A Varian Model 3700 gas chromatograph was used with a 0.32 mm i.d. x 15 m fused silica column coated with a 1 micron film of DB-5. The following oven conditions were employed: 5 min at 60°C then 5°C/min to 230°C and a final hold of 10 min. The column effluent was passed through an open split interface into a Finnigan Model 705 Ion Trap Mass spectrometer. Identifications were achieved by comparison of the generated spectra to those of the NBS Libarary Compilation or to published spectra. Relative concentrations of the products were determined using the Ion Trap quantitation program.

Optical densities (OD) were measured using a Beckman DU Model 70 scanning spectrophotometer. Measurements were made in the UV and visible range by scanning between 200 and 710 nm at a rate of 500 nm/min. The system was calibrated versus air and then standardized using the starting glucose/ proline solution. The optical density was determined at the lamda max of the major peak. The samples were diluted as necessary to give an OD between about 0.2 and 2.0. The OD, lamda max. and the degree of dilution were recorded.

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch020

Glucose was analyzed by ion exchange/high pressure liquid chromatography as per AOAC Method 979.23. Proline was analyzed by derivatization with phenylisothiocyanate folowed by reverse phase high pressure liquid chromatography as per Waters PICO-TAG (TM) method (25).

Several experimental conditions were repeated on separate days with reasonable reproducibilty. Inconsistent data was rejected.

Organoleptic Evaluation.

The odor qualities of two typical samples generated in this study (e.g. 180°C/ 1min and 200°C/ 1 min) are described separately (Gas Chromatography Olfactometry of Glucose - Proline Maillard Reaction Products by Roberts, D.D., and Acree, T.E., in this book).

DISCUSSION OF RESULTS

The total ion mass chromatogram for the proline/glucose system after 0.25 min and 2.0 min reaction times are given in Figure 1. After 0.25 min the internal standard, ethyl nonanoate, was the major peak and several products of the reaction at low concentration can be identified. However, after 2 min 5-acetyl-2,3-dihydro-1H-pyrrolizine (5-ADP) has become the primary component. Two other compounds, 2-acetyl tetrahydropyridine (2-AP) and maltoxazine (MO), were identified and were subsequently monitored as part of the kinetic study. The structures of these compounds are given below:

2-Acetyltetrahydro-pyridine (2-AP)

5-Acetyl-2,3-dihydro-1(H)-pyrrolizine (5-ADP)

Maltoxazine (MO)

The kinetic data for compound formation at 200°C is given in Table I as GC/MS counts relative to the internal standard, ethyl nonanoate. The data is plotted versus reaction time in Figures 2 and 3 and demonstrates that product formation is pseudo zero order which is consistent with the literature previously discussed (17-19). The rate constants (k) were generated from the slope of the regression equations obtained using the Lotus Freelance program.

The lower r^2 for MO and 2-AP compared to 5-ADP is probably due to errors in measurement of these components in the range of lowest detector sensitivity.

Single point rate constants for product formation were determined from the relative GC/MS counts at 1 min over a range of temperatures. These are given in Table II.

It is evident that the rate of formation of 5-ADP increases much more rapidly with temperature compared to 2-AP and MO. Figure 4 is the Arrhenius plot for the log of rate 5-ADP formation versus 1/T Kelvin. The activation energy for formation of 5-ADP was calculated

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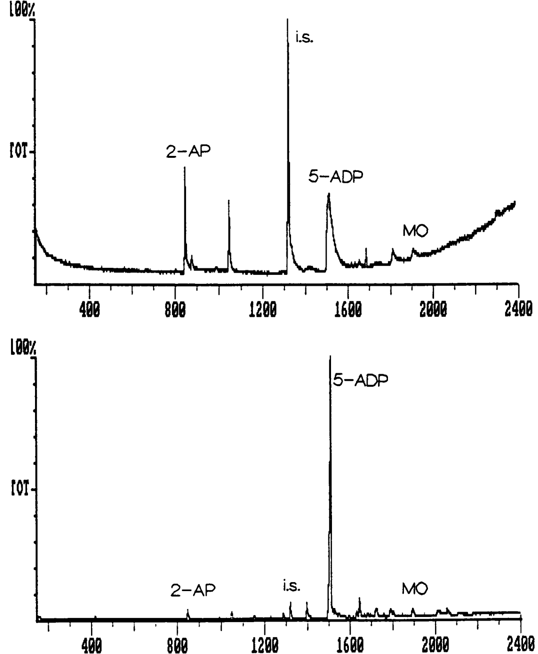

Figure 1. Total ion chromatogram of reaction at 200°C. Reaction times 0.25 min (upper curve) and 2.0 min (lower curve).

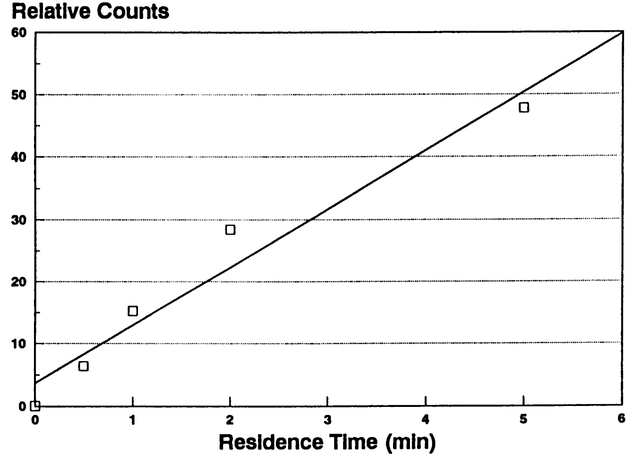

Figure 2. Formation of 5-acetyl-2,3-dihydro-1H-pyrrolizine versus residence time at 200°C.

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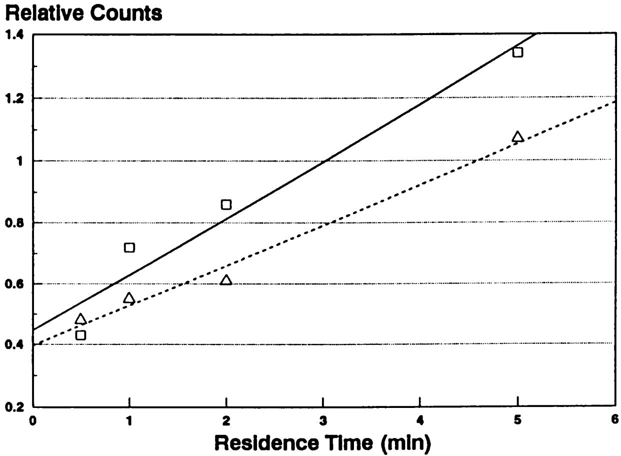

Figure 3. Formation of maltoxazine (upper curve) and 2-acetyl tetrahydropyridine (lower curve) versus residence time at 200°C.

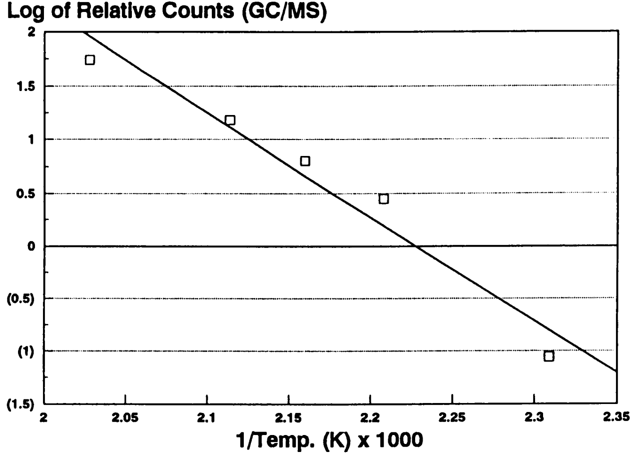

Figure 4. Arrhenius Plot: Log of rate of formation of 5-acetyl-2,3-dihydro-1H-pyrrolizine versus 1/Temperature.

from the slope generated by regression analysis as 45.0 kcal/mole (r^2= 0.955). Again because of the relatively low levels of 2-AP and MO generated within the temperature range studied, the activation energies determined from the Arrhenius plots appeared to be less reliable. The elimination of the data for 160°C (because of the low GC/MS counts) permited a rough estimate of the activation energy for these two compounds in the range of 6-15 kcal/mole.

The relatively low activation energies for the formation of MO and 2-AP suggests that they develop under mild conditions. This is supported by the literature. Tressl identified MO in malted barley which is typically dried at low temperatures and demonstated that MO could be formed in proline/glucose and proline/maltose model systems at boiling temperatures (12). On the other hand, 5-ADP has been identified as a major component in higher temperature reaction systems by Shigemasu et al (7) and Tressl et al (8).

Table III gives the results of analysis for glucose and proline remaining in the solution after the reaction at 200°C.

The linear plot obtained by plotting the log of the moles of glucose and proline remaining versus residence time (see Figure 5) indicated that the loss of reactants follow first order kinetics. These results are again consistent with the results of Warbier et al. (19). The rate constants were calculated by multiplying the slope of the line determined from regression analysis by -2.303. The results indicated that the rate of glucose loss (0.6328 min^{-1}) was almost three times that of the rate of proline loss (0.2269 min^{-1}). Warbier et al. also found a greater amount of glucose utilized in comparison to lysine in their study of an intermediate moisture system stored at 45°C. The ratio of rate of glucose loss to that of lysine typically varied between 1.3 and 1.8. They suggested that this may be due to regeneration of the amine in the initial reaction steps which can react with additional glucose. The greater loss ratio in our study may be the result of direct thermal breakdown of the glucose (caramelization) at higher temperatures.

Table IV presents the moles of glucose and proline remaining after reaction at various temperatures and the k (rate constants) calculated by solving the first order equation

$$k = 2.303/t\ (\log C_o/C_t)$$

where C_o= initial concentration
C_t= concentration after time t

The activation energies for the reaction of glucose and proline were determined from the slopes obtained by regression analysis of the Arrhenius plot data (log k versus 1/T Kelvin). They were 14.7 kcal/mole (r^2= 0.99) and 20.7 kcal/mole (r^2= 0.96) respectively.

Table V is a Summary for the optical density (OD) data at 300-304 nm for the glucose/proline reaction versus residence times at 170°, 185°and 200°C.

The plot of OD versus residence time confirmed pseudo zero order kinetics for formation of browning products as described earlier (19,20). The reaction rates at 170° C, 185° C, and 200° C as determined by measuring the slope of each of the OD plots were 0.143, 0.391 and 0.993 respectively. The Arrhenius plot based on

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Table I. Kinetic Data for Compounds Formed from Glucose/Proline at 200°C

Residence Time	Relative Counts		
	2-AP	5-ADP	MO
0.5 Min.	0.48	6.41	0.43
1.0 Min.	0.55	15.25	0.72
2.0 Min.	0.61	28.45	0.86
5.0 Min.	1.07	47.79	1.34
k (rel cts/min)	0.17	9.26	0.23
r^2	0.83	0.95	0.85

Table II. Rate Constants for Products (Rel. GC/MS Counts @ 1 min) versus Temperature

Temp. (°C)	k (2-AP)	k (5-AP)	k (MO)
160	0.08	0.09	0.05
180	0.49	2.81	0.23
190	0.57	6.36	0.69
200	0.55	15.25	0.72
220	0.91	55.29	1.05

Table III. Glucose and Proline Remaining after Reaction at 200°C

Time (min)	Moles Glucose Remaining	Moles Proline Remaining
0.0	0.0200	0.0200
0.5	0.0127	0.0180
1.0	0.0086	0.0158
2.0	0.0045	0.0134
5.0	0.0009	0.0069
rate of loss (min^{-1})	0.6328 (r^2=0.97)	0.2269 (r^2=0.96)

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Table IV. Moles Glucose and Proline Remaining after 1 Min Reaction at Various Temperatures and Calculated Rate Constants

	moles Reactant Remaining		Rate Constant, k	
Temp. (C)	Glucose	Proline	Glucose	Proline
control	0.0200	0.0200		
160	0.0176	0.0193	0.1277	0.0328
180	0.0145	0.0185	0.3181	0.0766
190	0.0125	0.0182	0.4737	0.0987
200	0.0086	0.0158	0.8484	0.2383
220	0.0013	0.0079	2.721	0.8267

Table V. Optical Density (300-304 nm) versus Residence Time for Glucose/Proline (Corrected to 1/100 dilution)

	OD		
Residence Time	(170°C)	(185°C)	(200°C)
0.5 Min.	0.024	0.135	0.548
1.0 Min.	0.091	0.413	1.51
2.0 Min.	0.252	1.03	2.95
5.0 Min.	0.213	2.18	5.24
10.0 Min.	1.476	3.81	
k (OD/min)	0.1428	0.3809	0.9934
r^2	0.87	0.99	0.96

these experimental k values indicated that the activation energy for the formation of browning products is 26.9 kcal/mole (r^2=0.99).

CONCLUSIONS

Most of the kinetic data in the literature for Maillard reactions has been obtained, for expedience, at relatively low temperature long time conditions. Yet many food processes (e.g coffee roasting, cereal puffing, and extrusion) in which desirable aromas are generated, occur at relatively high temperatures but short time conditions. This first study using a continuous flow reactor demonstrates that the kinetics of these types of processes can readily be determined in model systems. For the glucose/proline system we observed that one heterocyclic compound: 5-acetyl-2,3-dihydro-1H-pyrrolizine forms almost exclusively. The high energy of activation determined experimentially for the formation of this compound agrees with the preferential formation of this compound at higher temperatures. This approach should permit the study of the chemistry of the early stage of the Maillard reaction in other systems.

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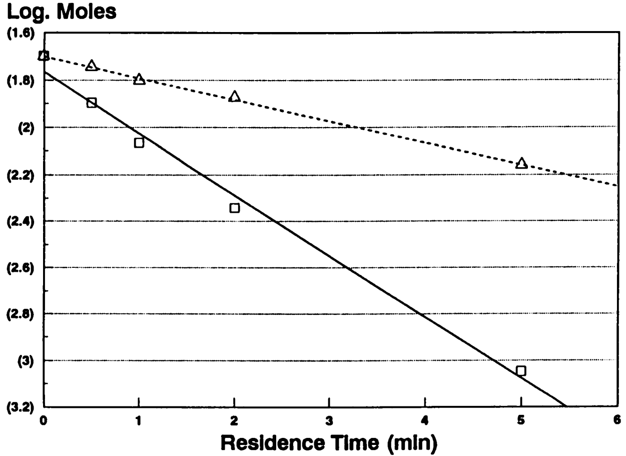

Figure 5. Log of moles of proline (upper curve) and glucose (lower curve) remaining versus residence time at 200°C.

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Chapter 21

Pyridoimidazoles, Histidine-Specific Reaction Products

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By reaction of histidine with glucose under the conditions of roasting (at 220°C) and autoclaving (at 120°C, 150°C and 180°C), 2-acetyl- and 2-propionyl-pyrido[3,4-*d*]imidazole were identified, along with the corresponding tetrahydropyrido derivatives. 2-Acetyl-pyrido[3,4-*d*]-imidazole was also formed by heating glucose with tuna fish. Glucose additionally reacts with histidines containing methyl substituents at the 1-, 2- or 3-position to form the corresponding methylated pyrido-imidazoles.

We recently observed the formation of 2-acetyl-pyrido[3,4-*d*]imidazole **III** by reaction of histidine with glucose under conditions of roasting or autoclaving foods. (*1*) The corresponding tetrahydro derivative **II** was also identified by GC/MS. The structure of **III** was elucidated by means of IR, MS and ^{1}H-NMR spectra. This compound class represents a new heterocyclic system which, to our knowledge, was not previously reported until now.

Experimental Procedure

Materials. Reagent grade L-histidine, D-glucose, and pyruvic aldehyde were obtained from commercial sources. Pyruvic aldehyde, diethyl ether, and dichloromethane were freshly distilled before use.

Model System Reactions. The following reaction conditions were utilized:

1. Reaction of D-glucose (0.04 mol) with histidine (0.04 mol) in 60 mL of 1.0 M phosphate buffer, pH 5.8.

2. Reaction of pyruvic aldehyde (4 mL) with histamine dihydrochloride (0.02 mol) in 60 mL of 1.0 M phosphate buffer, pH 5.8.

Reaction mixtures were individually heated and stirred at 120°, 150°, or 180°C for 1 h in a laboratory autoclave fitted with a Teflon insert. After cooling to room temperature, the mixtures were extracted with 5 x 40 mL of diethyl ether. The combined ether fractions were treated with aqueous sodium bicarbonate, and the residual water in the ether layer was frozen-out at -20°C. After filtration through a plug of cotton, the ether extracts were carefully concentrated to 2 mL using a Vigreux column.

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3. Reaction of tuna muscle with D-glucose. Fresh tuna muscle (18 g) was ground and mixed with 1 g of D-glucose. The mixture was heated at 150°C in 60 mL of 1.0 M phosphate buffer, extracted, and concentrated as described above. In order to remove residual fat, the concentrated ether extract (2 mL) was purified using gel permeation chromatography on a 450 x 25 mm SR 45 column, filled with 200-400 mesh SX 3 Bio Beads (Biorad, 2000 D), and equilibrated with dichloromethane. The fat was eluted with the first 100 mL of dichloromethane, and the components of interest were eluted in the subsequent 100 mL dichloromethane fraction, which was concentrated to a 2-mL volume using a Vigreux column. The composition of this fraction was investigated by GC/MS.

4. Roasting of D-glucose (0.004 mol) with histidine (0.004 mol). The reactants were ground in a mortar with 10% of 1.0 M phosphate buffer and 10% sand, and the resulting mixture was layered in a reaction tube with glass wool. After the tube was wrapped with heating tape and a contact thermometer was attached, it was connected to three cooling traps in series (20°C, 0°C, -196°C). Roasting was carried out by heating the tube at 20°C/min to a final temperature of 220°C, which was maintained for 10 min. The volatiles were purged into the cooling traps by nitrogen at a flow of 50 mL/min. The condensates were extracted with ether, and the combined ether extracts were treated as described above.

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch021

Preparation of Compound III. A concentrated ether extract from model system Reaction 2 (see above) was fractionated on a 30 x 2 cm column equipped with a cooling jacket and filled with 20 g of acidic alumina (ICN Biochemicals, deactivated with 10% water). A gradient elution was performed using pentane-diethyl ether, and the following fractions were collected which corresponded to the mobile phase ratios: 1) 90:10, 2) 80:20, 3) 70:30, 4) 60:40, 5) 50:50. Fraction 5 yielded about 1 mg of colorless crystals, identified as 2-acetyl-pyrido[3,4-*d*]imidazole by GC/MS.

Gas Chromatography–Mass Spectrometry (GC/MS). The following instrumental parameters were used:

Gas Chromatography: Carlo Erba Model 4130 equipped with a flame ionization detector. Retention indices were calculated according to van den Dool and Kratz (*6*) using *n*-alkane standards (C_8-C_{30}).

Column 1: 60 m x 0.32 mm i.d., 0.25 µm film thickness DB-WAX (J&W Scientific, Folsom, CA) fused silica capillary column; 5:1 split ratio; initial temperature 40°C, 5 min hold, then programmed at 2°C/min to 230°C, followed by a 60 min hold.

Column 2: 60 m x 0.32 mm i.d., 0.25 µm film thickness DB-1701 (J&W Scientific, Folsom, CA) fused silica capillary column; 5:1 split ratio; initial temperature 40°C, 5 min hold, then programmed at 3°C/min to 260°C, followed by a 50 min hold.

GC/MS System: Finnigan MAT Model 4500 mass spectrometer equipped with a Model 2010 interface and an INCOS 2100 data system. Ion source temperature: 120°C; ionization energy: 70 eV; closed coupling. Electron impact: source pressure: approx. 1 x 10^{-6} torr.; mass range 35–350 amu, 0.8 s scan rate. Chemical ionization: methane reactant gas; source press.: 0.7 torr.; mass range 80-350 amu, 0.8 s scan rate.

H/D-Isotope Exchange. Hydrogen/deuterium exchange was obtained on Column 1 (DB-WAX) by injection of the compounds of interest, after previous injection of CD_3OD.

Results and Discussion

In order to test the relevance of our results we reacted tuna fish muscle with glucose under the same conditions. Tuna meat is well known to contain relatively high

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amounts of histidine (up to 1%). As expected, exclusively 2-acetyl-pyrido[3,4-*d*]imidazole was identified after reaction of tuna muscle with glucose.

Useful to our investigations was the fact that this compound was also formed by reaction of histamine with pyruvic aldehyde. The tentative reaction pathway is illustrated in Figure 1.

We suggest that the aldehyde group of pyruvic aldehyde can react with the amine residue of histamine (**I**), yielding a Schiff base which forms **II** by cyclization, analogous to the isoquinoline pathway described by Pictet (2). Subsequent dehydration forms the pyrido-imidazole **III**. In our view, pyruvic aldehyde is the dehydration agent, because we identified relatively high amounts of 1,2-propandiol and hydroxyacetone in the volatiles from this reaction.

In Figure 2 our suggestion of the reaction mechanism for the formation of **III** via the Maillard reaction is illustrated. We assume that the first step of its formation is the condensation of pyruvic aldehyde with histidine. In the course of Strecker degradation, CO_2 is split-off forming compound **VIa** which, by scission of the Strecker aldehyde, should form the corresponding pyrazine. In our case, this reaction takes place but seems not to be prominent as in the case of most amino acids. Instead this structure **VIb** seems to be formed by tautomerism which offers two alternatives:

1) Hydrolysis to yield histamine, which can again react with pyruvic aldehyde.
2) Direct cyclization of **VIb** with subsequent dehydration to **III**.

The first pathway requires the presence of histamine in the reaction mixture. Since it was not present, we suggest that the second pathway should be considered.

In the gas chromatogram of the volatiles which were obtained after reaction of histidine with glucose, we identified among others four peaks in the mass spectra which represent a homologous series. The first two peaks with masses 161 and 165 were assigned to compounds **III** and **II** (Figure 3). The masses of the two other peaks were 175 and 179. The latter compounds had mass spectra which were very similar to those of **III,** except that the *m/z* 43 mass fragment was absent. Instead, M-57+1 (*m/z* 199) was the prominent base peak. Therefore, we assumed that these two compounds represented 2-propionyl-pyrido[3,4-*d*]imidazole and its tetrahydro derivative, respectively. Indeed, they were also formed by reaction of 2-ketobutyraldehyde with histamine. This reaction, in our view, should proceed via an analogous mechanism as described in Figure 1, with 2-ketobutyraldehyde as the starting reagent.

2-Ketobutyraldehyde is not a common degradation product of glucose. On the other hand, we detected relatively large amounts of 1-hydroxy-2-butanone in the volatiles from the reaction of histidine with glucose. Its formation can, in our opinion, possibly proceed *via* the 3-deoxyhexosone by dehydrogenation at the 4- and 5-positions to form 3,4-dideoxy-5-oxo-glucosone, followed by a carbonyl scission to yield 1-hydroxy-2-butanone.

Figure 1. Formation of 2-acetyl-pyrido[3,4-*d*]imidazole (**III**) by reaction of pyruvic aldehyde with histamine (**I**) *via* 2-acetyl-tetrahydropyrido[3,4-*d*]-imidazole (**II**).

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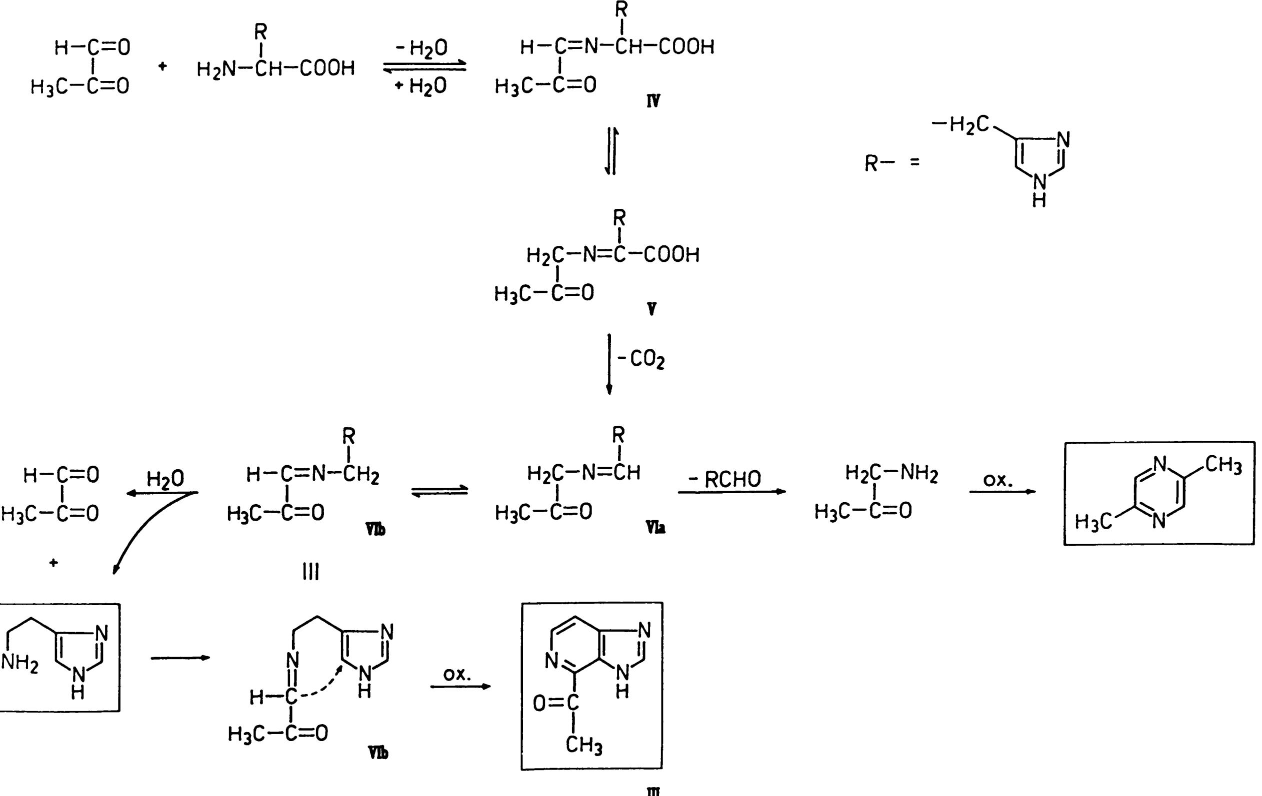

Figure 2. Mechanism proposed for the formation of 2-acetyl-pyrido[3,4-*d*]imidazole *via* Strecker degradation of histidine with pyruvic aldehyde.

The possibility of forming 2-ketobutyraldehyde by oxidation can be assumed because Maillard systems usually involve several redox reactions. Another possible pathway can include a 3-deoxyhexosone intermediate. Dehydration at the 3- and 4-positions and simultaneous retro-aldol scission should form 2-keto-3-butenal, which is presumed to produce 2-ketobutyraldehyde *via* hydrogenation. As reactions with 1-(^{13}C)-glucose showed, about 50% of the isotope was incorporated into the pyrido-imidazole ring system of the 2-acetyl- as well as the 2-propionyl derivative.

In order to additionally demonstrate the reactivity of histidine to form pyrido-imidazoles, we treated glucose with 1-, 2-, and 3-methylhistidine under the same conditions. 1-Methylhistidine occurs naturally as part of anserin, and 3-methylhistidine forms a dipeptide (balenine) with β-alanine (*3*). Indeed, the reaction of methylhistidines with glucose in an aqueous autoclave reaction system produced seven additional compounds of this type (Table 1). The principal products were in every case the 2-acetyl- and 2-propionyl-pyrido[3,4-*d*]imidazoles. From 1-

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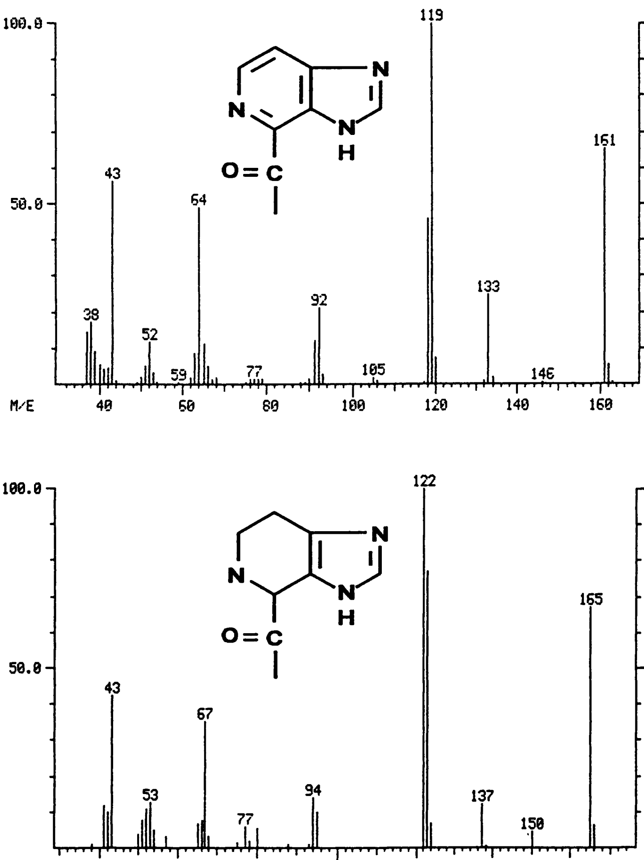

Figure 3. Mass spectra of 2-acetyl-pyrido[3,4-*d*]imidazole (III) (top), and the corresponding tetrahydro derivative II (bottom). GC-MS spectra were obtained after separation on a DB-WAX capillary column.

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Table I. Comprehensive Representation of Pyrido-imidazoles Formed by Autoclaving of Glucose (at 150°C) with Different Histidines

Glucose + Histidine	Glucose + 1-Methyl-histidine	Glucose + 2-Methyl-histidine	Glucose + 3-Methyl-histidine

methylhistidine, N-methyl-pyrido-imidazole and the 2-methylated derivative were additionally obtained. These cyclization products may have resulted from reaction with formaldehyde and acetaldehyde, respectively. Both of these aldehydes are known to be generated via sugar degradation pathways.

In order to study this reaction we treated phenylalanine with glucose, and phenylethylamine with pyruvic aldehyde, respectively. A cyclization product (isochinolin) could not be observed under our conditions. On the other hand, tryptamine and tryptophan react easily with glucose or suitable carbonyl compounds to form β-carbolins. (*4*) While the decarboxylation products of tryptophan and histidine are readily formed, the generation of phenylethylamine by Strecker degradation was evidently not the preferred pathway (*5*). Instead, the main product of this reaction was phenylacetic aldehyde. On the other hand, the Strecker aldehydes of histidine and tryptophan were formed only in poor amounts. Obviously, the stabilities of Schiff bases **VIa** and **VIb** are the key to the solution of this riddle.

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Chapter 22

Role of Cysteine in the Formation of 2-Methyl-3-furanthiol in a Thiamine–Cysteine Model System

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Labeled ^{34}S-cysteine was used to determine the amount of sulfur from cysteine incorporated into 2-methyl-3-furanthiol (MFT) in thermally processed thiamine/cysteine-glucose/xylose model systems. Four (I-IV) model systems comprised of water, monosodium glutamate, NaCl, inosine-5'-monophosphate, D-glucose, thiamine HCl^{I,II,IV}, cysteine HCl^{I}, ^{34}S-cysteine HCl^{II-IV}, and D-xylose^{III,IV} were reacted at 120°C for 1 hr in sealed glass vials. MFT was recovered from processed samples by direct extraction with CH_2Cl_2 and subsequent concentration under N_2 gas. Analysis was performed by gas chromatography/ mass spectrometry-selected ion monitoring and MFT was quantified by an internal standard method. The amounts of MFT formed in model systems I, II and IV ranged from 389 to 489 ng/33.3 g model system. No MFT was detected in system III. Only 7.5 to 8.0% of the MFT detected in systems II and IV contained ^{34}S.

The importance of MFT to meat-like flavors is well documented in the literature. Gasser and Grosch (*1-3*) identified MFT and its oxidation product bis-(2-methyl-3-furyl)disulfide (BMFD) as compounds important to the meaty aroma of boiled beef, pork and chicken meat. MFT also has been reported to be present in the steam distillate of processed tuna (*4*) and in yeast extracts (*5*).

Studies using model systems have demonstrated that MFT can be formed by the thermal degradation of thiamine and in thiamine-based model systems (*6-13*). Alternatively, MFT can form by a thermally induced reaction between a reducing sugar (commonly D-ribose) and a sulfur-containing compound such as cysteine or glutathione capable of liberating hydrogen sulfide (*13-18*).

MFT is formed from thiamine degradation by two possible pathways (Figure 1). One pathway involves cyclization of 5-hydroxy-3-mercaptopentan-2-one, followed by dehydration of the hemiacetal to form 2-methyl-4,5-dihydro-3-

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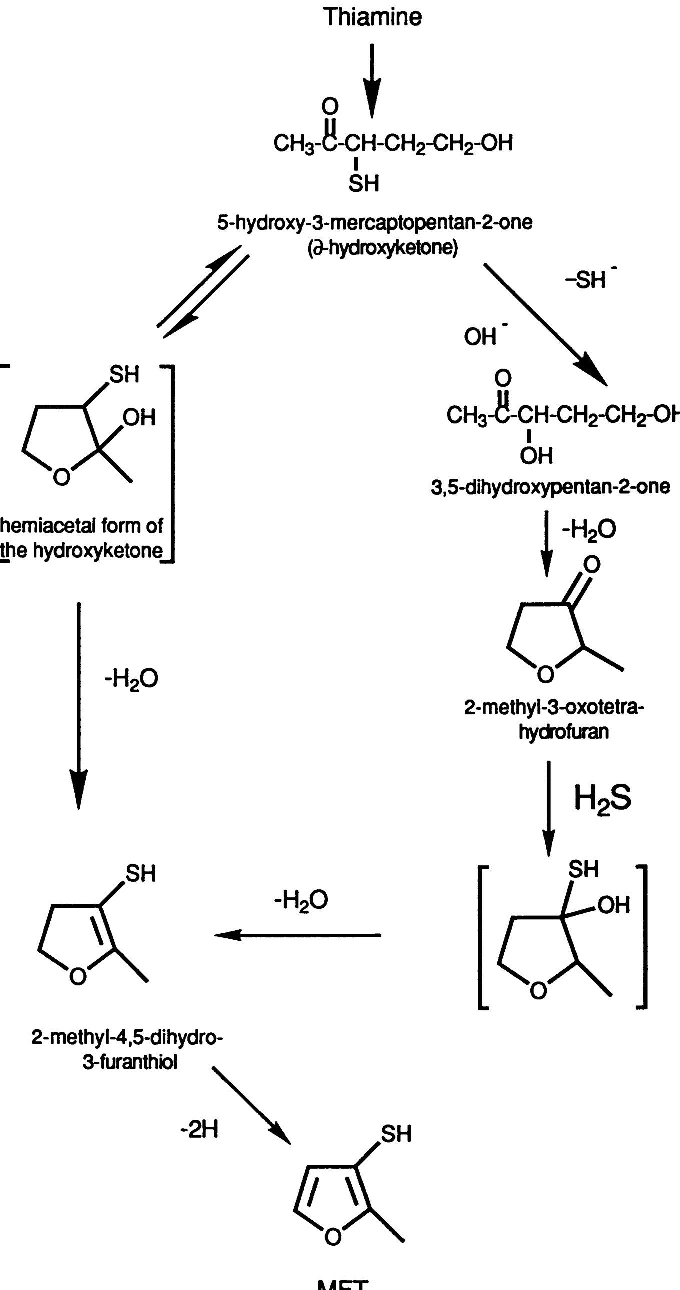

Figure 1. Formation of MFT during thiamine degradation (Adapted from Refs. *9* and *12*).

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furanthiol and subsequent oxidation to yield MFT (*6,19*). The second pathway involves the replacement of the SH group on 5-hydroxy-3-mercaptopentan-2-one by an OH-group. The 3,5-dihydroxypentan-2-one either cyclizes to form 2-methyl-3-oxotetrahydrofuran (*11,12*) or 2-methyl-4,5-dihydro-3-oxotetrahydrofuran (*6,19*). Hydrogen sulfide then reacts with the oxofuran which is subsequently oxidized to MFT (*6*).

In the absence of thiamine, Zhang and Ho (*18*) formed MFT under acidic conditions (pH 2.3) from inosine-5'-monophosphate (IMP provided ribose) and hydrogen sulfide (from cysteine). They (*18*) proposed a mechanism that involves an initial reaction of hydrogen sulfide with ribose in its open chain to form 5-hydroxy-3-mercaptopentan-2-one. MFT then forms from this precursor as in thiamine degradation (Figure 2).

Farmer et al. (*14*) suggested that MFT is formed from cysteine and ribose (at pH 5.7) by the reaction of H_2S and sugar-derived furanones by the mechanism proposed by van den Ouweland and Peer (*20*) for the formation of sulfur-substituted furans from H_2S and 4-hydroxy-5-methyl-3(2H)-furanone (Figure 2).

In dilute model systems (pH 5.7) containing thiamine, cysteine, glutathione and ribose, Grosch and Zeiler-Hilgart (*13*) determined that the amount of MFT formed increased in the following order: cysteine-ribose, thiamine, thiamine-ribose, thiamine-glutathione-cysteine-ribose, thiamine-cysteine-ribose, thiamine-glutathione-ribose and thiamine-cysteine. They suggested this trend may result by stabilizing the primary precursor (5-hydroxy-3-mercaptopentan-2-one) in a H_2S enriched environment and in the second possible pathway by a greater probability of H_2S adding to the oxofuran intermediate.

However, it is not known which mechanisms for MFT formation predominate in model systems that have the potential to proceed by all of the above proposed mechanisms. In previous studies, we found that thermal processing of our complex model system resulted in an intense meat-like aroma with sensory properties dependent on processing temperature and percent solids (*21*). The concentration of MFT increased with intensity of the perceived meat-like aromas. In addition, MFT and the characteristic meat-like aromas could not be produced without thiamine. The objective of this study was to determine the contribution of cysteine to the formation of MFT in this model system.

Materials and Methods

Model System. The following materials were used in the model systems investigated in this study; monosodium glutamate (MSG, food grade, Takeda Chemical, LTD., New York, NY), NaCl (food grade, Morton Salt, Chicago, IL), inosine-5'-monophosphate (IMP, food grade, Takeda Chemical, LTD., New York, NY), D-glucose monohydrate (USP, Sigma Chemical Co., St. Louis, MO), thiamine-HCl (USP, Sigma Chemical Co., St. Louis, MO), cysteine-HCl monohydrate (food grade, Takeda Chemical, TD., New York, NY) or ^{34}S labeled cysteine-HCl monohydrate (100% ^{34}S, synthesized at Nestle Research Center, Lausanne, Switzerland, *22*) and D-xylose (food grade, Takeda Chemical, LTD.,

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch022

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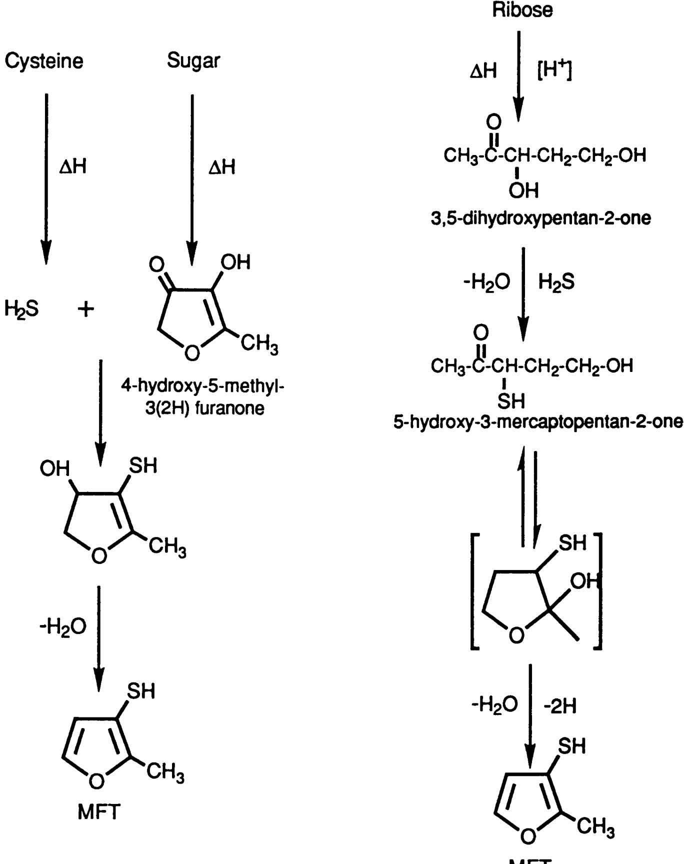

Figure 2. Formation of MFT from H_2S and pentose derived precursors (Adapted from Refs. *18* and *20*).

New York, NY). The solids composition of the model systems (I-IV) are presented in Table I.

The pH of the model system ranged from 5.5 to 5.8 before and after processing and the initial a_w of the system was 0.83 at ambient temperature.

Table I. Solids Composition of Model Systems (g/100 g)

Component	*I*	*II*	*III*	*IV*
MSG	45.19	45.19	45.19	45.19
NaCl	45.19	45.19	45.19	45.19
IMP	5.73	5.73	5.73	5.73
D-Glucose	2.16	2.16	2.16	2.16
Thiamine-HCl	1.08	1.08	-	1.08
Cysteine-HCl	0.43	-	-	-
^{34}S Cysteine-HCl	-	0.43	0.43	0.43
D-Xylose	-	-	0.22	0.22

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch022

Process. Samples were prepared in triplicate using 10 g of the model system and 23.3 g of distilled water (at 30% solids level complete solubility was observed) and were thermally processed at 120°C for one hour in 125 mL glass vials sealed with Teflon-lined septa and aluminum crimp caps. Post processing, samples were immediately diluted to 10% solids with distilled water.

Samples were extracted (5 x 20 mL) with dichloromethane (capillary GC/GC-MS solvent, American Burdick & Jackson, Muskegon, MI) using a 250 mL separatory funnel. Dodecane (Sigma Chemical Co., St. Louis, MO) was added as an internal standard at 1 ppm to the 100 mL-solvent extract. The solvent extract was dried with anhydrous $MgSO_4$ and then concentrated under N_2 gas to 0.10 mL.

Analysis by GC/MS. A splitless 2 μL injection of each extract was analyzed with a Hewlett Packard 5890 GC interfaced to a Hewlett Packard 5970 series low resolution mass selective detector in the selected ion monitoring mode. The following operating conditions were employed: initial oven temperature 35°C, initial time 2 min, program rate 3°C/min, final oven temperature 250°C, final time 20 min, carrier gas He, and 15 psi column head pressure. Separation was performed with a DB-5 (30 m x 0.32 i.d. x 1.0 μm film thickness) column (J&W Scientific, Folsom, CA).

MFT was identified by comparing its mass spectrum and retention time to that of a pure standard. An internal standard method was used to quantify MFT. The relative response factor of MFT/C_{12} was calculated from the area sum of three integrated ions from the SIM spectra of calibration solutions of dodecane (m/z 58, 85, 170) and MFT (m/z 71, 113, 114).

Results and Discussion

Previous studies demonstrated that MFT is formed from thiamine degradation (Figure 1) or by a reaction between a pentose and sulfur amino acid (Figure 2). The relative contribution of these mechanisms in more complex model systems has not been previously studied. In this study, labeled ^{34}S cysteine was used to determine the direct contribution of cysteine to the formation of MFT in a thiamine-cysteine-pentose based model system. By this approach, sulfur in MFT from ^{34}S labeled cysteine could be distinguished and quantified by mass spectrometry.

MFT was resolved and identified by GC/MS in model systems that contained thiamine (I, II and IV). The natural percent abundance of $^{34}S/^{32}S$ (m/z116/m/z114) in a pure MFT standard was found to be 4.5%. The quantity and percent (m/z 116/m/z 114) of MFT found in model systems I-IV is reported in Table II.

Our data confirm that sulfur from cysteine participated in the formation of MFT. A net 7.5 to 8.0% of the MFT formed contained labeled sulfur (model systems II and IV vs I). This amounts to approximately 29 to 39 ng per 33.3 g of processed model system. No trace of MFT was detected in III which lacked

Table II. Percent ^{34}S Incorporated into MFT Formed from Cysteine and ^{34}S Labeled Cysteine

Sample	*Replicate*	*Percent $^{34}S^{a}$*	*ng MFT/g[b]*
I. unlabeled	1.	4.54	330
cysteine	2.	4.52	444
no xylose	3.	4.68	393
		4.58 ± 0.09	389 ± 57
II. with ^{34}S	1.	11.66	405
cysteine	2.	13.11	477
no xylose	3.	12.75	585
		12.50 ± 0.76	489 ± 90
III. with ^{34}S	1.	not detected	-
cysteine	2.	-	-
no thiamine	3.	-	-
IV. with ^{34}S	1.	11.48	306
cysteine	2.	11.19	489
	3.	13.68	480
		12.11 ± 1.36	425 ± 103

[a]m/z(116/114) x 100
[b]ng MFT/g based on 33.3 g of thermally-processed sample.

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch022

thiamine. Two possible explanations for these results are: (1) MFT was formed in model system III at amounts below the detection limits of our methodology; and (2) MFT was formed primarily by thiamine degradation from 5-hydroxy-3-mercaptopentan-2-one.

If MFT was formed in model system III, but below detectable limits (ca. 10 ng), the amount formed is of little consequence. This quantity is very low compared to the ca. 440 ng formed in model systems I, II, and IV. Since MFT was detected only in the model systems containing thiamine, the primary mechanism for MFT formation involved thiamine. The quantity of MFT formed by the incorporation of H_2S into furans of carbohydrate origin (*20*) and from ribose (from IMP) (*18*) was insignificant under the conditions employed in this study. Of the two possible thiamine pathways (Figure 1), the direct contribution of cysteine to MFT must occur by the addition of $H_2{}^{34}S$ to the oxofuran intermediate.

Similar research on thiamine and cysteine model systems have reported results consistent with this study. Güntert et al. (*12*) and Werkhoff et al. (*11*) compared the volatiles formed in various meat model systems. MFT was identified only in their model systems which contained thiamine.

Shu and Ho (*23*) studied specifically the reaction of cystine with 2,5-dimethyl-4-hydroxy-3(2H)-furanone. During thermal processing cystine (the disulfide of cysteine) would liberate H_2S and would be expected to react with the dimethyl sugar-derived furanone to form furanthiols as proposed by van den Ouweland and Peer (*20*). The effect of pH, reaction medium, processing time, percent water, temperature and oxygen on the type of volatiles formed in this reaction were studied. However, no sulfur-containing derivatives proposed by the reaction of H_2S with 2,5-dimethyl-4-hydroxy-3(2H)-furanone were reported in their results (*23*).

In thiamine-cysteine model systems Grosch and Zeiler-Hilgart (*13*) demonstrated that the inclusion of cysteine into a thiamine-based model system increased the amount of MFT formed during heating. Since cysteine does not directly contribute to MFT formation via carbohydrate related pathways, H_2S from cysteine may act to stabilize the 3-mercaptopentan-2-one MFT precursor (from thiamine) and thereby increase the amount of MFT formed during heating (*13*).

Note also that model systems III and IV contained xylose. Hincelin et al. (*24*) reported that thermally processing thiamine with added xylose (pH 7.0, 140°C) increased the relative amount of MFT formed by 4 to 5 times that formed from thiamine degradation alone. In the absence of thiamine, ribose in combination with cysteine (or another source of sulfur) has been shown to produce MFT during heating (*13-18*). It is unlikely that xylose would react differently than ribose on heating (other than a potential difference in reaction rate); however, added xylose did not influence the amount of MFT formed in model systems III and IV.

The observed differences in the amounts of MFT formed in model systems I-IV compared to those obtained by other researches are likely due to the differences in precursor concentrations, pH, processing times and temperatures, and analytical methodologies.

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch022

For example, the cysteine-ribose model systems that produced MFT contained higher concentrations of cysteine and ribose and were reacted in closed systems at higher processing temperatures (130 and 140°C vs 120°C used in this study, *14-16*). In some cases the pH of the cysteine-ribose systems were the same as our model systems (pH 5.7), but phosphate buffer was used in these studies to maintain a constant pH.

The pH dependence of MFT formation in cysteine-ribose and thiamine model systems has also been demonstrated (*15,16,18,25*). In these studies MFT decreased in concentration with increasing pH in the range of 2.3 to 9.5.

Grosch and Zeiler-Hilgart (*13*) found that analytical methodology greatly influenced the quantities of MFT isolated from their model reaction systems. The authors reported a 4-5 fold increase in the amount of MFT recovered by a continuous Likens-Nickerson distillation-extraction procedure compared to a direct extraction procedure.

Consistent with our previous studies, only trace amounts of BMFD were detected in model systems I, II and IV (*21*). Only partial spectra of BMFD were obtained by GC/MS at the appropriate retention time and, as a result, data for BMFD are inconclusive and not included in this study.

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Conclusions

Studies with cysteine containing labeled sulfur (^{34}S) demonstrated that cysteine will contribute to MFT formation (ca. 8% of MFT formed) when heated in the presence of thiamine. However, under the conditions and model systems employed in this study, thiamine was required for the formation of MFT. Since no MFT was detected in the model system lacking thiamine, this indicates that pathways for MFT formation involving the substitution of H_2S onto thermally generated furans of pentose origin are of relatively minor significance. MFT was primarily formed in our model systems by direct cyclization of 5-hydroxy-3-mercaptopentan-2-one resulting from thiamine degradation in the pathway proposed by MacLeod (*19*).

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Chapter 23

Flavoring in Extrusion

An Overview

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Flavor is of critical importance to the quality of extruded products. Flavor stability, generation of desirable flavor notes, and generation of off-flavors are significant factors that influence product acceptance. Internal incorporation or topical application are important concerns when flavoring extruded products. Since various mechanisms are involved in the retention and generation of flavor, a better understanding of the reactivity of flavor compounds and further characterization of the thermal stability, diffusivity, and volatility of flavors is critical when determining approaches to generate high quality extruded products. Some of the aforementioned issues are discussed in this chapter.

Extrusion can have a significant effect on the flavor and aroma profiles of food products manufactured through this process. In fact, process parameters such as temperature, moisture content, amount of shear, pressure, and residence time can significantly affect flavor development, flavor and aroma retention, and degradation reactions undergone by flavor compounds during processing. Depending on composition, flavor development during the process may be an important consideration in product quality. Certain mechanisms such as non-enzymatic browning and lipid oxidation are considered to have significant implications in the flavor characteristics of food

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products. Oxidation and volatility of flavor compounds are also important factors to be taken into consideration.

The basic concept of an extruded food system is to provide a structure with pleasing textural attributes and appropriate flavor profile that enhance the overall quality of the product, and fit the particular class or category of product for which that profile is intended. Since the basic structure of many extruded foods is relatively devoid of flavor, being composed primarily of cereals, starches, and/or vegetable proteins, it is often required to add flavors as well as coloring agents to impart desirable flavor and appealing appearance. Generally, flavorings are applied to the surface of the product after extrusion. However, new technologies are looking more closely at incorporation of heat-stable flavors directly into the product during extrusion, or by some means, enhancing the natural flavor components of the basic structural ingredients to create a pleasing flavor and aroma.

When flavors are added to a formulation prior to extrusion, flavoring may present problems. While a low-temperature low-pressure cooking extruder will allow the incorporation of flavors internally, high-temperature high-pressure extruders do not. It is evident that fewer problems are expected in flavors with higher thermal stability such as onion, garlic, bacon, sausage, beef, chicken, cheese, and mushroom. Nevertheless, some of the components in the flavors on the aforementioned list, may undergo changes which can result in alteration of the flavor profile. Moreover, lipid-based flavors may undergo typical propagation mechanisms resulting in more evident changes in flavor during storage.

Whether flavors are incorporated internally or externally, the following are requirements for flavor applied to a snack food according to Blanchfield and Ovenden (*1*):

1. The flavor must be pleasant.
2. The flavor must mask, if possible, or complement any inherent base flavor of the extrudate.
3. The flavor should be evenly distributed over or throughout the product.
4. The flavor should not adversely affect the mouthfeel of the product.
5. If used internally, the flavor should not adversely affect the texture of the finished product.
6. It should be economical.

Flavorings used in extrusion may be classified into four main categories: (1) natural flavors (including various spices, nuts, chocolate, fats, and oils, etc.); (2) flavor concentrates (mostly from

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natural flavors in the form of a concentrate including essential oils, oleoresins, and isolates); (3) flavor enhancers (such as sugars, salt, monosodium glutamate [MSG], inosine 5'-nucleotide [IMP], etc.); and (4) synthetic or derived flavors (including acids, esters, aldehydes, ketones, ethers, amino acids, peptides, etc.)(*2,3*).

Cooking extrusion is often a high-temperature short-time process; hence, there is not enough time to form flavors normally developed during more traditonal cooking such as baking. By adding agents to catalyze formation of such baked flavors and aromas, it may be possible to generate some of the desired flavors directly into the extruded matrix. A great advantage of this approach is a more even distribution of flavor throughout the extruded product. Often times topical application of flavorings results in a totally bland center structure as in the case of expanded snacks and chips. Theoretically, addition of flavoring agents to the dry ingredients before extrusion would not only result in more even distribution within the product, but would also protect some of the more labile flavors from oxygen, thereby reducing their oxidative degradation, and avoiding the production of off-flavors. Furthermore, from a manufacturing point of view, incorporation of flavoring agents directly into the feed would streamline and simplify the overall process. Volatility, diffusivity, and thermal stability of the flavor and aroma compounds control the success of the aforementioned approach.

Flavor Loss

Two major aspects of a high-temperature short-time process, such as extrusion, must be considered when contemplating the final flavor profile. On one hand, despite the elevated temperatures present in extrusion, the short residence time may not allow some of the naturally occurring cooked flavors to be developed in products such as baked goods and simulated meat products. On the other hand, because of the high temperatures, despite the short residence times, flavorings that may be added to the feed prior to extrusion may be totally lost or degraded to produce off-flavors. Flavorings may either break down due to temperature effects or through interactions with other constituents in the feed; both can result in changes to the character of the flavor. In high-temperature high-pressure extrusion cooking rapid expansion of the extrudates emerging from the die, due to pressure differentials, results in an effective steam distillation which flashes off most of the flavor components; thereby, reducing their intensity in the final extruded product. According to Krukar (*4*), when adding flavoring systems containing hydrolyzed vegetable protein (HVP) at a 7% level

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(normally acceptable for external applications) to the feed, the finished product had a very weak meaty flavor. In order to produce an acceptable flavor, levels of up to 15% HVP were required; however, this was not economical. Delache (*5,6*) investigated the retention of 11 flavor substances incorporated into a corn base upon extrusion. Salt or lactic acid yeast were used as carriers for the flavor compounds. The author reported that steam distillation, rather than chemical reaction, was the most influential factor on flavor stability.

In some cases, this loss of volatiles due to steam distillation may be beneficial; such is the case with extruded whole raw soybeans, where vaporization of some of the off-flavors (in particular, hexanal, hexanol, and ethylvinylketone) may take place (*7*).

Flavor losses due to steam distillation as the extrudate emerges from the extruder die will, of course, depend upon the relative volatility of the flavors. One approach to reduce losses due to volatility is to encapsulate the flavors with some complex polysaccharide or hydrocolloid. Kinnison and Chapman (*8*) pointed out that even these methods would require relatively high initial concentrations to maintain sufficient flavor in the final product; although there would be a definite enhancement of the flavor, surface coatings with flavor would still most likely be required. Sadafian and Crouzet (*9*) studied the retention of aroma compounds including limonene, p-cymene, linalool, geraniol, terpenyl acetate, and beta-ionone during extrusion. Aroma compounds were incorporated in various forms including emulsions in water, oil solutions, microcapsules, or beta-cyclodextrin inclusion complexes. The authors observed losses of free volatiles in amounts greater than 90%. Retention was improved to less than or equal to 30% by natural or artificial encapsulation and the use of inclusion compounds. The use of multi-walled microcapsules achieved a retention greater than 90% for beta-ionone. Thermally induced degradation and interaction between aroma compounds and the matrix were observed. Thus, it is clear that a great percentage of volatile compounds will be lost unless proper encapsulation is provided. Multiple-layer encapsulation appears to result in a tremendous improvement in flavor retention; however, adequate and timely release of the flavor compounds upon chewing, then becomes an issue of concern.

A similar study was conducted by Mariani et al. (*10*), looking at the retention of some of the major components of orange flavor (limonene, n-decanal, linalool) during extrusion as affected by sugar concentration. Although analytical tests showed higher volatile retention with higher sugar concentration, organoleptic evaluations

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did not show significant differentiation, probably due to the high levels of loss (65 - 94%).

With the advancement of new technologies in the field of extrusion, the successful production of a desirably flavored snack product becomes more complex with an expanded array of flavoring ingredients and new techniques for their manufacture. The desire to incorporate flavors or flavor precursors in the feed prior to extrusion requires knowledge of their stability and potential to interact with other components in the system. Therefore, it is of utmost importance to have a full understanding of the behavior of the basic ingredients. With the incredibly large number of variables involved, whether composition or processing parameters, it is necessary to establish an appropriate experimental design. Lane (*11*) successfully applied surface response in determining formulation variables affecting the flavor of extruded snacks and crackers. His experiments more specifically explored interactions among grain components, internally and externally applied flavors in an extruded snack, and the effect of fat composition and flavor in a chicken-flavored snack cracker and a cheese-flavored extruded collet. The author showed that despite the skeptical outlook of internal application of flavors during extrusion, proper conditions can be chosen, using surface response techniques, so that internal application of flavors is a viable process, and that this concept can show improved product acceptability over externally applied flavor processes. The author found better flavor perception for cheese and chicken-flavored extruded snacks when utilizing internal application of flavors as compared with surface application. This surface response method provided a development scheme for proper flavoring profiles.

Chen et al. (*12*) developed two separate models for the prediction of loss of volatiles in a corn-based model system during extrusion. Four organic flavor compounds, including n-butanol, octane, benzaldehyde, and limonene, were tested. The extrusion unit used was a Brabender model 2003 with an L/D = 20:1, run at a range of screw speed from 140 to 170 rpm, moisture content from 15 to 25% and a final heating zone temperature of 140 to 160 °C. The first model was based on a thermodynamic approach, assuming complete equilibrium between solid and vapor phase; this model gave a poor estimation for n-butanol and predicted within 20-30% for limonene and benzaldehyde. The second model was based on more of an engineering approach, taking into account the volatility of the individual compounds relative to water. This model represented a reasonable approach for the prediction of retention of polar flavor compounds in the extrusion of cereals

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch023

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch023

Flavor Generation

The Maillard reaction is of great importance to extruded products. Non-enzymatic browning reactions can contribute to the development of flavor and color upon extrusion of materials containing the proper compounds. Yaylayan et al. (*13*) studied the in-situ generation of Maillard reaction flavors by extrusion using cellulose model systems containing glucose and tryptophan. The authors indicated that a controlled production of Maillard reaction flavor precursors by extrusion was possible.

Pyrazines have received a great deal of attention since they are present in many heat-processed foods such as breads, roasted nuts, coffee, and malt. Due to their low flavor threshold, only small amounts are required to give a sensory response.

In the case of beer production, one stage of particular importance for flavor formation is the preparation of the malt. Conventionally, malt is dried and roasted (a highly energy-consuming process). Extrusion of malt, therefore, should provide a more energy efficient alternative. Fors and Eriksson (*14*) investigated flavor production in partially dried green malt which was subjected to high-temperature short-time extrusion cooking as a function of processing temperature (130 - 190 °C). They found that a maximum concentration of pyrazines developed at 160 °C. The slight decrease in concentration at 190 °C was attributed to variations in the moisture content of the feed (see Figure 1). The pyrazines detected by gas chromatography were methyl-; 2,5-(and/or 2,6)-dimethyl-; ethyl-; 2,3-dimethyl-; 2-ethyl-5-methyl-; 2-vinyl-5/6-methyl-; and 2,5-dimethyl-3-ethyl-pyrazines. The authors also found that milling the malt prior to extrusion resulted in higher pyrazine concentration as compared with whole kernel samples. Increasing the surface area provided more availability of the precursors. As expected, an increase in brown color was found with increasing temperature, as well as with increasing the surface area. The enhanced formation of melanoidins resulted in a 10-fold increase in color over conventional lager malt. Concomitantly, a decrease in pH was monitored as temperature increased. As the amino groups are consumed in the browning reaction, the remaining carbonyl groups of the amino acids contributed to the fall in pH. Little if any formation of pyrazines was found at 130 °C.

