

ACS SYMPOSIUM SERIES 558

Lipids in Food Flavors

Chi-Tang Ho, EDITOR

Rutgers, The State University of New Jersey

Thomas G. Hartman, EDITOR

Rutgers, The State University of New Jersey

Developed from a symposium sponsored
by the Division of Agricultural and Food Chemistry
at the 205th National Meeting
of the American Chemical Society,
Denver, Colorado,
March 28–April 2, 1993



American Chemical Society, Washington, DC 1994

Lipids in food flavors



Library of Congress Cataloging-in-Publication Data

Lipids in food flavors / Chi-Tang Ho, editor, Thomas G. Hartman, editor.

p. cm.—(ACS symposium series, ISSN 0097-6156; 558)

“Developed from a symposium sponsored by the Division of Agricultural and Food Chemistry at the 205th National Meeting of the American Chemical Society, Denver, Colorado, March 28-April 2, 1993.”

Includes bibliographical references and indexes.

ISBN 0-8412-2922-8

1. Flavor—Congresses. 2. Lipids—Congresses.

I. Ho, Chi-Tang, 1944— . II. Hartman, Thomas G., 1958—
III. American Chemical Society. Division of Agricultural and Food Chemistry. IV. Series.

TP372.5.L57 1994
664'.5—dc20

94-13923
CIP

The paper used in this publication meets the minimum requirements of American National Standard for Information Sciences—Permanence of Paper for Printed Library Materials, ANSI Z39.48-1984.



Copyright © 1994

American Chemical Society

All Rights Reserved. The appearance of the code at the bottom of the first page of each chapter in this volume indicates the copyright owner's consent that reprographic copies of the chapter may be made for personal or internal use or for the personal or internal use of specific clients. This consent is given on the condition, however, that the copier pay the stated per-copy fee through the Copyright Clearance Center, Inc., 27 Congress Street, Salem, MA 01970, for copying beyond that permitted by Sections 107 or 108 of the U.S. Copyright Law. This consent does not extend to copying or transmission by any means—graphic or electronic—for any other purpose, such as for general distribution, for advertising or promotional purposes, for creating a new collective work, for resale, or for information storage and retrieval systems. The copying fee for each chapter is indicated in the code at the bottom of the first page of the chapter.

The citation of trade names and/or names of manufacturers in this publication is not to be construed as an endorsement or as approval by ACS of the commercial products or services referenced herein; nor should the mere reference herein to any drawing, specification, chemical process, or other data be regarded as a license or as a conveyance of any right or permission to the holder, reader, or any other person or corporation, to manufacture, reproduce, use, or sell any patented invention or copyrighted work that may in any way be related thereto. Registered names, trademarks, etc., used in this publication, even without specific indication thereof, are not to be considered unprotected by law.

PRINTED IN THE UNITED STATES OF AMERICA

American Chemical Society
Library

1155 16th St., N.W.

Washington, D.C. 20036

1994 Advisory Board

ACS Symposium Series

M. Joan Comstock, *Series Editor*

- | | |
|---|---|
| Robert J. Alaimo
Procter & Gamble Pharmaceuticals | Douglas R. Lloyd
The University of Texas at Austin |
| Mark Arnold
University of Iowa | Cynthia A. Maryanoff
R. W. Johnson Pharmaceutical
Research Institute |
| David Baker
University of Tennessee | Julius J. Menn
Western Cotton Research Laboratory,
U.S. Department of Agriculture |
| Arindam Bose
Pfizer Central Research | Roger A. Minear
University of Illinois
at Urbana–Champaign |
| Robert F. Brady, Jr.
Naval Research Laboratory | Vincent Pecoraro
University of Michigan |
| Margaret A. Cavanaugh
National Science Foundation | Marshall Phillips
Delmont Laboratories |
| Arthur B. Ellis
University of Wisconsin at Madison | George W. Roberts
North Carolina State University |
| Dennis W. Hess
Lehigh University | A. Truman Schwartz
Macalaster College |
| Hiroshi Ito
IBM Almaden Research Center | John R. Shapley
University of Illinois
at Urbana–Champaign |
| Madeleine M. Joullie
University of Pennsylvania | L. Somasundaram
DuPont |
| Lawrence P. Klemann
Nabisco Foods Group | Michael D. Taylor
Parke-Davis Pharmaceutical Research |
| Gretchen S. Kohl
Dow-Corning Corporation | Peter Willett
University of Sheffield (England) |
| Bonnie Lawlor
Institute for Scientific Information | |

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

FLAVOR, AN IMPORTANT ATTRIBUTE OF FOOD, is of major interest to food scientists and technologists. The public's perception of food taste and quality is dependent upon food flavor. Lipids, proteins, and carbohydrates are the major sources of flavor in foods. Of the three, lipids may play the most important role in flavor. Lipids are involved in the rancidity, oxidized flavors, and stale flavors of many lipid-containing foods and are also thought to be responsible for warmed-over flavor of meat. On the other hand, lipids are responsible for the desirable flavors of many fruits and vegetables, dairy products and deep-fat fried foods.

The past symposium on Lipids as a Source of Flavor (ACS Symposium Series No. 75) was held in 1977. Since then the mechanisms of lipid oxidation have been greatly upgraded, consequences of oxygen chemistry on food quality are better understood, the interaction of lipid oxidation and Maillard reaction has been discovered, and the analytical methodology for flavor research has advanced. The purpose of the symposium on which this book is based was to bring together scientific leaders in the field to present their latest findings on lipids in food flavors and to stimulate fellow scientists to participate in dialogues on current developments in enhancing food flavors.

We are indebted to the contributing authors for their worthy contributions. Without their dedication, expertise, and hard work, timely publication of this book would not have been possible.

CHI-TANG HO

Department of Food Science

Cook College

Rutgers, The State University of New Jersey

New Brunswick, NJ 08903

THOMAS G. HARTMAN

Center for Advanced Food Technology

Cook College

Rutgers, The State University of New Jersey

New Brunswick, NJ 08903

February 23, 1994

Chapter 1

Lipids in Food Flavors

An Overview

Chi-Tang Ho and Qinyun Chen

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

This article provides a brief introduction outlining the autoxidation, singlet oxygen oxidation and lipoxygenase mediated reaction of lipids. Important flavor compounds derived from lipid oxidation including aldehydes, ketones and furans are discussed. Recent studies on the interaction between the Maillard reaction and lipid degradation leading to the formation of desirable flavor compounds of foods are also discussed.

Lipids, proteins, and carbohydrates, the major structural components of living cells are also the major source of flavor in foods.

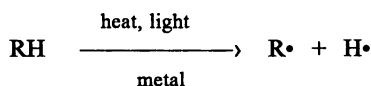
Generally, the negative qualities of food flavor are associated more closely with lipids than with proteins and carbohydrates. Lipids are responsible for rancidity in fats, oils and lipid-containing foods. On the other hand, lipids are also responsible for much of the desirable flavors of vegetables such as tomatoes, cucumbers, mushrooms and peas as well as many deep-fat fried foods such as French fried potatoes and fried chicken.

Formation of volatile flavor compounds from lipids is usually associated with either free radical-initiated or lipoxygenase-mediated oxidation.

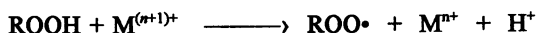
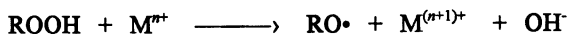
Free Radical Autoxidation of Lipids.

The reaction of unsaturated lipids with oxygen to form hydroperoxides is generally a free radical process involving three basic steps (1-2).

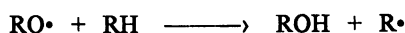
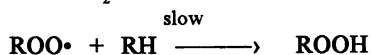
Initiation:



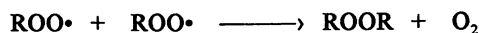
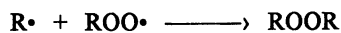
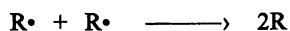
0097-6156/94/0558-0002\$08.00/0
© 1994 American Chemical Society



Propagation:



Termination:



RH, R•, RO•, ROO•, ROOH and M represent an unsaturated fatty acid or ester with H attached to the allylic carbon atom, alkyl radical, alkoxy radical, peroxy radical, hydroperoxide and transition metal, respectively.

The initiation reaction is the homolytic abstraction of hydrogen to form a carbon-centered alkyl radical in the presence of an initiator. Under normal oxygen pressure, the alkyl radical reacts rapidly with oxygen to form the peroxy radical which reacts with more unsaturated lipids to form hydroperoxide. The lipid-free radical thus formed can further react with oxygen to form a peroxy radical. Hence, the autoxidation is a free radical chain reaction. Because the rate of reaction between the alkyl radical and oxygen is fast, most of the free radicals are in the form of the peroxy radical. Consequently, the major termination takes place via the interaction between two peroxy radicals.

The rate of autoxidation increases with the degree of unsaturation. Linoleate is oxidized 10 times faster than oleate; linolenate 20-30 times faster (3).

Hydroperoxides of Fatty Acids or Their Esters

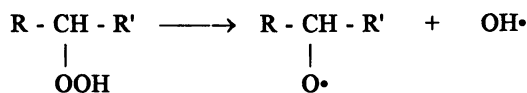
It is well-known that the free radical mechanism of hydroperoxy formation involves the abstraction of the hydrogen atom from the α -methylene group of a lipid molecule. This is favored due to the formation of a very stable allyl radical in which the electrons are delocalized over either three carbon atoms such as in the case of oleate, or five carbon atoms such as in the case of linoleate or linolenate. The mechanisms for the formation of isomeric hydroperoxides by autoxidation have been reviewed extensively (4-5)

For oleate, the hydrogen abstraction on C-8 and C-11 produces two allylic

radicals. These intermediates react with oxygen to produce a mixture of 8-, 9-, 10- and 11-allylic hydroperoxides. Autoxidation of linoleate involves hydrogen abstraction on the doubly reactive allylic C-11, with the formation of a pentadienyl radical. This intermediate radical reacts with oxygen to produce a mixture of conjugated 9- and 13-diene hydroperoxides. In the case of linolenate in which there are two separate 1,4-diene systems, hydrogen abstraction will take place on the two methylene groups, C-11 and C-14. These intermediate free radicals react with oxygen to form conjugated dienes with hydroperoxides on C-9 and C-13, or C-12 and C-16, with the third double bond remaining unaffected.

Decomposition of Hydroperoxides

Hydroperoxides of unsaturated fatty acids formed by autoxidation are very unstable and break down into a wide variety of volatile flavor compounds as well as nonvolatile products. It is widely accepted that (1) hydroperoxide decomposition involves homolytic cleavage of the -OOH group, giving rise to an alkoxy radical and a hydroxy radical.



The alkoxy radical undergoes β -scission on the C-C bond, with the formation of an aldehyde and alkyl or vinyl radical. A general reaction scheme with the formation of volatile aldehyde, alkene, alkane and alcohol is illustrated in Figure 1.

Aldehydes. Of the volatiles produced by the breakdown of the alkoxy radicals, aldehydes are the most significant flavor compounds. Aldehydes can be produced by scission of the lipid molecules on either side of the radical. The products formed by these scission reactions depend on the fatty acids present, the hydroperoxide isomers formed, and the stability of the decomposition products. Temperature, time of heating and degree of autoxidation are variables which affect thermal oxidation (6).

Some volatile aldehydes formed by autoxidation of unsaturated fatty acids are listed in Table I. The flavors of aldehydes are generally described as green, painty, metallic, beany and rancid, and are often responsible for the undesirable flavors in fats, oils and lipid-containing foods. Hexanal and 4-heptenal are found to be primarily responsible for the off-flavor in alligator meat (Chapter 13). Hexanal has long been used as an index of oxidative deterioration in foods (Chapters 8 and 18). Some aldehydes, particularly the unsaturated aldehydes, are very potent flavor compounds.

It should be pointed out that extremely low levels of many aldehydes

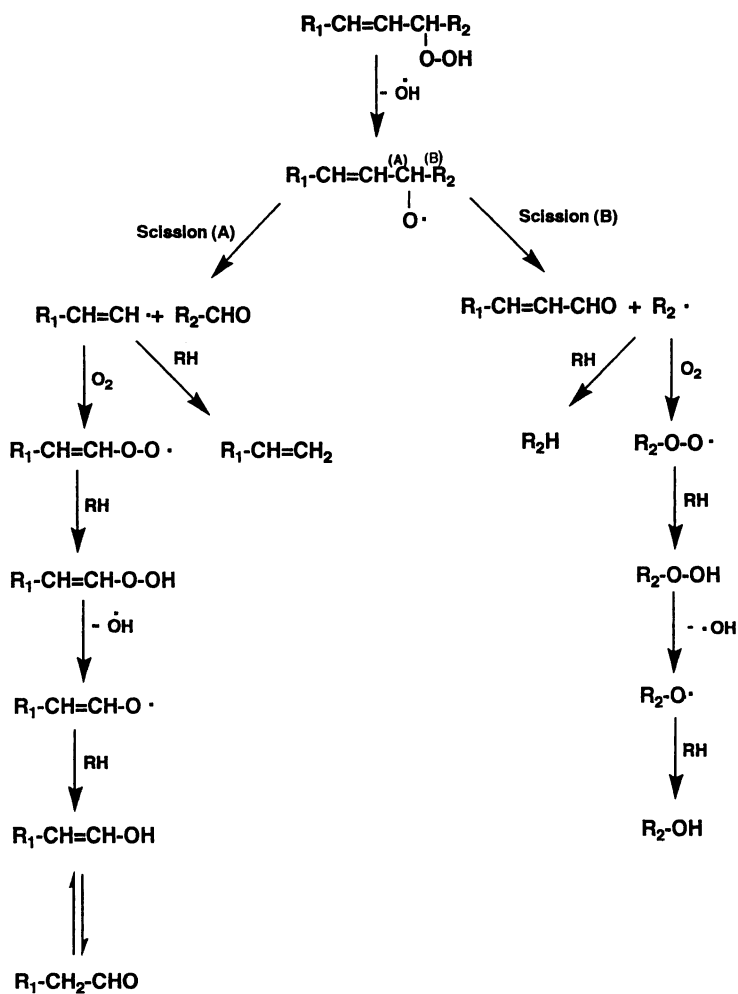


Figure 1. General reaction pathway for the homolytic cleavage of hydroperoxides of unsaturated fats.

Table I. Some Volatile Aldehydes Obtained from Autoxidation of Unsaturated Fatty Acids

Fatty acid	Monohydroperoxides	Aldehydes formed
Oleate	8-OOH	2-undecenal decanal
	9-OOH	2-decenal nonanal
	10-OOH	nonanal
Linoleate	11-OOH	octanal
	9-OOH	2,4-decadienal 3-nonenal
Linolenate	13-OOH	hexanal,
	9-OOH	2,4,7-decatrienal 3,6-nonadienal
	12-OOH	2,4-heptadienal 3-hexenal
Arachidonate	13-OOH	3-hexenal
	16-OOH	propanal
	8-OOH	2,4,7-tridecarienal 3,6-dodecadienal
	9-OOH	3,6-dodecadienal
	11-OOH	2,4-decadienal 3-nonenal
Eicosapentaenoate	12-OOH	3-nonenal
	15-OOH	hexanal
	5-OOH	2,4,7,10,13-hexadecapentaenal, 3,6,9,12-pentadecatetraenal
	8-OOH	2,4,7,10-tridecatetraenal 3,6,9-dodecatrienal
	9-OOH	3,6,9-dodecatrienal
	11-OOH	2,4,7-decatrienal 3,6-nonadienal
	12-OOH	3,6-nonadienal
	14-OOH	2,4-heptadienal 3-hexenal
	15-OOH	3-hexenal
18-OOH	propanal	

contribute to the desirable flavors of many foods. For example, the contributive role of aldehydes in poultry flavor has been well-recognized. Out of 193 compounds reported in the flavor of roasted chicken, 41 of them were lipid-derived aldehydes (7). When the aroma components of cooked chicken and cooked papain hydrolysates of chicken meat were qualitatively and quantitatively analyzed, 23 out of 66 compounds reported were lipid-derived aldehydes (8). Table II lists the quantitative data of selected aldehydes identified in these two studies. The most abundant aldehydes identified in chicken flavor were hexanal and 2,4-decadienal. In view of the much lower odor threshold of 2,4-decadienal (0.00007 mg/Kg) compared to hexanal (0.0045 mg/Kg) (9) the 2,4-decadienal should be the more important odorant for chicken flavor. Hexanal and 2,4-decadienal are the primary oxidation products of linoleic acid. The autoxidation of linoleic acid generates 9- and 13-hydroperoxides of linoleic acid. Cleavage of 13-hydroperoxide will lead to hexanal and the breakdown of 9-hydroperoxide will lead to 2,4-decadienal (10). Subsequent retro-aldol reaction of 2,4-decadienal will produce 2-octenal, hexanal and acetaldehyde (11). 2,4-Decadienal is known to be one of the most important flavor contributors to deep-fat fried foods (12). As shown in Table II, the enzymic hydrolysis of chicken with papain increased the concentration of 2,4-decadienal, as the aroma of cooked meat improved.

2,4-Decadienal can undergo further oxidation to produce *trans*-4,5-epoxy-*trans*-2-decenal. This compound was recently characterized as one of the most potent odorants of the crumb flavor of wheat bread and has a low odor threshold of approximately 1.5 pg/L (air) (13).

Ketones. Aliphatic ketones formed by autoxidation of lipids also contribute to the flavor of oils and food products. For example, Cadwallader et al. (Chapter 13) identified 1-octen-3-one as one of the odor-active compounds in the meat of wild alligators. This compound was described as metallic and mushroom-like. The reaction pathway for the formation of 1-octen-3-one from the arachidonate 12-hydroperoxide via the β -scission route is illustrated in Figure 2.

Furans. 2-Pentylfuran has been identified in many fats and oils and lipid-containing foods such as spray-dried dairy products (Chapter 7), mashed potatoes (Chapter 8) and alligator meat (Chapter 13). It is a well-known autoxidation product of linoleic acid and has been known as one of the compounds responsible for the reversion of soybean oil (14). Figure 3 shows the probable mechanism for its formation. The conjugated diene radical generated from the cleavage of the 9-hydroxy radical of linoleic acid may react with oxygen to produce vinyl hydroperoxide. The vinyl hydroperoxide will then undergo cyclization via the alkoxy radical to yield 2-pentylfuran (6).

Alcohols and Other Compounds. Cleavage of lipid hydroperoxides will also lead to alcohols, alkanes, alkenes and alkynes. The mechanism for the formation of 1-octen-3-ol which has a strong mushroom flavor is also shown in Figure 2.

Table II. Selected Aldehydes Identified in Chicken Flavor

Aldehyde	mg/Kg		
	Roasted chicken ^a	Cooked chicken meat ^b	Cook chicken meat ^b (papain treated)
butanal	0.133		
pentanal	0.319		
hexanal	1.804	25.6	17.2
heptanal	0.212	2.1	1.5
octanal	0.422	2.3	1.2
nonanal	0.467	1.7	1.3
decanal	0.052	0.3	0.3
undecanal	0.058		
dodecanal	0.022		
tridecanal	0.151		
tetradecanal	0.125	0.2	0.7
pentadecanal	0.383		
hexadecanal	19.788	1.4	9.8
heptadecanal	0.276	0.1	0.1
octadecanal	2.664		
<i>tr</i> -2-butenal	tr		
<i>cis</i> -2-pentenal	tr		
<i>tr</i> -2-pentenal	0.085	1.1	0.2
<i>cis</i> -2-hexenal	tr		
<i>tr</i> -2-hexenal	0.060	0.3	0.4
<i>tr</i> -2-heptenal	0.104	1.2	1.5
<i>cis</i> -2-octenal	0.004		
<i>tr</i> -2-octenal	0.195	3.7	2.0
<i>tr</i> -2-nonenal	0.084		
<i>cis</i> -2-decenal	0.003		
<i>tr</i> -2-decenal	0.139	1.0	1.2
<i>cis</i> -2-undecenal	0.002		
<i>tr</i> -2-undecenal	0.139	0.4	1.1
<i>tr</i> -2-dodecenal	0.002	0.3	0.1
<i>tr,cis</i> -2,4-nonadienal	tr		
<i>tr, tr</i> -2,4-nonadienal	tr	0.3	0.5
<i>tr,cis</i> -2,4-decadienal	0.051	1.0	2.7
<i>tr, tr</i> -2,4-decadienal	0.137	5.2	13.7
<i>tr, tr</i> -2,4-undecadienal	0.001	0.2	0.2

a. Noleau and Toulemonde, 1986.

b. Schroll et al., 1988.

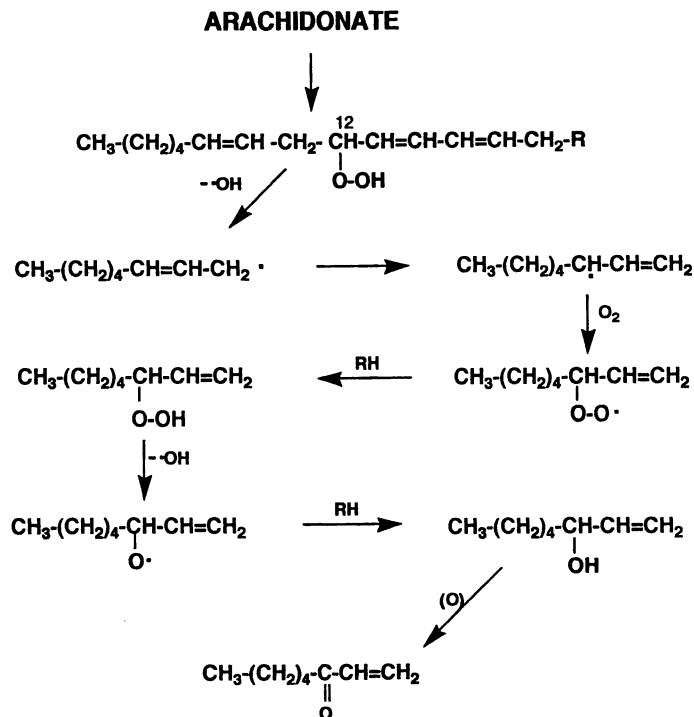


Figure 2. Mechanism for the formation of 1-octen-3-one from 12-hydroperoxide of arachidonate.

1-Octen-3-ol has been shown to be present in the volatiles of cooked pork in extremely high concentration (15-16). However, due to their relative high odor threshold, alcohols and hydrocarbons are generally not considered to be important contributors to the flavors of fats and oils and lipid-containing foods.

Singlet Oxygen Oxidation of Lipids.

Oxidation of lipid occurs in the presence of molecular oxygen in both the singlet and triplet states. Atmosphere oxygen that is in the triplet state contains two unpaired electrons, while oxygen in the singlet state has no unpaired electrons (17). The electron arrangement of triplet oxygen does not allow for a direct reaction of lipid molecules that exist in the singlet state. Singlet oxygen can be generated by the interaction of light, photosensitizers, and oxygen. Singlet oxygen

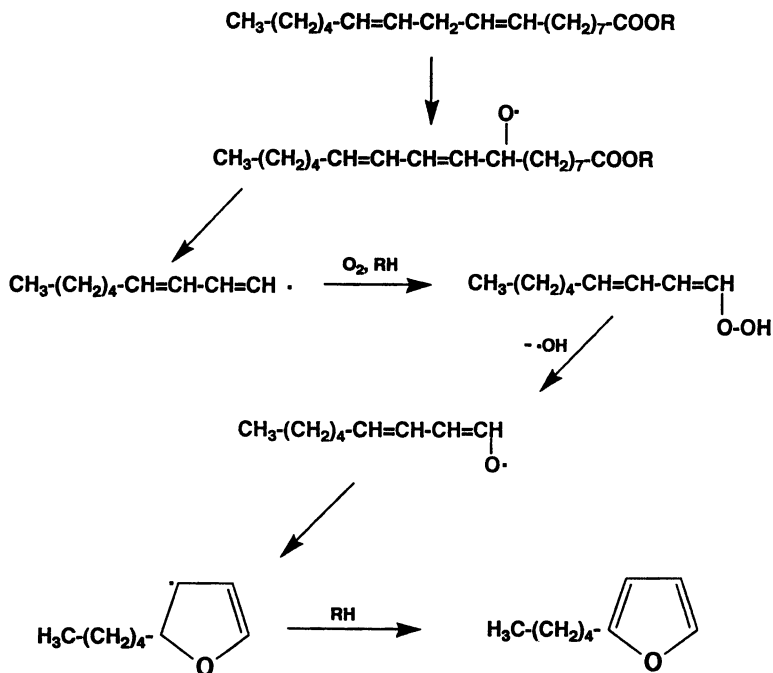


Figure 3. Mechanism for the formation of 2-pentylfuran.

has been suggested to be responsible for initiating lipid oxidation of food products due to its ability to directly react with the electron-rich double bonds of unsaturated fats (18). In fact, singlet oxygen reacts with linoleic acid at least 1450 times faster than triplet oxygen (19). The chemistry of singlet oxygen oxidation of foods is critically reviewed by Yang and Min in Chapter 2.

Formation of Flavor Compounds by the Lipoxygenase Pathway.

Many important flavor compounds in plants result from the enzymatic degradation of unsaturated fatty acids. The aerobic lipoxygenase cycle transforms fatty acids containing a *cis, cis*-1,4-pentadiene system into hydroperoxy-octadecadienoic acids by regio- and enantioselective reactions. Under oxygen deficient conditions, the cycle catalyzes the homolytic cleavage of the conjugated hydroperoxides to alkoxyl radicals, which are stabilized by further reaction such as oxidation, H-abstraction, β -cleavage and rearrangement to various products. The anaerobic cycle is related to the hemein catalyzed decomposition of conjugated

hydroperoxides. In plants, 9- and 13-hydroperoxyoctadecadienoates are further transformed into reactive intermediates by hydroperoxide lyases, hydroperoxide dehydrases, hydroperoxide epoxygenase and epoxide hydrolases (20). Recent investigations of the lipoxygenase pathway of plants have been extensively reviewed by Gardner (21). In this volume, Hsieh reviews the development of fresh and off-flavors in foods of plant and animal origins with lipoxygenase activity in Chapter 4.

Of particular interest is the recent study on the aromas of freshly-harvested fish (22). These aromas include both volatile alcohols and carbonyls which are derived from polyunsaturated fatty acids through lipoxygenase-mediated reactions. Figure 4 shows the proposed mechanism for the biogenesis of some fresh seafood aroma compounds from eicosapentaenoic acid (C20:5, n-3).

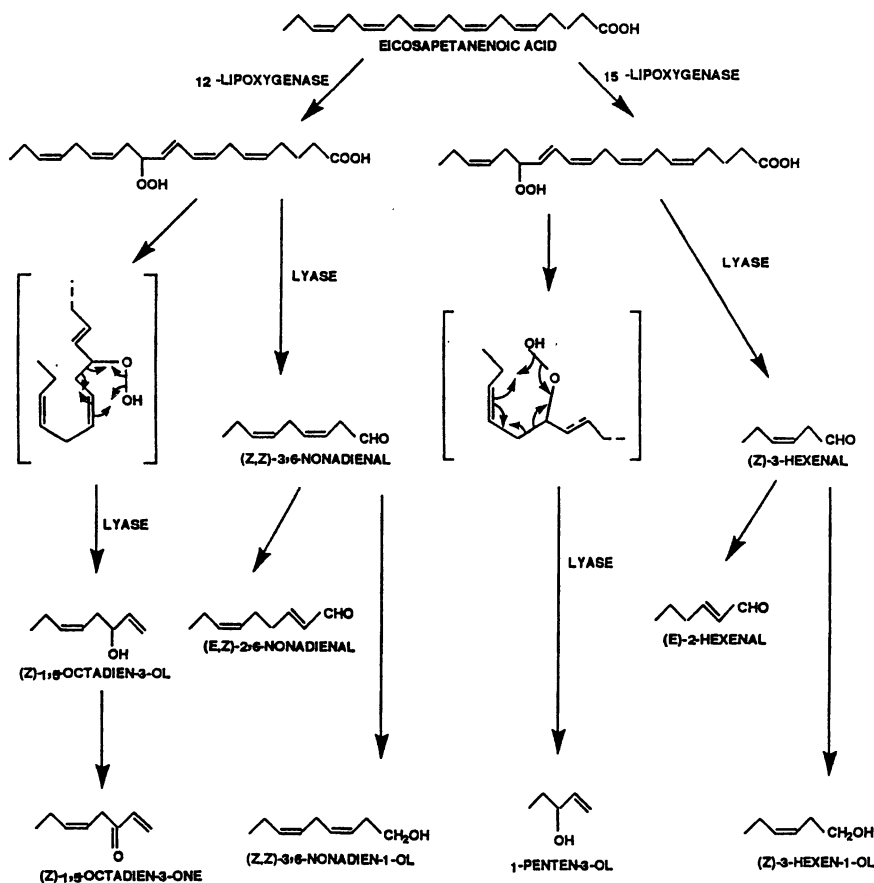


Figure 4. Proposed mechanism for the biogenesis of some fresh seafood aroma compounds from eicosapentaenoic acid (Reproduced with permission from ref. 22. Copyright 1986 American Chemical Society).

Volatiles from Interaction of Lipids and the Maillard Reaction.

Recent studies and interest in the volatile compounds formed from the interaction of the Maillard reaction and lipid degradation have resulted in an excellent review article by Whitfield (23). Studies with model systems have led to the identification of a number of volatile compounds that can be considered as products of Maillard/lipid interactions. The majority of these are heterocyclic compounds containing one or more atoms of nitrogen or sulfur and are characterized by the presence of a long-chain alkyl substituent with four or more carbon atoms. A reaction pathway has been proposed to account for the formation of many of these interaction products (23).

The identification of long-chain alkyl-substituted pyrazines in some cooked foods, particularly French-fried potatoes (24) and baked and extruded corn-based products (25-26) has prompted an investigation of the reaction of fatty aldehydes such as pentanal and hexanal, with 1-hydroxy-2-propanone (acetol) and ammonium acetate (27). Acetol is a possible hydrolysis product of the Maillard reaction. 2,5-Dimethyl-3-pentylpyrazine, 2,6-dimethyl-3-pentylpyrazine and 2,3,5-trimethyl-6-pentylpyrazine were formed in the reaction mixture with added pentanal; the corresponding hexylpyrazines were formed in the mixture containing added hexanal. A proposed mechanism for the formation of 2,5-dimethyl-3-pentylpyrazine is shown in Figure 5.

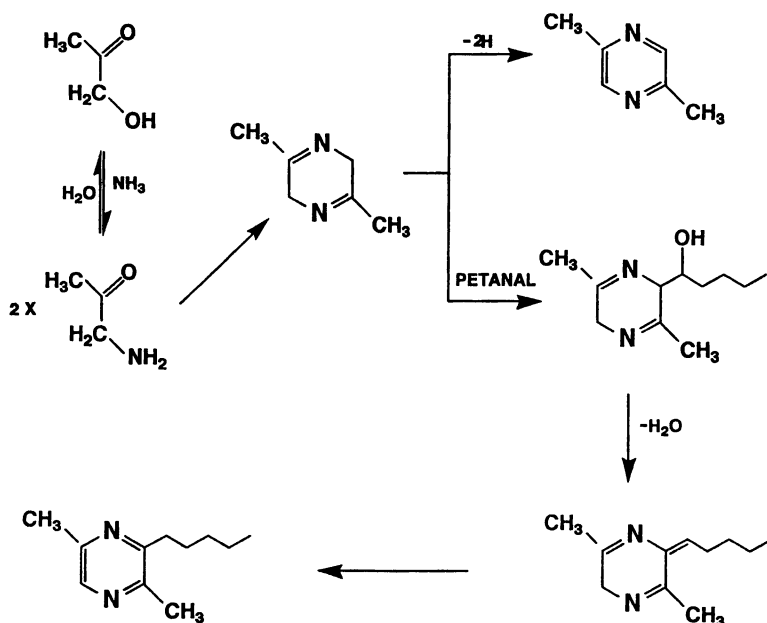


Figure 5. Proposed mechanism for the formation of 2,5-dimethyl-3-pentylpyrazine (Adapted from ref. 27. Copyright 1990 American Chemical Society)

Besides alkylpyrazines, other long-chain alkyl substituted heterocyclic compounds have been found in cooked foods. Of particular interest was the identification of 11 alkylthiazoles and 4 alkylloxazoles with long-chain alkyl substituents in the flavor of french fried potatoes and the fact that a number of these compounds were shown to possess important cooked food flavors (12).

Some recent data on the volatile flavor formation by lipid-mediated Maillard reactions involving a series of reducing sugars with selected amino acids, such as glycine, proline and cysteine, and peptide glutathione is discussed in Chapter 4.

The qualitative and quantitative effects that triglycerides and phospholipids have on the formation of volatile Maillard products have been extensively studied (28-32) and reviewed by Whitfield (23). In general, in model systems of reducing sugars and amino acids, many Maillard-type reaction products showed marked reductions in yields with the addition of lipids, particularly, in phospholipids.

Literature Cited

1. Chan, H. W.-S. In *Autoxidation of Unsaturated Lipids*; Chan, H. W.-S., Ed., Academic Press: London, UK, 1987, pp. 1-16.
2. Kochhar, S. P. In *Food Taints and Off-Flavours*; Saxby, M. J., Ed., Blackie Academic & Professional: London, UK, 1993, pp. 151-201.
3. Wong, D. W. S. *Mechanism and Theory in Food Chemistry*; Van Nostrand Reinhold: New York, NY, 1989, pp. 1-47.
4. Chan, H. W.-S.; Coxon, D. T. In *Autoxidation of Unsaturated Lipids*; Chan, H. W.-S., Ed., Academic Press: London, UK, 1987, pp. 17-50.
5. Frankel, E. N. In *Flavor Chemistry of Fats and Oils*; Min, D. B.; Smouse, T. H., Eds.; American Oil Chemists' Society: Champaign, IL, 1985, pp. 1-37.
6. Frenkel, E. N. *Prog. Lipid Res.* **1982**, *22*, 1-33.
7. Noleau, I.; Toulemonde, B. *Lebensm. -Wiss. u. -Technol.* **1986**, *19*, 122-125.
8. Schroll, W.; Nitz, S.; Drawert, F. Z. *Lebensm. Unters. Forsch.* **1988**, *187*, 558-560.
9. Van Germert, L. J.; Nettenbrijer, A. H. *Compilation of Odour Threshold Values in Air and Water*, National Institute for Water Supply: Voorburg, The Netherland, 1977.
10. Ho, C.-T.; Zhang, Y.; Shi, H.; Tang, J. *Food Rev. Int.* **1989**, *5*, 253-287.
11. Josephson, D. B.; Glinka, J. In *Thermal Generation of Aroma*; Parliment, T. H.; McGorin, R. J.; Ho, C.-T. Eds.; ACS Symp. Ser. No. 409, American Chemical Society: Washington D.C., 1989, pp. 242-246.
12. Ho, C.-T.; Carlin, J. T.; Huang, T. C.; Hwang, L. S.; Hau, L. B. In *Flavor Science and Technology*, Martens, M.; Dalen, G. A.; Russwurm Jr., H., Eds.; John Wiley & Sons: Chichester, 1987, pp. 35-42.
13. Schieberle, P.; Grosch, W. Z. *Lebensm. Unters. Forsch.* **1991**, *192*, 130-135.

14. Ho, C.-T.; Smagula, M. S.; Chang, S. S. *J. Amer. Oil Chem. Soc.* **1978**, *55*, 233-237.
15. Mottram, D. S. *J. Sci. Food Agric.* **1985**, *36*, 377-382.
16. Chou, C. C.; Wu, C. M. *The Volatile Compounds of Stewed Pork*. Research Report 285, Food Industry Research and Development Institute: Hsinchu, Taiwan, 1973.
17. Bradley, D. G.; Min, D. B. *Crit. Rev. Food Sci. Nutr.* **1992**, *31*, 211-236.
18. Korycka-Dahl, M. B.; Richardson, T. *Crit. Rev. Food Sci. Nutr.* **1978**, *10*, 209.
19. Rawls, H. R.; VanSanten, P. J. *J. Amer. Oil Chem. Soc.* **1970**, *47*, 121.
20. Tressl, R.; Albrecht, W.; Kersten, e.; Nittka, C.; Rewicki, D. In *progress in Flavour Precursor studies*, Schreier, P.; Winterhalter, P., Eds., Allured Publishing Corp.: Carol Stream, IL, 1993, pp. 1-28.
21. Gardner, H. W. *Biochi. Biophys. Acta.* **1991**, *1084*, 221-239.
22. Josephson, D. B.; Lindsay, R. C. In *Biogeneration of Aromas*, Prliment, T. H.; Croteau, R., Eds., ACS Sympoium Series N. 317, American Chemical Society: Washington, D. C., 1986, pp. 201-219
23. Whitfield, F. B. *Crit. Rev. Food Sci. Nutr.* **1992**, *31*, 1-58.
24. Carlin, J. T.; Jin, Q. Z.; Huang, T.-C.; Ho, C.-T.; Chang, S. S. *J. Agric. Food Chem.* **1986**, *34*, 621-623.
25. Huang, T. C.; Bruechert, L. J.; Hartman, T. G.; Rosen, R. T.; Ho, C.-T. *J. Agric. Food Chem.* **1987**, *35*, 985-990.
26. Bruechert, L. J.; Zhang, Y.; Huang, T. C.; Harman, T. G.; Rosen, R. T., Ho, C.-T. *J. Food Sci.* **1988**, *53*, 1444-1447.
27. Chiu, E.-M.; Kuo, M. C.; Bruechert, L. J.; Ho, C.-T. *J. Agric. Food Chem.* **1990**, *38*, 58-61.
28. Mottram, D. S.; Whitfield, F. B. In *Flavor Science and Technology*, Martens, M.; Dalen, G. A.; Russwurm Jr., H., Eds.; John Wiley & Sons: Chichester, 1987, pp. 29-34.
29. Whitfield, F. B.; Mottram, D. S.; Brock, S.; Puckey, D. J.; Salter, L. J. *J. Sci. Food Agric.* **1988**, *42*, 261-272.
30. Salter, L. J.; Mottram, D. S.; Whitfield, F. B. *J. Sci. Food Agric.* **1988**, *46*, 227-242.
31. Farmer, L. J.; Mottram, D. S.; Whitfield, F. B. *J. Sci. Food Agric.* **1989**, *49*, 347-368.
32. Farmer, L. J.; Mottram, D. S. *J. Sci. Food Agric.* **1990**, *53*, 505-525.

RECEIVED February 3, 1994

Chapter 2

Chemistry of Singlet Oxygen Oxidation of Foods

Wesley T. Yang and David B. Min

Department of Food Science and Technology, Ohio State University,
Columbus, OH 43210

The effects of β -apo-8'-carotenal, β -carotene, and canthaxanthin as well as α -, γ -, and δ -tocopherols on the chlorophyll photosensitized oxidation of soybean oil were studied by measuring peroxides, headspace oxygen, and conjugated diene content. As the number of conjugated double bonds of carotenoids increased, the antioxidant effects of carotenoids increased. α -Tocopherol showed the highest antioxidant effect, γ -tocopherol second, and then δ -tocopherol. Quenching mechanisms indicated that carotenoids and tocopherols quenched singlet oxygen to act as antioxidants in the chlorophyll photosensitized oxidation of soybean oil. The singlet oxygen quenching rate constants of β -apo-8'-carotenal, β -carotene, and canthaxanthin were 3.06×10^9 , 4.6×10^9 and $1.12 \times 10^{10} \text{ M}^{-1}\text{S}^{-1}$, respectively. The quenching rate constants of α -tocopherol were $2.7 \times 10^7 \text{ M}^{-1}\text{S}^{-1}$ by peroxide value and $2.6 \times 10^7 \text{ M}^{-1}\text{S}^{-1}$ by headspace oxygen.

The oxidation of lipids in food has been mainly responsible for the flavor stability, nutritional quality, and acceptability of lipid foods (1-15). Lipid oxidation is due to the combination of triple oxygen and singlet oxygen oxidations (3). Triplet oxygen lipid oxidation has been extensively studied to improve the oxidative stability of lipid foods during last 50 years (2, 4, 7, 13). However, it does not fully explain the initiation step of lipid oxidation (3, 6, 10). Recently, the role of singlet oxygen at the initiation stage of lipid oxidation was suggested because singlet oxygen can directly react with double bonds of fatty acids and its reaction rate with linoleic acid is at least 1,450 times higher than that of triplet oxygen (16).

Singlet oxygen can be formed by chemical, enzymatic, photochemical, and physical methods (6, 8, 10, 17, 18) and initiate the oxidation of lipids in foods. Photosensitized reaction, which is initiated by sensitizers, is the simplest and common pathway to generate a substantial amount of singlet oxygen in foods. This, in particular, has great impact on the oxidation of foods which contain sensitizers. Chlorophylls and their decomposition products in vegetable oils are known to be efficient photochemical sensitizers for singlet oxygen formation (19-27). Singlet oxygen directly reacts with unsaturated fatty acids of vegetable oils to form a mixture of conjugated and nonconjugated hydroperoxides (21, 24).

0097-6156/94/0558-0015\$08.00/0

© 1994 American Chemical Society

In Lipids in Food Flavors; Ho, C., et al.;

ACS Symposium Series; American Chemical Society: Washington, DC, 1994.

The decomposition of hydroperoxides produces off-flavor volatile compounds and potentially toxic oxidation compounds (28-38).

The undesirable singlet oxygen lipid oxidation can be minimized by preventing the formation of singlet oxygen and/or quenching singlet oxygen physically and chemically (23, 39). Physical quenching can be explained by energy and charge transfer mechanisms (39). Carotenoids quench the singlet oxygen through energy transfer from singlet oxygen to carotenoids with 9 or more conjugated double bonds. It is exothermic and these carotenoids are efficient singlet oxygen quenchers (40-47). The physical quenching of singlet oxygen by tocopherols is due to the charge transfer mechanism (43).

Chemical Properties of Triple Oxygen and Singlet Oxygen

Molecular oxygen which consists of two oxygen atoms has 5 bonding and 5 antibonding orbitals and 12 valence electrons (48). The electronic configuration of triplet state oxygen molecular orbitals is shown in Figure 1. Pauli exclusion principle states that no two electrons in an atom can have the same set of four quantum numbers. Hund's rule states that electrons with a number of equivalent orbitals first occupy all the orbitals singly with parallel spins before pairing in any orbital can occur. The two highest energy electrons of triplet state oxygen are located one each in the two degenerate π^*2p orbitals with parallel spins but with opposite angular momenta. The spin multiplicity used for spin states is defined as $2S + 1$, where S is the total spin quantum number.

The triplet state oxygen has three closely grouped energy states by the two unpaired electrons under a magnetic field. Therefore, triplet state oxygen has paramagnetic and diradical properties, and gives spin multiplicity of 3. As a result, it is characterized as triplet oxygen. The electronic configuration of singlet state oxygen molecular orbitals is shown in Figure 2, which displays no parallel characteristic of those electrons in π^*2p .

In virtue of cancellation of the opposite spin alignments of the two electrons, the total spin quantum number S in singlet state oxygen becomes 0 and the spin multiplicity is 1; therefore, the oxygen is named as singlet oxygen. Singlet oxygen exhibits diamagnetic and non-free radical nature and thus has no magnetic momentum. There are two types of singlet oxygen. The first is $^1\Sigma$ energy state and the second is $^1\Delta$ energy state. Both types violate Hund's rule which also implicates the maximum multiplicity principle. Electronic repulsion and low multiplicity of electronic arrangement of singlet oxygen result in the increase of potential energy. Consequently, both types of singlet oxygen have the energy levels of 37.5 and 22.4 Kcal/mole above triplet oxygen in ground state, respectively. The $^1\Sigma$ state singlet oxygen is so energetic that it rapidly converts to the $^1\Delta$ state singlet oxygen upon being produced. Therefore, singlet oxygen is generally referred to as $^1\Delta$. The lifetime of singlet oxygen is solvent dependent ranging from 50 to 700 μs (49); it is longer enough to initiate oxidation reactions with other molecules in food systems. Moreover, temperature has little effect on the reaction rate of singlet oxygen oxidation due to the low activation energy (0-6 Kcal/mole).

Triplet oxygen is reluctant to initiate the oxidation reaction of organic molecules which are naturally in singlet state because of a spin forbidden barrier. In contrast, singlet oxygen can readily and directly react with singlet state lipids in a spin allowed process to generate peroxides. Singlet oxygen with an vacant molecular orbital is highly electrophilic and eager to seek electrons to fill the

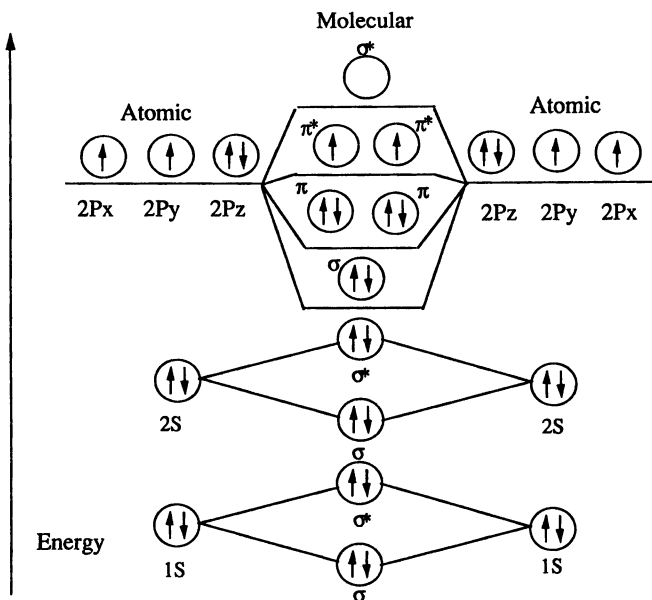


Figure 1. Electronic configuration of triplet oxygen molecular orbitals

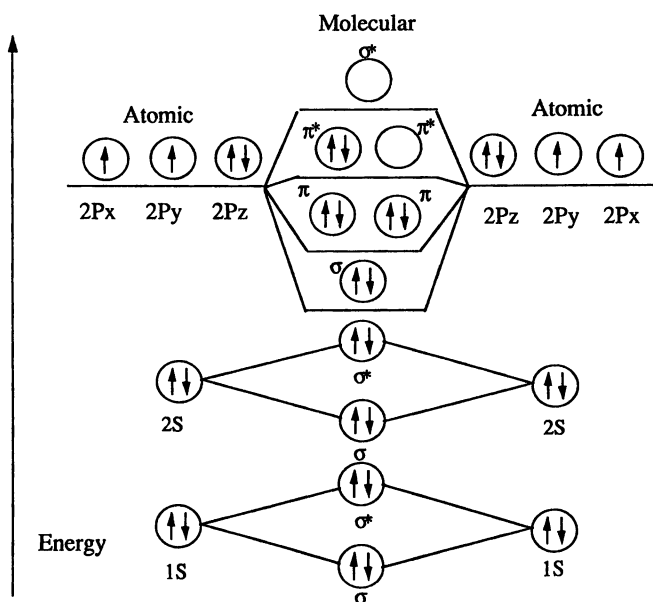


Figure 2. Electronic configuration of singlet oxygen molecular orbitals

July 15, 2012 | <http://pubs.acs.org>
 Publication Date: July 7, 1994 | doi: 10.1021/bk-1994-0558.ch002

orbital. Therefore, electron-rich olefinic and aromatic compounds, amines, and sulfides can easily react with singlet oxygen. Singlet oxygen can be detected by several methods such as chemical traps, luminescence, quenchers, and electron spin resonance. (43, 48, 50, 51).

Mechanisms of Photosensitized Oxidation

The effects of light on the flavor stability of lipid foods is explicable by photolytic autooxidation or photosensitized oxidation (10). Photolytic autooxidation which produces free radicals by ultraviolet irradiation is not the major concern in the oil industry because UV light is unlikely to reach lipids in systems except for inadvertent exposure to sunlight without a protective out-layer or container. However, photosensitized oxidation is important for the flavor stability of lipid foods. Photosensitized oxidation occurs in the presence of light, triplet oxygen, and photosensitizers. Photosensitizers include synthetic dyes such as acridine orange, crystal violet, eosin, erythrosin, methylene blue, proflavin, and rose bengal (52); naturally occurring pigments such as chlorophyll, flavin, and porphyrin (53), coenzymes and biochemicals such as pyridoxals and psoralens (54); metallic salts such as cadmium sulfide, zinc oxide, and zinc sulfide (54); polycyclic aromatic hydrocarbons such as anthracene and rubrene (55, 56); and transition metal complex such as ruthenium bipyridine (56). A sensitizer can absorb visible or near UV light and becomes an excited singlet state sensitizer ($^1\text{Sen}^*$) which has a short lifetime. The excited singlet state sensitizer ($^1\text{Sen}^*$) is rapidly converted to the ground state by emitting the fluorescence light or to the excited triplet state sensitizer ($^3\text{Sen}^*$) by inter-system crossing (ISC). The excited triplet state sensitizer ($^3\text{Sen}^*$) which has longer lifetime than the excited singlet state ($^1\text{Sen}^*$) decays to the ground state slowly by emitting the phosphorescence light. Thus, the efficient sensitizers for the generation of singlet oxygen are long-lived excited triplet state sensitizers ($^3\text{Sen}^*$) in high quantum yield (54). The excitation and deactivation of photosensitizers are illustrated in Figure 3.

The excited triplet state sensitizer ($^3\text{Sen}^*$) is produced via inter-system crossing during photosensitization. There are two reaction pathways, Type I and Type II, for the excited triplet sensitizer ($^3\text{Sen}^*$) to proceed (Figure 4). In Type I pathway (sensitizer-substrate), the sensitizer serves as a photochemically activated free-radical initiator. The excited triplet sensitizer ($^3\text{Sen}^*$) reacts with the substrate (RH) to produce radicals ($\text{R}\cdot$) or radical ions ($\text{RH}\cdot^+$) by hydrogen transfer or electron transfer, respectively. The resultant radicals react with the diradical triplet oxygen to produce the oxidized products (ROOH) which can breakdown to induce free radical chain autooxidation. In type II pathway, the excited triplet sensitizer reacts with triplet oxygen to generate the singlet oxygen by triplet-triplet annihilation reaction. The singlet oxygen thus produced reacts with substrates (RH) to form the oxidized products (ROOH) (57-62). There is also a chance of 1% that an electron can be transferred from the excited triplet sensitizer ($^3\text{Sen}^*$) to triplet oxygen to produce superoxide anion ($\text{O}_2\cdot^-$) and sensitizer radical ion ($\text{Sen}\cdot^+$) (63, 64). Singlet oxygen involvement in the photosensitized oxidation is in Type II which occurs rapidly and thus accounts for almost all photosensitized oxidation (65).

The participation of Type I or II and the intermediates depend on the chemical nature and concentrations of the sensitizer, substrate, and oxygen as well as the

reaction conditions such as pH and solvents (65). The change of reaction conditions can shift the pathway from Type I to Type II, or vice versa.

Generally, readily oxidizable (phenols and amines) or readily reducible (quinones) compounds favor Type I pathway, while compounds not readily oxidizable or reducible such as olefins, dienes, and aromatic compounds favor Type II pathway. However, amines, phenols, and other readily oxidizable compounds undergo Type II pathway under some conditions.

The oxygen solubility in solution is one of the most important factors which determine the predominant type of pathway. Oxygen is much more soluble in most organic solvents than in water (66). The high solubility of oxygen in organic solvents might favor Type II pathway, whereas the low solubility of oxygen in water might favor Type I pathway. The preferential solubility of the sensitizer in the solvents might decide the reaction type as well. Lipid soluble chlorophylls and hematoporphyrins might favor Type II pathway but water soluble riboflavin probably favors Type I pathway (65).

The competition between substrate and oxygen for a triplet sensitizer is another influential factor to determine whether Type I or Type II reaction occurs. As shown in Table I (65), Type I or Type II pathways compete efficiently for benzophenone in oxygen-saturated ethanol; however, the type II process predominates for the eosin under the same condition even at very low oxygen concentration.

Table I. Competition of Type I and Type II Processes*

Sensitizer	$K_I[S](\text{sec}^{-1})$	$K_{II}[S](\text{sec}^{-1})$
Benzophenone	3.2×10^7	2×10^7
Eosin	1.7×10^3	2×10^7

* In O₂ saturated ethanol as substrates

Reaction Mechanisms of Singlet Oxygen with Lipid Foods

Singlet oxygen is very electrophilic and involved in several reactions. These include 1,4-cycloaddition to dienes and heterocyclic compounds, the ene reaction with olefins, the oxidation of sulfides to sulfoxides, and the photosensitized oxidation of phenols to unstable hydroxy-dienones (57, 65, 67). Among these reactions, the ene reaction and photosensitized oxidation of phenols are the most important in singlet oxygen oxidation of foods. For the singlet oxygen ene reaction, there are five possible mechanisms: biradical intermediate, zwitterionic intermediate, concerted ene mechanism, pereperoxide intermediate, and perpendicular approach mechanisms. According to biradical intermediate and zwitterionic intermediate mechanisms, singlet oxygen attacks one end of the olefinic linkage to produce either a biradical (48) or a zwitterion (48, 50). These intermediates are changed to allylic hydroperoxides. In the concerted ene mechanism, a six-center transition state is involved in which attack of one end of the singlet oxygen molecule occurs at the α -olefinic carbon while the other end abstracts the γ -allylic hydrogen (58). In contrast, in the pereperoxide intermediate and perpendicular approach mechanisms, approach of the singlet oxygen is along the perpendicular bisector of the plane of p orbitals (68). The concerted six-membered ring formation process might yield a hydroperoxide from the cis addition of singlet oxygen. The ene reaction prefers hydrogen abstraction on the disubstituted side of trisubstituted olefins (48, 50). The geometry of possible six-membered transition state in the reaction between singlet oxygen and linoleate leads

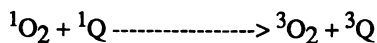
to the formation of conjugated and nonconjugated *cis* and *trans* compounds (18). The ene reaction producing conjugated and nonconjugated products can be used to distinguish singlet oxygen oxidation from the free radical autooxidation of lipid which does not produce the nonconjugated products. The pereperoxide mechanism is also responsible for the formation of nonconjugated isomers (68). Another important reaction of singlet oxygen in vegetable oils is photosensitized oxidation of phenols. This reaction is a quenching reaction of singlet oxygen by phenol compounds in the lipid. The electron transfer is the primary step which is followed by rapid proton transfer (13). It suggests the possibility of phenol oxidation by the combination of free radical autooxidation and singlet oxygen oxidation (69).

Photosensitized Oxidation of Lipid Foods

Biologically important lipids that are susceptible to photosensitized oxidation include unsaturated fatty acids, phospholipids, triglycerides, cholesterol, vitamin D, steroids, and prostaglandins (54). Photosensitized oxidation of unsaturated fatty acids and olefins is mainly responsible for the photooxidative degradation of lipid foods (54). Erythrosin showed the photosensitizing effect on the oxidation of pork lucheon meat and it accelerated the deterioration of the meat flavor during exposure to fluorescent lamps (55). Soybean phosphatidyl choline and synthetic dilinoleoyl phosphatidyl choline react with singlet oxygen in the presence of light and methylene blue as a sensitizer (24). Soybean and olive oils are photooxidized in the presence of light and chlorophyll as a sensitizer. Cholesterol is readily oxidized by singlet oxygen to form 3- β -hydroxy-5 α -hydroperoxy- Δ^6 -cholestene, the decomposition of which leads to free radical chain oxidation of unsaturated fatty acids (70).

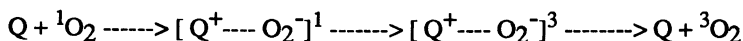
Quenching Mechanisms and Kinetics of Singlet Oxygen Lipid Oxidation

Quenching of singlet oxygen means both chemical and physical quenchings (43). Singlet oxygen reacts with quenchers to form oxidized quenchers in chemical quenching, but physical quenching degenerates singlet oxygen to triplet oxygen. Although chemical and physical quenchings can occur together, chemical quenching is a reaction rather than a quenching. Physical quenching can be explained by energy transfer and / or charge transfer mechanisms (43). Energy transfer quenching involves the formation of triplet oxygen and triplet quencher as follows:



The energy of the quencher in this process is very near or below that of singlet oxygen. The quenching of singlet oxygen by β -carotene is a good example of energy transfer quenching (40-47). The quenching rate constant of β -carotene is $3 \times 10^{10} \text{ M}^{-1}\text{S}^{-1}$ (42).

The compounds with low oxidation potentials and low triplet energies undergo the charge transfer quenching. In the charge transfer quenching, singlet oxygen reacts with electron donors to form a charge transfer complex as follows (71-74):



The complex of singlet state is relaxed to triplet state by inter-system crossing mechanism and then dissociates. The quenching rate constant of charge transfer quenching is below $10^9 \text{ M}^{-1}\text{S}^{-1}$. The involvement of electron transfer in this mechanism implies that the more easily oxidizable compounds are the better charge transfer quenchers. These types of quenchers are amines, phenols, sulfides, iodide, and azide (43).

The singlet oxygen lipid oxidation can be minimized by singlet oxygen quenching and / or triplet sensitizer quenching as shown in Figure 5 (43). Negligible singlet sensitizer quenching due to its short lifetime gives the following steady state kinetic equation for the lipid oxidation product (AO_2):

$$\frac{d[\text{AO}_2]}{dt} = K \left(\frac{K_o[{}^3\text{O}_2]}{K_o[{}^3\text{O}_2] + K_q[\text{Q}]} \right) \left(\frac{K_r[\text{A}]}{K_r[\text{A}] + (K_q + K_{ox-Q})[\text{Q}] + K_d} \right)$$

where AO_2 : oxidized lipid, K : rate constant of triplet sensitizer formation, K_r : reaction rate constant of lipid with singlet oxygen, A : lipid, k_q : reaction rate constant of physical singlet oxygen quenching by quencher Q , k_{ox-Q} : reaction rate constant of chemical singlet oxygen quenching by quencher Q , k_d : decaying rate constant of singlet oxygen.