From a more qualitative point of view, Wampler and Gould (*15*) investigated the utilization of distiller's spent grain (a major by-product produced from the fermentation residue after the distillation of alcohol) in extrusion processed doughs. The base flours tested were corn, wheat, rice, and potato with addition of up to 40% spent grain added. Extrusion was performed using a Bonnot

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch023

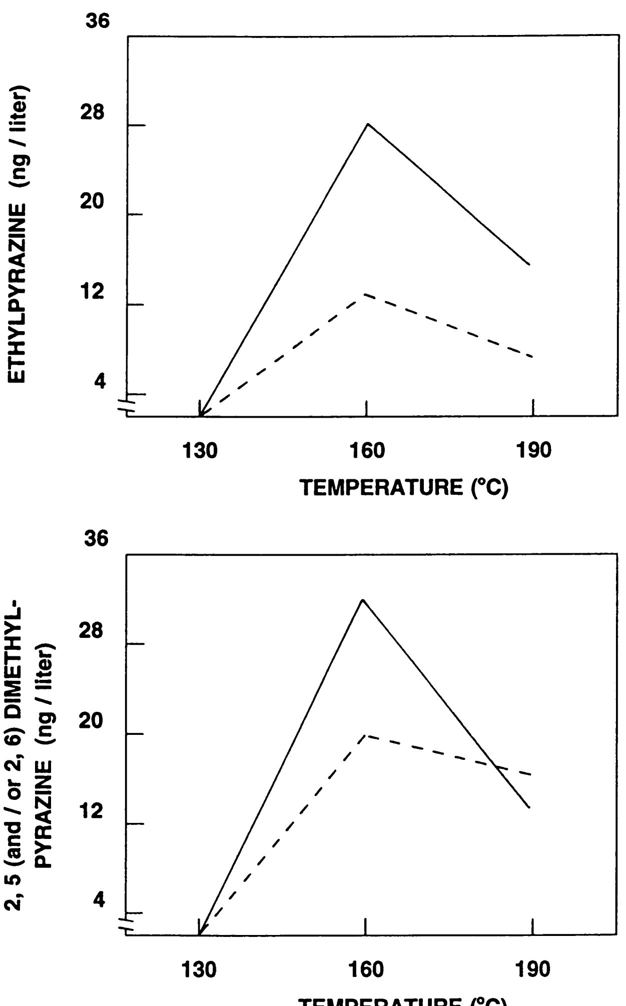

Figure 1. Pyrazine content in extruded green malt as affected by temperature and milling.
Legend: (—), milled kernels; (---), whole kernels.
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2.25 inch cooking extruder equipped with a 2.5 mm die. The screw was operated at a speed of 200 rpm and a barrel temperature profile of 10 °, ambient, 121 ° and 138 °C. They found that up to 20% of distiller's spent grain could be added to all the flours without any objectionable flavors or adverse reaction on the ability to produce a puffed product.

Precursors

There has been increasing interest in adding flavor precursors to the feed prior to extrusion which may enhance the natural production of flavors normally associated with particular cooked ingredients. This is an area that is currently being researched. The major difficulty is matching the optimal conditions for the development of flavor with those for optimal structural or textural development.

A great deal of interest has focused on addition of precursors to the extrusion feed to enhance development of flavors that normally form during cooking over extended periods of time. Yeast derivatives, or autolysed yeast extracts (AYE), are FDA approved natural flavoring agents (*16*). They are rich in peptides, free amino acids, nucleotides, and saccharides, making them a unique composite source of Maillard reactants with potential for producing flavor compounds (*17*). The volatile composition of an acid fraction of AYE provides a source of sulfur-bearing volatiles which are important in meat flavors (*18*). A basic fraction of AYE has been shown to contain predominantly alkylpyrazines as major volatile components (*19*). Cheftel (*20*) discussed the utilization of extrusion cookers as continuous reactors specifically for modification or enhancement of the volatile profile of such precursors. Research on the chemistry and control of Maillard flavor production was carried out by Izzo and Ho (*21*) who looked at this flavor technology coupled with AYE as a Maillard reactant source. These authors studied the effect of ammonia on the generation of a roasted aroma character in extruded AYE reaction flavors. This was accomplished by identification and quantification of pyrazine volatiles formed as well as by measuring the degree of brown color formation. Ammonium bicarbonate was used as a highly reactive nitrogen source. The initial materials included a spray dried extract from the autolysis of *Saccharomyces cerevisiae*, D-glucose, and ammonium bicarbonate. A type 2003 Brabender extruder with a 2 cm single screw was used. Process conditions included an L/D ratio of 20:1; a die of 0.6 cm, i.d.; a barrel temperature of 115 °C; and a screw speed of 140 rpm. Volatiles were analyzed by GC-MS. At least 24 volatile pyrazine compounds were identified and quantified. The major pyrazines found included methylpyrazine, ethylpyrazine, 2-ethyl-6-

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch023

methylpyrazine, 6-methyl-2-vinylpyrazine and 2,5-dimethyl-3-ethylpyrazine (Table I).

It may be seen in Table I that extrusion increased the pyrazine concentration up to 33 times the original concentration in the unextruded AYE. Addition of ammonium bicarbonate decreased the total pyrazines formed by approximately 58%; however, it was also shown that addition of ammonium bicarbonate enhanced browning, as measured spectrophotometrically.

The ammonia molecule is highly reactive. Addition of alkyl groups greatly decreases reactivity; therefore, the nitrogen of ammonium bicarbonate is more reactive than the nitrogen of amino acids. Due to this reactivity, ammonia participated readily in amino-carbonyl reactions. Ammonia attacked the carbonyl of one sugar moiety producing a primary amine which was still reactive to another sugar molecule, resulting in a series of reactions, thereby sequestering the reducing sugar. The end result was enhanced polymerization in the early stages of the Maillard reaction.

Maga and Sizer (*22*) pointed out that pyrazine volatiles are generated via traditional pathways, but addition of ammonia decreases the formation of pyrazines by reducing the activity of such a pathway. Izzo and Ho (*21*) concluded that the ammonia was too reactive, accelerating the Maillard reaction to the point where production of aroma is surpassed. More brown color was developed, but less roast aroma was generated. Perhaps more controlled addition of the ammonia or a somewhat less reactive nitrogen source may better regulate the extent of the reaction.

Stability and Retention

Of critical importance to the quality of extruded products is the stability of lipids during extrusion and the development of off-flavors during storage. Because of the rather severe conditions encountered during the process, lipid instability may become a problem. However, conditions encountered during extrusion may also help their stability. Inactivation of enzymes such as peroxidases, lipases, and lipoxygenases will enhance lipid stability. In fact, the potency of flavor compounds that can arise by decomposition of hydroperoxides generated by lypoxygenases is clear evidence that limited oxidation is needed to give origin to objectionable levels of flavor in fairly bland products. Moreover, complexation of lipids with the amylose fraction of starches, will also contribute to the stability of lipids. Transition metals such as iron, on the other hand, may act as inhibitors or catalysts for autoxidation reactions as indicated by Betts and Uri (*23*). Artz et al. (*24*) showed that an increase in extrusion temperature resulted in

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch023

an increase in the transition metal content of extrudates. Although the authors were unable to separate the effect of process temperature and the transition metal content on lipid stability, additional experiments indicated that the addition of ferrous acetate (50 ppm, dry weight basis) was more effective than BHA (50 ppm) in reducing oxidation in corn starch-based ground extrudates containing soybean oil. The authors suggested the possibility of reducing rancidity in extruded snacks by the incorporation of ferrous acetate at a suitable concentration. This same mechanism may contribute to the stability of lipid-soluble flavor compounds during storage. It should be stressed that damage to lipids and other lipid-like components during extrusion will result in the generation of free radicals, which in turn will accelerate off-flavor development during storage.

The sorption of vanillin on native and modified starches during extrusion was investigated by Schmidt and Maier (*25*). The authors reported an increase in sorbed vanillin upon an increase in starch swelling and degree of gelatinization. Moreover, the authors reported an extensive protection of vanillin towards oxidation upon binding. Thus, it is evident that the presence of binding sites will provide stability to flavor compounds. Hence, it is evident that lipid-soluble compounds will most likely exhibit a better stability as compared with water-soluble flavors and aromas. The presence of more binding sites available for their binding appears to be a critical issue. Delache (*5, 6*) indicated that the retention of flavor compounds such as alcohols, aldehydes, and esters in a corn paste upon extrusion, was influenced by composition. The model flavor compounds were 50% retained when the paste was supplemented with milk powder, while only 33% was retained when it was supplemented with NaCl.

Palkert and Fagerson (*26*) investigated flavor retention of pre-extrusion added flavor compounds in textured soy protein. Originally, in the early 1970's, it was expected that textured soy protein (TSP) would displace 15 - 20% of the meat in meat products in a period of a decade. By the early 1980's, however, this production was far lower than predicted. The major problem has been either the lack of meat-like flavors or the presence of off-flavors in the TSP. The model compounds used in their experiment have been reported as major contributors to meat flavors, including ethyl, propyl, and butyl disulfides; propyl and butyl sulfides; furfural and undecanal; and heterocyclic compounds such as pyrrole, 2,4-dimethylthiazole, and 2,5-dimethylthiophene. An experiment was designed such that to 400 g defatted soy flour were added 100 μl of each flavor component, followed by extrusion and subsequent drying. A Brabender type 125-20-8GR extruder with a screw speed

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch023

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch023

Table I. Pyrazines Identified in Extruded Autolyzed Yeast Extract Systems

Compound	I.k. (DB-1)	*Concentration (ppm)* UYE[a]	EYE[b]	EYE/A[c]	EYE/G[d]	EYE/G/A[e]
Methylpyrazine	798	t	0.55	0.39	3.80	6.09
2,6-Dimethylpyrazine	884	t	t	t	0.21	0.08
Ethylpyrazine	888	t	3.01	1.53	17.21	16.57
2,3-Dimethylpyrazine	891	t	0.24	0.04	0.83	0.50
2-Ethyl-5-methylpyrazine	974	t	0.72	0.38	2.91	1.97
2-Ethyl-6-methylpyrazine	980	0.03	2.06	0.72	6.40	3.19
2-Ethyl-3-methylpyrazine	982	t	0.65	0.26	1.89	0.93
6-Methyl-2-vinylpyrazine	992	0.02	0.03	t	5.22	4.67
2,5-Dimethyl-3-ethylpyrazine	1059	0.16	1.07	0.46	7.54	2.19
2,3-Diethylpyrazine	1063	t	t	t	0.54	0.50
2,3-Dimethyl-5-ethylpyrazine	1065	0.08	0.55	0.13	0.82	0.13
2-Methyl-6-propylpyrazine	1069	t	t	t	0.02	t
6-Ethyl-2-vinylpyrazine	1076	t	0.12	t	0.30	0.13
5-Methyl-2-acetylpyrazine	1088	t	t	t	2.53	2.33
6-Methyl-2-acetylpyrazine	1093	t	0.04	t	2.43	3.33
2-Methyl-5-butylpyrazine	1114	t	0.05	t	0.34	0.11
2-Methyl-6-butylpyrazine	1120	t	t	t	0.16	0.04

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch023

2-Methyl-3-butylpyrazine	1125	t	t	t	0.05	t
2,5-Diethyl-3-methylpyrazine	1136	t	0.05	0.01	0.22	0.03
2,6-Diethyl-3-methylpyrazine	1138	t	0.13	0.03	0.54	0.08
2,3-Diethyl-5-methylpyrazine	1140	t	0.15	0.04	0.59	0.11
2-Methyl-6-propenylpyrazine	1161	t	t	t	0.06	t
Ethylacetylpyrazine	1170	t	t	t	0.20	0.12
2,3-Dimethyl-5-butylpyrazine	1198	t	0.07	t	0.87	0.46

a Unextruded autolyzed yeast extract.
b Extruded autolyzed yeast extract.
c Extruded autolyzed yeast extract + ammonium bicarbonate.
d Extruded autolyzed yeast extract + glucose
e Extruded autolyzed yeast extract + glucose + ammonium bicarbonate
t Trace compound, less than 0.005 ppm.
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of 100 rpm and a temperature profile of 93, 140, 205, and 210 °C was utilized. Samples were dried at 105 °C. As may be seen in Table II, the resulting recoveries of the volatile flavor components was relatively low with a range of 4-22%, depending upon the particular flavor component.

Table II. Recovery of Extruded Flavor Compounds in a Defatted Soy Flour Base

Compound	Percent recovery
Butyl disulfide	4 ± 2
Butyl sulfide	8 ± 2
2,4-Dimethylthiazole	22 ± 3
2,5-Dimethylthiophene	5 ± 2
Ethyl disulfide	6 ± 2
Furfural	12 ± 3
Propyl disulfide	9 ± 2
Propyl sulfide	4 ± 2
Pyrrole	20 ± 3
Undecanal	4 ± 2

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Encapsulation

Although the previous information points out some of the problems associated with flavor and aroma retention during extrusion under controlled conditions extrusion may be used as a successful means for encapsulation. The basic idea behind encapsulation using extrusion is to create a molten mass in which the volatile is dispersed. Immediately after exiting the die, the conventional approach calls for immersion of the fibrils into a cold dehydrating liquid. Upon immersion in the solvent, the fibrils will solidify, trapping the volatile compounds. Because of the milder process conditions required for the process and the short residence times, extrusion presents great technical feasibility for its application in the encapsulation of heat sensitive compounds. Immersion of the material in a solvent is considered necessary for the removal of any surface active compound, thus preventing any rapid oxidation at the surface. Moreover, partial moisture removal may occur, thus minimizing the amount of water to be removed by other means.

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch023

As compared with other approaches for encapsulation such as spray drying and freeze-drying, extrusion is a rather new approach. However, some of the fundamental work was conducted by Schultz et al. in 1956 (*27*). The authors successfully encapsulated orange oil by emulsifying the oil in molten dextrose containing a small amount of corn syrup solids to retard crystallization. The molten material was poured on stainless steel sheets and allowed to solidify. Good retention of the volatile compound, gave origin to its implementation in an extruder by Swisher (*28*). Orange oil was incorporated into a molten corn syrup (42 D.E.) containing a minimum amount of water (3-8.5%) and an emulsifier to allow a good dispersion of the essential oil. Upon extrusion, the material was washed with isopropanol to remove any surface oil, followed by vacuum drying to reduce the moisture level. Refinements to this approach by Swisher (*29*) claimed the need for the use of glycerol to facilitate heat transfer, thus resulting in a more rapid melting of the sugar component and the need for a lower moisture content of the melt. The resulting material had increased plasticity, and thus more structural integrity.

Beck (*30*) suggested the use of sucrose and hydrolyzed cereal solids as an approach to reduce hygroscopicity of the powder. The D.E. of the hydrolyzate was recommended to be below 20. On the other hand, Miller and Mutka (*31*) introduced modifications to the overall process with regard to process temperature, moisture content, emulsifier level, and pressurization levels for loading levels up to 35%.

Although extrusion presents excellent potential for encapsulation, composition is of paramount importance. Loading levels, may be another major concern, primarily due to the limitation in matrix materials that can be used for encapsulation. Moisture content in the melt, is another key element determining success in encapsulation. Additional dehydration steps after extrusion operations may result in partial losses of volatile compounds depending on the porosity of the extruded product and the volatility and diffusivity of the active compound.

Summary

A great deal of research has yet to be accomplished in perfecting the "art" of flavoring extruded through a more scientific approach. A greater understanding of the chemistry of the major flavor components found in thermally induced reactions and their relative reactivity is required in order to predict retention of a given flavor note undergoing a specific process under defined conditions. As Maga (*32*) pointed out, further identification of individual flavor

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch023

compounds is also needed, a process that is currently being aided through computer modeling of probable structures. The concept of precursors and reaction accelerators for flavor development during extrusion also warrants further investigation.

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Chapter 24

Lipid Oxidation in Extruded Products

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An increase in the temperature at which corn meal or corn starch and soybean oil were extruded resulted in an increased oxidation of the extrudate during storage. Oxidation was measured as the conjugable oxidation products (COP), oxodiene values (OV), peroxide values (PV) and conjugated dienes (CD). At the end of the storage period there was an increase in the COP, OV, PV, and the CD that corresponded to an increase in extrusion temperature. Transition metal content, particularly iron (2 ppm to 6 ppm), also increased as extrusion temperature increased from 115 to 175 °C. In a separate experiment, corn starch and soybean oil were extruded with 50 ppm added ferrous acetate (dry weight basis) and with 50 ppm butylated hydroxyanisole (oil weight basis). The rate of oxidation during storage in the extrudate containing ferrous acetate was reduced relative to the control and the sample containing BHA.

The application of extrusion to food processing has resulted in a decrease in raw material costs and production time for such traditional foods as corn flakes (*1*), crispbreads (*2*), and chocolate (*3*). Ready-to-eat (RTE) cereals, pretzels, second-generation snacks coated with cheese or other savory coatings, and third-generation snacks, which are re-extruded and subsequently fried, are some of the extruded products which are now available in the marketplace (*4,5*). Oil is an important component with respect to product flavor and acceptance in many of these products. Extrusion processing has provided new and challenging problems in maintaining oxidative stability in these products. In addition, high fiber extruded products have recently been introduced. While many of the high fiber extruded food products have a reduced fat content, the low moisture content, high surface area and potential for metal contamination as a result of extrusion increases the product susceptibility to oxidation relative to products of the same composition that have not been extruded.

Perhaps the greatest concern for lipid instability is in the use of extrusion for the production of restructured muscle foods, instant soups, animal feeds, and weaning foods for third world countries. These products contain more fat than other pre-fried foods typical of extrusion (*6,7*). Weaning foods and animal feeds may be the primary source of nutrition for their respective target groups. Therefore, the stability of lipids in these foods may be a limiting factor in the bioavailability of essential fatty acids. The recent development of fourth-generation

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co-extruded or tube-filled products can also present special storage problems. In addition to increased moisture transfer between the various components, the materials composing the centers of these products may be more susceptible to oxidation.

Extrusion Effects on Lipids

Extreme conditions of temperature, pressure and shear are often employed during extrusion. These conditions can have a deleterious effect on lipid stability. Lipid oxidation can be induced (*8*) and accelerated (*9*) under certain extrusion conditions. There is even some question as to whether the effects of extrusion are always detrimental with respect to lipid oxidation. Smith (*10*) has suggested that lipid stability may increase as a result of extrusion due to lipid binding by starch. Daniels et al. (*11*) has also suggested that bound lipids were protected against peroxidation, since lipid peroxides were formed predominantly from free lipids.

The conversion of cis unsaturated fatty acids to trans can occur during extrusion (*12*). As the extrusion temperature increased from 155 °C to 171 °C, the percent trans fatty acids increased from 1 to 1.5%. The effect of extrusion conditions (moisture, temperature, and retention time) on lipid oxidation of the extrudate from a single screw extruder has been examined for full fat soy flour (*13*). An increase in the peroxide value during storage was related to an increase in either added moisture during extrusion and/or extrusion temperature. Shorter retention times in the extruder improved the lipid stability of the extrudate during storage. Extrusion also resulted in the inactivation of lipase and other deteriorative enzymes. However, some of the increase in lipid stability may be due to lipid binding by the starch (*10*). Lipids bound by starch exhibit increased resistance to oxidation, and mixing the dough in the absence of air can increase lipid binding (*14*).

Manioc starch, along with numerous additives, were extruded with a Creusot-Loire BC 45 twin screw extruder (*15*). Fatty acids, monoglycerides and several vegetable oils were added during extrusion. Analysis of the X-ray patterns indicated amylose-fatty acid complexes were produced with fatty acids and monoglycerides, but not with triglycerides. The percentage of water soluble fraction of extruded starch decreased with an increase in the length of the carbon chain of the fatty acids. An examination of manioc starch hydrolysis after extrusion indicated that hydrolysis of the macromolecular structure of starch was greater in the absence, rather than the presence of lipids (*16*). In the presence of triglycerides, the solubility of starch was greater than starch in the presence of lipids that readily complex, such as oleic acid and monoglycerides. Extrusion cooking reduced the amount of extractable lipid in wheat and maize to a greater extent than did other heat treatments (*17*). An increase in the relative amount of amylose in starch enhanced the formation of fatty acid-starch complexes upon extrusion. The complexation was analogous to the butanol-amylose complex, which exhibits a "V-type pattern" in its X-ray spectrum (*18*). The complex, which was resistant to alpha-amylase action, was dependent upon the amount of complex formed as well as the texture of the extrudate. Decreasing the solubility of starch by complexation reduced the "stickiness" of the extruded product, which reduced the susceptibility of the starch to alpha-amylase. It is likely, little, if any, complexation of triglycerides occurs.

Lipid oxidation of barley flour after extrusion with a Creusot-Loire twin screw extruder was monitored during storage (*19*). With an increase in storage temperature and water activity the unsaturated to saturated fatty acids ratio decreased and the amount of diene conjugation increased. The raw or non-extruded sample had a reduced unsaturated to saturated fatty acid ratio and increased diene conjugation as compared to the extruded samples.

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Lipid Oxidation

Unsaturated fatty acids are the lipid components most susceptible to oxidation. The effect of heating or thermal processing on the susceptibility of unsaturated fatty acids to oxidative deterioration has received substantial attention. The present understanding regarding the fundamental mechanisms of lipid oxidation is predominantly derived from the work conducted by Farmer et al. (*20-23*), Bolland and Gee (*24,25*) and Bateman et al. (*26*). The current theories related to the mechanisms of lipid oxidation are discussed in reviews by Frankel (*27-29*), Gardner (*30*), Paquette et al. (*31*), and Chan (*32*). It is widely accepted that the oxidation of unsaturated lipids proceeds via a free radical mechanism (*27*), although some of the details of the initiation, propagation, branching, and termination reactions are still debated. The hydroperoxides formed and the subsequent decomposition products differ depending upon the conditions of oxidation. Temperature, pressure, oxygen concentration and the presence of pro- or antioxidants can affect the rate of autoxidation, as well as the concentration and composition of the products formed. Frankel et al. (*33*) separated and identified the 8-, 9-, 10-, and 11-positional hydroperoxide isomers as the primary autoxidation products of methyl oleate. The concentration of the 8- and 11- hydroperoxides was slightly greater than that of the 9- and 10-hydroperoxides. Others have separated and identified the geometric and positional isomers of methyl oleate with HPLC-MS (*34*) and ^{13}C NMR (*35*). Allylic hydroperoxides can undergo changes in double bond position and geometric rearrangement (*36,37*), although different interpretations exist on the mechanism of rearrangement (*36-38*). Isomerization will also affect hydroperoxide distribution, and therefore, the distribution of subsequent decomposition products.

Linoleate autoxidation is usually initiated by the abstraction of hydrogen from the doubly allylic methylene group on the carbon 11 atom. The result is a stable, delocalized, pentadienyl radical. The 9- and 13-position carbon atoms are equivalent in terms of oxygen attack (*39*). Chan and Levett (*40*) have separated a series of linoleate isomers based on double bond position and geometric configuration. They (*40*) later reported that the rearrangement of the linoleate isomers was non-stereoselective based on the isomers separated from an equilibrium mixture. However, temperature does influence the distribution of the geometric isomers (*41*). Research has also focused on the elucidation of the stereochemical mechanisms of unsaturated hydroperoxide rearrangement (*38,39*). Two different mechanisms of rearrangement have been proposed (*36-38,44*). Chan et al. (*36*) favors exchange of the oxygen atoms of the hydroperoxide group with molecular oxygen after the carbon-oxygen bond of the peroxy group is broken. Porter et al. (*37,38,44*) proposed a scheme involving reversible oxygen addition to the intermediate pentadienyl carbon radicals.

The autoxidative mechanism of methyl linolenate is very similar to that of linoleate. Two pentadienyl radicals can be formed depending upon whether hydrogen is abstracted from the 11- or 14- position. Ground state triplet oxygen will readily attack the outer carbons of either pentadiene group radical. Frankel et al. (*45*) separated and identified eight positional and geometric hydroperoxide isomers of methyl linolenate with GC/MS, after reduction to the hydroxy stearates. Chan and Levett (46) used HPLC to separate linolenate hydroperoxide isomers. The outer (9- and 16-) positional isomers were present in significantly greater quantities than the inner (12- and 13-) isomers.

Frankel (*27-29*) and Paquette et al, (*42,43*) have reviewed the major pathways of hydroperoxide decomposition. Homolytic cleavage of the oxygen-oxygen bond of the hydroperoxy group results in the formation of alkoxy and hydroxy radicals. An aldehyde, in addition to another free radical, can be formed from carbon-carbon scission of the alkoxy radical on either side of the radical group carbon. Alcohols can be formed if a proton is abstracted from another

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch024

molecule, which will also produce a new free radical. The radicals produced will propagate additional chain reactions. Monomolecular decomposition during branching involves the release of an hydroxy radical. The remaining free radical is an alkoxy radical. Bimolecular decomposition involves the elimination of reactive free radicals by either the addition of two free radical species or the formation of a stable radical, which results in termination of the chain reaction (*47*).

Terao et al. (*48*) showed that isomerization occurs competitively with secondary oxidation during the decomposition of linoleate monohydroperoxides, while secondary oxidation reactions predominate during the decomposition of linolenate hydroperoxides. This may explain the relative stability of linoleate over linolenate hydroperoxides and the increased complexity of linolenate hydroperoxide decomposition products. In 1975, Terao and Matsushita (*49*) reported di- and tri-oxygenated compounds during the decomposition of linoleate hydroperoxides at 37 °C. Ketodienes, epoxyhydroxymonoenes, di- and trihydroxy compounds and epoxyenes have also been identified as minor products from oxidized linoleate (*39,50*).

Neff et al. (*51*) found that 6-membered cyclic peroxides may be formed by the photosensitized oxidation of methyl linoleate monohydroperoxides produced during autoxidation. While these compounds have not been reported in autoxidized samples, their formation is possible as a result of singlet oxygen produced by the decomposition of a postulated intermediate, a tetroxide (*52*). Neff et al. (*51*) characterized the volatile thermal decomposition products for cyclic peroxides. Six membered cyclic peroxides and five membered cyclic hydroperoxy epidioxides will produce a strongly positive TBA test result (*53*).

Linolenate monohydroperoxides can undergo free radical cyclization to produce monocyclic endoperoxides, which are non-volatile precursors to malondialdehyde (MDA) (*54*). The thiobarbituric acid (TBA) test has long been used to quantitate MDA. The TBA test has often been criticized as specific only for the limited number of lipid oxidation products capable of actually forming MDA (*55*). Frankel and Neff (*55*) have developed a method for determining whether a compound might be a precursor to MDA. Five-membered epidioxides and 1,3-dihydroperoxides were the most important precursors of MDA. The 1,4-dihydroperoxides formed very little MDA, while monohydroperoxides formed almost none. Six-membered epidioxides, 1,7- and 1,8- dihydroperoxides did not form MDA.

Neff et al. (*56*) found that hydroperoxy cyclic peroxides were formed to the same extent as were monohydroperoxides from autoxidized linolenate. The cyclic peroxides are formed by the intramolecular cyclization of the internal hydroperoxide isomers (10- and 12-) of autoxidized linolenate. Formation is so rapid that they are regarded as primary products of linolenate autoxidation. This may explain the reduced level of detection for the 10- and 12-hydroperoxide isomers in autoxidized samples, which was once thought to be due to preferential oxygen attack at the outer pentadienyl carbons. Dihydroperoxides of linolenate are capable of forming small but significant quantities of MDA and are considered important oxidation products in linolenate autoxidation (*55*). In the absence of singlet state oxygen, linoleate, unlike linolenate, cannot form the precursors necessary for MDA formation (*55*).

Thermal decomposition of primary unsaturated fatty acid hydroperoxides, which consist of a single hydroperoxy group, results in carbon-carbon scission on either side of the alkoxy radical (*57*). The temperature during autoxidation will affect the composition of the decomposition products formed by altering the cleavage position of the primary hydroperoxides (*58,59*). With an increase in temperature during heating, splitting of the C-C bond on the side opposite the olefinic linkage of the oxygen atom of the alkoxy radical is favored (*60*). Apart from temperature, pressure can have a significant effect on the rate constant of the reaction and the compounds formed (*61*). Gas phase oxidation, which can involve

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch024

secondary oxidation of several decomposition products, is also influenced to a great extent by the applied pressure. The rate of oxidation at 22 atmospheres of pressure and 100 °C is 50 times the rate at one atmosphere and 100 °C (*62*). This has important implications with respect to extrusion and its high temperature, high pressure conditions.

The oxidation of methyl oleate at 80 °C produced two groups of compounds. One group consisted primarily of the decomposition products of methyl oleate hydroperoxides formed during autoxidation. The other group consisted of methyl oleate epoxides, dihydroperoxides and dimers (*58*). Further study of methyl oleate oxidation by Lercker et al. (*59*) indicated the presence of alpha,beta-unsaturated hydroxy and keto esters. The presence of epoxides was also confirmed. Shifting of the double bond of oleic acid during heating of 1-oleyl dipalmitin can also occur (*63*).

Oxidative dimers were formed from the condensation of free radicals produced as a result of the cleavage of primary hydroperoxides (*64*). Thermal dimers were formed by Diels-Alder type condensation reactions (*65*). An increase in either temperature or the percentage of unsaturated fatty acids resulted in increased formation of cyclic monomers in heated vegetable oils (*66*).

Rao and Artz (*67*) oxidized methyl oleate under conditions of elevated temperature and pressure. The volatiles were trapped and then identified by capillary gas chromatography and mass spectrometry. Several compounds not usually reported, including bicyclics, suggested that different pathways may be favored under conditions of elevated temperature and pressure. A variety of aromatic and alicyclic compounds, heterocyclic compounds, aldehydes and ketones were observed. Several mechanisms have been proposed to explain the formation of ring structures produced as a result of oxidation (*68-72*). Wheeler and White (*71*) suggested that the bicyclic and tricyclic structures found may be formed as a result of free radical coupling. Formation of heterocyclic compounds can occur due to peroxy radical decomposition and cyclization (*62*). Another possibility is the formation of an endoperoxide (*31*) via singlet oxygen, which can, upon thermolysis, produce heterocyclic compounds.

Decomposition products isolated from oxidized methyl oleate included aldehydes, ketones, heterocyclic and aromatic compounds. Compounds with similar functional groups and structures have been noted in soybean oil, cottonseed oil, corn oil, methyl linoleate, and synthetic triglycerides during simulated frying: heating for extended periods of time at high temperatures (185-200 °C, 30-200 h) (*73-78*). Henderson et al. (*72*) identified not only aldehydes and oxo-alkanoates, but benzene derivatives, such as propylbenzene and some of the alkylphenyl ethers upon thermal oxidation of ethyl linoleate. Earlier reviews on the mechanism of thermal oxidation of unsaturated fatty acids have suggested that the oxidation products formed at elevated temperatures may be different from those involved in autoxidation at ambient temperatures (*79*). Many of the same principles which apply to thermally oxidized oils might be applicable to lipids contained in extruded products.

Michael (*69,70*) characterized the aromatic and non-aromatic methyl esters produced from the thermal oxidation of methyl linoleate. The aromatic esters omega-(o-alkylphenyl) alkanoic acids, 7-(2'-pentyl-phenyl) heptanoate and methyl 8-(2'-butylphenyl) octanoate were isolated and identified from thermally oxidized linoleate. Single ring closure via a free radical allylic hydrogen abstraction was proposed as a possible mechanism for formation of non-cyclic compounds. Sebedio et al. (*80,81*) isolated cyclic fatty acid monomers (CFAM) from sunflower and linseed oils heated under nitrogen at 275 °C (12 hrs) and from thermally oxidized sunflower oil heated at 200 °C (48 hrs). The linoleic rich sunflower oil contained less than one-tenth (ca. 1%) the amount of CFAM as did the linolenic rich linseed oil heated under the same conditions. While the thermally oxidized (200 °C, 48 hrs) sunflower oil exhibited greater changes, only 0.5% CFAM was found (*80,81*).

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch024

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Metal Catalysis of Lipid Oxidation

Metal catalysis of lipid oxidation can be broadly divided into two types, one involving an one electron transfer, which is referred to as homolytic catalysis, and another type referred to as heterolytic catalysis involving a two electron process (*82*). In the first case, the transition metal accelerates the rate of the chain reaction of lipid oxidation by generating free radicals, either by a reduction or an oxidation mechanism (*83-85*). The overall rate of the reaction depends on the rate of the catalytic reaction of metal with hydroperoxides, which is dependent upon the redox potential of both the oxidized and the reduced states of the metal. The reduction reaction involving hydroperoxide decomposition is faster than the oxidation reaction, since fatty acid hydroperoxides are better oxidizing than reducing agents. For example, with the oxidation of chromium from Cr^{+2} to Cr^{+3}, no regeneration of the Cr^{+2} state was observed (*86*), suggesting that Cr^{+2} does not catalyze the degradation of alkylhydroperoxides.

With copper, due to a low redox potential for the Cu^{+2}/Cu^{+3} couple, regeneration of Cu^{+} can occur through pathways such as the oxidation of alkyl radicals. Alkyl radicals can be formed by the reduction of alkoxy radicals. Decomposition of tertiary alkoxy radicals can also produce alkyl radicals. Alkyl radicals can react with Cu^{+2} regenerating Cu^{+} and a cyclic catalytic process is possible (*87*). Metal catalysis depends on the relative concentrations of metal and hydroperoxide. The nature of the solvent in which catalysis takes place and the presence of radical scavengers also influences the catalytic process (*88*).

Mo, W, V and Ti promote heterolytic catalysis, unlike Co, Mn, Fe and Cu, which promote homolytic catalysis and decomposition of alkyl hydroperoxides. Transition metals facilitating heterocatalysis characteristically form epoxides (*89*). Polar solvents can bind with the metal at active sites reducing the catalytic action (*90*). In addition, water can exhibit a pro- as well as an antioxidant effect on lipid oxidation in food, which can be quite different depending upon whether transition metals are present (*91*) or absent (*92*).

Work done in the early 1960s outlined the specificity and selectivity of iron and copper salts with respect to oxidation of hydrocarbons (*87,93*). With an excess of the metal the resultant increase in the rate of termination reactions due to the reaction of free radicals with the metal catalyst can inhibit further autoxidation of the hydrocarbon substrate (*94*). Fe^{+3} will oxidize, while Fe^{+2} will reduce alkyl radicals.

Inhibition of Lipid Oxidation by Transition Metals

Transition metals, such as iron, can exhibit a concentration dependent conversion from catalyst to inhibitor of hydrocarbon autoxidation reactions (*95*). The effect of transition metal on autoxidation reactions is strongly catalytic, until the metal concentration exceeds that of the hydroperoxide. At that point the excess metal begins to react with and subsequently deactivate, peroxy radicals (*96*). The application of that phenomena, the conversion of transition metal catalyst to inhibitor, has not been fully explored in food systems.

Iron catalyzed autoxidation occurs through the formation of an iron-hydroperoxide complex, with the subsequent breakdown of the hydroperoxide to form free radicals (*97*). Catalysis of hydroperoxide decomposition by all transition metals takes place through the formation of similar complexes (*98,99*). Alkyl hydroperoxides, upon metal catalyzed decomposition, will produce both peroxy and alkoxy radicals. The catalytic effect of transition metals are observed at low metal concentrations (*95,100*). As the metal concentration increases in the catalytic concentration range, the rate of autoxidation increases. This increase continues until the metal concentration reaches a critical concentration, which is equivalent to

the hydroperoxide concentration. As the metal concentration is increased above the hydroperoxide concentration, inhibition will occur. The conversion from catalyst to inhibitor is due to the reaction of the excess transition metal with free radicals. This results in conversion of free radicals to ionic species with the subsequent inactivation of these radicals (*96*). This phenomena has been observed with manganese and cobalt (*101*). Another example of the catalysis to inhibitor conversion is exhibited by excess heme in the oxidation of unsaturated fatty acids (*102,103*). In media of high polarity, this inhibition is not usually observed. The metal catalyst preferentially binds with the solvent forming a complex, thereby reducing the concentration of the metal-hydroperoxide complex, limiting the rate of the reaction and inhibition does not occur (*104,105*). Deactivation of the catalyst upon precipitation as an insoluble oxide or hydroxide may occur in non-polar media, if the catalyst is sensitive to polar substances formed during autoxidation (*83*).

The present study was designed to evaluate the effects of extrusion temperature on lipid autoxidation, with respect to storage stability, In addition, the feasibility of utilizing iron compounds for the inhibition of lipid oxidation during extrudate storage was explored.

Experimental Conditions

All chemicals were reagent grade, unless otherwise noted. Degermed corn meal was purchased from the Lauhoff Grain Co. (Danville, IL), while soybean oil without added antioxidants, was purchased from A.E. Staley Mfg. Co. (Decatur, IL) and from Archer Daniels Midland Co. (Decatur, IL). A Werner and Pfleiderer twin-screw extruder, model ZSK 30 (Ramsey, NJ) with screw components made of nitrited steel, was used. The temperature profiles for each experiment are given in Table I, while the screw configuration is in Table II. The feed rate was 200 g/min and the screw speed was 200 rpm. Degermed corn meal was used in the first series of experiments, while corn starch was used in the second. An extrusion die with twin dies, 8 mm i.d., 7 mm in length, was used. Moisture was added in the first extruder barrel section (29%, w/w, dry weight basis) and oil was added in the second section at (5%, w/w, dry weight basis). The extrudate was frozen in liquid nitrogen after extrusion (<5 sec) and dried in a Vacudyne freeze-drier (Vacudyne Corp., Chicago, IL) overnight to a moisture content of 7.0 ± 0.5%. Each sample was ground in a hammer mill (W.J. Fitzpatrick Co., Chicago, IL) and fractions between 30 and 60 mesh in size were used for the storage study.

Extrudate samples containing corn starch and added soybean oil were extracted with petroleum ether prior to storage for the active oxygen method (AOM) assay (*106*). For the storage study, freeze-dried, ground extrudate samples were placed in petri plates to a uniform depth of 7 mm. The petri plates were loosely covered with filter paper to allow oxygen to diffuse readily and then stored at 37 °C in the dark. After storage for selected intervals, the oil was extracted with petroleum ether. The solvent was removed under a stream of nitrogen and the peroxide value (PV) (*107*) and conjugable oxidation product (COP), plus the oxodiene value (OV) were determined (*108*). The lipid in the corn starch/soybean oil extrudate was also extracted after storage, the solvent removed and the lipid analyzed for peroxide value (*107*) and percent conjugated dienes (*109*).

For transition metal analysis (Co, Cr, Cu, Fe, Mn, No, Ni, Ti, Zn), freeze dried, extrudate samples were dried at 130 °C in a forced convection oven and ashed overnight in a muffle furnace. About 0.1 g of ash was accurately weighed and dissolved in 1.0 mL of acid (HNO_3:HCl:H_2O, 1:3:1, v:v:v) on a steam bath

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch024

Table I. Extruder Barrel Temperature (°C) Profiles

Experiment	Sections[a] 1	2	3	4	5
		corn meal-oil			
1	35	50	125	125	125
2	35	50	140	150	150
3	35	50	140	175	175
		corn starch-oil			
4	35	50	115	115	115
5	35	50	135	135	135
6	35	50	155	155	155
7	35	50	175	175	175
		corn starch-oil-iron			
8	35	50	150	150	150

a: Sections 1 through 5, starting at the feeder.

Table II. Extruder screw configuration

Section number	Screw element type X/Z[a] KB[b]/A°/Y/Z	Number of screw elements	Length of each element Z (mm)
1	S/10	1	10
	20/10	1	10
	42/21	5	21
	42/42	3	42
	28/28	2	28
2	28/28	10	28
3	28/28	2	28
	20/20	5	20
4	20/20	6	20
	KB/45°/5/14	2	28
5	20/20	5	20
	KB/45°/5/14	2	14
	20/20	4	20
	14/14	1	14
	20/20	1	20

a: X = distance (mm) to make one complete revolution.
Z = length of screw element (mm).
b: KB = Kneading Block;
A° = angle of flight;
Y = number of discs;
Z = length of screw element (mm).

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch024

for 2 h. After cooling, 0.5 mL of HF was added to dissolve any silicates and the solution was brought up to volume (10 mL) with saturated boric acid. The sample solutions were analyzed with an inductively coupled plasma spectrometer (*8*) (Jarrel-Ash Atom Comp. 110 Analyzer, Allied Analytical Systems, Waltham, MA).

To examine the effects of iron on extrudate oil stability, ferrous acetate was added to the extrudate during extrusion of corn starch and 5% (w/w) soybean oil. The extrusion temperature was 150 °C. The ferrous acetate was added to the extrudate feed water (boiled, distilled water, stored under nitrogen) at 50 ppm of iron (extrudate dry weight basis). Samples were also extruded with butylated hydroxyanisole (BHA), which was added to the soybean oil at 50 ppm (oil weight basis).

Each sample was extruded and prepared for storage and analysis as previously outlined, i.e. extruded into liquid nitrogen, freeze dried and ground. The samples were placed in plastic containers to a depth of approximately 2 cm, covered with filter paper and stored at room temperature. The lipid in the extrudate was extracted with petroleum ether, which was removed under a stream of nitrogen. The extracted oil was analyzed for peroxides (*107*) and conjugated dienes (*109*). Statistical analysis was done on a IBM 3081-GX mainframe computer with the software package SAS (Cary, NC), utilizing the MTEST statement, which is designed to test hypotheses in multivariate regression models. Samples were done in duplicate.

Extrusion Temperature and Lipid Oxidation during Storage

The quantitation of peroxides immediately after extrusion did not provide meaningful information with respect to the effect of extrusion temperature on lipid oxidation; generally the peroxide value decreased with an increase in temperature from 115 to 175 °C. This was not unexpected, since peroxides decompose rapidly at the elevated temperatures encountered during extrusion (*110*).

Statistical analysis of the conjugable oxidation product values, the oxodiene values, and the peroxide values of the lipid from the corn meal-soybean oil extrudate as a function of storage time and extrusion temperature (Figs. 1-3) indicated a significant temperature effect. Similar analysis for the results from the corn starch-soybean oil extrudate storage study (Figs. 4 and 5) also indicated a significant effect with respect to extrusion temperature and suggested that the increase in oxidation was not due to components in the corn meal. An increase in peroxide value after storage for 10-12 days was observed with an increase in extrusion temperature from 135 to 175 °C (Fig. 4). After an induction period of approximately 8 to 12 days, the differences between samples with respect to oxidative stability increased and became readily apparent. Other investigators have found the same general increase in lipid oxidation with an increase in extrusion temperature. An increase in PV with an increase in moisture content and residence time was observed for extrudates produced in a single-screw extruder (*13*). Although an increase in extrusion temperature was expected to have a deleterious effect on lipid stability, it seemed unlikely the result could be attributed entirely to temperature effects. Transition metals, such as Mo, Mn, Cr, and Fe, can substantially increase the rate of oxidation (*111-113*). A substantial increase in transition metal concentration, particularly iron, was observed with an increase in extrusion temperature (Fig. 6). Chromium, manganese and molybdenum exhibited much smaller increases; Cr, Mn and Mo increased from 0.07 to 0.14, 0.048 to 0.066, 0.11 to 0.15, mg/kg extrudate, respectively, with an increase in extrusion temperature from 115 °C to 175 °C. The iron concentration for the sample extruded at 175 °C was nearly 6 times that of the unextruded corn starch/soybean oil mixture. Most of the metals are present in concentrations that are highly catalytic with respect to lipid oxidation (0.1 to 5 ppm). The reduced stability of the

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch024

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch024

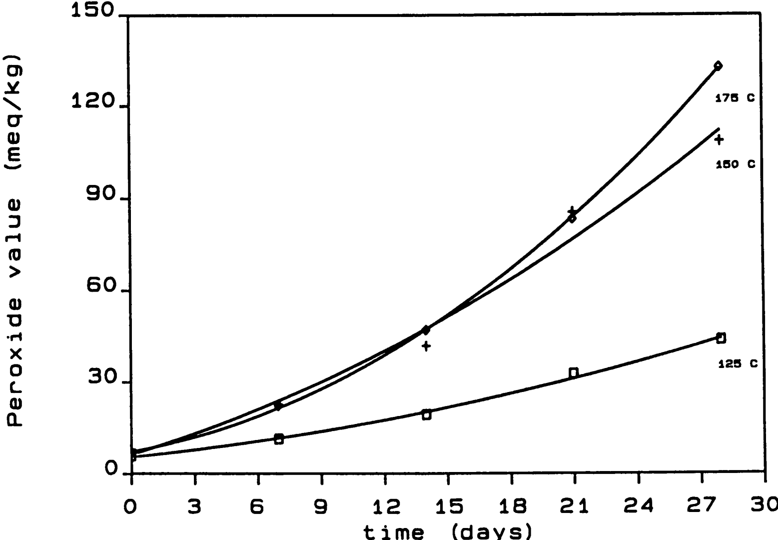

Figure 1. The peroxide values of oil extracted from corn meal and soybean oil extrudate after storage of the ground extrudate in air at 37°C. Reproduced with permission from ref. 9. Copyright 1989 Institute of Food Technologists, Chicago, IL.

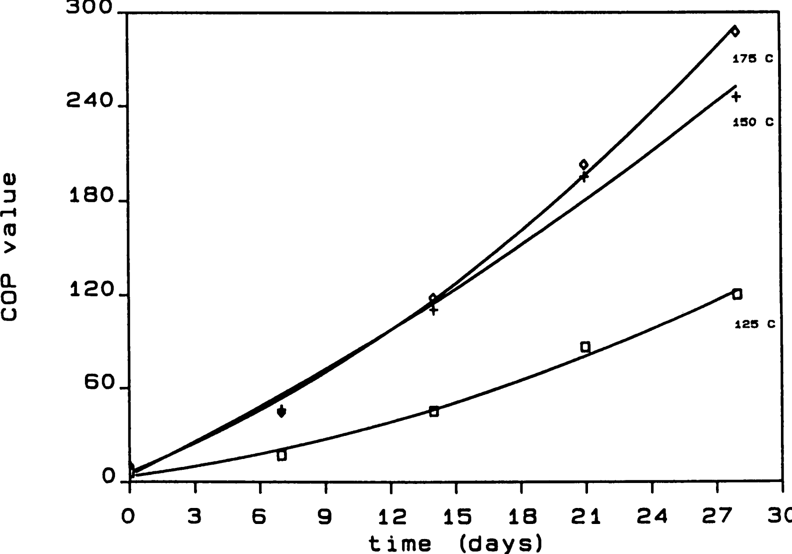

Figure 2. The conjugated oxidation product (COP) values of oil extracted from corn meal and soybean oil extrudate after storage in air at 37°C. Reproduced with permission from ref. 9. Copyright 1989 Institute of Food Technologists, Chicago, IL.

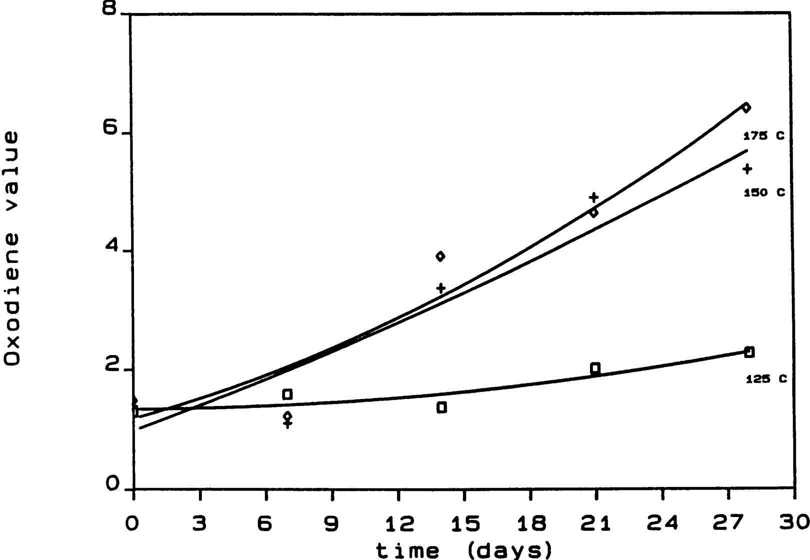

Figure 3. The oxodiene values of oil extracted from corn meal and soybean oil extrudate after storage in air at 37°C. Reproduced with permission from ref. 9. Copyright 1989 Institute of Food Technologists, Chicago, IL.

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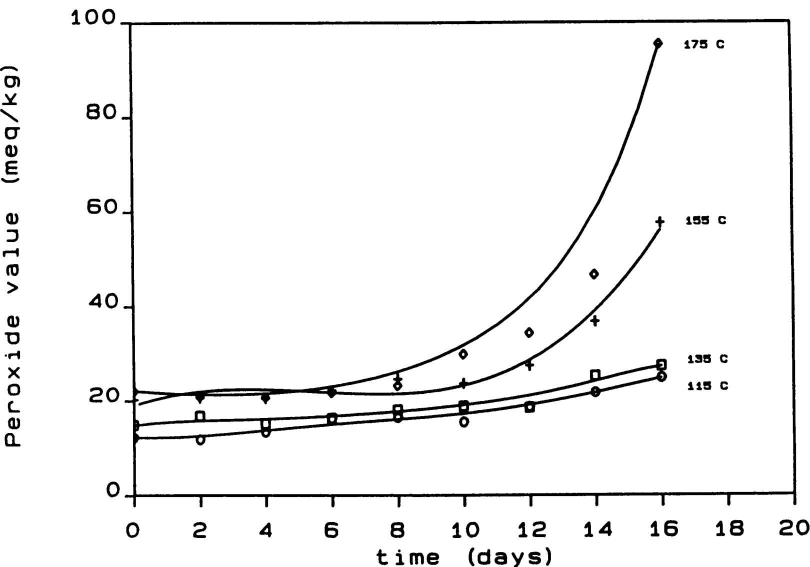

Figure 4. The peroxide values of oil extracted from corn starch and soybean oil extrudate after storage in air at 37°C. Reproduced with permission from ref. 9. Copyright 1989 Institute of Food Technologists, Chicago, IL.

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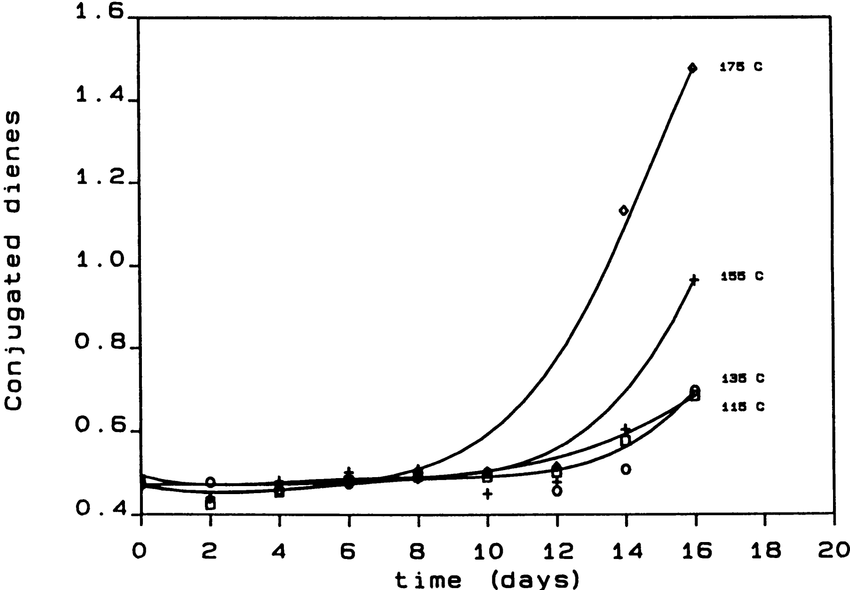

Figure 5. The percent conjugated dienes in oil extracted from corn starch and soybean oil extrudate after storage in air at 37°C. Reproduced with permission from ref. 9. Copyright 1989 Institute of Food Technologists, Chicago, IL.

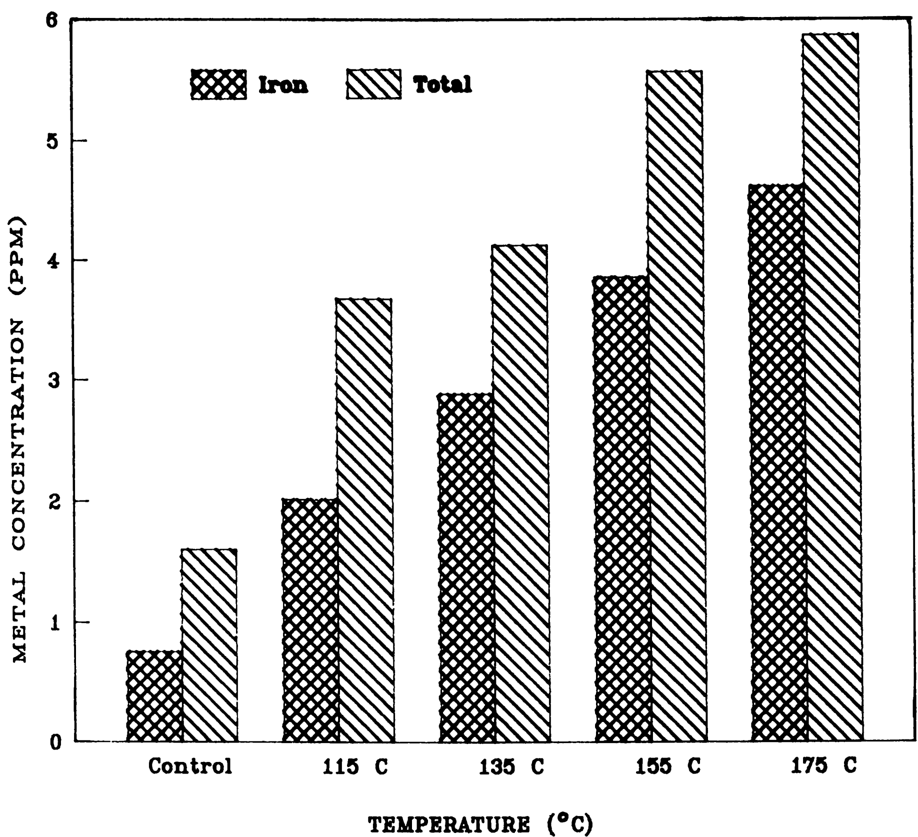

Figure 6. The iron and total transition metal content from corn starch and soybean oil before extrusion and after extrusion at 115°C, 135°C, 155°C and 175°C. Reproduced with permission from ref. 9. Copyright 1989 Institute of Food Technologists, Chicago, IL.

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch024

lipid in the extrudate after extrusion appears to be due, to a large degree, to the increased metal content.

With an increase in extrusion temperature there was generally an increase in the torque and the back pressure at the die, both of which indicate increased shear. This suggests the source of the iron (extruder components) and explains the increase in iron content with an increase in extrusion temperature. In contrast, after single screw extrusion of whole soybeans, there was no significant increase in the iron content of the extrudate (*114*). It may be that twin screw extrusion in general results in greater component wear than single screw extrusion. It may also be that the particular combination of product and extruder in the twin screw experiments (corn starch-soy oil, Werner-Pfleiderer) results in greater extruder component wear than the product/extruder combination in the single screw experiments (whole soybeans, Insta-Pro) (*114*).

To examine the effects of extrusion temperature on lipid stability independent of the corn meal and corn starch matrix, lipid was extracted immediately after freeze drying and prior to any storage, then analyzed using the active oxygen method (AOM). The AOM PV are shown in Fig. 7. Although there was an obvious deleterious effect as a result of extrusion, i.e., AOM values generally increased with an increase in extrusion temperature, there was no significant difference between any of the extruded samples. Extrusion also eliminated the induction period observed with the control.

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch024

Inhibition of Lipid Oxidation with Ferrous Acetate

The amount of oxidation in the extrudate was significantly less than the control, when BHA or iron was used (Fig. 8). The antioxidant effect of iron was even greater than that of BHA. This was probably due, at least in part, to the loss of BHA as a result of volatilization during extrusion, which may be a substantial advantage in commercial applications.

The theoretical basis for the catalyst to inhibitor conversion was discussed in depth by Black (*96*). Transition metals, including iron, catalyze the unimolecular decomposition of hydroperoxides resulting in the formation of free radicals. Although iron preferentially reacts with hydroperoxides, an excess of iron relative to the hydroperoxides results in inhibition due to the reaction of excess metal with free radicals, e.g., (peroxy, ROO• and/or alkyl, R• radicals), followed by formation of non-radical species.

Ferrous acetate was used since studies with other food systems have indicated that ferrous acetate exhibited antioxidant activity as determined by a reduction in the rate of oxygen uptake (*115*). The application of transition metals as antioxidants in food will have limited application, since the phenomena doesn't readily occur in the presence of polar solvents.

The present study indicates that within the temperature range examined, lipid stability generally decreased with an increase in extrusion temperature. The PV, COP value, OV, as well as the AOM PV, generally increased with an increase in extrusion temperature. An increase in extrusion temperature also resulted in an increase in the transition metal content of the extrudate. The effects of extrusion temperature and transition metal content on lipid stability, however, could not be separated.

It may be possible to reduce rancidity in extruded snack products by incorporation of ferrous iron at a suitable concentration (*116*). An inhibitory effect on lipid oxidation greater than that due to BHA was observed during storage of the extrudate. An added advantage is that iron fortification of extruded foods provides a much needed increase in a nutrient that is deficient in the diet of many Americans. Iron is considered one of the nutrients that is seriously deficient in the American diet (*117*). A 50 g sample of snack food containing 50 ppm of iron would provide approximately 2.5 mg or 14% of the RDA for women.

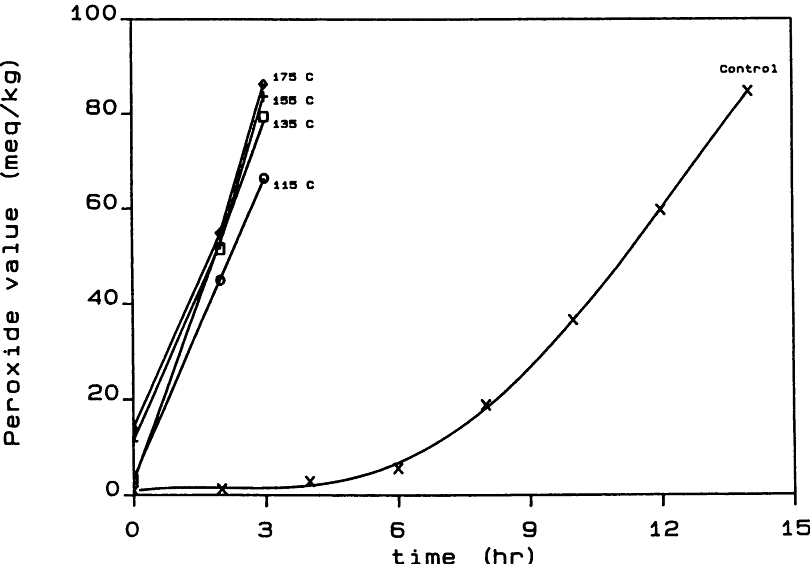

Figure 7. The active oxygen method peroxide values of oil extracted from corn starch and soybean oil extrudate. Reproduced with permission from ref. 9. Copyright 1989 Institute of Food Technologists, Chicago, IL.

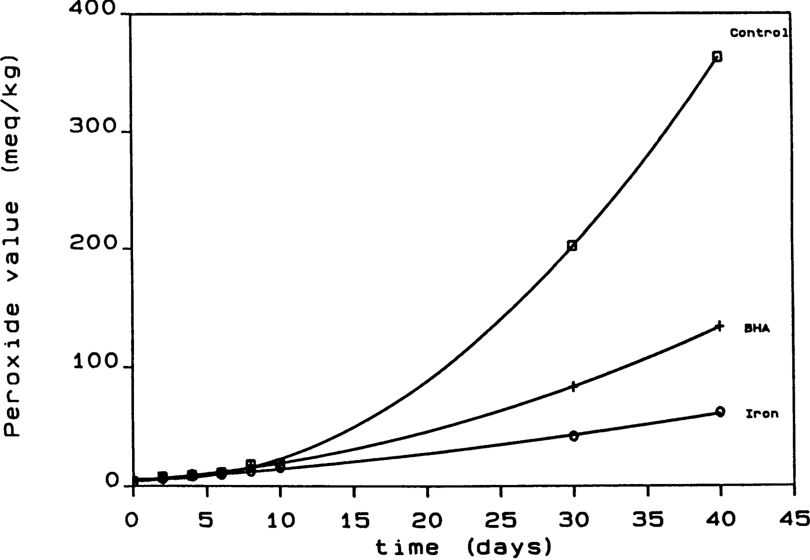

Figure 8. The peroxide values of oil extracted from corn starch and soybean oil extrudate after storage in air at room temperature. Samples containing butylated hydroxyanisole, ferrous acetate and a control without added antioxidants were extruded under the same conditions. Reproduced with permission from ref. 9. Copyright 1989 Institute of Food Technologists, Chicago, IL.

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Chapter 25

Maillard Reaction Volatile Compounds and Color Quality of a Whey Protein Concentrate–Corn Meal Extruded Product

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Samples containing five to twenty percent whey protein concentrate mixed with corn meal were extruded in an APV Baker twin-screw extruder at varying screw speeds, specific feeding loads, and moisture contents. Volatile compounds generated in this system included lipid oxidation and Maillard reaction products. Important volatiles included 2,6-dimethylpyrazine, 2-ethyl-2,5-dimethylpyrazine, 2-ethyl-3-methylpyrazine, and dimethyldisulfide. Production parameters used were important in the formation of these compounds and in color development, which is believed to be highly related to flavor.

Extrusion cooking has been practiced as a food processing procedure for over 50 years, and a large number of products from this type of processing are available on the market. Extrusion serves several functions in one operation, including mixing, cooking, foaming, puffing, and drying (*1*).

The extruder is considered to be a short-term, high temperature bioreactor that transforms raw materials into a variety of finished products (*2*). Product characteristics are greatly influenced by machine processing parameters such as the feed rate of raw materials, screw configuration, screw speed, and die conformation. Important features include the temperature generated by the processing procedure, moisture content, and nature of the ingredients or chemical constituents.

The flavor of extruded products is largely dependant upon the chemical ingredients and temperature of the extrudate. The large surface of product exposed to the processing machinery favors lipid oxidation, which can drastically influence flavor. However, the most important influence on flavor and color development is the Maillard reaction, which readily occurs at the low moisture and high temperatures generated in extruded foods. Maillard reactions usually produce product darkening, which is directly related to

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formation of flavor constituents. This is a highly desirous reaction for most extruded foods, particularly cereal products.

The Maillard reaction involves the coupling of a sugar and an amine to form 1-amino-1-deoxy-2-ketose (Amadori compound) from aldo sugars such as glucose and 2-amino-2-deoxyaldose (Heynes compound) from ketoses such as fructose. These products are important precursors for the formation of flavor compounds. Subsequent mechanisms for reactions during the elimination of the amines to form reactive flavor compounds by dehydration and fission have been proposed by many authors. The most acceptable pathways are those based on the brilliant work of Hodge (*3*). They are: (1) the elimination of the amine and dehydration to form a 1-deoxyosone that further dehydrates to compounds such as 4-hydroxy-5-methyl-3(2H)-furanone (HM-fone) from pentoses and the 2,5-dimethyl homolog furaneol (HD-fone), isomaltol, maltol, 2-acetylfuran, and cyclotene from hexoses; (2) a 1-deoxyosone equilibrium product, 1-deoxyreductone, which can retroaldo degrade by fission to yield a number of very reactive carbonyls such as pyruvic acid, diacetyl, dihydroacetone, glyoxal hydroxyacetal, and acetic acid; and (3) 1,3-enolization of the amine (Amadori or Heynes compound) to yield 3-deoxyosones that dehydrate to furfural from pentoses and 5-hydroxymethyl furfural from hexoses.

Considered by many to be the fourth part of the Maillard reaction, Strecker degradation involves oxidative deamination of amino acids by dicarbonyl compounds, such as those from 1-deoxyreductone fission, to produce aldehydes, with one less carbon atom than the original amino acid; carbon dioxide; and an α-aminoketone. The reaction of Strecker degradation dicarbonyls with other intermediates of the Maillard reaction is very important for the formation of browning flavor volatiles including heterocyclics such as furans, pyrazines, pyrroles, imidazoles, pyridines, and oxazoles.

Little data has been reported in the literature on extrusion flavor formation or the influence of protein on flavor of cereal products (*4*). Aguilera and Kosikowski (*5*) reported on the extrusion of corn-soy-whey mixtures, but were primarily interested in the stability of essential amino acids. Maga and Kim (*4*) studied the influence of several proteins on the flavor volatiles from extruded products made from corn starch. They did not identify or quantify volatile compounds, but concluded that a greater number of volatiles at higher relative concentration were produced with increasing levels of protein. They also found that low temperature and high moisture extrusion conditions resulted in low quantities of volatiles, and that high temperatures and low moisture resulted in high quantities of volatiles.

Ho et al. (*6*) studied the formation of volatile compounds from extruded corn-based model systems; they found that lipid-derived aldehydes contributed to the formation of pyrazines by Maillard reaction in a system containing zein, corn amylopectin, and corn oil. Although zein has good functional properties for extruded products, it is limited in lysine and methionine, which may decrease in concentration during heating. Whey protein is produced in surplus quantities by the cheese industry, and although it has poor extrusion characteristics, it can supply adequate amounts of lysine, methionine, and other sulfur amino acids (*7*).

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Results reported in the present paper are concerned with the identity and quantities of volatile compounds and the color of a whey protein concentrate (WPC)-corn meal product extruded under several processing conditions.

Experimental

Preparation of Food Mixture. WPC and corn meal were mixed by using a paddle mixer (Model L-100DA, Leland Detroit Mfg. Co., Detroit, MI) to prepare the feed material for extrusion. The WPC contained 34% protein, 3% lipid, 50% lactose, and 4% moisture; the corn meal contained 7% protein, 0.7% lipid, 0.5% fiber, and 12% moisture. WPC contents of corn meal mixtures prepared were 5, 10, 15, and 20%, respectively.

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Color Measurement. Colors of the extrudates were measured with a Hunter D25-PC2 colorimeter (Hunter Associates Laboratory, Inc., Reston, VA). The extrudates were ground and sieved through a 32 mesh sieve (no. 35, Soiltest Incorporated, Chicago, IL). The ground samples were packed in petri dishes, and the "L" (lightness), "a" (redness) and "b" (yellowness) values were measured. A second reading was taken after the samples had been rotated 90°.

Extrusion. An APV Baker MPF 50/25 intermeshing, co-rotating twin-screw extruder (APV Baker, Inc., Grand Rapids, MI) with a K-tron T-35 twin-screw volumetric feeder (K-tron Corp., Pitman, NJ) was used. Moisture content of the feed was controlled by a single speed, variable stroke, positive displacement water pump with an 8 mm head (Model N-P 33, Bran and Lubbe, Chicago, IL). The extruder barrel was heated by cartridge heaters in five zones. Each zone temperature was controlled electronically. The barrel temperatures were set at 26.7, 51.7, 93.3, 121.1, and 131 °C. Screw speeds were 200, 250, 300, 350, and 400 rpm; the specific feeding loads were 1.89×10^{-3}, 2.27×10^{-3}, 2.65×10^{-3}, 3.03×10^{-3}, and 3.41×10^{-3} kg/rev; and the feed moisture contents were 18, 19, 20, 21, and 22%. A die plate at the end of the barrel was 3.18 mm in diameter. A four-bladed central die face cutter was used to cut the extrudate at 350 rpm. Samples collected were dried in a fluidized bed drier at 65 °C to a constant moisture content of 7%.

Quantitative Gas Liquid Chromatography of Volatile Compounds. Headspace sampling was used to collect volatiles from a 5 g sample during refluxing in a 500 mL flask containing 25 mL of distilled water. The flask was heated at 120 °C surface temperature (100 °C product temperature) for 1 h while the sample was purged with nitrogen gas at a flow rate of 50 mL/min. Volatiles were trapped into a tube (9 X 90 mm) containing 250 mg Tenax GC (80-100 mesh) attached to the top of the condensor. The precision of this method for collecting a series of standard aldehydes, ketones, and alcohols was studied by Shin-Lee (*8*).

Trapped volatiles were desorbed in a thermal tube desorber Model 890 (Dynatherm Analytical Instruments, Inc.), and quantitation was by the method of Suzuki and Bailey (*9*) using 2-methyl-4-octanone as an internal standard.

The gas chromatograph used was a Perkin-Elmer 8500; the column was a fused silica capillary (50 m X 0.32 mm, 0.51 microns thick) SE54 (5% phenylmethyl-silicone). The split ratio of the sample was 1:10; starting temperature was 35 °C for 5 min; ramp-programmed 8 °C/min to 200 °C, then at 2 °C/min to 250 °C; flow rate (He) 2.0 mL/min. Integration of peaks was by a Perkin-Elmer LCI-100 integrator.

Gas Liquid Chromatography/Mass Spectrometry Identification of Volatiles. Separation of volatiles trapped by headspace sampling for qualitative purposes was accomplished on the same capillary column described above, except that the gas chromatograph was a Carlo Erba 4160, and the column outlet was positioned directly into the source of a double-focussing Kratos MS-25 mass spectrometer. The ionization voltage of the mass spectrometer was 70 eV; the accelerating voltage was 2 KeV; the source temperature was 200 °C; and the resolution was 600 (10% valley). The data system used was a Kratos DS-55 with a NIH/EPA data base and supplementary library systems. Supplementary library systems included the Wiley/NBS registry of mass spectral data (*10*) and the bench top/PBM version 1.0 software based on the Wiley registry of mass spectral data (*11*).

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Results and Discussion

Flavor, color, and texture are important quality features of extruded food products. Temperature changes influence all of these characteristics, and the temperature is highly dependent upon extrusion characteristics such as screw speed, moisture content, and specific ingredients of the product. In these experiments, temperature of the product was not a controlled variable since it is difficult to adjust precisely, but it was highly related to processing variables such as WPC content as shown in Table I, which is a list of the regression coefficients relating the system variables and the melting zone temperature of the product. The variables listed accounted for 93% of the variation in product temperature. There were strong negative relationships between the WPC content and the moisture content relative to temperature. This is shown in Figure 1, which is a surface response curve relating these three variables.

Higher product temperature occurred in samples containing low WPC and low moisture content. The temperature was more dependant upon the WPC content than on the moisture content. Temperature decreased from 163.5 to 142.4 °C when the WPC content was increased from 0 to 20%.

Identification and Influence of Processing Parameters on Concentrations of Volatile Compounds. GLC/MS was used to identify 71 and quantify 68 volatile compounds. These included 12 aldehydes, 10 ketones, 6 alcohols, 2 esters, 6 aromatics, and 10 hydrocarbons from lipids; and 11 pyrazines, 4 furans, 5 other heterocyclics, and 2 sulfur compounds that were probably formed by the Maillard reaction.

Volatile compounds identified in these products and their concentrations for samples containing varying amounts of WPC are listed in Table II. The

aliphatic compounds were mostly formed from the oxidation of unsaturated fatty acids and generally contribute to undesirable flavor of foods. It has been shown (*12*) that lipid stability generally decreases with increase in temperature in the range of 115 to 175 °C. This may be important during storage of corn products. Some of the saturated and unsaturated aldehydes have "green" or "tallowy" odors. The ketones, alcohols, and esters have a sweet and fruity character and aroma, and benzeneacetaldehyde has a honey-like odor (*13*).

Table I. Regression Coefficients Between Extrusion Parameters and Melting Zone Product Temperature

Parameter	*Temperature*
intercept	158.043[a]
whey protein concentrate (WPC)	-5.792[a]
screw speed (SS)	3.433[a]
moisture content (MC) of product	-4.108[a]
specific feeding load (SFL)	1.125
WPC X SS	0.900
SFL X WPC	-0.537
MC X SS	-0.250
MC X SFL	0.163
MC X WPC	0.025
WPC^2	-0.144[b]
SS^2	-0.943
SFL^2	0.193
MC^2	-0.369

[a]$P < 0.01$
[b]$P < 0.05$

2-Heptenal, 7-octen-3-ol, 1-octanol, hexanal, and 1-heptanal or associated compounds were listed by Wade (*14*) as contributing to corn odor. They may have been formed metabolically, but most were undoubtedly formed by oxidation. Many, but not all, aliphatic compounds increased in concentration in the presence of WPC, but the changes were erratic and probably influenced by other processing parameters.

Possibly the most important volatiles contributing to corn flavor are those produced by the Maillard reaction. Those identified in these experiments are also listed in Table II, and the influence of temperature on the concentrations of these compounds is revealed in Table III. The pyrazines are the most prominent volatiles of this group, and several have been implicated in the flavor of corn products. They are undoubtedly formed by the Maillard reaction, and most of them were influenced by WPC concentration and temperature.

The concentrations of pyrazines, furans, and other heterocyclics generally increased in samples relative to the amount of WPC in the product (Table II). There were dramatic increases in methylpyrazine, pyrazine, tetrahydrofuran, and N-methyl-1-H-imidazole-ethanolamine in product that contained 5-10%

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch025

Table II. Influence of WPC Concentration on Quantities (ppb) of Lipid and Maillard Reaction Volatiles

Name of Compound	*WPC Concentration*		
	0%[a]	*5-10%*[b]	*15-20%*[c]
acetaldehyde	46.8	123.6	150.0
ethanol	tr	tr	tr
butane	807.1	661.7	616.1
tetrahydrofuran/2-methyl propanal	46.4	786.8	770.8
pentane/2-pentanone	294.1	1342.7	2686.1
ethyl acetate	25.9	41.5	49.4
pentanal	155.1	1623.4	3601.3
3-propoxy-1-propene	144.2	413.5	727.0
pyrazine	13.4	165.6	133.1
dimethyl disulfide	16.3	59.1	73.6
1-H-pyrrole	8.4	33.8	37.2
methylbenzene	103.5	85.5	108.8
2,3-dihydro-4-methylfuran	19.2	12.4	25.4
hexanal	779.7	391.1	626.9
dihydro-2-methyl-3(2H)-furanone	22.5	58.5	98.3
hydrocarbon	tr	11.3	12.8
methylpyrazine	10.0	495.8	350.7
N-methyl-1-H-imidazole-ethanolamine	4.8	243.2	464.2
trimethyloxazole	29.1	28.3	40.6
2-furanmethanol	8.1	26.5	29.9
ethyl benzene	53.0	61.8	94.9
1-hexanol	2.4	1.9	20.8
1,3-dimethylbenzene	7.4	18.0	42.3
2-H-pyron-2-one	25.3	40.0	70.0
2-heptanone	39.8	40.3	62.0
1-heptene	67.2	63.5	93.7
heptanal	12.5	58.7	79.2
3-methylthiopropanal	62.2	315.5	282.0
1-(2-furanyl)-ethone/2,6-dimethylpyrazine	8.2	81.8	38.7
5-methyl-2-pyridinamine	3.8	54.2	43.7
2,6-dimethylpyrazine	2.3	66.0	66.2
ethenylpyrazine	14.2	25.9	23.6

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Table II. *Continued*

Name of Compound	*WPC Concentration*		
	0%[a]	*5-10%*[b]	*15-20%*[c]
2,3-octanedione	6.2	9.7	26.1
2-heptenal (E)	121.9	51.6	66.6
1-heptanol	98.2	152.4	205.8
7-octen-3-ol	71.3	31.0	52.0
6-methyl-5-hepten-2-one	37.4	27.1	40.8
2-pentyl furan	82.7	80.9	110.1
2-ethyl-5-methylpyrazine	29.6	20.4	26.7
2-ethyl-6-methylpyrazine	33.9	39.8	26.2
2-ethyl-3-methylpyrazine	29.3	50.5	65.9
1-(2-furanyl)-1-propanone	80.6	14.7	49.9
2-ethenyl-6-methylpyrazine	18.8	244.8	413.8
2-ethenyl-5-methylpyrazine	55.1	40.4	114.9
1-methyl-4(1-methylethenyl)-cyclohexene	27.3	14.7	26.8
3-ethyl-2-methyl-1,3-hexadiene	21.6	24.3	31.3
benzeneacetaldehyde	78.1	194.8	137.6
2-decenal	108.7	40.8	52.9
1-octanol/3,5-octadiene-2-one	36.1	15.6	26.9
2-ethyl-2,5-dimethylpyrazine	19.0	20.8	27.0
2-nonanone	31.3	37.5	20.9
3,5-octadiene-2-one	32.0	22.5	43.8
nonanal	128.3	114.6	320.1
2,3-dihydroindole	11.5	8.5	11.0
3-methoxy-3-methyl-2-butanone	13.8	12.3	16.2
2-nonenal	42.7	35.8	54.5
decanal	13.5	13.6	28.7
2,6,6-trimethyl-1,3-cyclohexadiene-1-carboxaldehyde	13.2	16.3	22.6
2,6,6-trimethyl-1,5-cyclohexadiene-1-carboxaldehyde	10.0	14.4	17.1
hydrocarbon	27.9	16.5	38.5
2-undecanone	28.1	19.6	36.1
2,4-decadienal (E,E)	101.2	109.4	112.1
hydrocarbon	21.8	22.2	64.5
hydrocarbon	21.9	23.0	41.1

Continued on next page

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch025

In Thermally Generated Flavors; Parliment, T., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 1993.

Table II. *Continued*

Name of Compound	*WPC Concentration*		
	0%[a]	*5-10%*[b]	*15-20%*[c]
4-(2,2-dimethyl-2-cyclohexene-1-yl)-3-buten-2-one	58.6	42.7	64.8
6,10-dimethyl-5,9-undecadien-2-one	133.6	120.0	164.6
pentadecane	4.7	9.4	18.1
4-(2,6,6-trimethyl-2-cyclohexene-1-yl)-3-butene-2-one	11.1	24.0	37.1
hydrocarbon	3.1	35.9	73.0
tetradecanal	6.6	23.3	42.0
hydrocarbon	7.1	11.6	16.3

tr = trace (less than 1 ppb)
[a]N = 4
[b]N = 6 (3-5% WPC + 3-10% WPC)
[c]N = 6 (3-15% WPC + 3-20% WPC)

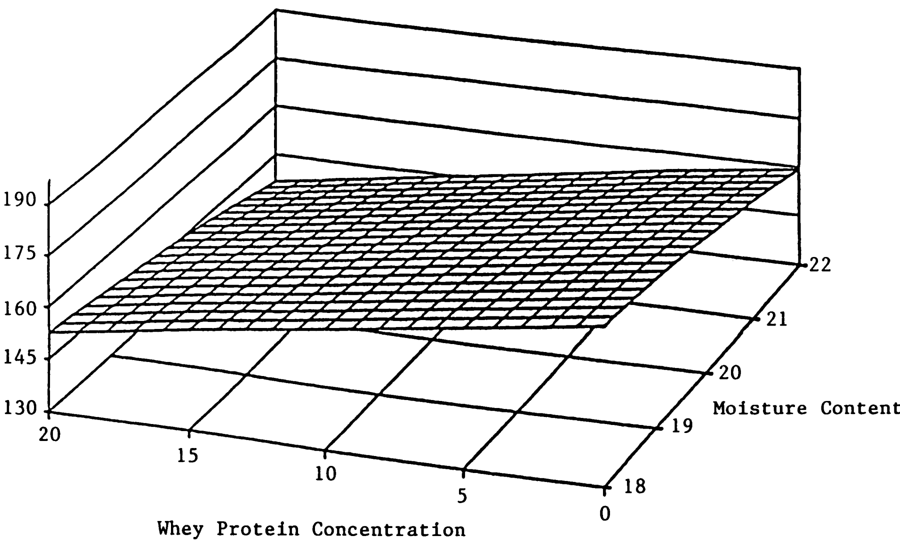

Figure 1. Response surface of melting zone product temperature (TM) versus whey protein concentrate (WPC) concentration and feed moisture content (water) of extruded WPC-corn meal products.

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch025

WPC; but the increase in other important volatiles such as 2,6-dimethylpyrazine, 2-ethyl-3-methylpyrazine, 2-ethyl-2,5-dimethylpyrazine, and dimethyldisulfide were influenced less by the concentration of the WPC.

2,6-Dimethylpyrazine, 2-ethyl-2,5-dimethylpyrazine, and 2-ethyl-5-methylpyrazine have been recognized as having a sweet, corn-like odor (*15*). The formation of pyrazines during extrusion cooking has been studied in potato flakes by Maga and Sizer (*16*), who found that pyrazine formation increased with increase in temperature up to 160 °C, but at 190 °C they decreased in concentration. The pyrazines contribute to roasted flavors and have low flavor thresholds.

Table III. Influence of Melting Zone Product Temperature on Concentration of Maillard Reaction Volatiles

Name of Compound	*Concentration (ppb)*	
	140-152 °C[a]	*160-174 °C*[b]
Pyrazines		
pyrazine	67.0	204.9
methylpyrazine	168.4	598.8
2,6-dimethylpyrazine	34.1	82.3
2-ethyl-6-methylpyrazine	23.8	47.2
2-ethyl-3-methylpyrazine	46.1	61.8
2-ethenyl-5-methylpyrazine	71.5	75.6
2-ethyl-2,5-dimethylpyrazine	25.7	18.7
Furans		
tetrahydrofuran	228.7	1237.7
2,3-dihydro-4-methylfuran	20.1	17.3
2-furanmethanol	21.9	27.7
dihydro-2-methyl-3(2H)-furanone	64.6	71.1
Other Heterocyclics		
1-H-pyrrole	20.2	45.0
N-methyl-1-H-imidazole-ethanolamine	186.5	430.0
trimethyloxazole	28.7	40.4
2-furanmethanol	21.9	27.7
5-methyl-2-pyridinamine	38.2	42.5
2,3-dihydroindole	9.0	11.7
Sulfur Compounds		
dimethyldisulfide	42.5	77.1
3-methylthiopropanal	208.4	315.9

[a]N = 7
[b]N = 9

The formation of pyrazines can readily be accounted for by the Maillard reaction as outlined in Figure 2. Amadori compounds formed by amine-

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Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch025

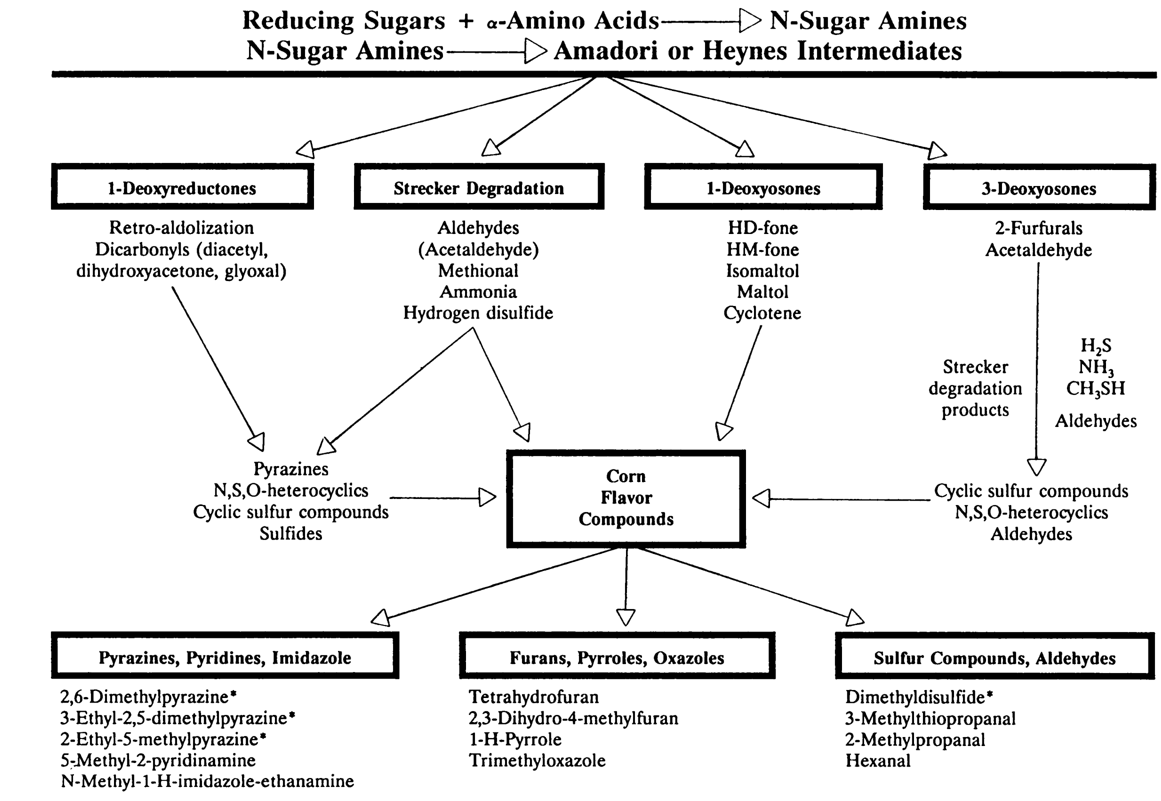

Figure 2. Formation of some important Maillard reaction volatile compounds from whey protein concentrate-corn products by extrusion.

carbonyl coupling can be transformed into α-dicarbonyls, which can react with amino acids by Strecker degradation to form α-aminoketones. The latter are dehydrated and oxidized to substituted pyrazines (*17*).

Furans can arise from sugar or lipid degradation. Tetrahydrofuran and its derivatives are widespread and have been found in roasted foods such as coffee, cocoa, and heated vegetable proteins (*18*). They usually have caramel-like odors and are very reactive with other components such as ammonia and hydrogen sulfide.

Furanones such as dehydro-2-methyl-3(2H) furanone are intermediates for many secondary reactions in the Maillard series (Figure 2). They readily react with hydrogen sulfide to form thiophenes and thiophenones.

Dimethyldisulfide (DMS) is an important contributor to corn flavor, and it has been identified in corn products by many workers including Dignan and Wiley (*19*). It could have been produced via extrusion by the Maillard reaction, or by enzymatic degradation of 5-methyl-L-cysteine sulfoxide which occurs in some foods (*20*). The latter mechanism appears unlikely since DMS is not found in raw corn but does appear at ppm levels upon cooking (*21*). The threshold for DMS 12 ppb (*22*).

Chen et al. (*23*) reported that the toasted corn taste of extrudates was affected significantly by temperature and by interaction between temperature and moisture. Increasing the temperature and decreasing the moisture resulted in a marked increase in toasted corn taste due to Maillard reactions. Whey proteins with a high percentage of lactose contributed significantly to Maillard reaction during extrusion cooking.

Extrusion and Color. Changes in product color during processing can provide important information concerning degree of thermal treatment provided by the extrusion process, and are directly related to the flavor of the product. Some of the color of the product is due to the corn pigments, but the colors of the samples heated at the higher temperature with high levels of WPC were due to Maillard browning.

Color change was strongly related to temperature of heating, moisture content, and WPC as shown in Table IV. Samples containing high WPC and high moisture had greater "L" values, lower "a" values, and higher "b" values than the samples containing high WPC and low moisture. The latter samples were darker (lower "L" value) in color and also more red (higher "a" value) in color than samples having no WPC. Samples containing high moisture and low WPC were lighter in color (low "L" value) and more yellow (higher "b" value) in color. Moisture content had a greater overall effect on color than WPC concentration.

Conclusions

WPC-corn meal product flavor compounds are formed from oxidation and Maillard reactions. Aldehydes, alcohols, pyrazines, and dimethyldisulfide are important compounds formed by these reactions.

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The degree of flavor and color development is determined by the extruder parameters that influence temperature, such as screw speed, WPC concentration, and percent moisture.

Table IV. Parameter Estimates for Lightness, Redness and Yellowness of Extruded WPC-Corn Meal Products

Parameter	*Parameter Estimate*		
	L[a]	*a*[b]	*b*[c]
intercept	55.0286[d]	6.9286[d]	25.0714[d]
whey protein concentrate (WPC)	-1.7375[e]	1.0625[d]	-1.2750[e]
screw speed (SS)	0.9875	-0.4375[d]	-0.1667
specific feeding load (SFL)	1.0042	-0.3542[d]	0.1750
feed moisture content (MC)	3.7042[d]	-0.7708[d]	2.0583[d]
WPC X SS	-0.4063	0.2063	0.0625
SFL X WPC	0.0563	0.1688	0.1625
SFL X SS	-0.4438	0.1438	0.2125
MC X WPC	1.0688	-0.2938	-0.0250
MC X SS	-0.4813	0.1813	-0.0250
MC X SFL	-0.6438	-0.0063	-0.4000
WPC^2	2.7960[d]	-0.8499[d]	1.3551[d]
SS^2	-0.3165	0.2251[f]	-0.0574
SFL^2	-1.1040[f]	0.2376[e]	-0.6449
MC^2	0.3585	-0.3249[d]	-0.0824
Total R^2	0.88[d]	0.88[d]	0.81[e]

[a]Lightness
[b]Redness
[c]Yellowness
[d]$P < 0.001$
[e]$P < 0.01$
[f]$P < 0.05$

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Chapter 26

Ammonium Bicarbonate and Pyruvaldehyde as Flavor Precursors in Extruded Food Systems

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Extrusion technology is fast becoming one of the most important mechanisms to add value to a variety of food products. One very important class of extruded products is "ready-to-eat" breakfast cereals. An attempt to enhance the flavor quality of breakfast cereals has resulted in investigating the application of flavor precursors to extruded food systems. The effects of the addition of ammonium bicarbonate and pyruvaldehyde on the aroma profile produced in extruded wheat flour were examined. The results indicate that the addition of these compounds yielded enhanced levels of heterocyclic pyrazines which imparted more of a toasted aroma character to the product.

Extrusion cooking is classified as a high temperature short time process (HTST) in which a raw material is fed into a heated barrel and conveyed through it by the action of a rotating screw. Camire (*1*) summarized extrusion as an operation which combines the processes of heating, transport, mixing, working, and forming. Extrusion has been used to alter the texture and appearance of raw food materials and is used to produced a wide array of "ready-to-eat" breakfast cereals and snack foods (*2*). Extruders offer the food processor the convenience and flexibility of a continuous process that requires little space and capital investment.

However, extrusion is not without its shortcomings, especially in reference to flavor generation (*3*). As was mentioned previously, the process is continuous. This leaves little time for favorable roasted, toasted, or bakery-type aromas to develop in food systems. Therefore the extent of flavor formation is minimal, resulting in products of a bland or uncooked aroma character. It is for this reason that alternative forms of flavoring extruded foods have been applied.

Extruded products can be flavored either before or after extrusion. If flavoring is added to the mix before extrusion, then the product exhibits a more uniform flavor character. However, high losses of flavor are incurred due to thermal degradation and flash-off at the die (*4*). For this reason overdosing the flavor system 10 to 50 times is usually necessary. Depending on the flavor one is trying to achieve

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in the product, this can become a costly endeavor. To avoid these problems, flavoring can be added to the product after emerging from the die. This can be done by either applying dry flavor mixtures or spraying the product with flavoring agents dissolved in oil. Even when these applications are successfully achieved, the interior of the product still exhibits a bland taste devoid of toasted flavors.

Given the shortcomings of these two techniques, novel research focuses on the use of reactive flavor precursors added to the extruder feed before processing. The hypothesis behind this is governed by the idea that the extruder will provide the precursors with enough thermal and mechanical energy to induce the formation of the aroma chemical during the process. During extrusion starch and protein are fragmented (*5*), resulting in the formation of simple sugars and free amino acids. These compounds then react to form various dicarbonyl and amino carbonyl compounds that condense during the final stages of the Maillard reaction to generate heterocyclic flavor compounds. Since the residence time in the extruder is limited, this sequence of chemical events may not progress to completion. By adding flavor precursors to the mix before extrusion, the production of volatiles is enhanced because the initial events that lead to flavor formation are surpassed. The objective of this research was to exploit this idea in an extruded wheat flour system in an attempt to remove the "raw dough" flavor character. This work focuses on the effect of extrusion on flavor formation in wheat flour, as well as how the addition of an exogenous nitrogen and dicarbonyl source effects the final aroma of the product.

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Experimental

Materials. High gluten wheat flour (14% protein) was obtained from the Bay State Milling Company (Clifton, NJ) and is sold under the trade name *Bouncer*. Pyruvaldehyde and ammonium bicarbonate were purchased from the Sigma Chemical Co. (St. Louis, MO).

Formulation of Precursor Solutions. Precursor solutions were mixed immediately prior to extrusion. The first solution consisted of ammonium bicarbonate in distilled water at a 154 mg/mL level. The second solution consisted of pyruvaldehyde and ammonium bicarbonate at levels of 46 mg/mL and 154 mg/mL, respectively. Solutions were kept cool until used.

Extrusions. All extrusions were carried out on a Werner Pfliederer ZSK-30 co-rotating twin-screw extruder (Werner Pfliederer, Ramsey, NJ). The unit was equipped with a die exhibiting two 3 mm diameter openings. The length and diameter of each screw were 900 mm and 30 mm, respectively. The barrel was induction heated and possessed five independently controlled heating zones. Product temperatures were recorded by a thermocouple inserted at the die plate. Wheat flour was fed into the unit with a K-Tron series 7100 volumetric feeding system (K-Tron Corp., Pitman, NJ). A metering pump (U.S. Electric Motors, Milford, CT) was used to add the liquid feed (precursor solutions).

A total of three extrudates were produced: extruded wheat flour (no precursor added), extruded wheat flour + ammonium bicarbonate, extruded wheat flour + ammonium bicarbonate + pyruvaldehyde. All extrudates were produced under identical conditions of temperature, feed moisture, and screw speed (180°C melt temperature, 16% feed moisture, 450 rpm). To facilitate the production of the precursor-added samples, the extruder was equilibrated with water, fed via a reservoir. Once proper conditions were met, the liquid feed was switched by way of a valve to a second reservoir containing the reactants. Samples were then collected 5

minutes after the switch. All extrudates were stored at 10°C in one-quart Mason jars until analyzed.

Volatile Isolation. Each extrudate was ground in the presence of dry ice in a benchtop grinder (Glen Mills, Maywood, NJ) and passed through a 24 mesh sieve to achieve uniform particle size. Ten grams (dry basis) of each sample was weighed into a glass cylinder which was connected to a thermal desorption sample-collecting system (Scientific Instrument Services, Ringoes, NJ). At this time an internal standard (1 mL of 1 mg/mL toluene-d_8 in methanol) was spiked directly into the matrix of the solid sample to facilitate quantitative analysis. Purge and trap isolation was then performed on the sample and the volatiles were trapped onto a polymer cartridge consisting of Tenax (Alltech) and Carbotrap (Supelco). The conditions for trapping were as follows: 80°C heating block temperature, 40 mL/ min nitrogen gas flow, and 1 hour purge time. After trapping was completed, the polymer cartridges were purged an extra 30 minutes with nitrogen (40 mL/min) at ambient temperature to remove excess water.

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Volatile Analysis. The trapped volatiles were desorbed directly into the GC column (220°C, 5 min, helium flow 1 mL/min) using a Model TD-1 short-path thermal desorption apparatus (Scientific Instrument Services, Ringoes, NJ) (*6*). Separation of the volatiles was accomplished using a Varian 3400 gas chromatograph equipped with a nonpolar fused silica capillary column (60 m x 0.32 mm i.d., 0.25 mm film thickness, DB-1; J&W Scientific). The GC was operated with an injector temperature of 250°C, a helium carrier gas flow rate of 1 mL/min, and a split ratio of 10:1. The program for volatile separation was as follows: initial column temperature of -20°C with a 5 minute hold during thermal desorption and a temperature increase of 10°C/min from -20°C to 280°C with a 20 minute isothermal hold. The separated volatiles were then detected and identified with a Finnigan MAT 8320 high resolution mass spectrometer. The ionization was set at 70 eV and the source temperature was 250°C with a filament emission current of 1 mA. Spectra obtained were identified by utilizing an on-line computer library (NBS) and the Eight-Peak Mass Spectra Series (*7*). Linear retention indices were determined through the use of a C_5-C_{26} *n*-parrafin standard (Alltech Associates) according to the method of Majlat et al. (*8*).

Results and Discussion

A summary of the volatiles produced from the extrusion of wheat flour with and without added precursors is depicted in Table I. Each compound is presented with its corresponding retention index and resulting semiquantitative data. A total of 23 volatile compounds were identified in the study. These included 8 aldehydes, 4 alcohols, 3 ketones, 2 furans, 5 pyrazines, and 1 sulfur-containing compound. It is interesting to note that the majority of the aroma compounds present in the unextruded wheat are aldehydes and alcohols, products derived mainly from lipid degradation. The flour itself however is very low in lipid content, only about 4%. It is possible that the concentration of such volatiles was enhanced during the milling process leaving these components as the major volatiles. When the flour is extruded however, with or without added precursors, the concentration of volatile aldehydes and alcohols diminishes dramatically, especially for hexanal and hexanol.

When the flour is extruded, heterocyclic pyrazines are generated which are derived from amino-carbonyl interactions. Pyrazines possess a roasted, toasted, or

Table I. Volatiles Generated in Extruded Wheat Flour Systems

COMPOUND	R.I.	UNEX [a]	EX [b]	EX+AB [c]	EX+AB+PA [d]
		Volatile Concentration (ppm)			
Aldehydes					
3-Methylbutanal	641	-	.0208	.0059	.0198
Pentanal	680	.3114	.0423	.0221	.0212
Hexanal	785	4.6571	.8382	.5559	.3591
Heptanal	890	.1334	.0169	.0171	.0094
Benzaldehyde	944	-	.1382	.0735	.0789
Ethylpentanal	1048	.1139	.0472	.0242	.0103
Nonanal	1098	.3233	.0178	.0609	.1218
2-Nonenal	1153	-	-	-	.0286
Alcohols					
Pentanol	781	.5949	-	-	-
2-Furanmethanol	855	-	-	-	.0174
Hexanol	867	7.1221	.0345	.0430	.0248
1-Octen-3-ol	979	.2846	.0312	.0191	.0144
Ketones					
5-Methyl-2-hexanone	870	-	.0102	.0107	-
2-Heptanone	880	.1549	.0172	.0116	.0077
3-Octen-2-one	1025	.1948	.1140	.0568	.0191
Furans					
2-Propylfuran	676	.0603	.0148	.0060	.0202
2-Pentylfuran	995	.4249	.0666	.0870	.0640
Pyrazines					
Pyrazine	720	-	.0119	.1000	.3231
Methylpyrazine	819	-	.0261	.0210	.8595
2,6 - Dimethylpyrazine	905	-	-	-	.2582
2,5 - Dimethylpyrazine	911	-	-	-	.0205
2-Vinyl-5-methylpyrazine	1005	-	-	-	.0107
Sulfur-Containing					
Dimethyl trisulfide	959	-	-	-	.0096

[a] UNEX = Unextruded wheat flour; [b] EX = Extruded wheat flour; [c] AB = Ammonium bicarbonate added; [d] PA = Pyruvaldehyde added

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nutty aroma character and are important flavor components in a variety of heated foods (*9*). When wheat flour is extruded with ammonium bicarbonate, a more reactive ammonia source, the amount of total pyrazines increases. Presumably, this is because the reactive ammonia introduced into the sample is participating in the formation of Amadori products, catalyzing sugar degradation and contributing nitrogen moieties to the pyrazine structure. In the sample which contains wheat flour, ammonium bicarbonate, and pyruvaldehyde, the concentration of pyrazines increased dramatically. This illustrates the importance of the presence of both a reactive carbonyl source and a reactive amino source to the "time limited" formation of such compounds in an extruded system.

Pyrazine Formation. If one looks more closely at the trends of pyrazine formation in each of the extruded samples, it can be seen that in the case of the wheat flour sample alone and that with ammonium bicarbonate, only unsubstituted pyrazine and methylpyrazine are formed. The sample with added ammonium bicarbonate produced higher levels of unsubstituted pyrazine. This means that the degradation of any sugars hydrolyzed from the starch backbone during extrusion produced two-carbon and three-carbon fragmentations, which in turn resulted in the production two- and three-carbon amino carbonyl fragments. It is these fragments which are known to condense to form pyrazine and methylpyrazine (*10*). The question then arises as to why no dimethylpyrazine was detected in these samples, since this compound can be formed by the condensation of two three-carbon amino carbonyl fragments. A possible explanation for this can be hypothesized by examining studies on the kinetics of alkylpyrazine formation (*11*). It has been shown that the energy of activation for the formation of pyrazine and methylpyrazine is lower than that of dimethylpyrazine and other highly-substituted pyrazines. Therefore, it is possible that in this system the condensation of similar two-carbon amino carbonyl fragments or the condensation of a two- carbon fragment with a three-carbon fragment is more chemically favored than is the condensation between two three-carbon amino carbonyl fragments. In the sample containing ammonium bicarbonate and pyruvaldehyde, these two pyrazines are the major components, probably due to the fact that their formation is kinetically favored. However, 2,6-dimethylpyrazine, 2,5-dimethylpyrazine, and 2-vinyl-5-methylpyrazine are also produced. The fact more pyrazine and methylpyrazine are generated in this sample, coupled with the appearance of other substituted pyrazines, shows that the addition of reactive ammonia and dicarbonyl sources is needed to produce greater quantities and diversities of these compounds.

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Conclusions

The data suggest that the addition of precursors to extruded food systems can enhance the formation of pyrazine aroma compounds and thus boost toasted aroma character. The addition of ammonium bicarbonate to the wheat flour systems did not enhance the formation of pyrazines as much as did the addition of both ammonium bicarbonate and pyruvaldehyde. This suggests that even though starch fragmentation and protein hydrolysis occur during extrusion, the levels of macromolecular breakdown are still too low to facilitate adequate flavor generation.

After breakdown, the resulting sugars and amino acids released from the macromolecules must be further degraded to form amino carbonyl fragments, and finally aroma compounds. Time in the extruder is too short to complete these sets of reactions. Since little can be done during extrusion to lengthen the duration of

reaction, we must increase the reactivity as well as the amount of available reactants so as to achieve more concentrated and diversified levels of pyrazines. Consequently, the addition of reactants in the form of specific Maillard precursors is a promising way to enhance and control the aromas generated in extruded foods.

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Chapter 27

Collection and Characterization of Volatile Compounds Released at the Die during Twin Screw Extrusion of Corn Flour

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A specially designed collection apparatus was used to collect the volatile compounds released at the extruder die during twin screw extrusion of corn flour. The collected volatile compounds were analyzed using GC-MS. Among the 91 compounds identified, furans, pyrazines, pyridines, thiazoles, thiophenes and pyrrolizines were the major aroma compounds released at the die of the extruder during processing. Two sensory significant proline-specific Maillard reaction products, 5-acetyl-2,3-dihydro-1*H*-pyrrolizine and 5-propionyl-2, 3-dihydro-1*H*-pyrrolizine were identified. Several carotenoid decomposition products such as α-ionone, dihydro-β ionone, β-ionone and dihydroactinodiolide were also identified. The volatile compounds released at the extruder die were compared with the volatiles recovered by purge and trap analysis of the extrudates. The volatile profiles show significant differences in their chemical composition and provide additional information on possible mechanisms of flavor generation during extrusion cooking.

Extrusion cooking has gained immense popularity in the food industry in recent years. The versatility, energy efficiency and economical advantage of extrusion processing has led to the utilization of extruders in the manufacture of numerous foods such as breakfast cereals, snacks, and precooked products as well as many pet foods.

There is a need to continously maximize the quality of extruded foods. Many processors rely on post-extrusion flavor application due to the high temperature and pressure inside the extruder as well as volatile losses that occur at the extruder die during processing. Addition of flavorants prior to extrusion and flavor encapsulation methods are currently being studied to simplify and make extrusion processing more economical.

It is important to determine the volatile compounds that are released at the die during extrusion. Characterization of these compounds in comparison to the compounds that are recovered from extrudates can provide information on the qualitative and quantitative distribution of the thermally generated volatiles. In addition, the information also aids in understanding flavor retention and its release as influenced

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by the chemical structure of the biopolymers in the matrix and also provides a scientific basis for the optimization of extrusion conditions to improve product quality.

The mechanism of flavor losses that lead to decreased volatile recovery from the product has been previously explained (*1*). As the extruder transports the material and forces it against the exit die by means of rapidly rotating screws, a high amount of pressure and shear are generated inside the extruder. As the cooked product is forced out at the die, the material passes from a "high pressure" region to atmospheric pressure. This process is analogous to the application of vacuum. Water is flashed off as steam during this process which simultaneously results in loss of volatile compounds with the water vapor at the die of the extruder.

Few authors have studied the recovery of model compounds from extrudates. It has been suggested that volatility in aqueous solutions is a complex phenomenon and that the retention of particular volatile compounds would depend on the molecular size and the volatility of the compound (*2*). The recovery of preextrusion added model flavor compounds has been studied (*3*). Approximately 90% of the added flavor compounds could not be recovered from the extrudates.

There is very limited information available to date on the volatiles released at the extruder die. A thermally controlled collection chamber connected to the extruder die was used to study recovery and losses of preadded model flavors (*4*). The authors evaluated different models for volatile losses that occur at the die during extrusion cooking. The chamber allowed the collection of the extruded solids while the volatile compounds that accumulated in the headspace of the chamber were flushed into cold traps by nitrogen. This procedure collects volatile compounds that are released at the die and may also include volatiles that are simultaneously released from the solid extrudate in the chamber, due to the flushing of the chamber with nitrogen.

A specially designed collection apparatus was constructed and used to study the loss of preadded volatile compounds in corn meal (*5*). The apparatus was reproducible and allowed the collection of volatiles directly released with the steam at the extruder die.

The present study involved the collection of thermally generated volatiles released at the extruder die during processing of corn flour using the same apparatus as that used by Chen *et al* (*5*).

The objectives of this study were to collect volatiles released at the die and characterize these compounds in comparison to the volatiles recovered from the corresponding solid extrudate matrix. The extrudate sample utilized for the characterization of volatiles was a high amylose corn flour extrudate selected based on its sensory properties from a series of extrusion experiments.

Materials and Methods

Extrusion. High amylose corn flour (Microcrisp) containing 70% amylose (National Starch and Chemical Co., Bridgewater, NJ) was used as the feed material. Two types of adsorbents, Tenax TA (Alltech Associates, Inc., Deerfield, IL) and Carbotrap (Supelco, Inc., Bellefonte, PA), were used for purge and trap analysis of volatiles from the extrudates.

A ZSK-30 twin screw extruder (Werner and Pfleiderer, Ramsey, NJ) with a screw configuration having six kneading blocks and two reverse elements with a L/D ratio of 30 was used for the extrusion. Extrusion conditions were as follows : moisture content (12.5 %), total mass flow rate at the die (200 g/min), screw speed (500 rpm), die temperature (178°C), specific mechanical energy (1709 kJ/kg), torque (63%) and average residence time (46 sec). The moisture content of the raw flour was 9.5%. During extrusion, it was adjusted to 12.5% using a positive displacement pump. Final moisture content of the extruded sample was 4%. The moisture contents reported are on wet basis and were determined by the standard AOAC method. Extrudates were not

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subjected to further drying. The extruded samples, were cut into shapes with a pelletizer, allowed to cool to room temperature, and were stored in gas-tight glass containers at -20° C until further analysis.

Collection and Analysis of Volatiles Released at the Die. The collection apparatus consisted of a sample container, a collection adapter, a condenser and two cold traps in series at -60°C and -90°C, respectively. A relatively low vacuum of approximately 200-400 mm Hg was used to trap the volatiles flashed off with the steam at the extruder die during a collection time of approximately 15 minutes. A schematic representation of the collection apparatus is shown in Figure 1. This apparatus had been previously used in order to study the recovery of model volatile compounds (*5*). A water condenser, connected to the extruder die and cooled by circulating tap water (10°-25°C), collects most of the condensed steam along with the volatiles released off at the die. The cold traps were each washed with 100 mL methylene chloride immediately after the collection period and combined with the water condensate in the condenser. The sample was extracted by steam distillation-extraction using the Lickens-Nickerson method for 3 hours. The extract was further concentrated to 3 mL using a Kuderna-Danish apparatus and 1 µL of the extract was analysed by direct injection - GC-MS.

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Analysis of Volatiles from Extrudates. Volatiles from the extrudates were analysed by the purge and trap - thermal desorption method. The frozen extrudate sample was allowed to equilibrate to room temperature and the extrudate was ground to 20 mesh size in a grinding mill (Thomas Scientific, U.S.A). The ground extrudate (4.61 g) was filled in a prebaked glass tube (0.5 in. o.d. x 0.36 in. i.d. x 14 in. long) and sealed at either end with glass wool plugs. The tube was placed in a short path thermal desorption oven (Scientific Instrument Services, Inc., Ringoes, NJ) and purged at 50°C with nitrogen at a flow rate of 40 mL/min for 1 hour. The volatiles were trapped in a silanized and preconditioned steel absorbent tube containing 100 mg each of Tenax TA and Carbotrap. The steel trap was backflushed with nitrogen for 40 min to remove the water condensed on the trap. Then the steel trap was placed in a short-path thermal desorption unit (SIS Model 1, Scientific Instrument Services, Inc., Ringoes, NJ) connected to the injection port of a Varian 3400 GC. Desorption was carried out at 220°C for 5 minutes followed by GC-MS analysis. Quantitative reproducibility studies of selected volatile compounds from cereal products by dynamic headspace-gas chromatography has been reported earlier. (*6*).

Gas Chromatography-Mass Spectrometry. A 8230 Finnigan mass spectrometer in the electron ionization mode (70ev) coupled to a Varian 3400 gas chromatograph was employed for the identification of volatile compounds. A DB-1 column, 60 m long and 0.32 mm i.d. (J&W Scientific, Folsom, CA), was used for the analysis. GC conditions for analysis of volatiles from the extrudates by purge and trap-thermal desorption method were : -20°-280°C at 10°C/min with a 5 min final hold time at split ratio of 10:1. Conditions for analysis of volatiles concentrated from the collection apparatus by direct injection were : 35°-115°C at 2°C/min with a 10 min initial hold time and 115°-280°C at 4°C/min with 10 min final hold time at split ratio of 50:1. The injector temperature was held at 250°C. Identifications were made by on-line comparison of the mass spectra of the unknowns with the National Bureau of Standards computerized data base. A Finnigan Mat SS300 data system was used for the data acquisition.

Results and Discussion

Chemical Classes of Identified Compounds. Table I lists the different classes of identified compounds and the relative number identified in each class. Figure 2a shows a volatile profile of the compounds released at the die while Figure 2b shows the

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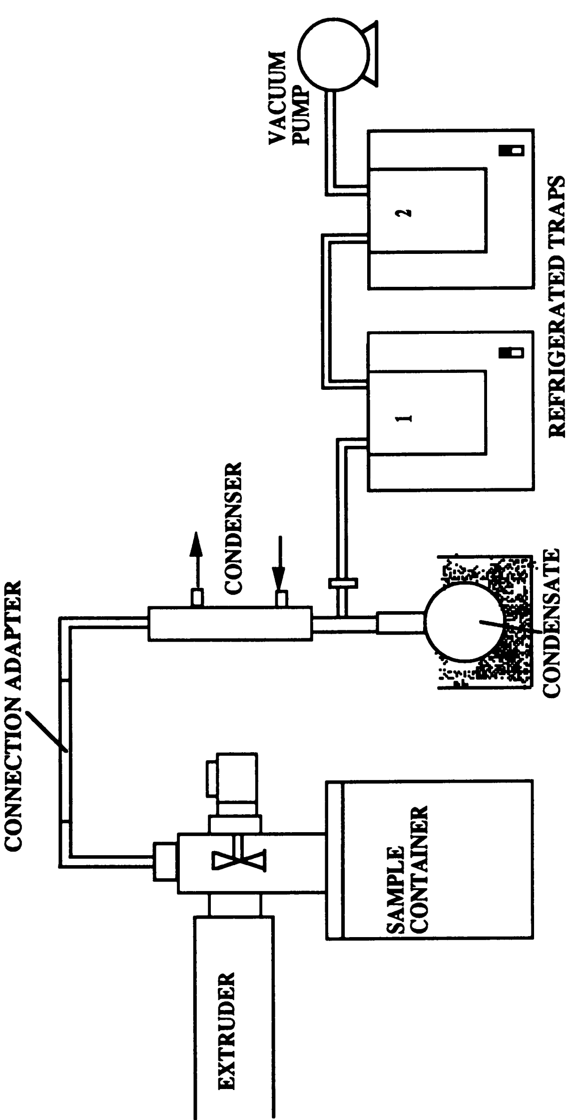

Figure 1. Apparatus for collecting volatiles at the extruder die.

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Table I. Classes of Volatile Compounds

Compounds	*Recovered from Extrudates*	*Released at the die*
Furans	9	12
Sulfur Compounds	7	8
Pyrazines	7	9
Pyridines	2	2
Other Nitrogen Compounds	5	2
Nonaromatic Hydrocarbons	4	11
Ketones	7	13
Aldehydes	10	13
Alcohols	1	1
Esters	1	13
Acids	0	4
Aromatic Hydrocarbons	3	3

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch027

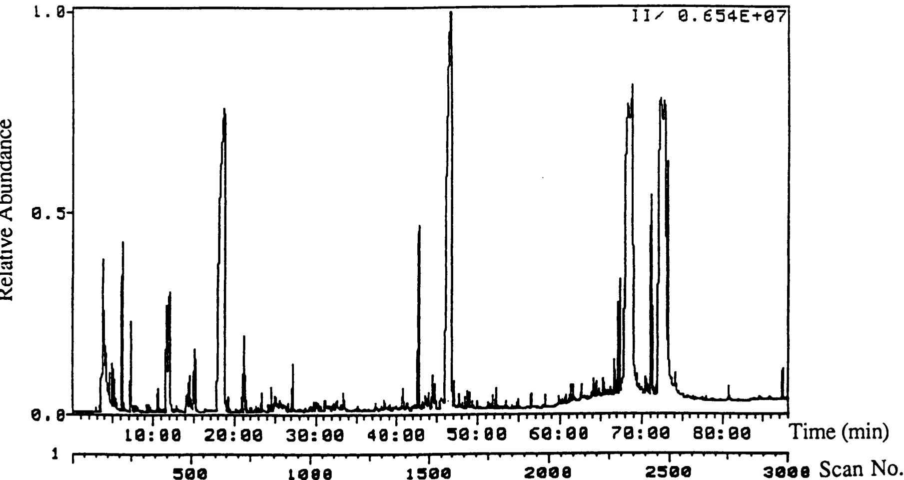

Figure 2a. EI-GC-MS of extruder condensate.

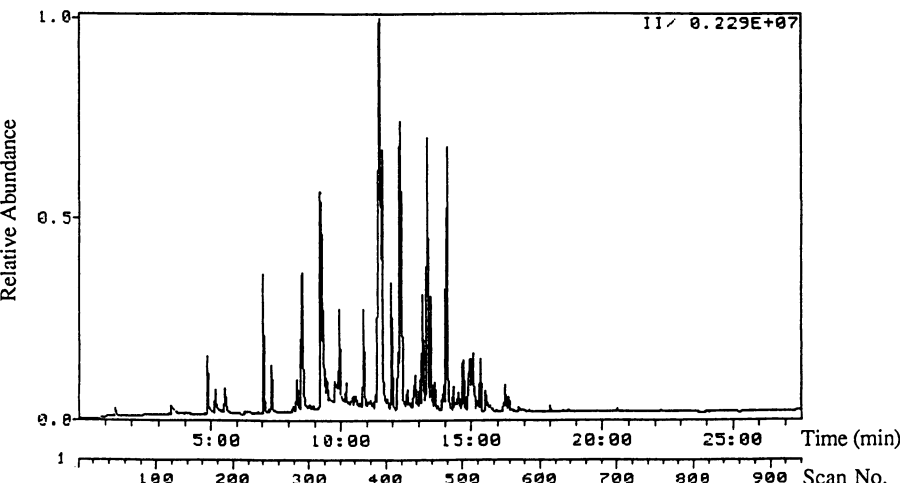

Figure 2b. EI-GC-MS of extrudate.

volatile profile of the compounds recovered from extrudates. A total of 91 compounds were identified among the volatile compounds collected at the extruder die whereas 56 compounds were identified from the corresponding extrudate sample. More than half of the total number of volatiles generated during extrusion belonging to various chemical classes of compounds are released at the extruder die with the steam. It is observed that the volatile compounds released at the die differ significantly from those recovered from the extrudates. The difference in composition is most probably caused by the difference in their volatility with water vapor. Higher levels of nonaromatic hydrocarbons, ketones, aldehydes, acids and esters were identified at the die. It was observed that the amount of volatiles released at the die was two times higher than the amounts recovered from extrudates. However, the different methodologies used for the purge and trap experiments and collection at the die make it difficult to directly compare the quantities of these two fractions. Other information on the volatiles which are generated during extrusion but lost at the die during processing is not available to date .

It is observed that there are a significant number of pyrazines, sulfur compounds, furans, aldehydes and ketones among the volatile compounds recovered from the extrudates. No acids were identified from the extrudates whereas one ester was identified, compared to 13 esters which were identified in the extruder condensate. At least 61 compounds have been previously identified in corn meal extrudates (*7*). Among those identified include hydrocarbons, aldehydes, acids, ketones, esters, alcohols, pyrazines, furans and sulfides. Fewer numbers of compounds were identified belonging to volatile classes such as pyrazines, furans and sulfur compounds which included 2-methyl pyrazine, 2,5-dimethyl pyrazine, 2-furaldehyde, 2-pentyl furan, dimethyl disulfide and dimethyl trisulfide. Our studies indicate that at least 7 pyrazines, 7 sulfur compounds and 9 furans were identified from the extrudates. Different levels of amylose in the corn-based raw material and different extrusion processing parameters may explain the variations in recovery of different classes of volatiles in extrudates. Moisture levels of 12.5% were used in our experiments as compared to moisture levels ranging from 20%-30% in the previous study (*7*).

Pyrazines, furans, ketones, aldehydes and esters form the major classes of volatile compounds identified at the extruder die. 2,4-Decadienal and 2,4-nonadienal were among the unsaturated aldehydes released at the extruder die. Decomposition of linoleic acid, a major component of corn oil, is responsible for the formation of 2,4-decadienal which is notable for its fried flavor (*8*). Low recovery has been reported for this compound in corn extrudates. Significant losses of 2,4-decadienal at the extruder die may explain the absence or low recovery levels of this compound in extrudates.

Comparison of the volatile compounds identified from the extrudate and those released at the die indicate that 16 compounds, which include 4 pyrazines, 3 sulfur compounds, 3 ketones, 2 furans and one compound belonging to each of the following classes such as aldehydes, esters, aromatic hydrocarbons and pyridines, were common to both samples. Most of these compounds have been known to be important flavor contributors. It has been speculated that decomposition or the high volatility of some of the generated compounds results in their low retention in the extrudate after extrusion (*8*). The above observations confirm that there is a significant loss of major volatiles with the water vapor during extrusion cooking at the extruder die, particularly during processing at low moistures and high temperatures.

Qualitative Differences Among Volatile Compounds. Table II lists the tentative identification of the volatile compounds released at the die and those recovered from the corresponding extrudate. The scan numbers of the volatiles in the Table identify the position of the volatiles in the chromatogram.

The furans identified in the extrudates differ from those released at the extruder die. Compounds such as 5-methyl-2-furfural have been identified as those steam distilled at the die with the water vapor during extrusion cooking as well as in the

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch027

TABLE II. Tentative Identification of Volatile Compounds

Scan Number	*Volatiles released at the die*	*Scan Number*	*Volatiles recovered from extrudates*
	Acids		Acids
2134	Tetradecanoic acid		
2328	Hexadecanoic acid		
2348	Octadecanoic acid		
2404	Heptanoic acid		
	Alcohols		Alcohols
952	4-Ethyl-2-methoxy phenol	223	2-Pentanol
	Aldehydes		Aldehydes
172	2-Butenal	110	2-Methyl propanal
177	3-Methyl pentanal	251	Isovaldehyde
268	2-Methyl-2-butenal	258	3-Methyl butanal
281	2-Pentenal	262	Butanal
713	Benzaldehyde*	266	2-Methyl butanal
929	Benzacetaldehyde	292	Pentanal
1002	2-Octenal	377	Hexanal
1141	Nonanal	451	Heptanal
1276	2-Nonenal	486	Benzaldehyde*
1379	2,4-Nonadienal	632	Decanal
1601	2,4-Decadienal		
1661	2-Undecenal		
1685	2-Butyl-2-octenal		
	Esters		Esters
220	Ethyl acetate*	221	Ethyl acetate*
385	Ethyl butanoate		
495	Ethyl-2-methylbutanoate		
501	Ethyl-3-methylbutanoate		
638	Ethyl pentanoate		
1129	Butyl pentanoate		
1391	Ethyl octanoate		
1502	2,4-Dimethyl heptanoate		
1517	Ethyl nonanoate		
1525	Ethyl,4-methyloctanoate		
1598	Ethyl benzoate		
1806	Geranyl acetate		
2754	Isooctylphthalate		

*compounds common to extrudate and die-released fraction

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Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch027

Table II. Continued

Scan Number	*Volatiles released at the die*	*Scan Number*	*Volatiles recovered from extrudates*
	Furans		Furans
340	2(3*H*)-furanone	192	2-Methyl furan
360	Dihydro-2-methyl-3(2*H*)furanone	309	2,5-Dimethyl furan
415	2-Furfuryl alcohol*	382	Furfural
486	2-Furanmethanol	432	2-Furfuryl alcohol*
519	3-Furanmethanol	456	Acetyl furan
561	Dihydro-2(3*H*)furanone	490	5-Methyl-2-furfural*
707	2 Propionyl furan	548	2-Butanoyl furan
724	5-Methyl-2-furfural*	567	2,2'-(Methylene-bis)-furan
767	Furancarboxylic acid,methyl ester	681	Difurfuryl ether
782	Isomaltol		
1629	Dihydro-5-pentyl-2(3*H*)furanone		
1866	Dihydroactinidiolide		
	Nonaromatic hydrocarbons		Nonaromatic hydrocarbons
213	Cyclohexene	4	Pentane
920	Cyclopentane	252	2-Ethenyloxy propane
1658	2,3-Nonadiene	349	Acetoin
1747	Tetradecane	399	3-Ethyl cyclopentene
1872	2,9-Dimethyl undecane		
2041	2-Methyl-8-propyl dodecane		
2089	Heptadecane		
2099	2,6,10-Trimethyl hexadecane		
2186	Octadecane		
2199	Hexadecane		
2278	Nonadecane		
	Aromatic hydrocarbons		Aromatic hydrocarbons
318	Toluene*	355	Toluene*
1200	1,2,3,4-Tetramethyl benzene	434	Ethyl benzene
1653	1,2-Dihydro-1,1,6-trimethyl naphthalene	785	Butylated hydroxytoluene

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch027

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch027

Table II. Continued

Scan Number	*Volatiles released at the die*	*Scan Number*	*Volatiles recovered from extrudates*
	<u>Ketones</u>		<u>Ketones</u>
146	2-Butanone*	181	2-Butanone*
261	3-Penten-2-one	203	2,3-Butanedione
349	2-Hexanone*	294	2,3-Pentanedione
441	3-Methyl-3-penten-2-one	368	3-Hexanone
730	1-(Acetyloxy)-2-butanone	371	2-Hexanone*
957	3-Octen-2-one	445	2-Heptanone
1313	1-(4-Methyl phenyl)ethanone	548	3,4,4-Trimethyl,2-cyclohexen-1-one*
1344	3,4,4-Trimethyl,2-cyclohexen-1-one*		
1751	α-Ionone		
1768	Dihydro-β-ionone		
1783	Meryl lactone		
1825	β-Ionone		
2068	2-Pentadecanone		
	<u>Pyrazines</u>		<u>Pyrazines</u>
250	Pyrazine*	322	Pyrazine*
402	2-Methyl pyrazine*	408	2-Methyl pyrazine*
656	Vinyl pyrazine	465	2,5-Dimethyl pyrazine
839	2-Ethyl-6-methyl pyrazine*	470	2,3-Dimethyl pyrazine
849	2-Ethyl-3-methyl pyrazine	518	2-Ethyl-6-methyl pyrazine*
863	Propyl pyrazine	523	2-Ethyl-5-methyl pyrazine
984	Isopropenyl pyrazine*	579	Isopropenyl pyrazine*
1013	Acetyl-3-methyl pyrazine		
1082	2,5-Dimethyl-3-ethyl pyrazine		
	<u>Pyridines</u>		<u>Pyridines</u>
826	3-Pyridine methanol	338	Pyridine
907	2-Acetylpyridine*	535	2-Acetylpyridine*

Continued on next page

Table II. Continued

Scan Number	*Volatiles released at the die*	*Scan Number*	*Volatiles recovered from extrudates*
	Other N compounds		Other N compounds
1672	5-Acetyl-2,3-dihydro-1 *H*-pyrrolizine	330	3-Methyl-2-butamine
1798	5-Propionyl-2,3-dihydro-1 *H*- pyrrolizine	347	1 *H*-pyrrole
		385	3-Ethyl-1*H* pyrrole
		515	5-Methyl-2-formyl pyrrole
		618	1-2(Furfuryl) pyrrole
	S-compounds		S-compounds
273	Dimethyl disulfide	391	4-Methyl thiazole*
370	2-Methyl thiazole	424	Trimethyl oxazole
388	4-Methyl thiazole*	502	2-Formyl thiophene
771	2-Thiophene carboxaldehyde	508	2-Formyl thiophene
1023	2-Acetyl thiophene*	561	2-Acetyl thiophene*
1032	2-Formyl-5-methyl-thiophene*	564	2-Formyl-5-methyl-thiophene*
1042	3-Acetyl thiophene	595	3,5-Dimethyl-1,2,4 trithiolane
1349	2-Acetyl-3-methyl thiophene		

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extrudate sample. This compound has been identified in heated model systems of zein with glucose/fructose (*9*). Furans generally contribute to a nutty, fruity, caramel odor. Those identified in our studies such as acetylfuran and 5-methyl-2-furfural are known to be derived from thermal degradation or caramelization of sugars (*10*). All the furanones identified were released at the die while none could be identified in the extrudates. It is possible that the volatility of furanones in an aqueous medium is responsible for their release at the die with the escaping steam. Furanones possibly have a greater affinity for an aqueous environment which might lead to their loss at the extruder die after generation under specific conditions.