In the case where there is only singlet oxygen quenching ($k_Q[\text{Q}] \ll k_o[{}^3\text{O}_2]$), the equation is as follows:

$$\frac{d[\text{AO}_2]}{dt} = K \left(\frac{K_r[\text{A}]}{K_r[\text{A}] + (K_q + K_{ox-Q})[\text{Q}] + K_d} \right)$$

where, K : rate constant of singlet oxygen formation

The plot of $(d[\text{AO}_2]/dt)^{-1}$ vs. $[\text{A}]^{-1}$ at various concentrations of $[\text{Q}]$ gives constant y-intercept of K^{-1} which is independent of $[\text{Q}]$. When there is no quencher the slope (S_o) becomes $K^{-1}(k_d/k_r)$ and the ratio of S_o to y-intercept gives k_d/k_r . Since k_d is known for the solvent, k_r can be calculated from k_d/k_r . If there is a quencher, the slope (S_Q) is equal to $K^{-1}\{(K_{ox-Q}[\text{Q}] + k_q[\text{Q}] + k_d)/k_r\}$ and thus $[\text{Q}]$ dependent. The ratio of slope (S_Q) to y-intercept gives $\{(K_{ox-Q}[\text{Q}] + k_q[\text{Q}] + k_d)/k_r\}$. The slope of S_Q /y-intercept vs. $[\text{Q}]$ has another y-intercept of k_d/k_r and slope of $(K_{ox-Q} + k_q)/k_r$ from which the total singlet oxygen quenching rate constant ($K_{ox-Q} + k_q$) of the quencher can be determined (75).

In the case where there is only triplet sensitizer, $(K_{ox-Q} + k_q)[\text{Q}] \ll K_r[\text{A}] + k_d$, the reaction equation becomes as follows:

$$\frac{d[\text{AO}_2]}{dt} = K \left(\frac{K_o[{}^3\text{O}_2]}{K_o[{}^3\text{O}_2] + K_q[\text{Q}]} \right) \left(\frac{K_r[\text{A}]}{K_r[\text{A}] + K_d} \right)$$

where, K : rate constant of triplet sensitizer formation

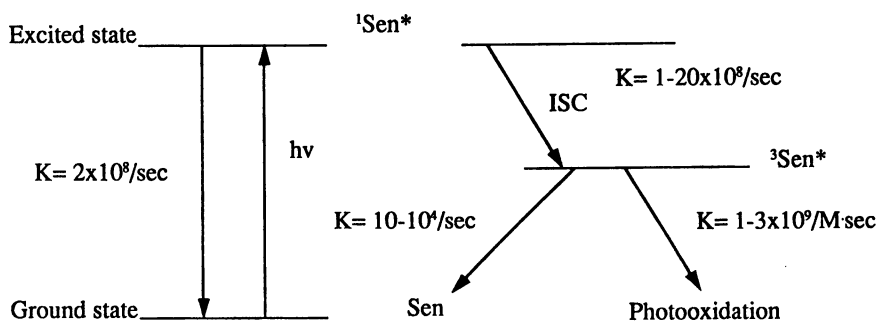


Figure 3. Excitation and deactivation of photosensitizers

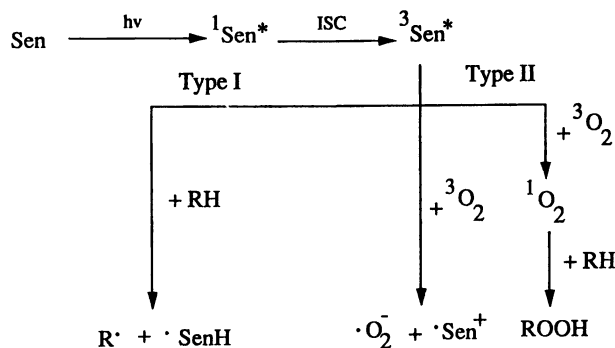


Figure 4. Type I and Type II pathways

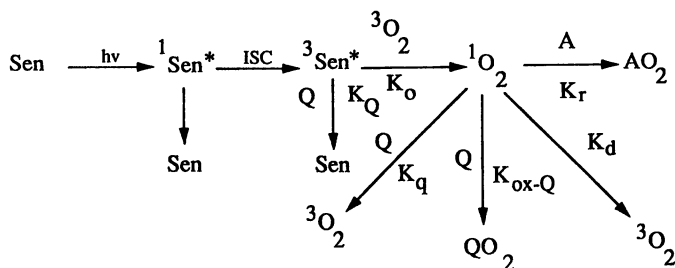


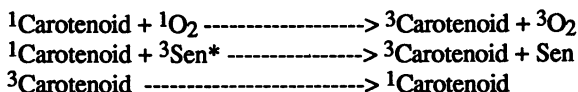
Figure 5. Scheme for the quenching of triplet sensitizer and singlet oxygen

The plot of $(d[AO_2]/dt)^{-1}$ vs. $[A]^{-1}$ at various concentrations of $[Q]$ gives y-intercept equal to $K^{-1}(1 + K_Q[Q]/k_Q[{}^3O_2])$. The ratio of slope to the y-intercept of this plot is k_d/k_r and independent of $[Q]$.

Singlet Oxygen Quenchers in Vegetable Oil Oxidations

Carotenoids. Carotenoids, which are responsible for many of the yellow and red colors of plants and animal products, have been known to minimize singlet oxygen oxidation (6, 8, 23). Carotenoids include a class of hydrocarbons called carotenes and their oxygenated derivatives called xanthophylls.

The energy transfer quenching mechanism is responsible for the minimization of singlet oxygen oxidation of lipids by β -carotene (36-43). Electron excitation energy is transferred from singlet oxygen to singlet state carotenoid, generating triplet state carotenoid and triplet oxygen, which is called singlet oxygen quenching (58).



Energy is also transferred from excited triplet state sensitizer (${}^3\text{Sen}^*$) to the singlet state carotenoid, which is called triplet sensitizer quenching. Foote (65) reported that only 10% of the triplet state chlorophyll would survive carotenoid quenching within intact chloroplasts with a local concentration of β -carotene at 2×10^{-2} M. The triplet state carotenoid is changed to the singlet state carotenoid without any radiation. The energy transfer from singlet oxygen (22 Kcal/mole) to carotenoids with nine or more conjugated double bonds (< 22 Kcal/mole) is exothermic (42). Carotenoids with fewer than nine conjugated double bonds have triplet energies above that of singlet oxygen and are less efficient singlet oxygen quenchers. Carotenoids with eleven or more conjugated double bonds quench at a diffusion-controlled rate of oxygen. However, the quenching activity decreases with nine or fewer conjugated double bonds.

Beta-carotene is an efficient singlet oxygen quencher with the quenching rate constant of $1.3 \times 10^{10} \text{ M}^{-1}\text{S}^{-1}$ in benzene (76, 77). One molecule of β -carotene can quench 250 to 1,000 molecules of singlet oxygen (65). Flavor deterioration of soybean oil initiated by light can be minimized effectively by β -carotene at levels from 5 to 10 ppm (78). The high concentration of β -carotene is not desirable because it produces off-flavors and facilitates oxidation by breaking down into secondary oxidation products that can initiate and promote free radical autooxidation (23). The rate constants for quenching singlet oxygen by carotenoids are as follows: β -apo-8'-carotenal, 3.06×10^9 ; β -carotene, 4.6×10^9 ; and canthaxanthin, $1.12 \times 10^{10} \text{ M}^{-1}\text{S}^{-1}$ (79).

The quenching effectiveness of β -carotene on the chlorophyll sensitized photooxidation of soybean oil is proportional to the increment of β -carotene concentration, as manifested by the headspace oxygen depletion in the oil containing bottle (Figure 6). The same y-intercept of regression lines of the samples containing 0, 5, 10, and 20 ppm β -carotene in Figure 6 suggests that quenching effect of β -carotene is due to the singlet oxygen quenching only.

Although carotenoids are excellent quenchers in singlet oxygen lipid oxidation, they can be destroyed via radical routes or prolonged irradiation, and may react with singlet oxygen (39, 43, 80). By the photosensitized oxidation, β -

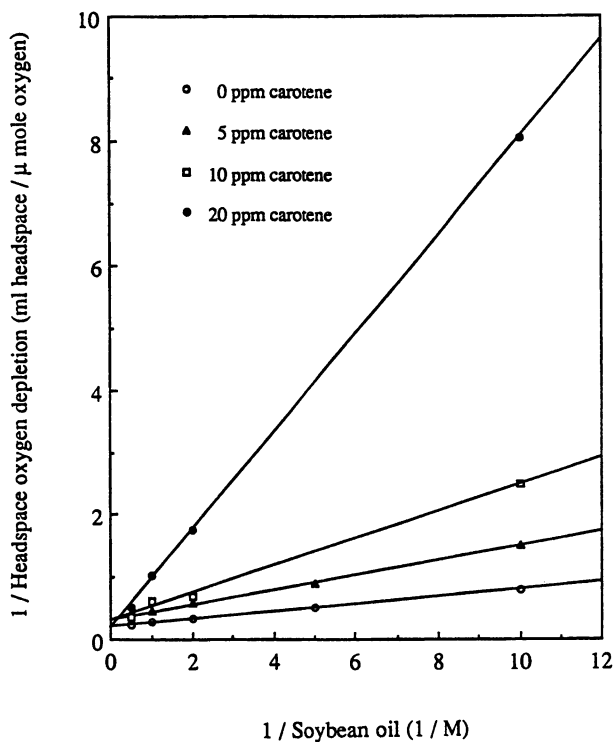


Figure 6. Effects of β -carotene on the headspace oxygen depletion of soybean oil in methylene chloride containing 4 ppm chlorophyll under light storage at 10°C for 1 day

carotenoid is changed to form β -ionone and produces dihydroactinidiolide (81). The decomposition of β -carotene implies three important meanings: first, the color change in the products may result in alteration of product acceptability; second, the quenching effect decreases and photosensitized oxidation increases, and finally, the vitamin A activity is lost (82). Fortunately, the decomposition of β -carotene can be prevented by the co-existence of tocopherols (83). Thus, the stability of β -carotene may be high in the oil containing high amount of tocopherols. Min and Lee (23) also reported that the minimization of soybean oil oxidation by β -carotene was partially attributed to light filtering effect.

Tocopherols. Tocopherols are free radical scavengers and singlet oxygen quenchers (65, 56). Tocopherols minimize free radicals produced by singlet oxygen oxidation of free fatty acids and quench singlet oxygen (84, 85). Tocopherols not only quench singlet oxygen by physical quenching mechanism but also react with singlet oxygen by chemical quenching. Foote et al. (41-43) reported that α -tocopherol quenched singlet oxygen physically and chemically, and α -tocopherol was an effective antioxidant against photooxidation mediated by singlet oxygen. The proportion of physical quenching vs. chemical quenching is structure dependent and possibly solvent system dependent. The ratio of physical quenching to chemical quenching is 13.5 in methanol (41-43) but 120 in pyridine (56). Physical quenching is the major mechanism in the tocopherols (43). Tocopherols deactivate about 120 singlet oxygen molecules before they are destroyed (80). Carlsson et al. (17) proposed that tocopherols underwent singlet oxygen oxidation and formed hydroperoxides. Yamauchi and Matsushita (85) isolated and identified two isomers of 8- α -hydroperoxy tocopherones as the primary products from the oxidized tocopherols in ethanol by singlet oxygen oxidation. Therefore, the oxidized tocopherols could be prooxidant by initiating new free radical chain reactions (17).

Figure 7 shows the quenching effects of α -tocopherol on the headspace depletion of samples containing 3 ppm chlorophyll and 0.03, 0.06, 0.1, and 0.16 M soybean oil in methylene chloride. As the tocopherol amount in oil increases, the headspace depletion of samples containing chlorophyll decreases. Tocopherol minimized the lipid oxidation by quenching the chlorophyll and / or singlet oxygen formed by the chlorophyll (23).

Jung et al. (86) reported that singlet oxygen quenching rate constants of α -tocopherol were $2.7 \times 10^7 \text{ M}^{-1}\text{S}^{-1}$ by peroxide value and $2.6 \times 10^7 \text{ M}^{-1}\text{S}^{-1}$ by headspace oxygen and that α -tocopherol showed the highest antioxidant effect, γ -tocopherol second, and then δ -tocopherol. The quenching ratios of α -tocopherol, γ and δ -tocopherols were 100: 26: 10, respectively (84). Since the reaction rates between tocopherols and singlet oxygen are high, tocopherols may not be good antioxidants in the photosensitized oxidation of lipid foods, although in a model system δ -tocopherol acted as an inhibitor in chlorophyll-sensitized photooxidation of methyl linoleate (87). The stability of tocopherols during photosensitized oxidation showed that α -tocopherol in methyl linoleate disappeared completely after 12 hour irradiation, and γ and δ -tocopherols remained 34 and 48 % after 48 hours, respectively (83). The higher stability of γ and δ -tocopherols to photosensitized oxidation enabled their quenching activities to last for a long period of time (85).

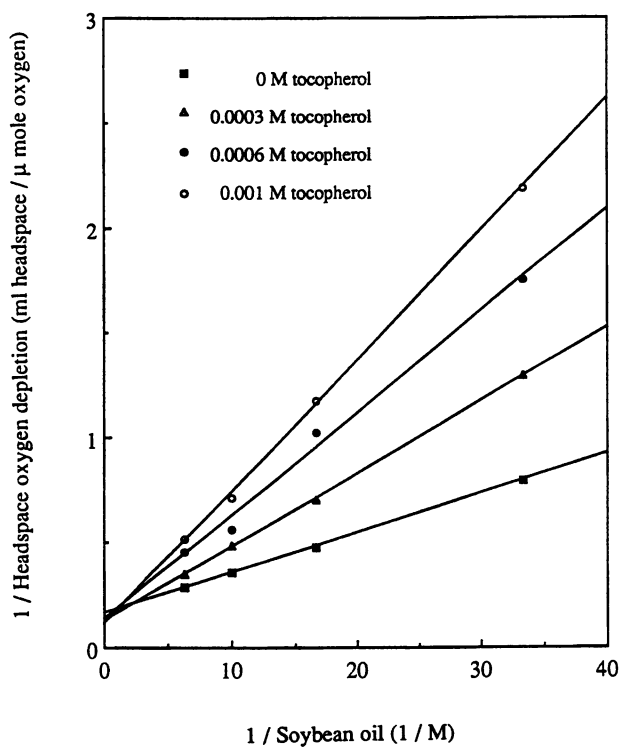


Figure 7. Effects of α -tocopherol on the headspace oxygen depletion of soybean oil in methylene chloride containing 3 ppm chlorophyll under light storage at 25°C for 2 hours

Literature Cited

1. Awl, R. A.; Frankel, E. N.; Weisleder, D. *Lipids* 1987, 22, 721.
2. Frankel, E. N. *Prog. Lipid Res.* 1980, 19, 1.
3. Frankel, E. N.; Warner, K.; Moulton, K. J. Sr. *JAOCS* 1985, 62, 1354.
4. Labuza, T. P. *CRC Crit. Rev. Food Technol.* 1971, 2, 355.
5. Lee, S. H.; Kim, Z. U. *J. Korean Agri. Chem.* 1986, 28, 138.
6. Lee, S. H.; Min, D. B. *J. Agri. Food Chem.* 1990, 38, 1630.
7. *Autooxidation and Antioxidants*; Lundberg, W. O., Ed.; Interscience Publishing: New York, NY, 1961, Vol. I; 1962, Vol. II.
8. Min, D. B.; Lee, E. C.; Lee, S. H. In *Flavor Chemistry of Lipid Foods*; Min, D. B.; Smouse, T. H., Eds.; AOCS: Champaign, IL, 1989, 57-97.
9. Min, D. B.; Lee, S. H.; Lindamood, J. B.; Chang, K. S.; Reneccius, G. A. *J. Food Sci.* 1989, 54, 1222.
10. *Flavor Chemistry of Fats and Oils*; Min, D. B.; Smouse, T. H., Eds.; AOCS: Champaign, IL, 1985.
11. Pearson, A. M.; Gray, J. I., Wolzak, A. M.; Horenstein, N. A. *Food Technol.* 1983, 37, 121.
12. Sattar, A.; deMand, J. M.; Alexander, J. C. *Can. Inst. Food Sci. Tech.* 1976, 9, 108.
13. *Autooxidation in Food and Biological Systems*, Simic, M. G.; Karel, M. Eds.; Plenum Press: New York, NY, 1980.
14. Stevenson, S. G.; Vaisey-Genser, M.; Eskin, N. A. M. *JAOCS* 1984, 61, 110.
15. Warner, K.; Frankel, E. N.; Synder, J. M.; Porter, W. L. *J. Food Sci.* 1986, 51, 703.
16. Rawls, H. R.; van Santen, P. J. *JAOCS* 1970, 47, 121.
17. Carlsson, D. J.; Suprunchuk, T.; Wiles, D. M. *JAOCS* 1976, 53, 656.
18. Clements, A. H.; Den Engh, R. H.; Frost, D. J.; Hoogenhout, K.; Nooi, J. R. *JAOCS* 1972, 50, 325.
19. Endo, Y.; Usuki, R.; Kaneda, T. *Agri. Biol. Chem.* 1984, 48, 985.
20. Endo, Y.; Usuki, R.; Kaneda, T. *JAOCS* 1984, 61, 781.
21. Frankel, E. N.; Neff, W. E.; Bessler, T. R. *Lipids* 1979, 14, 961.
22. Jen, J. J.; MacKinney, G. *Photochem. Photobiol.* 1970, 11, 303.
23. Min, D. B.; Lee, E. C. In *Frontiers of Flavor*; Charalambous, G. Ed.; Elsevier, Amsterdam, The Netherlands, 473-498.
24. Terao, J.; Matsushita, S. *Agri. Biol. Chem.* 1977, 41, 2467.
25. Usuki, R.; Suzuki, T.; Endo, Y.; Kaneda, T. *Yukagaku* 1983, 32, 321.
26. Usuki, R.; Suzuki, T.; Endo, Y.; Kaneda, T. *JAOCS* 1984, 61, 785.
27. Usuki, R.; Suzuki, T.; Endo, Y.; Kaneda, T. *Agri. Biol. Chem.* 1984, 48, 991.
28. Frankel, E. N.; Neff, W. E.; Bessler, T. R. *Lipids* 1979, 14, 961.
29. Frankel, E. N.; Neff, W. E.; Selke, E. *Lipids* 1981, 16, 279.
30. Frankel, E. N.; Neff, W.E.; Selke, E.; Weisleder, D. *Lipids* 1982, 17, 11
31. Frankel, E. N.; Neff, W.E.; Selke, E. *Lipids* 1983, 18, 353.
32. Frankel, E. N.; Neff, W.E.; Weisleder, D. *J. Chem. Soc. Chem. Commun.* 1982, 59.
33. Frankel, E. N. In *Flavor Chemistry of Fats and Oils*; Min, D. B.; Smouse, T. H., Eds; AOCS: Champaign, IL, 1985, 1-37.
34. Gunstone, F. D. *JAOCS* 1984, 61, 441.
35. Neff, W. E.; Frankel, E. N. *Lipids* 1980, 15, 587.
36. Neff, W. E.; Frankel, E. N.; Weisleder, D. *Lipids* 1982, 17, 780.

37. Neff, W. E.; Frankel, E. N.; Selke, E.; Weisleder, D. *Lipids* 1983, 18, 868.
38. Neff, W. E.; Frankel, E. N. *Lipids* 1984, 19, 952.
39. Bellus, D. In *Singlet Oxygen- Reaction with Organic Compounds and Polymers*; Randy, B.; Rabek, J. F., Eds.; Wiley: New York, NY, 1978, 61-110.
40. Besasson, R.; Land, E. J.; Maudinas, B. *Photochem. Photobiol.* 1976, 23, 189.
41. Foote, C. S. *J. Am. Chem. Soc.* 1968, 90, 6233.
42. Foote, C. S.; Chang, Y. C.; Denney, R. W. *J. Am. Chem. Soc.* 1970, 92, 5216.
43. Foote, C. S. In *Singlet Oxygen*; Wasserman, H. H.; Murray, R. W., Eds.; Academic Press: New York, NY, 1979, 139-171.
44. Herkstroeter, W. G. *J. Am. Chem. Soc.* 1975, 97, 3090.
45. Herkstroeter, W. G. *J. Am. Chem. Soc.* 1975, 97, 4161.
46. Mathis, P.; Kleo, J. *Photochem. Photobiol.* 1973, 18, 343.
47. Mathis, P.; Vermiglio, A. *Photochem. Photobiol.* 1972, 15, 157.
48. Korycka-Dahl, M. B.; Richardson, T. *Crit Rev. Food Sci. Nutr.* 1978, 10, 209.
49. Ogilby, P. R.; Foote, C. S. *J. Am. Chem. Soc.* 1983, 105, 3423.
50. Krinsky, N.I. *TIBS* 1977, 2, 35.
51. Whang, K.; Peng, I. C. *J. Food Sci.* 1988, 53, 1863.
52. Garstone, B. *J. Soc. Dyers Colourists* 1980, 96, 535.
53. Jori, G.; Spikes, J. D. In *Topics in Photomedicine*; Smith, K.C., Ed.; Plenum Press: New York, NY, 1984, 183.
54. Spikes, J. D. In *The Science of Photobiology*, Smith, K.C., Ed.; Plenum Press: New York, NY, 1977, 87-112.
55. Chan, H. W. S. *JAOCs* 1977, 54, 100.
56. Fahrenholtz, S. R.; Doleiden, F. H.; Trozzolo, A. M.; Lamola, A. A. *Photochem. Photobiol.* 1974, 20, 505.
57. Foote, C. S. *Accounts Chem. Res.* 1968, 1, 104.
58. Foote, C. S.; Denny, R. W. *Pure Appl. Chem.* 1971, 27, 635.
59. Kearns, D. R. *Chem. Rev.* 1971, 71, 395.
60. Wayne, R. P. *Advan. Photochem.* 1969, 7, 311.
61. Wilson, T.; Hastings, J. W. *Photophysiology* 1970, 5, 49.
62. Politzer, I. R.; Griffin, G. W.; Laseter, J. *Chem. Biol. Interact.* 1971, 3, 73.
63. Balny, C.; Douzu, P. *Biochem. Biophys. Res. Commun.* 1974, 56: 386.
64. Kepka, A.; Grossweiner, L. I. *Photochem. Photobiol.* 1972, 14, 621.
65. Foote, C. S. In *Free Radicals in Biology*; Pryor, W. A., Ed.; Academic Press: New York, NY, 1976, 85-133.
66. Wilhelm, E.; Battino, R. *Chem. Rev.* 1973, 73, 1.
67. Rao, V. J.; Muthuramu, K.; Ramamurthy, V. *J. Org. Chem.* 1982, 47, 127.
68. Fencical, W.; Kearns, D. R.; Radlick, P. *J. Am. Chem. Soc.* 1969, 91, 7771.
69. Grams, G. W.; Eskins, K.; Inglet, G. E. *J. Am. Chem. Soc.* 1972, 94, 866.
70. Doleiden, F. H.; Fahrenholtz, S. R.; Lamola, A. A.; Trozzolo, A. M. *Photochem. Photobiol.* 1974, 20, 519.
71. Ogryzlo, E. A. In *Singlet Oxygen-Reactions with Organic Compounds and Polymers*; Ranby, B.; Rabek, J. F., Eds.; Wiley: New York, NY, 1978, 17.
72. Ounnes, C.; Wilson, T. *J. Am. Chem. Soc.* 1968, 90, 6528.
73. Young, R. H.; Brewer, D. R. In *Singlet Oxygen-Reactions with Organic Compounds and Polymers*; Ranby, B.; Rabek, J. F., Eds.; Wiley: New York, NY, 1978, 36.

74. Young, R. H.; Martin, R. L. *J. Am. Chem. Soc.* 1972, 94, 5183.
75. Higgins, R.; Foote, C. S.; Cheng, H. *Adv. Chem. Ser.* 1968, 77, 102.
76. Foote, C. S.; Denny, R. W. *J. Am. Chem. Soc.* 1968, 90, 6233.
77. Farmilo, A.; Wilkinson, F. *Photochem. Photobiol.* 1973, 18, 447.
78. Warner, K.; Frankel, E. N. *JAOCS* 1987, 64, 213.
79. Jung, M. Y.; Min, D. B. *JAOCS* 1991, 68, 653.
80. Spikes, J. D.; Swartz, H. M. *Photochem. Photobiol.* 1978, 28, 921.
81. Isoe, S.; Hyeon, S. R.; Sakan, T. *Tetrahedron Letters* 1969, 4, 279.
82. Francis, F. J. In *Food Chemistry*, Fennema, O.R., Ed.; Marcer Dekker: New York, NY, 1979, 139-171.
83. Matsushita, S.; Terao, J. In *Autooxidation in Food and Biological Systems*; Simic, M. D.; Karel, M., Eds.; Plenum Press: New York, NY, 1980, 27-44.
84. Grams, G. W.; Eskins, K. *Biochem.* 1972, 11, 606.
85. Yamauchi, R.; Matsushita, S. *Agric. Biol. Chem.* 1977, 41, 1425.
86. Jung, M. Y.; Choe, E.; Min, D. B. *J. Food Sci.* 1991, 56, 807.
87. Terao, J.; Matsushita, S. *JAOCS* 1977, 54, 234.

RECEIVED March 10, 1994

Chapter 3

Contribution of Lipoxygenase Pathway to Food Flavors

Rudolf Juuping Hsieh

Campbell Research and Development, Campbell Soup Company,
1 Campbell Place, Camden, NJ 08103

Lipoxygenase is widely distributed in many plant, animal and fish tissues. Depending on its origin, lipoxygenase catalyzes the insertion of oxygen into polyunsaturated fatty acids to generate position specific hydroperoxides. The hydroperoxides can be further metabolized into various secondary oxidation products. The lipoxygenase pathway is highly associated with the development of fresh and off flavors in many plant and animal food systems. Its secondary metabolites have been documented to have significant impacts on the physiology and pathology of plant and animal tissues. Lipoxygenase activity can be controlled by free radical quenching and metal chelating mechanisms. Heat stability of lipoxygenase is also discussed.

Lipid oxidation affects the quality attributes (color, odor, flavor and texture), nutritional value, safety and physiological effects of food components. Low levels of oxidative degradation of the unsaturated fatty acids of food lipids may be beneficial in some foods in generating low levels of desirable flavorful carbonyl compounds. However more concerns are the free radical intermediates formed. Free radicals are reactive and can interact and alter other constituents such as proteins, nucleic acids, carbohydrates, pigments and minerals. Typical impacts of lipid oxidation on food qualities are flavor deterioration, color bleaching and textural changes.

The lipoxygenase pathway participates in oxidative degradation of biological systems as an initiator. Lipoxygenase can catalyze the peroxidation of polyunsaturated fatty acid to produce position specific hydroperoxide. The enzyme is of interest to food scientists due to its role in the biogenesis of flavor and aroma in plant products (1,2). Lipoxygenase-produced flavor and aroma compounds are desirable in many foods, but may also give rise to offensive

0097-6156/94/0558-0030\$08.00/0
© 1994 American Chemical Society

flavors in some plant systems. Lipoxygenase activity is also associated with degradation of colors in unblanched vegetables (1,2) and rheological properties in dough products (3).

Various aspects concerning plant lipoxygenases have been reviewed extensively (1-3). Interest in animal and fish lipoxygenases have been growing rapidly in the past two decades (1). However the direct correlation between animal/fish lipoxygenase activity and food quality is still to be established. This review concentrates on the lipoxygenase pathway in flavors in various biological systems.

Sources of Lipoxygenases

Lipoxygenases are ubiquitous in many plant, animal, poultry and fish tissues (Table I). The amount of lipoxygenases in tissues varies widely and can differ among cultivars and strains of the same organisms, with age, tissue and with environmental factors.

Plant Lipoxygenase. Soybean lipoxygenase has been studied quite extensively because its activity is highly associated with the development of off-flavor. Lipoxygenase is found primarily in the cytosol of the storage parenchyma of mature soybean seeds (4,5). On germination, however, lipoxygenase decreases in the storage parenchyma and increases in the abaxial hypodermis, epidermis and vascular bundle sheaths (5).

There are four isozymes of lipoxygenase in soybean. Relative amounts of the isozymes differ among cultivars. Lipoxygenase-3 is the most abundant isozyme in mature soybean on a protein basis, lipoxygenase-1 is almost as abundant, with lipoxygenase-2 least abundant. However lipoxygenase-2 has the highest specific activity, so that on an activity basis there are similar amounts of the three isozymes in soybeans (6).

Lipoxygenase-1 has optimum pH at 9 whereas lipoxygenase-2, 3 and 4 has pH optima of 6.5-7.0 (7). Isozymes 1-3 are similar in amino acid sequences (8,9). The isozymes are different in the products produced from linoleic acid. Lipoxygenase-1 produces 13-hydroperoxide almost exclusively (95%), while lipoxygenase-2 produces approximately equal amounts of the 13- and 9-hydroperoxides and lipoxygenase-3 produces more 9-hydroperoxides (65%) than 13-hydroperoxides (35%) (10). Generally speaking, lipoxygenase-2 and -3 are sometimes referred to as type 2 lipoxygenase while lipoxygenase-1 is referred to as type 1 lipoxygenase. Type-2 lipoxygenases are much more widespread in plants than type-1 (11).

Soybean seeds contain more lipoxygenase than do English green peas and green beans. Four isozymes were separated from pea seed extracts (12,13). Lipoxygenase activities have been reported in black bean, navy bean, pinto bean, kidney bean, lima bean and mung bean, etc. (14).

Cereal seeds generally have less lipoxygenases than legumes. Wheat has

been reported to contain up to four isozymes (15), barley two after germination (16), maize two after germination (17), rice three (18), rye three (19) and oats two (20,21). Lipoxygenase in cereal seeds appears to be associated primarily with the germ, although the subcellular location is not known. The germ and bran of wheat were found to contain seventeen- and four-fold more activity, respectively, than the endosperm (22). Lipoxygenase activity varied more than ten-fold in wheat samples of different varieties and conditions of cultivation (23).