One of the benzofuranones, dihydroactinidiolide, was released at the die. This compound is a thermal degradation product of carotenoids. Ketones, such as α-ionone, dihydro-β-ionone, β-ionone, 3,4,4-trimethyl 2-cyclohexen-1-one, and 1,2-dihydro-1,1,6-trimethyl naphthalene are the carotenoid decomposition compounds identified among the compounds released at the extruder die. Toluene was identified as being released at the die and was also found in the corresponding extrudate. Dihydroactinidiolide and other ionones which have important flavor characteristics were the predominant compounds identified during heating of carotene at temperatures between 155° and 210°C for 1-4 hours (*11*). Toluene and other aromatic hydrocarbons including substituted naphthalenes have been proposed to be formed by fragmentation of the carotene molecule.

Among the sulfur containing compounds identified from the extrudates and the extract collected at the die, three compounds which included 4-methyl thiazole, 2-acetyl thiophene and 2-formyl-5-methyl thiophene were common to both samples. In addition, 2-acetyl-3-methyl thiophene, as well as 2-thiophene carboxaldehyde, were identified among the compounds released at the die. Compounds such as 2-thiophene carboxaldehyde and 2-acetyl thiophene were previously identified in the reaction of cysteine with IMP (*10*). The formation of thiophenes may be catalyzed by Maillard browning. Thiophenes formed from the reaction of cysteine with α-dicarbonyl compounds/sugars have been identified in popcorn and roasted coffee (*12*). Long chain substituted thiophenes are formed by reaction of cysteine with lipid oxidation products such as 2,4-decadienal.

Two thiazoles were identified as released from the die and one of them, 4-methyl thiazole, was also identified in the extrudate. Sulfur-containing amino acids such as cysteine are precursors of thiazoles and other heterocyclic compounds (*12, 13*). Low moisture contents of 15% resulted in increased formation of thiazoles while higher moistures favored the formation of trithiolanes and thiophenes (*14*). The latter compounds were formed favorably at medium temperatures, while higher temperatures favored the formation of thiazoles, cyclopentenones and other heterocyclic compounds. In addition to these sulfur-containing compounds, it was observed that esters and furanones which contribute to bready, caramel aromas were dominant at 100°C (*14*). Dimethyl disulfide, which was one of the sulfur-containing compounds identified as that released at the die, has been previously identified in corn extrudates processed under different extrusion conditions. This compound, which is associated with cooked corn aroma, was not observed among the sulfur-compounds recovered from our extrudates. Methionine has been known to be a precursor of dimethyl disulfide (*7*). Release of hydrogen sulfide from proteins due to thermal effects of extrusion explains the formation and release of sulfur-containing compounds at the die.

Many of the alkyl-substituted pyrazines such as 2-methylpyrazine, 2-ethyl-6-methyl pyrazine, 2-ethyl-3-methylpyrazine, 2,5-dimethyl-3-ethylpyrazine, 3-methyl-acetylpyrazine and isopropenylpyrazine were identified at the die. Most of these compounds contribute to a peanut-type, slightly roasted, bready-type flavor. 2-Methyl pyrazine and 2,5-dimethyl pyrazine were identified in extruded corn meal (*7*). Identification of pyrazines released at the die indicates that a number of important flavor compounds are lost at the extruder die during cooking. Pyrazines in foods such

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as peanuts, popcorn, toasted barley, roasted pecans and cocoa products have been reviewed in detail (*15*). Pyrazines are found in toasted or roasted foods or those in which high heats are involved, including toasted barley, roasted pecans, popcorn and cocoa products. An example of an important pyrazine is 2,5-dimethyl pyrazine which is associated with potato flavor and has a threshold value of 35 ppm in water. Ammonium salts and different amino acids react differently to form pyrazines (*16*). The extrusion of yeast extract increases the pyrazine concentration by 33% (*17*). However, it was also observed that identical extrusion of yeast extract in the presence of ammonium bicarbonate decreases the pyrazine concentration by 55%. It was concluded that addition of ammonia suppresses aroma formation but promotes increased browning of the product. The high amounts of pyrazines identified in our experiments suggest that nonenzymatic deamidation of proteins leads to the release of ammonia which may be an important precursor leading to optimum formation of roasted aroma in extruded products.

Two prolines with important sensory characteristics such as 5-acetyl-2,3-dihydro-1*H*-pyrrolizine and 5-propionyl-2,3-dihydro-1*H*-pyrrolizine were identified among the compounds released at the die. Proline interaction resulting in the formation of substituted pyrrolizines via the Maillard reaction has been identified in bready aromas (18). These two pyrrolizines identified as being released at the die were not previously identified in corn extrudates.

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Conclusions

These studies suggest that significant losses of volatiles generated during extrusion occur with the steam released at the exit die. This phenomenon is a major hindrance to the efficient utilization of the extruder for the retention of desirable flavors which may be generated during processing or flavors that are added before extrusion of the dough. In order to obtain further insight on the mechanisms of volatile generation and retention, the extruder condensate collected at the die will need to be studied in greater detail.

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Chapter 28

Formation and Degradation of Tryptophan Amadori Products during Extrusion Processing

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In situ generation of Maillard reaction intermediates by extrusion processing was investigated using a model system consisting of 15% (w/w) glucose, 5% (w/w) tryptophan and 80% (w/w) inert microcrystalline cellulose under pre-selected conditions of temperature, moisture content, screw speed and feed rate. Samples were analyzed by HPLC for the presence of Amadori rearrangement products (ARP), hydroxymethyl furfural (HMF) and maltol. Two temperature ranges were investigated, one 70-90°C and the other 80-100°C. When components were optimized individually using the Generalized Distance Approach (GDA) algorithm, in both temperature ranges ARP formation was highest at lower temperatures, HMF production was favored at about 16°C above the ARP optimum in both temperature ranges, while maltol production was favored at a temperature about 3-4°C lower than the HMF optimum temperature. Slightly higher moisture contents favour the production of ARP, while lower values favour HMF and maltol. Maltol shows the largest increase in yield in the second temperature range, indicating that ARPs are decomposed preferentially to produce maltol. Similar trends were observed when the formation of all components were optimized simultaneously: the yield of ARP dropped somewhat in the higher temperature range whereas maltol doubled, and HMF increased slightly. The results indicate that a controlled continuous production of Maillard reaction flavor precursors is possible by extrusion.

Extrusion combines various unit operations such as mixing, cooking and texturizing into a single continuous process *(1)*, which makes it an attractive option for the food processor and as a result, it has been exploited extensively by the food industry. In terms of flavor, extrusion tends to be limiting because of chemical degradation due to oxidation, hydrolysis and other reactions occurring under high temperature short time (HTST) extruder operating conditions *(2)* and also due to the volatiles being flashed off at the die. On the other hand, systems containing reducing sugars and proteins or amino acids can rapidly undergo the Maillard reaction *(3)*, one of the key flavor-producing reactions occurring in food systems during extrusion. As such, the extruder could serve as a continuous reactor for the *in situ* production of flavor

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precursors from selected sugar/amino acid mixtures. There is no information in the literature regarding the application of extrusion processing to produce Maillard reaction flavors *per se*, most studies deal with the stability of added aroma compounds *(4, 5)*. Some work has been done by Ho et al. *(6)* on the formation of volatile compounds in corn-based model systems and determining Maillard reaction products by gas chromatography/mass spectrometry (GC/MS). They noted that formation of flavour related compounds, including pyrazines were favored at higher temperatures and lower moisture conditions. Cheftel *(7)* indicated that the Maillard reaction is favored at temperatures greater than 180°C and high shear conditions (>100 rpm) in combination with moisture contents of 15% or less. A recent review by Maga *(2)* summarizes flavor formation and retention during extrusion.

Sugar/amino acid model systems have served as a common means of studying the development of Maillard flavor and color precursors, specifically the Amadori rearrangement products (ARP). The objectives of this study were:

i) To assess production and yield of tryptophan ARP and its key degradation products in a model system using the extruder as a continuous reactor;
ii) To assess the effect of key process variables (i.e., temperature, moisture content, screw speed) on extrusion dependent variables (product temperature, pressure at the die, specific energy consumption) and Maillard reaction product yield;
iii) To predict the optimum reaction conditions for product formation using the Generalized Distance Approach algorithm *(8)* and
iv) To compare the results with the aqueous model decomposition system.

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Materials and Methods

Tryptophan, D-glucose, hydroxymethyl furfural (HMF), maltol, and microcrystalline cellulose were obtained from Aldrich Chemical Company (Milwaukee, WI) and used without further purification. All the solvents were of HPLC grade (BDH, U.S.A), water was obtained from a Milli-Q reagent grade water system (Millipore Corp., Bedford, MA) and all HPLC mobile phases were degassed under vacuum. Tryptophan Amadori product was synthesized as reported by Yaylayan and Forage *(9)* and the extrudates analyzed by HPLC.

Extrusion Processing and Optimization. Tryptophan (5 kg), glucose (15 kg) and cellulose (80 kg) were mixed in a vacuum dispersion mixer (Day Mixing, Cincinnati, Ohio) to obtain a homogeneous mass. The moisture content of the mixture was determined to be 4.2% by oven drying (2 hr at 60°C). The extruder used was a Baker Perkins MPF-50D (APV Baker, Inc., Grand Rapids, MI) co-rotating intermeshing twin screw extruder with the barrel configured in a 20:1 L/D ratio (barrel length to screw diameter), and screw profile was designed to obtain good mixing and conveying performance. The screw configuration in sequence from the feeder to the die consisted of: 300 mm, feed screw; 50 mm, 30° forwarding paddles; 50 mm, short pitch screw; 50 mm, single lead screw; 37.5 mm, 60° forwarding paddles; 37.5 mm, 30° reversing paddles; 100 mm, single lead; 50 mm, 60° forwarding paddles; 37.5 mm, 30° reversing paddles; 100 mm, single lead; 50 mm, 90° paddles; 75 mm, single lead; 37.5 mm, 60° forwarding paddles and 75 mm, single lead. A die having two 9 mm circular orifices was used, and the temperature was controlled over nine zones along the barrel. Pressure, product temperature at the die and torque were measured, and the specific energy consumption (SEC) was calculated *(10)*.

Preliminary experiments were carried out to determine viable operating ranges (i.e., screw speed, temperature, moisture) from which the basic experimental design was developed, which lays out the operating conditions for the extrusion experiments. A simple coded factorial design varying only moisture and temperature

was used for two separate extrusion runs having distinct temperature ranges (70-90°C and 80-100°C) but common moisture variation (55-63%), with the feed rate and screw speed held constant. A separate experiment was carried out to study the effect of screw speed. The extruder was run at each set of design conditions until the operating conditions stabilized. Samples were then taken, placed in plastic bags after cooling and frozen at -25°C until analyzed. The response data (i.e., the concentration of ARP, maltol and HMF produced) was then used to determine the optimal conditions for their formation by the Generalized Distance Approach using a design radius of 1.0 *(8, 11)*. Compromise optima in turn were obtained from the GDA data using the Multiple Response (MR) program developed by Conlon and Khuri *(12)*.

HPLC Analysis. Extrudate samples were taken under selected conditions of temperature, moisture content, screw speed and feed rate for analysis of HMF, maltol and ARP. Two grams of each sample was diluted to 100 mL with distilled water and filtered through a 0.45 mm, type HA Millipore filter (Waters Scientific) before injection onto a HPLC column equipped with a Rheodyne injector having a 20-μL loop. A Beckman System Gold modular HPLC was used for analyses, consisting of a Model 166 variable wavelength UV detector set at 280 nm and a model 110B solvent delivery system controlled by a NEC lap-top computer and connected to a Shimadzu CR-18 integrator (10-mV full scale). Analyte HPLC separation was carried out using either a 5μ, 2.0x150 mm Beckman C-18 Ultrasphere column or a Merck 5μ, 2.0x150mm C-18 Lichrosphere, 100-RP-18 column, equipped with a Licro CART 4-4 guard column. Both columns were operated at ambient temperature, 20 μL was injected for analysis and the values obtained represent the average of three injections. The analyte peaks were identified and confirmed by comparison of their retention times with commercial or synthesized standards using two mobile phases, wavelength ratioing, and spiking *(9)*. Quantitation was performed by the use of appropriate calibration curves.

Results and Discussion

Processing Conditions. The model system chosen was tryptophan/glucose, which has been studied previously by the authors *(9, 13)* in an aqueous matrix. Because of the extensive raw material requirements and inherent expense imposed by full scale extrusion experimentation, the sugar/amino acid mixture was diluted by microcrystalline cellulose which does not interfere with the Maillard reaction *(14)* and is insoluble in water, making it simple to extract the reaction products from the extrudate. Tryptophan Amadori compound (a reactive intermediate) and its breakdown products, HMF and maltol were used as indicator compounds of the extent of Maillard reaction. Initial experimental designs were predicated on using relatively high temperatures and low moisture conditions as suggested by Cheftel *(7)*; however, preliminary trials indicated that viable extrusion conditions could only be obtained using >55% moisture and temperatures lower than 110°C. These limiting conditions were likely due to the effect of the microcrystalline cellulose matrix (~80%) rather than the amino acid/sugar mixture.

Based on the experimental design conditions, the barrel temperature did not affect torque, pressure at the die or specific energy consumption (SEC); however, moisture was a limiting variable. Temperature, which normally affects viscosity, has no effect in this system as cellulose is insoluble and does not melt. Moisture acts as a lubricant, reducing torque, pressure and energy consumption. The effect of screw speed on torque, SEC, and pressure was studied independently. Although the torque decreases exponentially as screw speed increases, SEC reaches a minimum around 200 rpm while the pressure tends to maximize at the screw speed. Screw speed was also studied and has a complex effect on torque, specific energy and pressure, which were related to Maillard product formation *(10)*.

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Maillard Reaction. The Maillard reaction, known to be an important flavor producing reaction in food systems, is usually associated with baked and roasted products, and is used commercially by the flavor industry to produce reaction flavors *(15)*. The flavor charactersistics produced depends primarily on the precise nature and ratio of the amino acids and sugars, reaction conditions (primarily time, temperature and moisture) and the nature of any thermal degradation reactions which may contribute to the overall flavor profile. Some of the steps of the reaction mechanism are well known based on model system studies; however, the accurate prediction of flavors produced is still largely an art.

The degradation of tryptophan ARP produces a complex mixture of products. Maltol and HMF can be used as indicator compounds, although they may also be formed at much slower rates from the decomposition of glucose alone *(9)*. ARP decomposition basically takes place *via* two well established pathways: a) 1,2-enolization producing mainly HMF leading to browning, and b) 2,3-enolization producing maltol and leading mainly to flavor formation *(15)*. However, other mechanisms have also been proposed *(9)*. The information available on this reaction in the model aqueous matrix is presented below.

Considering the reaction of tryptophan with glucose, it is important to point out that the measured values of the Amadori products represent the accumulated amounts (the difference between the amount of ARP formed and the amount decomposed). Hence the concentrations of ARP, HMF and maltol produced are interrelated, ARP accumulation and decomposition being a dynamic process and the optimization of any one compound automatically implies reduced levels of the others.

Decomposition of Tryptophan ARP in a Model Aqueous System. The products formed from the thermal decomposition of tryptophan Amadori product alone have been studied previously in an aqueous matrix, at two temperatures (110°C, 140°C) and concentrations (1.53 mg/mL, 1.33 mg/mL) *(9, 13)*.

Table I. Relative Rates of Decomposition of Tryptophan ARP

Temperature (°C)	*Concent. of ARP (mg/mL)*	*Relative Rates*
110→140	1.53	3.5 x
110→140	1.33	4.7 x
110	1.33→1.53	1.4 x
140	1.33→1.53	1.08 x

→ change to

The results from these studies have shown that the first order rate constant for the disappearance of ARP in the absence of sugar was dependent on the temperature and the water content of the reaction mixture. The rate of decomposition of the more concentrated solution was 3.5 times faster at 140°C than at 110°C and 4.7 times faster in the less concentrated solution at 140°C than at 110°C. Comparing the rates at the same temperature but at different concentrations indicated that at 110°C, the rate is 1.4 times faster in the more concentrated solution and at 140°C the rate is faster by only 1.08 times (see Table I). These results indicate that in dilute solutions,

decomposition of ARP proceeds by hydrolytic rate-determining reactions, while thermally induced non-hydrolytic reactions (such as C-C and C-N bond cleavages) become important as the temperature is increased. The value of the activation energy (Ea) for the decomposition of ARPs was found to depend on the water content of the reaction mixture; higher the water content, the higher is the value of Ea. Consequently, dilute solutions of Amadori products are more sensitive to changes in temperature than concentrated solutions.

Formation of HMF and Maltol from Tryptophan ARP in a Model Aqueous System. The formation of HMF and maltol from ARP was also studied *(9)*. The generation of hydroxymethyl furfural (HMF) in food or in model systems is a useful indicator of the extent of Maillard reaction. Maltol on the other hand, is associated with caramel flavor and is used as a flavor potentiator in non-alcoholic beverages. According to Table II, increasing the temperature by 30°C increases the rate of formation of HMF by 1.5 times at both ARP concentrations. However, increasing the water content increases the rate at both temperatures by 2.5 times, indicating that a hydrolytic reaction is involved in the rate determining step. However, activation energies at both water concentrations are the same; these observations indicate that the reaction is more sensitive to the water content than to variations in temperature. On the other hand, increasing the temperature in the more concentrated solution of the ARP by 30°C increases the rate of formation of maltol by 1.5 times, and by 2.8 times in the less concentrated solution, indicating that thermally induced non-hydrolytic reactions (such as C-C and C-N bond cleavages) might be involved in the rate-determining step. However, increasing the water content, decreases the rate at 110°C by 2.5 times and by 1.4 times at 140°C, indicating that dehydration steps are also involved in the formation of maltol from the ARP. The same conclusion can be reached by comparing the activation energies at two concentrations. Both HMF and maltol can also be formed directly from the degradation of glucose alone, however at much slower rates.

Table II. Relative Rates of Formation of HMF and Maltol from TRP-ARP

Temperature (°C)	*Concentration of ARP (mg/mL)*	*Rate of formation of HMF*	*Rate of formation of maltol*
110→140	1.53	1.5 x ↑	1.6 x ↑
110→140	1.33	1.5 x ↑	2.8 x ↑
110	1.53→1.33	2.5 x ↑	2.5 x ↓
140	1.53→1.33	2.5 x ↑	1.4 x ↓

→ change to , ↑ increase, ↓ decrease

Separate Optimization of Maltol, HMF and ARP Formation in the Extruder. Using the Generalized Distance Approach (GDA), one can predict the optimum concentrations and processing conditions for each component from the concentrations of the selected Maillard intermediates and the experimental design process conditions. The mathematical basis for this procedure was developed well over a

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decade ago *(8)*, however it has only recently been applied to assist in the development and optimization of multivariate extrusion processes *(16)*. GDA permits one to find compromise conditions for the input variables that are somewhat favorable to all responses. This implies that the multiresponse function deviates as little as possible from the individual optima, and the deviation is formulated as a distance function which is minimized over the experimental region. By evaluating the Maillard reaction system by this means, one can determine whether the selected processing conditions favor the 1,2 or 2,3 enolization degradation pathway, based on whether HMF or maltol is dominant. The method also permits the prediction of compromise conditions where all components are optimized simultaneously, by formulating responses as a distance function minimized over the experimental region.

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Table III. Optimum Concentrations Calculated by Generalized Distance Approach for Each Maillard Reaction Component and the Respective Processing Conditions Required to Produce the Optima Listed

Response	*Optimum (g/L)*	*Yield (%)*	*Moisture (%)*	*Temperature (°C)*
		(Temperature Range 70-90°C)		
ARP[a]	0.04760	2.70	58.6	70.0
HMF[b]	0.00116	0.16	55.9	86.3
MAL[c]	0.03880	5.50	55.3	83.4
		(Temperature Range 80-100°C)		
ARP[a]	0.03450	1.95	58.6	80.0
HMF[b]	0.00138	0.19	56.1	97.2
MAL[c]	0.06970	9.87	55.2	93.3

[a]ARP = Amadori rearrangement product, [b]HMF = Hydroxymethylfurfural, [c]MAL = Maltol
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Table III provides a summary of the optimum concentrations and yield for each Maillard component and the respective processing conditions for each of the two temperature sets. It can be seen that in both temperature ranges ARP development is optimized at lower temperatures, although the optimum shifts from 70 to 80°C in the higher temperature range and the yield drops slightly. HMF production is favored at about 16°C above the ARP optimum in both temperature

ranges, while maltol production is favored at a temperature about 3-4°C lower than HMF production. Slightly higher moisture contents favour the production of ARP, while lower values favour HMF and maltol. Maltol shows the largest increase in the second temperature set, with the yield almost doubling indicating that ARP is decomposing preferably to maltol, thereby reducing the total ARP present. This observation is consistant with the results obtained in the aqueous model studies where a similar trend was observed (see Table II).

Table IV. Compromise Optima Calculated for the Two Temperature Sets and the Associated Extrusion Conditions Required

Response	*Compromise Optimum (g/L)*			
	70-90°C	*%Yield*	*80-100°C*	*%Yield*
Temperature (°C)	72.9		97.2	
Moisture (%)	56.2		56.2	
ARP (g/L)	0.04200	2.40	0.03100	1.75
HMF (g/L)	0.00098	0.14	0.00138	0.19
MAL (g/L)	0.03170	4.49	0.0631	8.94

[a]ARP = Amadori rearrangement product, [b]HMF = Hydroxymethylfurfural, [c]MAL = Maltol
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Simultaneous Optimization of Maltol, HMF and ARP Formation in the Extruder. Table IV presents the calculated compromise optimum data, where all components are optimized simultaneously. In this situation, one is balancing the production of the three components against each other; however, similar trends are observed as before: ARP dropping somewhat in the higher temperature range, maltol doubling, and HMF increasing, but being produced only in relatively small amounts. These results are consistant with those from the aqueous decomposition studies.

Moisture and temperature are but two of many variables which can be controlled during the extrusion process *(1)* and the GDA procedure has been successfully applied to develop and optimize the production of sodium caseinate for three product characteristics dictated by six simultaneous operating variables *(17)*. As such, the GDA has been proven to be a very useful optimization technique; however, the derived data are only valid within the original experimental design parameters and cannot be extrapolated.

Conclusion

A twin screw extruder can be used as a continuous reactor for the Maillard reaction. The composition of reaction products can be based on the judicious adjustment of extrusion conditions to optimize the formation of ARP, HMF and maltol individually or simultaneously as a compromise optimum. One limitation of this work was the need to use microcrystalline cellulose as a diluent, which strongly affected the extruder operating conditions (torque, specific energy and pressure). It is

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unlikely that the basic reaction pattern would change dramatically if run under the same conditions without the filler; however, the elimination of the filler would undoubtedly expand the range of operating conditions (i.e., temperature and moisture), which could increase yield and provide more direction to controlling the reaction. In order to elucidate whether there would be any commercial potential for the production of Maillard reaction intermediates for a particular sugar/amino acid mixture, additional work should be carried out without the diluent, including reaction kinetics study that requires the assessment of the residence time distribution in the extruder. Albeit somewhat limited, sufficient information has been obtained to confirm the basic premise that Maillard flavor intermediates may be produced by extrusion processing, and that some control can be exercised over the reaction by manipulating the extruder parameters.

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Chapter 29

Flavor Properties of Extrusion Cooked Mechanically Deboned Pork

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Mechanically deboned pork was blended with varying levels of soy protein concentrate (SPC), non-glutinous rice flour (NGRF) and Chinese five-spice flavor. The mixtures were extruded at 80°-85°C using a single-screw Brabender laboratory extruder equipped with a 3:1 compression screw operating at 100 rpm. Resulting extrudates were air-dried for one hour then soaked in varying concentrations of soy-based marinade at 4°C for 8 hours. Resulting products were then dried at 45°C for two hours. A sensory panel utilized a 9-point hedonic scale to evaluate color, texture, flavor and overall acceptability. Optimum product acceptability resulted with 0.1% added flavoring, 8% SPC and 15% NGRF.

Extrusion technology is a versatile, continuous, high-temperature short-time process that offers new alternatives to conventional food technologies. Foods produced by extrusion have greatly increased in recent years.

Based on the attractive characteristics of the extrusion process, food processors have attempted to develop meat-containing products by extrusion cooking (*1-2*). The potential products include meat analogs, intermediate-moisture meat products and meat-based snacks. Some extrusion systems have been designed to produce meat-based processed foods including skinless sausages; logs of beef, pork, poultry and fish; and dried meat-based products (*3-5*).

In some areas of the world, animal production has been reduced because it is considered a high pollution-producing industry. Meat has become very expensive because of the increasing costs of labor and feeds. Thus, in order to maintain a steady supply of meat at a reasonable price, the greatest concern of the meat industry is to obtain maximum production from each animal. Including the amounts of underutilized edible by-products, any edible meat remaining on the carcass after deboning can be used to accomplish this purpose.

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The production of mechanically deboned meat (MDM), which can be regarded as a new meat source, is interesting. The world potential production of mechanically deboned pork (MDP) was estimated at 1.9 million metric tons in 1989. Since the carcass weight of pigs is approximately 75% of their live weight and about 83% of the carcass is meat (*6*), MDP production is equal to about 30 million pigs. These statistics imply that a significant amount of food resources can be saved in the animal industry, and in turn, used for human consumption.

Extrusion is capable of producing acceptable texturized meat products by using MDM. Most published reports are centered on mechanically deboned poultry. No reports were found using MDP to produce restructured meat products by extrusion cooking. Other researchers have concluded that non-meat ingredients are required to produce non-expanded moist meat products to improve their texture, binding and flavor. The effects of soy protein products, wheat gluten, gums and starches on the chemical and physical properties have been investigated. However, no applications of rice flour with MDP were found in the literature.

Therefore, the overall objective of this study was to develop a non-expanded pork-based product using extrusion processing. Specific objectives include the evaluation of optimum extrusion conditions required to produce the most palatable product texture, optimum non-meat ingredient levels, evaluation of the influence of ingredient mixing order, and the evaluation of texture, color, flavor and overall acceptability of the resulting products.

Materials and Methods

Materials. Fresh pork bones including the vertebral column and ribs were stored at -18°C. Prior to mechanical deboning, the bones were tempered at 1° to 4°C for 24 hours. Then the bones were ground through a 12 mm plate of a Weiler grinder and deboned using an AUX Beehive mechanical deboner with 0.86 mm holes in the cylinder head. The MDP was mechanically mixed for 3 minutes, vacuum packaged in 2 kg packages and stored at -18°C. The yield of MDP was 34.2%. The proximate composition was 55.6% moisture, 15.3% protein, 25.8% fat and 2.8% ash. Fresh pork back fat containing 9.0% moisture and 89.5% fat at a temperature of -5°C was ground through a 3.2 mm plate twice using a Univex Model PB2 grinder. Before the second grinding, the back fat was frozen to a temperature of -5°C. The finely-ground back fat was then vacuum packed and stored at -18°C. Fat levels evaluated in the formulations were 12, 15, 18, 20 and 21%. The frozen MDP and back fat were thawed to -2° to -5°C prior to being mixed with other ingredients.

NGRF was obtained by grinding U.S. #1 California medium grain rice to a particle size distribution of 0.0-2.0, 13.0-19.0, 5.5-9.5, 16.0-22.0 and 16.5-20.5% retained on U.S. sieve # 50, 80, 100, 140, and 200, respectively, and 35.0-41.0% through a U.S. sieve #200. Glutinous rice flour (GRF) was obtained by grinding sweet rice to a particle size distribution of 0.0-1.0, 9.0-15.0, 2.5-8.5, 12.0-18.0 and 12.0-18.0% retained on U.S. sieve #50, 80, 100, 140 and 200, respectively, and 49.0-55.0% through a U.S. #200 sieve. Initial trials incorporating 0, 15, 17, 20 or 23% of either NGRF or GRF clearly demonstrated that from a textural standpoint, NGRF performed better than GRF and that a level of 15% was the most appropriate.

SPC (STA-PRO[R] 3000) was obtained from Central Soya Company. This product is accepted by the USDA for use in meat and poultry products. Initial levels

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of SPC evaluated were 0, 5 and 8%, and it was concluded that 8% SPC gave the best textural results.

The spice used as a flavoring material was commercial Chinese five-spice powder containing star anise, fagara (wild pepper), cinnamon, cloves and fennel seeds. Initial flavoring levels evaluated were 0, 0.1, 0.4 and 0.7%.

Extrusion Cooking. All extrusion cooking was accomplished in a laboratory Brabender single screw extruder (Model PL V500) with a 1.92 cm barrel diameter and a 20:1 length-to-diameter ratio. The extruder barrel was equipped with eight 0.79 x 3.18 mm longitudinal grooves. The desired cooking temperature was controlled by two electrically heated zones, which were in turn controlled by compressed air-cooled barrel collars. Zone one was in the compression section and zone two was in the metering section of the extruder barrel.

Samples were prepared 16 hours prior to extrusion cooking. The resulting extrudates were collected and cooled to room temperature and then placed in plastic bags and refrigerated (1° to 4°C) until analyses were performed.

Preliminary studies were devoted to the role of zone one/zone two extrusion temperatures on extrudate textural properties. Zone one temperatures evaluated were 80° and 100°C while zone two temperatures evaluated were 80°, 90° and 100°C. It was concluded that a zone one temperature of 80°C and also a zone two temperature of 80°C gave the best results.

Extrusion Dies. Three different die configurations were initially evaluated. The first was a flat opening die measuring 6.4 x 1.5 x 25.2 mm. The second was a small round die opening measuring 4.6 mm in diameter by 15.8 mm in length, and the third was a large round die that had an opening of 8 mm and was 70 mm long.

Compression Screws. Initially both 3:1 and 1:1 compression screws were evaluated, and from these observations it was concluded that the 3:1 screw produced products that had better textural properties than those resulting from the 1:1 screw.

The influence of screw speed was also considered, and it was concluded, based on extrudate textural properties, that 100 rpm produced better results than 80 rpm.

Ingredient Mixing Order. In an attempt to influence extrudate textural properties, ingredient mixing order was evaluated. In one series of experiments, the NGRF and SPC were first manually dry-blended, then the MDP was added and the complete mixture manually blended for 8 minutes. This was called the rice/soy/pork (RSP) procedure. Another technique evaluated involved first manually mixing the SPC with the MDP for 4 minutes, then adding the NGRF and mixing for an additional 4 minutes (PSR). The last mixing procedure evaluated involved first mixing the NGRF with the MDP for 4 minutes, followed by the addition of the SPC and additional mixing for 4 minutes (PRS).

Post-Extrusion Treatments. Several drying techniques were evaluated on the resulting extrudates. In one series, the extrudates were permitted to air dry at room temperature for 24 hours. This was compared to oven drying the extrudates for 2 hours at 45°C.

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch029

In another series, the influence of soaking extrudates in two types of marinades was evaluated. The first marinade was called "non-soy" and was composed of 10% sugar and 1% salt in water while the second marinade was composed of 10% soy sauce, 10% sugar and 1% salt, with the remainder being water ("soy"). In both systems, the extrudate was soaked at 4°C for 8 hours, then the extrudate was removed and air dried at 45°C for 2 hours.

Sensory Evaluation. The properties of hardness and binding were analyzed using a six-point sensory evaluation method utilizing food references as anchor points (*4, 7-8*). Six panel members consisting of three males and three females from the Chinese Student Association at Colorado State University were trained to provide scores by comparing the samples used as reference points. The actual foods used are summarized in Table I.

For evaluation of extrudate color, texture, flavor and overall acceptability, the same panel used the hedonic scale shown in Table II.

Table I. Reference Scale for Evaluating Hardness and Binding

Score	*Hardness*	*Binding*
1	Very Soft (Cream Cheese)	Raw Ground Beef
2	Soft (Cooked Egg White)	
3	Slightly Hard (Frankfurter)	
4	Moderately Hard (Olives)	Frankfurters
5	Hard (Cheddar Cheese)	
6	Very Hard (Peanuts)	Beef Stick

Table II. Hedonic Scale Utilized for Sensory Evaluation

Descriptor	*Score*
Extremely Desirable	9
Very Desirable	8
Moderately Desirable	7
Slightly Desirable	6
Neither Desirable Nor Undesirable	5
Slightly Undesirable	4
Moderately Undesirable	3
Very Undesirable	2
Extremely Undesirable	1

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Analytical Methods. Standard AOAC (*9*) methods were used for a duplicate measurement of proximate composition including moisture, protein, fat and ash.

Results and Discussion

Proximate Composition. For informational purposes, the proximate composition and pH values for the major ingredients utilized in this study are summarized in Table III.

Table III. Proximate Composition (%) and pH of Major Ingredients

Ingred	*Moisture*	*Protein*	*Fat*	*Ash*	*pH*
MDP[1]	55.68	15.32	25.83	2.78	6.7
GRF[2]	11.44	7.00	2.08	1.02	6.3
GRF[3]	11.54	7.01	2.57	1.01	6.4
SPC[4]	6.20	69.32	2.15	3.67	6.7

[1]MDP=Mechanically Deboned Pork, [2]GRF=Glutinous Rice Flour, [3]NGRF=Non-Glutinous Rice Flour, [4]SPC= Soy Protein Concentrate

As can be seen, the composition of the two rice flours was essentially the same, and as expected, the SPC was approximately 70% protein. It is interesting to note that the MDP used in this study was close to 26% fat. If MDP was extruded alone, its high fat content would make extrusion difficult due to product slippage in the extruder barrel. Its relatively high fat content is one reason that non-meat ingredients were incorporated. Non-meat ingredients serve two functions. First they dilute the fat content, and secondly, they can tie up a portion of the fat thus minimizing its functional properties.

Roles of Rice Type/Amount and Drying on Extrudate Hardness Scores. As can be seen in Figure 1, the type of rice flour utilized had little if any influence on resulting hardness scores. Since NGRF is usually less expensive than GRF, all further studies were conducted with NGRF.

As would also be expected, it can be seen that the extrudates that were dried had higher hardness scores than those that were not. Apparently the drying process removed moisture from the system thereby creating a harder product.

It can also be seen that increasing the rice flour content from 17 to 23% had little, if any, influence on extrudate hardness. Studies conducted with 15% rice flour displayed similar results, thus further studies were conducted with 15% rice flour since some panel members mentioned detecting a cereal taste in extrudates containing more than 15% rice flour.

Sensory Binding Scores as Influenced by Rice Type/Amount and Screw Speed. Sensory binding scores were not dramatically influenced by rice type or the amount of rice used, especially at 17 or 20% rice levels. Binding of product was probably directly related to the degree of starch gelatinization that occurred during the extrusion process, and apparently in this study, similar degrees of gelatinization occurred.

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These data are shown in Figure 2. Also, little influence was evident for extrusion rpm.

Hardness and Binding Scores Versus Ingredient Mixing Order. As can be seen in Table IV, the order in which the ingredients were mixed prior to extrusion did influence product textural properties. Dry blending the two powdered ingredients before adding the moist MDP produced the highest hardness score, while mixing the MDP with the rice flour first then adding the SPC, produced the highest bind score. Competition for the moisture in the MDP by the rice flour and SPC probably accounted for these observations.

Table IV. Extrudate Hardness and Binding Scores as Influenced by Ingredient Mixing Order (15% NGRF, 8% SPC, 100 rpm, 80°C)

Order	*Hardness*	*Binding*
RSP[1]	4.0	3.2
PSR[2]	3.8	3.0
PRS[3]	3.9	3.8

[1]RSP=Rice/Soy/Pork, [2]PSR=Pork/Soy/Rice, [3]PRS=Pork/Rice/Soy

Influence of Fat Content. Without question, one would expect that the amount of fat in an extrudate would have a direct influence on its hardness and binding properties. As shown in Figure 3, both of these scores decreased as fat content increased. However, as can be seen, the extrudate containing 12% had hardness and binding scores that were approximately twice that of a product containing 21% fat. From these data it can be concluded that a moderate amount of fat can be incorporated along with non-meat binders to technically produce an extrudate but one must expect rather soft textural properties if this is done.

Influence of Spice Levels and Drying. As is apparent in Figure 4, both the amount of added flavoring and the method of drying influenced the sensory properties of the extrudates.

In the case of texture, drying actually decreased overall textural properties as compared to nondried samples. The panel felt that the drying process produced an extrudate that was quite hard and thus not acceptable. However, the panel reported that the drying process actually improved extrudate color, flavor and overall acceptability. A flavor addition level in the range from 0.1 to 0.4% gave the most desirable results.

Spice Level/Marinade Type. As shown in Figure 5, the use of a soy-based marinade was preferred for overall extrudate acceptability and flavor. However, the type of marinade utilized did not seem to have much effect on extrudate color and texture. Again, an added spice level of 0.1 to 0.4% gave the most desirable results. The naturally-occurring flavor potentiators present in soy sauce probably was responsible for improving overall extrudate acceptability.

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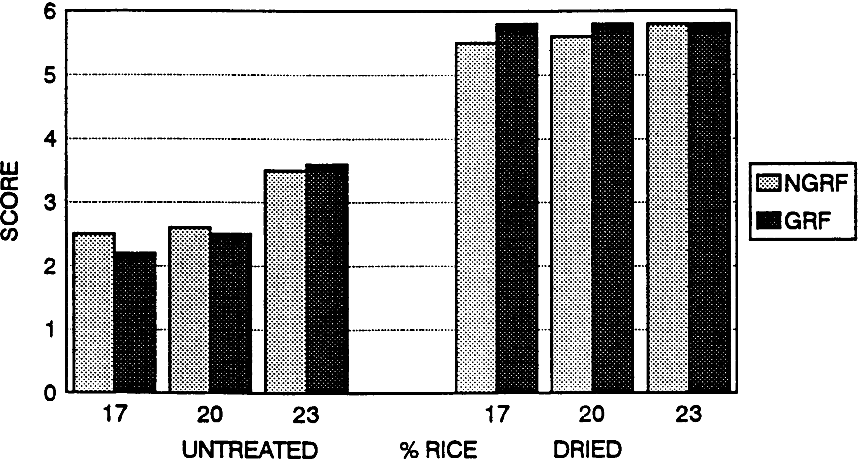

Figure 1. Extrudate hardness scores as influenced by rice type/amount and drying (100 rpm, 80°C).

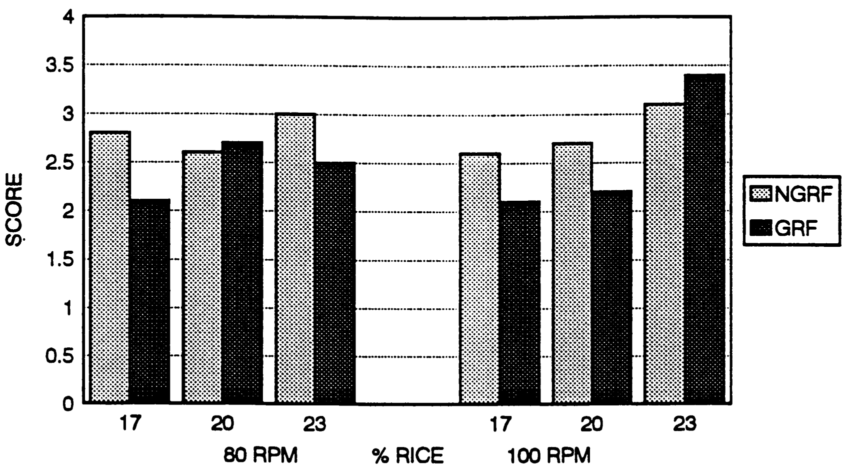

Figure 2. Extrudate binding scores as influenced by rice type/amount and screw speed (80°C).

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch029

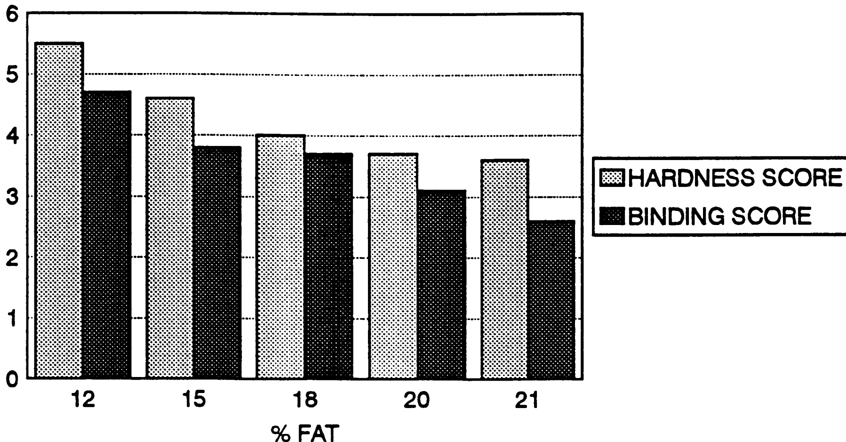

Figure 3. Extrudate hardness and binding scores as influenced by fat content (15% NGRF, 8% SPC, 100 rpm, 80°C).

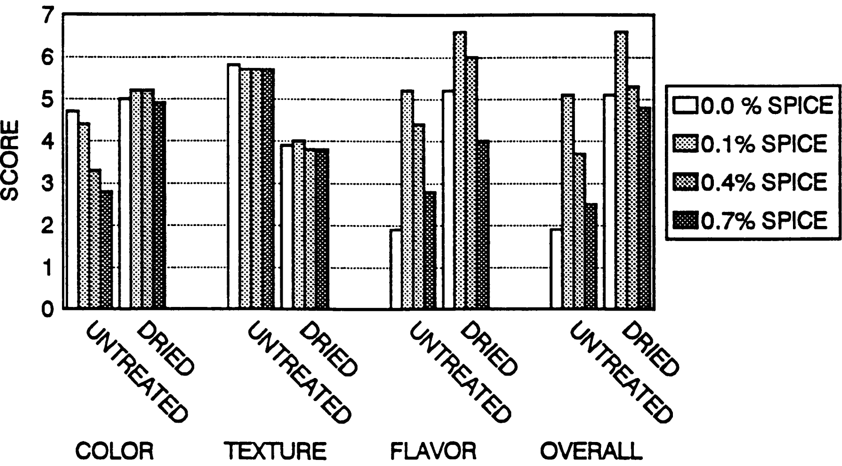

Figure 4. Extrudate panel scores as influenced by spice level and drying.

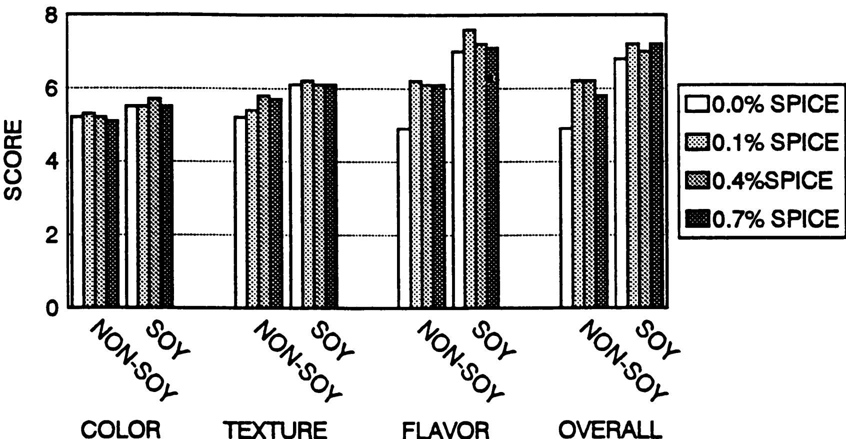

Figure 5. Extrudate panel scores as influenced by spice level/marinade type (15% NGRF, 8% SPC, 100 rpm, 80°C).

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Chapter 30

Extruded Taro (*Colocasia esculenta*) Volatiles

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Taro corms were peeled, sliced, dried, ground and extruded under conditions to produce maximum expansion. The volatiles from the resulting extrudate were extracted and identified using GC/MS. A series of common aliphatic hydrocarbons, acids and alcohols was identified along with Strecker degradation and Maillard reaction products. A total of 41 compounds were identified, the most abundant being octane, eicosanoic acid, 2-methylbutanal and pyridine.

Taro is an ancient crop that originated in Asia but is now grown in many parts of the world where it is also known as *eddo*, *dasheen* or *cocoyam*. It is a member of the family *Araceae* and is one of the most important edible aroids (*1*). Currently, it is primarily grown as a subsistence crop and eaten as a staple because of its high starch content (*2*). The cooked corms have a relatively bland flavor similar to that of potatoes or cereals and its volatile composition has been recently characterized (*3*).

Due to its high starch content, taro is postulated to have good expansion properties, and thus perhaps would have application as an expanded snack product that could be extrusion processed. Therefore, the major objectives of this study were to determine the optimum extrusion temperature required to maximize taro expansion and to identify and semi-quantitate the volatiles associated with such a product.

Materials and Methods

Taro Preparation. Fresh taro corms averaging 1 kg each were directly imported from the Caribbean. Upon receipt, they were manually peeled and mechanically sliced into 0.5 mm slices. The resulting slices were placed in a heated forced-air dehydrator operating at 90°C and held for eight hours. The dehydrated product was ground to pass through a 2 mm screen. The moisture content of the resulting flour was determined using standard gravimetric procedures and was adjusted to 15% using tap water.

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Extrusion Conditions. The moisture-adjusted flour was extruded using a single-screw Brabender model PL V500 laboratory extruder equipped with a 3:1 compression screw operating at 100 ppm. The unit was equipped with a die having an opening of 3.75 mm. Dough temperatures just before the exit die were maintained at 80°, 100°, 120° or 140°C. The resulting extrudates were permitted to air dry overnight. Expansion ratio was determined by dividing the average extrudate diameter by the die diameter.

Volatile Compound Extraction/Identification. Representative samples of extrudate were ground to pass through a 0.5 mm screen. The ground extrudate was extracted for 4 hours in a Likens/Nickerson apparatus using pentane as the solvent. The recovered solvent was concentrated under a stream of nitrogen and injected into a Hewlett Packard Model 5890 gas chromatograph equipped with a 10 m x 0.32 mm i.d. capillary column coated with Carbowax 20M. Helium was the carrier gas. An oven temperature of 50°C was maintained for 7 minutes, then the oven temperature was increased 2°/minute until a final temperature of 210°C was obtained. The injection port was operated at 230°C, and the detector was maintained at 250°C. For compound identification, the gas chromatograph was connected to a Hewlett Packard Model 5970 mass selective detector.

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Results and Discussion

Taro Composition. Upon receipt, the proximate composition of the fresh peeled taro was determined using standard analytical procedures. These data are shown in Table I.

Table I. Raw Peeled Taro Composition

Component	*Percent*
Moisture	71.3
Protein (N x 6.25)	1.6
Lipid	0.3
Carbohydrate	25.4
Fiber	0.9
Ash	1.4

As can be seen, the major functional component was starch. However, it should be noted that a small amount of protein and lipid material were also present that could serve as precursors for the thermal formation of flavor compounds.

Extrudate Expansion. The influence of extrusion temperature on resulting extrudate expansion ratio is summarized in Figure 1. As can be seen, extrudate expansion continued as the temperature increased to 120°C and then expansion decreased at 140°C. With any starch-based food, optimum expansion temperature needs to be determined. Product moisture content, which in this study was held constant at 15%,

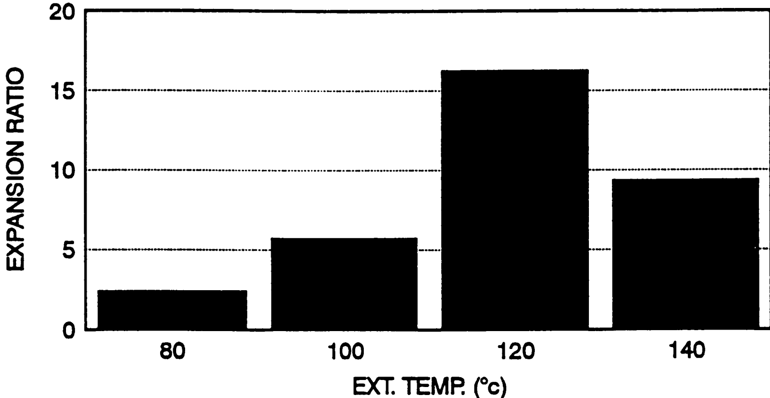

Figure 1. Influence of extrusion temperature on taro extrudate expansion ratio.

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as well as the type and amount of starch are important contributors to overall product expansion. A maximum expansion ratio of slightly over 16 was observed, thus demonstrating that taro has very good expansion properties normally associated with an extruded snack product.

Volatiles Identified. The sample extruded at 120°C was used for volatile identification/quantitation. A total of 41 compounds were identified, including the hydrocarbons shown in Table II. Octane was the major volatile identified.

Three fairly common acids were also identified which represented 10.9% of the total volatiles. The nine aldehydes shown in Table II were also identified, with 2-methyl butanal being the most prevalent at 4.1%. Due to thermal action on various classes of precursors, a series of heterocyclics was identifed with pyridine being the most abundant. Heterocyclics represented 6.70% of the total volatiles. The eight alcohols shown in Table II were identified with hexadecan-1-ol being the most prominent at 1.6%. Several ketones were also present, with the compound decan-2-one being the most dominant. Several miscellaneous compounds including two lactones and a phenol were also identified.

Formation Pathways. The formation of all of the compounds identified can be explained by commonly accepted pathways. For example, the Maillard reaction could explain the formation of furans, thiophenes, thiazoles, and pyrroles. Sugar degradation can account for ketones and certain furans. Amino acids are likely precursors for the aldehydes identified, and lipids can account for the presence of the hydrocarbons, acids, alcohols, aldehydes and lactones.

Conclusion

The extrusion of taro at 120°C and 15% moisture produced optimum product expansion and resulted in the identification of 41 typical flavor volatiles.

Table II. Volatiles Identified in Extruded Taro (120°C)

Class/Compound	Relative %
Hydrocarbons	
Octane	16.3
Tetracosane	1.5
Pentacosane	3.7
Total	*21.5*
Acids	
Nonanoic	1.4
Octadecanoic	2.6
Eicosanoic	6.9
Total	*10.9*
Aldehydes	
2-Methylpropanal	0.2
2-Methylbutanal	4.1
3-Methylbutanal	0.4
Hexanal	1.1
Nonanal	0.1
Decanal	0.1
5-Methyl-2-phenylhex-2-enal	0.2
Phenylacetaldehyde	0.2
Benzaldehyde	0.4
Total	*6.8*
Heterocyclics	
2-Methylthiophene	0.5
Thiophene-2-carboxaldehyde	0.05
Thiophene-3-carboxaldehyde	0.05
Pyridine	3.5
N-Methylpyrrole	0.2
2-Pentylfuran	0.1
2,3-Dihydrobenzofuran	0.3
2-Furaldehyde	0.5
Benzothiazole	0.1
2-Acetylthiazole	1.4
Total	*6.7*
Alcohols	
2-Methylbutan-2-ol	0.1
2-Methylbut-3-en-2-ol	1.1
3-Methylbutan-1-ol	0.2
Pentan-1-ol	0.2
Hexan-1-ol	0.2
(Z)-Hex-3-en-1-ol	0.1
Oct-1-en-3-ol	0.2
Hexadecan-1-ol	1.6
Total	*3.7*

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Table II. Continued

Class/Compound	Relative %
Ketones	
Butanedione	0.02
Pentane-2,3-dione	0.2
Nonan-2-one	0.05
Decan-2-one	0.1
3,5,5-Trimethylcyclohex-2-en-1-one	0.05
Total	*0.42*
Miscellaneous	
γ-Decalactone	0.04
δ-Decalactone	0.06
2-Methoxy-4-vinylphenol	2.1
Total	*2.2*

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Chapter 31

Glycoside as a Flavor Precursor during Extrusion

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The application of glycosides as flavor precursors during extrusion cooking was evaluated using phenyl-β-glucoside as a model compound. The glycoside was added to cornmeal, and the amount of phenol generated during twin-screw extrusion was measured as a function of different temperatures and screw speeds. The extrudate was extracted with methanol, followed by water-saturated butanol and quantified by HPLC analysis. The analytical results indicated that phenol could be produced from its glycoside during extrusion cooking. Extrusion temperature had more significant effects on phenol generation than screw speed. Higher temperatures, in general, resulted in more generation of phenol.

Flavor generation during extrusion cooking using twin-screw extruders has recently attracted the interest of many researchers. One of the key flavor technology issues is the loss of flavors, especially those added before extrusion (*1*-*2*). Several studies concerning the application of the so-called "extrusion stable flavors" have been reported (*3*).

In recent years, the importance of glycosidically-bound volatile constituents to fruit and vegetable aromas is receiving increased attention by many investigators (*4*-*9*). It has been noted by many authors that these glycosidically-bound volatiles in fruits and plant tissues will subsequently release their free aroma constituents by enzymatic and/or chemical hydrolysis and increase the yield of essential oils during fruit ripening and climatic conditions (*10*). The release of free aroma compounds such as vanillin through the pyrolysis of its glycosidic precursor has been reported (*11*).

[3]Current address: Takasago International Corporation (USA), 100 Green Street, Teterboro, NJ 07608

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Glycosidically-bound aroma compounds may possibly be used as stable flavor precursors in extruded foods. The thermal and mechanical energy applied during extrusion may break down the glycoside linkage of the added precursors and generate the desirable flavor for the extruded products. Therefore, the objective of this research is to study the generation of phenol from its β-glucoside, which is added to cornmeal before twin-screw extrusion, and to evaluate the effects of the extrusion conditions on its formation.

Experimental

Extrusion Conditions. Degerminated corn meal (CC 400, Lauhoff Grain Co., Danville, IL) was extruded using a twin-screw extruder (ZSK-30, Werner & Pfleider, Ramsey, NJ) with two circular opening dies (each diameter was 3 mm). Table I shows the screw configurations used for this study. Three extrusion temperatures (165°C, 175°C and 185°C at the die plate), and three screw speeds (300, 400 and 500 rpm) were used to evaluate the effects of extrusion conditions on the formation of three phenolic acids from their glycosidic precursors.

Water was fed to the corn meal by a metering pump (U.S. Electric Co., Milford, CT) from a barrel opening adjacent to a volumetric feeder (K-Tron series 7100, K-Tron Corp., Pitman, NJ). For all experiments, the moisture content of the corn meal was maintained at 18% during extrusion by determining its moisture content beforehand. The feed rate of the corn meal at 18% moisture content was 300 g/min.

Phenyl-β-glucoside. Phenyl-β-glucoside (3.5 g, Sigma Chemical Co., St. Louis, MO) was mixed with 0.04 g of red dye (FD&C Red No. 40, Hilton Davis Co., Cincinnati, OH) and formed into a pellet by a hydraulic press. The pellets were added to the corn meal through the opening at the connection between the feeder and the barrel after the stable extrusion outputs were observed.

Extraction of Phenol and Phenyl-β-glucoside - Determination of Residence Time Distribution. The extruded corn meal was collected every 15 seconds for 3.35 minutes, starting from the moment the phenyl-β-glucoside pellet was added. Each extrudate sample was powdered by a Waring blender (Dynamics Corporation of American, New Hartford, CT) and passed through sieves. The powder passed through a sieve with 0.589 mm openings, but was held by the 0.180 mm openings, and was collected. Using the standard AOAC method, the moisture content of the powder was measured before the phenolic quantifications were determined.

For all experimental extrusion conditions, preliminary HPLC analysis was performed after methanol extraction. After it was determined that the majority of added phenyl-β-glucoside was converted to phenol within 0-60 seconds of the sampling period, the powdered extrudates were combined. Next, a total of 20 g (dry weight basis) of the powdered material was made from the combined material to evaluate extrusion conditions.

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Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch031

Table I. Screw Configuration for ZSK-30 Extruder

Elements	Screw Type	Pitch/Length (mm)	No.
Drive end			
1*	Right-handed	42/21	x 1
1*	Right-handed	42/42	x 2
1*	Right-handed	42/21	x 2
	Igel (Mixing)	42/42	x 1
1*	Right-handed	28/28	x 3
	Igel (Mixing)	42/42	x 1
1*	Right-handed	28/28	x 2
2*	Right-handed	45 degree (5 discs)/28	x 1
1*	Right-handed	28/14	x 3
2*	Right-handed	45 degree (5 discs)/14	x 1
1*	Right-handed	20/20	x 5
2*	Right-handed	45 degree (5 discs)/20	x 1
1*	Left-handed	20/10	x 1
1*	Right-handed	20/10	x 5
2*	Right-handed	45 degree (5 discs)/20	x 1
1*	Left-handed	20/10	x 1
1*	Right-handed	20/10	x 2
1*	Right-handed	14/14	x 2
2*	Right-handed	45 degree (5 discs)/14	x 1
2*	Left-handed	45 degree (5 discs)/14	x 1
1*	Right-handed	14/14	x 6
Die plate			

1* : All transition elements (1*) are double-flighted screws
2* : Kneading blocks

Extraction to Evaluate Extrusion Conditions. Twenty g (dry weight basis) of the powder was mixed with 300 mL of methanol (A452, Fisher Scientific, Pittsburgh, PA) and stored overnight at room temperature. The mixture was filtered using Whatman No. 2 paper (Whatman, International, Ltd., Maidstone, England), and the extract was evaporated to 3 mL by a rotary evaporator (Flash evaporator, Buchler Instruments, Fort Lee, NJ) for HPLC analysis. The residual powder after methanol extraction was further extracted by adding 200 mL of water saturated with *n*-butanol (Fisher Scientific, Pittsburgh, PA). The mixture was agitated for 6 hr at 75° C in a shaker apparatus (New Brunswick Scientific, Edison, NJ). After filtration under vacuum, the extract was concentrated to 12 mL for further HPLC analysis.

HPLC Analysis. HPLC conditions for the quantification of phenolic compounds were based on those reported by Wu et al. (*8*). A solvent delivery system (Model 6000A) and an absorbance detector (Model 440, Waters Associates, Milford, MS) with a 280 nm wavelength kit were used. The column was a PartiSphere C_{18} reverse phase column (Whatman, Inc., Clifton, NJ).

An aliquot of the concentrated extracts was injected into the column using an injector with a 20 µL loop for each analysis. The mobile phase consisted of 0.05 M sodium acetate (pH = 4.0):methanol (80:20) and its flow rate was 0.8 mL/min.

Quantification was done by comparing the integrated peak area from the extracts with those of known standards using an integrator (Model 4270, Varian Associates, Walnut Creek. CA). The phenol was purchased from Mallinckrodt Chemical Works (St. Louis, MO). A typical retention time for the phenol and phenyl-β-glucoside were ca. 18 and 8 minutes, respectively. The presence of phenol in the methanol extract was confirmed by HPLC-mass spectrometric analysis. No phenols were observed in the extracts from the unextruded corn meal by either methanol or water-saturated *n*-butanol.

Color Measurement. The color of the powder made from extruded corn meal was measured by absorbance of the methanol extract at 499.2 nm using a spectrophotometer (Model U-3110, Hitachi Instruments, Inc., Tokyo, Japan).

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch031

Results and Discussion

Distribution of Phenyl-β-glucoside and Phenol during Extrusion. Because a large quantity of material is required to perform the experiments on a twin-screw extruder, it is difficult to study a particular chemical reaction, especially when the chemicals are expensive. In order to solve this problem, we mixed the expensive phenyl-β-glucoside with the intensely colored red #40 dye, which was pressed into a pellet. The pellet was added into the extruder when the desired extrusion conditions for the corn meal were met. Distribution of phenyl-β-glucoside in the extrudates (at 165°C and 300 rpm) which were collected every 15 seconds from the dies after the pellet was added is shown in Figure 1. The results indicate that the majority of the glucoside added was distributed within 1 minute. In the case of the extrudate processed under 185°C and 500 rpm, a substantial distribution of phenyl-β-glucoside was observed between 0 and 15 seconds (Figure 2). In both cases, all the extracted phenol were observed within these periods (Figure 3).

It would be interesting to observe a relationship between the dye and the phenyl-β-glucoside in terms of residence time behavior during extrusion. Color intensity measured by absorbance showed a good linear relationship (Figure 4). The results indicate the dye and glucoside would behave almost identically during extrusion, even though they were not chemically attached to each other.

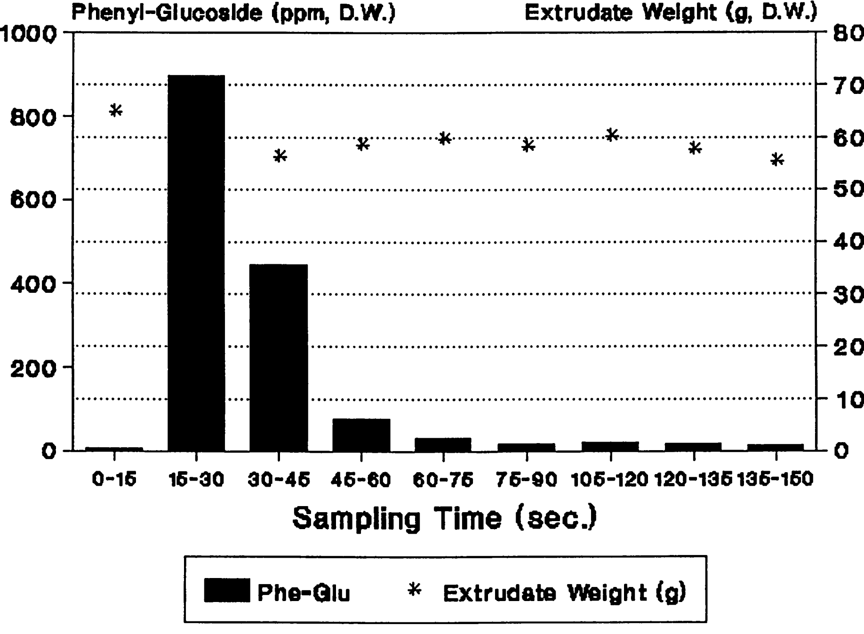

Figure 1. Distribution of glucoside in sample extruded at 165°C and 300 rpm.

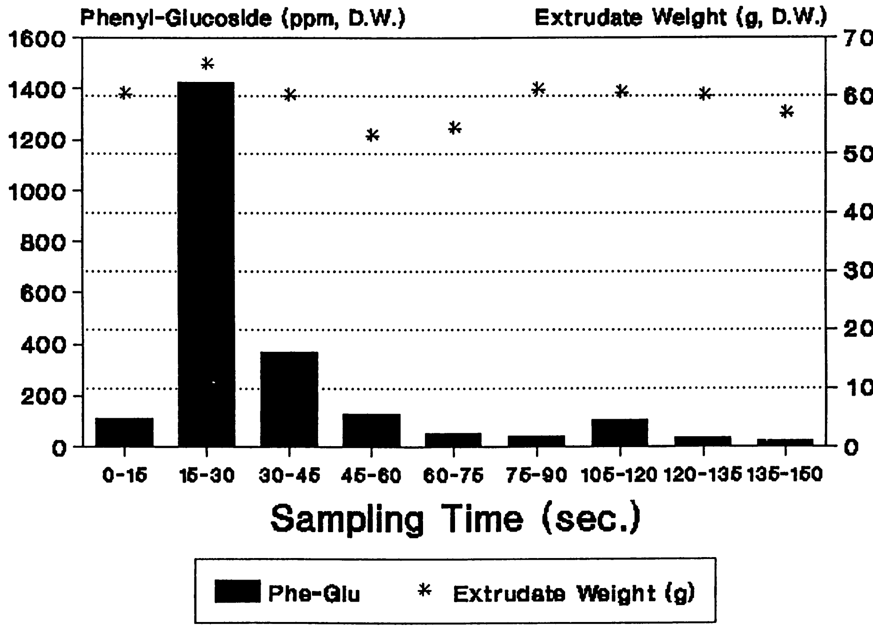

Figure 2. Distribution of glucoside in sample extruded at 185°C and 500 rpm.

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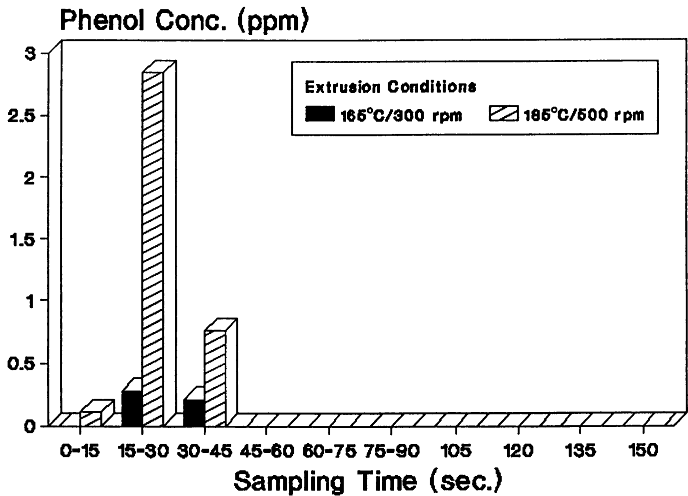

Figure 3. Phenol distribution (ppm in extrudates, dry weight basis)

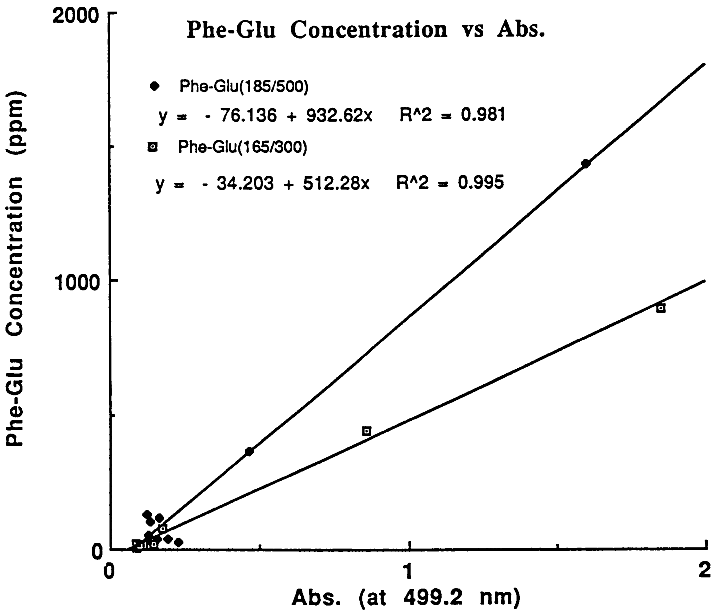

Figure 4. Correlation between phenyl-glucoside concentration and absorbance of the methanol extract.

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Formation of Phenol from Phenyl-β-glucoside during Extrusion. Figure 5 shows changes in the phenol concentration of the extrudates due to the difference in the extrusion conditions. Differences in the quantification results from methanol and butanol extractions showed that a majority of phenol formed was extracted by methanol. Generally, the concentration of phenol increased as the extrusion temperature and screw speed were increased. The highest concentration (6.96 ppm, dry base) of phenol was observed in the extrudate at 185°C with a screw speed of 400 rpm, and the lowest (0.37 ppm) under the conditions of 165°C and 300 rpm combination. As shown in Figure 5, however, the concentration of phenol extruded at 185°C and 500 rpm decreased. This might result from the loss to the atmosphere through evaporation of the steam at the die opening, and the higher extrusion conditions might have favored the dissipation. Actually, the odor of phenol was detected under the higher extrusion conditions mentioned above.

The effects of extrusion (die) temperature and screw speed on the concentration of phenol were analyzed by regression analysis using an SAS package. The results indicated that the extrusion temperature exerted a significant ($p = 0.01$) effect on the formation of phenol from the glucoside, however, the screw speed showed no significant effect.

The recovery of the phenyl-β-glucoside by the two solvents is shown in Figures 6, 7 and 8. It was found that the higher the extrusion temperatures and screw speeds used, more glycosides were recovered by water-saturated butanol than methanol. This might result from the difference in the degree of the entrapment of the glycosides by the extrudates formed by higher temperatures and screw speed. A recent study (*12*) concerning the fluorescence anisotrophy measurement of the extruded corn meal described the possible involvement of phenolic acids in the formation of crosslinking among the starch-protein matrix. It indicated that this immobilization of the innate phenolic acids could be responsible for the regidification of the extruded materials.

Attempts were made to calculate the conversion of extractable phenol from the glycoside by treating the extrudate with starch enzymes (amyloglucosidase, for example) to increase the recovery. However, cleavage of the glycoside in conjunction with the cleavage of the starch matrix was observed.

If 90% of 3.5 g of phenyl-β-glucoside added was assumed to be distributed within the first 60 seconds, the concentration of the glucoside was estimated ca. 1.3% (dry weight) of the extrudate (185°C and 400 rpm). Since the observed concentration of phenol was 6.96 ppm (dry basis) in the extrudate, its estimated conversion (mole basis) could be 0.15%.

Conclusion

Formation of phenol from phenyl-β-glucoside which was added extrinsically to corn meal was observed during cooking utilizing a twin-screw extruder. Evaluation of the extrusion conditions indicated extrusion temperature had a more significant effect than screw speed.

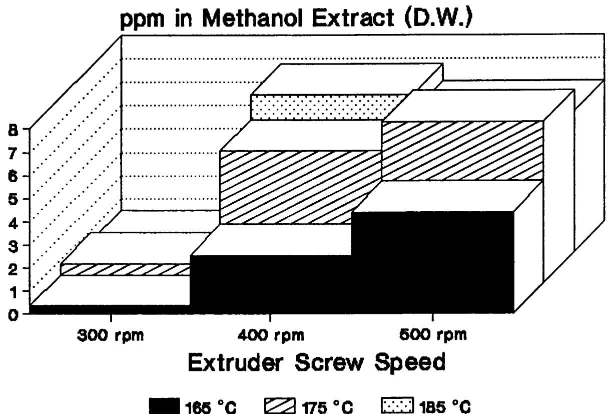

Figure 5. Effects of extrusion conditions on phenol concentration.

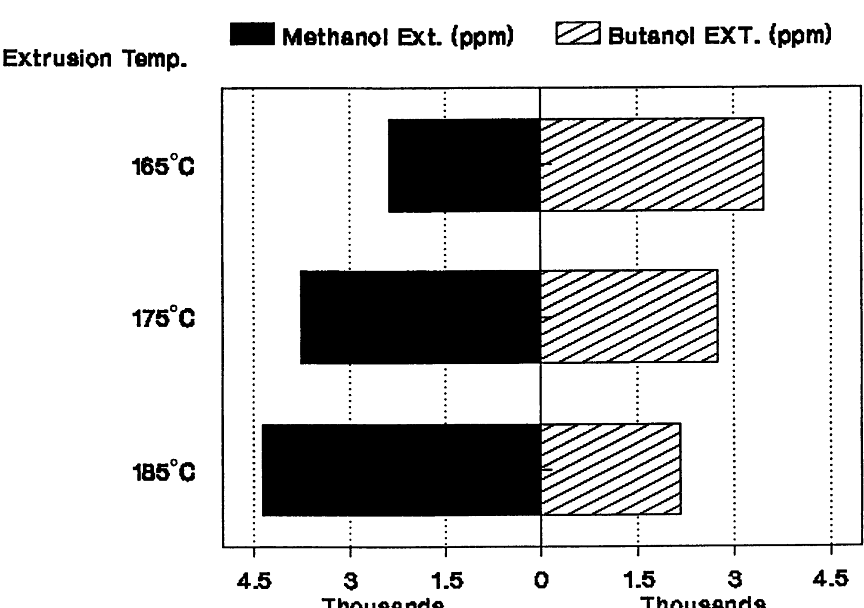

Figure 6. Glucoside concentration in samples extruded at 300 rpm.

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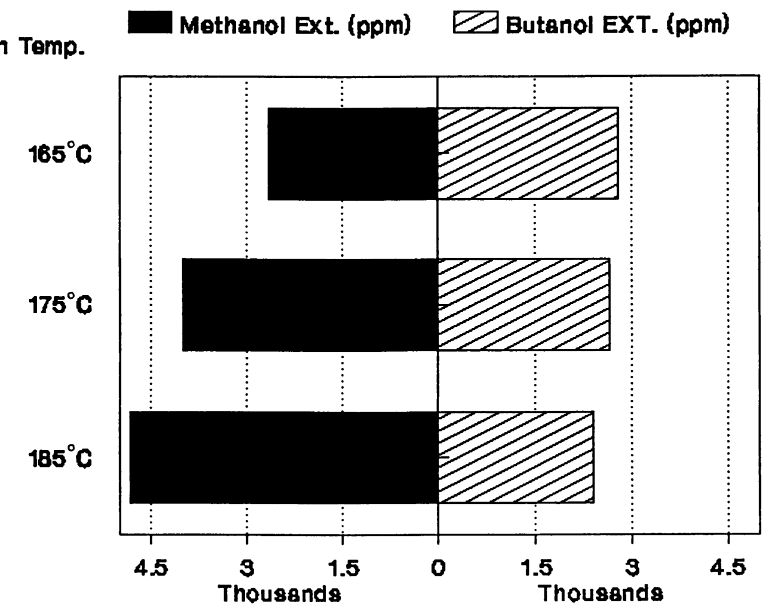

Figure 7. Glucoside concentration in samples extruded at 400 rpm.

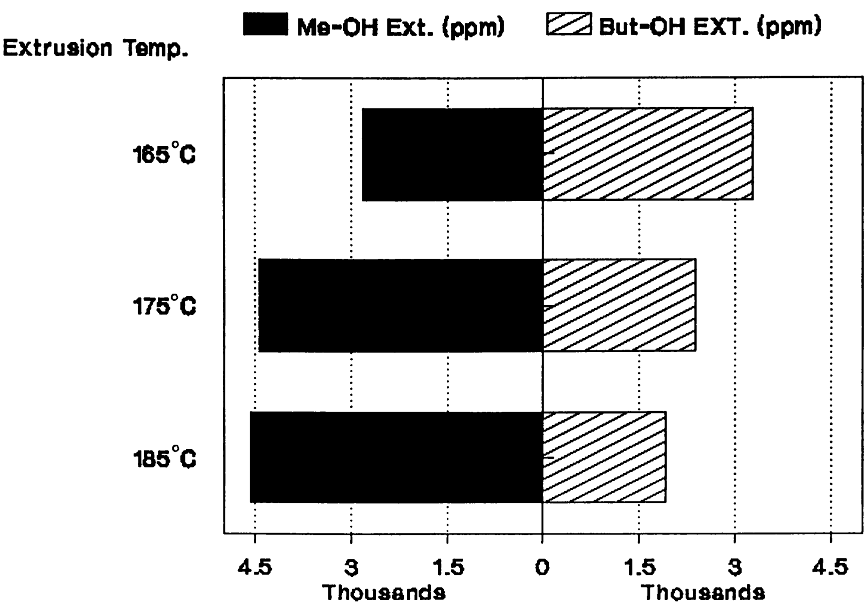

Figure 8. Glucoside concentration in samples extruded at 500 rpm.

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch031

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Chapter 32

Chain Length and Functional Group Impact on Flavor Retention during Extrusion

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A series of C_6, C_8 and C_{10} acids, alcohols and aldehydes were added to high amylose corn starch, the mixture adjusted to 24% moisture and extruded at 115°, 125° or 135°C using a 3:1 compression screw operating at 100 rpm. Residual added volatiles were extracted from the extrudates and analyzed using gas chromatography. Total retention was greatest for alcohols and lowest for aldehydes. For all compound classes, total retention increased with increasing chain length.

The flavoring of extruded foods has traditionally been a problem due to the limited stability of some flavorants during extrusion and their volatility upon exiting the die (*1*). Surprisingly few studies have specifically addressed this important issue (*2-7*).

Consequently, the major objective of this study was to add a series of compounds varying in chain length and functional group to a high amylose starch and to extrude at increasing temperature. The anticipated outcome was that this information would be useful in providing a better understanding of flavor compound retention during extrusion.

Materials and Methods

Materials. High amylose corn starch (Hylon V, National Starch and Chemical Corp., Bridgewater, NJ) was used as the base material. Hexanol, octanol, decanol, hexanal, octanal, decanal, and hexanoic, octanoic and decanoic acids were commercially obtained (Sigma Chemical Co., St. Louis, MO). Theramyl 120L was used for enzymatic starch digestion and was obtained from Novo Laboratories, Wilton, CT.

Feed Material Preparation. Each alcohol, aldehyde and acid was added at a level of 200 ppm to the starch and manually blended for 10 min. The moisture content of the starch was determined and then adjusted to 24% with tap water. The resulting mixture of flavor compound, starch and water was manually blended for an additional 10 min., transferred to moisture- proof containers, and stored at 22°C for eight hours

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to insure equilibrium. Blending was performed at 4°C to minimize volatile compound losses.

Extrusion. A Brabender Plasticorder Model PL V500 single screw laboratory extruder with a barrel length-to-diameter ratio of 20:1 was used. It was equipped with a 4.80 mm die opening and a 3:1 compression screw operating at 100 rpm. Dough temperature just before the die exit was maintained at 115°, 125° or 135°C. The ratio of measured extrudate diameter to the die diameter resulted in expansion ratio values. In addition, a series of control samples, without added flavorants, were obtained at each of the three extrusion temperatures.

Compound Extraction. Extrudates were permitted to air dry at room temperature for 24 hours, then were frozen and ground to pass through a 0.5 mm screen. Two 0.5 g ground samples of each extrudate were placed in screw cap culture tubes and suspended in 4 mL of deionized water. No enzyme was added to one set of tubes while enzyme was added to the other set. Both sets of tubes were sealed and incubated at 50°C for one hour in a vibrating water bath. The tubes were permitted to cool to room temperature and then extracted with 4 mL of ether. The extraction was repeated twice, and the ether extracts were combined and concentrated under a stream of nitrogen. The set of tubes with no added enzyme represented the "free" volatiles that were not bound to starch. The second set of tubes represented the "total" volatiles that were released by the enzyme. The "bound" volatiles were represented by the difference between the "free"" and "total" volatiles. Preliminary informal subjective analysis demonstrated that the starch/water mixture remaining after extraction contained no detectable amount of added flavorants.

Gas Chromatographic Analysis. A Hewlett Packard Model 5830A gas chromatograph was used to separate and quantitate the added compounds. A 2 m glass column packed with 5% Carbowax 20M on 80/100 mesh GasChrom P was used. Known quantities of the added compounds were used for identification/quantitation purposes. All quantitation data were converted to percent extrudate retention.

Statistical Design and Analysis. A complete factorial design involving all variables was used. All extrusion runs were repeated and all analyses were performed in duplicate for each of the two runs. All resulting data were then combined and averaged.

Results and Discussion

One could argue that the degree of expansion that an extrudate undergoes immediately after exiting the extruder die can influence volatile retention with the greater the expansion, the larger the surface area that would be available for volatilization.

Extrudate Expansion. As can be seen in Figure 1, extrusion temperature and type of added flavor compounds apparently did influence extrudate expansion. As expected, overall expansion increased from 115° to 125°C, where maximum expansion occurred. However, at the higher extrusion temperature (135°C), dough viscosity was not ideal to promote optimum expansion.

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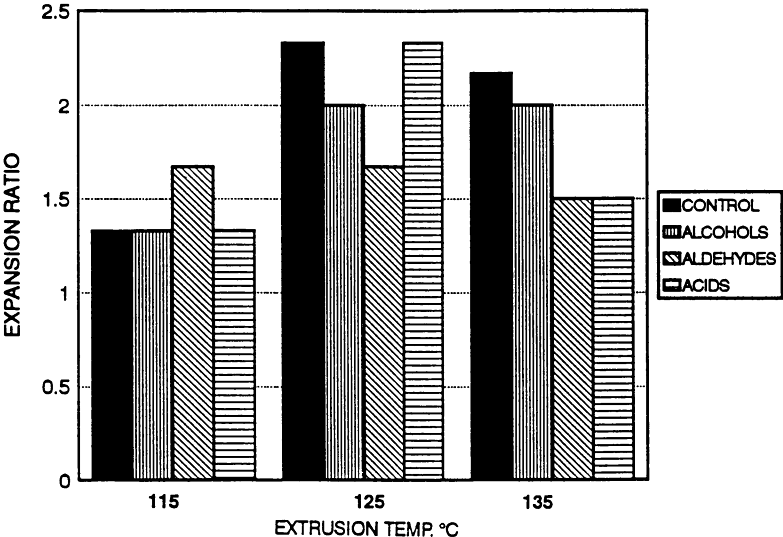

Figure 1. Extrudate expansion as influenced by extrusion temperature and functional group additions.

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When added compound functional groups are compared to a no-additive control, temperature also displayed significant interaction. At a low extrusion temperature (115°C), it can be seen in Figure 1 that alcohols and acids did not influence extrudate expansion, but the presence of aldehydes actually increased extrudate expansion. However, at the highest extrusion temperature (135°C), it can be seen that the addition of all compounds resulted in lower expansion ratios than a no-additive control, with aldehydes and acids resulting in less expansion than alcohols. Alternatively, at 125°C, it can be seen that acid additions did not influence extrudate expansion, whereas alcohols and aldehydes also lowered extrudate expansion. The data clearly demonstrate that most of these compounds can apparently interact with starch during extrusion, thereby influencing expansion.

Standard deviation data are not shown in Figure 1 since the maximum noted deviation was less than 7%.

Compound Retentions. As can be seen in Figures 2, 3 and 4, overall compound retention increased as compound chain length increased. This is probably best explained on the basis of volatility, with shorter chain compounds in a homologous series having more volatility than longer chain ones.

It can also clearly be seen in Figures 2, 3 and 4 that the ratio of "free" to "bound" compound retention was influenced by functional group. In the case of added acids, (Figure 2) most of the retention was in the "free" form. In fact, at the highest extrusion temperature (135°C), no "bound" C_6 or C_8 acids were found. These data indicate that under the conditions of this experiment acids do not bind to starch during extrusion. However, in the case of alcohols (Figure 3), more binding regardless of chain length can be noted. Aldehydes (Figure 4) behaved more like acids,

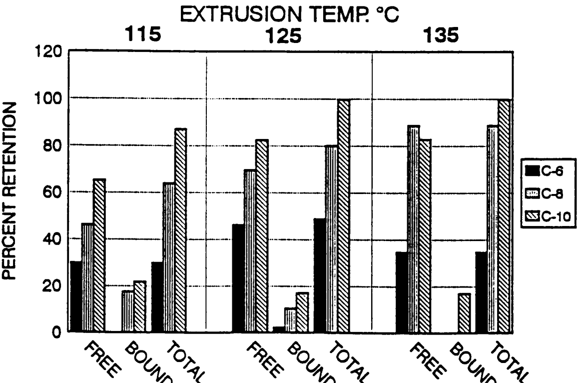

Figure 2. Retention of added acids as a function of chain length and extrusion temperature.

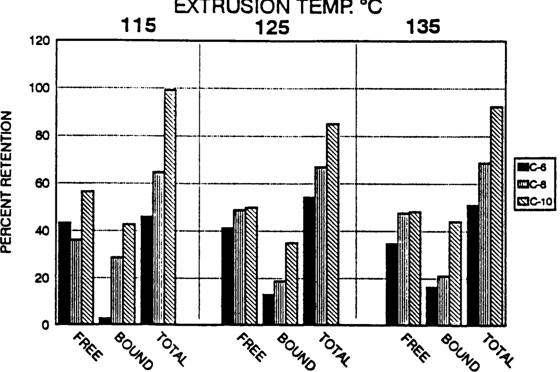

Figure 3. Retention of added alcohols as a function of chain length and extrusion temperature.

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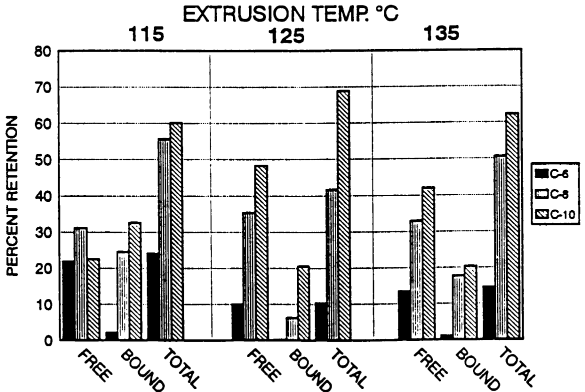

Figure 4. Retention of added aldehydes as a function of chain length and extrusion temperature.

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especially at the two higher extrusion temperatures, since the "bound" form of the compounds was quite low.

If one looks at the total amount of added compounds retained, it can be seen that at all extrusion temperatures, alcohols had the greatest retention (85-98%) while aldehydes had the least (60-68%). Total retention of added acids was also relatively high ranging from approximately 85 to 98%.

The presence of "free" and "bound" flavor compounds in extruded products are both quite important in that in theory the "free" compounds can influence product acceptability before actual product ingestion, while "bound" compounds can modify product acceptability during product mastication.

Conclusion

This study demonstrates that compounds of the same chain length but possessing different functional groups behave differently relative to their retention during extrusion processing.

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Chapter 33

Critical Factors in Microwave-Generated Aromas

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In many cases microwave ovens are not conducive for the development of aromas when heating foods. Much of this is due to the extreme speed of heating and the low ambient temperatures in the oven. However, other factors also play a role such as evenness of heating, varying depths of penetration into foods, and most importantly water pumping which maintains the food surface at a temperature of or near 100°C and a water activity of 1.0, neither of which is suitable for Maillard aromas. It remains for the food technologist to overcome these problems through special formulations and the addition of suitable flavors.

It took more than 25 years after the invention of the microwave oven in 1945 by Percy Spencer of Raytheon for consumers to begin purchasing them in any significant quantity. It took another ten years for the food industry to realize that there was an opportunity to market foods for microwaving. In the interim, oven owners were mostly on their own in preparing microwave meals.

What happened in the USA is somewhat unusual because, while microwave manufacturers were selling these appliances as cooking devices, most consumers saw them simply as convenient food reheaters. Despite the often well written and beautifully illustrated cookbooks provided with the ovens, offers of free cooking classes, and growth of special microwave cooking schools, consumers resisted cooking in the microwave oven. If they did try roasting meat, for example, they came away confused and disappointed. The roast looked different, and the wonderful aroma of roasting meat didn't come across, and so it was hard to tell when it was done. The

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cooking of many items required following recipes with care, doing some things that were unusual and then frequently having to accept food that just didn't appear or taste right. Anyone who has ever tried to bake a microwave cake may end up with something that doesn't brown, may be too moist or collapse, or may be overheated and turn dark brown or black on the inside, while the surface still looks golden yellow.

All these problems, plus the need to learn new microwave cooking techniques and to accept compromises in flavor, texture and appearance of the food, have made microwave cooking something of a rarity. While over 90% of US adults use their microwave ovens to reheat leftovers, less than 30% cook meat or fish and less than 15% bake cakes, pastries or cookies (1).

Much of the fault for this failure can be laid at the doorstep of the microwave oven manufacturers for providing an appliance that is essentially a steamer or poacher and expecting consumers to follow unusual procedures to simulate broiling, baking, roasting, braising and other cooking procedures which require high surface temperatures to provide browning and crisping. Further, oven manufacturers provided an appliance that heats so quickly that it is difficult to control in terms of uniformity of heating, development of internal structures in such products as cakes, and the development of proper flavors and aromas. How much better the situation would have been if those early microwave oven manufacturers had produced only combination microwave/hot air ovens. Such ovens with lower power output to allow both heat sources to be used simultaneously would provide more even cooking but still significantly faster than conventional cooking. An option for microwave-only heating at higher power for reheating or cooking vegetables, etc. rapidly could have been provided. Microwave only ovens are cheaper and easier to sell, and so in the U.S. today there are more than 100 million microwave ovens of which fewer than 5% are combination ovens with both microwave and hot air heating. By contrast, some European countries sell at least 50% combination ovens in their markets. No wonder Europeans believe that Americans like their meat gray and tasting stewed instead of roasted.

Microwave Factors in Aroma Development

Yeo and Shibamoto (2) indicated that "The short cooking times and low temperatures achieved during microwave irradiation usually do not induce the Maillard reaction, which produces many flavor components. Furthermore, the rapid loss of water during microwave irradiation causes distillation effects'; resulting in the loss of volatile flavor compounds, especially those with polar

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characteristics". These same authors went on to describe the development of various compounds that appear to be developed during microwaving of meats and baked products that contribute to off flavors and aromas. The chemistry of the various aromas developed by microwaving foods will be covered in subsequent chapters, however, the focus here will be those microwave factors which contribute to flavor development. We may divide these into two broad groups: characteristics of the ovens themselves and effects due to the manner in which microwaves interact with foods.

Oven Characteristics: It is essential to understand that it is not possible to speak of microwave ovens as representing a single appliance as one might do with a standard electric range, toaster, or pressure cooker. Microwave ovens differ in so many ways, as shown in Table I (3), that there are 50 or more different types of microwave ovens whose heating and, therefore, cooking characteristics vary widely.

Table I. Microwave ovens vary in a number of different ways

a) Power output: 400 to 750 watts
b) Cavity size: 0.4 to 1.8 cubic feet
c) Microwave feed system: mode stirrer rotating antenna, rotating waveguide
d) Location of microwave input into the oven: at top or bottom of oven cavity, on both top and bottom of the oven or the sides of the oven,
e) Cavity wall construction: stainless steel or painted cold-rolled steel
f) Presence or absence of turntable
g) Use of glass shelf or metal rack in some ovens
h) Microwave only or microwave-convection or microwave with a browning element

In addition, the power output of a microwave oven diminishes with age and usage. Those factors which most affect the development of aromas are:

- speed of heating
- uniformity of heating
- ambient conditions in the oven

The first two are intimately related, while the latter profoundly affects the surface temperature of the food.

Speed of Heating: The rate at which any material is heated by microwaves is dependent upon the electric field strength generated within the food and the dielectric and thermal properties of the food as seen in Equation 1:

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch033

$$\text{Rate of temperature increase} = \frac{kfE^2\varepsilon''}{\rho C_p} \qquad (1)$$

where, k = constant to express units of temperature and time
f = frequency
E = electric field strength
ρ = density of the material
ε'' = dielectric loss factor
C_p = specific heat

The output power of the magnetron is the primary contribution to the heating rate but other factors such as the type and location of the microwave feed system, the materials of construction of the oven and the size of the oven cavity also play an important role as does the food itself. It is a mistake to look at the manufacturers' power rating for an oven and extrapolate from that to what the heating rate will be. In other words, not all 700 watt ovens heat at the same rate. Not only that, but an oven's rating is only an average number and actual power may vary by ± 20%; i.e. a "700 watt" oven may actually produce between 560 and 840 watts. We must also understand that the power ratings are always made into large loads of water; (i.e. one or two Liters) which gives a significantly higher number than if the measurement were made into a smaller load, i.e. 250 mL which is more representative of commonly cooked or heated foods in terms of water equivalency. It is also possible to design ovens with lower power ratings, i.e. 400 or 500 watts, which couple energy better into small loads and heat these faster than an oven rated at 700 watts.

Microwave ovens generally will heat most foods in 1/4 to 1/3 of the time necessary to accomplish the same degree of heating in conventional ovens. This is primarily due to the direct coupling of the electromagnetic field with the food and its conversion directly into heat within the food. There is no need to heat an intermediary heat transfer medium such as air. This rapid heating may interfere with the cooking process if one looks upon cooking in chemical and physical terms. Cooking, and hence aroma development, is a result of a complex series of chemical reactions and physical changes which must occur in a controlled sequence. If the heating rate is too high, reactions such as the Maillard reaction, may not have time to go to completion. The rapid cooking may also reduce the effectiveness of conductive heat transfer. This will affect the uniformity of temperature distribution and make it

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch033

necessary to heat for a longer period to raise the temperature in the cooler zones while at the same time continuing to heat, sometimes excessively, in the hot zones. This may lead to excessive evaporation, drying, and the development of burnt flavors and aromas, often accompanied by undesirable hardening.

Uniformity of Heating: From an oven perspective, the uniformity of temperature or lack thereof, in foods is due to several factors. First, all ovens have hot and cold zones, that is areas of high or low electric field, as a result of standing wave patterns. These may, in turn, produce areas of high and low heat within a food. The electric fields also cause edge heating effects and concentrations of energy in corners. Energy which must reach the bottom center of a food in a tray may be prevented from doing so in a top or side feed oven by the inability of the waves to be reflected from the bottom to enter the food from below. This is especially true in cases of a food which is thick, (>5 cm) or large in surface area (more than 20 cm x 20 cm). Thus, a rectangular tray will typically show its highest temperatures, or zones of most intensive microwave energy in the corners and the coolest (low energy) zones in the center. If the food is heated in a metal container or upon a metal turntable, the food closest to the metal will be heated only by conductive heat transfer due to shielding restrictions of electric field energy at metal boundaries.

With uneven heating we can expect to find uneven development of aromas, both good and bad. The foods themselves will also contribute to these effects as will be described below.

Ambient Conditions in the Oven: By far, the most peculiar situation one encounters in a microwave oven is the cool ambient conditions. Air is transparent to microwave energy and, unless one has added some form of auxiliary heater, will rise to approximately 50°c due to heat loss from the food and warming of the cooling air for the magnetron. The result is that it is very difficult for the surface of high moisture foods to exceed 100°C. This prevents high temperature reactions from occurring which then prevents the formation of the associated aromas and flavors. The cool air also condenses water reaching the surface so that the surface tends to remain at or close to saturation, with a water activity of 1.0. Food surface temperatures are limited to ≤ 100°C as long as moisture is present at the surface. This is not conducive for the Maillard reaction since it keeps the reactants in a highly diluted state as well as maintains temperature lower than desirable for the reaction to proceed in a reasonable time. Sometimes, when microwave cooking large portions of meat with significant fat on the surface for a long cooking time,

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch033

it is possible to obtain the high surface temperatures required for Maillard browning, flavor and aroma. This is in part due to a reduction of water loss from the meat and drying of meat surfaces as it becomes thoroughly cooked. In fact, it is possible under such circumstances for the surface temperature of meat to exceed 100°C.

Microwave Interactions with the Food: Once again, too rapid heating and uneven heating are major effects which affect aroma development. To these we must add one more power effect: water pumping.

Water Pumping: In conventionally heated foods moisture migrates to the surface in a somewhat passive manner. As water evaporates from the surface, water from below migrates to the surface, usually through capillarity, in order to maintain a moisture equilibrium between a drying surface and a wet interior. The rate at which water is transported is diffusion rate dependent. Such movement can be slow enough to permit the water activity of the surface to decrease below 1.0. This allows the surface temperature to climb above 100°C and the heat plus the concentration of Maillard reactants promotes browning with its associated aroma, flavor and color effects.

In a microwave oven, water movement is due to the generation of an internal vapor pressure which actively pumps water to the surface. No longer is water movement diffusion rate limited, but may continue at such a rate to maintain the surface at a saturated condition, and evaporative cooling will maintain the temperature at or below 100°C. Water pumping acts to defeat crisping as well as Maillard browning.

Heating in the Microwave Oven

Rapid Heating: Referring again to Equation 1, above, we note that the rate of heating is directly related to the dielectric loss properties and inversely proportional to the specific heat of the food. Foods of high dielectric loss such as ham, gravy and most salty foods heat rapidly. Foods with low specific heats, such as high sugar or oil containing foods, will also heat rapidly. Sometimes these foods may exhibit runaway heating since their loss factors increase with temperature as seen in Figure 1 (4). Such rapid heating is accompanied by water pumping and edge heating effects which may cause areas within the food to dehydrate and burn.

Another property which influences the rate of heating is the mass of food being heated. The smaller the food mass, the faster it will heat despite the fact that it may couple energy less efficiently. One cannot expect much aroma development in a 100 gm portion of food

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch033

which may be heated in one minute as compared to the 40 or 60 minutes required to cook a 3lb. chicken.

Uneven heating: In addition to the oven effects described above, the dielectric loss properties of foods themselves contribute to uneven heating. Consider a three component meal in which there may be meat, gravy, vegetables and potatoes. All the components are likely to heat at different rates. The fastest would probably be the gravy which might burn around the edges by the time the other components were hot. This would especially be true when dealing with frozen foods due to the tremendous difference in the dielectric properties of ice and water. These differences may result in runaway heating of portions as they thaw (Table II).

Table II. Dielectric Properties of Water and Ice at 2,450 MHz

	Relative Dielectric Constant	Loss Tangent	Loss Factor
Water (25°C)	78	0.16	12.48
Ice	3.2	0.0009	0.0029

The mass of the food may also play a role in a multicomponent meal. If one portion is particularly large, e.g. a frozen chicken breast, compared to the other components, the smaller portions may become overheated and develop burnt aromas and flavors by the time the chicken is properly heated.

Depth of Heating The dielectric loss properties have another profound effect in controlling the depth of penetration of microwave energy and hence the temperature profile. Equation 2 shows the formula to determine the half-power depth, D_{50}, which is the depth at which 50% of the energy has been attenuated.

$$D_{50} = \frac{0.347\,\eta_o \sqrt{\varepsilon'}}{\pi\,\varepsilon''} \quad (2)$$

where, ε' = relative dielectric constant
η_o = wavelength in free space
ε'' = dielectric loss factor

Figure 2 shows the effect of penetration depth upon the temperature profile of equal thicknesses of ham and peas. In Table III (6) we see that the penetration depth for both foods varies with temperature, however, in the opposite way for each food.

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch033

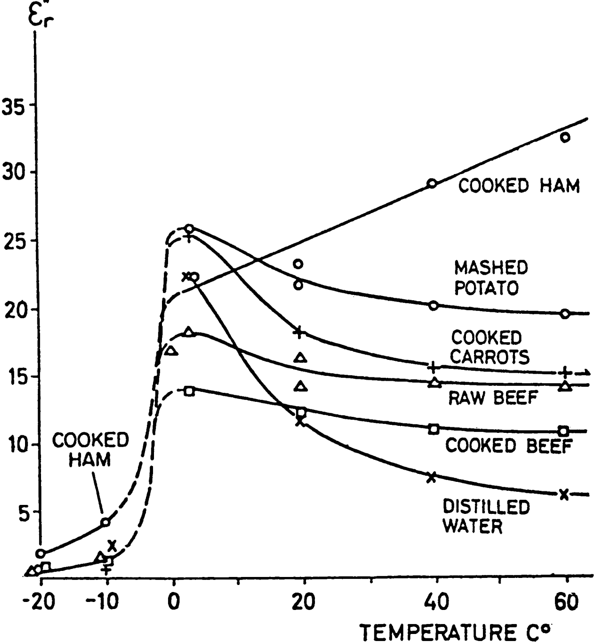

Figure 1: Temperature dependence of dielectric loss factor (ε") at 2.8 GHz. (4)

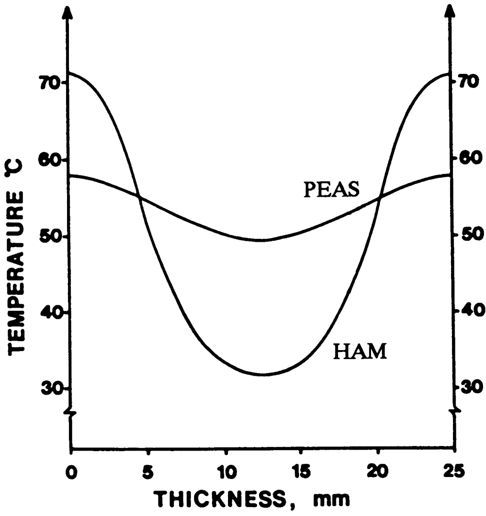

Figure 2: Penetration of microwaves into 25 mm thick slabs of peas and ham at 2,450 MHz (5)

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Table III. Half Power Depth (CM) of Carrots and Ham

	10°C	60°C
Carrots	0.5	0.7
Ham	0.4	0.3

Addition of salt to a food seriously affects the penetration depth so that most of the heating will be at and near the surface. In order to raise interior temperatures it may be necessary to overheat the surface.

It is also possible to achieve conditions in which the highest temperatures are in the interior as occurs in small jars of baby food or a microwave baked potato. The former can present a serious high temperature hazard to the child; the latter may lead to interior burning accompanied by burnt aromas and taste.

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch033

Conclusion

The development of successful microwavable foods is a great challenge for the food technologist. Similarly, the development of the correctly balanced and appropriate aromas and flavors is a significant challenge for aroma and flavor chemists. It is a problem - not only of trying to hit a moving target, but one which may move in different directions at different times or under different circumstances. It is unlikely that superior results can be achieved every time in every microwave oven, but rather one should aim for a spectrum of results, hopefully from good to excellent, and experience shows that every now and then a poor to fair result will also occur. In order to optimize the better results it is essential that the food scientists and technologists understand microwave and microwave ovens in depth, otherwise the targets will never be hit.

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Chapter 34

Flavor and Flavorings in Microwave Foods

An Overview

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An overview is presented on the influence of microwave heating on flavor. Differences between microwave and conventional heating are used to illustrate how these procedures effect flavor. It is concluded that, the difference in time-temperature profile can account for the flavor related phenomena. This difference changes how the food matrix influences flavor retention and generation. Criteria for selecting and modifying flavor systems for microwave foods are also discussed.

Flavor, in the limited sense, is the perception that originates in the brain from aroma and taste stimuli perceived by the nose, tongue, and palate. It is important to realize that character and intensity of volatile flavor (aroma) depend exclusively on the concentration of single flavoring substances in the gaseous phase. Any factor that influences the mechanism of release of volatile compounds unavoidably leads to a corresponding change, either positive or negative, in flavor profile and intensity. Most substances that cause taste perception are non-volatile; hence, intensity of taste perception depends on the concentration of the triggering substance in the aqueous phase (e.g. saliva). Processing and preparation of food products have a great influence on aroma profile; whereas, the taste profile depends mainly on the basic composition of the food. In a food product, the largest influences on flavor impact are the interaction of the flavor compounds with other components of the food, and the food preparation procedure.

The physico-chemical interaction of flavor substances with other ingredients of the food system can change the way the flavor compounds are released into the gaseous phase. The interactions take place in the form of reversible and irreversible binding of the flavor chemicals to carbohydrates, proteins, fats, and ions. This

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binding is very substance specific, and can alter the relative proportions of flavoring substances that are in the gaseous phase, which can lead to a flavor profile that is unbalanced. The generally occurring interactions of flavoring substances in the foods can be summarized as follows:

With carbohydrates:	Adsorption Inclusion compounds
With proteins:	Adsorption Hydrogen bonding Hydrophobic interactions Covalent bonding (carbonyl compounds)
With fats:	Adsorption Hydrophobic interactions Dissolving of non-polar/less polar flavor compounds

The other major influence on the flavor profile of a food product is the preparation or processing procedure. Processing and preparation methods may generate flavor compounds (e.g. by fermentation, roasting, cooking, baking, etc.), but these methods may also result in the loss of desirable flavor substances by physical means (e.g. evaporation, steam distillation, etc.). The later effect is especially important in the case of microwave heating, as will be discussed later.

Effect of Microwave Heating on the Properties of Flavor Compounds

In conventional cooking, heat is transferred from the environment to the food product by conduction, convection, or infrared radiation. Oil or fat is often used as a heat transfer medium in order to reach surface temperatures higher than 100 °C in a short time. This results in a temperature gradient from the surface to the inside of the food. In meat, for example, the surface temperature may reach 120 - 130 °C, whereas the center does not surpass 65 - 75 °C. The cooking effect a time-temperature relationship depends on the type and size of the oven, the size and weight of the food, and the heat conductivity of the food.

In microwave cooking, the factors relevant to generation and performance of flavoring substances in foods can be summarized as follows:

- The alternating electromagnetic field sets polar molecules into a flip-flopping action, thereby increasing their temperature.
- Heating occurs throughout the whole food simultaneously, although the rate of heating may not be uniform throughout the food.
- A high temperature is reached within a short time.
- Food products tend to dry out as no crust formation takes place. At the same time the surface remains moist and soggy.

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- Browning reactions occur to a very limited extent, due to lower surface temperatures and higher surface water activity.

The concept that heating occurs from the inside to the surface of the product is erroneous. The basis for this misconception is that temperatures at the surface tend to be lower than those inside the product, which is a result of moisture migration and evaporative cooling.

Heat development in a food exposed to microwaves depends on the appliance and the composition of the food. The appliance dependent factors are:

- The oven setting, which determines the magnetic field strength. In current household appliances maximum power settings are between 400 and 1200 watts.
- The magnetic field distribution, which may be influenced by auxiliary means such as wave stirrers, turntables, etc.
- Oven geometry and size.

The factors related to food composition are:

- The dielectric properties of the food and its specific heat capacity, which are determined by the foods composition. Water, fat, salt content, and their distribution have a great influence on temperature level and distribution.
- Homogeneity of the food. Liquid foods such as beverages, soups, and sauces have a more uniform temperature profile than ready-made meals, which contain pieces of meat, vegetables, potatoes, etc. together with a sauce. Deep-frozen foods, during defrosting, are especially problematic. At the point in which both ice and water are present there can be run-away heating and hot spots.
- Form, size, and weight of the food product.

In the case of microwave heating the composition of the food product has a much greater influence on the temperature distribution, and consequently on the cooking effect, than in the case of conventional cooking.

A very important effect of microwave heating relating to flavor is water vapor migration to the surface of the food. Many flavoring substances are steam volatile. During microwave heating these compounds evaporate with the water at temperatures well below their boiling points. If no barrier is present they are transported to the surrounding atmosphere, and they are lost from the food. A further effect of vapor migration is to make the surface conditions unfavorable for flavor generation (*1*).

The influence of moisture migration in microwave cooking is best illustrated by comparison to conventional cooking.

In a conventional oven the ambient air has a high temperature (ca. 180 °C) and low relative humidity. Heating of the food results in a temperature gradient and a corresponding water vapor pressure gradient from the surface to the center. In the initial stage of cooking, water evaporation cools the surface of the food, and the region of maximum temperature and vapor pressure lies directly below the surface. This means the water vapor generated inside the food cannot escape to the outside, and flavoring substances present or generated within the food are confined. There is also a driving

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch034

force for moisture to migrate from the region of high water vapor pressure to the outside; however, water in the surface area is driven out quickly, and the surface dries out. At the high ambient temperatures near the surface of the food protein denaturation, starch gelatinization, caramelization, etc. take place. The result is formation of a crust, which acts as a moisture and flavor barrier. Even if the level of maximum water vapor pressure is shifted closer to the center of the food during prolonged cooking, water vapor and the flavoring substances carried with it are hindered from evaporating into the ambient air by this crust.

Conditions during microwave cooking are quite different: the ambient air temperature is low (ca. 25 °C) and the relative humidity is high. The region of maximum temperature and consequently maximum vapor pressure generally lie deeper below the surface than in conventional cooking. Therefore, the main driving force for moisture migration is directed toward the surface, and water vapor generated within the food, along with flavoring substances, is driven toward the surface. As the rate of water evaporation is not sufficient to dry out the surface, and surface moisture is continually replaced by the vapor migrating from the interior, the inside of the product dries out while the surface remains moist. The low temperature and high water activity near the surface have the following consequences: No crust formation takes place, and Maillard browning reactions do not occur to an appreciable extent.

The discussion so far has focused on the thermal effects of microwave heating. Attention can now shift to the athermal effects of microwave energy. The quantum energy of microwave radiation is to small by 3 to 5 log values to induce molecular breakdown or to catalyze chemical reactions in foods (See Table I.). The energy content of microwaves is lower than the radiation energy in an infrared grill. Therefore, radiation-chemical effects have to be regarded as theoretically highly improbable if not impossible. Nevertheless, there have been reports claiming such effects: isomerization of fatty acids, changes in the amino acid profile of vegetables, isomerization of L to D amino acids in milk, and the breakdown of citral into a dozen artifacts (*2*). However, precise details of the time and temperature profiles of the systems are not included in these reports. Without this information, the occurrence of hot spots can not be ruled out, and these claims must be considered with caution.

Practical experience and theoretical considerations lead to the conclusions that, the difference in generation and performance of flavoring substances in microwave foods can be explained satisfactorily by differences in the heating pattern, the corresponding differences in water vapor migration, and the resulting physical changes which occur at the surface of the food.

Flavor Generation in Foods by Microwave Heating

Food products, both natural and fabricated, have an intrinsic flavor in the uncooked state that is very often rather subdued. This "primary" flavor is a consequence of the food's composition. It is only after preparation by heat treatment that the often far more intense

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch034

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch034

Table I: Quantum Energy of Electromagnetic Radiation at 2,450 MHz vs. Binding Energy and Activation Energy of Chemical Reactions in Foods

		Molar Energy kJ/Mol
Equivalent quantum energy of electromagnetic radiation at 2450 Mhz:		ca. 0.001
Activation energy of most chemical reaction in foods:		59 - 118
Binding Energy of different types of bonds:		
--Covalent bonds	atom--atom	42 - 460
	-O-O-	147
	-S-S-	230
	-C-C-	248
	-C-H-	365
	-C=C-	428
	-O-H-	460
Hydrogen bonds	O...H...O	6 -34
	in H_2O	19
	in CH_3COOH	ca. 31
	in peptides	ca. 34
	in peptides (aqueous Solution)	ca. 6
Hydrophobic Interaction		0.025/Å^2 surface
	ala...ala	3
	val...val	8
	leu...leu	9
	phe...phe	13

"secondary" flavor is generated. The phenomena that occur on or directly below the surface of the food, especially in baking, frying, and roasting processes, determine the flavor, and it is at the surface where the biggest differences between microwave and conventional heating are observed.

Ideal conditions for Maillard browning reactions are temperatures above 100 °C and water activities between 0.60 and 0.80. As discussed in the previous section, these conditions are easily attained in conventional cooking procedures. However, due to lower surface temperatures, short cook times, and higher surface water activity (ca. 1.00), Maillard browning reactions, if they occur at all, proceed at a much slower rate. Moreover, the reactions between the flavor precursors present in the food are of a different kind, leading to reaction products with a different chemical structure and flavor profile (*3*).

Linking thermal flavor generation exclusively to Maillard reactions is an over simplification. Reaction of fats are also of great importance to thermally generated flavors. As in the case of Maillard reactions, these reactions are also suppressed, resulting in an incomplete flavor profile. In addition caramelization reactions, which require more severe conditions than Maillard reactions with the generation of the corresponding flavoring and coloring substances are essentially not possible under microwave cooking conditions.

A number of approaches to overcome the insufficient flavor generation associated with microwave cooking have been attempted. First, modification to the cooking environment has been accomplished by the use of special microwave browning pans or microwave absorbing susceptor sheets. These specialty products change the thermal environment at the surface of the food. Combination ovens, in which microwave energy is applied either simultaneously or intermittently with a conventional heat energy source, appear to be gaining in popularity.

Another way to compensate for the lack of flavor is through addition of commercial flavorings. The success of this approach is dependent on the flavor profile desired and the specific application. Addition of flavorings appears to be most successful in systems that require flavor chemicals with higher boiling points (less volatile). Crust type flavors are an example of reaction flavors that are a unique challenge. These flavors are localized on or near the surface of the food, but are not in the interior. Special methods are needed to apply these flavors so that their effects are limited to the outer parts of the product. Recent research, especially in the US., is pursuing topical flavor systems that not only provide flavor precursors, but compounds which modify the dielectric property of the surface of the food product. Alteration of the surface dielectric properties make it possible to generate sufficient heat for the precursors to react. These systems make it possible to generate the desired flavor and color profiles *in situ* (*4-8*).

Starchy foods (e.g. cakes) present yet another set of problems. As the starch gel forms in these high moisture foods, polar flavor compounds can dissolve and be trapped in the gel. In addition, non-polar flavoring substances can form inclusion complexes with the amylose. In both cases the flavoring substances are bound

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch034

preventing them from volatilizing and being perceived in the flavor. This binding phenomenon is not exclusive to microwave cooking.

Loss of flavor compounds is a major problem in microwave cooking. As described earlier, the outward migration of water vapor carries away volatile flavor compounds. The only way to avoid this is to apply suitable coating systems that will form a film at surface temperatures of ca. 100 °C. This film performs the same function as the crust formed during conventional cooking. In addition to the functional ingredients (e.g. carboxymethylcellulose, xanthan gum, polysaccharides, modified starches, etc.) required to form the moisture barrier, the coating system can contain flavor compounds, precursors, and coloring substances. Such coatings are often also required to mask off-notes, which originate from starch or frying fat due to the short cooking times.

Flavorings for Microwave Food Products

Flavoring compositions are added to microwave foods for the same reasons as in other types of fabricated foods:

- To impart the desired flavor character.
- To enhance or compliment the food product's intrinsic flavor.
- To compensate for losses resulting from processing, preparation, or storage.

Migration of water vapor to the surface of the food is the most important factor influencing flavor retention. Flavorings used for microwave applications should, therefore, have low water vapor volatility. The opposite is true if the flavor is intended to create an 'oven aroma" to mask off-flavors generated during microwave cooking.

The first step in selecting a suitable microwave-stable flavorings is to choose those that do not contain highly volatile flavoring substances. This is not always possible, especially in the case of fruit flavorings; in fruit flavorings the character impact components (e.g. aldehydes, lower aliphatic esters, etc.) are often very volatile, and they cannot be replaced with less volatile compounds without impairing the desired flavor profile.

To help food technologists select suitable components for microwave stable flavorings, various model experiments have been reported on the behavior of single flavoring substances under microwave conditions. The theory behind these experiments (9) was that dielectric properties and specific heat of the substance determine its behavior under microwave conditions. However, the theory was not successful in predicting behavior of flavor compounds under more realistic conditions (*10,11*). For example, aliphatic aldehydes and alcohols have low specific heats and dielectric constants. In spite of this heavy losses are observed during microwave exposure. In addition, dielectric properties change as a function of temperature. Therefore, this seems to be a questionable method for predicting the behavior of flavoring substances under microwave conditions. Probably specific heat and boiling point resp. water vapor volatility are more suitable parameters for the selection of flavoring substances.

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch034

An important question in this regard is whether there is any practical relevance in studying the behavior of individual compounds under microwave conditions. The total quantity of flavoring substances in a food - whether added or naturally occurring - seldom surpasses hundreds of parts per million. Individual flavoring substances are present at levels of a few ppm and often even a few ppb. This relatively small number of flavor molecules is distributed throughout the water, fat, protein, and carbohydrate in the food matrix. In a water containing food large quantities of water vapor are generated during microwave heating. Heating effects caused by heating the water are certainly greater than those that could result from microwave interaction with individual flavoring molecules. It is therefore safe to assume that, the external influence of the food matrix on flavoring substances is more important than the behavior of these substances in an alternating electromagnetic field.

Based on the foregoing arguments it can be concluded: testing the microwave susceptibility of individual flavor compounds has no real practical value, as the interactions with major components of the food matrix are not taken into account.

Nevertheless, flavoring compositions do change after microwave exposure, with fruity type flavorings being more problematic than savory types. The character impact compounds of fruity type flavorings tend to be low molecular weight steam volatile substances such as citral, cis-3-hexenal, etc., which have not proven to be microwave stable. Moreover, fruit flavorings often are of a more hydrophilic character; therefore, a great part of the flavoring substances migrate into the aqueous phase of the food, which selectively absorbs the greater part of the microwave energy.

Excessive loss of these steam volatile flavoring substances leads to unbalanced flavor profiles, which lack impact. It has been my experience, that in microwave cakes a three fold excess of flavor is sometimes necessary to achieve an impact comparable to conventionally prepared product. Modifying the composition of flavorings (flavor engineering), e.g. by exchanging single-fold citrus oils with their more concentrated, less volatile, terpeneless derivatives, may be necessary in those cases where the profile is unbalanced by selective loss of key components. Further modification may also be necessary to mask off-notes. These off-notes are especially noticeable in starch based products (microwave cakes), where uncooked notes of the matrix (floury, batter-like, etc.) detract from the flavor profile (*12*).

In contrast to fruit flavors, savory flavors are not usually characterized by impact components. These flavors, which are generated by thermal processing of a precursor mixture at high temperatures, tend to be more heat stable and microwave resistant.

It has been suggested that flavorings for microwave applications be intentionally unbalanced to compensate for the selective loss of flavor chemicals. The idea is that such a flavoring would provide the desired balanced profile after microwave treatment. The difficulties with this approach are:

- Most products are designed for conventional as well as microwave preparation.

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- There are vast differences in microwave ovens. This will influence the heating profile, and hence the volatiles lost.
- The volatiles that are lost during preparation may impart unpleasant or off-aromas to the cooking environment.

A more practical approach is to try to improve flavor retention under microwave conditions. It is my experience that physical properties and form of flavoring compounds influence flavor retention in microwave foods. Spray dried flavorings often perform better than the liquid flavors from which they are derived, especially when gum arabic is the carrier. This is explained by the superior degree of flavor retention of gum arabic compared to other carrier materials such as maltodextrins, modified starches, etc. (*13,14*). Improved flavor retention of the gum arabic systems is observed even after the flavors are dissolved in water, which has been attributed to microfine distribution in the form of an emulsion.

Cyclodextrin inclusion complexes also appear to improve flavor retention. However, legal restrictions and the high cost impede the practical use of this carrier, at the present time.

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Conclusions

Flavor performance in microwave foods is very product specific, and depends on the composition of the food matrix. The influence of matrix composition on flavor is much greater in microwave foods than in those prepared by conventional cooking. Foods with high moisture content lose significant amounts of flavoring substances through steam distillation. In addition, the lower temperatures and higher water activities at the surface of microwave foods are not conducive to flavor generation, especially those flavors associated with browning reactions. Athermal effects of microwave heating have not been established.

The most practical approach to improving flavor performance is to enhance retention of flavor volatiles. This can be done by selecting chemicals that are not readily steam distilled and by altering the mechanism of interaction between flavor chemicals and the matrix (i.e., spray drying with gum arabic).

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Chapter 35

Microwave Volatilization of Aroma Compounds

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Dielectric constants and dielectric loss factors were measured for a series of seven and eight carbon alcohols, aldehydes, ketones and esters, having the similar boiling points. The microwave heating rates of these compounds were also measured and found to be proportional to the microwave power absorption calculated from the measured dielectric properties. Both the microwave absorption and microwave heating rates were found to decrease in the series benzaldehyde, octanone, heptanol, octanal, octanol, methyl hexanoate. Volatilization of these compounds by microwave heating was determined by headspace analysis of mixtures dispersed in fat and heated in a microwave oven. For all mixtures, the headspace concentrations of microwave heated samples were the same as control samples heated on a steam bath. There was no selective microwave volatilization of the chemicals even though the microwave heating rates of the individual compounds varied by a factor of ten.

The concept that microwave heating occurs through the absorption of microwave energy by specific molecules has long enticed flavor chemists into compounding microwave flavors on the basis of dielectric and microwave heating properties.

A prominent example of this approach is the Delta T theory proposed by Shaath (*1*). Shaath has cataloged over 500 flavor compounds on the basis of relative microwave heating rates of the pure flavor compounds.

Other studies suggest that the dielectric properties of the pure flavor compounds are not important. Steinke et al (*2,3*), in a study of the microwave volatilization of organic acids, concluded that the chemical and physical properties of the complete food system controlled the volatilization of individual flavor compounds.

The purpose of this study is to understand the role of dielectric properties (*4*) in microwave volatilization. The dielectric constants and dielectric loss factors for

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six flavor compounds possessing similar boiling points were measured. The microwave heating rates of the individual flavor compounds were measured and related to the dielectric properties and microwave absorption. The volatilization of these flavor compounds was determined by measuring the relative headspace concentrations of the flavor compounds over mixtures heated in a microwave oven and on a steam bath.

Materials and Methods

Materials The flavor compounds were purchased from Aldrich Chemical Co. (Milwaukee, WI) and used without further purification. The boiling points reported by Aldrich are: 1-heptanol 176°, methyl heptanoate 172-173.5°, benzaldehyde 178-179°, 2-octanol 174-181°, 2-octanone 173°, and octanal 171°. The fat used was hydrogenated coconut/palm kernal oil manufactured by Karlshamns Food Ingredients (Columbus, OH).

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch035

Dielectric Measurements The dielectric constants and dielectric loss factors were measured at 51 frequencies between 0.3GHz and 20GHz using a Hewlett-Packard HP8720A network analyzer equipped with a HP85070A dielectric probe kit. All measurements were made at 25°C.

Microwave Heating of Pure Flavors Ten grams of each flavor compound in a 25mL glass vial were heated in a Litton Generation II microwave oven (630w) at full power for two minutes. 200g of room temperature water in a 400mL glass beaker were heated simultaneously with each flavor compound to serve as a simulated food load. Both the sample vial and the water load were positioned in exactly the same location in the microwave oven for each heating test. The temperatures of the flavor compounds were measured continuously during microwave heating using a Luxtron model 750 Fluoroptic Thermometry System (Mountain View, CA) equipped with Luxtron MIW fiber optic probes. Two probes were used in each flavor compound vial; one in the center and one on the side, each 1cm above the bottom of the vial. The surface temperatures of the vial were also monitored continuously during microwave heating using a Hughes Aircraft Co. (Carlsbad, CA) model 4100 Probeye infra red video camera to detect uneven heating patterns across the sample vial. No uneven heating was detected.

Heating of Flavors in Fat Matrix Equal weight binary, ternary, and quaternary mixtures of the flavor compounds were mixed with the hydrogenated fat so that each flavor compound was present at 2%(w/w) of the total flavor-fat mixture.

10g of each flavor-fat mixture were sealed in a serum screw cap 25mL vial and heated either on a steam bath for 3.5 minutes (to 95°C) or in the Litton microwave oven containing a 60g water load for 3.5 minutes (also to 95°C). The 60g water load was used to make the microwave heating rate of the flavor-fat mixture comparable to the steam bath heating rate.