Lipoxygenase was not detected in mitochondria of wheat seedlings (24). It has been found in the chloroplast of a number of plants, including soybean leaves (5), pea leaves (25), wheat (26,27) and possibly cauliflower and calabrese florets (28,29).

Lipoxygenases of the seeds of soybeans, English peas, green beans and corn are responsible for aroma and flavor deterioration (30,31), while lipoxygenases in cauliflower and broccoli appear to be of little or no consequence (32).

Animal Lipoxygenase. The history of the studies of lipoxygenases in animal tissues is relatively shorter than that of plant origin. Much information about enzymes, their metabolites and physiological significance has been accumulated in the past ten years (33). However more knowledge about physical and chemical structure, kinetics and mechanism of animal lipoxygenases is still needed (1). Like plant lipoxygenase, animal lipoxygenase can utilize linoleic acid as a substrate. However lipoxygenase activity in polyunsaturated fatty acids such as arachidonic, eicosapentaenoic and docosahexaenoic acids is of more interest to animal physiology.

12-Lipoxygenase, identified in mammalian platelet (34,35), lung (36,37) and skin (38), catalyzed the conversion of arachidonic acid into 12-hydroperoxy-eicosatetraenoic acid. Eicosapentaenoic and docosahexaenoic acids can also be metabolized by platelet lipoxygenase into their corresponding 12- and 14-hydroperoxy derivatives (39-41). Under certain conditions, the above hydroperoxy product is further metabolized to a dihydroperoxy derivative by an additional lipoxygenase (42), or nonenzymatically by iron-catalyzed peroxidation to a mixture of trihydroxy derivatives via hydroxy epoxide intermediates (43-45). In leukocytes (46), 5-lipoxygenase activity was found. It oxidized arachidonic acid into a 5-hydroperoxy derivative and further metabolized into different leukotrienes (47). Reticulocytes (48) and human eosinophils (49) converted arachidonic acid into a 15-hydroperoxy derivative via a 15-lipoxygenase and further metabolized into a 15-series of leukotrienes (50).

Poultry Lipoxygenase. Lipoxygenase from chicken muscle exhibited oxygenation activity at the n-6 position in arachidonic acid (51). Optimum pH for the enzyme was around 7.0, with little activity observed below pH 5.0 and above pH 8.5. The enzyme was stable in frozen storage, and activity was almost completely preserved after twelve months of frozen storage at -12°C. It is suggested that lipolysis may

provide the substrate for lipoxygenase which may be responsible for some of the oxidative and rancidity changes during frozen storage of chicken meat (52).

Fish Lipoxygenase. Lipoxygenase activity in fish skin caused postharvest bleaching of carotenoids (53). Carotenoids are responsible for the bright red and orange hues of some fish skins. Lipoxygenase activity has been identified in gill and skin tissues in fish in several laboratories (Table I) (54-59). Some lipoxygenase activity was reported in light and dark muscles of lake herring (60).

Fish lipoxygenase has an optimum pH at 7.0 and is stable over the pH range of 6-9 (56,61). Two lipoxygenases have been partially purified from the gill tissue of marine rockfish and freshwater trout (58,62). One introduced oxygenation into arachidonic acid at the n-9 position while the other possessed activity at the n-6 position. In these two kinds of fish gill tissues, n-9 lipoxygenase is much more preponderant than the n-6 enzyme (58). In sardine skin tissue, n-6 lipoxygenase is the predominant type (59,61). Results from chiral phase HPLC indicated that oxidation products from n-9 and n-6 lipoxygenases had the S configuration (58,59,62). This 12(S) structure is consistent with that produced by 12-lipoxygenase in mammalian platelets and lungs (62).

Table I. Plant and Animal Sources of Lipoxygenase

PLANTS				
Apple	Alfalfa	Banana	Barley	Beans
Broccoli	Cauliflower	Cucumber	Egg Plant	Grape
Maize	Mango	Melon	Mushroom	Oat
Peas	Pear	Potato	Rice	Rye
Soybean	Tobacco	Tomato	Wheat	Watermelon
MAMMALS				
Platelets		Lungs		Leukocytes
Skins		Reticulocytes		
FISHES				
Drum	Perch	Emerald Shiner	Bass	Bullhead
Sheephead	Trout	Catfish	Salmon	Blue Gill

July 15, 2012 | http://pubs.acs.org
 Publication Date: July 7, 1994 | doi: 10.1021/bk-1994-0558.ch003

Mechanism of Lipoxygenase Reaction

Lipoxygenase catalyzes the insertion of oxygen into polyunsaturated fatty acids. The basic *cis,cis*-1,4-nonconjugated diene system is required for all unsaturated fatty acids. The normal product from the action of animal and plant lipoxygenases on such a substrate is a *cis,trans*-conjugated hydroperoxy fatty acid (63). Most available information concerning the mechanism of catalysis has been obtained from experiments with soybean lipoxygenase-1 (64).

The nonheme iron has been suggested to be around the active site of soybean lipoxygenase and is bound tightly. It cannot be removed without denaturation of the enzyme (65). According to absorption and fluorescence spectroscopy studies, there are at least three catalytic forms of lipoxygenase, the native ferrous form, the yellow activated ferric form and the purple enzyme-hydroperoxide complex form, during its catalytic cycle (66,67).

There are some indications for the presence of methionine (68,69), tyrosine and tryptophan (65,66,70) in the neighborhood of the catalytically active site of soybean and reticulocyte lipoxygenases. Calcium cation may influence the physical state of the fatty acid substrate in solution, particularly at low pH (71). Calcium cation have also been reported to inhibit soybean lipoxygenase activity (72)

The activation of lipoxygenase can be initiated by either ferric or ferrous lipoxygenase in the presence of oxygen. The activation of lipoxygenase requires the presence of fatty acid hydroperoxide (73,74). In a simplified scheme, there are four major steps in the lipoxygenase initiated oxidation of polyunsaturated fatty acids in both animal and plant tissues (48,75): (a) activation of enzyme from ferrous state to ferric state by the presence of trace amounts of hydroperoxide; (b) hydrogen abstraction from the substrate unsaturated fatty acids; (c) stereospecific introduction of an oxygen molecule and (d) formation of fatty hydroperoxy anion and regeneration of ferric enzyme for the next cycle. In this reaction scheme, the lipoxygenase reaction catalysis is initiated by hydrogen abstraction of fatty acid, not by activation of oxygen. This is supported by the fact that, even with the removal of oxygen from the reaction mixture, fatty acid dimers arise from the radical intermediates of free fatty acids generated via the anaerobic lipohydroperoxidase reaction.

Substrate Specificity. Lipoxygenases are generally defined in terms of their ability to dioxygenate polyunsaturated fatty acids possessing a 1,4-*cis,cis*-pentadiene system. Among the naturally occurring n-3 and n-6 fatty acids that meet this structure requirement (48,63), the following are most frequently used as lipoxygenase substrates: linoleic, α -linolenic, γ -linolenic, arachidonic, *bis*-(homo)- γ -linolenic, 5,8,11-all-*cis*-eicosatrienoic, eicosapentaenoic and docosahexaenoic acids.

Fatty acids containing a *trans* double bond, such as linoleic acid, are not oxygenated and are competitive inhibitors of linoleate oxygenation by soybean

lipoxygenase (76). Both *cis* and *trans* fatty acids are bound to the enzyme, but the initial hydrogen abstraction is sterically hindered in the *trans* structure. Moreover, fatty acids with a conjugated diene system are strong competitive inhibitors of linoleate oxygenation.

Many lipoxygenases attack not only unesterified polyenoic fatty acids, but also their methyl esters. The rate of oxygenation of methyl linoleate were consistently 25% of that for free linoleic acid for lipoxygenases in reticulocytes, pea seeds and wheat (77). Soybean lipoxygenase-1 attacks methyl linoleate with low reactivity and low regio- and stereospecificity as compared to the reactions with free linoleic acid (48). Unfractionated soybean oil, mono- and dilinolein (78) can be oxygenated by soybean lipoxygenase. 15-Lipoxygenases from reticulocytes and polymorphonuclear leukocytes may attack unsaturated fatty acids in phospholipid (79). The specificity of lipoxygenase is a function of substrate fatty acids, pH, and other incubation conditions, e.g., the presence of calcium (10,48,80).

Product Specificity. For the formation of one conjugated diene radical during a lipoxygenase cycle, there are always two possible positional isomers of oxygenation products. The preference formation of one of the two may be explained in two ways: (a) stabilization of the mesomeric structures of the fatty acid radical intermediate and (b) the orientation of the dioxygen introduced at the active site (48). According to the position of oxygenation, lipoxygenase may be categorized into two groups: (1) the position of hydrogen removal is determined by the distance from the methyl end of the fatty acid and oxygenation by a (+2) rearrangement, as catalyzed by lipoxygenase from soybean (81), reticulocytes (82), and bovine blood (35); or (2) the position of hydrogen removal is determined by the distance from the carboxyl end of the fatty acid and oxygenation by a (-2) rearrangement, such as occurs with the lipoxygenase from potato (83), corn (81), tomato (84), wheat (85), and neutrophil (46). Dual positional specificity has been found in lipoxygenases from soybean (86), potato (87), wheat (85), human blood platelet (88), and reticulocytes (89).

Fresh and Off-Flavors Associated with Lipoxygenase Activity

A lipoxygenase/lyase pathway has been demonstrated in the production of many important volatile carbonyl compounds in watermelon seedlings (90), tea chloroplast (91), alfalfa and cucumber seeds (92), pears (93) and apples and tomatoes (94). The reaction usually involves the cleavage of hydroperoxides by hydroperoxide lyase and/or subsequent isomerization by isomerase (95-97). Cleavage of 13-hydroperoxy linoleic or linolenic acids by hydroperoxide lyase generated twelve-carbon compounds, 12-oxo-*cis*-9-dodecenoic acid, and six-carbon aldehydes, hexanal or *cis*-3-hexenal. The 12-oxo-*cis*-9-dodecenoic acid and *cis*-3-hexenal undergo isomerization to the more stable 12-oxo-*trans*-10-dodecenoic acid and *trans*-2-hexenal (98,99). Cleavage of 9-hydroperoxy fatty acids by

hydroperoxide lyase results in the formation of nine-carbon oxo fatty acids and aldehydes. The volatile products from the 9-hydroperoxides of linoleic and linolenic acids are *cis*-3-nonenal and *cis,cis*-3,6-nonadienal, respectively. The differences in the type and proportion of volatile compounds produced have an impact on the distinctive flavor and aroma of a particular fruit or vegetable and are due to the nature and properties of the enzymes involved in the oxidative degradation of linoleic and linolenic acids.

Soybean. Lipoxygenase represents about 1-2% of the protein in soybeans (10). Soybean oil contains approximately 55% linoleic and 8% linolenic acids which are substrates for lipoxygenase. Volatile degradation products of these fatty acid hydroperoxides by lipoxygenase pathway are n-hexanal, *cis*-3-hexenal (100-102). These compounds have been associated with grassy, beany and rancid off-flavors in soybeans (103,104) which reduce the consumer acceptability of soybean products. Flavor problems can occur in soybean protein as well as oil products because undesirable compounds can bind covalently to proteins during processing (104,105). Some of these aldehydes can be reduced by the action of alcohol dehydrogenase to alcohols leading to less undesirable flavors because of higher alcohol threshold values (106,107).

Tomato. The characteristic flavor and aroma of tomatoes are produced by a large group of volatile compounds including aldehydes, ketones and alcohols (108,109). The principal volatiles produced from the breakdown of fatty acids in tomatoes are hexanal and *cis*-3-hexenal (110-112). Tomato lipoxygenase, which has been purified and characterized (113), forms 9- and 13-hydroperoxides. 9-Hydroperoxide is the predominant product in quantity. However only 13-hydroperoxide is susceptible to the action of the tomato hydroperoxide lyase and is converted to the six-carbon aldehydes (94,114). The metabolic fate of the 9-hydroperoxides is not well understood. An isomerase can transform *cis*-3-hexenal to *trans*-2-hexenal, although the level of this isomerase in tomato is low (11). Alcohol dehydrogenase can then reduce these aldehydes to the corresponding alcohols. All the six-carbon aldehydes and alcohols contribute a green note to the flavor of fresh tomato. During the processing of tomato paste, *cis*-3-hexenal and hexanal concentrations are greatly diminished (116), thereby causing a loss of fresh tomato aroma. Nonspecific oxidation pathways such as autooxidation and light-induced oxidation are also involved in the development of tomato flavors (117-119).

Apple. Lipoxygenase has been extracted from Golden Delicious apples and then partly purified (120). Its activity was reported the highest in the core and peel (121). Hexanal and *trans*-2-hexenal are the principal products of the action of lipoxygenase in fatty acids in apples; lesser amounts of hexanal, *trans*-2-hexenol, *cis*-3-hexenal and *cis*-3-hexenol are also formed (122-124).

Cucumber. Cucumber peel contains twice as much lipoxygenase activity as the flesh (125). Lipoxygenase, hydroperoxide lyase and *cis*-3-hexenal isomerase have been demonstrated to be involved in the genesis of volatiles in cucumber (95-97). The principal aroma compounds in cucumber are nine-carbon volatiles although six-carbon volatiles are also present (95). These volatiles are not found in intact cucumber tissue. Cutting or chewing of the tissue induces the production of nine-carbon carbonyl compounds, among which, *trans*-2-*cis*-6-nonadienal provides most of the characteristic aroma of cucumber (126,127). In addition, twelve-carbon and eighteen-carbon aldehydes are present in the volatile fractions of cucumber homogenate (128,129) which results from the action of an α -oxidation enzyme system.

Mushroom. A major contribution to the flavor of mushrooms comes from 1-octen-3-ol and 1-octen-3-one (130). These are derived from the 10-hydroperoxide of linoleic acid, presumably formed as a result of lipoxygenase activity (131). The R(-)-enantiomer of 1-octen-3-ol has the typical mushroom flavor while S(+)-1-octen-3-ol has a mouldy, grassy flavor note (132). 1-Octen-3-ol is present in much larger amounts in mushroom than 1-octen-3-one (133). However the ketone has a higher aroma value and is a more important flavor volatile. Dehydration causes massive loss of 1-octen-3-ol, while cooking leads to an increase in both 1-octen-3-ol and 1-octen-3-one, especially the ketone (134,135).

Banana. During the ripening process, the content of fatty acids, e.g. palmitic, oleic and linoleic acids, decreases. The decrease in fatty acid content is associated with the oxidative activity of lipoxygenase activity and subsequent generation of flavors. Green banana contains *trans*-2-nonenal, *trans*-2-*cis*-6-nondienal and 9-oxonanoic acid. Bananas treated with ethylene, due to induced lipoxygenase activity, contains hexanal, *trans*-2-hexenal and 12-oxo-*trans*-10-dodecenoic acid (136).

Green Peas. Lipoxygenase has been found in pea cotyledon and skin tissues (137). Lipoxygenase activity is associated with off-flavor development. The addition of purified lipoxygenase to blanched peas produces the characteristic off-flavors (30,138).

Winged Beans. Hexanal and 2-pentylfuran are the principal volatile components responsible for the strong beany off-flavors in winged beans (139). Mature winged beans possess lipoxygenase at levels comparable to soybeans (72). These authors purified two major isozymes of lipoxygenase from winged beans. Both were thermostable (72).

Green Beans. Significant contributions to the acceptable flavors of green beans are made by volatiles such as 1-octen-3-ol, *cis*-3-hexenol, *trans*-2-hexenal, hexanal and hexanol (140). Lipoxygenase (141) and hydroperoxide isomerase (142)

activities have been detected in bean seeds. Direct relationship between lipoxygenase activity and flavor formation is still lacking. However blanching to inactivate lipoxygenase has been shown to prevent off-flavor formation in beans (143).

Potato. The action of lipoxygenase on linolenic acid in potatoes produced its 9-hydroperoxide (144,145). A breakdown compound from the 9-hydroperoxy derivative of linolenic acid, *trans*-2-*cis*-6-nonadienal, has been shown to be associated with cucumber-like off-flavors in potato and formed through the lipoxygenase pathway (146,147). This off-flavor is removed during boiling because the nonadienal undergoes double bond hydration and retro-aldol condensation to *cis*-4-heptenal. *cis*-4-Heptenal contributes to the boiled potato flavor (148).

Grape. Grape lipoxygenase produces mainly 13-hydroperoxides (80%) and 9-hydroperoxides (20%) (149). Its hydroperoxide lyase like the tomato isozyme attacks only 13-hydroperoxide, not 9-hydroperoxide (150,151), to generate six-carbon aldehydes (149,152).

Corn. Off-flavor development in frozen corn-on-the-cob was attributed to lipoxygenase activity in the germ and cob (153). Compared to that in peas and beans, lipoxygenase in immature maize seeds was less active by a factor of 10 (154). Germination increased lipoxygenase activity in germ considerably (17). Main volatiles originating from maize lipoxygenase oxidation of linoleic acid were pentanal, hexanal, *trans*-2-heptenal, ethanal and heptanal (155).

Tea. Lipoxygenase was reported in tea leaf chloroplast (156,157). Its enzymic activity oxidized linoleic acid to its 13-hydroperoxide, then hexanal and linolenic acid to *cis*-3-hexenal and *trans*-2-hexenal (158). Lipoxygenase activity in tea leaves increases after plucking (159) and there is a further increase in subsequent dehydration. Lipoxygenase activity is higher in leaves used for making high quality tea than those used for lower quality products.

Fish. Various aliphatic aldehydes contribute strongly to the characteristic aroma of oxidized fish lipids. These carbonyl compounds impart flavors which have been frequently described as rancid, cod-liver-oil-like and painty. Typical oxidized fish-like flavors are 4-heptenal, 2,4-heptadienal, 2,4-decadienal and 2,4,7-heptatrienal (160-162). These flavors, due to autoxidation of polyunsaturated fatty acids, tend to increase with time and temperature of frozen storage (160,162).

Fresh fish is characterized by a plant-like, green grassy aroma, reflecting the combination of six-carbon, eight-carbon and nine-carbon carbonyl and alcohol compounds (85,163,164). These are hexanal, 2-octenal, 1-octen-3-one, octen-3-ol, 1,5-octadien-3-ol, 2,5-octadien-1-ol, 2-nonenal and 2,6-nonadienal. The origin of these volatile compounds has been demonstrated to be associated with

lipoxygenase activity in polyunsaturated fatty acids (57,165-167). The generation of such flavors can be suppressed by flavonoids specific in lipoxygenase activity (57,165). Lipoxygenase activity was reported to be associated with the generation of flavors, 5,8,11-tetradecatrien-2-one, in heated shrimp (168). Its activity also helped to generate surimi and other fresh seafood flavors (55).

The characteristic aromas of fresh fish vary among different species (164). Nine-carbon aldehydes are present in freshwater fish whereas eight-carbon alcohols occur in some species of freshwater and also saltwater fish (84,85,163,164). The nine-carbon carbonyl compounds appeared to be associated with species inhabiting cold waters. These species also appear to be more vulnerable toward handling damage than species that do not contain these compounds (164).

Animal Tissues. Although there is no direct correlation between lipoxygenase activity and flavor generation in muscle foods, the potential of lipoxygenase activity in causing flavors and off-flavors should not be deemphasized. Hydroperoxides of fatty acids are unstable and can be further metabolized into various biological active compounds. The other route is that they may become decomposed thermally or via metal ion catalysis to generate various alkyl and alkoxy radicals. These free radicals may react with hydrogen or hydroxyl radicals to generate various flavor compounds, or they may attack other available compounds resulting in autoxidation and development of off-flavors and other quality deterioration (169).

Physiological Roles of Lipoxygenase Pathway

In Animal Tissues. Physiological roles for some animal lipoxygenase metabolites have been widely studied. Lipid hydroperoxide products of lipoxygenase are potent inhibitors specifically of prostacyclin synthetase (170). This indicates that lipoxygenase activity could significantly alter the balance between antagonistic prostaglandins. 5-Lipoxygenases are involved in the biosynthesis of bioactive oxeicosanoids, such as leukotriene. Leukotrienes have been reported to be chemotaxins and inflammatory mediators of human neutrophils (171,172) and probably in allergic brochopasm (47). 12-Lipoxygenase activity was reported to modulate intercellular interactions (173,174). 15-Lipoxygenase plays a key role in the maturation of reticulocytes (48).

In Plant Tissues. Several investigators have observed an increase in lipoxygenase activity during seed germination and early seedling growth (78,175-177). Its activity in germinating seedlings accelerates the disruption of cellular membranes and facilitates the transport of storage products to the developing embryos.

Lipoxygenase is involved in the synthesis of jasmonic acid which has been reported to promote plant cell senescence and inhibit growth (178-180). Jasmonic acid has growth-retarding properties in plant tissues similar to those of abscisic

acid. Lipoxygenase may be involved in the production of ethylene (181-183) which initiates fruit ripening and regulates many aspects of plant growth, development and senescence, although other evidence does not support a role for lipoxygenase in the synthesis of ethylene (184-186).

Lipoxygenase-mediated lipid oxidation has been postulated to be responsible for the membrane damage observed associated with infection in potato tubers (187) and tobacco leaves (188). The resulting hydroperoxy radicals and some of the secondary volatile oxidation products are toxic to plant pests (75,189). The increase in lipoxygenase activity has generally been much larger in resistant plants than in susceptible ones. Additionally lipoxygenase catalyzes the production of traumatin (12-*oxo-trans*-dodecenoic acid) in wounded plant tissues. Traumatin was reported to be involved in wound healing in plant tissues (190).

Control of Lipoxygenase Activity

Stability of Lipoxygenase. Lipoxygenase activity is usually inhibited by the presence of hydrogen peroxide (66,191) or by its oxygenated substrate (192,193). The self-inactivation of lipoxygenase may be caused by oxidation and accumulation of excess amounts of enzyme-generated hydroperoxides (194). The stability of enzyme can be significantly improved by the addition of thiols such as glutathione and 2-mercaptoethanol (56,195). The stabilization effect may be ascribed to the maintenance of essential sulfhydryl groups in lipoxygenase in the reduced state and minimize self-inactivation (196). A critical role of sulfhydryl groups for enzyme activity has also been reported in lipoxygenases from platelet (38), soybean (197) and guinea pig skin (198). However the requirement of low levels of hydroperoxides (<10nM) for activation of lipoxygenase has been reported by many animal and plant tissues (63).

Control by Free Radical and Metal Chelation Mechanisms. It is well documented that iron-oxygen complex and free radicals are involved in the catalytic cycle of many lipoxygenase activities (48,75,199). Free radical quenching and metal chelating capabilities of flavonoids have been attributed to control lipoxygenase pathways (200-202). Traditional antioxidants such as BHA, PG and NDGA (nordihydroguaiaretic acid) inhibit lipoxygenases from various sources while BHT does not (48,203-205). These findings suggest that the oxidation potential of an antioxidant does not predict its inhibitory potency on lipoxygenase. The effectiveness of such compounds in the inhibition of lipoxygenase comes from their structure and accessibility toward the catalytic site in the enzyme (48,205).

Quercetin functions as a hydrogen donor and is an inhibitor of soybean lipoxygenase-dependent oxidation of linoleic acid (206,207). Kaempferol, caffeic acid and chlorogenic acid are effective inhibitors of soybean lipoxygenase (208). Esculetin, with a catechol structure, is a potent inhibitor of lipoxygenases (205,209). Glycosidation at one of the hydroxy groups of the catechol structure converts esculetin to esculin, which is much less in inhibitory potency on

lipoxygenase (205). Flavonoids with a catechol structure can form complexes with ferric ion in the active site of 12- and 5-lipoxygenases and cause inhibition (86,198,210). Pathophysiology and the reaction mechanism of flavonoids toward lipid oxidation needs to be further understood before its extensive use in foods.

Heat Stability. There are two stages during the inactivation of purified pea lipoxygenase at 70°C (211). The first stage was dependent on the concentration of linoleic acid hydroperoxide. This is followed by a slow, first-order inactivation which is independent of hydroperoxides. Inactivation of purified soybean lipoxygenase follows first-order kinetics at 65-77.5°C (212). Resistance to heat inactivation was in the order of soybean isozyme-1 > -2 > -3 (213). The presence of other soybean constituents enhances the stability of the enzyme. Purified lipoxygenase from potato tubers follows a first-order kinetics of inactivation at 50-60°C while crude enzyme preparations and reconstituted isoenzyme mixture followed a second-order reaction (214). Fish lipoxygenase is inactive at 50-60°C (56). Microwave heating was explored to inactivate lipoxygenase in soybean (215) and wing bean (216). Soaking of the seeds prior to microwave heating considerably reduced the heating time required to inactivate the enzyme (216).

Literature Cited

1. Whitaker, J.R. In *Oxidative Enzymes in Foods*; Robinson, D.S.; Eskin, N.A.M., Ed.; Elsevier Sci. Pub. Ltd.: London, 1991, pp 175-215.
2. O'Connor, T.P.; O'Brien, N.M. In *Food Enzymology*; Fox, P.F., Ed.; Elsevier Sci. Pub. Ltd.: London, 1991, Vol. 1; pp 337-372.
3. Gardner, H.W. In *Advances in Cereal Science and Technology*; Pomeranz, Y., Ed.; Amer. Assoc. Cereal Chem.: St. Paul, MN, 1989, Vol. IX; pp 161-215.
4. Vermooy-Gerritsen, M.; Leunissen, J.L.M.; Veldink, G.A.; Vliegthart, J.F.G. *Plant Physiol.* **1984**, *76*, 1070-1079.
5. Vermooy-Gerritsen, M.; Bos, A.L.M.; Veldink, G.A.; Vliegthart, J.F.G. *Plant Physiol.* **1983**, *73*, 262-267.
6. Hildebrand, D.F.; Hamilton-Kemp, T.R.; Legg, C.S.; Bookjans, G. *Curr. Topics Plant Biochem. Physiol.* **1988**, *7*, 201-219.
7. Christopher, J.P.; Pistorius, E.K.; Axelrod, B. *Biochim. Biophys. Acta* **1972**, *284*, 54-62.
8. Shibata, D.; Steczko, J.; Dixon, J.E.; Hermodson, M.; Yazdanparast, R.; Axelrod, B. *J. Biol. Chem.* **1987**, *262*, 1080-1085.
9. Yenofsky, R.L.; Fine, M.; Liu, C. *Mol. Gen. Genet.* **1988**, *211*, 215-222.
10. Axelrod, B.; Cheesbrough, T.M.; Laasko, S. *Met. Enzymol.* **1981**, *71*, 441-451.
11. Yoon, S.; Klein, B.P. *J. Agric. Food. Chem.* **1979**, *27*, 955-962.
12. Antis, P.J.P.; Friend, J. *Phytochem.* **1974**, *13*, 567-573.
13. Reynolds, P.A. *Diss. Abst. Int. B.* **1982**, *42*, 3623.
14. Chang, P.R.Q.; McCurdy, A.R. *Can. Inst. Food. Sci. Technol. J.* **1985**, *18*, 94-96.

15. Guss, P.L.; Richardson, T.; Stahmann, M.A. *Cereal Chem.* **1967**, *44*, 607-610.
16. Yabuuchi, S. *Agric. Biol. Chem.* **1976**, *40*, 1987-1992.
17. Vick, B.A.; Zimmerman, D.C. *Plant Physiol.* **1982**, *69*, 1103-1108.
18. Shastry, B.; Raghavendra Rao, M. *Cereal Chem.* **1975**, *52*, 597-603.
19. Heimann, W.; Klaiber, V. *Lebensm. Unters. Forsch.* **1977**, *165*, 131-136.
20. Heimann, W.; Dresen, P.; Schreier, P. *Lebensm. Unters. Forsch.* **1973**, *152*, 147-151.
21. Heimann, W.; Dresen, P.; Klaiber, V. *Lebensm. Unters. Forsch.* **1973**, *153*, 1-5.
22. Averman, L.Y.; Popov, M.P.; Dubtsov, G.G.; Samsonov, M.M. *Prikl. Biokhim. Mikrobiol.* **1971**, *7*, 678-681.
23. Colas, A.; Chargelegue, A. *Ann. Technol. Agric.* **1974**, *23*, 323-334.
24. Goldstein, A.H.; Anderson, J.O.; McDaniel, R.G. *Plant Physiol.* **1980**, *66*, 488-493.
25. Haydar, M.; Hadziyev, J. *J. Sci. Food. Agric.* **1973**, *24*, 1039-1053.
26. Douillard, R.; Bergeron, E. *Physiol. Plant.* **1981**, *51*, 335-338.
27. Douillard, R.; Bergeron, E. *Physiol. Veg.* **1979**, *17*, 457-476.
28. Wardale, D.A.; Galliard, T. *Phytochem.* **1977**, *16*, 333-338.
29. Wardale, D.A.; Galliard, T. *Phytochem.* **1975**, *14*, 2323-2329.
30. Williams, D.C.; Lim, M.H.; Chen, A.O.; Pangborn, R.M.; Whitaker, J.R. *Food Technol.* **1986**, *40*, 130-140.
31. Velasco, P.J.; Lim, M.H.; Pangborn, R.M.; Whitaker, J.R. *Biotech. Appl. Biochem.* **1989**, *11*, 118-127.
32. Lim, M.H.; Velasco, P.J.; Pangborn, R.M.; Whitaker, J.R. *ACS Symp. Ser.* **1989**, *405*, 72-83.
33. Hansson, G.; Malmsten, C.; Raadmark, O. In *Prostaglandins and Related Substances*; Pace-Asciak, C.R.; Granstrom, E. Ed.; Elsevier Sci. Pub. Ltd.: Amsterdam, 1983, pp 127-169.
34. Hamberg, M.; Samuelsson, B. *Proc. Natl. Acad. Sci. USA* **1974**, *71*, 3400-3404.
35. Nutgeren, D.H. *Biochim. Biophys. Acta* **1975**, *380*, 299-307.
36. Hamberg, M. *Biochim. Biophys. Acta* **1976**, *431*, 651-654.
37. Pace-Asciak, C.R.; Mizuno, K.; Yamamoto, S. *Biochim. Biophys. Acta* **1981**, *665*, 352-354.
38. Ruzicka, T.; Vitto, A.; Printz, M.P. *Biochim. Biophys. Acta* **1983**, *751*, 368-374.
39. Powell, W.S.; Gravelle, F. *Biochim. Biophys. Acta* **1985**, *835*, 201-211.
40. Corey, E.J.; Shih, C.; Cashman, J.R. *Proc. Natl. Acad. Sci. USA* **1983**, *80*, 3581-3584.
41. Alvadano, M.I.; Sprecher, H. *J. Biol. Chem.* **1983**, *258*, 9339-9343.
42. Borgeat, P.; Picard, S.; Vallerand, P.; Sirois, P. *Prostaglandins Med.* **1981**, *6*, 557-570.
43. Pace-Asciak, C.P. *J. Biol. Chem.* **1984**, *259*, 8332-8337.
44. Pace-Asciak, C.P.; Granstrom, E.; Samuelsson, B. *J. Biol. Chem.* **1983**, *258*, 6835-6840.
45. Bryant, R.W.; Bailey, J.M. *Prostaglandins* **1979**, *17*, 9-18.