<u>Headspace Analysis</u> Immediately after heating, 1mL of headspace was removed from the vial with a gas-tight syringe and injected into a Perkin Elmer model 3920 gas chromatograph. A 30m x 0.53mm i.d. fused column coated with a 1μm film of DB225 (cyanopropyl phenyl methyl silicon) was used. The following gas chromatograph oven parameters were employed: 2 minutes at 80°C then 4°C/minute to 130°C with a final hold of 1 minute. Peak area analysis was performed by a Perkin Elmer Nelson model 2600 Chromatography Data System. Experiments were performed in duplicate and the results averaged.

Results and Discussion

The six compounds were chosen for this study since they are used or found in various flavors in food products. They represent six chemical classes yet possess the one characteristic of similar boiling point.

The frequency dependence of the dielectric constants of the aldehydes and ketone is shown in Figure 1. Benzaldehyde has the highest dielectric constant in this frequency range. The dielectric constants for the alcohols and ester are shown in Figure 2. These dielectric constants of the alcohols and ester are lower then those of the aldehydes and ketone shown in Figure 1. The frequency dependence of the dielectric constants of the alcohols is much different from the other compounds. Instead of having a characteristic dispersion at about 8 to 10 GHz, the two alcohols have a much lower frequency dispersion out of the measuring range of the instrument.

The corresponding frequency dependence for the dielectric loss factors of the aldehydes and ketone is shown in Figure 3. Again, benzaldehyde has the highest dielectric loss factors rising to a maximum value of 8.1 at its critical frequency (*4*) of 5.7GHz. Octanone has a maximum dielectric loss factor of 4.2 at a critical frequency of 8.0GHz. Octanal has a maximum dielectric loss factor of 2.4 at a critical frequency of 8.6GHz. Figure 4 shows the dielectric loss factors for the alcohol and ester. The dielectric loss factors of the alcohols rise towards a maximum value at a critical frequency that lies outside the measuring range of the instrument. This suggests that the dipolar rotational motion of the alcohols is much slower than the other compounds in this study which is consistent with the ability of the alcohols to form intermolecular hydrogen bonds.

Table I lists both the dielectric constant ε' and the dielectric loss factor ε'' at 2.45GHz for the six flavor compounds. Benzaldehyde has the highest dielectric constant and loss factor. 2-Octanol has the lowest dielectric constant and methyl heptanoate has the lowest dielectric loss factor.

A useful way to relate the dielectric properties to the absorption of microwave energy is through the concept of penetration depth (*5*). The half-power penetration depth is the distance into the absorbing material at which half the microwave power has been absorbed. The half power penetration depth is a convenient measure to compare the relative microwave absorbing characteristics of materials and to understand readily the effects of dielectric properties and geometry on microwave heating.

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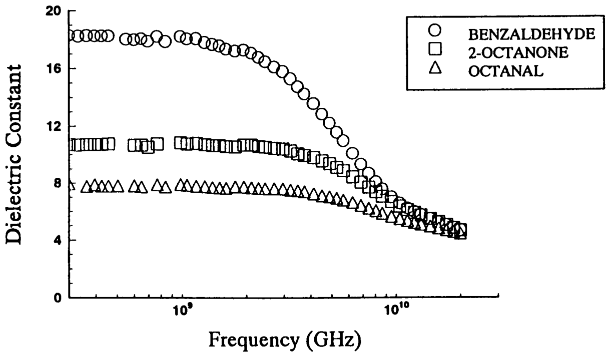

Figure 1. Dielectric constants of benzaldehyde, 2-octanone and octanal as a function of frequency at 25°C.

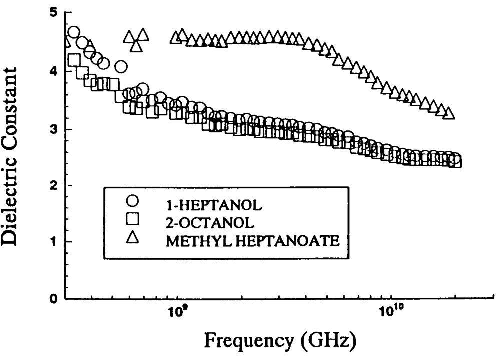

Figure 2. Dielectric constants of 1-heptanol, 2-octanol and methyl heptanoate as a function of frequency at 25°C.

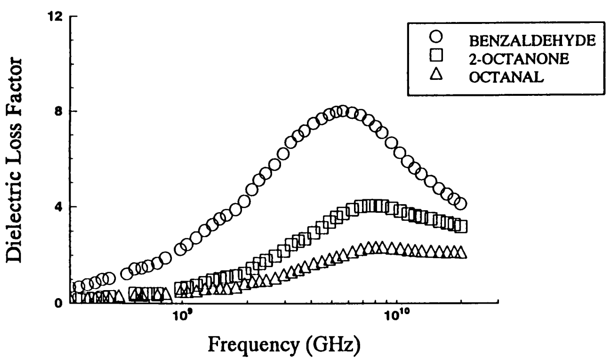

Figure 3. Dielectric loss factors of benzaldehyde, 2-octanone and octanal as a funtion of frequency at 25°C.

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch035

Table I. Dielectric Properties at 2.45GHz and 25°C, Calculated Microwave Penetration Depths, and Microwave Heating Rates of Flavor Compounds

Flavor Compound	Dielectric Constant ε'	Dielectric Loss Factor ε"	Half Power Penetration Depth (cm)	Heating Rate (°C/sec)
Benzaldehyde	16.42	5.34	1.04	2.98
2-Octanone	10.54	1.78	2.48	1.34
1-Heptanol	3.14	0.79	3.06	1.13
Octanal	7.61	1.00	3.73	0.77
2-Octanol	2.76	0.58	4.05	0.76
Methyl Heptanoate	4.59	0.31	9.43	0.28

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The half power penetration depth in centimeters $d_{p/2}$ is calculated from the measured dielectric properties by the following equation:

$$d_{p/2} = 0.9563\,\epsilon' \left[\sqrt{1+\left(\frac{\epsilon''}{\epsilon'}\right)^2} - 1\right]^{-1/2} \qquad (1)$$

in which ε' is the dielectric constant and ε" is the dielectric loss factor.

The half power penetration depths were calculated for the six flavor compounds from the measured dielectric properties at 2.45GHz and 25°C and are listed in Table I. Short penetration depths indicate that the microwave power is absorbed more readily. Benzaldehyde has the shortest penetration depth; methyl heptanoate has the longest penetration depth. Table I lists the flavor compounds in order of increasing penetration depth or decreasing microwave power absorbance. Note that the penetration depth or power absorbance is primarily dependent on the dielectric loss factor.

The microwave heating rates of the pure flavor compounds are shown in Figures 5 and 6. Benzaldehyde heats the fastest reaching 130°C in 1 minute. Methyl heptanoate heats the slowest with the temperature rising only 15° in 1 minute. Heating rates were calculated as the initial slopes of the curves in Figures 5 and 6. These heating rates are listed in the fourth column of Table I and are expressed in degrees per second. The heating rates have the same rank order as the microwave power absorption expressed as the penetration depth.

These heating rates are graphed as a function of the reciprocal half power penetration depth in Figure 7. The reciprocal half power penetration depth is directly proportional to the microwave power absorbed per distance. Figure 7 clearly shows that the microwave heating rates of these six flavor compounds are

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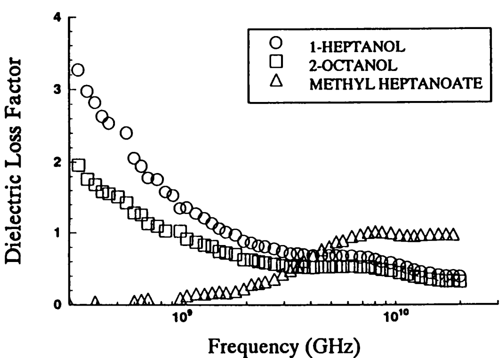

Figure 4. Dielectric loss factors of 1-heptanol, 2-octanol and methyl heptanoate as a function of frequency at 25°C.

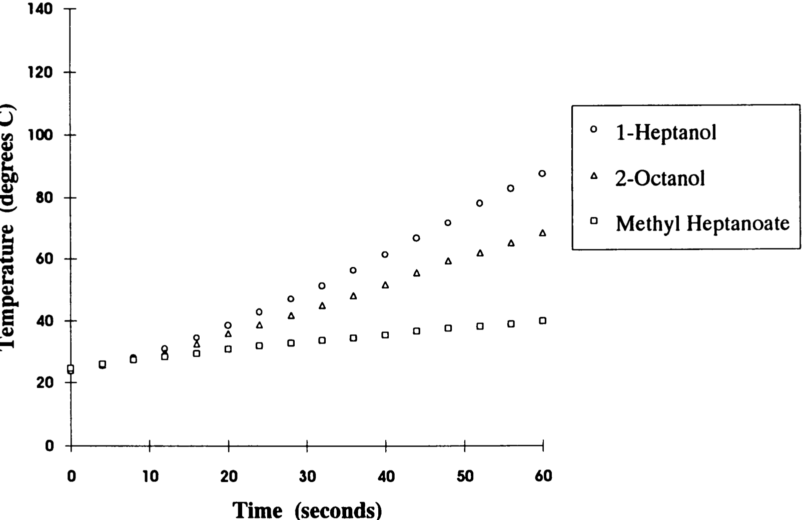

Figure 5. Microwave heating rates of heptanol, octanol and methyl heptanoate.

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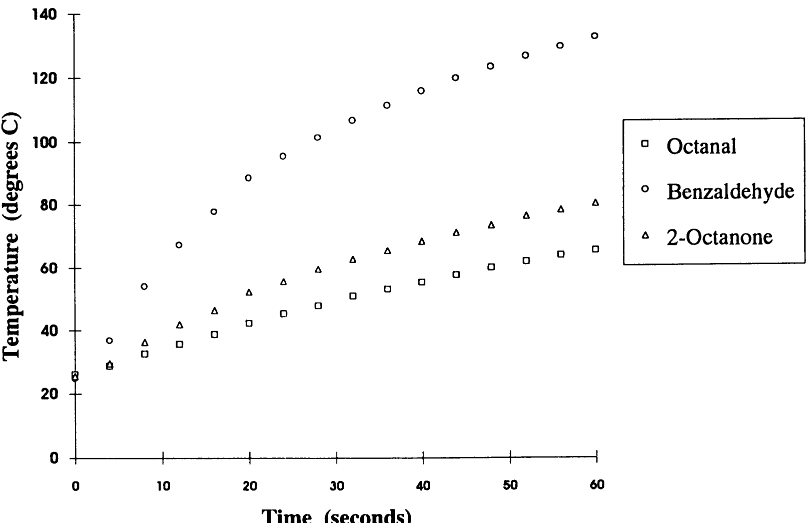

Figure 6. Microwave heating rates of octanal, benzaldehyde and octanone.

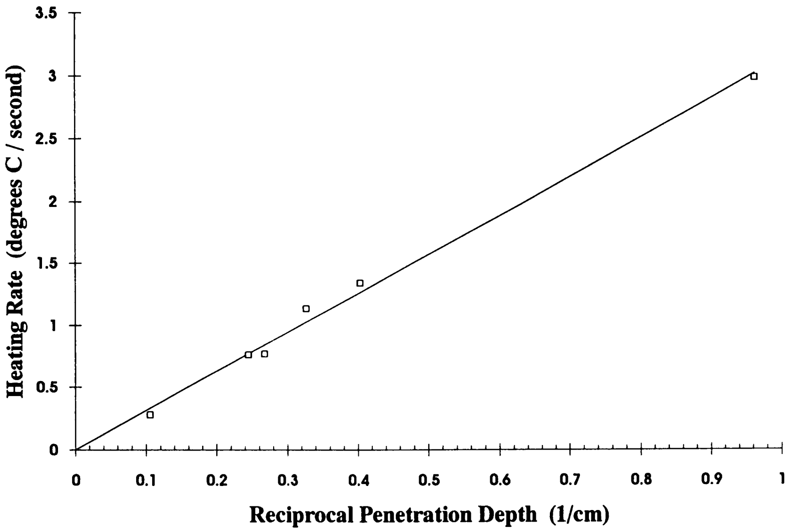

Figure 7. Microwave heating rates as a function of the reciprocal of the half power penetration depth.

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch035

directly related to the microwave power absorbed, calculated from the dielectric properties.

Table II lists the normalized gas chromatograph peak areas of the headspace over the flavor-fat mixtures heated to 95°C (3.5 minutes) on either a steam bath or microwave oven. Data for four different mixtures are shown. In the binary mixture (Mixture 1) of benzaldehyde and methyl heptanoate, the ratio of benzaldehyde to methyl heptanoate in the headspace is essentially the same whether the flavor compounds were volatilized on a steam bath or in the microwave oven. If anything, the benzaldehyde concentration decreases in the microwave where it would be expected to increase. Similar results are found for Mixtures 2, 3, and 4. In each case where the flavor compounds are dispersed in fat, the headspace concentrations of flavor compounds are the same regardless of the form of heating. Compounds having higher microwave absorption and higher microwave heating rates are not preferentially volatilized by microwave heating compared to heating over a steam bath.

Table II. Normalized GC Peak Areas of Headspace over Four Flavor Compound Mixtures Heated to 95°C (3.5 minutes) on Steam Bath or in Microwave Oven

Mixture Number	Composition	Normalized Peak Areas: Steam Bath	Normalized Peak Areas: Microwave Oven
1	Benzaldehyde	67	63
	Methyl Heptanoate	100	100
2	Benzaldehyde	62	64
	2-Octanol	24	25
	Methyl Heptanoate	100	100
3	Benzaldehyde	67	68
	2-Octanone	85	87
	1-Heptanol	34	31
	Methyl Heptanoate	100	100
4	Benzaldehyde	64	64
	Octanal/2-Octanol	95	93
	Methyl Heptanoate	100	100

Conclusions

These results demonstrate that the microwave heating rates of pure flavor compounds are dependent on the dielectric properties of the compounds. The

measured heating rates were found to be related to the dielectric constant and loss factor in a way described by the equations for microwave power absorption by dielectric materials. Across this series of flavor compounds having similar specific heats, those having high dielectric loss factors heat more rapidly than compounds having low dielectric loss factors.

The volatilization by microwave heating of the flavor compound mixtures dissolved in fat does not depend on the relative microwave heating rates of the pure compounds. The headspace concentrations of the flavor compounds is dependent on the temperature of the total system rather than the absorption of microwave energy by individual molecules.

The aroma of foods heated in a microwave oven will be dependent on the temperature profile of the food itself and not on the dielectric properties of the individual aroma compounds.

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Chapter 36

Flavor Volatilization in Microwave Food Model Systems

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Flavor differences are often observed between microwave and conventionally heated foods. Disproportionate volatilization or degradation of flavor components by microwave heating has previously been attributed to microwave-specific effects. The present model study compares the relative losses of aqueous solutions of aldehydes, ketones, and esters heated under carefully controlled conventional and microwave conditions. Volatiles remaining after heating were quantitated using equilibrium headspace sampling coupled with gas chromatography. Variable losses of flavor components were observed with changes in solubility/hydrophobicity, and heating of single versus multiple component solutions. No microwave-specific effects were observed. Data collected in this study disprove the previous "Delta-T" theory of microwave-induced flavor loss. Disproportionate flavor losses were consistent with positive deviations from Raoult's law. Understanding flavor volatilization processes in a microwave environment will enable delivery of conventional flavors for microwave prepared foods.

Foods heated in conventional and microwave ovens often deliver significantly different flavor profiles. Variations in flavor development result from an alternate set of thermal and volatilization pathways which occur during microwave heating. (*1*,*2*) Typical roasted, browned, and baked flavors are not developed via Maillard or Strecker reactions because of relatively higher water activity and lower temperature at food surfaces. Additionally, individual flavor components are often lost at variable rates through volatilization or degradation, leading to loss of top-notes or unbalancing and distortion of the desired flavor profile.

There is little available literature regarding losses of flavor compounds from food systems during microwave heating. A recent study described the relative losses of Strecker aldehydes, fatty acids, diacetyl, and acetoin in a series of food model systems as a function of temperature, salt concentration, and water content. (*2*)

A number of microwave effects have been prematurely concluded from experiments in which heating conditions were not adequately monitored or controlled, or sample concentrations and heating methods were not applicable to food systems. The "Delta-T" theory proposed that the microwave heating behavior of pure flavor

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compounds would predict the volatilization and loss of flavor compounds at low concentration levels (typically < 50 ppm) in food systems. (*3,4*) However, it is difficult to conceive how the relatively low concentration of these flavorants can influence the bulk heating properties of foods in a microwave field.

The purpose of the current study was two-fold:

1) Investigate the loss of flavor volatiles under carefully monitored and controlled heating conditions (microwave vs. conventional) to test for microwave-specific effects at realistic flavor levels in open systems.
2) Conduct experiments to determine relationships between the chemical identity of flavorants and their volatile losses.

Experimental Procedures

Materials. Water and methanol solvents were HPLC grade and Optima grade, respectively (Fisher Scientific, Fair Lawn, NJ). Reagent grade 2,3-butanedione (diacetyl), benzaldehyde, *trans*-cinnamaldehyde, *trans*-2-hexenal, hexanal, octanal, 2-pentanone, 2-heptanone, 2-octanone, 2-nonanone, ethyl butyrate, ethyl hexanoate and ethyl octanoate were purchased from Aldrich Chemical Co. (Milwaukee, WI) and used without further purification.

Solutions. A stock solution (25 mg/mL) of each flavor compound was prepared by weighing 2.500 g ± 0.010 g into a 100-mL volumetric flask, and diluting to volume with methanol. Working standards (2.500, 5.000, 7.500, 12.500 mg/mL) were obtained by diluting 5, 10, 15, and 25 mL, respectively, of the stock solution to a 50-mL volume with methanol. Flavor standard solutions (10, 20, 30, 50 ppm) were prepared by 1:250 dilutions of the respective working standards with water. Binary and multiple-component flavor solutions were obtained from stock solutions, containing a mixture of 2.500 g ± 0.010 g of each compound, prepared by dilution to a 100-mL volume with methanol; subsequent serial dilutions with methanol and water were performed as described above. The concentration of methanol in the final solutions was 0.4% (v/v); its presence at a low level was necessary to ensure solubility for hydrophobic flavor compounds. All solutions were observed with a microscope at 100x to verify that no undissolved droplets were present. Solutions were freshly prepared, stoppered, and used within 6 hours to assure that volatiles were not lost or degraded prior to performing the heating experiments.

Heat Treatments. Stock solutions (5.000 g ± 0.001 g) were weighed into open 20-mL glass scintillation vials (Fisher Scientific, Pittsburgh, PA) immediately prior to heating. A small sample size was chosen to minimize sample heating nonuniformity. The relatively large vial height minimized solvent losses. Typical losses, monitored by sample weight differences before and after heating, were 0.220 g ± 0.050 g. Three replicates of each sample were heated and analyzed for volatile losses. If a sample lost more than 0.270 g during heat treatment, it was discarded.

Sample temperatures were monitored with fluoroptic thermometry probes at three locations within the sample, spaced approximately 1 cm apart to verify time-temperature profile heating rates and uniformity (see Figure 1). A Model 750 fluoroptic thermometer with MIC probes (Luxtron, Santa Clara, CA) was used to monitor temperature (± 0.1°C accuracy).

For sample heating, a time-temperature profile of 95°C for 150 sec was chosen to model the short times at elevated temperatures which prepared food products typically encounter during microwave heating. The authors chose 95°C (instead of 100°C) for two reasons:

1) To avoid boiling, solvent vaporization, and subsequent concentration effects.
2) To avoid superheating, "bumping", and sample boil-over.

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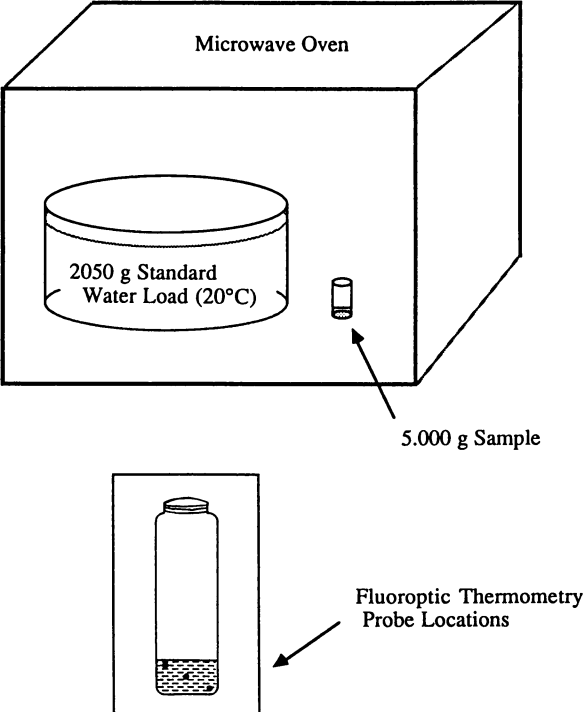

Figure 1. Relative placement of sample and standard water load within microwave oven cavity, with close-up of sample temperature monitoring sites.

Conventional. Samples were weighed into scintillation vials, placed in a constant-temperature oil bath, heated to 95°C, held for 150 sec, transferred to an ice bath, rapidly cooled to 25°C, and reweighed. A 1.000 g ± 0.001 g aliquot of each heat-treated sample was weighed into a 20-mL headspace vial, which was immediately sealed with a Teflon-coated butyl rubber septum and aluminum crimp-cap (Perkin Elmer, Norwalk, CT) for subsequent headspace analysis within 12 hr of heat treatment.

Microwave. Samples were weighed into vials, placed into a standardized microwave oven environment, heated to 95°C at the same heating rate as oil bath samples, held at 95°C for 150 sec by manually pulsing the microwave power on and off to maintain a constant temperature, transferred to an ice bath, rapidly cooled to 25°C, and reweighed. Aliquots (1.000 g ± 0.001 g) of the heat-treated samples were weighed into headspace vials, immediately sealed with rubber septa as described above, and analyzed within 12 hr of heat treatment.

Careful monitoring of both sample time-temperature profiles and sample weight losses are critical to ensure equivalent microwave and conventional heat treatments. A commercial microwave oven environment was modified to provide sample heating profiles equivalent to those of samples heated in the oil bath used in this study. This standardized microwave oven environment was produced as follows:

1) A Variac Model W20MT3A auto transformer (Technipower, Danbury, CT) was used to regulate electrical outlet voltage to the microwave oven (set at 120V ± 2V while the oven was in operation). The microwave oven magnetron was preheated by heating approximately 2 kg of ice water in the microwave oven for 15 minutes. The oven floor and walls were cooled to room temperature using an ice pack followed by a dry towel prior to running samples. If the oven was unused for more than a period of 1 hour, the preheating procedure was repeated. This step of the procedure is key to providing consistent sample-to-sample heating rates because microwave oven "cold starts" and AC line voltage fluctuations can cause microwave oven power output to vary significantly.

2) A "sweet spot" for sample placement within the microwave oven was located using a thermal map technique to ensure uniform microwave field flux at the sample site. This procedure along with other microwave oven calibration techniques is described elsewhere. (*5*)

3) Microwave sample heating rates were matched to oil bath sample heating rates from 20°C to 95°C by placing a large water load in the oven with the sample. A PYREX 190 x 100 mm crystallizing dish (No. 3140) contained the 20°C ± 0.5°C standard water load (see Figure 1). The microwave oven used was a Litton Generation II Model 2924 oven which required a 2050 g ± 1 g standard water load to match the oil bath heat treatment in this study. The standard water load absorbs most of the microwave power, attenuating the microwave field intensity at the sample site to a low level, thereby reducing the sample heating rate. Most commercial microwave ovens could be substituted for the one used in this study, provided that the weight of the standard water load is adjusted appropriately. Precise placement of both the sample and standard water load at fixed positions within the oven, and minimum variation in both the weight (± 1 g) and initial temperature (± 0.5°C) of the standard water load are important to ensure consistent sample-to-sample heating rates.

Headspace Gas Chromatography. Flavor volatiles were collected and quantitated using an equilibrium (static) headspace technique in conjunction with gas chromatography. (*6,7*) A Model 7000 equilibrium headspace system with cryofocusing module and a Model 7050 autosampler (both from Tekmar, Cincinnati, OH) were interfaced to a Hewlett Packard Model 5890 Series II gas chromatograph (Palo Alto, CA), equipped with a flame ionization detector, heated at 300°C. The GC oven was equipped with a 10 m x 0.32 mm i.d., 10 µm film thickness PoraPLOT Q

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch036

(Chrompack, Raritan, NJ) fused silica capillary column. The carrier gas was helium, supplied directly by the headspace sampler at a 2.5 mL/min flow rate (59 cm/sec average linear velocity).

Each 1-g sample of flavor solution was equilibrated in a 20-mL vial at 95°C for 30 min prior to injection. The injection sequence was 1.25 min vial pressurization at 7.5 psi, and 0.25 min injection time, during which a 5-mL portion of headspace was withdrawn. Volatile components were cryofocused onto a fused silica transfer line for 6 min at -150°C, rapidly heated to 250°C, then transferred over a 5-min interval into the GC injection port (250°C). The GC oven temperature was maintained at 80°C during the vial injection and volatile transfer (12 min), then increased at 5°/min to 250°C, and held for 26 min. Chromatographic data were collected on a Beckman PeakPro data system capable of automatic calibration, plotting, and post-run analysis calculations.

Calibration standards were used to determine the relative GC response factors for each flavorant. Peak area quantitation was linear over a 1 to 50 ppm range. The limit of detection for flavor components used in this study was experimentally determined to be between 0.1 and 0.5 ppm. The relative standard deviation was 13% for replicate analyses on the same (split) sample, and 15% for duplicated experiments.

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch036

Dielectric Measurements. Dielectric constants (ε') and loss factors (ε'') of neat flavor liquids and aqueous flavor solutions were measured at 20°C using a Model 8720C network analyzer equipped with a Model 85070A probe (Hewlett Packard, Santa Rosa, CA). The instrument is capable of scanning dielectric responses at frequencies between 0.2 and 20.0 GHz.

Results and Discussion

Homologous series of aldehydes, ketones, and ethyl esters which are typically used in commercial dairy, fruit, and confection flavor systems were selected for this study. Several other aldehydes were chosen to overlap with compounds studied by Shaath and Azzo in their "Delta-T" publication. (*3*) These flavorants cover a range of boiling points and dielectric properties (Table I). Aqueous solutions at realistic flavor levels (10 to 50 ppm) were heated open to the atmosphere to mimic the conditions a volatile flavorant would typically encounter in a precooked high moisture food which is heated uncovered to serving temperature.

Carefully matched and controlled time-temperature profiles for microwave and conventional heat treatments were crucial to test for microwave-influenced flavor volatile losses. The relationship between microwave power setting and actual watts of power delivered is not necessarily one-to-one and must be determined experimentally. (*5,8*) Nonlinearities or curvatures in calibration are known to differ from oven to oven. Previous studies reported in the literature have utilized microwave heat treatments which were not well defined and/or not matched to conventional heat treatments used as controls. Insufficient characterization of experimental heat treatment methods in these papers makes it impossible for other researchers to reproduce published findings. In this study, heating procedures are described in sufficient detail to enable them to be reproduced in other laboratories.

A conventional heat treatment was selected, the sample time-temperature profile was quantitatively measured, and the microwave heat treatment was determined by settings which yielded an equivalent time-temperature profile (described in the Experimental Section above). In addition, sample weight losses were also monitored and matched to ensure equal heat energy delivery to both microwave and conventional samples. Typical heating profiles are shown in Figure 2. No significant differences were observed in the heating behavior of samples with varying flavor components or concentrations.

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch036

Table I. Physical Chemical Properties of Flavorants

Flavor Component	Mol. Wt. *(g/mole)*	Boiling Pt.[a] *(°C)*	Henry's Constant[b] *(– log)*	Dielectric Constant[c] ε'	Loss Factor[c] ε''
Ketones					
Diacetyl	86	88	5.093*	3.8	3.1
2-Pentanone	86	102	2.465[d]	15.8	1.3
2-Heptanone	114	151	2.183[d]	12.3	1.6
2-Octanone	128	173	2.114[d]	10.9	1.7
2-Nonanone	142	195	1.824[d]	9.6	1.8
Esters					
Ethyl butyrate	116	122	1.766*	5.1	0.3
Ethyl hexanoate	144	168	1.529*	4.6	0.3
Ethyl octanoate	172	208	1.283*	4.2	0.3
Aldehydes					
Hexanal	100	128	4.184*	7.4	1.1
t-2-Hexenal	98	146	2.962[e]	7.4	1.1
Octanal	128	171	2.699[f]	7.6	1.0
Benzaldehyde	106	178	2.060[d]	16.4	5.3
t-Cinnamaldehyde	132	253	1.678[d]	5.8	4.2

[a]Values obtained from Ref. 9.

[b]Values represent the –log of the unitless Henry's law constant at 20°C for each flavorant at dilute concentrations in water. Entries are calculated from experimental literature data, except those indicated with an asterisk (*) which were estimated using the procedure described in Ref. 10.

[c]Values were measured for neat flavor liquids at 2450 MHz, 20°C. For the flavor solutions in this study, $\varepsilon' = 78.2–78.5$, $\varepsilon'' = 10.8–10.9$; for 0.4% methanol/water solvent $\varepsilon' = 78.5$, $\varepsilon'' = 10.8$; for distilled water $\varepsilon' = 78.8$, $\varepsilon'' = 10.8$.

[d]Constants calculated using data from Ref. 11.

[e]Constant calculated using data from Ref. 12.

[f]Constant calculated using data from Ref. 13.

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch036

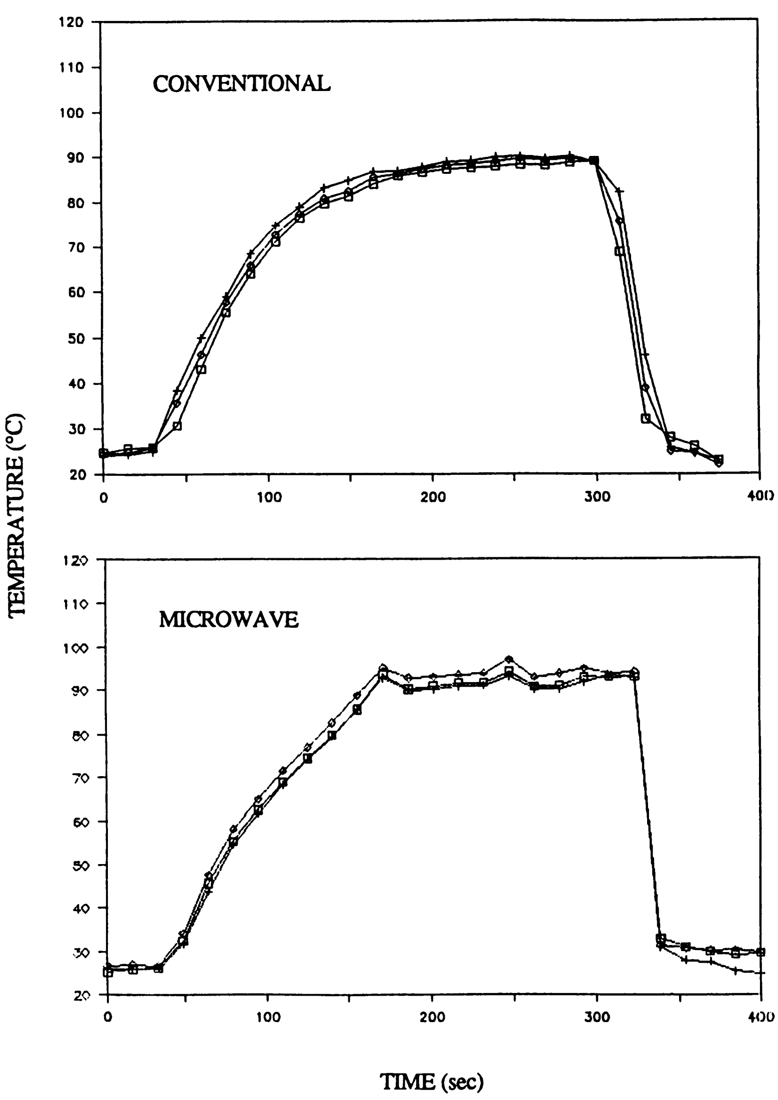

Figure 2. Typical time-temperature sample heating profiles for conventional (top) and microwave (bottom) heat treatments. Each heating profile shows temperatures at three monitoring sites within the sample. Example is 20 ppm diacetyl.

The dielectric properties, thermal properties, and subsequent microwave heating behavior of solutions were determined by the solvent system and not the flavor components. The dielectric properties of the solutions in this study were virtually identical with those of the 0.4% methanol/water solvent system. The measured range for all solutions was $\varepsilon' = 78.2–78.5$ and $\varepsilon'' = 10.8–10.9$, vs. solvent system values of $\varepsilon' = 78.5$ and $\varepsilon'' = 10.8$. At low concentrations, flavorants which absorb microwaves more strongly than the surrounding medium quickly dissipate heat to the bulk phase and flavor volatility is not significantly affected by the dielectric properties of the flavorants. Conversely, flavorants which absorb microwaves less strongly than the surrounding medium are quickly heated by thermal energy transfer from the surrounding bulk phase medium and again flavor volatility is not significantly affected by the dielectric properties of the flavorants.

Large flavor losses were observed following conventional and microwave heat treatments. Sample concentrations between 1 and 10 ppm yielded post-heating flavorant levels too low to detect by equilibrium headspace sampling (< 0.1–0.5 ppm). Therefore pre-heating concentration levels between 10 and 50 ppm were chosen.

Equilibrium headspace gas chromatography is a convenient and sensitive procedure to rapidly monitor large numbers of flavor samples. (*6,7*) In this technique, the flavor sample is placed in a sealed septum-capped vial and heated; once equilibrium has been established between the sample and the vapor phase, a portion of the headspace volatiles is withdrawn via syringe and injected onto the GC column. After evaluation of other flavor measurement techniques, this analytical method was chosen because it offers the following advantages:

1) High-resolution capillary GC columns can be used.
2) Reproducible measurements of volatile flavor compounds can be quantitatively obtained in the presence of water.
3) Automated operation enables a set of results to be obtained within 12 hr after heat treatment. This ensured that volatile degradation or losses did not occur in post-heating samples prior to GC analysis.

In addition to studying volatile losses of single component solutions, losses from selected binary and multiple component flavor mixtures were also investigated to explore possible synergistic effects. The flavorants and their concentrations used in this study are shown in Tables II and III.

Single Component Solutions. Large flavor losses were observed following both microwave and conventional heat treatments. Flavorants initially at the same concentration in solution lost disproportionate amounts of volatiles dependent upon the identity of each flavorant and its initial concentration. These losses are reported in Tables II and III as percent flavor remaining relative to its concentration prior to heating.

Typical gas chromatographic profiles for single component solutions before and after heat treatments are shown in Figures 3 and 4. Peak areas are proportional to individual flavorant response factors. Sample concentrations were determined using calibration procedures described in the Experimental Section above. Ethyl esters were most volatile with post-heating losses exceeding 90% of the initial flavor concentrations. Ketone losses were also very high except for diacetyl, which is soluble in the solvent system used. Diacetyl solubility in water is 25 g per 100 g at 20°C, and it is infinitely soluble in alcohol. (*9*)

Aldehydes were less volatile than esters and ketones. Volatile losses were lower for benzaldehyde and *t*-cinnamaldehyde, which are again more soluble than the aliphatic aldehydes which exhibited relatively higher volatilities. Benzaldehyde solubility in water is 0.3 g per 100 g, and it is infinitely soluble in alcohol. (*9*) Cinnamaldehyde solubility is 50 g per 100 g alcohol, and it is slightly soluble in water. (*9*)

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch036

Table II. Observed Post-Heating Flavor Retention for Single Component Solutions

Component	Conventional (% Flavor Retention)				Microwave (% Flavor Retention)			
Initial Concentration, ppm	10	20	30	50	10	20	30	50
Ketones								
Diacetyl	34	47	38	25	25	14	16	11
2-Pentanone			8				1	
2-Heptanone	37		7		1		0.3	
2-Octanone	3		5		2		0.3	
2-Nonanone	4		3		2		0.3	
Esters								
Ethyl butyrate	7		2		0		0.3	
Ethyl hexanoate	1		1		0		< 0.3	
Ethyl octanoate			0.3				0.6	
Aldehydes								
Hexanal	22				5			
t-2-Hexenal	24				16			
Octanal	15				7			
Benzaldehyde	27		32		31		35	
t-Cinnamaldehyde			88				89	

Table III. Observed Post-Heating Flavor Retention for Multiple Component Solutions

Component Mixture	Conventional (% Flavor Retention)		Microwave (%Flavor Retention)	
Initial Concentration, ppm	20	30	20	30
Two-Component:				
Diacetyl	36		8	
Benzaldehyde		41		10
Four-Component:				
2-Pentanone		5		0.7
2-Heptanone		2		0.3
2-Octanone		2		0.3
2-Nonanone		1		0.3
Seven-Component:				
2-Pentanone		5		1
2-Heptanone		2		0.3
2-Octanone		2		0.3
2-Nonanone		1		0.3
Diacetyl		26		8
Benzaldehyde		31		11
t-Cinnamaldehyde		68		58

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch036

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch036

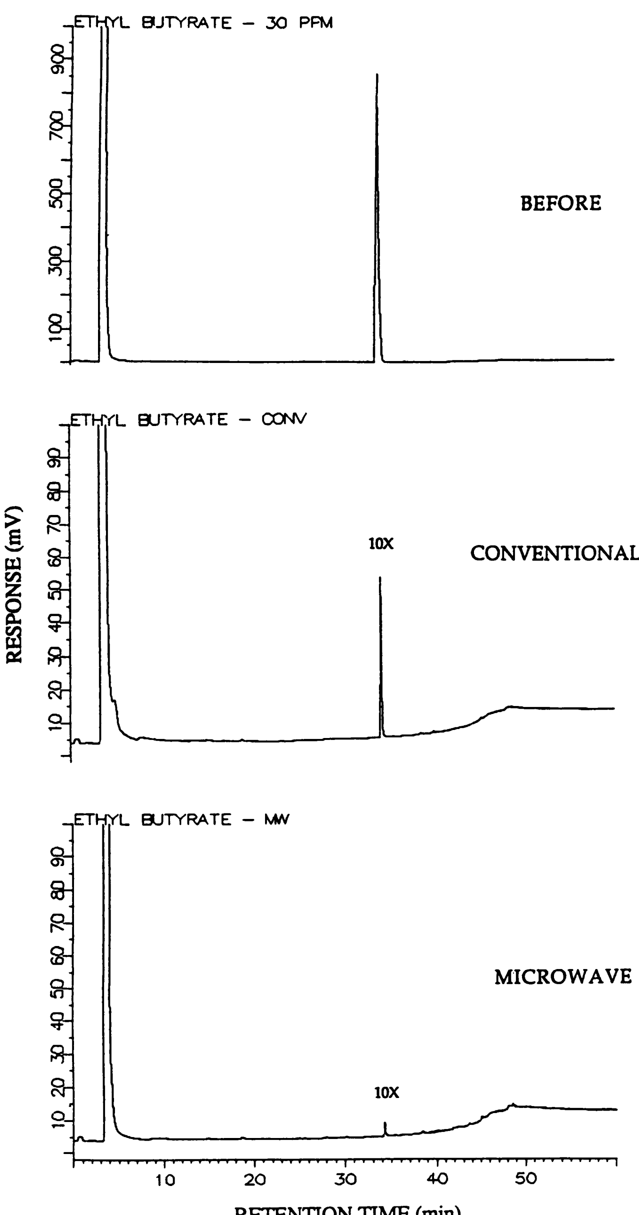

Figure 3. Representative gas chromatographic (GC) profiles obtained on PoraPLOT Q, showing relative flavor concentrations for 30 ppm ethyl butyrate, before and after heat treatments.

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch036

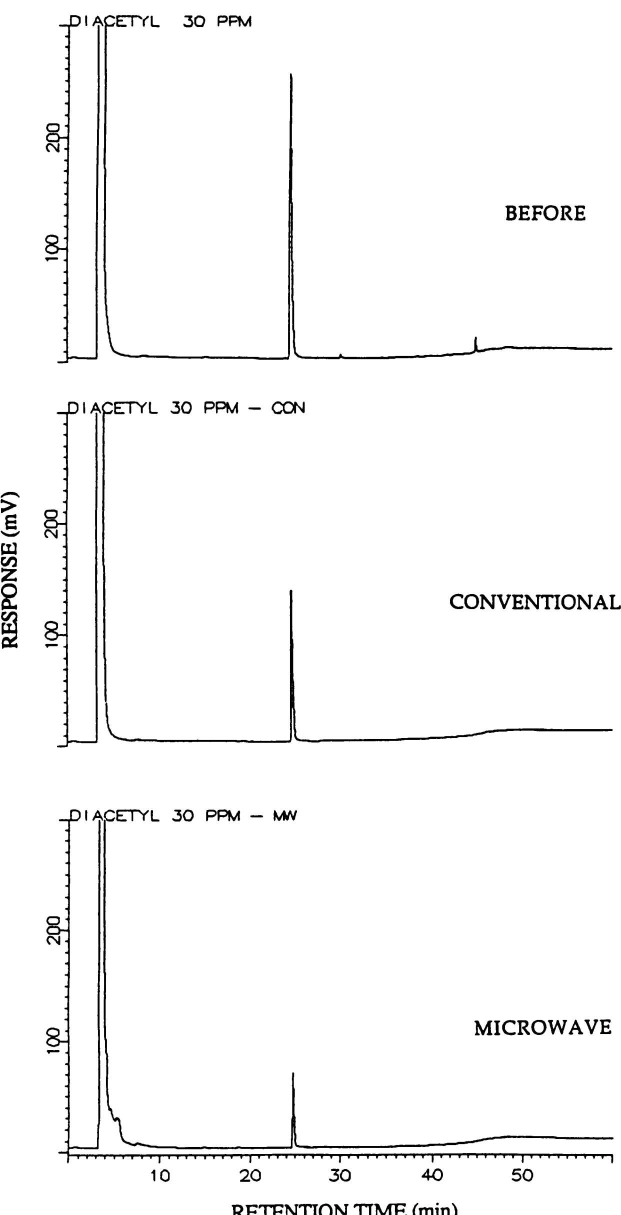

Figure 4. Representative gas chromatographic (GC) profiles obtained on PoraPLOT Q, showing relative flavor concentrations for 30 ppm diacetyl, before and after heat treatments.

Flavor losses increased within a homologous series as carbon number increased and water solubility decreased, as shown in Figure 5. Influence of initial flavor concentration was investigated for diacetyl. The relative percent of volatiles increased slightly with concentration (see Figure 6).

Volatile losses were significantly higher for microwave heated vs. conventionally heated samples. At first glance, this appears to be a microwave effect. However, volatile loss predictions based on microwave properties and boiling points of the pure flavor compounds are in the *opposite* direction from what was observed for solutions!

If boiling point were related to flavorant volatile loss, it would be expected that volatility would increase as boiling point decreased. However, it can be seen from the data in Table II that volatile losses *increased* with boiling point within a homologous series of ketones or esters.

If flavorant dielectric properties were related to volatile loss, it would be expected that volatility would increase as the value of the dielectric loss factor, ε'', increased. However, the flavors in this study with the *lowest* loss factor values (the ethyl esters) were most volatile. Therefore, the observed losses are *not* due to a microwave-specific effect.

The "Delta-T" theory proposed that volatilization and loss of flavor compounds at low concentration levels in foods would be predicted by the microwave heating behavior of the pure flavor compounds. (*3,4*) Volatile losses are predicted to increase with increased ΔT values. However, the results of the current study clearly provide evidence directly opposite "Delta T" predictions. As an example, volatile losses for a series of aldehydes measured in dilute solutions are shown in Table IV, along with the ΔT values which were measured for the neat liquids as reported by Shaath and Azzo. (*3*) A graph of this data is presented in Figure 7. Volatile losses strongly *decrease* with increased ΔT values. Volatile loss from dilute solutions is opposite the heating behavior of the neat flavor liquids. Moreover, these results disprove microwave-specific volatile loss because the same behavior is observed for conventionally heated samples as well.

Table IV. Observed Post-Heating Flavor Retention for Single Component Solutions and "Delta T" Values for Neat Flavor Components

Component	Flavor Retention (% vs. Initial)		"Delta T" Value[a] (ΔT)
	Microwave	Conventional	
t-Cinnamaldehyde	89	88	2.58
Benzaldehyde	35, 31	32, 27	2.19
t-2-Hexenal	16	24	1.50
Water	–	–	1.00
Hexanal	5	22	0.93
Octanal	7	15	0.81

[a]Values obtained from Ref. 3.

Higher volatile losses observed for microwave-heated samples can be attributed to subtle differences in sample heating uniformity vs. conventionally-heated samples. During conventional heat treatment, sensible heat is conducted through the glass sample vial to the outside sample surface, followed by heat transfer which takes place within the sample via conduction/convection processes.

In microwave heat treatment, microwaves incident on sample surfaces are absorbed as they pass through the sample towards the center. Sensible heat is

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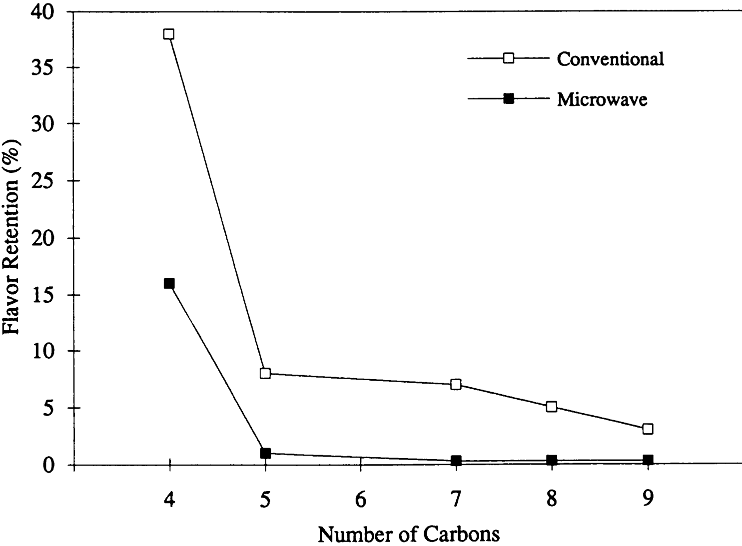

Figure 5. Post-heating flavor retention for ketones in single component solutions (30 ppm), showing volatile loss increase with hydrophobicity.

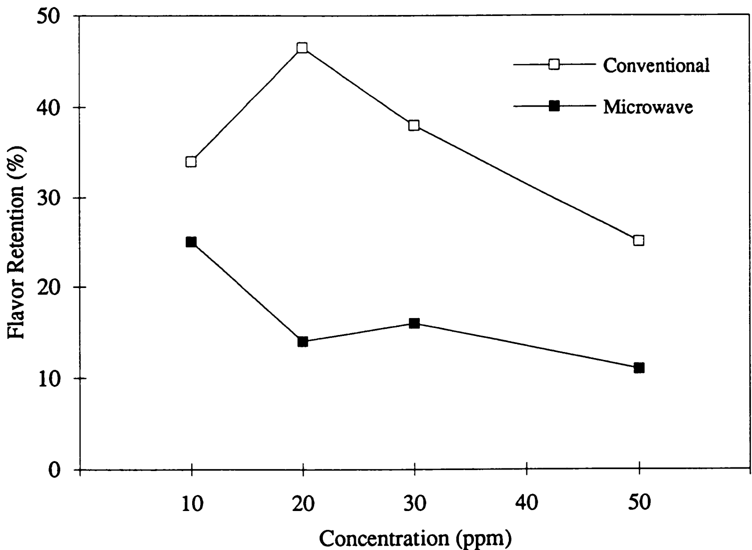

Figure 6. Concentration dependence for post-heating flavor retention of diacetyl.

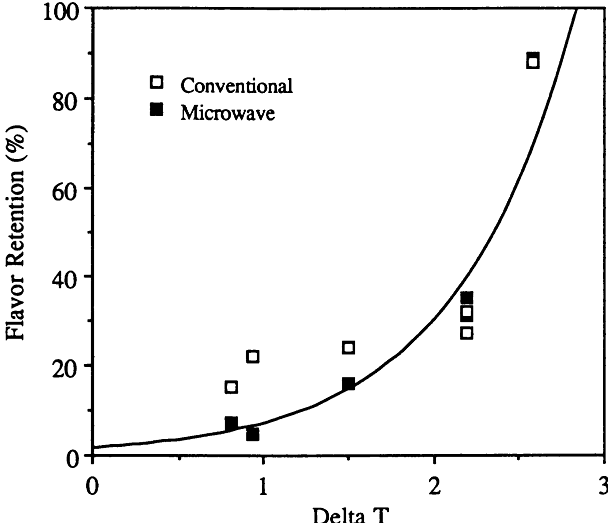

Figure 7. Post-heating flavor retention for aldehydes in solution vs. Delta T values.

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch036

generated where microwave absorption takes place followed by heat transfer as above. The extent to which microwave power is attenuated once it enters the sample is determined by both ε' and ε''. The amount of sensible heat formed is determined by ε'' only. The shape of the sample has a major impact on microwave heating uniformity, since microwaves are incident on all sample surfaces.

In this study, microwave power is attenuated to 50% of its initial value after traveling a 1.1-cm distance through the sample. The samples are cylindrically shaped (ca. 1 cm high and 2.54 cm diameter) and microwave focussing is likely to occur near the center of the sample, since the half-power point occurs at approximately 50% of the diameter of the sample. (*14*) It is highly possible that localized superheating occurred in samples during microwave heating, even though it was not detected at the three temperature probe locations. Superheating of microwave samples has been documented in other studies. (*15*) Thermal gradients generated within the sample would account for the net increased loss of flavor volatiles for microwave vs. conventional heating.

The above results are consistent with flavor solubility/hydrophobicity impact on the total vapor pressure of the solution, and the propensity of flavor components to disproportionately partition between the solution and vapor phases. This partitioning process is governed by classical physical chemistry vapor–liquid equilibrium relationships.

Raoult's law of partial pressures states that for ideal solutions, losses of volatile compounds are predicted by the relative mole fractions of N solution components. The total vapor pressure of the system is a colligative property, dependent upon the total number of solute particles in a given amount of solvent. (See Refs. *16–18* for further discussion). The partial pressure of each component, P_i, is defined by:

$$P_i = X_i P_{oi}$$

Where X_i is the mole fraction and P_{oi} is the equilibrium vapor pressure of each component. The total vapor pressure of the solution is equal to the sum of partial pressures:

$$P = \sum_{i=1}^{N} X_i P_{oi}$$

Raoult's law is true only for solutions of liquids which are totally miscible and have equivalent vapor-liquid equilibrium relationships for all components.

Non-ideal behavior of dilute liquid solutions is frequently expressed in terms of Henry's law, which states that the partial pressure of each component is equal to a constant, C_i, multiplied by the mole fraction and the equilibrium vapor pressure of the pure component.

$$P_i = C_i X_i P_{oi}$$

$$P = \sum_{i=1}^{N} C_i X_i P_{oi}$$

The Henry's law constant for a flavorant can be determined experimentally by measuring the ratio of its equilibrium concentration in the vapor phase to its concentration in a dilute water solution. This ratio, or air-to-water partition coefficient, is usually known as a unitless Henry's law constant. The negative logarithm of the Henry's law constants for flavor compounds in this study are shown in Table I.

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch036

Solutions which contain chemically and physically similar components (i.e., components with extremely high solubilities) tend to be nearly ideal, and the Henry's law constants are close to unity (e.g., hexane–heptane). Positive deviations from Raoult's law occur as solution components become more dissimilar (i.e., components with low solubilities). Solution components repel each other, become immiscible, and have large Henry's law constants (e.g., the Henry's law constants for a hexane–water system are >100 for each component). If solution components form complexes, Henry's law constants are less than 1, and the system exhibits a negative deviation from Raoult's law (e.g., the Henry's law constants for a chloroform-acetone system are <1 for each component).

Henry's law explains why flavor volatile losses in this study are linked to water solubility and not boiling point. It also explains why volatile losses are greater at higher initial concentrations. The flavorants in this study have very low solubility in the solvent system used, with the exception of diacetyl, benzaldehyde, and cinnamaldehyde. Large positive deviations from Raoult's law are expected. The observed volatile losses follow Henry's law predictions, and increase with hydrophobicity. Consequently, the ethyl esters show the highest volatile losses (> 95%) as predicted by the Henry's law constants, which are largest for this homologous series. Ketone losses exceed 90%, except for diacetyl which has higher water solubility.

In summary, the overall disproportionate flavor losses observed from single component solutions were favored by microwave vs. conventional heating, increased hydrophobicity, and higher initial concentration. Losses were consistent with increases in volatility caused by positive deviations from Raoult's law and *not* caused by microwave-specific effects.

Multiple Component Solutions. Large flavor losses were observed following both microwave and conventional heat treatments. Typical gas chromatographic profiles for multiple component mixtures before and after heat treatments are shown in Figures 8 through 11.

Disproportionate flavor losses for microwave and conventionally heated multiple component solutions were consistent with patterns identified for single component solutions. Post-heating ketone losses were very high except for diacetyl. Flavor losses increased within a homologous series as carbon number increased and water solubility decreased. (See Figure 12.) Aldehydes were less volatile, especially benzaldehyde and cinnamaldehyde.

For most flavorants, the relative percent of volatiles lost was increased by the presence of other flavorants. Ketone retention following conventional heating was reduced from 8%, 7%, 5%, and 3% flavor retention (pentanone, heptanone, octanone, and nonanone respectively) in single component solutions to 5%, 2%, 2%, and 1% flavor retention in the four-component ketone solution. Addition of three water-soluble flavorants (diacetyl, benzaldehyde, and cinnamaldehyde) in the seven-component solution had no further impact on ketone volatile loss. The same behavior is observed for the analogous sets of microwave heated ketone samples. This is additional proof that differences between conventional and microwave heated samples are not caused by microwave-specific effects.

The behavior of flavorants with higher water solubilities was more complex. The disproportionate change in volatile levels seen in single component solutions appears to be further influenced by types and levels of additional flavorants present. The volatile losses observed for multiple component solutions suggest that introduction of one flavorant influences how the solvent interacts with other flavorants in multiple component solutions. This is consistent with the colligative behavior of the total vapor pressure of the system as predicted by Henry's law.

In the two-component solution diacetyl retention is reduced by the presence of benzaldehyde, but benzaldehyde retention is increased by the presence of diacetyl.

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch036

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch036

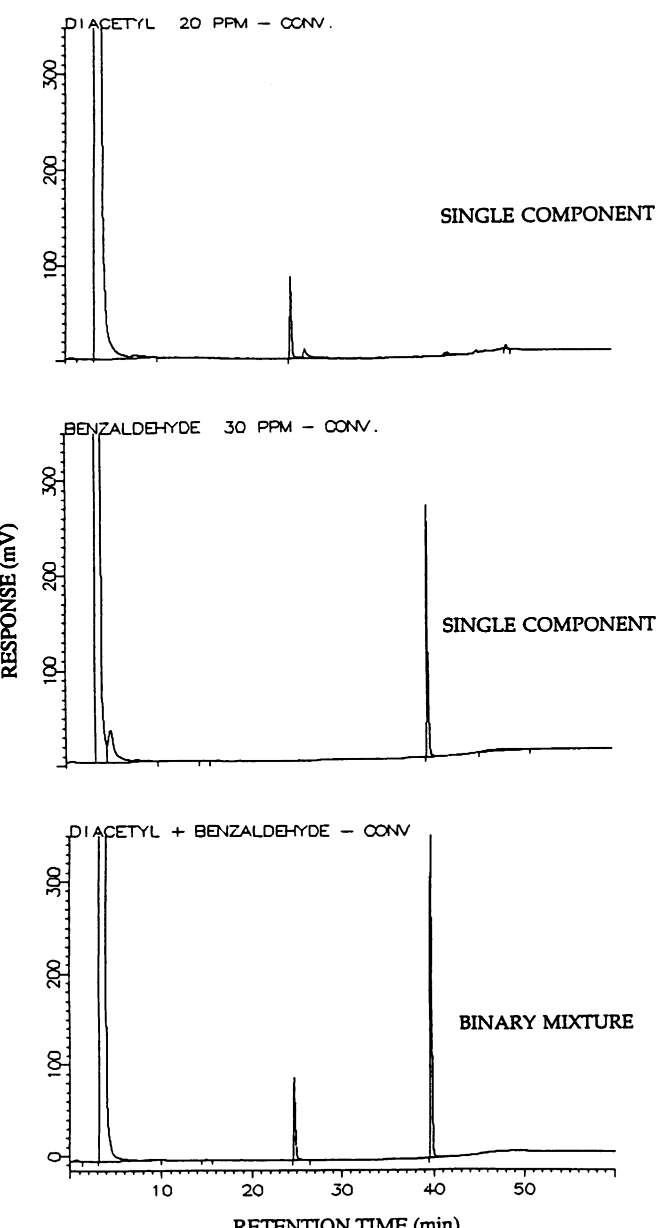

Figure 8. Post-conventional heating GC profiles for binary vs. single component solutions of 20 ppm diacetyl (24.7 min retention time), and 30 ppm benzaldehyde (39.7 min retention time).

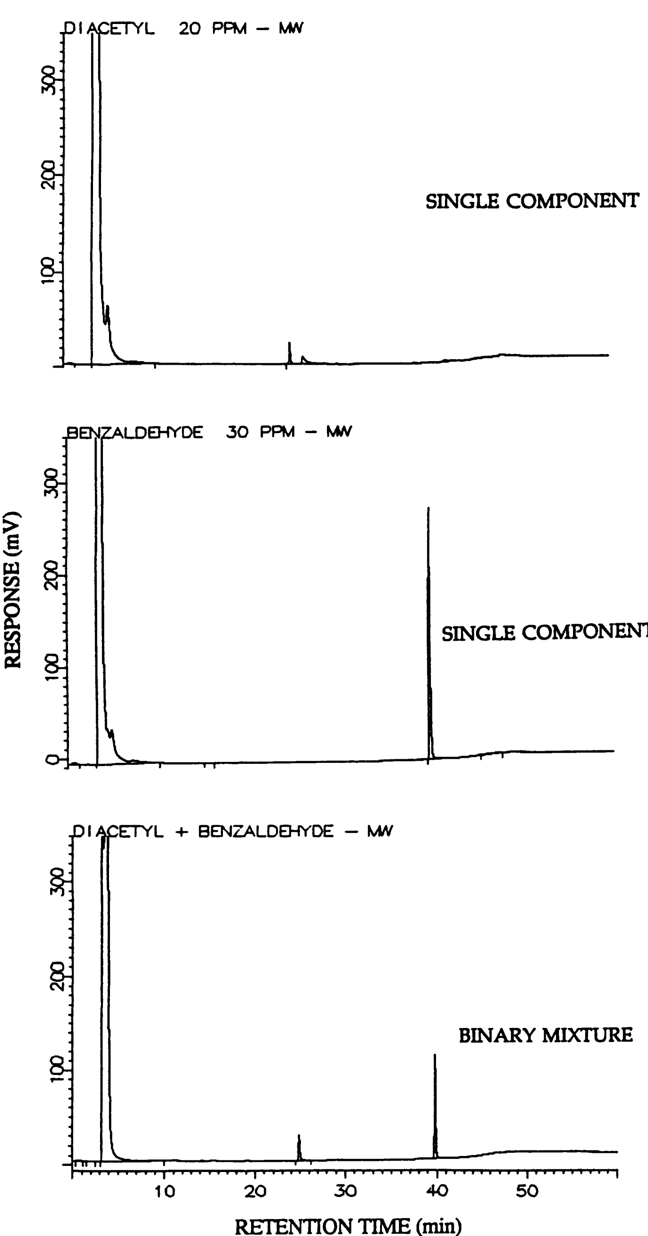

Figure 9. Post-microwave heating GC profiles for binary vs. single component solutions of 20 ppm diacetyl and 30 ppm benzaldehyde.

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch036

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch036

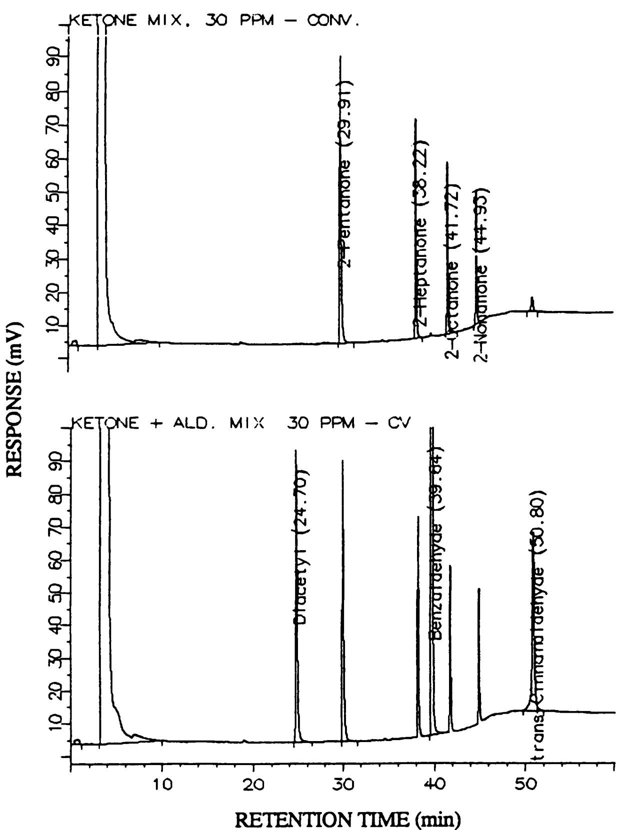

Figure 10. Post-conventional heating GC profiles for four- and seven-component solutions, 30 ppm initial concentrations.