46. Borgeat, P.; Hamberg, M.; Samuelsson, B. *J. Biol. Chem.* **1976**, *251*, 7816-7820.
47. Drazen, J.M.; Austen, K.F.; Lewis, R.A. *Proc. Natl. Acad. Sci. USA* **1980**, *77*, 4354-4358.
48. Schewe, T.; Rapoport, S.M.; Kuhn, H. *Adv. Enzymol.* **1986**, *58*, 191-273.
49. Turk, J.; Maas, R.L.; Brash, A.R.; Robert II, L.J.; Oates, J.A. *J. Biol. Chem.* **1982**, *257*, 7068-7076.
50. Dutilh, C.E.; Haddeman, E.; Jouvenaz, G.H.; Ten Hoor, F.; Nugteren, D.H. *Lipids* **1979**, *14*, 241-246.
51. Grossman, S.; Bergman, M.; Sklan, D. *J. Agric. Food. Chem.* **1988**, *36*, 1268-1270.
52. Sklan, D.; Tenne, Z. *Poult. Sci.* **1984**, *63*, 76.
53. Tsukuda, N. *Bull. Jap. Soc. Sci. Fish.* **1970**, *36*, 725-733.
54. German, J.B.; Kinsella, J.E. *J. Agric. Fd. Chem.* **1985**, *33*, 680-683.
55. Josephson, D.B.; Lindsay, R.C. In *Biogeneration of Aromas*; Parliment, T.H.; Croteau, R., Eds., ACS Symp. Ser. No. 317; American Chemical Society: Washington, DC, 1986, pp 200-217.
56. Hsieh, R.J.; German, J.B.; Kinsella, J.E. *J. Agric. Food. Chem.* **1988**, *36*, 680-685.
57. Hsieh, R.J.; Kinsella, J.E. *J. Agric. Food. Chem.* **1989**, *37*, 279-286.
58. German, J.B.; Creveling, R.K. *J. Agric. Food. Chem.* **1990**, *38*, 2144-2147.
59. Mohri, S.; Cho, S.; Endo, Y.; Fujimoto, K. *Agric. Biol. Chem.* **1990**, *54*, 1889-1891.
60. Wang, Y.J.; Miller, L.A.; Addis, P.B. *J. Amer. Oil Chem. Soc.* **1991**, *68*, 752-757.
61. Mohri, S.; Cho, S.; Endo, Y.; Fujimoto, K. *J. Agric. Food. Chem.* **1992**, *40*, 573-576.
62. Winkler, M.; Pilhofer, G.; German, J.B. *J. Food. Biochem.* **1991**, *15*, 437-448.
63. Papatheofanis, F.J.; Lands, W.E.M. In *Biochemistry of Arachidonic Acid Metabolites*; Lands, W.E.M., Ed.; Nijhoff: The Hague, 1985, p 9.
64. Vliegthart, J.F.G.; Veldink, G.A.; Boldingh, J. *J. Agric. Food. Chem.* **1979**, *27*, 623.
65. Klein, B.P.; King, D.; Grossman, S. *Adv. Free Radical Biol. Med.* **1985**, *1*, 309-343.
66. Egmond, M.R.; Finazzi-Agro, A.; Fasella, P.M.; Veldink, G.A.; Vliegthart, J.F.G. *Biochim. Biophys. Acta* **1975**, *397*, 43-49.
67. de Groot, J.J.; Garssen, G.J.; Veldink, G.A.; Vliegthart, J.F.G.; Boldingh, J. *FEBS Letter* **1975**, *56*, 50-54.
68. Rapport, S.M.; Hartel, B.; Hausdorg, G. *Eur. J. Biochem.* **1984**, *139*, 573-576.
69. Zakut, R.; Grossman, S.; Pinsky, A.; Wilchek, M. *FEBS Lett.* **1976**, *71*, 107-110.
70. Finazzi-Agro, A.; Avigliano, L.; Veldink, G.A.; Vliegthart, J.F.G.; Boldingh, J. *Biochim. Biophys. Acta* **1973**, *326*, 462-470.
71. Zimmerman, G.; Snyder, H. *J. Agric. Food. Chem.* **1974**, *22*, 802-805.

72. Den, T. van, Raymundo, L.C. and Mendoza, E.M.T. *Food Chem.* **1982**, *8*, 277-89.
73. Smith, W.L.; Lands, W.E.M. *J. Biol. Chem.* **1972**, *247*, 1038-1047.
74. Sekiya, J.; Aoshima, H.; Kajiwara, T.; Togo, T.; Hatanaka, A. *Agric. Biol. Chem.* **1977**, *41*, 827-832.
75. Hildebrand, D.F. *Physiol. Plant.* **1989**, *76*, 249-253.
76. Veldink, G.A.; Vliegthart, J.F.G.; Boldingh, J. *Prog. Chem. Fats Other Lipids* **1977**, *15*, 131-166.
77. Regdel, D.; Schewe, T.; Rapport, S.M. *Biomed. Biochim. Acta* **1985**, *44*, 1411.
78. Guss, P.L.; Richardson, T.; Stahmann, M.A. *J. Amer. Oil Chem. Soc.* **1968**, *45*, 272.
79. Jung, G.; Yang, D.C.; Nakao, A. *Biochem. Biophys. Res. Commun.* **1985**, *130*, 559.
80. Bild, G.; Ramadoss, C.; Axelrod, B. *Lipids* **1977**, *12*, 732-735.
81. Hamberg, M. *Anal. Biochem.* **1971**, *43*, 515-526.
82. Kuhn, H.; Pliquet, F.; Wunderlich, S.; Schewe, T.; Krause, W. *Biochim. Biophys. Acta* **1983**, *735*, 283.
83. Corey, E.J.; Albright, J.O.; Barton, A.E. *J. Amer. Chem. Soc.* **1980**, *102*, 1435.
84. Matthew, J.A.; Chan, H.W.-S.; Galliard, T. *Lipids* **1977**, *12*, 324.
85. Kuhn, H.; Heydeck, D.; Wiesner, R.; Schewe, T. *Biochim. Biophys. Acta* **1985**, *830*, 25-29.
86. Hamberg, M. *Prostaglandins Leukotrienes Med.* **1984**, *13*, 27.
87. Shimizu, T.; Radmark, O.; Samuelsson, B. *Proc. Natl. Acad. Sci. USA* **1984**, *81*, 689.
88. Hamberg, M. *Biochem. Biophys. Res. Commun.* **1983**, *117*, 593.
89. Bryant, R.W.; Bailey, J.M.; Schewe, T.; Rapport, S.M. *J. Biol. Chem.* **1982**, *257*, 6050-6055.
90. Vick, B.A.; Zimmerman, D.C. *Plant Physiol.* **1976**, *57*, 780-788.
91. Hatanaka, A.; Kajiwara, T.; Sekiya, J.; Fujimara, K. *Agric. Biol. Chem.* **1979**, *43*, 175-176.
92. Sekiya, J.; Kajiwara, T.; Hatanaka, A. *Agric. Biol. Chem.* **1979**, *43*, 969-980.
93. Kim, I.S.; Grosch, W. *J. Agric. Food. Chem.* **1981**, *29*, 1220-1225.
94. Schreier, P.; Lorenz, G. *Z. Naturforsch* **1982**, *37c*, 165-73.
95. Galliard, T.; Philips, D.R.; Reynolds, J. *Biochim. Biophys. Acta* **1976**, *441*, 181-192.
96. Galliard, T.; Philips, D.R. *Biochim. Biophys. Acta* **1976**, *431*, 278-287.
97. Phillips, D.R.; Matthew, J.A.; Reynolds, J.; Fenwick, G.R. *Phytochem.* **1979**, *18*, 401-404.
98. Hatanaka, A.; Kajiwara, T.; Sekiya, J. *Chem. Phys. Lipids* **1987**, *44*, 341-361.
99. Vick, B.A.; Zimmerman, D.C. *Plant Physiol.* **1987**, *85*, 1073-1078.
100. Matoba, T.; Hidaka, H.; Kitamura, K.; Kaizuma, N.; Kito, M. *J. Agric. Food. Chem.* **1985**, *33*, 856-858.

101. Matoba, T.; Hidaka, H.; Narita, H.; Kitamura, K.; Kaizuma, N.; Kito, M. *J. Agric. Food. Chem.* **1985**, *33*, 852-855.
102. Olias, J.; Rios, J.J.; Valle, M.; Zamora, R.; Sanz, L.C.; Axelord, B. *J. Agric. Food. Chem.* **1990**, *38*, 624-630.
103. Mattick, L.R.; Hand, D.R. *J. Agric. Food. Chem.* **1969**, *17*, 15-17.
104. Rackis, J.J.; Sessa, D.J.; Honig, D.H. *J. Amer. Oil Chem. Soc.* **1979**, *56*, 262-271.
105. Gardner, H.W. *J. Agric. Food. Chem.* **1979**, *27*, 220-229.
106. Matoba, T.; Sakurai, A.; Taninoki, N.; Saitoh, T.; Kariya, F.; Kuwahata, M.; Yukawa, N.; Fujino, S.; Hasegawa, K. *J. Food Sci.* **1989**, *54*, 1607-1610.
107. Tihanyl, K.; Talbot, B.; Brzezinski, R.; Thirion, J.P. *Phytochem.* **1989**, *28*, 1335-1338.
108. Yu, M.; Olson, L.; Salunkhe, D. *Phytochem.* **1967**, *6*, 1457-1467.
109. Dalal, K.B.; Salunkhe, D.K.; Olson, L.E.; Do, J.Y.; Yu, M.H. *Plant Cell Physiol.* **1968**, *9*, 389-400.
110. Kazeniac, S.J.; Hall, R.M. *J. Food Sci.* **1970**, *35*, 519-530.
111. Jadhav, S.; Singh, B.; Salunkhe, D.K. *Plant Cell Physiol.* **1972**, *13*, 449-459.
112. Stone, E.J.; Hall, R.M.; Kazeniac, S.J. *J. Food Sci.* **1975**, *40*, 1138-1141.
113. Zamora, R.; Olias, J.; Mesias, J. *Phytochem.* **1987**, *26*, 345-347.
114. Galliard, T.; Matthew, J.A.; Wright, A.J.; Fishwick, M.J. *J. Sci. Food Agric.* **1977**, *28*, 863-868.
115. Schreier, P. In *Quality in Stored and Processed Vegetables and Fruits*; Goodenough, P.W.; Atkins, R.K. Ed.; Acad. Press: New York, 1981, pp 355-471.
116. Buttery, R.G.; Teranishi, R.; Ling, L.C.; Turnbaugh, J.G. *J. Agric. Food. Chem.* **1990**, *38*, 336-340.
117. Schormuller, J.; Kochmann, H.J. *Leb. Unters. Forsch.* **1969**, *136*, 271-278.
118. Schreier, P.; Drawert, F.; Junker, A. *Leb. Unters. Forsch.* **1977**, *165*, 23-27.
119. Tressl, R.; Bahri, D.; Engel, K.H. *ACS Symp. Ser.* **1981**, *170*, 213-232.
120. Kim, I.S.; Grosch, W. *J. Agric. Food. Chem.* **1979**, *27*, 243-245.
121. Feys, M.; Naessens, W.; Tobback, P.; Maes, E. *Phytochem.* **1980**, *19*, 1009-1011.
122. Flath, R.A.; Black, D.R.; Guadagni, D.G.; McFadden, W.H.; Schultz, T.H. *J. Agric. Food. Chem.* **1967**, *15*, 29-33.
123. Drawert, F.; Tressl, R.; Heimann, W.; Emberger, R.; Speck, M. *Chem. Mikrobiol. Technol. Lebensm.* **1973**, *2*, 10-22.
124. Schreier, P.; Drawert, F.; Schmid, M. *J. Sci. Food Agric.* **1978**, *29*, 728-736.
125. Wardale, D.A.; Lambert, E.A. *Phytochem.* **1980**, *19*, 1013-1016.
126. Forss, D.A.; Dunstone, E.A.; Ramshaw, E.H.; Stark, W. *J. Food. Sci.* **1962**, *27*, 90-93.
127. Fleming, H.P.; Cobb, W.Y.; Etchells, J.L.; Bell, T.A. *J. Food. Sci.* **1968**, *33*, 572-576.
128. Kemp, T.R. *J. Amer. Oil Chem. Soc.* **1975**, *52*, 300-302.
129. Galliard, T.; Matthew, J.A. *Biochim. Biophys. Acta* **1976**, *424*, 26-35.

130. Pyysalo, H.; Suihko, M. *Lebensm. Wiss. Technol.* **1976**, *9*, 371-373.
131. Wurzenburger, M.; Grosch, W. *Biochim. Biophys. Acta* **1984**, *794*, 25-30.
132. Mosandl, A.; Heusinger, G.; Gessner, M. *J. Agric. Food Chem.* **1986**, *34*, 119-122.
133. Fischer, K.; Grosch, W. *Lebensm. Wiss. Technol.* **1987**, *20*, 233-236.
134. Maga, J.A. *J. Agric. Food Chem.* **1981**, *29*, 1-4.
135. Maga, J.A. *J. Food Proc. Pres.* **1981**, *5*, 95-101.
136. Tressl, R.; Drawert, T. *J. Agric. Food. Chem.* **1973**, *21*, 560-565.
137. Eriksson, C.E. *J. Food. Sci.* **1967**, *32*, 438-441.
138. Henderson, H.M.; Kanhai, J.; Eskin, N.A. *Food Chem.* **1984**, *13*, 129-137.
139. Mtebe, K.; Gordon, M.H. *Food Chem.* **1987**, *23*, 175-182.
140. de Lumen, B.O.; Stone, E.J.; Kazeniac, S.J.; Forsynthe, R.H. *J. Food. Sci.* **1978**, *43*, 698-702.
141. Adams, J.B.; Ongley, M.H. *Food Chem.* **1989**, *31*, 57-71.
142. Kermasha, S.; Metche, M. *J. Sci. Food Agric.* **1987**, *40*, 1-10.
143. Adams, J.B. *Campden Food and Drink Research Association Technical Memorandum No. 322*; CFDR: Gloucestershire, UK, 1983
144. Galliard, T.; Phillips, D.R. *Biochem. J.* **1971**, *124*, 431-438.
145. Berkley, H.D.; Galliard, T. *Phytochem.* **1976**, *15*, 1481-1485.
146. Galliard, T. *J. Sci. Food Agric.* **1973**, *24*, 617-622.
147. Grosch, W.; Laskawy, G.; Weber, F. *J. Agric. Food. Chem.* **1976**, *24*, 456-459.
148. Josephson, D.B.; Lindsay, R.C. *J. Food Sci.* **1987**, *52*, 328-331.
149. Cayrel, A.; Crouzet, J.; Chen, H.W.S.; Price, K.R. *Amer. J. Enol. Vitic.* **1983**, *34*, 77-82.
150. Galliard, T.; Matthew, J.A. *Phytochem.* **1977**, *16*, 339-343.
151. Valentin, G.; Crouzet, J. In *Actualites Oenologiques VI Symposium International d'Oenologie de Bordeaux*: Dunod, Paris, 1989.
152. Crouzet, J.; Nocolas, M.; Molina, J.; Valentin, G. In *Progress in Flavor Research*; Adda, J. Ed.; Elsevier Sci. Pub.: Amsterdam, The Netherlands, 1985, pp 401-408.
153. Wagenknecht, A.C. *Food Res.* **1959**, *24*, 539-547.
154. Rhee, K.S.; Watts, B.M. *J. Fd. Sci.* **1966**, *31*, 664-668.
155. Leu, K. *Lebensm. Wiss. Technol.* **1974**, *7*, 98-100.
156. Hatanaka, A.; kajiwara, T.; Sekiya, J. *Phytochem.* **1976**, *15*, 1125-1126.
157. Sekiya, J.; Numa, S.; Kajiwara, T.; Hatanaka, A. *Agric. Biol. Chem.* **1976**, *40*, 185-190.
158. Hatanaka, A.; Kajiwara, T.; Sekiya, J.; Imoto, M.; Inouye, S. *Plant Cell Physiol.* **1982**, *23*, 91-99.
159. Takeo, T.; Tsushida, T. *Phytochem.* **1980**, *19*, 2521-2522.
160. McGill, A.S.; Hardy, R.; Gunstone, F.D. *J. Sci. Food. Agric.* **1977**, *28*, 200.
161. Swoboda, P.A.T.; Peers, K.E. *J. Sci. Food. Agric.* **1977**, *28*, 1010.
162. Ke, P.J.; Ackman, R.G.; Linke, B.A. *J. Food. Technol.* **1975**, *12*, 37.
163. Josephson, D.B.; Lindsay, R.C.; Stuibler, D.A. *J. Agric. Food. Chem.* **1983**, *31*, 326-330.

164. Josephson, D.B.; Lindsay, R.C.; Stuiber, D.A. *J. Agric. Food. Chem.* **1984**, *32*, 1344-1347.
165. German, J.B.; Berger, R.G.; Drawert, F. *Chem. Mikrobiol. Technol. Lebensm.* **1991**, *13*, 19-24.
166. Zhang, C.; Hirano, T.; Suzuki, T.; Shirai, T. *Nippon Suisan Gakkaishi* **1992**, *58*, 559-565.
167. Josephson, D.B.; Lindsay, R.C.; Stuiber, D.A. *J. Food. Sci.* **1987**, *52*, 596-600.
168. Kuo, J.; Pan, B.S. *Agric. Biol. Chem.* **1990**, *55*, 847-848.
169. Frankel, E.N. *J. Amer. Oil Chem. Soc.* **1984**, *61*, 1908.
170. Salmon, J.A.; Smith, D.R.; Flower, R.J.; Moncada, S.; Vane, J. *Biochim. Biophys. Acta* **1978**, *52*, 250.
171. Goldman, D.W.; Pickett, W.C.; Goetzel, E.J. *Biochem. Biophys. Res. Commun.* **1983**, *117*, 282-288.
172. Lee, T.H.; Sethi, T.; Crea, A.E.; Peters, W.; Arm, J.P.; Horton, C.E.; Walport, M.J.; Spur, B.W. *Clin. Sci.* **1988**, *74*, 467-475.
173. Gale, P.H.; Egan, R.W. In *Free Radicals in Biology*; Pryor, W.A. Ed.; Acad. Press: N.Y. **1983**, Vol. 6, p. 1.
174. Kulmacz, R.J.; Lands, W.E.M. *Prostaglandins* **1983**, *25*, 531-539.
175. Hildebrand, D.F.; Hymowitz, T. *Bot. Gaz.* **1983**, *144*, 212-216.
176. Peterman, T.K.; Siedlow, J.N. *Plant Physiol.* **1985**, *78*, 690-695.
177. Ohta, H.; Ida, S.; Mikami, B.; Morita, Y. *Agric. Biol. Chem.* **1986**, *50*, 3165-3171.
178. Dathé, W.; Ronsch, H.; Preiss, A.; Schade, W.; Sembdner, G.; Schreiber, K. *Planta* **1981**, *153*, 530-535.
179. Vick, B.A.; Zimmerman, D.C. In *Biochemistry of Plants: A Comprehensive Treatise*; Stumpf, P.K. Ed.; Acad. Press: Orlando, FL, **1987**, Vol. 9, pp 53-90.
180. Vick, B.A.; Zimmerman, D.C. *Plant Physiol.* **1984**, *75*, 458-461.
181. Wooltorton, L.S.C.; Jones, J.D.; Hulme, A.C. *Nature* **1965**, *207*, 99-101.
182. Legge, R.L.; Thompson, J.E. *Phytochem.* **1983**, *22*, 2161-2166.
183. Bousquet, J.F.; Thimann, K.V. *Proc. Natl. Acad. Sci. USA* **1984**, *81*, 1724-1727.
184. Pirrung, M.C. *Biochem.* **1986**, *25*, 114-119.
185. Mack, A.J.; Peterman, T.K.; Siedow, J.N. *Isozymes: Current Topics in Biol. Med. Res.* **1987**, *13*, 127-154.
186. Wang, T.T.; Yang, S.F. *Planta* **1987**, *170*, 190-196.
187. Galliard, T. In *Biochemistry of Wounded Plant Tissues*; Kahl, G. Ed.; Watter de Gruyter: Berlin, Germany, **1978**, pp 155-201.
188. Lupu, R.; Grossman, S.; Cohen, Y. *Physiol. Plant Pathol.* **1980**, *16*, 241-248.
189. Hildebrand, D.F.; Rodriguez, J.G.; Brown, G.C.; Luu, K.J.; Volden, C.S. *J. Econ. Entomol.* **1986**, *79*, 1459-1465.
190. Zimmerman, D.C.; Caudron, C.A. *Plant Physiol.* **1979**, *63*, 536-541.
191. Mitsuda, H.; Yasumoto, K.; Yamamoto, A. *Agric. Biol. Chem.* **1967**, *31*, 853-860.

192. Rapport, S.M.; Schewe, T.; Wiesner, R.; Halang, K.W.; Ludwig, P.; Janicke-Hohne, M.; Tannert, C.; Hiebsch, C.; Klatt, D. *Eur. J. Biochem.* **1979**, *96*, 545.
193. German, J.B.; Kinsella, J.E. *Biochim. Biophys. Acta* **1986**, *879*, 378-387.
194. Egan, R.W.; Gale, P.H.; Kuehl, F.A. *J. Biol. Chem.* **1979**, *254*, 3295.
195. Bell, J.G.; Cowey, C.B.; Youngson, A. *Biochim. Biophys. Acta* **1980**, *795*, 91-99.
196. Flohe, L. In *Free Radicals in Biology*; Pryor, W.A. Ed.; Acad. Press: New York, 1982, Vol 5; p 223-249.
197. Siegel, M.I.; McConnell, R.T.; Porter, N.A.; Cuatrecasa, P. *Proc. Natl. Acad. Sci. USA* **1980**, *77*, 308.
198. Spaapen, L.J.M.; Verhagen, J.; Veldink, G.A.; Vliegthart, *Biochim. Biophys. Acta* **1980**, *618*, 153-162.
199. Vliegthart, J.F.G.; Veldink, G.A. In *Free Radicals in Biology*; Pryor, W.A. Ed.; Acad. Press: New York, 1982, Vol. 5; p 29.
200. Baumann, J.; Wurm, G.; Bruchhausen, F.V. *Arch. Pharmacol.* **1980**, *313*, 330.
201. Havsteen, B. *Biochem. Pharmacol.* **1983**, *32*, 1141.
202. Hsieh, R.J.; Kinsella, J.E. *Adv. Food. Nutr. Res.* **1989**, *33*, 233-341.
203. Agradi, E.; Petroni, A.; Socini, A.; Galli, C. *Prostaglandins* **1981**, *22*, 255.
204. van Wauwe, J.; Goossens, J. *Prostaglandins* **1983**, *26*, 725.
205. Hsieh, R.J.; German, J.B.; Kinsella, J.E. *Lipids* **1988**, *23*, 322.
206. Takahama, U. *Photochem. Photobiol.* **1983**, *38*, 363.
207. Takahama, U. *Phytochem.* **1985**, *24*, 1443-1446.
208. King, D.L.; Klein, B.P. *J. Food. Sci.* **1987**, *52*, 220-221.
209. Sekiya, K.; Okuda, H.; Arichi, S. *Biochim. Biophys. Acta* **1982**, *713*, 68.
210. Neichi, T.; Koshihara, Y.; Murota, S. *Biochim. Biophys. Acta* **1983**, *753*, 130.
211. Svensson, S.; Eriksson, C. *Lebensm. Wiss. Tech.* **1972**, *5*, 124-127.
212. Shibasaki, K.; Hukano, S.; Okubo, K. *J. Food. Sci. Tech. (Nihon, Shokuhin Kogyo Gakkai-shi)* **1973**, *20*, 415-420.
213. Ediriweera, N.; Akiyama, Y.; Saio, K. *J. Food. Sci.* **1987**, *52*, 685-690.
214. Park, K.M.; Kim, Y.M.; Lee, C.W. *J. Agric. Food. Chem.* **1988**, *36*, 1012-1015.
215. Wang, S.H.; Toledo, M.C.F. *J. Food. Sci.* **1987**, *52*, 1344-1347.
216. Esaka, M.; Suzuki, K.; Kubota, K. *J. Food. Sci.* **1987**, *52*, 1738-1739.

RECEIVED May 24, 1993

Chapter 4

Volatile Formation by Lipid-Mediated Maillard Reaction in Model Systems

Yuangang Zhang¹, William J. Ritter¹, C. Craig Barker¹, Paul A. Traci¹,
and Chi-Tang Ho²

¹Universal Flavors Corporation, 5600 W. Raymond Street,
Indianapolis, IN 46241

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

Maillard reactions are perhaps the most important set of reactions in the development of cooked food flavors. A lot of research has been carried out to study the mechanism of this reaction in an aqueous system. However, lipids have been recently recognized as important in flavor development in conjunction with Maillard reactions. This paper discusses the volatile flavor formation by lipid-mediated Maillard reactions involving a series of reducing sugars with selected amino acids, such as glycine, proline and cysteine, and peptide glutathione.

Thermal processing of food products provides an essential function for flavor development in addition to ensuring the safety of food. Under thermal processing conditions, a wide range of chemical reactions occurs simultaneously, such as protein denaturation, enzyme deactivation, oxidation of lipid and Maillard reactions, which have substantial effects on the quality of processed food.

The Maillard, or nonenzymatic browning reaction occurs as a result of a condensation reaction between a reducing sugar and the primary amino group from an amino acid, peptide or protein. The initial stage is the formation of a labile *N*-substituted aldoylamine product. The isomerization of the labile compound via Amadori rearrangement gives a stable *N*-substituted deoxyketose, the so-called Amadori rearrangement product (ARP). The following Strecker degradation, retroaldolization, fragmentation and further interaction lead to the formation of various volatile compounds (*1*).

Lipids, on the other hand, have been well-known to contribute to the sensory quality of foods. Similar to the Maillard reaction, they provide food with desirable as well as undesirable flavors. As one of the three major food components coexisting with proteins and carbohydrates, lipids are inevitably involved in thermal interactions during food processing. It moderates the reaction

0097-6156/94/0558-0049\$08.00/0
© 1994 American Chemical Society

environments for the Maillard reaction, such as temperature, water content and substrate concentration, as a reaction medium, such as for deep-fat frying of foods. It can also provide lipid-derived carbonyl compounds as reactive intermediates, and as special component phospholipids can catalyze or modify Maillard products formation (2-6). It is the intent of this paper to upgrade and review such information as the effects of lipids on the Maillard reaction.

Effect of Lipids on the Maillard Reaction as a Medium

Role of Lipids in Deep-fat Frying Flavors. Lipids are commonly used as a frying medium to provide a high temperature for preparation of deep-fat fried food, which involves the continuous submersion of moisture-containing food in oil which has been heated to approximately 180°C. During the frying process, moisture is rapidly evaporated and decomposition of oil occurs.

Table I represents the major volatile decomposition products of frying oil isolated from a Maillard reaction model, which mimics deep-fat frying conditions. Cysteine, proline and lactose were dissolved in water and this solution was injected into cotton balls (80 % water by weight). The balls were fried in corn oil at 170°C for 4 minutes (three batches). Volatiles remaining in the frying oil were isolated by vacuum steam distillation followed by acid-base fractionation and solvent extraction. As shown in Table I, all the volatiles are the lipid derived compounds. The major peak, 2,4-decadienal, which accounted for about 47% of the total volatiles isolated, is a typical oxidation product of linoleate decomposition *via* a 9-hydroperoxide (7). It was identified as a major component of carbonyl compounds in deodorization distillates from cottonseed oil, soybean oil, beef tallow and lard (8). It is potent as a flavor compound and odor quality is best described as deep-fried (8). Surprisingly this percentage is very close to what was reported (46.1%) from a thermal degradation of the isomeric hydroperoxide of linoleate in a model study (7). Hexanal, 2-pentylfuran, and 4,5-epoxy-2-*cis/trans*-decenal are also formed from the same precursor. Other major components, 2-*trans*-decenal and 2-*trans*-undecenal, are among the major oxidation products of oleate (7). The high percentage of 2,4-decadienal probably indicates a rapid oxidation and decomposition of corn oil under frying conditions.