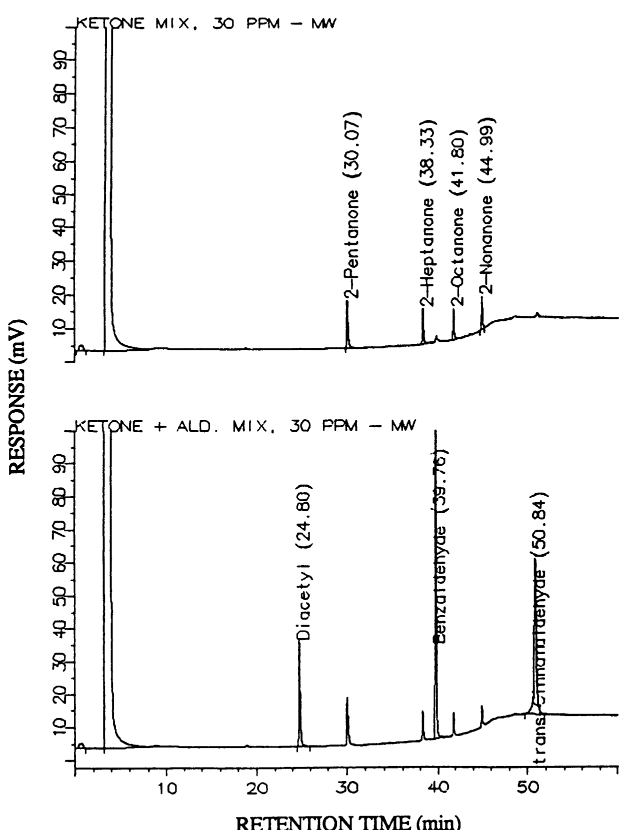

Figure 11. Post-microwave heating GC profiles for four- and seven-component solutions, 30 ppm initial concentrations.

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch036

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch036

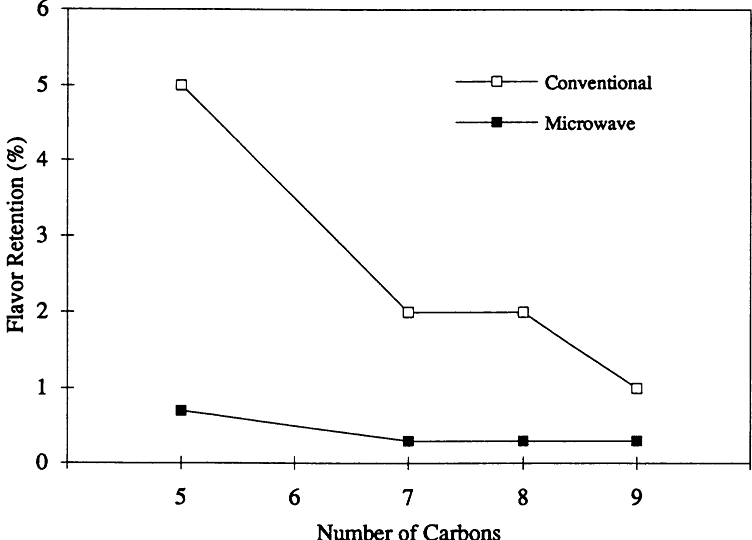

Figure 12. Post-heating flavor retention of four-component ketone mixture showing volatile loss increase with hydrophobicity.

For conventionally heated samples, 36% and 41% retention levels were observed for diacetyl and benzaldehyde respectively vs. 47% and 32% retention in single component solutions. The same behavior was observed for microwave heated samples, presenting further evidence against microwave-specific effects: 8% and 10% retention of diacetyl and benzaldehyde in the two-component solution vs. 14% and 35% retention in single component solutions.

In the seven-component solution, diacetyl retention is reduced, benzaldehyde retention is reduced or unchanged, and cinnamaldehyde retention is reduced by the presence of the combination of other flavorants present.

Again, similar volatile loss patterns were observed for microwave vs. conventional heated samples, providing further evidence against microwave-specific effects and in support of effects related to positive deviations from Raoult's law.

In summary, the overall flavor losses from multiple component solutions were favored by microwave vs. conventional heating, increased hydrophobicity, and the presence of other flavorants, which appeared to have both positive and negative impact on a given flavor's volatility, depending upon their relative hydrophobicities.

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch036

Conclusions

We observed *no* microwave-specific effects in this study, although disproportionate volatile losses were observed and total volatile losses were greater for microwave vs. conventionally heated samples. Disproportionate losses of flavor volatiles were observed in *both* microwave and conventionally heated samples. The enhanced volatile losses were in the opposite direction form those predicted by flavorant microwave properties.

Losses for individual flavorants were related to solubility/hydrophobicity and interactions with other flavorants present in a given sample. Volatile losses were consistent with the colligative impact of flavor components on the total vapor pressure of the systems studied, as predicted by classical physical chemistry of solutions (e.g., vapor pressure elevation, positive deviations from Raoult's law, and Henry's law constants). Thermal gradients generated within the sample account for the net increased loss of flavor volatiles for microwave vs. conventional heating.

The implications for increasing the retention of a specific flavorant within a food system to prevent flavor loss or distortion in microwave-heated foods are two-fold:

1) Substitution of a more water-soluble form of the flavorant.
2) Addition of ingredients to the food base which increase the solubility of flavorant in the aqueous phase of the food.

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Chapter 37

Nonequilibrium Partition Model for Prediction of Microwave Flavor Release

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During microwave cooking of food many flavor components are rapidly flashed off. Such selective volatilization may result in off-odors, flavor profile distortion, or even a complete lack of flavor. A rigorous physicochemical model was developed to predict flavor retention. This mathematical description accounts for volatility, hydrophobicity, and compartmentalization of the flavor compound within the food matrix. The model lends itself to (1) predict the behavior of a flavor mixture in food during microwave cooking; (2) design a flavor composition and delivery system for unique microwave applications; and (3) optimize the food formulation to enhance the intended flavor functionality. Creative exploitation of the synergistic interactions between flavor and food is paramount to the systematic engineering of microwave foods with superior flavor performance.

Demographic changes and increasing affluence in Western societies have resulted in a growing consumer demand for convenience foods, particularly microwaveable single serve items. Current market penetration of the microwave oven in the US is estimated at 90% of all households and the market for microwaveable foods is predicted to reach about 28 billion dollars per year by 1993 (*1*). Despite their request for convenience, consumers are unwilling to compromise taste and texture quality. This provides a serious technological challenge but also a competitive business opportunity to the sophisticated food processor and ingredient supplier.

A number of flavor problems often limit the use of microwave ovens (*2,3*). Generation of Maillard browning is mostly absent during microwave cooking due to vast differences in the mechanism of heat transfer. High ambient temperatures in convection ovens dehydrate the food product surface and create an environment conducive to flavor generation, i.e. high temperature at a low water activity for an extended time period. During microwave cooking, however, the product is heated internally and moisture migrates to the food surface, causing evaporative cooling and

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high water activity. Development of baking flavors will not occur under these unfavorable conditions and at the drastically reduced cook times.

This lack of flavor and aroma generation necessitates addition of flavors to microwave food to increase palatability and consumer acceptance. However, microwave cooking of food results in rapid loss of many flavor components at variable rates, leading to flavor profile distortion or even a complete lack of flavor. In addition to these flavor problems, microwave heating often generates off-odors due to volatilization of minor constituents that are essential to the overall flavor quality yet are malodorous as a single chemical, such as butyric acid in a butter flavor. Finally, with some products consumers desire sustained aroma release throughout the entire cook cycle - instead of an intense initial burst - that mimicks baking in the traditional convection oven.

There exists only a limited number of publications on the preparation of microwave food flavors (*4-7*). Most of this research has been of empirical nature and often entails some form of flavor immobilization by enrobement. Currently, the only systematic approach to describing the behavior of a flavor in a microwave food is the Delta-T theory (*8,9*). Shaath and Azzo proposed a model in which the release of a flavor can be predicted from its heat capacity and dielectric properties. Unfortunately, all of the microwave absorptivity measurements were performed on pure compounds and the results are completely irrelevant to a flavor diluted to 10 ppm or less. Flavor compounds with high dielectric constants absorb more microwave radiation than those with low dielectric constants. The absorbed radiation is then dissipated to the surrounding medium as heat energy, but flavor volatility is not affected. Due to the high dilution of most flavors, their dielectric properties fail to measurably influence the temperature of the solution. A similar conclusion concerning the inadequacy of the Delta-T theory was reached previously (*10*).

The Delta-T theory assigns absolute microwave volatility constants to flavors and does not recognize any effects of the medium on the flavor volatility. Steinke et al. demonstrated the large dependence of aroma release on the type of solvent (*11,12*). In fact, we will show below that the numeric value of the partition coefficient of a flavor compound between two solvents is directly proportional to the ratio of its volatility from the same two solvents. Therefore, aroma release of a very hydrophobic compound from water may exceed its release from oil by a factor of 10,000.

The current paper presents a rigorous physicochemical mathematical model that takes into account flavor volatility, hydrophobicity and its compartmentalization in the food matrix. This novel nonequilibrium partition model lends itself to accurately predict the behavior of a flavor mixture in any food during microwave cooking, to prevent some of the aforementioned problems specific to microwave flavors, and to customize food systems with unique flavor performances.

Materials and Methods

Determination of Partition Coefficients. Oil-to-air partition coefficients were measured at 25°C, 55°C, 80°C, and 100°C using capillary tubes packed with XAD-4 beads according to the method of Etzweiler et al. (*13*).

Water-to-air partition coefficients were determined at 25°C, 90°C, and 95°C by monitoring the decrease in aqueous concentration of the flavor compounds during stripping with nitrogen gas at a flow rate of 12.5 ml/min. To avoid evaporation of water from the solution the nitrogen was presaturated with water vapor. Samples of

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200 µl were analyzed by HPLC after 15, 60, 120, and 240 min. The partition coefficients were calculated from the fraction of the flavor retained in the aqueous solution. Partition coefficients at 100°C were calculated by extrapolation using the Clausius-Clapeyron equation.

Microwave Cake Preparation. Microwave cake batters (2000 g) - 24.9% flour, 24.6% sugar, 2.9% vegetable shortening, 1.1% Tween 60 (emulsifier), 0.1% methanolic flavor solution (10 to 30 ppm of 5 different flavor compounds in batter), variable amounts of water (24.0% to 46.4%), and variable amounts of soy oil (0% to 22.4%) - were prepared by creaming the sugar with the shortening and then blending in the remaining ingredients to uniformity. The combined amount of water and oil was kept constant at 46.4%.

Triplicate batter aliquots of 100 g were placed in glass dishes and baked in the microwave oven for 1, 2, and 3 minutes at 640 watts (calibrated using 2000 g of water). Batter temperature was monitored continuously using a fluoroptic fiber probe (Luxtron instrument). Moisture loss was determined gravimetrically at the end of each time interval by measuring total weight loss.

Some samples were also baked in a convection oven at 230°C for 20 and 40 minutes. Interior cake temperature was determined with a thermometer after both time intervals.

The following 5 flavor compounds were selected for the microwave release study: 2,3-dimethylpyrazine, indole, naphthalene, α-ionone, and δ-2-decenolactone. Criteria for choosing these flavor compounds included their range of volatility and hydrophobicity, as well as their ease of analytical extraction, separation, and detection. Similarly, the above microwave cake model was developed to (1) allow for a wide range of water-to-oil ratios without phase separation; (2) use a pourable system to minimize variability in sample weight, volume, and geometry; and (3) to evaluate aroma release in a real food product in order to eliminate any potential colligative and other interactive solute effects of the flavor constituents that might cause boiling point elevations or depressions and steam distillation in a pure liquid.

Determination of Flavor Retention in Cake Batter. Cake batter (50 g) was weighed accurately into a separatory funnel. An 18% aqueous NaCl solution (120 ml) was added, followed by methanol (160 ml), dichloromethane (320 ml), and 0.4% (w/v) methanolic m-dimethoxybenzene (50 µl). The mixture was stirred vigorously for 30 min and the resulting slurry was allowed to separate into two phases. The organic phase was centrifuged - if necessary - and concentrated by distillation through a Vigreux column until the temperature started to rise. Dichloromethane (250 ml) was added and distillation was continued to complete azeotropic removal of methanol. The concentrate (10 ml) was filtered and analyzed by HPLC. The method for the flavor determination in the cake was the same as that in batter, except water was added prior to extraction to compensate for moisture loss during baking.

Quantitative flavor analyses were performed by reverse phase HPLC on a 250 x 4 mm Ultrasphere ODS column with a guard column. The compounds were eluted at 30°C at a flow rate of 1 ml/min using the following linear gradient systems: $CH_3CN:H_2O$ (1:4) for 2.5 min, $CH_3CN:H_2O$ (3:1) for the subsequent 22.5 min, and 100% CH_3CN for the last 10 min. The eluate was monitored at 235 and 270 nm with a multiple wavelength detector. The column was washed with methylene chloride.

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Results and Conclusions

When a flavor compound is allowed to equilibrate between an emulsion and air in a closed system, the fraction of the compound present in the liquid phase is given by its mass balance (equation 1). From this mass balance we can easily derive the mathematical extraction equilibrium based on partition coefficients (equation 2) by substituting partition coefficients for flavor concentrations ($c_a = P_{aw}c_w$, $c_o = P_{aw}c_w/P_{ao}$) and by subsequently multiplying the numerator and denominator by P_{ao}/c_w. In the case of a single-phase liquid system, such as pure water, the term V_o in equation 2 becomes zero and the extraction equilibrium for a water-air system can be reduced to equation 3. In equation 2 either P_{ao} or P_{aw} can also be expressed in terms of P_{ow} according to equation 4, since a complete mathematical description of all three partition equilibria in a closed ternary system requires the measurement of only two partition coefficients. The partition coefficient P_{ow} is directly proportional to the hydrophobicity of the flavor compound, while P_{aw} and P_{ao} are a direct measure of its volatility in water and in oil, respectively.

$$f = \frac{c_o V_o + c_w V_w}{c_a V_a + c_o V_o + c_w V_w} \qquad (1)$$

$$f = \frac{V_w P_{ao} + V_o P_{aw}}{V_a P_{aw} P_{ao} + V_w P_{ao} + V_o P_{aw}} \qquad (2)$$

$$f = \frac{V_w}{V_a P_{aw} + V_w} \qquad (3)$$

$$\log P_{ow} = \log P_{aw} - \log P_{ao} \qquad (4)$$

where:

f	=	fraction of flavor retained in liquid or food
V_w	=	volume of water (ml)
V_o	=	volume of oil (ml)
V_a	=	volume of air (ml)
P_{ow}	=	oil-to-water partition coefficient ($[c]_o/[c]_w$)
P_{ao}	=	air-to-oil partition coefficient ($[c]_a/[c]_o$)
P_{aw}	=	air-to-water partition coefficient ($[c]_a/[c]_w$)
$[c]_w$	=	flavor concentration in water (g/l)
$[c]_o$	=	flavor concentration in oil (g/l)
$[c]_a$	=	flavor concentration in air (g/l)

The pertinence of equation 2 to a real food system was tested in a whole milk model. Five flavor compounds with a wide range of volatility and hydrophobicity were dissolved in both water and olive oil and their liquid-to-air partition coefficients were determined from headspace analyses. These partition coefficients were employed to calculate flavor retention in milk using equation 2. Milk was assumed to consist of a

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch037

4% oil-in-water emulsion. Table I demonstrates excellent agreement between observed and predicted flavor retention in milk. This example clearly illustrates the general validity of the above flavor equilibrium partition model for estimation of aroma release from food products.

In order to apply the static equilibrium equation to dynamic nonequilibrium microwave systems, we used the multiple extraction model which is premised on the following assumptions: During baking in the microwave oven the batter or cake is extracted consecutively with infinitesimal volumes of air or water vapor. During each successive extraction, food-to-air equilibrium is achieved only at the interphase. However, continuous flavor uniformity throughout the food and oil-to-water equilibrium of flavor compounds are maintained by the mass transfer of moisture due to rapid steam generation. The fraction of a flavor compound remaining in the cake after n extractions is given by equation 5.

$$f = \left[1 - f_e + f_e\left(\frac{V_w P_{ao} + V_o P_{aw}}{V_a P_{aw} P_{ao} + V_w P_{ao} + V_o P_{aw}}\right)\right]^n \qquad (5)$$

where:

f_e	=	fraction of food being extracted
n	=	number of successive extractions

For each food the variables f_e, n and V_a must be determined by measuring the retention of a test sample of flavor compounds with known partition coefficients. In principle, n is very large and f_e infinitely small. Therefore, in practice, we substitute for f_e a very low value (<0.01) and then determine the values of n and V_a from the set of equations obtained with the known test sample. V_a in equation 5 denotes the part of vapor phase that is in equilibrium with the food surface.

Although the partition coefficients in equation 5 change substantially between room temperature and the boiling point of water, as shown in Table II, we only used their value at 100°C. Typical foods like the microwave cake used in this study reach 100°C within 45 to 60 seconds, high oil contents accelerating the rate of heating as demonstrated in Figure 1. During this initial cooking period moisture loss also undergoes a lag phase (Figure 2) which accounts for the low level of flavor volatilization at the beginning of microwave cooking. Effective aroma release requires high temperature and steam formation to facilitate mass transfer throughout the product. Steam also serves as a vehicle to transport volatile aromas across the product-air boundary. This assumption is consistent with the observation that flavor release is related to the amount of water that evaporates during microwave cooking.

Equation 5 assumes that water and oil are the only food constituents that affect the volatility and release of the flavor compounds. In general, the hydrophobicity of proteins and polysaccharides is too low to significantly influence the hydrophobic-hydrophilic balance in the food as long as water and oil are present. However, interactions with food polymers and acid-base equilibria of some flavor compounds may account for their departure from the release predicted by their volatility in model oil-water systems. Examples of such deviation from ideality include (1) unexpectedly high retention of phenols in low fat cakes, and (2) slow release of vanillin from starch-containing foods.

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Table I. Observed vs Predicted Flavor Retention in Milk

	Fraction of Flavor Retained			
	Water	Olive Oil	Milk Observed	Milk Predicted
Acetic acid	0.94	0.43	0.94	0.94
Ethyl acetate	0.01	0.03	0.01	0.01
Hexyl acetate	0.01	0.79	0.13	0.14
Vanillin	1.00	1.00	1.00	1.00
Linalyl acetate	0.02	0.98	0.68	0.67

Table II. Temperature Effect on Partition Coefficients

	log P_{aw}		log P_{ao}	
	25°C	100°C	25°C	100°C
2,3-Dimethylpyrazine	-3.87	-2.27	-4.01	-2.85
Indole	-4.08	-2.54	-5.69	-4.35
delta-2-Decenolactone	-4.92	-2.54	-6.84	-4.68
Naphthalene	-1.86	-0.69	-5.41	-3.65
alpha-Ionone	-2.92	-1.32	-6.22	-4.33

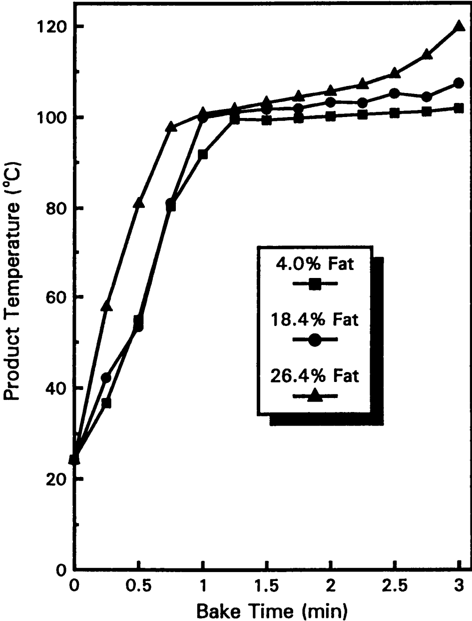

Figure 1. Effect of fat level on heating rate of MW cake. Internal temperature was monitored continuously using a fluoroptic probe.

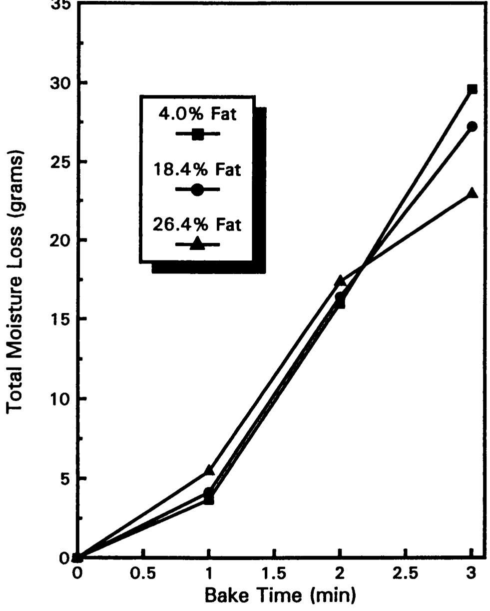

Figure 2. Effect of fat level on moisture loss from cake during MW baking. Moisture content was calculated from total weight loss after 3 time periods.

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As a further refinement of the nonequilibrium model, microwave flavor retention can be approximated by the product of a series of fractions retained during consecutive baking intervals as expressed in equation 6. This equation corrects for any moisture loss in the food during cooking by employing the average volume fraction of water for each time interval as calculated from the analytical weight loss.

$$F = f_1 \times f_2 \times f_3 \tag{6}$$

where:

F	=	total fraction of flavor retained in food
f_1	=	fraction of flavor retained during first minute
f_2	=	fraction of flavor retained during second minute
f_3	=	fraction of flavor retained during third minute

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In order to validate the proposed nonequilibrium partition model for the microwave flavor release, we predicted the retention of five flavor compounds in a microwave cake heated for three different time intervals. Figure 3 demonstrates the excellent agreement between experimental and calculated retention values, with an overall correlation coefficient for the 15 pairs of data points of 0.96. The water and oil levels of the cake samples prepared for Figure 3 were 32.0% and 18.4%, respectively.

Figure 3 clearly illustrates the principle of microwave flavor distortion. A completely balanced flavor loses various constituents at different rates depending on their partition coefficients. This differential volatilization may result in a completely unacceptable flavor. Correction of such flavor distortion may require initial adjustments to the level of individual components to customize the final profile or selection of different flavor chemicals with similar partition coefficients in order to prevent profile alterations. The approach largely depends on the customer's request, e.g. aroma generation versus finished product flavor.

The microwave nonequilibrium partition equation 6 was equally applicable to cake formulas containing high or low levels of oil and water (Figure 4). These results illustrate the relative insensitivity of most flavors to a wide range of water-to-oil ratios, provided the food contains enough of each phase to completely dissolve the flavor. Computer simulations (Figure 5) further demonstrate that the retention of hydrophilic flavor compounds is not noticeably affected by the oil level, while it is strongly dependent on moisture content but only under conditions nearing complete dehydration. Analogous behaviour is observed for hydrophobic compounds, which explains the common difficulty in formulating no-fat foods. Therefore, matrix composition affects aroma release primarily in extremely low oil or moisture zones. Such boundary conditions can be created in discrete areas of the food to exploit food-flavor synergies that enhance the quality of microwave products.

The flavor nonequilibrium partition model was also evaluated under a variety of heating conditions, such as lower wattage microwave ovens and traditional convection heating. In each case the predicted flavor profile was in good agreement with experimentally observed values. The model demonstrates that, while minor differences due to crust formation may exist, the same physicochemical principles govern flavor retention during both microwave and convection heating. Independent of the heating mode, the rate of aroma volatilization is determined by the partition coefficients of the flavor constituents, the chemical and physical properties of the food matrix, the total amount of heat input, and moisture loss.

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch037

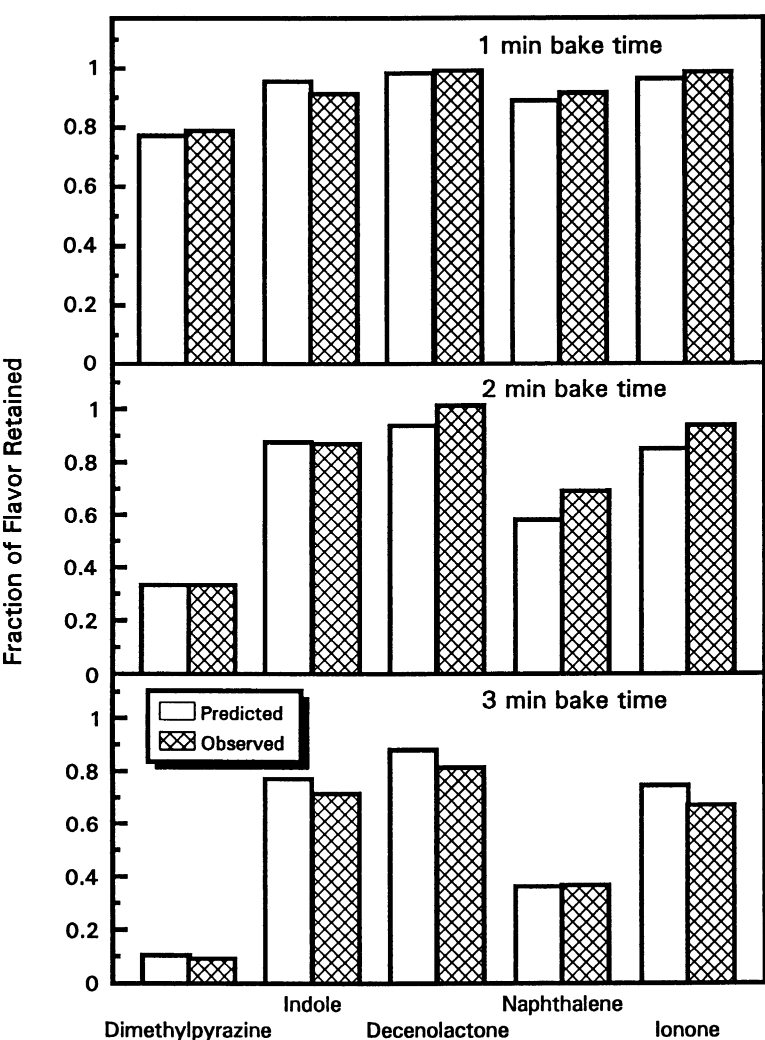

Figure 3. Flavor retention in MW cake as determined by HPLC and as predicted by the nonequilibrium partition model. The flavor compounds were 2,3-dimethylpyrazine, indole, δ-2-decenolactone, naphthalene, and α-ionone. The cumulative correlation coefficient for all 15 pairs of data points was 0.96.

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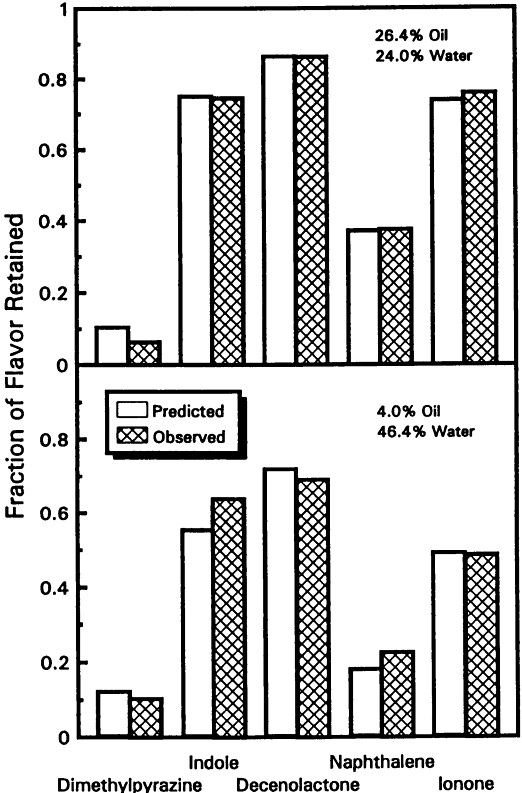

Figure 4. Effect of water-to-oil ratio on flavor retention in cake during MW baking as determined by HPLC and as predicted using the equilibrium partition model. The 5 flavor compounds were the same as those in Figure 3.

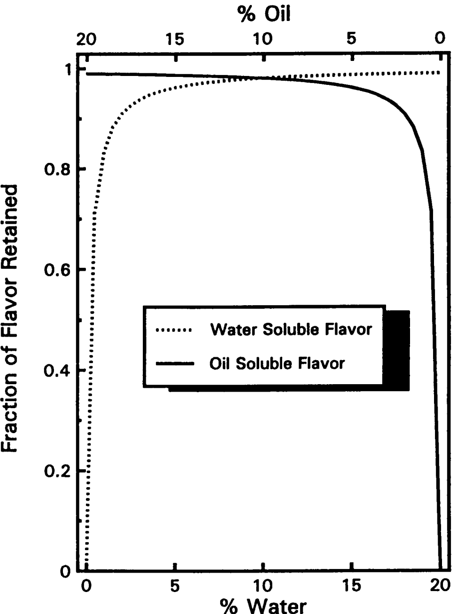

Figure 5. Effect of water-to-oil ratio on the retention of a hydrophilic and a hydrophobic compound from a food containing 20% of a water-oil mixture. The fraction retained was calculated by computer simulation using equation 2.

The general validity and ease of practical use of the proposed flavor equilibrium partition model allows for accurate analytical prediction of aroma release and the customization of a flavor composition and delivery system to enhance the intended flavor functionality. The proposed concept lends itself to prevent several of the microwave related flavor problems mentioned above and to design foods with unique flavor performance. Examples include high flavor retention in bakery products, sustained aroma release throughout the entire cook cycle that mimicks traditional baking in the convection oven, decrease in flavor distortion, and prevention of off-odor generation by extremely volatile chemicals like methyl butyrate. Furthermore, the nonequilibrium partition model will also predict flavor performance in low fat food systems, calculate flavor release from chewing gum (*14*) and it may even be applicable to understanding flavor loss during extrusion cooking.

Our results from the current study revealed the major effects of food matrix composition on the volatility of a flavor in a microwave food. Therefore, creative exploitation of the synergistic interactions between flavor and food is paramount to the systematic engineering of microwave foods of superior flavor performance. The design of microwave foods of a specific flavor functionality requires a collaborative development effort from both the flavor supplier and the food processor.

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Chapter 38

Microwave and Thermally Induced Maillard Reactions

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The effect of amino acid type on the generation of Maillard aromas under microwave irradiation in an open system was evaluated by mixing different combinations of amino acids with the same reducing sugars and characterizing the aromas produced as caramel, meaty, nutty, fragrant, vegetable and baked. The amino acids were divided into five categories; aliphatic, aromatic, basic, acidic and sulfur-containing. Certain trends emerged after analysis of the results that relate the presence of specific amino acid category in the reaction mixture to a corresponding aroma note produced after microwave heating. The presence of amino acids with alkyl side chains were found to be essential for the generation of caramel notes, sulfur-containing amino acids for meaty type notes and basic amino acids for nutty and baked notes. Selected formulations were also subjected to conventional heating and their sensory properties and chemical composition (by GC/MS analysis) were compared to those from microwave treated samples. No significant differences were observed between the two samples.

Developing natural flavors and colors for the microwave poses one of the next challenges to the food industry. The lack of Maillard flavor development during microwaving and the loss of added flavors are primary factors which contribute to the low acceptability of many microwaved food products *(1)*. However, in spite of these shortcomings, the sales of microwave food products have experienced a larger growth compared to overall food sales in the last few years. The most common approach used today in the industry to promote flavor development in the microwave is the use of susceptor packaging. While susceptors are effective in promoting surface browning, they do not allow the full development of flavors *(2)*. On the other hand, the "Delta T" theory provides some guidelines as to the type of added flavors that can be used in microwave formulations to minimize their loss *(3)*. To improve the quality of microwave foods, and to better understand the differences between microwave and conventional heating, the chemical composition of model systems subjected to both modes of heating have been compared. Parliment *(4)* studied the products of the Maillard reaction between glucose and proline formed under microwave and conventionally heated systems and found that certain products were predominant in

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the microwave treated samples and others in the conventionally heated mixtures. Yeo and Shibamoto *(5)* reviewed the chemical composition of microwaved and conventionally heated foods and model systems. Some of the conclusions drawn from different studies were in contradiction, such as the relative amounts of pyrazines and sugar fragmentation products in microwave and conventionally heated samples. On a more fundamental level, microwave irradiation has been used to perform variety of organic single-step reactions such as esterification, hydrolysis, cyclization, Diels-Alder, S_N2-type reactions etc., and the results have been compared to classical reflux method by different researchers *(6, 7)*, according to these studies there are no fundamental differences between microwave and reflux methods of heating, the reactions proceeds through the same mechanisms, to produce the same products, in comparable yields, however, if the microwave heating is done under closed system, then the rate of the microwave reaction becomes faster up to several hundred times, due to the superheating of the solvent. For example, the percent yield of n-propyl benzoate after heating equal amounts of reactants for 4 min in an oil bath at 160°C and in the microwave oven in an open vessel was 29 and 25 % respectively. The same reaction produces a yield of 79% for 6 min of microwave heating in closed system, compared to 4 h refluxing which produces the same yield. In a closed system microwave increased the rate of the esterification reaction by 40 times *(7)*.

Materials and Methods

Sample Preparation. Vials containing aqueous solutions of Maillard precursors were subjected to microwave irradiation or to conventional heating or both, in an open system in a domestic dual microwave/conventional oven, operated at full power (640 W). The samples were irradiated by microwave until all the water was evaporated (2-4 minutes) and the residue was dark brown, to mimic actual cooking conditions that require surface drying of foods. Thus, the compounds identified by GC/MS represent the volatiles that were trapped in the residue or undecomposed non-volatile components. In order to insure that both treatments produced the same extent of Maillard reaction for comparison purposes, the conventional heating time was adjusted such that after similar dilutions, both samples absorbed to the same extent at 460 nm in the spectrophotometer. The samples were diluted with 1.0 mL of methanol, and the absorbance was measured (460 nm) prior to injection into the GC/MS. Similarly, the same samples were also subjected to conventional heating until most of the water has evaporated (20-50 minutes), diluted to 1.0 mL with methanol and the absorbance at 460 nm was measured. The process was repeated at different time intervals, until the same absorbance was achieved as for the microwave treated sample. On the average, one minute of microwave heating time was equivalent to 12 minutes of conventional heating time to produce the same extent of browning.

GC/MS Analysis. A Hewlett Packard GC/Mass selective detector (5890 GC/5971B MSD) was used for the separation and acquisition of the electron impact (70 eV) mass spectra of the compounds. Two µL of the methanol solution was injected in the split mode (15:1) on to a fused silica capillary column (HP Ultra-2, 50-m x 0.2 mm i.d. x 0.33 µm film thickness). The column was held at 70°C for 2 min., then increased at a rate of 5°/min to 250°C and held for 5 minutes. Carrier gas (helium) flow rate was 0.85 cm^3/min; injection port temperature was 250°C. Tentative identification of the compounds were achieved by comparison of the fragmentation patterns of the unknowns with those in the Wiley/NBS mass spectral library through a PBM library search routine.

Model systems. Model systems were prepared by mixing the same reducing sugar(s) with different combination of amino acids (see Table I) from each group with all

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch038

possible permutations in specific ratios. The mixtures were subjected to microwave heating in an open vial until the development of brown color and evaporation of most of the water. The sensory evaluation by an untrained panel of the resulting aromas were than recorded.

Results and Discussion

There are no satisfactory theoretical explanations or practical solutions to the problem of Maillard flavor generation during microwave heating. Attempts have been made to compare the chemical composition of microwaved and conventionally heated Maillard model systems, however, this type of comparisons can be extremely misleading due to the variations in the time-temperature exposure of the two systems, specially in closed containers where due to extreme high pressures, the solvent can superheat and accelerate the rate of the chemical reactions (7). In order to draw meaningful conclusions about the real differences between microwave and conventional heating, the two systems should undergo Maillard reaction to the same extent. In addition, due to variations in the time-scale between the two modes of heating, complex multistep reactions such as Maillard reaction, can remain incomplete in the microwave if enough time is not allowed for the reaction to proceed. However, during a chemical reaction, the yield of a particular product can be increased by increasing the reactivity and /or the concentrations of the reactants, specially if time is a limiting factor which is the case in the microwave. If one of the reasons for the failure of microwave to develop flavors is the absence of reactive Maillard precursors in sufficient concentrations to effect browning and flavor formation in the limited time-scale of microwave, than adding reactive precursors in sufficient concentrations to the food products, the Maillard reaction can be initiated at microwave conditions. Fresh baked, roasted, and chicken flavor formulations have been already developed in our laboratories using this strategy that can produce the desired effect in the microwave oven *(8)*. However to facilitate the choice of amino acids, the effect of different amino acids was evaluated in terms of their potential to produce specific aroma notes in the microwave.

The Effect of Amino Acid Side Chain on the Type of Aroma Produced in the Microwave. In order to find trends within specific groups of amino acids, and their effect on the type of aromas produced in the microwave, the amino acids were divided into five categories based on the chemical nature of their side chains (Table I). The aroma produced by the model Maillard systems were categorized into eight aroma types listed in Table I which shows a partial list of different mixtures of amino acids examined. Inspection of the results reveals that meaty, nutty and baked aroma notes are the most common in the Maillard reaction mixtures subjected to microwave heating, this is the same trend observed in conventional heating. An interesting type of aroma that can be produced in the microwave is the fragrance reminiscent of different fruits.

Generally it was found that amino acids with alkyl side chains (Aa: glycine, alanine, leucine, isoluecine and valine) were essential for the production of caramel notes. This is mainly due to the fact that Amadori products formed from such amino acids produce relatively large amounts of maltol by dehydration reactions. Maltol is known to produce a caramel type of aroma. Sulfur containing amino acids seem to be essential components in model systems producing meaty type aromas. Under conventional heating, sulfur containing compounds such as mercaptans, thiazoles and thiophenes are predominant in meaty aromas. The characteristic feature of the amino acid requirement for the nutty notes was the presence of either basic amino acids (Bc category: glutamine and asparagine) or the presence of aliphatic amino acids (Ab category: serine and threonine). One of the main components of roasted nutty aromas are the derivatives of pyrazines, and this fact might explain the requirement of basic amino acids for the generation of these aromas.

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch038

Table I. Different Aromas Produced by the Model Amino Acid-Sugar Mixtures

Caramel	Meaty	Nutty	Meaty+Veg.
Aa	S	Bc+Ba	S+Ar
Aa + Ab	S+Aa	Bc+Ba+Bb	S+Ar+Ac
Aa + Ba	S+Bb	Bc	S+Ar+Ba+Bb+Bc
	S+Ar+Ab	Ab+Bb	
	S+Ar+Bb	Ab+Ba	
Fragrant	**Roasted vegetable**	**Baked potato**	**Baked**
Ar	Ar+S+Ac+Aa+Bb	S+Ab	Ba
Ar+Aa	Ar+S+Ac+Aa+Bc	S+Ac+Aa+Ab	Ba+Bb
Ar+Ab		S+Ac+Ar+B	Ba+Bb+Bc+Ac
Ar+Ba			Ba+Ac

Aa (glycine, alanine, leucine, isoluecine, valine) *Bb (lysine, arginine, histidine)*
Ab (serine and threonine) *Bc (glutamine and asparagine)*
Ar (tryptophan, phenylalanine and tyrosine) *Ac (glutamic acid and aspartic acid)*
Ba (proline, hydroxyproline) *S (cysteine, cystine and methionine)*

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch038

All the model systems producing mixed meaty vegetable aroma notes contained both sulfur and aromatic amino acids. Such aroma notes are usually characterized by the presence of sulfur containing and aromatic compounds. Aromatic amino acids are essential for the production of fruity aromas in combination with aliphatic or basic amino acids. The importance of aromatic amino acids lies in the fact that they can be converted into aromatic aldehydes and ketones essential for fruity-type flavors, such as benzaldehyde. On the other hand, the two model systems that produced roasted vegetable notes contained aromatic, sulfur, acidic and aliphatic (Aa) amino acids in addition to either Bb category amino acids (lysine+arginine+histidine) or Bc (glutamine+asparagine). Acidic, aliphatic and sulfur-containing amino acids seem to promote baked potato notes in Maillard model systems. The essential feature of the Maillard model systems producing baked aroma notes under microwave conditions is the presence of basic amino acids especially Ba category amino acids (proline + hydroxy proline). This is in accordance with observations under conventional heating conditions. Analysis of baked flavors have shown that many important aroma compounds contain a proline derived moiety, such as 2-acetyl-pyrroline.

Comparison of the Microwave and Thermally Induced Flavors. Based on the information gathered on the effect of amino acid type on the generation of Maillard aromas in the microwave, six formulations were optimized (samples 1-6 in Table II) for microwave generation of aromas that require surface drying for their formation (baked and roasted). The same formulation was subjected both to microwave irradiation and to conventional heating in an open system as described under "Materials and Methods". The compounds identified in all the samples by GC/MS are shown in Table II. As expected, only a small number of components (between 9 and 17) were identified in each sample, for a total of 31 compounds in all samples. Under the experimental conditions at which the flavors were produced, there was no significant difference in the type of compounds generated between microwave

Table II. Compounds Identified in Microwave (M) and Conventionally (C) Heated Flavor Mixtures

Name	*M*	*C*	*Sample*
Furan Derivatives			
Furan	+	+	6
2-Furan carboxaldehyde	+	+	1, 2, 3, 4, 5,
5-Methyl-2-furan carboxaldehyde	+	+	2, 4, 5
2-Furan methanol	+	+	1, 2, 3, 4, 5
HMF	+	+	1, 2, 3, 4, 5
2,3-Dimethyl-4-hydroxy-3(2H)-furanone	-	+	1
2-(5H)-Furanone	+	+	3, 4
2-Methylfuran	+	+	6
Furanylmethylpyrrole	+	+	3, 5
2,3-Dihydro-4-hydroxy-2,5-dimethyl-3-furanone	+	+	5
1-(2-Furanyl)ethanone	+	-	5
4-Hydroxy-but-2-enoic acid lactone	+	-	5
2,5-Furandione	+	-	5
Pyran Derivatives			
2,3-Dihydro-3,5-dihydroxy-6-methyl 4(H)pyran-4-one	+	+	1, 2, 3, 4, 5
3,5-Dihydroxy-2-methyl-4(H)pyran-4-one	+	+	3, 4
N-Heterocycles			
2-(1H)-Pyridinone	-	+	1
3-Methylpyrrole	+	+	5
Pyrazine	-	+	5
S-Containing Compounds			
3-Methylthio propanal	+	+	3
Dimethyl disulfide	+	+	6
Methanthiol	+	+	6
Dimethyl trisulfide	+	-	6
Aliphatic			
Acetic acid	+	+	1, 2, 3, 4, 5
Formic acid	+	-	2
Acetaldehyde	-	+	6
Butanal	+	+	3
2-Methylpropanal	+	+	2, 6
3-Methylbutanal	+	+	2, 6
2-Methylbutanal	+	+	2, 3, 6
1-Hydroxy-2-propanone	+	+	2, 4
Aromatic			
Benzene acetaldehyde	+	+	2

Sample 1 Meat aroma, Sample 2 Fragrant, Sample 3 Potato aroma, Sample 4 Baked aroma, Sample 5 Bread aroma, Sample 6 Baked potato aroma

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch038

irradiated and of conventionally heated samples. 1-(2-Furanyl)ethanone, 4-hydroxy-but-2-enoic acid lactone, 2,5-furandione and dimethyl trisulfide were present only in the microwave treated samples, whereas acetaldehyde, 2-(1H)-pyridinone and pyrazine were only present in the conventionally heated samples. However dimethyl trisulfide was also detected in a different sample that was heated conventionally in our laboratories. MacLeod and Coppock *(9)*, studied volatile extracts of boiled beef cooked conventionally and by microwave irradiation and found that six of the eight pyrazines identified were formed in higher amounts in the microwave. Most likely these differences are due to time-temperature exposure variations rather than differences in mechanistic pathways as suggested by others.

Half of the identified compounds were either furan or pyran derivatives, there were only four N-containing heterocyclic compounds and four S-containing compounds. All of the aldehydes identified could arise by Strecker degradation from corresponding amino acids (Table III), it seems Strecker aldehydes could form in the microwave as easily as under conventional heating.

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch038

Table III. Strecker Aldehydes Identified in Maillard Flavor Formulations

Strecker Aldehyde	*Amino acid source*
Acetaldehyde	Alanine
Benzene acetaldehyde	Phenylalanine
3-Methylthiopropanal	Methionine
2-Methylpropanal	Valine
3-Methylbutanal	Leucine
2-Methylbutanal	Isoleucine

Figure 1 shows compounds that were identified in all of the microwave and conventionally treated samples. 2,3-Dihydro-3,5-dihydroxy-6-methyl-4(H)-pyran-4-one, has been shown *(10)* to form directly from Amadori products during mass spectrometric fragmentation in the electron impact mode by a process termed "*ortho*-elimination". It has also been shown that it is the direct precursor of maltol, by mass spectrometric linked-scan experiments. Figure 2 shows the proposed mechanism of formation of maltol and the simultaneous release of an amino acid moiety based on mass spectrometric studies. In the initial step, the Amadori product undergoes dehydration to form a double bond between C-2 and C-3 of the sugar residue, this is followed by a cleavage of the C-N bond by thermally induced *ortho*-elimination reaction to release the amino acid and a sugar residue. The latter isomerizes to form the pyranone, a more stable product and can than undergo further dehydration to form maltol.

Conclusion

Compensation for the time-temperature variations between microwave and conventionally heated samples can be achieved by increasing the concentrations of specific reactive Maillard precursors in model systems or in formulations that can be applied to microwavable foods. In additon, surface drying of foods appears to be necessary to achieve browning.

2-Furancarboxaldehyde
(R_1=H, R_2= CHO)
5-Methyl-2-furan carboxaldehyde
(R_1= CH_3, R_2= CHO)
2-Furan methanol
(R_1=H, R_2= CH_2OH)
HMF
(R_1= CHO, R_2 = CH_2OH)

2,3-Dihydro-3,5-dihydroxy - 6-methyl-4(H)-pyran-4-one

Figure 1. Common O-heterocyclic compounds identified in all of the samples studies

Maltol

Figure 2. *Ortho*-elimination of Amadori products

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch038

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Chapter 39

Flavor in the Cysteine–Glucose Model System Prepared in Microwave and Conventional Ovens

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Flavors created by cooking in a microwave oven and a conventional oven are significantly different. The difference is due to the formation, both in quality and quantity, of heterocyclic compounds. The volatile heterocyclic compounds generated from an aqueous D-glucose/L-cysteine Maillard system upon microwave irradiation or conventional heating were isolated and identified by gas chromatography and mass spectrometry. Heterocyclic compounds were formed in considerably higher amounts in the microwave-irradiated samples. They included thiazole, 2,5-dimethylthiazole, 4,5-dimethyloxazole, 2-methylpyridine, and 2,3-dihydro-3,5-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one. Compounds formed in significantly higher amounts in the conventionally heated samples were 2-methylpyrazine, 2,6-dimethylpyrazine, 2-furanmethanol, and 4-hydroxy-2,5,-dimethyl-3(2H)-furanone. The lack of desirables cooked flavors in microwaved foods may be due to the absence of or decreased formation of pyrazines and furans. The flavor profiles of microwaved and conventionally heated foods suggest that the formation mechanism of compounds, especially of pyrazines, are different. Various factors such as electrolytes, pH, and moisture content have been shown to alter the generation of volatiles and degree of browning in microwaved systems.

Foods cooked in the microwave oven often lack the desirable flavors and browning which are normally found in foods cooked by conventional heating. This may be due to a short cooking time and low achievable cooking temperature. With the increasing popularity of the microwave oven, understanding the differences in the chemistry and flavor profiles of foods generated in the presence of microwave irradiation and conventional heating will be valuable for the development of appealing microwaved food products. Furthermore, techniques to enhance the degree of browning and flavor production in microwaved products should be explored. Some attempts in this regard have been made by the use of susceptor packagings and food coatings (*1*).

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In this paper, comparative studies were performed on the flavor compounds formed from a Maillard model system upon microwave irradiation and conventional heating. In addition, enhancement of the flavor compounds formed by microwave irradiation by the control of the electrolyte concentration, pH, and moisture content of the Maillard system upon microwave irradiation was investigated.

Experimental

The Maillard system consisting of L-cysteine and D-glucose was used as a model to investigate the chemistry of flavor compounds under various cooking conditions. The above reaction mixture was subjected to microwave irradiation at the HIGH setting of a 700-watt microwave oven for 15 min, or thermally heated at 100°C for 40 hours under reflux (*2, 3*). The above conditions were determined by the onset of browning and aroma formation since the heating rate, temperature, and cooking times of the two methods are different. The resulting heterocyclic flavor compounds such as pyrazines, thiazoles, furans, and oxazoles were isolated and identified by gas chromatography-mass spectrometry.

Flavor Products Formed by Microwave Irradiation and Thermal Heating

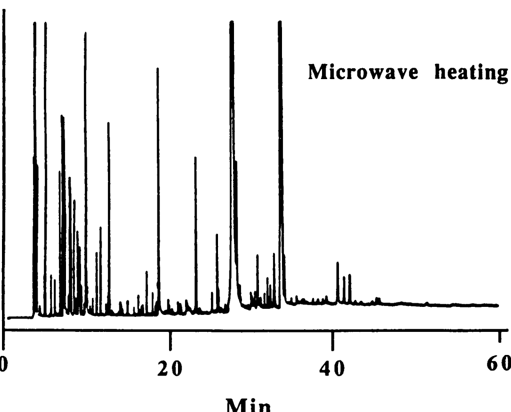
Figure 1 shows a typical gas chromatogram of the volatiles generated from the L-cysteine/D-glucose model system upon microwave irradiation and conventional heating. There is a distinct difference in the profile of heterocyclic flavor compounds generated by the two cooking methods. The volatiles generated from microwave and conventionally heated systems are shown in Table I.

The microwave samples produced a nutty, roasted, and meaty flavor, with strong popcorn characteristics. However, these samples also gave a distinct pungent, raw, and burnt aroma. The major compounds identified in these microwaved samples were oxazoles, pyridines, thiazoles, and pyran. 4,5-Dimethyloxazole (*4*) and 2-methylpyridine (*5*) have been associated with green- and vegetable-like flavors. The oxazoles and pyridines may explain the off-flavors present in the microwave samples. The sample also generated high levels of thiazole and 2,5-dimethylthiazole which have meaty and roasted flavors (*6*). The significant amount of 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one in the microwaved samples suggests a limited degree of sugar fragmentation during microwave irradiation, probably due to the low energy involved in microwave irradiation.

Conventionally heated samples generated similar popcorn and nutty flavors as did the microwaved samples. In contrast, the thermal samples lacked the distinct off-flavors that were found in the microwave samples. Compounds formed in significantly higher levels in the conventionally heated samples were the pyrazines and furans. It is known that 2-methylpyrazine contributes to a roasted flavor in cooked foods (*5*) and 2,6-dimethylpyrazine possesses a sweet and toasted corn-like aroma (*7*). The presence of these compounds may explain the popcorn and nutty flavors in both the conventional and microwave samples.

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch039

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch039



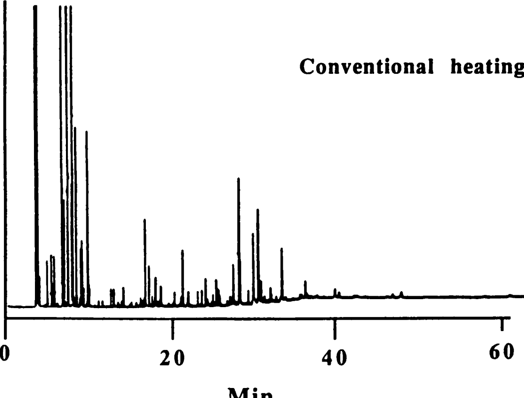


Figure 1. Typical gas chromatograms of the volatiles generated from the L-cysteine/D-glucose model system upon microwave irradiation and conventional heating

Table I. Heterocyclic Flavor Compounds Formed by Microwave Irradiation and Conventional Heating in the L-Cysteine/D-Glucose Model System at pH 9

Class	*GC Peak Area Ratio*[a]		
	Peak No.	*Thermal*	*Microwave*
Oxazole			
4,5-Dimethyloxazole	1	0.18	13.44
Trimethyloxazole	2	1.59	1.68
Thiazole			
Unsubstituted	5	0.12	1.26
2,5-Dimethylthiazole	16	—	5.06
Pyrazine			
2-Methylpyrazine	6	66.20	4.26
2,5-Dimethylpyrazine	11	5.90	5.40
2,6-Dimethylpyrazine	12	30.40	3.84
2-Ethylpyrazine	13	0.38	3.81
2,3-Dimethylpyrazine	14	6.61	4.94
2-Ethyl-6-methylpyrazine	17	2.26	3.27
Trimethylpyrazine	19	7.33	10.75
Tetramethylpyrazine	24	0.23	2.92
Pyridine			
2-Methylpyridine	4	0.09	0.54
Furan			
2-Acetylfuran	25	0.76	5.46
2-Furanmethanol	29	2.25	—
4-Hydroxy-2,5-dimethyl-3(2H)-furanone	37	49.93	8.12
Thiophene			
2-Thiophenethiol	26	0.87	—
3-Thiophenethiol	27	0.83	—
Pyrrole			
2-Acetylpyrrole	36	0.98	3.88
Pyran			
2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	55	—	76.10

[a] GC peak area of product/GC peak area of internal standard. Values are means of two replicates.

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The reduced quantities of pyrazines and furans in the microwave samples may be due to their low rates of formation (*8*) and may explain the lack of desirable flavors in microwaved foods. The differences in the flavor profiles of microwaved and heated systems suggest different formation mechanisms of these compounds, especially for pyrazines and oxazoles. Various factors such as pH, electrolyte concentration, and moisture content have been shown to alter the generation of volatiles and the reaction mechanisms of flavor formation in microwaved systems (*3, 9, 10*).

Factors Affecting Flavor Formation in Microwave Irradiated Systems

Differences in the flavor profiles between systems subjected to microwave irradiation and conventional heating led us to study the factors for flavor production by the microwave oven. Various parameters such as pH, electrolyte concentration, and moisture content of the Maillard system during microwave irradiation were manipulated, and the flavor production and browning intensity were investigated.

pH. Previous studies have demonstrated that pH plays an important role in the production of heterocyclic flavor compounds in the Maillard model system. Its role in microwaved Maillard systems was investigated by subjecting the system to various pH conditions (*3*). Figure 2 shows the effect of pH on the total volatiles and the degree of browning (as measured by the absorbance at 420 nm) in microwaved samples. It was found that the total amount of volatiles increases as pH increases. The degree of browning of the resulting samples, however, did not correspond to the total volatiles produced. The sample at pH 2 had the darkest brown color, whereas the sample at pH 5 had a very light yellow color. Table II summarizes the volatiles produced by microwave irradiation under different pH conditions (pH 2, 5, 7, and 9). Of the four pH samples studied, only the sample at pH 9 produced a distinct nutty, roasted, and meaty flavor, with a pungent, raw, and burnt aroma. The samples at pH 5 and 7 gave a strong, pungent, and sulfurous odor characteristic of rotten eggs. The GC profiles of these latter two samples were very similar.

The sample at pH 2 generated the fewest volatiles. Like the samples at pH 5 and 9, the pH 2 sample also possessed a pungent odor and lacked the desirable roasted and nutty attributes. Samples at pH 2, 5 and 7 did not yield any detectable pyrazines. The lack of desirable flavors found in microwave heating may be due to the absence of these pyrazines.

Electrolyte concentration. Microwave radiation is non-ionizing and it interacts with dielectric materials such as water and electrolytes to generate heat. When Maillard reaction mixtures were subjected to microwave irradiation in the presence of various electrolytes, an enhancement in the volatile production and browning intensity were observed.

Figure 3 shows the browning intensity versus concentration of electrolytes (*9*). NaCl promoted the highest degree of browning among the electrolytes with chloride anions, followed by $CaCl_2$, and $FeCl_2$. For electrolytes with Na cations, Na_2SO_4

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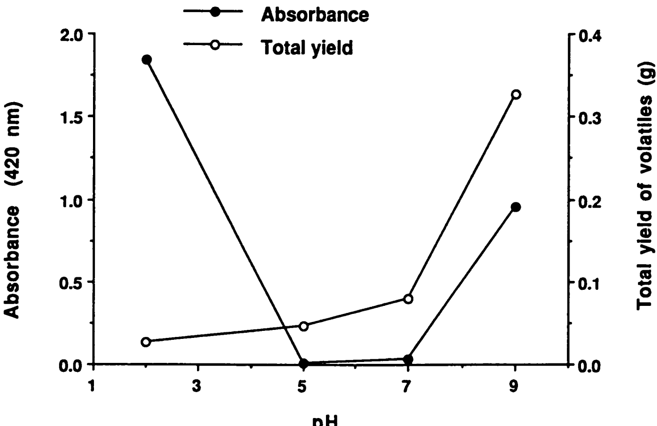

Figure 2. Effects of pH on the total GC peak area of volatiles (o) and the degree of browning (•) in the L-cysteine/D-glucose model system upon microwave irradiation

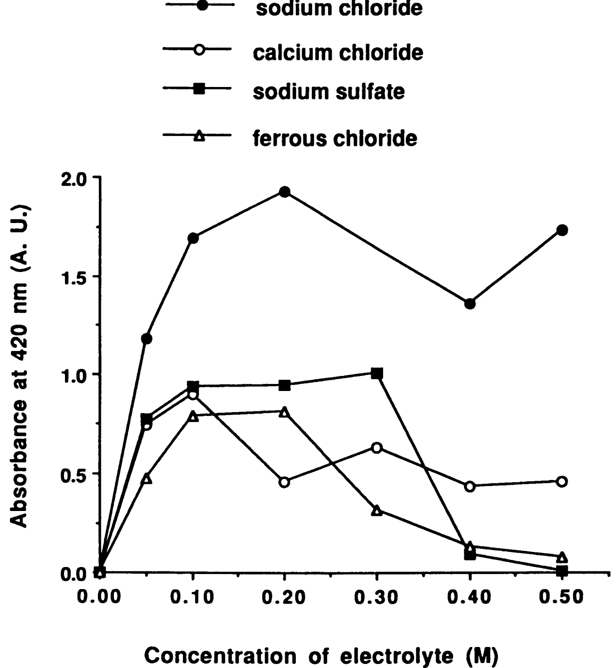

Figure 3. Effects of electrolytes on the degree of browning in the L-cysteine/D-glucose model system upon microwave irradiation

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch039

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch039

Table II. Volatiles formed by Microwave Irradiation in the Cysteine/Glucose Model System

Class	*GC Peak Area Ratio*[a]			
	pH 2	*pH 5*	*pH 7*	*pH 9*
oxazoles	*b*	0.27	1.08	15.12
thiazoles	2.07	0.68	0.92	6.32
pyrazines	—	—	—	39.19
pyridines	—	0.11	0.08	0.54
furans	9.08	32.52	44.78	13.58
thiophenes	0.79	1.03	1.07	—
pyrroles	2.79	3.60	5.64	3.88
pyrans	0.92	4.31	13.01	76.10

[a] GC peak area of product/GC peak area of internal standard. Values are means of two replicates.
[b] Not detected.

showed a lower enhancement in the browning intensity than NaCl. Figure 4 shows the total volatiles obtained from the Maillard system in the presence of different electrolytes. The total quantity of volatiles increased with the addition of electrolytes. The samples with NaCl gave the highest quantity of volatiles, followed by $CaCl_2$, and $FeCl_2$. However, the increase in production of volatiles may not necessarily correspond to the formation of desirable flavors.

The chemical inhibition or promotion mechanism of these electrolytes on the Maillard browning reaction is not well understood. In the case of microwave irradiation, the dielectric properties of the electrolyte solutions, such as size and charge of the ions may cause an overall increase in the degree of browning and generation of volatiles.

Water Content. Water plays an important role in a microwave food product. The movement of water molecules in an alternating electromagnetic field causes heat production. We have recently shown (*10*) that a threshold level of 10% moisture is required to cause browning during microwave irradiation of the Maillard model system (Figure 5). This observation suggests that a threshold level of moisture is required to serve as a medium for chemical reactions and for absorption of the microwave irradiation, but not cause inhibition of the forward Maillard reaction, especially Schiff base formation.

Conclusions

Flavors formed in a model system by microwave irradiation were shown to be chemically different from those formed by conventional heating. Microwaved foods

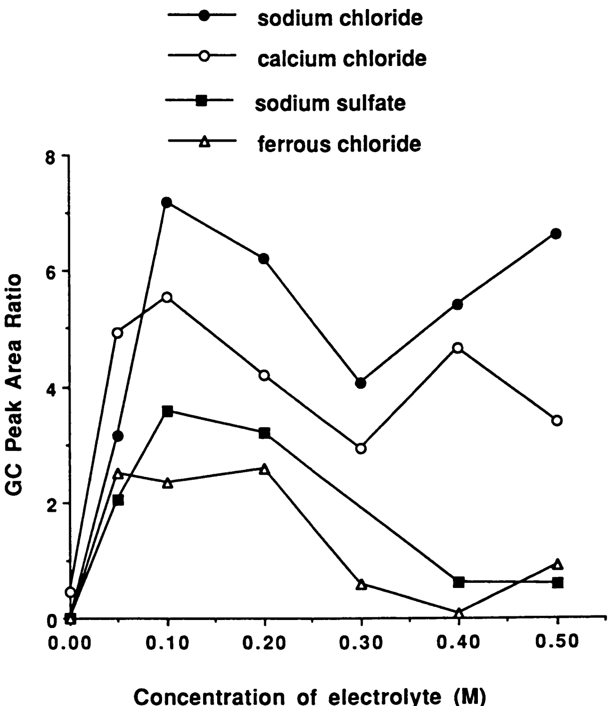

Figure 4. Effects of electrolytes on the total yield of volatiles in the L-cysteine/D-glucose model system upon microwave irradiation

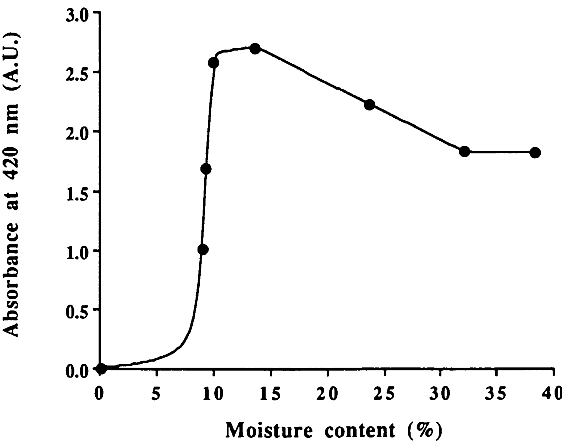

Figure 5. Degree of browning of the L-cysteine/D-glucose model system as a function of moisture content upon microwave irradiation.

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generally lack desirable flavors and browning which are found in conventional heating.

Various factors such as pH, electrolyte concentration, and moisture content were explored using the Maillard system as a model food system. The pH and electrolytes were found to affect browning and flavor production during microwave irradiation. Although some of these conditions are unrealistic for food systems, these examples may suggest applications such as food coatings to enhance browning and desirable flavors.

The enhancement of flavors and browning in the Maillard system does not necessary imply similar observations in real food systems. Foods are complex systems which include lipids in addition to sugar and amino acids. Further research is needed to study the chemistry of flavor chemicals between various precursors in foods prepared by microwave irradiation and to determine factors which enhance the flavor of microwaved foods. The ultimate factor determining consumer acceptance of a product is its sensory properties and more research is needed in this area.

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Chapter 40

Flavor Formation during Frying and Subsequent Losses during Storage and Microwave Reheating in Pancakes

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Based on aromagram data, the key aroma compounds contributing to pancake flavor were found to be the 2,4-decadienals (in batter and formed during frying), methionine (Maillard product), 2-acetyl pyrazine (Maillard product) and linalool (in batter). While a relatively large amount of furans and a pyranone were formed during frying, these components are considered to make little contribution to flavor due to their high sensory thresholds. The losses of individual flavor compounds during microwave reheating of fried pancakes were considerable ranging from 10 to 56%. The losses of flavor compounds (blueberry) added to batter are also considerable during frying (0-86%), frozen storage (10-30% during 7 weeks) and microwave reheating (1-17%). The losses during microwave reheating would likely have been greater if most of the highly volatile flavor components were not already lost by the time the pancakes were reheated.

The Maillard reaction is well known for its contribution to the flavor of a variety of foods during cooking or baking. Certain classes of compounds are generally known to be formed via the Maillard reaction. The specific flavor impact compounds vary with different types of food and the reactants available. A detailed review of nonenzymatic browning will not be presented as part of this chapter as it is covered in other chapters of the book.

The products investigated in this research were microwave pancakes. While there has been substantial research on flavor development in flour-based food systems during heating (e.g. cake *1*, sponge cake *2*, bread *3-6*, and crackers *7*), little information is available on the flavor of fried flour-based foods.

The first part of this study focussed on determining the flavor compounds present in uncooked pancake batter and which compounds formed during frying. The fate of volatile compounds contributing to pancake flavor was determined

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as a function of frozen storage and microwave reheating. The final part of this research examined the retention of a blueberry flavor added to the batter before cooking.

Experimental

Sample Preparation. Flavor compounds were determined in raw pancake batter (unfried), fried pancakes, frozen stored pancakes and blueberry flavored (model blueberry flavor) pancakes. The pancake batter was prepared from a commercial mix (flour, vegetable oil, eggs, sugar, buttermilk, salt and leavening) by simply combining the mix with equal weights of water and blending by hand until lump free. The batter was fried on a Teflon coated griddle (no additional fat) at 190C until golden brown in color. A sample of the fried pancakes was analyzed immediately for volatile compounds while the remainder of the pancakes were placed in Zip Lock bags and frozen (-20C) overnight prior to microwave reheating. The microwave reheated pancakes were also analyzed for volatiles.

The blueberry flavored pancakes were prepared by combining 600 g pancake mix, 600 g water and 20 mL blueberry model flavor solution. The blueberry model flavor consisted of ethyl-2-methyl butyrate, ethyl valerate, maltol, γ-decalactone, cis-3-hexenol, linalool, anethole and α-terpineol. This model system was made up in acetone with each compound at a concentration of 0.6 mg/mL. The compounds chosen are typical of what might be found in an artificial blueberry flavor. A 60 g sample of batter was saved for analysis and the remainder was fried at 200C. A portion of these pancakes was analyzed immediately for volatiles while the remainder were placed in Zip Lock bags and frozen (-20C). The frozen pancakes were analyzed weekly over a seven week period for volatiles and the remaining samples (after seven weeks frozen storage) were reheated in a microwave oven and again analyzed for volatiles.

Both flavored and unflavored pancakes were reheated in a home microwave oven (Litton II, 650 watts). The pancakes were reheated individually for 1.5 min to reach serving temperature.

Flavor Isolation. Flavor isolation was done by distillation/solvent extraction under vacuum to obtain qualitative and quantitative data on the unflavored pancakes. Losses of added blueberry flavor were quantified using direct solvent extraction.

Distillation/Solvent Extraction. For the raw batter, 200 g dry pancake mix was combined with 1300 g distilled water and mixed well. This dilute batter was added to a rotary evaporator and then distilled (47C water bath under 640 mm Hg vacuum) until 300 mL of distillate was collected. The distillate was extracted twice with 100 mL methylene chloride in a separatory funnel. The extract was dried with anhydrous magnesium sulfate, filtered and concentrated to a volume of approximately 100 uL under a stream of nitrogen.

The fried pancakes were treated similarly, except for quantitative

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch040

purposes an adjustment had to be made for moisture loss during the frying operation. Thus 400 g pancake batter (1:1 mix:water) was fried as noted earlier and the resultant pancakes were weighed and moisture loss determined. After cooling, the pancakes were blended well with 1100 g (plus the amount of water lost during frying) distilled water in a Waring blender. This mixture was distilled in the rotary evaporator as described for the pancake batter.

Quantitative data were obtained by adding known amounts of pure reference standards to the batter or pancakes. Thus recoveries as well as losses during concentration etc. could be accurately determined. All analyses were replicated four times and averaged.

Direct Solvent Extraction. Pancake batter was analyzed for added blueberry flavor compounds by slowly adding 35 mL acetone (containing ethyl hexanoate as an internal standard) to 15 g of the batter while mixing on a magnetic stirplate. The resultant mixture was then filtered and the filtrate frozen at -70C for 90 min to solidify the fat. The liquid portion was recovered by filtering, dried with anhydrous magnesium sulfate and filtered again. This extract was concentrated to approximately 1 mL under nitrogen and analyzed by gas chromatography.

The cooked pancakes were analyzed immediately after frying, frozen storage (over night) and microwave reheating. The water loss was calculated during cooking, storage and microwave reheating. Samples were taken to represent 7.5 g dry pancake mix and water added to reach a total sample weight of 15 g. The amount of water added was dependent on the water lost during heating and storage. The sample was mixed with 35 g acetone solution containing internal standard and prepared as described for the batter. The samples were analyzed by GC and the percent retention relative to the initial batter were calculated.

Flavor Analysis. Flavor isolates were analyzed by gas chromatography (GC), mass spectrometry (MS) and odor analysis (aromagram).

Gas Chromatography/Mass Spectrometry. Flavor isolates were analyzed (1 uL) by GC/MS using a Hewlett Packard model 5890 GC coupled to a model 5970 Mass Selective Detector. A 30 m x 0.32 mm i.d. (1 um phase thickness) DB-5 column (5% phenyl substituted methyl silicone, from J&W Scientific, Folsom, CA) was used. The GC temperature program was started at 40C (0.5 min post injection hold) and then programmed at 3C/min to a final temperature of 170C (final time 10 min).

The compounds were tentatively identified by comparison to either our in-house or National Bureau of Standards libraries. Identity was confirmed by co-chromatography with reference compounds.

Aromagram Analysis. Following identification of the volatile compounds in the pancakes, the extracts were used to produce aromagrams. The GC effluent was sniffed directly from the end of the capillary column (passed

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch040

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch040

Table I. Compounds Identified In Pancake Batter and/or Fried Pancakes

Compound	*Batter*	*Fried*	*Sensory Properties*
1,2-Propanediol[a]	X	X	Sweet
1-Pentanol	X	X	Faint stale
Hexanal	X	X	Grassy, green
Furfural[b]		X	Bready
Furfuryl Alcohol[b]		X	Candy
Hexanol	X	X	Faint sweet
2H-Pyran-2-one[a,b]		X	------[c]
Methional[b]		X	Potato
2-Acetyl Pyrazine[a,b]		X	Popcorn
2-Acetyl Furan[b]		X	Coffee
t-2-Heptenal	X	X	Oily
5-Methyl Furfural[b]		X	------
Benzaldehyde	X	X	Cherry
Heptanol	X	X	Mushroom
1-Octen-3-ol	X	X	Mushroom, fishy
1,3,7-Octatriene	X	X	------
t-2-Octenal	X	X	Metallic, sour
2-Octen-1-ol	X	X	------
2,3-Pentanediol[a]	X	X	------
6-Me-5-Methylene Heptanone[a]	X	X	Cucumber
Linalool	X	X	Floral
2-Undecene[a]	X	X	------
2-Undecenal[a]	X	X	Fatty
2,4-Decadienal (Isomer)	X	X	Fatty, tallowy
2,4-Decadienal (Isomer)	X	X	Fatty, tallowy

[a] Compound identity was not confirmed by co-chromatography with a pure reference compound.
[b] Compounds found only in the fried pancakes.
[c] No odor associated with this peak at concentration found in product.

through a heated exit port) and the character of each eluting compound was recorded. Successive dilutions were made to determine the compounds having the greatest flavor and aroma impact (*8,9*).

Results and Discussion

Fried Pancakes vs. Batter. The volatile compounds identified in pancake batter and fried pancakes as well as their sensory properties are listed in Table I. Eighteen of the twenty five volatile compounds identified in the fried pancakes

were originally present in the raw pancake mix. The majority of the volatiles in raw pancake mix are known to be products of lipid oxidation e.g. 1-pentanol, hexanal, hexanol, 1-octen-3-ol, t-2-heptenal, heptanol, 1,3,7-octatriene, t-2-octenal, 2-undecene, 2-undecenal and the 2,4-decadienals (*10-13*). While lipid oxidation products may be enhanced by heating, it is obvious that many are present prior to any heating of the product. Compounds such as benzaldehyde and linalool probably were present as natural components of the flour.

Frying resulted in the formation of seven compounds which are assumed to be produced via the Maillard reaction. The majority of these are oxygenated heterocyclic compounds associated with sugar degradation under mild heating. These furans and pyranones have been found in other flour-based foods (e.g. bread, *10*). Methional and 2-acetyl pyrazine likely arose from the Strecker degradation and condensation of Maillard products, respectively.

Quantitative changes in some of these volatiles are presented in Table II. It should be noted that the quantitative data on fried pancakes represents the amounts of these compounds in the batter plus any additional amounts formed during frying less any losses that may have occurred via volatilization.

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Table II. Concentration of Flavor Compounds in Batter and Fried Pancakes

Compound	*Batter*	*Fried*	*Change (%)*
	----------ppb----------		
1-Pentanol	454	109	76
Hexanal	689	310	55
Furfural	0	5,844	--
Furfuryl Alcohol	0	5,824	--
Hexanol	32	16	49
2-Acetyl Furan	0	495	--
t-2-Heptenal	61	27	56
5-Methyl Furfural	0	514	--
Heptanol	6	4	28
1-Octen-3-ol	64	16	75
Linalool	94	59	27
2,4-Decadienal (Total)	12,249	11,147	9

For compounds such as 1-pentanol and 1-octen-3-ol, approximately 75% of the amount present in the batter was lost during frying. The 2,4-decadienals changed little in concentration during frying since they are formed during heating which would offset losses due to volatilization. The significance of the Maillard reaction is obvious in the case of the furans. Furfural and furfuryl alcohol went from undetected in the batter to be major volatiles in the fried pancakes.

Frying and Microwaving Pancakes. The data presented in Table III give an overall comparison of flavor loss in pancakes due to frying and their subsequent reheating from the frozen state in a microwave oven. More compounds are included in this Table than in Table I since these data are based on normalized GC peak areas rather than absolute quantities (i.e. pure standards were not required to obtain these data). Losses for compounds which were not present in the raw batter but formed during frying are based on the amount in the fried pancakes.

The main observation that can be made from these data is that there is significant loss of flavor compounds not only during frying but also during the brief microwave reheating. The aroma profile that is obtained in fried pancakes represents the true character of pancakes - the combination of flavor losses and formation during frying yields an aroma that is pancake. The changes induced by microwave reheating may result in an imbalanced aroma profile and less than ideal product. Flavor losses from only microwaving ranged from 10 to 56%.

Aromagram Results. Aromagrams were generated to determine which volatile compounds had the most significant impact on the aroma/flavor of pancakes. At the highest aroma extract dilution, five compounds could still be detected by effluent sniffing. These were two isomers of 2,4-decadienal, methional, 2-acetyl pyrazine and linalool. As noted earlier, the decadienals are from the thermal degradation of fat while methional (a Strecker aldehyde) and 2-acetyl pyrazine are from the Maillard reaction. The source of the linalool is not known with certainty but likely comes from the flour. Based on aromagram theory, these data indicate that the compounds most important for the flavor of pancakes are a combination of those from thermal fat degradation and the Maillard reaction.

The group of compounds which were evident in the aromagram in the next to final dilution were phenyl ethyl alcohol (based on its retention time and odor character), 2-undecenal, t-2-octenal and two oily/fatty compounds which were not identified. The phenyl ethyl alcohol is likely formed from another Strecker aldehyde, phenyl acetaldehyde, and the other compounds are from fat degradation, reinforcing the two significant contributors to the flavor of pancakes.

Blueberry Flavored Pancakes. Blueberry is the most common flavor added to pancakes. This flavor must be added prior to frying of the batter, and thus must withstand the frying and microwave reheating processes. The last section of this research investigated the losses of model blueberry flavor compounds added to

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch040

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch040

Table III. Changes in Concentration of Flavor Compounds Due to Frying and Reheating

Compound	*Batter*	*Cooked*	*Microwaved*
	--------% change-----------------		
1,2-Propanediol	100	64	NQ[a]
1-Pentanol	100	76	91
Hexanal	100	55	80
Furfural	ND[b]	100	70
Furfuryl Alcohol	ND	100	49
Hexanol	100	49	89
2H-Pyran-2-one	ND	100	NQ
2-Acetyl Furan	ND	100	68
t-2-Heptenal	100	56	84
5-Methyl Furfural	ND	100	80
Benzaldehyde	100	19	NQ
Heptanol	100	28	72
1-Octen-3-ol	100	75	85
1,3,7-Octatriene	100	28	NQ
t-2-Octenal	100	48	78
2-Octen-1-ol	100	28	84
2,3-Pentanediol	100	30	NQ
6-Me-5-Methylene Heptanone	100	26	NQ
Linalool	100	37	49
2-Undecene	100	13	NQ
2-Undecenal	100	43	NQ
2,4-Decadienal	100	9	47

[a] NQ - Not quantified in microwaved product.
[b] ND - Not detected in this product.

pancakes in order to gain some insight into the loss of flavor compounds characteristic of blueberry. Since it is unlikely that the flavor compounds used in blueberry flavoring would be present in significant quantities in either the batter or produced during frying, it was felt that we could evaluate flavor losses due to volatilization during both frying and microwave reheating.

The losses of eight different compounds typically used in artificial blueberry flavor are presented in Table IV. The data show that there are substantial losses of these compounds during frying, storage and reheating of pancakes. It is interesting to note that for ethyl-2-methyl butyrate and ethyl valerate, 69% and 86% respectively, were lost during the original frying step. Of the small amount left in the pancakes, there were additional losses during frozen storage and microwaving, resulting in essentially complete loss of the compounds in the microwaved pancakes. Very little α-terpineol, maltol and anethole were lost during initial frying while ca. 25% was lost during extended frozen storage. This is expected considering that these types of products have minimal packaging. There were additional losses of these compounds during microwave reheating but these losses were minor.

Table IV. Loss of Added Blueberry Flavor compounds

		----------Frozen---------------		
Compound	Fried	1-Week	7-Weeks	Microwaved
	---------------% loss*---------------------------			
Et-2-Me-Butyrate	69	90	97	98
cis-3-Hexenol	51	65	74	91
Et Valerate	86	93	96	99
Linalool	21	26	49	65
α-Terpineol	2	3	28	32
Maltol	0	10	30	32
Anethole	8	16	27	38
γ-Decalactone	11	14	35	33

* Average values of four replicates.

The magnitude of losses during microwave reheating are reasonable considering previous research (*14-16*). Compounds such as α-terpineol, maltol, anethol and γ-decalactone are fat soluble and of low vapor pressure. There would not be significant losses of these compounds during microwave reheating. We would have expected greater losses of the very volatile compounds (ethyl butyrate, cis-3-hexenol and ethyl valerate) however, most of these volatiles were lost prior to microwaving and thus, their loss had already occurred.

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch040

Conclusions

The key aroma compounds contributing to pancake flavor were found to be both indigenous to the batter and formed during frying. The batter contributed fatty/fried notes (2,4-decadienals and other unsaturated aldehydes) and a floral note (linalool). Quantitatively, the major compounds formed during frying were furans and a pyranone. However, based on the aromagram data, these compounds made little or no contribution to pancake flavor. The most important flavor compounds formed during frying were Strecker aldehydes (methional and indirectly, phenyl acetaldehyde) and 2-acetyl pyrazine. While frying likely resulted in the formation of additional unsaturated aldehydes, we could not distinguish their formation from that which was initially present in the batter (loss during frying vs formation from heat).

Flavor losses during microwave reheating of fried pancakes were considerable and ranged from 10 to 56%. One would expect these losses to influence sensory perception of the product. The losses of flavor compounds (blueberry) added to batter are also considerable during frying (0-86%), frozen storage (10-30% during 7 weeks) and microwave reheating (1-17%). The losses during microwave reheating would likely have been greater if most of the highly volatile flavor components were not already lost by the time the pancakes were reheated.

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch040

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RECEIVED February 9, 1993

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ch040

Author Index

Acree, Terry E., 71
Ames, Jennifer M., 228
Apriyantono, Anton, 228
Arnoldi, Anna, 240
Artz, William E., 296
Ba, T. Huynh, 270
Bailey, Milton E., 315
Baltes, W., 192,263
Bolton, T. A., 270
Bouter, N., 142
Cheng, Chien-Wei, 315
Daun, Henryk, 334
de Kok, P. M. T., 158
de Roos, Kris B., 437
Dinnauer, M., 55
Eichner, K., 42,218
Eiserich, J., 55
Feather, Milton S., 127
Fichtali, J., 348
Forage, N. G., 449
Gerhardt, Klaus O., 315
Gi, U. S., 263
Graf, Ernst, 437
Gutheil, Richard A., 315
Hartman, Thomas G., 328
Hawkes, James G., 280
Heinzler, M., 218
Ho, Chi-Tang, 328,334,370
Holscher, Wilhardi, 206
Hsieh, Fu-Hung, 315
Izzo, Henry V., 328
Jennings, W., 55
Karwe, Mukund V., 334,370
Katz, Ira, 2
Kim, C. H., 380
Li, H.-C., 466
Liardon, R., 270
Lin, Lawrence J., 7
Lindstrom, Ted R., 405
Liu, M. B., 356,365
Ludwig, S. Peter, 26
Maga, J. A., 356,365,380
Mandeville, S., 449
Manley, Charles H., 16
McGorrin, Robert J., 414
Morello, Michael J., 95
Mottram, Donald S., 104,180
Nair, Malathy, 334
Parliment, Thomas H., 251,405
Pos, A., 142
Rao, Suresh K., 296
Reineccius, G. A., 270,466
Reutter, M., 42
Risch, S. J., 466
Roberts, Deborah D., 71
Rosing, E. A. E., 158
Schiffmann, Robert F., 386
Schnee, R., 218
Shi, Zulin, 334
Shibamoto, Takayuki, 55,457
Song, C., 192
Stahl, Howard D., 251
Stanford, Marlene A., 414
Steely, J. S., 80
Steinhart, Hans, 206
Tanaka, Shigeru, 370
Tjan, S. B., 142
Valois, P. J. de, 142
van de Voort, F. R. , 348
van Eijk, Tony, 395
Villota, Ricardo, 280
Vonk, H., 142
Weenen, H., 142
Whitfield, Frank B., 180
Wittmann, R., 42
Yaylayan, V. A., 348,449
Yeo, Helen, 457
Zeller, K. R., 80

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ix001

Affiliation Index

Colorado State University, 356,365,380
Commonwealth Scientific and Industrial Research Organisation, 180
Cornell University, 71
Darby & Darby P.C., 26
Dragoco Gerberding GmbH, 395
Food and Drug Administration, 7
Golden Valley Microwave Foods Inc., 466
Hershey Foods Corporation, 80
International Flavors and Fragrances, Inc., 2
J&W Scientific, 55
Jacobs–Suchard, 206
Kraft General Foods, 251,280,405,414
McGill University, 348,449
Nestle Research Center, 270
The Quaker Oats Company, 95
Quest International, 142
R.F. Schiffmann Associates, Inc., 386
Rutgers, The State University of New Jersey, 328,334,370
Takasago International Corporation (USA), 16
Tastemaker, 437
Technical University of Berlin, 192,263
Unilever Research Laboratory Vlaardingen, 158
Università di Milano, 240
Universität Münster, 42,218
University of California, Davis, 55,457
University of Hamburg, 206
University of Illinois, 280,296
University of Minnesota, 270,466
University of Missouri, 127,315
University of Reading, 104,180,228

Subject Index

A

Abhexone, role in roasted coffee aroma, 211,212*f*
Absolute novelty rule, description, 37
Advanced-phase Maillard reaction, flavor formation, 160
Alcohols, identification as aroma compounds in wheat bread, 200
Aldehydes, Strecker, role in roasted coffee aroma, 211
Aliphatic hydrocarbons, identification as aroma compounds in wheat bread, 200
Amadori compounds
  amino acid analytical detection, 44,45*f*
  decomposition, 42
  degradation, 130–134
  HPLC detection, 44,46*t*,47*f*
  Maillard reaction, indicators, 44
  role as aroma precursors, 224–226*f*
Amino acid analysis, Amadori compound detection, 44,45*f*
Amino acid effect on Maillard aroma generation during microwave irradiation
  experimental conditions, 451
  GC–MS analytical procedure, 450
Amino acid effect on Maillard aroma generation during microwave irradiation—*Continued*
  microwave vs. thermally produced flavors, 452–455*f*
  model systems, 450–452*t*
  previous studies, 449–450
  sample preparation, 450
  side chain vs. type of aroma produced, 451–452
Ammonium bicarbonate
  role in flavoring in extrusion, 287–288,290–291*t*
  use as flavor precursor in extruded food systems, 328–332
Aroma, thermal generation, 16
Aroma compounds, microwave volatilization, 405–412
Aroma compounds in wheat bread
  alcohols, 200
  aliphatic hydrocarbons, 200
  analytical methodology, 194–198
  bread preparation procedure, 194,195*f*
  carbonyl compounds, 200,202*f*
  dynamic headspace analytical procedure, 194,197*f*

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ix001

Affiliation Index

Colorado State University, 356,365,380
Commonwealth Scientific and Industrial Research Organisation, 180
Cornell University, 71
Darby & Darby P.C., 26
Dragoco Gerberding GmbH, 395
Food and Drug Administration, 7
Golden Valley Microwave Foods Inc., 466
Hershey Foods Corporation, 80
International Flavors and Fragrances, Inc., 2
J&W Scientific, 55
Jacobs–Suchard, 206
Kraft General Foods, 251,280,405,414
McGill University, 348,449
Nestle Research Center, 270
The Quaker Oats Company, 95
Quest International, 142
R.F. Schiffmann Associates, Inc., 386
Rutgers, The State University of New Jersey, 328,334,370
Takasago International Corporation (USA), 16
Tastemaker, 437
Technical University of Berlin, 192,263
Unilever Research Laboratory Vlaardingen, 158
Università di Milano, 240
Universität Münster, 42,218
University of California, Davis, 55,457
University of Hamburg, 206
University of Illinois, 280,296
University of Minnesota, 270,466
University of Missouri, 127,315
University of Reading, 104,180,228

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ix002

Subject Index

A

Abhexone, role in roasted coffee aroma, 211,212*f*
Absolute novelty rule, description, 37
Advanced-phase Maillard reaction, flavor formation, 160
Alcohols, identification as aroma compounds in wheat bread, 200
Aldehydes, Strecker, role in roasted coffee aroma, 211
Aliphatic hydrocarbons, identification as aroma compounds in wheat bread, 200
Amadori compounds
 amino acid analytical detection, 44,45*f*
 decomposition, 42
 degradation, 130–134
 HPLC detection, 44,46*t*,47*f*
 Maillard reaction, indicators, 44
 role as aroma precursors, 224–226*f*
Amino acid analysis, Amadori compound detection, 44,45*f*
Amino acid effect on Maillard aroma generation during microwave irradiation
 experimental conditions, 451
 GC–MS analytical procedure, 450
Amino acid effect on Maillard aroma generation during microwave irradiation—*Continued*
 microwave vs. thermally produced flavors, 452–455*f*
 model systems, 450–452*t*
 previous studies, 449–450
 sample preparation, 450
 side chain vs. type of aroma produced, 451–452
Ammonium bicarbonate
 role in flavoring in extrusion, 287–288,290–291*t*
 use as flavor precursor in extruded food systems, 328–332
Aroma, thermal generation, 16
Aroma compounds, microwave volatilization, 405–412
Aroma compounds in wheat bread
 alcohols, 200
 aliphatic hydrocarbons, 200
 analytical methodology, 194–198
 bread preparation procedure, 194,195*f*
 carbonyl compounds, 200,202*f*
 dynamic headspace analytical procedure, 194,197*f*

Aroma compounds in wheat bread—*Continued*
GC–MS procedure, 198
GC procedure, 194,198
identification, 198,200
nitrogen heterocycles, 200–202*f*
oxygen heterocycles, 200
static headspace analytical procedure, 194,196*f*
sulfur compounds, 201,203–205
yeast effect, 198,199*f*
Aroma development, microwave factors, 387–391
Aroma extraction–dilution analysis, 71
Aroma precursors, use of Amadori compounds, 224–226*f*
Aroma volatiles from extruded oat ready-to-eat cereal, 95–101
Aroma volatiles from meatlike Maillard systems
compounds, 181,182*f*
furanthiols, 181,183,184*f*
4-hydroxy-5-methyl-3(2*H*)-furanone–cysteine reaction, 183,185–187*f*
pH effect on formation, 185,187–189*t*
Aromagrams
application to Maillard model systems, 247–249*f*
description, 244
Asparagine, use to study pyrazine formation, 142
Autolyzed yeast extracts
description, 287
role in flavoring in extrusion, 287–291*t*

B

Blackstrap, description, 80–81
Bread production, steps, 192,193*f*

C

Carbohydrate degradation products, role in roasted coffee aroma, 213,214*f*
Carbonyl compounds, identification as aroma compounds in wheat bread, 200,202*f*
Carotene, role in roasted coffee aroma, 215,216*f*
Chain length effect on flavor retention during extrusion
compound extraction procedure, 381
experimental materials, 380
extrusion procedure, 381
extrusion temperature vs. compound retention, 382–384*f*
feed material preparation, 380–381
GC analytical procedure, 381
statistical design and analytical procedure, 381
CharmAnalysis, description, 71
CharmAnalysis of glucose–proline Maillard reaction products, *See* Gas chromatography– olfactometry of glucose–proline Maillard reaction products
Chemical compounds, rules of priority for invention, 34
Cocoa aroma
degree of roasting vs. aroma characteristics, 218
formation, 218
indicator compounds and precursors for formation, 219–226
volatile aroma compound formation, 218
Cocoa beans, Amadori compound detection, 51*t*,52*f*
Cocoa roasting, degree, evaluation using indicator compounds, 219–224
Cocoyam, 365–369
Coffee volatiles, studies, 206
Color, importance of improvements for microwave and extrusion cooking, 6
Color of whey protein concentrate–corn meal extruded product, influencing factors, 325,326*t*
Commercial preparation and use, process flavors, 16–24
Composition of bread aroma, studies, 192
Composition of matter, description, 30
Conception, description, 33
Convenience foods, consumer demand, 437
Conventional ovens, flavor in cysteine–glucose model system, 457–461
Cooked meat, flavor characteristics, 180
Cooking, good flavor effect, 240
Corn flour, collection and characterization of volatile compounds release at die during twin screw extrusion, 334–346

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ix002

Corn meal–whey protein concentrate extruded product, *See* Whey protein concentrate–corn meal extruded product
Cysteine, reaction with 2-hydroxy-5-methyl-3(2*H*)-furanone, 183,185–187*f*
Cysteine effect on 2-methyl-3-furanthiol formation in thiamin–cysteine model system
experimental procedure, 272,274
extraction procedure effect, 277
GC–MS analytical procedure, 274
heating effect, 276
influencing factors, 276
percent ^{34}S incorporation, 275*t*,276
pH effect, 277
solid composition of model system, 274*t*
temperature effect, 277
xylose effect, 276
Cysteine–glucose–lipid reactions
aromagrams, 247–249*f*
experimental procedure, 241
volatiles formed vs. lipid, 244–246*f*
Cysteine–glucose model system, flavor from microwave and conventional ovens, 457–464
Cysteine–ribose model systems, aroma volatile formation, 183

D

Dasheen, 355–369
Decompression pressure effect, supercritical CO_2 extraction of oat ready-to-eat cereals, 99,100*f*
Defatting, aroma effect, 240
Degree of cocoa roasting, evaluation using indicator compounds, 219–224
Delta T theory
description, 405,414–415,438
limitations, 438
1-Deoxy-2,3-dicarbonyl intermediates, formation, 131–134*f*
3-Deoxy-1,2-dicarbonyl intermediates, formation, 130–132*f*
Depth of heating in microwave
definition, 392
salt effect, 394
temperature effect, 392–394*t*
Developments in chemistry and food science, principles for protection, 26–38
Dicarbonyl sugar derivatives, role in Maillard reaction, 127–139
1,4-Dideoxy-2,3-dicarbonyl intermediates, formation, 131,134*f*
2,3-Dihydro-3-hydroxymaltol, evaluation of degree of cocoa roasting, 219–221*f*
2,3-Dihydro-1*H*-pyrrolizines
formation mechanism, 233–235*f*
pH effect on formation, 231–233*t*
Diligence, definition and description, 33–34
Dimeric furans, pH effect on formation, 237,238*t*
Dimethylpyrazines, evaluation of degree of cocoa roasting, 219,222*f*
Disulfides, formation from Maillard reaction, 116

E

Early-phase Maillard reaction, mechanistic aspects, 159–163
Eddo, 355–369
Efficiency, influencing factors, 62,64–67*f*
Encapsulation, use of extrusion, 292–293
Extraction pressure effect, supercritical CO_2 extraction of oat ready-to-eat cereals, 97–99
Extraction time effect, supercritical CO_2 extraction of oat ready-to-eat cereals, 97–99
Extruded food systems, ammonium bicarbonate and pyruvaldehyde as flavor precursors, 328–332
Extruded products
flavor, 315–316
flavoring processes, 328–329
lipid oxidation, 296–310
Extruded taro volatiles
extrusion conditions, 366
extrusion temperature vs. extrudate expansion ratio, 366,367*f*
formation pathways, 367
identification, 367–369*t*
taro composition, 366*t*
taro preparation, 365
volatile compound extraction and identification procedure, 366

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ix002

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ix002

Extruder, description, 315
Extrusion
advantages, 348,356
chain length and functional group effect on flavor retention, 380–384
food processing, 296
flavor and aroma effect, 280
flavor production via Maillard reaction, 348–349
glycoside as flavor precursor, 370–378
lipid oxidation, 297
volatile compound determination, importance, 334–335
Extrusion cooked mechanically deboned pork, flavor properties, 356–364
Extrusion cooking
advantages, 4,328,334
applications, 328,334
description, 4–5,315,328
disadvantages, 328
flavor development, 5
flavor losses, mechanism, 335
potential products, 356
relationship to Maillard browning and microwave cooking, 2
Extrusion of corn flour, twin screw, collection and characterization of volatile compounds released at die, 334–336
Extrusion processing, formation and degradation of tryptophan Amadori products, 348–354

F

Factors in microwave-generated aromas
heating, 391–394*t*
microwave interactions with food, 391
oven characteristics, 388*t*–391
Fats, good flavor effect, 240
Federal Food, Drug, and Cosmetic Act, regulatory requirements of food additives, 7
Ferrous acetate, inhibition of lipid oxidation, 309,310*f*
First molasses, description, 80
Flavor
conventional and microwave heating, comparison, 414
description, 395
Flavor—*Continued*
microwave and extrusion cooking, importance of improvements, 6
influencing factors, 395
interaction effect, 395–396
legal definition, 18
lipid effect, 240–249
preparation procedure effect, 396
Flavor and Extract Manufacturers' Association, purpose, 17
Flavor and manufacturing practices
art-to-science evolutionary path, 18–19
chef vs. grandma, 17–20*f*
establishment, 17
regulation of process flavors, 17
Flavor applied to snack food, 281
Flavor compounds
generation in foods by microwave heating, 398,400–401
microwave heating vs. properties, 396–399*t*
Flavor compounds formed during Maillard reaction
classification, 105–106
early stages of reaction, 106–108*f*
influencing factors, 118–119
late stages of reaction, 109
lipid interaction, 119–123
nitrogen-containing aroma compounds, 112–113,115*f*
oxygen-containing aroma compounds, 109,112
Strecker degradation, 109–111*f*
sulfur-containing aroma compounds, 113–118
Flavor formation and losses in pancakes
aromagram analytical procedure for flavor analysis, 468–469
direct solvent extraction procedure for flavor isolation, 468
distillation–solvent extraction procedure for flavor isolation, 467–468
experimental description, 466–467
frying and heating, comparison, 471,472*t*
GC–MS procedure for flavor analysis, 468
loss of added blueberry flavor compounds, 471,473*t*
sample preparation, 467
volatile(s) affecting flavor and aroma, 471
volatile compound concentration in batter and fried pancakes, 470*t*,471

Flavor formation and losses in pancakes—*Continued*
volatile compound identification in batter and fried pancakes, 469*t*,470
Flavor generation during extrusion cooking using twin screw extruders, interest, 370
Flavor in cysteine–glucose model system
electrolyte concentration effect in microwave-irradiated systems, 461–464*f*
experimental procedure, 458
pH effect in microwave-irradiated systems, 461–463*t*
volatiles generated in microwave and conventional ovens, 458–461
water content effect in microwave-irradiated systems, 463,464*f*
Flavor precursors, use of glycoside during extrusion, 370–378
Flavor precursors in extruded food systems
extrusion procedure, 329–330
precursor solution formulation, 329
pyrazine formation, 322
volatile analytical procedure, 330
volatile identification, 330–332
volatile isolation procedure, 330
Flavor properties of extrusion cooked mechanically deboned pork
analytical methods, 360
compression screws, 358
drying effect, 361,363*f*
experimental materials, 357–358
experimental objectives, 356
extrudate hardness vs. rice type and amount and drying, 360,362*f*
extrusion cooking procedure, 358
extrusion die configurations, 358
fat content effect, 361,363*f*
ingredient(s), proximate composition, 360*t*
ingredient mixing order, 358
marinade type effect, 361,364*f*
postextrusion treatment procedure, 358–359
sensory binding vs. rice type and amount and screw speed, 360–362*f*
sensory evaluation procedure, 359*t*
spice level effect, 361,363*f*
Flavor retention during extrusion, chain length and functional group effect, 380–384
Flavor volatilization in microwave food model systems
dielectric measurement procedure, 418
experimental materials and objectives, 415
GC profiles for multiple-component solutions, 429–433*f*
GC profiles for single-component solutions, 421,423–424*f*
headspace GC procedure, 417–418
heat treatment procedures, 415,416*f*,417
partitioning between solution and vapor phases, 428–429
physical and chemical properties of flavorants, 418,419*t*
postheating flavor retention
single-component solutions, 421,422*t*
vs. concentration, 425,426*f*
previous studies, 414–415
solution preparation, 415
time–temperature profiles, 418,420*f*,421
volatile loss
single-component solutions vs. delta T values, 425*t*,427*f*
vs. hydrophobicity for multiple-component system, 429,434*f*,435
vs. hydrophobicity for single-component system, 425,426*f*
Flavorants, stability problem, 380
Flavoring(s) for microwave food products, 401–403
Flavoring in extrusion
classifications, 281–282
encapsulation, 292–293
experimental design, 284
flavor generation, advantages over flavor addition, 282
future work, 293–294
generation, 285–287
hydrolyzed vegetable protein effect on loss, 282–283
influencing factors, 280–281
loss, 282–284
precursors, 287–288,290–291*t*
pyrazine effect on generation, 285–287
retention of preextrusion-added flavor compounds, 289,292*t*
short residence time effect on loss, 282
stability of lipids, 288–289
steam distillation effect on loss, 283
sugar concentration effect on loss, 283–284

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ix002

Flavoring in extrusion—*Continued*
temperature effect on loss, 282
volatile loss predication models, 284
Food additive, definitions, 8,19
Food ingredient, regulatory requirements, 7
Food processing, advantages of extrusion, 296
Food selection and purchase, reasons, 3
Formation pathways for primary roasted coffee aroma compounds
abhexone, 211
breakdown products of lipid oxidation, 207
carotene, 215,216*f*
carbohydrate degradation products, 213,214*f*
character impact compounds, 207–209*t*
experimental procedure, 207
2-furanmethanethiol, 213,214*f*
methanethiol, 211
methional, 211
2-methyl-3-furanthiol, 215,216*f*
2-methylindole, 215
phenols, 215*f*
prenyl alcohol derivatives, 213,214*f*
pyrazines, 207,209,210*f*,212*f*
sotolone, 211,212*f*
Strecker aldehydes, 211
volatile acids, 211
Fructose–alanine, role as aroma precursor, 224–226*f*
Frying, flavor formation in pancakes, 466–471
Functional group effect on flavor retention during extrusion
compound extraction procedure, 381
experimental materials, 380
extrusion procedure, 381
extrusion temperature vs. extrudate expansion, 381,382*f*
feed material preparation, 380–381
GC analytical procedure, 381
statistical design and analytical procedure, 381
Furan(s)
dimeric, pH effect on formation, 237,238*t*
monocyclic, pH effect on formation, 234,236*t*,237
2-Furanmethanethiol, role in roasted coffee aroma, 213,214*f*
Furanthiols, formation from Maillard reactions, 116,181,183,184*f*

G

Gas chromatographic analysis of Maillard reaction products from microwave heating
efficiency, 62,64–67*f*
experiment materials, 56
instrumentation, 56–57
retention, 60,62,63*f*
sample preparation, 56
selectivity, 57–61
specific detection systems, 64,68–69*f*
Gas chromatographic–mass spectrometric–sniffing, identification of coffee aroma compounds, 207
Gas chromatography, Amadori compound detection, 48–52
Gas chromatography–olfactometry of glucose–proline Maillard reaction products
capillary column effect, 73,76*f*
capillary GC–MS procedure, 73
chemicals, 72
GC–olfactometry procedure, 72
odor-active compounds, 73–77*f*
odor vs. amount, 75,77*f*
sample preparation, 72
temperature effect, 75,76*f*
Generally recognized as safe concept
determination, 21
development, 19
threshold levels for components, 19–21
Generally recognized as safe substances
categories, 10
concept development, 9–11
legal definitions, 8
regulatory requirements, 7
Glucose
conversion in early phase of Maillard reaction, 159–163
reactivity
Gly-X dipeptides, 170,172,173*f*
glycine homopolymers, 168–171,173
Met-X dipeptides, 172–174
Pro-X dipeptides, 174,175*f*
Glucose–amino acid–lipid reactions
aromagrams, 244,247–249*f*

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ix002

Glucose–amino acid–lipid reactions—*Continued*
experimental procedure, 241
volatiles formed vs. lipid
cysteine systems, 244–246*f*
lysine systems, 241–244
Glucose–amino acid reactions
aromagrams, 247–249*f*
experimental procedure, 241
Glucose–cysteine model system, flavor from microwave and conventional ovens, 457–464
Glucose–histidine reaction, pyridoimidazole formation, 263–269
Glucose–proline Maillard reaction products, GC–olfactometry, 71–77
Glucose–proline model system, high-temperature short-time kinetics for Maillard product formation, 251–260
Gly-X dipeptides, reactivity with glucose, 170,172,173*f*
Glycine homopolymers, reactivity with glucose, 168–171,173
Glycoside as flavor precursor during extrusion
color measurement procedure, 373
extrusion conditions, evaluation, 372
experimental objective, 370
extrusion conditions, 371,372*t*
extrusion conditions vs. phenol concentration, 376,377*f*
glucoside concentration
vs. absorbance of methanol extract, 373,375*f*
vs. solvent, 376–378*f*
glucoside distribution vs. temperature and screw speed, 373,374*f*
HPLC analytical procedure, 373
phenol distribution, 373,375*f*
phenyl β-glucoside preparation, 371
residence time distribution determination, 371
Glycosidically bound volatile constituents to fruit and vegetable aromas, 370,371

H

Heated foods, Amadori compound detection, 42–52
Heating in microwave oven
depth of heating, 392–394*t*
flavor compound property effect, 396–399*t*
flavor generation in foods, 398,400–401
rapid heating, 391–393*f*
speed vs. aroma development, 388–390
uneven heating, 392*t*–394*t*
uniformity vs. aroma development, 390
Heterocyclic amines
isolation in processed foods, 12–14
safety evaluation, 24
High-pressure liquid chromatography (HPLC), Amadori compound detection, 44,46*t*,47*f*
High-temperature short-time kinetics for Maillard product formation in proline–glucose model system
activation energies, 257,259
analytical procedure, 253–254
compound formation, kinetic data, 254,258*t*
experimental description, 252–253
formation vs. residence time, 254–256*f*
glucose and proline remaining
after reaction, 257,258*t*
vs. residence time, 257,260*f*
vs. temperature, 257,259*t*
optical density vs. residence time, 257,259*t*
organoleptic evaluation procedure, 254
previous studies, 251–252
rate constants for glucose and proline vs. temperature, 257,259*t*
rate constants for products vs. temperature, 254,258*t*
rate of formation vs. temperature, 254,256*f*,257
reaction conditions, 253
structure of products, 254
total ion chromatogram vs. reaction time, 254,255*f*
Histidine–glucose reaction, pyridoimidazole formation, 263–269
Hydrolyzed vegetable proteins, safety, 24
Hydroperoxide decomposition, pathways, 298–299
4-Hydroxy-5-methyl-3(2*H*)-furanone, reaction with cysteine, 183,185–187*f*

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ix002

I

Imported molasses, description, 80
Improvement, description, 30
Indicator compounds and precursors for cocoa aroma formation
  Amadori compounds as aroma precursors, 224–226*f*
  cocoa roasting, evaluation of degree, 219–224
Inhibition, Maillard reaction, 135,137,139*f*
Interference practice, resolution of conflicting claims to inventorship, 32
Invention
  contents of disclosure, 34–35
  notebook records, adequate, 35–36
  rules of priority for new chemical compounds, 34
  steps, 32–34
Invention disclosure, contents, 34–35
Inventor, determination, 23–34

K

Kinetics for Maillard product formation in proline–glucose model system, *See* High-temperature short-time kinetics for Maillard product formation in proline–glucose model system

L

Labeling regulation, process flavors, 21–23*t*
Linoleate autoxidation, mechanism, 298
Lipid(s), flavor effect on reactions with glucose and amino acid, 240–249
Lipid–Maillard reaction interaction
  amino acid, ribose, and lipid containing systems, 120–122*f*
  products, 119–120
  volatiles in foods, 121–123
Lipid oxidation in extruded products
  experimental procedure, 302,304
  extrusion effects, 297
  extrusion temperature effect, 304–307*f*
  inhibition by ferrous acetate, 309,310*f*
  inhibition by transition metals, 301–302
  lipid stability vs. extrusion temperature, 309,310*f*
Lipid oxidation in extruded products—*Continued*
  mechanisms, 298–300
  metal catalysis, 301
  problem, 296–297
  screw configuration, 302,303*t*
  storage time effect, 304–307*f*
  temperature profiles for experiments, 302,303*t*
  transition metal content vs. extrusion temperature, 304,308*f*,309
Lysine–glucose–lipid reactions
  aromagrams, 247–249
  experimental procedure, 241
  volatiles formed vs. lipid, 241–244
Lysine–xylose model system, pH effect on volatile compound formation, 228–238

M

Machine, description, 29
Maillard browning
  relationship to microwave cooking and extrusion cooking, 2
  studies, 71
Maillard products, high-temperature short-time kinetics for formation in proline–glucose model system, 251–260
Maillard reaction
  Amadori compound(s)
    degradation, 130–134
    use as indicators, 44
  ascorbic acid, 131,135–138*f*
  description, 104,127–128,158
  dicarbonyl sugar derivatives, 127–139
  flavor and color development effect on extruded products, 315–316
  flavor compounds formed, 105–123
  flavor contribution, 466
  flavor formation in advanced phase, 160
  history, 104
  influencing factors, 228
  inhibition, 135,137,139*f*
  initial stages, 128,129*f*
  mechanistic aspects of early phase, 159–163
  pathways, 42,43*f*
  peptides, reactivity, 160,164–178
  pH effect on volatile compound formation, 228–238

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ix002

Maillard reaction—*Continued*
phosphate effect, 159
process, 316
significance for food, 104–105
stages, 105
Maillard reaction flavors
current regulatory status, 11–12
optional and precursor ingredients, 8
production, 7–8
regulations, 8–11
safety, 8,12–14
Maillard reaction products
chemical properties, 55
detection systems, 55–56
glucose–proline, GC–olfactometry, 71–77
importance of identification, 55
Maillard reaction products from microwave heating of model systems, GC analysis, 55–69
Maillard reaction volatile compounds of whey protein concentrate–corn meal extruded product, formation, 315–325
Maillard systems, meatlike, aroma volatiles, 180–189
Malondialdehyde, precursors, 299
Malts
Amadori compound detection, 48,50*f*
extrusion, 285–287
Manufacture, description, 29
Manufacturing guidelines, process flavors, 21,23*t*
Manufacturing practices, flavor, *See* Flavor and manufacturing practices
Meatlike Maillard systems, aroma volatiles, 180–189
Mechanically deboned meat
flavor properties, 357–364
importance, 356
production, 357
Mechanisms, pyrazine formation, 142–156
Met-X dipeptides, reactivity with glucose, 172–174
Metal catalysis, lipid oxidation in extruded products, 301
Methanethiol, role in roasted coffee aroma, 211
Methional, role in roasted coffee aroma, 211
Methyl linoleate autoxidation, mechanism, 298
Methyl linoleate thermal oxidation, products, 300
Methyl oleate oxidation, mechanism, 300
2-Methyl-3-furanthiol
cysteine, role in formation, 272–277
formation, 270
H_2S formation pathway, 272,273*f*
meatlike flavors, 270
roasted coffee aroma, 215,216*f*
thiamin degradation formation pathways, 270–272
3-Methylindole, role in roasted coffee aroma, 215
Methylpyrazines, evaluation of degree of cocoa roasting, 219,223*f*,224
Microwave cooking
color effect, 3–4
convenience, 3
flavor effect, 3–4
flavor problems, 437–438,457
oven standardization, 4
problems, 386–387
relationship to Maillard browning and extrusion cooking, 2
texture effect, 3–4
Microwave factors in aroma development
ambient conditions in oven, 390–391
microwave interactions with food, 391
oven characteristics, 388*t*–391
speed of heating, 388–390
uniformity of heating, 390
Microwave flavor release, prediction using nonequilibrium partition model, 437–447
Microwave-generated aromas, critical factors, 386–394
Microwave heating
compounding flavors using dielectric and heating properties, 405
flavor compound property effect, 396–399*t*
flavor generation in foods, 398,400–401
Microwave heating of model systems, GC analysis of Maillard reaction products, 55–69
Microwave-induced Maillard reactions, amino acid effect on aroma generation, 449–455
Microwave ovens
flavor in cysteine–glucose model system, 457–464

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ix002

Microwave ovens—*Continued*
history of specialized food development, 386
Microwave reheating, flavor loss in pancakes, 471,473*t*
Microwave volatilization of aroma compounds
dielectric constants
alcohols and ester, 407–409*t*
aldehydes and ketone, 407–409*t*
dielectric loss factors
alcohols and ester, 407,409*t*,410*f*
aldehydes and ketone, 407–409*t*
dielectric measurement procedure, 406
experimental objectives, 405–406
GC peak areas of headspace, 412*t*
half-power penetration depths, 409*t*
headspace analytical procedure, 407
heating of flavors in fat matrix, 406
heating of pure flavors, 406
heating rates
alcohols and ester, 409,410*f*
aldehydes and ketone, 409,411*f*
vs. reciprocal of half-power penetration depth, 409,411*f*,412
Microwaved food(s)
flavor and flavorings, 395–403,449
foods, market, 437
Microwaved food model systems, flavor volatilization, 414–435
Microwaved pancakes, flavor formation and loss, 466–473
Model systems, Amadori compound detection, 51,52*f*
Modifier concentration effect, supercritical CO_2 extraction of oat ready-to-eat cereals, 99,100*f*
Moisture, Maillard reaction effect, 118–119
Molasses, 80-81
Molasses flavor, using sulfur chemiluminescence detection
advantages and disadvantages, 92–93
components, 81
detection procedure, 84*t*,85
detector performance, 84
dynamic headspace chromatogram, 85,91*f*
dynamic headspace purge and trap and thermal desorption procedures, 85
instrumentation, 84
limit of detection, 92
Molasses flavor, using sulfur chemiluminescence detection—*Continued*
sample vs. sulfur compounds, 85,88–90*f*
sulfur compounds,
detection, 81–83,85–87*f*
identification, 89
sulfur peak identification, 85,92*t*
Monocyclic furans, pH effect on formation, 234,236*t*,237
Monosaccharide–proline reactions, 71

N

New Orleans molasses, description, 80
Nitrogen-containing aroma compounds from Maillard reaction, 112–113,115*f*
Nitrogen-containing reaction products, pH effect on formation, 232*t*,234
Nitrogen heterocycles, identification as aroma compounds in wheat bread, 200–202*f*
Nonequilibrium partition model for prediction of microwave flavor release
advantages, 438
applications, 447
fat level
vs. heating rate, 441,443*f*
vs. moisture loss during heating, 441,443*f*
flavor retention in cake batter, determination procedure, 439
fraction of flavor compound remaining vs. number of extractions, 441
mass balance, 440
mathematical extraction equilibrium, 440
microwave cake preparation procedure, 439
flavor retention in cake, observed vs. predicted, 444,445*f*
flavor retention in milk, observed vs. predicted, 440–442*t*
oil-to-water partition coefficient, 440
partition coefficient determination procedure, 438–439
temperature effect on partition coefficients, 441,442*t*
total fraction of flavor retained in food, 441,444
validity, 444,447
water-to-oil ratio
vs. flavor retention, 444,446*f*

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ix002

Nonequilibrium partition model for prediction of microwave flavor release—*Continued*
water-to-oil ratio—*Continued*
vs. retention of hydrophilic and hydrophobic compound, 444,447*f*
Notebook records, maintenance for determination of inventorship, 35–36

O

Olfactory–gas chromatography of glucose–proline Maillard reaction products, *See* Gas chromatogaphy–olfactometry of glucose–proline Maillard reaction products
Oxazoles, formation from Maillard reaction, 113
Oxazolines, formation from Maillard reaction, 113
Oxygen-containing aroma compounds from Maillard reaction, formation, 109,112
Oxygen heterocycles, identification as aroma compounds in wheat bread, 200

P

Pancakes, microwave, flavor formation and loss, 466–473
Partial pressure, definition, 428
Patent
definition, 28
harmonization of laws worldwide, 37–38
priority rules, 32
types, 28–29
Patent protection
avoiding loss, 36–37
benefits, 30
comparison to trade secret protection, 31*t*,32
composition of matter, 30
disadvantages, 28
improvement, 30
journal publication effect, 37
pH
Maillard reaction effect, 119
volatile formation effect in Maillard systems, 185,187–189*t*
pH effect on volatile compounds formed in xylose–lysine model system
2,3-dihydro-1*H*-pyrrolizines, 231–235
dimeric furans, 237,238*t*
experimental procedure, 229
monocyclic furans, 234,236*t*,237
nitrogen-containing reaction products, 232*t*,234
yields and amounts of compounds vs. pH control, 229–231
Phenols, role in roasted coffee aroma, 215*f*
Phenyl β-glucoside, use as flavor precursor during extrusion, 370–378
Phosphate catalysis, nature, 174,176–178
Polysulfur heterocyclics, formation from Maillard reaction, 114,116,117*f*
Precursors, role in flavoring in extrusion, 287–288,290–291*t*
Precursors in extruded food systems, *See* Flavor precursors in extruded food systems
Prediction of microwave flavor release
delta T theory, 438
nonequilibrium partition model, 438–447
Prenyl alcohol derivatives, role in roasted coffee aroma, 213,214*f*
Primary unsaturated fatty acid hydroperoxide thermal decomposition, 299–300
Prior-sanctioned substances
legal definition, 9
regulatory requirements, 7
Pro-X dipeptides, reactivity with glucose, 174,175*f*
Process, description, 29
Process flavors
current regulatory status, 11–12
description, 17
generally recognized as safe concept, 19–21
labeling regulation, 21–23*t*
manufacturing guidelines, 21,23*t*
manufacturing practices, 17–20*f*
market, 17
production, 7–8
reason for development, 22
regulations, 8–11
safety, 8,12–14
safety evaluation, 22,24
threshold levels for components, 19–21
Product, description, 29

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ix002

Proline–carbohydrate system, aroma production, 251
Proline–glucose Maillard reaction products, GC–olfactometry, 71–77
Proline–glucose model system, studies on Maillard product formation, 251–252
Protection for new developments in chemistry and food science
avoiding loss of intellectual property rights, 36–37
forms, 26–32
priority rules, 32–34
worldwide patent harmonization, 37–38
Publications in scientific journals, patentability effect, 37
Pyrazine(s)
flavor generation effect, 285–287
formation from Maillard reaction, 112
formation in extruded food systems with flavor precursors, 332
from C_2 and C_3 precursors, formation mechanism, 152,156
from [1-^{13}C]glucose and -fructose, formation mechanism, 144–150
from [2-^{13}C]glucose, formation mechanism, 144,151
roasted coffee aroma, 207,209–212*f*
Pyrazine formation mechanism
abbreviations, 143
carbohydrate cleavage mechanisms, 143
2,5-dimethylpyrazine vs. 2,6-dimethylpyrazine formation, 152,155
GC–MS procedure, 143–144
GC procedure, 143
NMR procedure, 143
pyrazines from C_2 and C_3precursors, 152,156
pyrazines from [1-^{13}C]glucose and fructose, 144–150
pyrazines from [2-^{13}C]glucose, 144,151
retroaldolization vs. other cleavage mechanisms, 144,152–154
Pyridoimidazoles
experimental procedure, 263–264
formation mechanism, 265,266*f*
formation reaction pathway, 265*f*,267,269
model system reactions, 263–264
MS, 265,267*f*
role of glucose, 267–269
Pyrroles, pyrrolidines, and pyrrolines, formation from Maillard reaction, 113,115*f*
Pyruvaldehyde, use as flavor precursor in extruded food systems, 328–332

R

Reactivity of peptides in Maillard reaction
analytical method, 167
experimental procedure, 165–166
glycine homopolymer reactivity with glucose, 168–171,173
phosphate catalysis, 174,176–178
reaction conditions, 166–167
reactivity of Gly-X dipeptides with glucose, 170,172,173*f*
reactivity of Met-X dipeptides with glucose, 172,173*f*,174
reactivity of Pro-X dipeptides with glucose, 174,175*f*
studies, 160,164–165
Ready-to-eat cereals
comparison of distillation–extraction and supercritical CO_2 extraction for aroma volatile isolation, 95–101
criteria for volatile isolation procedure selection, 95
sales, 95
Reduction to practice, description, 33
Regulations for processed flavors
definitions, 8–9,11
generally recognized as safe concept development, 9–11
Regulatory requirements, food ingredient, 7
Regulatory status of processed flavors, generally recognized as safe determination, 11–12
Relative retention, definition, 57
Resolution, definition, 57
Retention, influencing factors, 60,62,63*f*
Retention of preextrusion-added flavor compounds, role in flavoring in extrusion, 288,292*t*

S

Safety evaluation, process flavors, 22,24
Safety of processed flavors
heterocyclic amine isolation, 12–13
risk assessment, 13–14

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ix002

Second molasses, description, 80
Secrecy, description, 27
Selectivity, stationary phases, 57–61
Solute partition ratio, definition, 60
Sotolone, role in roasted coffee aroma, 211,212*f*
Specific detection systems, role in component identification, 64,68–69*f*
Stability of lipids, role in flavoring in extrusion, 288–289
Strecker aldehydes, role in roasted coffee aroma, 211
Strecker degradation
  flavor compound formation during Maillard reaction, 109–111*f*
  process, 316
Sulfides, formation from Maillard reaction, 116
Sulfur chemiluminescence detector
  evaluation, 83
  molasses flavor analysis, 84–93
  procedure, 82–83
Sulfur compound(s), identification as aroma compounds in wheat bread, 201,203–205
Sulfur compound detection, 81–82
Sulfur-containing aroma compounds from Maillard reaction, formation, 113–118
Supercritical carbon dioxide extraction of oat ready-to-eat cereals
  advantages, 95–96
  comparison to vacuum steam distillation solvent extraction, 99*t*,101
  decompression pressure effect, 99,100*f*
  extraction pressure effect, 97–99
  extraction time effect, 97–99
  modifier concentration effect, 99,100*f*
  procedure, 96–97

T

Taro, 365–369
Temperature, Maillard reaction effect, 118
Texture, importance of improvements for microwave and extrusion cooking, 6
Thermal generation of aroma, 16
Thermally induced Maillard reactions, amino acid effect on aroma generation, 452–455*f*
Thiamine–cysteine model system, role of cysteine in 2-methyl-3-furanthiol formation, 270–277
Thiamine degradation, aroma volatile formation, 183,184*f*
Thiazoles and thiazolines, formation from Maillard reaction, 114,115*f*
Thiophenes, formation from Maillard reaction, 116,118
Thiophenethiols, formation from Maillard reaction, 116
Third molasses, description, 80
Trade secret protection
  avoiding loss, 36
  benefits, 27–28
  comparison to patent protection, 31*t*,32
  definition, 26–27
  disadvantages, 28
Transition metals, inhibition of lipid oxidation, 301–302
Tryptophan Amadori products during extrusion processing
  decomposition, 351*t*,352
  experimental objectives, 349
  extrusion processing and optimization procedure, 349–350
  formation, 352*t*
  HPLC analytical procedure, 350
  Maillard reaction, 351
  processing conditions, 350
  separate optimization, 352–354
  simultaneous optimization, 354*t*
Twin screw extrusion of corn flour, collection and characterization of volatile compounds released at die, 334–346

V

Vacuum steam distillation–solvent extraction of oat ready-to-eat cereals
  advantages and disadvantages, 95
  comparison to supercritical CO_2 extraction, 99*t*,101
  decompression pressure effect, 99,100*f*
  extraction pressure effect, 97–99
  extraction time effect, 97–99
  modifier concentration effect, 99,100*f*
  procedure, 96

Publication Date: November 30, 1993 | doi: 10.1021/bk-1994-0543.ix002

Volatile acids, role in roasted coffee aroma, 211
Volatile compounds formed in xylose–lysine model system, pH effect, 228–238
Volatile compounds released at die during twin screw extrusion of corn flour
 analytical procedure for volatiles from extrudates, 336
 chemical classes of identified compounds, 336,338*t*–340
 collection and analytical procedure, 336,337*f*
 experimental objectives, 335
 extrusion procedure, 335–336
 GC–MS procedure, 336
 previous studies, 335
 qualitative differences, 340–346
 volatile profile of extrudate, 336,339*f*,340
 volatile profile of extruder condensate, 336,339*f*,340
Volatilization in microwave food model systems, flavor, *See* Flavor volatilization in microwave food model systems
Volatilization of aroma compounds, microwaves, *See* Microwave volatilization of aroma compounds

W

Water pumping, aroma development effect in microwave-generated food, 391
Wheat bread, aroma compounds, 192–205
Wheat bread aroma, complexity, 192
Whey protein concentrate–corn meal extruded product
 color measurement procedure, 317
 experimental description, 317
 extrusion procedure, 315
 factors affecting color, 325,326*t*
 food mixture preparation, 317
 Maillard reaction volatile compounds, formation, 323–325
 gas–liquid chromatographic–MS procedure, 318
 identification of volatile compounds, 318–322*t*
 previous studies, 316
 quantitative gas–liquid chromatographic procedure, 317–318
 regression coefficients between extrusion parameters and melting zone product temperature, 318,319*t*
 temperature effect, 319,323*t*
 whey protein concentrate vs. moisture content vs. temperature, 318,322*f*

X

Xylose–lysine model system, pH effect on volatile compound formation, 228–238

Y

Yeast, bread aroma effect, 198,199*f*

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