An attempt has also been made to isolate the interaction products of sugars, amino acids and lipids. Only two proline-specific Maillard products, N-methylpyrrole and N-acetylpyrrole (9), were identified in trace amounts. The lack of interaction products between sugars, amino acids and lipids is probably due to two main reasons: 1) the reaction time is short thus limiting volatile compound formation and interactions among them; 2) during the isolation, the predominance of lipid oxidation products may have masked the interaction products which have already formed. Therefore, an extended reaction time and relatively more stable oil is suggested for the model system and new isolation techniques, even partial purification, are necessary for future research.

Table I. Major Volatile Compounds Identified from a "Cotton-ball" Model System Containing Cysteine, Proline and Lactose Fried in Corn Oil

I_B (DB-1)*	Structure	Area %
355	pentanol	0.53
392	hexanal	2.60
551	2- <i>trans</i> -heptenal	4.54
	1-octen-3-ol	0.61
620	2-pentylfuran	1.21
625	2,4-heptadienal	0.51
654	2- <i>trans</i> -octenal	1.21
759	2- <i>trans</i> -nonenal	1.01
863	2- <i>trans</i> -decenal	2.13
894	2- <i>cis</i> -4- <i>trans</i> -decadienal	3.76
897	2- <i>trans</i> -4- <i>trans</i> -decadienal	43.03
967	2- <i>trans</i> -undecenal	3.41
970	4,5-epoxy-2- <i>trans</i> -decenal	1.56
972	4,5-epoxy-2- <i>cis</i> -decenal	0.71
	palmitic acid	6.91
Sum		73.73%

* Linear retention indices on DB-1 column calculated in relation to ethyl ester of carboxylic acids.

Effect of Water Content on Maillard Reactions. Since moisture loss is a typical feature of most thermal processing of foods, further research was conducted to study the water content effect on the volatile formation through thermal reactions. A mixture of 600 mg cysteine or 1500 mg glutathione and 100 g of a solvent mixture consisting of water: diglyme (prepurified diethylene glycol dimethyl ether) in proportions of 0, 20, 50, 80 and 100% water was heated in a closed system. As Table II shows, eleven volatile sulfur-containing compounds were identified as major products from the thermal degradation of cysteine and glutathione. The maximum amount of volatiles was produced between 20% - 50% water content for both of them and the major volatile compounds produced were 3,5-dimethyl-1,2,4-trithiolanes for cysteine and 4,7-dimethyl-1,2,3,5,6-pentathiepanes for glutathione (Figure 1). Above 50% water content, a limited amount of volatile compounds was formed and this may be due to the substrate dilution effect or inhibition of condensation steps. The same effect of high water activity on nonenzymatic browning was observed by other researchers (10-11) and on meat flavor simulation (12). Below a 20% water content, there was a sharp decline in volatile production for both model systems. The effect of water content on the final pressure of the closed systems was observed by Hartman et al. (12). As the water content increases, so do the vapor pressures. At lower water content, pressure was decreased thus reducing the formation of volatiles.

Table II. Sulfur-containing Compounds Identified in Thermal Degradation of Cysteine and Glutathione at 180 °C (Quantity Unit: mg/mol)

Water Content (%) Compounds	100		80		50		20		0	
	Cys	GSH	Cys	GSH	Cys	GSH	Cys	GSH	Cys	GSH
Thiazole	1.3	1.5	4.1	0.4	1.1	0.4	0.3			
2-Methylthiazole	1.4	1.6	7.5	1.0	9.9		29.1	1.7	148.5	2.3
3,5-Dimethyl-1,2,4-trithiolane	267.1	9.0	577.7	25.0	495.4	17.5	643.7	37.1	5.4	2.4
3,5-Dimethyl-1,2,4-trithiolane	320.3	2.7	708.3	26.6	597.7	23.4	780.5	18.9	6.9	1.8
3,6-Dimethyl-1,2,4,5-tetrathiane	9.2		6.6	14.2	5.2	110.7	5.5	86.4		
3,6-Dimethyl-1,2,4,5-tetrathiane	10.9		9.4	20.2	5.6	98.4	6.8	125.1		
4-Methyl-1,2,3,5-tetrathiane	30.3		19.0	1.5	16.5	9.3	30.1	4.0	6.2	
3,5,7-Trimethyl-1,2,4,6-tetrathiepane				1.2	0.7	2.6				10.1
3,5,7-Trimethyl-1,2,4,6-tetrathiepane	5.2		5.6	0.9	3.5		5.0			0.7
4,7-Dimethyl-1,2,3,5,6-pentathiepane	55.9	7.1	101.1	36.0	182.5	496.3	96.6	483.0		3.5
4,7-Dimethyl-1,2,3,5,6-pentathiepane	30.6		64.0		94.3		79.1			

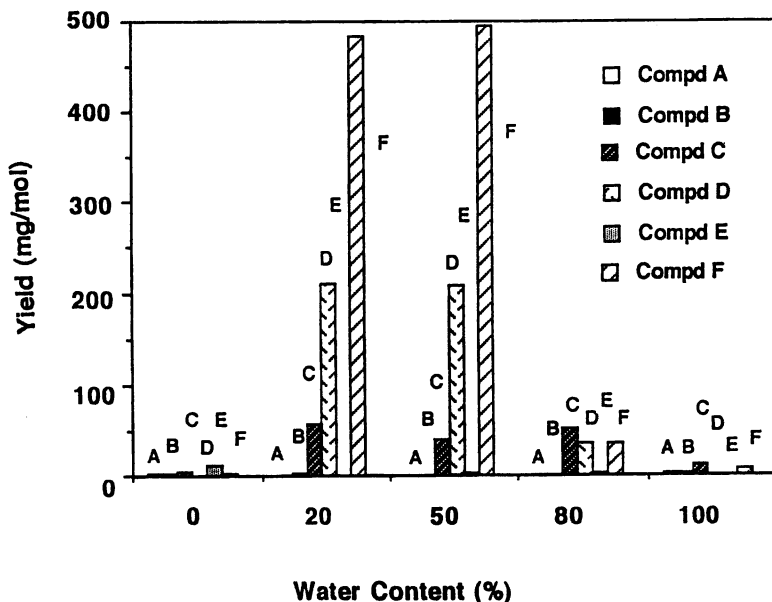


Figure 1. Effect of water content on the formation of sulfur-containing compounds from the thermal degradation of glutathione at 180°C; Compd A: thiazole; B: 2-methylthiazole; C: 3,5-dimethyl-1,2,4-trithiolane; D: 3,6-dimethyl-1,2,4,5-tetrathiane; E: 3,5,7-trimethyl-1,2,4,6-tetrathiepane; F: 4,7-dimethyl-1,2,3,5,6-pentathiepane.

However, different agents used for manipulating the water content affect the maximum volatile yield, also. When a solvent mixture of glycerol and water was used in the reaction of cysteine and 2,5-dimethyl-4-hydroxy-3(2H)-furanone (DMHF) (13), both the best sensory results and volatile production were obtained at 75% water content.

Effect of Lipids on the Sensory Characteristics of Maillard Reaction Products. Oil medium changes the formation of volatile compounds and their sensory characteristics. The sensory characteristics of Maillard reaction products produced by glucose/amino acids model systems are shown in Table III. The sensory descriptors for an aqueous medium represents the products formed by heating a 1:1 molar ratio of glucose and amino acid at 180°C (1), and that in an oil medium represents the products formed by heating a 1:1 w/w ratio of glucose and amino acid at 160°C for 5 minutes. It shows that the flavor of the reaction product was dependent on the amino acid involved.

Table III. Comparison of Sensory Characteristics of Maillard Reaction Products Produced in Aqueous and Oil Media

Amino acids	Aqueous Medium	Oil Medium
Gly	burnt sugar syrupy,	old oil
Val	penetrating chocolate	candy
Leu	burnt cheese	painty
Thr	burnt	coffee
Met	potato	fried vegetable
Phe	violet	rose, honey
Pro	bakery	corn, bready
Lys	bread	corn tortilla
Gln	butter	sweet nut
Cys	meaty	chicken

Lipids, in general, modify the sensory characteristics of the final products by contributing oxidized oil, painty or fried characteristics among them.

The differences in flavor quality of the Maillard products was also reported for a model study of L-cysteine with hydroxyacetone in water, triglyceride and glycerol (14); and cysteine reacted with 2,5-dimethyl-4-hydroxy-3(2H)-furanone (DMHF) in water, glycerol, and a mixture of water and glycerol (13). Dimethylpyrazines were identified as major volatiles in the glycerine and triglyceride systems but not in water. 2-Acetylthiazole in the triglyceride system and 2-acetylthiophene in the glycerol system were the secondary abundant products. The sensory results indicated that the triglyceride and glycerol systems yielded more roasted notes and the water system had more sulfury notes (14). In the reaction of cysteine and DMHF, after one-half hour, the aroma of the product was judged to be roasted, or pot-roasted in an aqueous medium, and roasted and biting in glycerol. The best aroma was produced in a water:glycerol mixture with 75% water, which was described as most the balanced and meaty.

Effect of Lipid-derived Carbonyl Compounds on the Maillard Reaction

Lipids upon autoxidation produce carbonyl compounds which react with amino compounds to form brown, high-molecular weight products. This type of browning has been recently reviewed by Pokorny (15). The discussion here intends to update volatile production from Maillard reactions as affected by lipid-derived carbonyls.

Effect on Pyrazine Formation. The effect of lipids on the formation of pyrazines through Maillard reactions was first reported by Huang et al. (16). As part of the investigation of the effect of lipids on volatile formation in corn-based extrusion products, they heated a mixture composed of zein, corn starch, corn oil and water

from 120°C to 180°C for 30 minutes. Two new dimethyl- *n*-pentyl trisubstituted pyrazines were identified which were believed to be the interaction products of lipids, proteins and carbohydrates. The authors suggested that lipid-derived aldehydes reacted with a Maillard reaction intermediate, a disubstituted 2,5-dihydropyrazine, to form the corresponding alkylated pyrazines. Therefore, the substitution of the long-chain alkyl group in the pyrazine indicated the involvement of lipids or lipid-derived products in the Maillard reactions (17).

A typical reaction scheme of involvement of lipid-derived carbonyl compounds in the formation of pyrazines through a Maillard type reaction was shown in a model system (18) using hydroxyacetone, ammonium acetate and different aliphatic aldehydes. With ammonium acetate used as an ammonia source which represents a protein or an amino acid, hydroxyacetone yields an α,β -dicarbonyl compound from carbohydrate and aldehydes formed by lipid oxidation. The pentyl- or hexyl-substituted pyrazines were formed from the model systems with the pentanal and hexanal, respectively, thus indicating the interaction of lipid with Maillard reaction products. In a recent study on the contribution of peptides to the formation of volatile compounds through the Maillard reaction, Zhang et al. (19) identified several novel alkyl-substituted pyrazines, which could be possibly formed from the same suggested mechanism, but the corresponding aldehydes are the Strecker degradation products from amino acids methionine, leucine, and phenylalanine.

Long-chain alkyl substituted pyrazines have so far been identified in a few food systems including fried chicken (20), baked potato (21,22) and French fries (17).

Effect on the Formation of Sulfur-containing Compounds. Lipid-derived aldehydes participated in the formation of long-chain sulfur-containing heterocyclic compounds in the Maillard reaction when cysteine or cysteine-containing peptide glutathione were present. In a model system mimicking the contribution of lipids to the formation of meat flavors, Zhang et al. (23) conducted an interaction of 2,4-decadienal and cysteine (model system A) or glutathione (model system B). A formation pathway for the selected alkyl-substituted sulfur-containing compounds is presented in Figure 2. The major volatile compounds formed in these systems were 2,4,6-trimethylperhydro-1,3,5-dithiazines (A1, 10.8%) and 2,4,6-trimethylperhydro-1,3,5-thiadiazines (A2, 31.5%), the condensation and oxidation products of acetaldehyde, hydrogen sulfide and ammonia, which are derived from the thermal degradation of cysteine or glutathione. Acetaldehyde, however, could also be derived from lipid oxidation via retroaldolization of 2,4-decadienal (24). Hexanal is also primarily derived from the retroaldolization of 2,4-decadienal. Other lipid-derived aldehydes, pentanal and butanal probably come from the thermal degradation of 2,4-decadienal. Interaction products of all these lipid-derived aldehydes with cysteine degradation products were observed to form the long-chain alkyl substituted heterocyclic sulfur-containing compounds. For example, the formation of 2-methyl-4-butyl-6-pentylperhydro-1,3,5-dithiazine (A4, <0.1%) involves the interaction of pentanal, hexanal, acetaldehyde and hydrogen sulfide. Except for 3-methyl-5-pentyl-1,2,4-trithiolane (A7, 0.5%) identified in

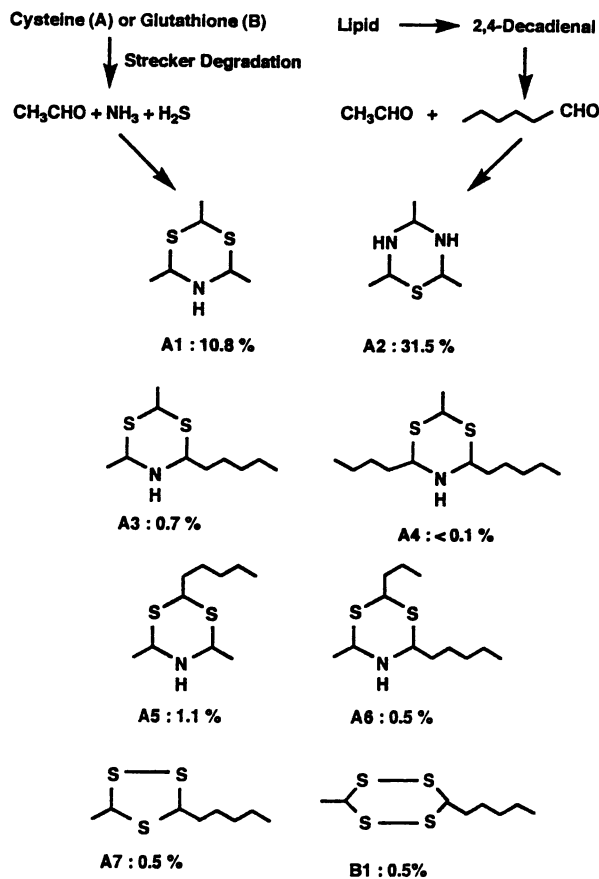


Figure 2. Formation of long chain sulfur-containing heterocyclic compounds from lipid-derived aldehydes.

fried chicken (20), none of the other long-chain alkyl-substituted sulfur-containing heterocyclic compounds shown in Figure 2 have ever been identified in foods.

Another cysteine-specific reaction with lipid-derived aldehydes reported recently by Rizzi et al. (25) also involves the Schiff-base formation mechanism. The reaction starts with formation of a 2-substituted carboxythiazoline by a condensation reaction of Schiff-base derived from cysteine and lipid-derived aldehydes (Figure 3). The length of the 2-substituted alkyl group is dependent on the chain length of lipid-derived aldehydes. This intermediate either undergoes decarboxylation to form a 2-substituted thiazoline or is decomposed into thioaldehydes and vinylamines. Although decarboxylation is not a main pathway at pyrolysis temperature (25), it has been shown to be the pathway for the formation of the major product, 2-methylthiazoline, in cysteine-corn oil frying conditions (26). Thioaldehydes are extremely unstable and predictably undergo

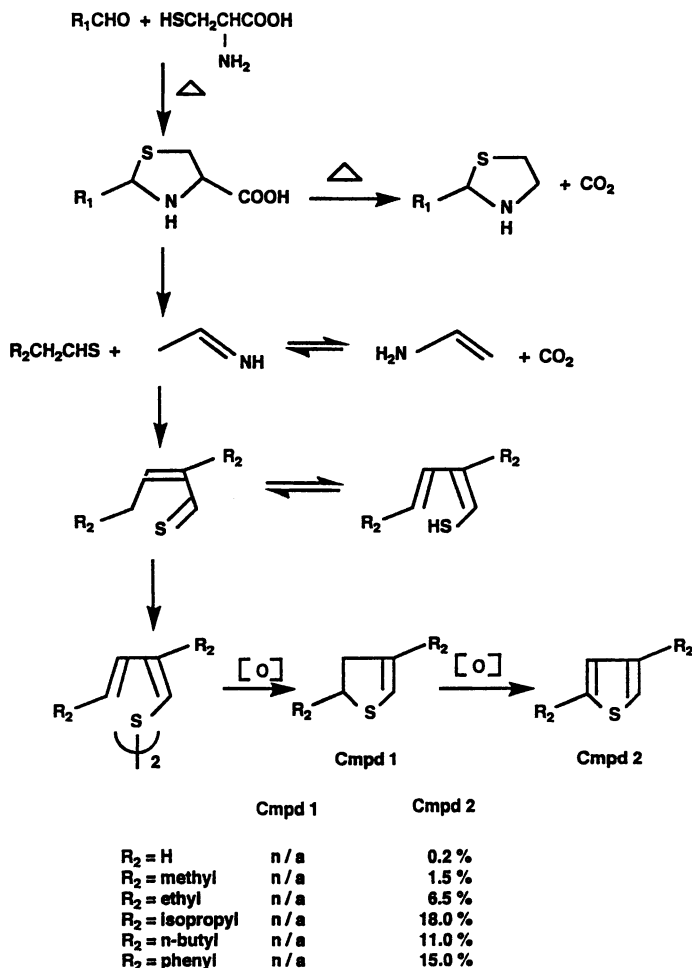


Figure 3. Formation of 2,4-dialkylthiophenes from lipid-derived aldehydes. (Adapted with permission from ref. 25. Copyright 1992 Elsevier Science Publishers BV)

further reactions. The dimer of the thioaldehydes from the aldol condensation may react with oxygen to form a predictably more stable disulfide. However, under pyrolytic conditions such a disulfide can undergo homolytic S-S bond cleavage, cyclization and chain transfer protonation to form dihydrothiophenes. Further oxidation or disproportionation of this dihydrothiophene finally leads to the formation of 2,4-dialkylthiophenes. Five different 2,4-dialkylthiophenes from the corresponding aldehydes with cysteine were characterized and their percentage yield was presented in the same figure. In an independently study, 2,4-dibutylthiophene was identified only in the reaction of 2,4-decadienal with cysteine but not glutathione (27).

On the Formation of other Heterocyclic Flavor Compounds. Lipid-derived aldehydes are directly involved in the Schiff-base formation with the primary free amino group in an amino acid or peptide in Maillard reactions, which affect further Maillard reactions. In the same study, Zhang et al. (27) observed the formation of a larger quantity of 2-pentylpyridine in the model system containing glutathione instead of cysteine. By comparing the volatiles formed from the interaction of 2,4-decadienal with cysteine and glutathione, the authors suggested that 2,4-decadienal was involved in a direct Schiff-base formation with the amino group in cysteine or glutathione, followed by electrocyclic reaction and aromatization to form pentylpyridine. The relatively more stable glutathione rather than cysteine provided more primary free amino groups for the Schiff-base formation thus leading to the production of larger amounts of 2-pentylpyridine. This compound has been identified in numerous thermal reaction model systems containing oil and amino acids (2,5,6,23,26) and in deep-fat fried foods, such as fried chicken (20) and potato (21,22).

Effect of Phospholipid on the Maillard Reaction

Phospholipids have been reported to affect the formation of meat flavors and the formation of Maillard reaction products (4,5,28,29). In general, the addition of a phospholipid to the simple Maillard reaction mixtures exerted some quenching effect on the production of those heterocyclic compounds derived solely from the reaction of amino acids with reducing sugars. For example, the alkylpyrazines were produced in slightly lower concentration in the presence of a phospholipid. This effect was assumed to be due to a preferential reaction of free ammonia or the amino nitrogen with compounds derived from lipids (5). Reactions were carried out to study the effect of phosphoryl residue on the formation of alkylpyrazines. An equimolar mixture of lysine and glucose (0.05 mol) and 200 ml water with or without a phosphorylcholine chloride (PCC) was placed in a closed system and heated in an oil bath at 180° C for 2 hr. The volatile components from the reaction were isolated by simultaneous steam distillation and solvent extraction. Shown in Table IV, the results indicated that the phosphoryl residue did not suppress the formation of alkylpyrazines and their amount increased by approximately five times in the presence of PCC. It was reported that phosphate buffer catalyzes the conversion of glucosylamine into ARP in the Maillard reaction (3), which may explain the increases for the formation of alkylpyrazines in the model system containing PCC. Therefore, the suppression of alkylpyrazine formation involving phospholipids was probably due to the lipid residue instead of the phosphate residue.

Conclusion

In conclusion, lipids modify the environment of the Maillard reaction thus changing the volatile compound formation and sensory quality of the products.

Table IV. Quantitation of Pyrazines from Model Systems (mg/ mol.)

Pyrazines	pH	I _k (DB-1)*	LG	LG/PCC
			9.1	9.8
pyrazine		707	107.3	389.4
methylpyrazine		799	175.6	746.3
2,5-dimethylpyrazine		890	231.3	1905.5
2-ethylpyrazine		893	4.2	12.2
2,3-dimethylpyrazine		897	25.4	243.9
2-ethyl-5-methylpyrazine		982	58.3	433.2
2-ethyl dimethylpyrazine		1119	8.7	42.8
Sum			610.8	3670.0

LG: lysine/glucose; PCC: phosphoryl choline chloride.

*: Linear retention indices on DB-1 column calculated in relative to hydrocarbons.

In addition, the oxidation and decomposition of lipids occur during thermal processing, which provides lipid-derived carbonyl compounds. The carbonyl compounds affect the Maillard reaction by directly reacting with the free amino group available in the system and produce unique long-chain alkyl-substituted heterocyclic compounds.

Literature Cited

- Hodge, J.E. In *The Chemistry and Physiology of Flavors*; Schultz, H.W.; Day, E.A.; Libbey, L.M., Eds.; The AVI Pub.: Westport: CT, 1967, pp.465.
- Farmer, L.J.; Mottram, D.S. *J. Sci. Food Agric.* **1990**, *53*, 505-525.
- Potman, R.P.; van Wijk, T.A. In *Thermal Generation of Aromas*; Parliment, T.H.; McGorin, R.J.; Ho, C.-T., Eds.; ACS Symposium Series 409; American Chemical Society: Washington, DC, 1989; pp. 182-195.
- Mottram, D.S.; Salter, L.J. In *Thermal Generation of Aromas*; Parliment, T.H.; McGorin, R.J.; Ho, C.-T., Eds. ACS Symposium Series 409; American Chemical Society: Washington, DC, 1989; pp. 442-451.
- Whitefield, F.B.; Mottam, D.S.; Brock, S.; Puckey, D.J.; Salter, L.J. *J. Sci. Food Agric.* **1988**, *42*, 261-272.
- Salter, L.J.; Mottram, D.S.; Whitefield, F.B. *J. Sci. Food Agric.* **1988**, *46*, 227-242.
- Frankel, E.N. *Prog. Lipid Res.* **1982**, *22*, 1-23.
- Patton, S.; Barnes, I.J.; Evans, L.E. *J. Amer. Oil Chem. Soc.* **1959**, *36*, 280-283.

9. Tressl, R.; Helak, N.M.; Kersten, E. In *Thermal Generation of Aromas*; Parliment, T.H.; McGorin, R.J.; Ho, C.-T., Eds. ACS Symposium Series 409; American Chemical Society: Washington, DC, 1989; pp. 156-171.
10. Labuza, T.P.; Tannebaum, S.R.; Karel, M. *Food Technol.* **1970**, *24*, 543-550.
11. Eichner, K.; Karel, M. *J. Agric. Food Chem.* **1972**, *20*, 218-221.
12. Hartman, G.J.; Scheide, J.D.; Ho, C.-T. *J. Food Sci.* **1984**, *49*, 607-613.
13. Shu, C.K.; Ho, C.-T., In *Thermal Generation of Aromas*; Parliment, T.H.; McGorin, R.J.; Ho, C.-T., Eds. ACS Symposium Series 409; American Chemical Society: Washington, DC, 1989; pp. 229-241.
14. Okumura, J.; Yanai, T.; Yajima, I.; Hayashi, K. *Agric. Biol. Chem.* **1990**, *54*, 1631-1638.
15. Pokorny, C. *Prog. Food Nutri. Sci.* **1981**, *5*, 421-428.
16. Huang, T.C.; Bruechert, L.J.; Hartman, T.G.; Rosen, R.T.; Ho, C.-T. *J. Agric. Food Chem.* **1987**, *35*, 985-990.
17. Ho, C.-T.; Carlin, J.T. In *Flavor Chemistry: Trends and Developments*; Teranishi, R.; Buttery, R.G.; Shahidi, F., Eds., ACS Symposium Series 388; American Chemical Society: Washington, DC, 1989, pp. 92-104.
18. Chiu, E.M.; Kuo, M.C.; Bruechert, L.J.; Ho, C.-T. *J. Agric. Food Chem.* **1990**, *38*, 58-61.
19. Zhang, Y.; Dorjpalam, B.; Ho, C.-T. *J. Agric. Food Chem.* **1992**, *40*, 2467-2471.
20. Tang, J.; Jin, Q.Z.; Shen, G.H.; Ho, C.-T.; Chang, S.S. *J. Agric. Food Chem.* **1983**, *31*, 1287-1292.
21. Coleman, E.C.; Ho, C.-T. *J. Agric. Food Chem.* **1980**, *28*: 66-68.
22. Coleman, E.C.; Ho, C.-T.; Chang, S.S. *J. Agric. Food Chem.* **1981**, *29*, 42-48.
23. Zhang, Y.; Ho, C.-T. *J. Agric. Food Chem.* **1989**, *37*, 1016-1020.
24. Josephson, D.B.; Glinka, J. In *Thermal Generation of Aromas*; Parliment, T.H.; McGorin, R.J.; Ho, C.-T., Eds. ACS Symposium Series 409; American Chemical Society: Washington, DC, 1989; pp. 242-246.
25. Rizzi, G.P.; Steimle, A.R.; Patton, D.R. In *Food Science and Human Nutrition*; Charalambous, G., Ed., Elsevier Science Publishers B.V.: Amsterdam, 1992, pp.731-741.
26. Macku, C.; Shibamoto, T. *J. Agric. Food Chem.* **1991**, *39*, 1987-1989.
27. Zhang, Y., Ph.D. Dissertation, Rutgers University, New Brunswick, NJ, 1991.
28. Whitfield, F.B. *Crit. Rev. Food Sci. Nutri.* **1992**, *31*, 1-58.
29. Farmer, L.J.; Mottram, D.S. In *Flavour Science and Technology*; Bessièrè, Y.; Thomas, A.F., Eds., John Wiley & Sons, Ltd. : Chichester, UK, 1990, pp. 113-116.

RECEIVED November 4, 1993

Chapter 5

Volatile Compounds Generated from Thermal Interaction of 2,4-Decadienal and the Flavor Precursors of Garlic

Tung-Hsi Yu¹, Min-Hsiung Lee², Chung-May Wu³, and Chi-Tang Ho¹

¹Department of Food Science, Cook College, Rutgers, The State University of New Jersey, New Brunswick, NJ 08903

²Department of Agricultural Chemistry, National Taiwan University, Taipei, Taiwan, Republic of China

³Food Industry Research and Development Institute, Hsinchu, Taiwan, Republic of China

Alliin and deoxyalliin, two important nonvolatile flavor precursors of garlic, were reacted with 2,4-decadienal in a model system to simulate the deep-oil fried condition of garlic. The volatile compounds generated in these model systems were analyzed by GC and GC-MS. They can be divided into three groups: those generated from the thermal degradation of alliin or deoxyalliin, those generated from the thermal degradation of 2,4-decadienal, and those generated from the interactions of 2,4-decadienal and alliin or deoxyalliin. Allyl alcohol was found to be the major volatile degradation product of alliin. Allyl sulfide, 1,2-dithiacyclopentane, allyl disulfide, allyl mercaptan and (allylthio)acetic acid were found to be the major volatile degradation products of deoxyalliin. Hexylthiophenes, 2-pentylpyridine, 2-pentylbenzaldehyde, and 5-formyl-2-pentylthiophene were found to be the major interaction products of 2,4-decadienal and alliin or deoxyalliin.

γ -Glutamyl alk(en)yl cysteine dipeptides and alk(en)yl cysteine sulfoxides are two important groups of nonvolatile flavor precursors of garlic in intact garlic cloves. During cold storage or the sprouting of the garlic cloves, γ -glutamyl alk(en)yl cysteine dipeptides can be transformed to relative alk(en)yl cysteines by the action of γ -glutamyl transpeptidase. By the action of hydrogen peroxidase, alk(en)yl cysteines can then be oxidized to relative alk(en)yl cysteine sulfoxides. During the physical breakdown of the garlic cells, alk(en)yl cysteine sulfoxides can be transformed to alk(en)yl thiosulfinates, the primary flavor compounds of garlic, by

0097-6156/94/0558-0061\$08.00/0
© 1994 American Chemical Society

the action of allinase through the dehydration process and accompanied by the formation of ammonia and pyruvic acid (1-8).

Three γ -glutamyl alk(en)yl cysteines, i.e. γ -glutamyl allyl-, γ -glutamyl (*E*)-1-propenyl-, and γ -glutamyl methyl cysteine, have been found in intact garlic cloves with the former two compounds to be the predominant compounds. Three alk(en)yl cysteine sulfoxides, i.e. allyl-, (*E*)-1-propenyl-, and methyl cysteine sulfoxide, have also been found in intact garlic cloves with the first one to be the predominant compound. During heating at boiling temperature, γ -glutamyl allyl cysteine was broken down to allyl cysteine (deoxyalliin), γ -glutamyl (*E*)-1-propenyl cysteine was transformed to (*E*)-1- and (*Z*)-1-propenyl cysteines, and alliin was destroyed completely in eight hours (5,8,9).

Most attention of garlic flavor research has been focused on the enzymatic generation of flavor compounds from nonvolatile flavor precursors of garlic and the stability of these flavor compounds. In the study of fried garlic flavor, we (10), however, found that the nonenzymatic degradation of nonvolatile flavor precursors of garlic contributed significantly to the characteristic aroma of fried garlic.

Isomers of 2,4-decadienal have been found to be the major lipid oxidation products of vegetable oils such as soybean oil, corn oil and sunflower oil (12). The interaction of 2,4-decadienal with sulfur-containing amino compounds such as cysteine and glutathione has also been reported (13). It is therefore interesting to know if the nonvolatile flavor precursors of garlic, which are the derivatives of cysteine, can interact with lipid or lipid oxidation products to contribute to the formation of some fried garlic flavor compounds.

Experimental

Synthesis of Deoxyalliin and Alliin. Deoxyalliin and alliin were synthesized according to the procedures of Iberl et al. (14) with a slight modification as shown below.

Synthesis and Purification of L-deoxyalliin (S-allyl-L-cysteine). L-cysteine (1 mole) was suspended in 3 L absolute ethanol and kept in an ice bath. Allyl bromide (1.1 mole) (99 %, Aldrich Chemical Co., Milwaukee, WI) was added to the stirred suspension followed by the addition of 3.5 mole NaOH as a 20 M aqueous solution. After stirring for 1 hr, a solution of crude deoxyalliin formed and was acidified to pH 5.3 by glacial acetic acid at 30°C. After crystallization at 4°C, the white needle-like crystals were filtered, washed twice with absolute ethanol, dried at 50°C and then recrystallized at 4°C. This was done by dissolving the substance in a small portion of boiling water containing 1% glacial acetic acid. This solution was poured into a 15-fold amount of boiling ethanol. The solvent immediately turned turbid with the appearance of small plates of deoxyalliin. After cooling down to room temperature, the solution was left to stand at 4°C. Filtration was followed by washing with ethanol and drying at 50°C. This product was the purified L-deoxyalliin.

Synthesis and Purification of L-Alliin (S-allyl-L-cysteine sulfoxide). L-deoxyalliin

(0.3 mole) was dissolved in 500 ml distilled water. Hydrogen peroxide (0.6 mole) (30% w/w, Aldrich Chemical Co.) was added slowly to the stirred solution, and stirring was continued for 24 hours at room temperature. The solvent was evaporated at 60°C under vacuum. The dry, white residue was dissolved in a 500 ml mixture of acetone : water : glacial acetic acid = 65 : 34 : 1 at boiling temperature. While cooling down to room temperature, long white needles started to grow. To complete precipitation of the oxidized amino acid, the solution was kept at 4°C overnight. After filtration of the precipitate, it was washed with the solvent mentioned above and followed by washing with absolute ethanol. The precipitates were then dried at 50°C. The product was pure L-alliin.

Thermal Interaction of 2,4-Decadienal and Alliin or Deoxyalliin. Alliin (0.005 mole) or deoxyalliin (0.005 mole) synthesized was mixed with 0.001 mole of (E,E)-2,4-decadienal (Aldrich, 85% purity) in 100 ml of distilled water. The solution was adjusted to pH 6 using 2N NaOH and then added to a 0.3 liter Hoke SS-DOT sample cylinder (Hoke Inc., Clifton, NJ) and sealed. This cylinder was then heated at 180°C in a GC oven for 1 hr. After being cooled down to room temperature, a reaction mass of 2,4-decadienal and alliin or deoxyalliin was obtained. For the control reaction, 0.001 mole of the same (E,E)-2,4-decadienal was put into water, adjusted to pH 6 and heated in the cylinder in the same manner as described above.

Isolation of the Volatile Compounds. The total reaction mass was simultaneously distilled and extracted into diethyl ether using a Likens-Nickerson (L-N) apparatus. After distillation, 5 ml of heptadecane stock solution (0.0770 g in 200 ml diethyl ether) was added to the isolate as the internal standard. After being dried over anhydrous sodium sulfate and filtered, the distillate was concentrated to about 5 ml using a Kurdena-Danish apparatus fitted with a Vigreux distillation column, then further concentrated under a stream of nitrogen in a small sample vial to a final volume of 0.2 ml.

Gas Chromatographic Analysis. A Varian 3400 gas chromatograph equipped with a fused silica capillary column (60 m x 0.25 mm i.d.; 1 µm thickness, DB-1, J & W Inc.) and a flame ionization detector was used to analyze the volatile compounds. The operating conditions were as follows: injector temperature, 270 °C, detector temperature, 300°C; helium carrier flow rate, 1 ml/min; temperature program, 40°C (5 min), 2°C/min, 260°C (60 min). A split ratio of 50:1 was used.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis. The concentrated isolate was analyzed by GC-MS using a Hewlett-Packard 5840A gas chromatograph coupled to a Hewlett-Packard 5985B mass spectrometer equipped with a direct split interface and the same column used for the gas chromatography. The operating conditions were the same as described above. Mass spectra were obtained by electron ionization at 70 eV and an ion source temperature of 250°C.

Identification of the Volatile Compounds. Identification of the volatile compounds in the isolate was mostly based on gas chromatography-mass spectrometry (GC-

MS), and information from the GC retention index (I_R) used a C_5 - C_{25} mixture as a reference standard. The structural assignment of volatile compounds was accomplished by comparing the mass spectral data with those of authentic compounds available from the Browser-Wiley computer library, NBS computer library or previously published literature (15-20). The retention indices were used for the confirmation of structural assignments.

Results and Discussion

Table I shows the final pH, final appearance, and flavor description of the model reaction system. The color development in the reaction system clearly shows that the degradation and/or the interaction of reactant(s) did proceed drastically.

The gas chromatographic separation of the volatile compounds generated from the model reaction systems are shown in Fig. 1. The identification and quantification of the volatile compounds generated from the model system of 2,4-decadienal + alliin, 2,4-decadienal + deoxyalliin, and 2,4-decadienal only are shown in Tables II, III, and IV, individually.

Table I. Final pH, Final Appearance and Flavor Description of Model Reaction Systems

Model System	Final pH	Final Appearance	Flavor Description
2,4-D* + Alliin	5.3	dark green-brown	green-oily, fried-lard like
2,4-D + Deoxyalliin	6.6	yellow-brown	green-oily, pungent-garlic
2,4-D	4.3	slightly yellow	green-oily, fatty

* 2,4-D : 2,4-decadienal

Volatile Compounds Generated in the Model System of Alliin and 2,4-Decadienal.

As shown in Table II, volatile compounds generated in the model system of alliin and 2,4-decadienal can be grouped into: those generated from thermal degradation of alliin, those generated from thermal degradation of 2,4-decadienal and those generated from the interactions of 2,4-decadienal and alliin. Allyl alcohol was found to be the predominant volatile compound in those compounds generated from the thermal degradation of alliin. Allyl alcohol was proposed to derive from alliin through the [2,3]-sigmatropic rearrangement and followed by the reduction process (11). In those proposed mechanisms, cysteine was also thought to be generated. Ammonia, hydrogen sulfide and acetaldehyde were then proposed to be generated from the degradation of cysteine or alliin directly. The formation mechanisms of most of those compounds degraded from alliin have been reported (11).

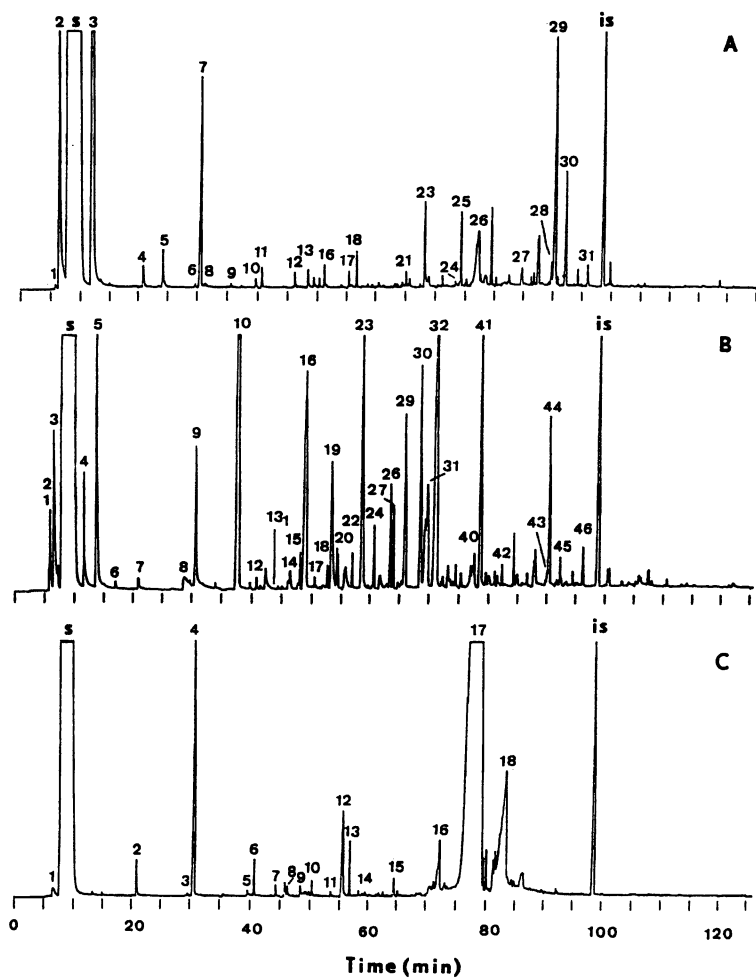


Figure 1. Gas chromatograms of volatile compounds isolated from (A) 2,4-decadienal + alliin, (B) 2,4-decadienal + deoxyalliin, and (C) 2,4-decadienal model systems.

Table II. Volatile Compounds Identified from the Thermal Reaction of Alliin and (*E,E*)-2,4-Decadienal

Peak No.*	Compounds	RI**	mg/mole of Alliin
Compounds generated from thermal degradation of alliin			
1	1-Propene	<500	4.5
3	Allyl alcohol	558	2193.6
5	Thiazole	713	42.5
8	2-Methylthiazole	784	1.3
13	1,2-Dithiacyclopentane	962	15.9
14	3-Formylthiophene	972	6.0
16	2-Acetylthiazole	990	16.2
19	3,5-Dimethyl-1,2,4-trithiolane	1126	6.7
20	Methyl-1,2,3-trithiacyclopentane	1140	4.0
27	1,2,3,4-Tetrathiepane	1368	16.5
31	4-Ethyl-6-methyl-1,2,3,5-tetrathiane	1561	24.9
Compounds generated from thermal degradation of 2,4-decadienal			
4	Pentanal	677	25.4
6	1-Pentanol	771	4.4
7	Hexanal	780	272.7
9	2-Pentanol	831	2.7
10	2-Heptanone	872	9.8
11	Heptanal	882	17.1
12	Benzaldehyde	937	11.8
15	2-Pentylfuran	982	5.2
26	(<i>E,E</i>)-2,4,-decadienal	1296	238.9
Compounds generated both from thermal degradation of alliin and 2,4-decadienal			
2	Acetaldehyde	<500	544.5
Compounds generated from thermal interaction of alliin and 2,4-decadienal			
17	Allyl caproate	1038	14.9
18	2-Butylthiophene	1053	31.5
21	4-(3-hydroxy-1-propenyl)phenol	1153	1.9
22	2-Pentylthiophene	1155	6.1
23	2-Pentylpyridine	1185	78.4
24	Methyl,pentylthiophene	1249	2.7
25	2-Hexylthiophene	1261	61.1
28	2-Hexanoylthiophene	1469	31.5
29	2-Pentylbenzaldehyde	1477	394.1
30	5-Formyl-2-pentylthiophene	1505	110.2

* Peak number refers to that shown in Figure 1 (A).

** Calculated Kovat's retention indices.

Table III. Volatile Compounds Identified from the Thermal Reaction of Deoxyalliin and (*E,E*)-2,4-Decadienal

Peak No.*	Compounds	RI**	mg/mole of Deoxyalliin
Compounds generated from thermal degradation of deoxyalliin			
1	Hydrogen sulfide	<500	30.1
2	1-Propene	<500	35.1
4	Allyl alcohol	545	58.0
5	Allyl mercaptan	581	288.6
6	Methylthiane	630	4.5
8	1-Mercapto-2-propanol	762	37.3
10	Allyl sulfide	854	1059.0
15	Mercaptomethylcyclopentane	959	22.1
16	1,2-Dithiacyclopentane	969	356.5
18	4-Methyl-1,3-dithiacyclopentane	1005	13.9
19	(Allylthio)acetic acid	1014	179.1
20	2-Methyl-1,3-dithiane	1024	23.1
21	(Allylthio)propanal	1041	26.7
23	Allyl disulfide	1074	374.8
24	1,4-Dithiepane	1095	39.4
25	2-(Mercaptoethyl)tetrahydrothiophene	1106	19.4
26	2-Ethyl-1,3-dithiane	1128	78.4
27	3,6-Dimethyl-1,3-dithiane	1134	51.2
28	Methyl-1,2,3-trithiacyclopentane	1144	3.0
29	2-Methyl-1,4-dithiepane	1158	188.8
31	2,4,6-Trimethylperhydro-1,3,5-dithiazine	1198	101.7
32	unknown (C ₆ H ₁₂ S ₂)	1221	567.9
33	3,5-Dimethyl-1,2,4-trithiolane	1235	11.4
34	5-Hydroxy-1,2-dithiacyclooctane	1246	23.9
35	3,5-Dimethyl-1,2,4-trithiolane	1256	6.3
37	3,5-Dimethyl-1,2,6-trithiane	1274	12.3
38	4-Methyl-1,2,5-trithiepane	1293	27.9
39	1,2,5-Trithiacyclooctane	1296	22.2
42	1,2,3,4-Tetrathiepane	1366	13.1
43	4,6-Dimethyl-1,2,5-trithiepane	1470	4.1
46	4-Ethyl-6-methyl-1,2,3,5-tetrathiane	1565	29.9

Table III. Continued on next page.

Table III. Continued

Peak No.*	Compounds	RI**	mg/mole of Deoxyalliin
Compounds generated from thermal degradation of 2,4-decadienal			
7	Pentanal	678	9.1
9	Hexanal	781	146.8
10	2-Heptanone	872	2.7
12	2-Butylfuran	883	6.1
14	Benzaldehyde	941	20.2
17	2-Pentylfuran	983	5.7
40	(<i>E,E</i>)-2,4,-decadienal	1300	32.4
Compounds generated both from thermal degradation of deoxyalliin and 2,4-decadienal			
3	Acetaldehyde	<500	181.6
Compounds generated from thermal interaction of deoxyalliin and 2,4-decadienal			
13	<i>n</i> -Hexanethiol	910	0.4
22	2-Butylthiophene	1054	17.2
30	2-Pentylpyridine	1188	202.6
36	2-Hexylthiophene	1262	17.6
41	3-Hexylthiophene	1315	308.0
44	2-Pentylbenzaldehyde	1479	150.9
45	5-Formyl-2-pentylthiophene	1504	16.7

* Peak number refers to that shown in Figure 1 (B).

** Calculated Kovat's retention indices.

Table IV. Volatile Compounds Identified from the Thermal Degradation of (*E,E*)-2,4-Decadienal

Peak No.*	Compounds	RI**	mg/mole of 2,4-decadienal
1	Acetaldehyde	<500	45.2
2	Pentanal	677	133.6
3	1-Pentanol	771	10.7
4	Hexanal	782	2050.0
5	2-Heptanone	871	15.4
6	Heptanal	882	128.2
7	3-Nonen-2-ol	918	29.3
8	Benzaldehyde	939	37.1
9	Hexanoic acid	962	35.7
10	2-Pentylfuran	982	47.7
11	3-Ethyl-2-methyl-1,3-hexadiene	1015	17.6
12	2-Octenal	1037	669.9
13	<i>n</i> -Butylbenzene	1053	170.9
14	Acetal	1082	12.8
15	2-Nonenal	1141	99.1
16	2,4-Decadienal	1235	520.5
17	(<i>E,E</i>)-2,4,-decadienal	1322	49770.3
18	2,4-Decadienal	1379	3182.1

* Peak number refers to that shown in Figure 1 (C).

** Calculated Kovat's retention indices.

Hexanal was found to be the predominant compound in those compounds generated from thermal degradation of 2,4-decadienal. It is known that two consecutive retro-Aldolizations of 2,4-decadienal will yield one molecule of hexanal and two molecules of acetaldehydes (21-22). Acetaldehyde can also come from the degradation of alliin as already mentioned.

Ten volatile compounds, i. e. allyl caproate, 2-butylthiophene, 4-(3-hydroxy-1-propenyl)phenol, 2-pentylthiophene, 2-pentylpyridine, methylpentylthiophene, 2-hexylthiophene, 2-hexanoylthiophene, 2-pentylbenzaldehyde, and 5-formyl-2-pentylthiophene, are thought to generate from the interactions of 2,4-decadienal and alliin. All the thiophenes and 2-pentylpyridine identified here also have been identified in the model system of cysteine and 2,4-decadienal (13). Figure 2 gives the proposed mechanisms for the formation of 2-hexylthiophene from the interactions of 2,4-decadienal and hydrogen sulfide, one of the degradation volatile compounds of alliin. Figure 3 gives the proposed mechanisms for the formation of 2-pentylpyridine from the interactions of 2,4-decadienal and alliin or ammonia, one of the degradation volatile compounds of alliin.

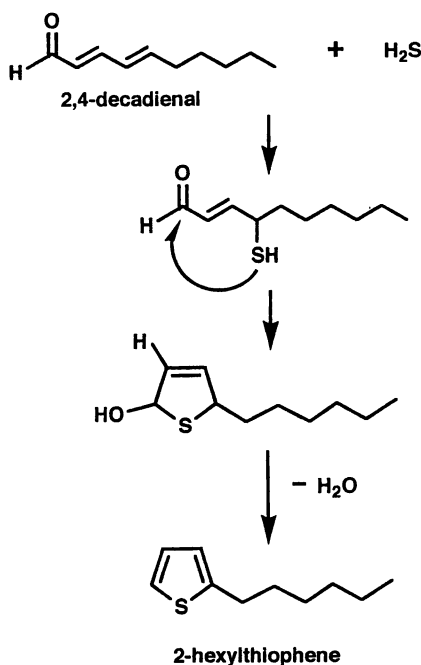


Figure 2. Proposed mechanism for the formation of 2-hexylthiophene from the interaction of 2,4-decadienal and H₂S.

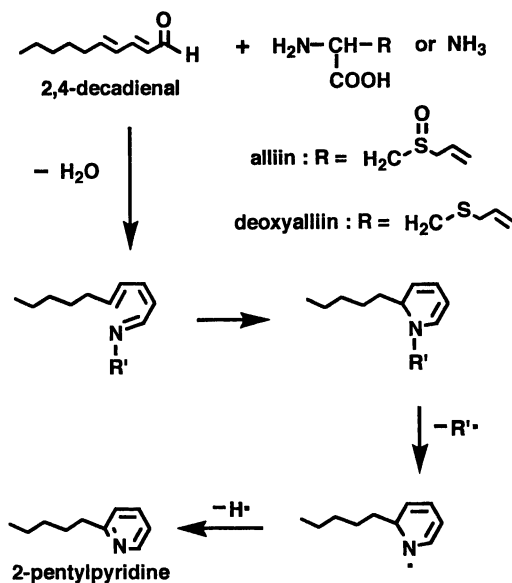


Figure 3. Proposed mechanism for the formation of 2-pentylpyridine from the interaction of 2,4-decadienal and alliin, deoxyalliin, or ammonia.

2-pentylbenzaldehyde was found to be the predominant compound in those compounds generated from the interactions of 2,4-decadienal and alliin. Figure 4 shows the mass spectrum of 2-pentylbenzaldehyde. The proposed mechanisms for the formation of 2-pentylbenzaldehyde from the interactions of 2,4-decadienal and acetaldehyde, one of the degradation products of alliin or 2,4-decadienal, were shown in Figure 5. This compound, however, was not identified in the degradation products of 2,4-decadienal (Table IV). The reason for the absence of 2-pentylbenzaldehyde in the degradation products of 2,4-decadienal probably is that the amount of acetaldehyde generated in that system was too small or that 2-pentylbenzaldehyde could further break down to generate benzaldehyde.

The allyl caproate identified probably came from the interactions of allyl alcohol, the predominant degradation product of alliin, and hexanoic acid, the oxidation product of hexanal. 2-Butylthiophene was proposed to generate from the interactions of hydrogen sulfide and 2-octenal, one of the degradation products of 2,4-decadienal. 5-Formyl-2-pentylthiophene was proposed to come from the interactions of 2,4-decadienal and hydrogen sulfide directly.

Volatile Compounds Generated in the 2,4-Decadienal + Deoxyalliin Model System. As shown in Table III, volatile compounds generated in the 2,4-

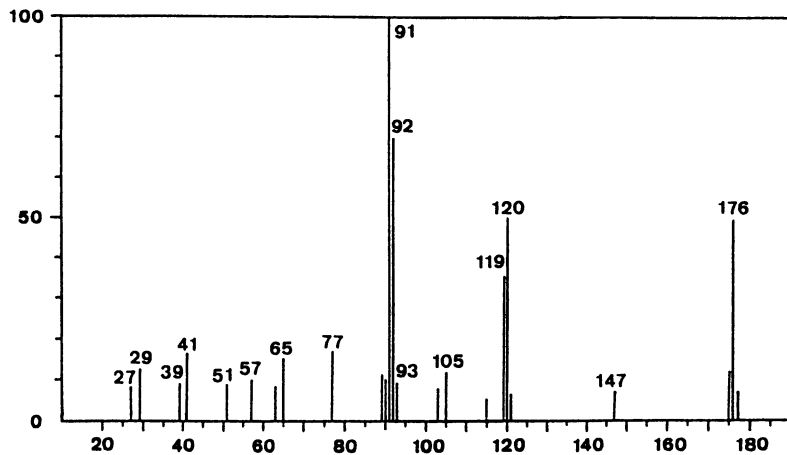


Figure 4. Mass spectrum of 2-pentylbenzaldehyde.

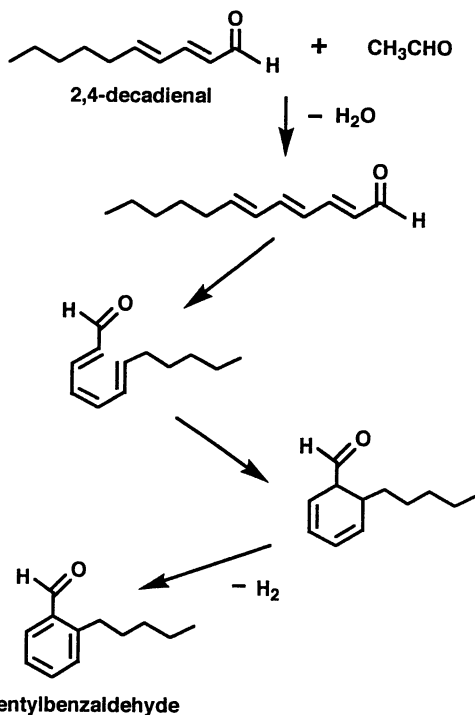


Figure 5. Proposed mechanism for the formation of 2-pentylbenzaldehyde from the interaction of 2,4-decadienal and acetaldehyde.

decadienal + deoxyalliin model system can be grouped into: those generated from thermal degradation of deoxyalliin, those generated from thermal degradation of 2,4-decadienal, those generated both from thermal degradation of 2,4-decadienal and deoxyalliin, and those generated from the interactions of 2,4-decadienal and deoxyalliin. Allyl sulfide, one unknown compound (Figure 6 shows its mass spectrum), allyl disulfide, allyl mercaptan, 1,2-dithiacyclopentane, (allylthio)acetic acid, and 2-methyl-1,4-dithiane were found to be the predominant volatile compounds in those compounds generated from the thermal degradation of deoxyalliin. Allyl mercaptan was proposed to generate from deoxyalliin through the hydrolysis process; allyl sulfide and allyl disulfide could then generate from allyl mercaptan through the oxidation process or free radical rearrangement.(11). (Allylthio)acetic acid was also proposed to generate from deoxyalliin through the deamidation and decarboxylation process. Besides ammonia, hydrogen sulfide and acetaldehyde were also proposed to be generated from the degradation of deoxyalliin. The formation mechanisms of most of those compounds degraded from deoxyalliin were also reported (11).

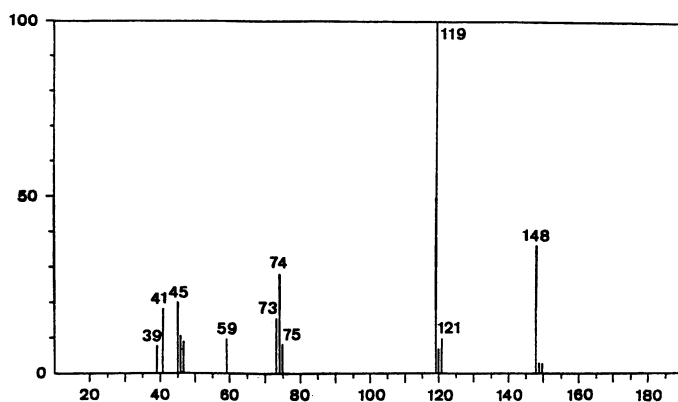


Figure 6. Mass spectrum of the known volatile compound peak No. 32 in Figure 1 (B).

Hexanal again was found to be the predominant compound in those compounds generated from thermal degradation of 2,4-decadienal. Acetaldehyde identified in this model system is thought to be generated both from the degradation of deoxyalliin (11) and the retro-aldol degradation of 2,4-decadienal (21-22).

Seven volatile compounds, i.e. n-hexanethiol, 2-butylthiophene, 2-pentylpyridene, 2-hexylthiophene, 3-hexylthiophene, 2-pentylbenzaldehyde, and 5-formyl-2-pentylthiophene are thought to generate from the interactions of 2,4-decadienal and alliin. Among these compounds, 3-hexylthiophene, 2-

pentylpyridine, and 2-pentylbenzaldehyde were found to be the dominant compounds. Besides 3-hexylthiophene, all thiophenes and 2-pentylpyridine identified here also have been identified from the 2,4-decadienal and cysteine (13), and 2,4-decadienal and alliin model system. Figures 2, 3 and 4 give the proposed mechanisms for the formation of 2-hexylthiophene, 2-pentylpyridine, and 2-pentylbenzaldehyde from the interactions of 2,4-decadienal and deoxyalliin, respectively.

The proposed mechanisms for the formation of 3-hexylthiophene from the interaction of 2,4-decadienal and deoxyalliin were shown in Figure 7. In these proposed mechanisms, 2,4-decadienal was broken down to acetaldehyde and 2-octenal through the retro-aldol degradation process first. After the addition of hydrogen sulfide to these aldehydes and followed by the condensation and dehydration, 3-hexylthiophene was formed.

n-Hexanethiol was proposed to generate from the interactions of hexanal, one of the degradation volatile compounds of 2,4-decadienal and hydrogen sulfide, 2-Butylthiophene was proposed to come from the interactions of hydrogen sulfide and 2-octenal, one of the degradation products of 2,4-decadienal. 5-Formyl-2-pentylthiophene was proposed to be the interaction product of 2,4-decadienal and hydrogen sulfide.

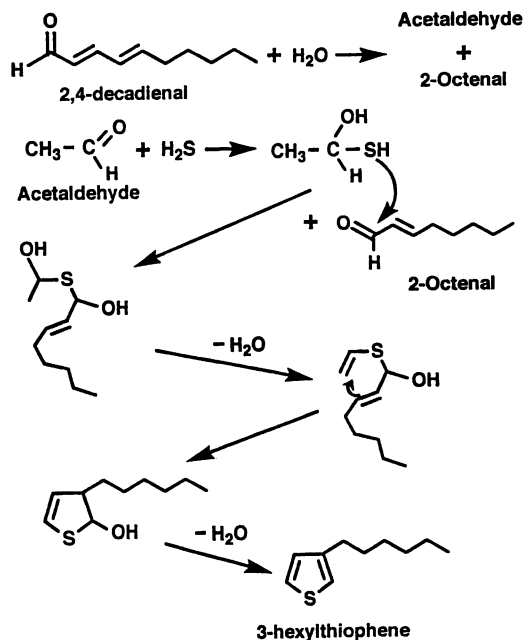


Figure 7. Proposed mechanism for the formation of 3-hexylthiophene from the interaction of 2,4-decadienal and H₂S.

Volatile Compounds Generated from the Degradation of 2,4-Decadienal. As shown in Table IV, fifteen compounds were found to be generated from the degradation products of 2,4-decadienal with hexanal and 2-octenal to be the predominant compounds. Most of these compounds have been reported to be the retro-aldol or oxidation products of 2,4-decadienal (21,22). The total amount of 2,4-decadienal remained plus that of its degradation products shown in Table IV is far higher than the total amount of 2,4-decadienal which remained, plus that of its degradation and interaction products as shown in Table II or Table III. This difference may suggest that the interaction of 2,4-decadienal and alliin or deoxyallin not only can generate volatile flavor compounds but probably can also generate some nonvolatile compounds.

Acknowledgements

This is publication No. D-10205-3-93 of the New Jersey Agricultural Experiment Station supported by the State Funds and Regional Project NE-116. We thank Mrs. Joan B. Shumsky for her secretarial aid.

Literature Cited

1. Fenwick, G. R. and Hanley, A. B. *CRC Crit. Rev. Food Sci. Nutr.* **1985**, *22*, 273-271.
2. Carson, J. F. *Food Rev. Internat.* **1987**, *3*, 71-103.
3. Iberl, B.; Winkler, G.; Muller, B.; Knobloch, K. *Planta Med.* **1990**, *56*, 320-326.
4. Lawson, L. D.; Wood, S.G.; Hughes, B.G. *Planta Med.* **1991**, *57*, 263-270.
5. Lawson, L. D.; Wang Z-Y J.; Hughes, B.G. *J. Nat. Prod.* **1991**, *54*, 436-444.
6. Block, E.; Naganathan, S.; Putman, D.; Zhao, S.-H. *J. Agric. Food Chem.*, **1992**, *40*, 2418-2430.
7. Block, E.; Putman, D.; Zhao, S.-H. *J. Agric. Food Chem.*, **1992**, *40*, 2431-2438.
8. Block, E. *Angew. Chem.* **1992**, *31*, 1135-1178.
9. Block, E.; Naganathan, S.; Putman, D.; Zhao, S.-H. *Pure & Applied Chem.* **1993**, *4*, 625-632.
10. Yu, T. H.; Wu, C. M.; Ho, C.-T. *J. Agric. Food Chem.* **1993**, *41*, 800-805.
11. Yu, T. H.; Wu, C. M.; Rosen, R. T.; Hartman, T. G.; Ho, C.-T. Submitted to *J. Agric. Food Chem.* for publication, **1993**.
12. Snyder, J. M.; Frankel, E. N.; Selke, E.; Warner, K. *J. Amer. Oil Chem. Soc.* **1988**, *65*, 1617-1620.
13. Zhang, Y.; Ho, C.-T. *J. Agric. Food Chem.* **1989**, *37*, 1016-1020.
14. Iberl, B.; Winkler, G.; Muller, B.; Knobloch, K. *Planta Med.* **1990**, *56*, 320-325.
15. Kawai, T. *Thiadiazine and Dithiazines in Volatiles from Heated Seafoods, and Mechanisms of Their Formation*; Shiono Koryo Kaisha, Ltd.: Yodogawaku, Osaka, Japan, **1991**, pp. 14-88.

16. Zhang, Y. *Thermal generation of food flavors from cysteine and glutathione*, Ph.D. Dissertation, Rutgers University, New Brunswick, NJ, 1991.
17. Heller, S. R.; Milne, G. W. A. *EPA/NIH Mass Spectra Data Base*. US Dept. of Commerce: Washington, DC. 1978.
18. TNO *Compilation of mass spectra of volatile compounds in food*, Central Institute for Nutrition and Food Research-TNO, The Netherlands, 1988.
19. Heller, S. R.; Milne, G. W. A. *EPA/NIH Mass Spectra Data Base*. US Dept. of Commerce: Washington, DC. 1980.
20. Block, E.; Iyer, R.; Grisoni, S.; Saha, C.; Belman, S.; Lossing, F. P. *J. Amer. Chem. Soc.* **1988**, *110*, 7813-7827.
21. Matthews, R. F.; Scanlan, R. A.; Libbey, L. M. *J. Agric. Food Chem.* **1971**, *48*, 745-747.
22. Josephson, D. B.; Lindsay, R. *J. Agric. Food Chem.* **1987**, *52*, 1186-1190, 1218.

RECEIVED November 4, 1993

Chapter 6

Relationship of Temperature to the Production of Lipid Volatiles from Beef

A. M. Spanier, A. J. St. Angelo, C. C. Grimm, and J. A. Miller

Southern Regional Research Center, Agricultural Research Service,
U.S. Department of Agriculture, 1100 Robert E. Lee Boulevard,
New Orleans, LA 70124

Analysis of flavor volatiles is typically performed by gas chromatography utilizing variously coated columns for separation. This method is fraught with many potential problems such as artifacts that arise due to the amount of water contained in the sample, the preparation and end-point cooking temperature of the sample, the temperature used to sparge the volatiles from the sample, and the capillary oven temperature and rate of elution or retention of the volatiles on the capillary column. This paper presents data demonstrating the effect of end-point cooking temperature and sparging/purging temperature on the development of volatile profiles in cooked/stored beef. Both types of heating produced different volatile profiles. Based on these data, the manuscript describes how various analytical methods can lead to potentially faulty impressions of the true perceivable meat flavor volatiles when the temperature parameters are not fully considered.

MEAT FLAVOR PERCEPTION

Human perception of flavor is a fine balance between the sensory input of both desirable and undesirable flavors. It involves a complex series of biochemical and physiological reactions that occur at the cellular and subcellular levels (1). The final sensory response or perception to food is both initiated and transduced via stimulation of two major neural networks, the olfactory and gustatory systems (the smell and taste systems, respectively) by the food's lipids, carbohydrates, proteins, and their reaction products. While all three major flavor producing groups are important to the overall flavor perception, this report will focus on those flavor components that are principally olfactory and thus volatile in nature.

This chapter not subject to U.S. copyright
Published 1994 American Chemical Society

The flavor of food is a main driving factor involved in a consumer's decision to purchase a food item. Therefore, it is necessary that the food technologist have a thorough understanding of how food flavor deteriorates if s/he is to prepare products that consumers will purchase repeatedly. Such knowledge is particularly important in meat and meat products, since the deterioration of meat flavor is a serious and continual process (1-5) that involves both the loss of desirable flavor components (5, 6) and the formation of off-flavor compounds (7-10). Many of these flavor producing components are associated with the process of lipid oxidation (11).

The term "meat" encompasses a wide variety of foods of muscle origin; these include, and are not limited to, beef, poultry, pork, lamb, and their associated products. It stands to reason that this impressive list of muscle foods constitutes a commercially important food product. It is for this reason that muscle foods have long deserved, and still warrant, a great deal of research effort designed to reveal their flavor secrets. The species specific flavor of muscle foods (beef in particular) and the largest proportion of muscle food flavor come from volatile components primarily of lipid origin. While much is known as a result of prior research, much of the chemistry of meat flavor still remains a mystery to flavor chemists.

Meat flavor development and deterioration have been shown to be affected by the actions and interactions of several antemortem and postmortem factors. Antemortem factors include, and are not limited to, the age, breed, and sex of the animal as well as the animal's nutritional status, and fat level and composition. Postmortem factors include the length of aging, the manner of cooking, and the manner of storage after cooking. The manner of cooking can include items such as wet vs. dry cooking, convection vs microwave, the rate of heating, the final end-point cooking temperature, and the animal's final fat level and composition. The manner of storage after cooking also affects the final flavor of the beef and includes refrigerated vs. frozen, aerobic vs anaerobic. Of all these factors, temperature is perhaps the most important in the production of flavor in beef. The effect of temperature on meat flavor is a function of both the cooking temperature and the analytical temperature used to determine the composition of the meat's flavor components. This chapter will present information that should shed some valuable light into areas where much of the previous research efforts may have been sidetracked. The remainder of this report will address the effect of these two temperature factors on meat flavor development and analysis.

COOKING TEMPERATURE

Top round (*semimembranosus*) muscle was used in this investigation. It was either USDA select or USDA choice having a fat content of 3.22% and 4.55%, respectively (12). Select cuts were used for the intact roast model while choice was used in studies utilizing ground beef.

Effect of end-point cooking temperature (Packed Columns): One inch cubed (40 gram) mini-roasts were prepared from top round obtained from

Angus-cross beef. **Figure 1** shows representative examples of the mini-roasts cooked to end-point temperatures of 125 °F, 140 °F, 155 °F, and 170 °F (or 52 °C, 60 °C, 68 °C, and 77 °C, respectively). The photograph indicates that there is a geometric progression of temperature into the interior of the roast and the coloration changes to a more "well done" appearance as the temperature increases. These photographs of roasted meat, by virtue of their different structural and color appearance, suggest that the internal portion of the roast may have undergone different chemical reactions and thus has a different chemical composition than the outer portion of the roast. This would suggest that the inner and outer regions of the roast might share differences in flavor.

The direct effect of temperature on the enzymatic activity of a roast has been demonstrated previously (3). These earlier studies demonstrated the progressive inactivation of specific proteins and enzymes (notably hydrolases) with cooking of beef to higher end-point temperatures (EPT). In addition to measurement of enzyme activity in cooked beef, evaluation of the protein profile/composition of extracts of meat cooked to various EPT revealed that the protein composition of the muscle, measured by gel electrophoresis, changed as a function of increased EPT (3). The presence of significant remaining proteolytic activity along with the increased proportions of low molecular mass protein material appearing in cooked meat suggests that increases in cooking temperatures are responsible for the production of large numbers of flavor components as well as flavor precursors. The latter, particularly the sulfur containing peptides, can react with reducing sugars to form Maillard reaction products and other heterocyclic flavor components upon cooking or re-cooking.

The process of cooking disrupts the tissue and membranes that serve as a source of many of the lipid precursors and lipid volatiles associated with desirable and undesirable flavors (3, 7, 10, 11, 13-15). In addition to the membrane alterations due to cooking temperatures and proteases activation (3), lipases could also be activated and thereby contribute to the production of additional flavor precursors or reactions. Cooking exposes or liberates various lipid components from their normal subcellular localization (via thermal denaturation and dissociation), making them more accessible to oxygen and other catalysts of lipid oxidation such as free iron. The process of lipid peroxidation should be measurable by assessment of the volatile profile and by assessment of the levels of thiobarbituric acid reactive substances (TBARS). These analyses were performed on samples taken from inner and outer portions of the minicube roast model. Examination of the data (**Figure 2**) reveals that storage leads to a progressive increase in TBARS and in lipid volatiles (as demonstrated by the increase in hexanal levels), no matter what the final EPT. The higher the EPT (155 °F/68 °C > 125 °F/52 °C) the greater the level of these lipid oxidation markers in the meat. The inner portion of the roast (**Figure 1**), having a lower temperature than the outer region of the roast (1) always shows lower levels of these markers than does the outer layer of the roast such that $155^{\circ}\text{F}_{(\text{outer})} > 155^{\circ}\text{F}_{(\text{inner})} > 125^{\circ}\text{F}_{(\text{outer})} > 125^{\circ}\text{F}_{(\text{inner})}$.

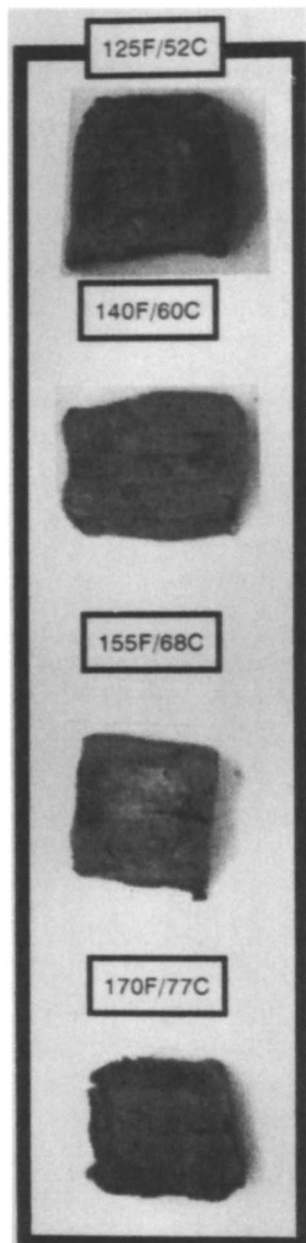


Figure 1: Photos of mid-section of beef minicube roasts [*semimembranosus* muscle; top round; 40 grams/whole minicube; ~3.8 cm/edge (55.3 cm³)] cooked to the end-point temperature identified in the photo. Cooking was in a typical convection oven set to 350° F/177° C.

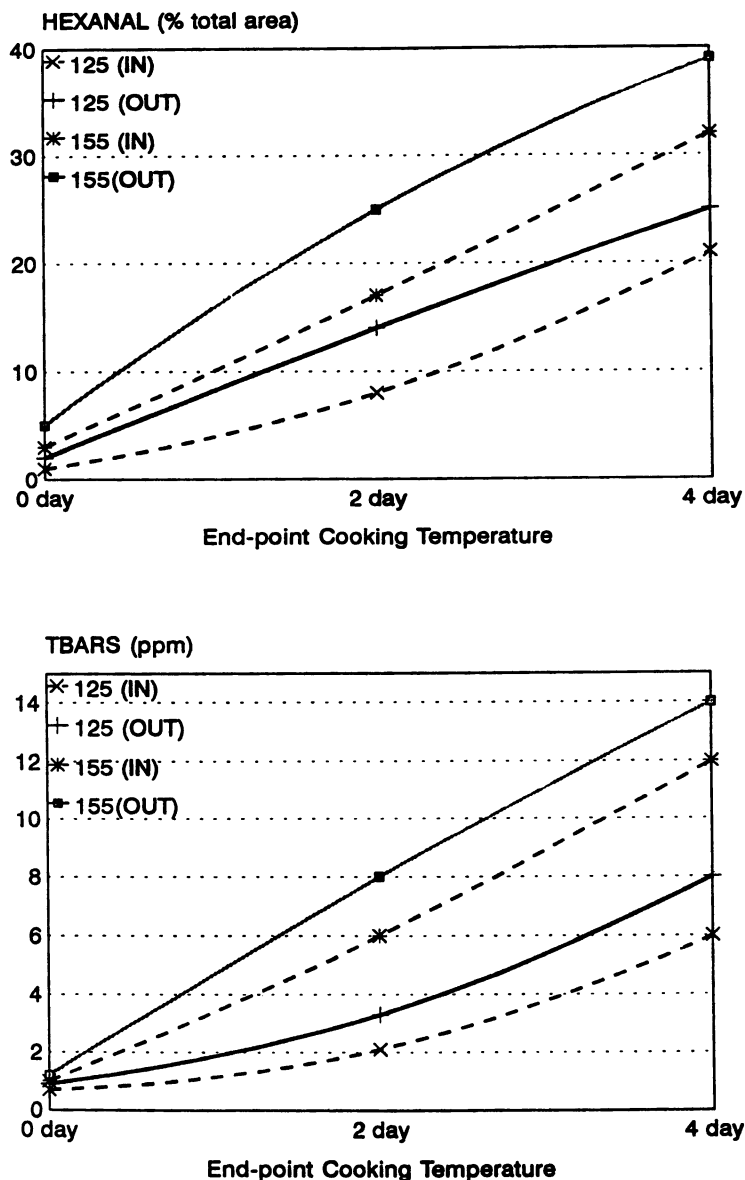


Figure 2: Effect of storage (40°F/4°C) on the development of off-flavor volatiles (hexanal) and markers (thiobarbituric acid reactive substances; TBARS) in the core (IN) and outer (OUT) sections of minicube roasts cooked to end-point temperatures of 125°F/52°C and 155°F/68°C. Storage was for 0, 2, and 4 days (see 4 for details).

The temperature dependency in off-flavor volatile production is shown to occur through the range of 125 °F to 155 °F. On the other hand, at 170 °F, the TBARS and hexanal levels appear to decrease (Figure 3). This apparent decline in measurable volatile (hexanal) level in the miniroast is thought to be due to the release of these volatiles from the sample into the environment, much like the bouquet of odors one would perceive if one walked into the kitchen before dinner is served. Another observation is to note that these temperature-dependent changes appear to become readily evident or measurable only after a period of storage (compare front two rows of data in Figure 3 with back two rows of data) since no appreciable difference is seen in the samples of freshly cooked meat. This suggests that while the structure and chemistry of the meat are affected immediately during the initial cooking process (Figure 1), the development and/or appearance of off-flavor volatiles is seen only after the meats are stored. In other words, increased EPT causes both structural (Figure 1) (13) and compositional (2, 3, 7, 13, 14) changes in beef whereby the structure has been modified and precursors are available for oxygen and other free-radical initiating components (e.g., free iron; 4) to drive the peroxidation of lipids and thus enhance the undesirable flavor in meat after storage. Storage dependent changes are well documented and are discussed briefly below (4-7, 10, 13-15).

Precooked beef products, often referred to as "convenience" and "institutional" foods, comprise 35% of all the beef sold and consumed in America today; this represents almost \$10 billion in consumer expenditures on meat. Therefore, a thorough understanding of the flavor of beef and what factors affect the flavor would be critical to continued sales in this large market. Hornstein and Crowe (16) and others (17-19) suggested that, while the fat portion of muscle foods from different species contributes to the unique flavor that characterizes the meat from these species, the lean portion of meat contributes to the basic meaty flavor thought to be identical in beef, pork, and lamb. The major differences in flavor between pork and lamb result from differences in a number of short chain unsaturated fatty acids that are not present in beef. Even though more than 600 volatile compounds have been identified from cooked beef, not one single compound has been identified to date that can be attributed to the aroma of "cooked beef." Therefore, a thorough understanding of the effect of storage on beef flavor and on lipid volatile production would be helpful to maintain or expand that portion of the beef market.

Storage of precooked meat leads to the production of flavor volatiles typically associated with off-flavors (15). These volatiles include, but are not limited to, pentanal, hexanal, heptanal, 2,3-octanedione, 2-pentylfuran and nonanal when examined in packed columns. Figure 4 presents data from a typical chromatographic profile for precooked/stored ground beef patties.

Effect of purging temperature (Packed Column): The data presented above raises a very important question. If temperature will affect the volatiles produced by the beef, can the temperature used to sparge, purge and desorb the

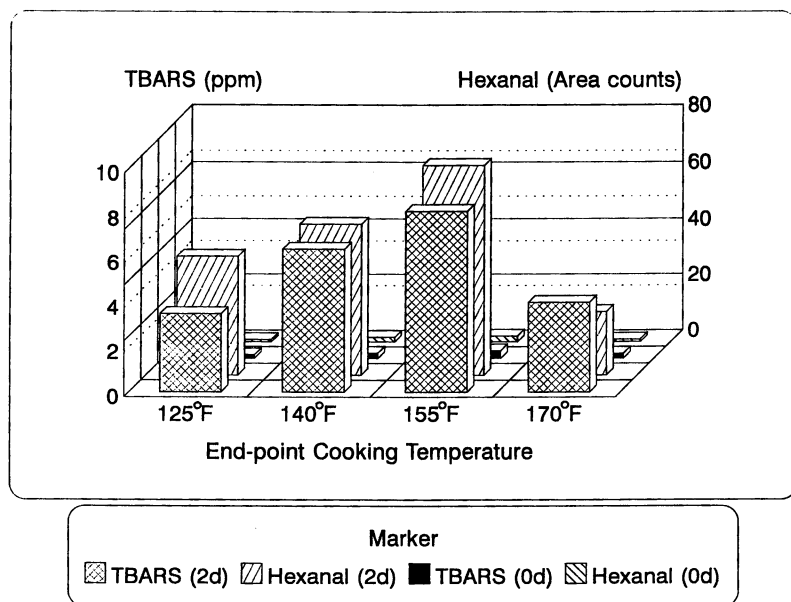


Figure 3: Effect of cooking temperature on the production of lipid oxidation products in freshly cooked (0d) and cooked/stored (2d) whole beef mini-roasts (40 grams/cube).

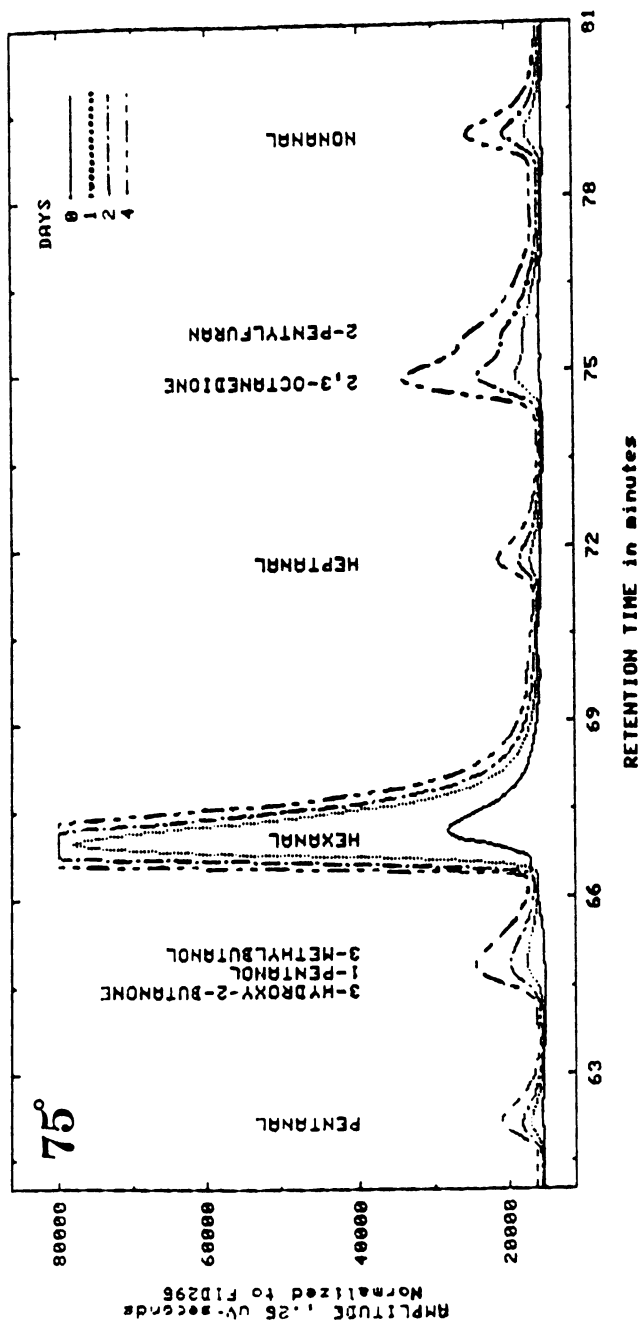


Figure 4: Packed column (Tenax GC - 8% polymethaphenyl ether; 10 ft. x 1/8") gas chromatographic (GC) profile of volatiles from either freshly cooked and unstored ground beef (top round), or pre-cooked and then stored (40° F/4° C) for 1, 2, and 4 days. Volatiles identified and noted directly on the figure.

July 15, 2012 | <http://pubs.acs.org>
 Publication Date: July 7, 1994 | doi: 10.1021/bk-1994-0558.ch006

volatiles from their matrix (the meat and the column) also affect the types and kinds of volatiles seen during analysis? To answer this question, we utilized dynamic purge-and-trap gas chromatography using a packed column. Packed columns were chosen because of their tolerance for high levels of water such as that found in muscle foods.

Freshly cooked/reground beef patties, and those stored at 4 °C for 0, 1, 2, and 4 days, were analyzed by dynamic purge-and-trap gas chromatography (Figure 5). Volatile detection was via a Tracor Model 100AT detector, described by Brown et al. (20) for egg volatiles. This detector combines flame photometric detection (FPD; used in sulfur mode) with flame ionization detection (FID). A Tekmar 25 mL needle impinger-assembly was used to substitute for the generally accepted Tekmar semi-automatic purge and trap concentrator (Model LSC-3; 21). One (1.0) gram of freshly cooked/reground beef patty was sparged for 30 minutes at either 50 °C, 75 °C or 100 °C within the needle impinger assembly. Nitrogen was used as a carrier gas at flow rate of 20 mL/min. The beef volatiles, after passing through a transfer line and a six port, 1/16", Valco™ valve (Valco Instruments, Houston, TX 77255), were trapped/concentrated directly onto a packed Tenax GC - 8% poly-metaphenyl ether column (10 ft x 1/8") held at ambient temperature (20° - 21 °C). Samples were allowed to concentrate on the column for 30 minutes. Volatiles were eluted from the column by purging with nitrogen for a total of 60 minutes (nitrogen flow: 20 mL/min; column heated from 25 °C to 250 °C at 3 °C per minute). The sample tube was removed from the injection port after completion of volatile stripping/sparging process and replaced with a tube containing a few milliliters of water to effect steam distillation. The six port valve permitted switching to a valve purge position that allowed the valve and transfer lines to be cleaned between runs by steam distillation from an impinger tube containing water alone.

The carbonyl components found in precooked/stored meat sparged at three different temperatures (50°, 75°, 100°C) were analyzed (Figure 6). Volatile profiles were similar to those seen in Figure 4 with the exception that the intensities and areas of various peaks differed significantly. Notable variability in the temperature-dependent quantitation of the volatiles was seen with low carbon number materials, i.e., from methanol to 2,3-butanedione, while the typical increases in volatile levels with storage were seen for volatiles ranging in mass from 3-methyl butanal to decanal. These latter carbonyl compounds showed their lowest levels and most difficulty in quantitation at sparging temperatures of 50 °C. Replicability, peak heights, and peak patterns appeared to reach their optimum level at sparging temperatures of 75 °C; their patterns at 100 °C were similar to that at 75 °C (Figure 7). Previous studies have shown similar results (6).

Unlike the carbonyl compounds, sulfur containing components show a very high degree of temperature dependence (Figure 8). Precise assessment of the content and composition of sulfur compounds is essential in the study of meat flavor since the sulfur containing compounds [long known to be involved in the generation of Maillard reaction products, MRPs (22)] play a vital role in

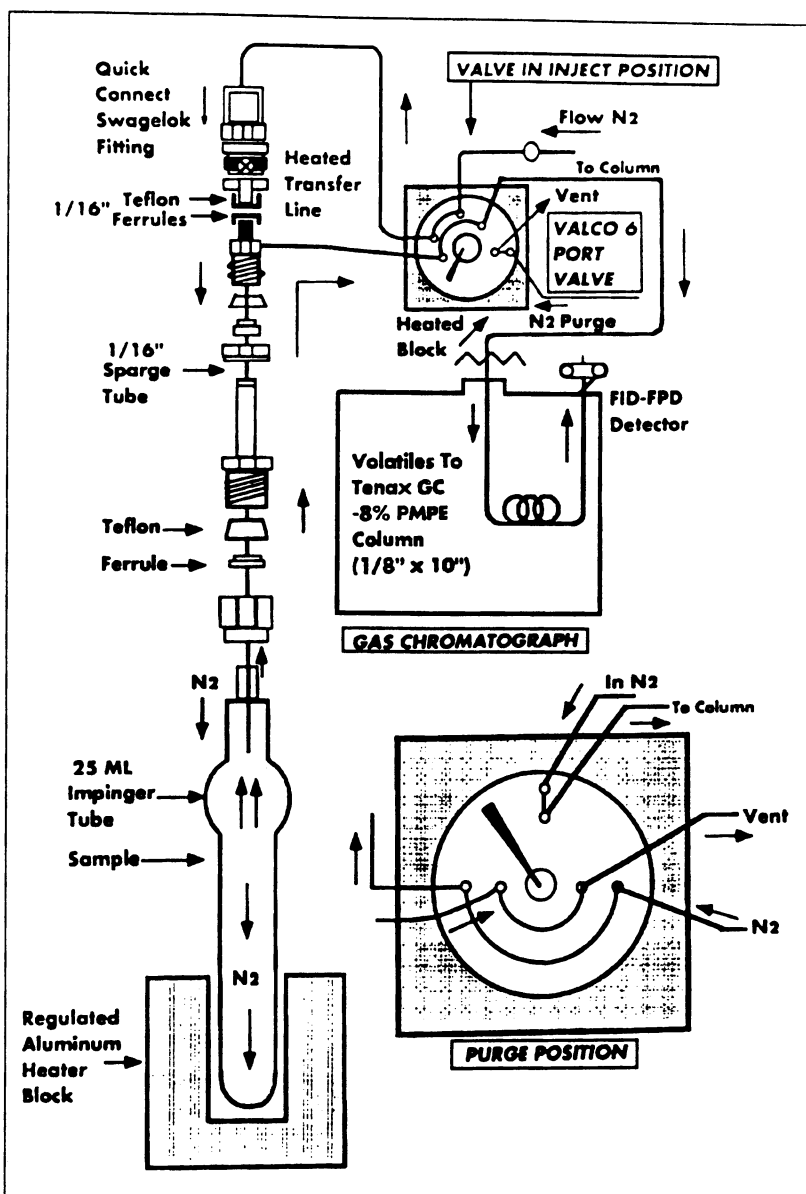


Figure 5: Diagram of the dynamic purge-and-trap GC setup for the packed column experiments.

July 15, 2012 | http://pubs.acs.org
 Publication Date: July 7, 1994 | doi: 10.1021/bk-1994-0558.ch006

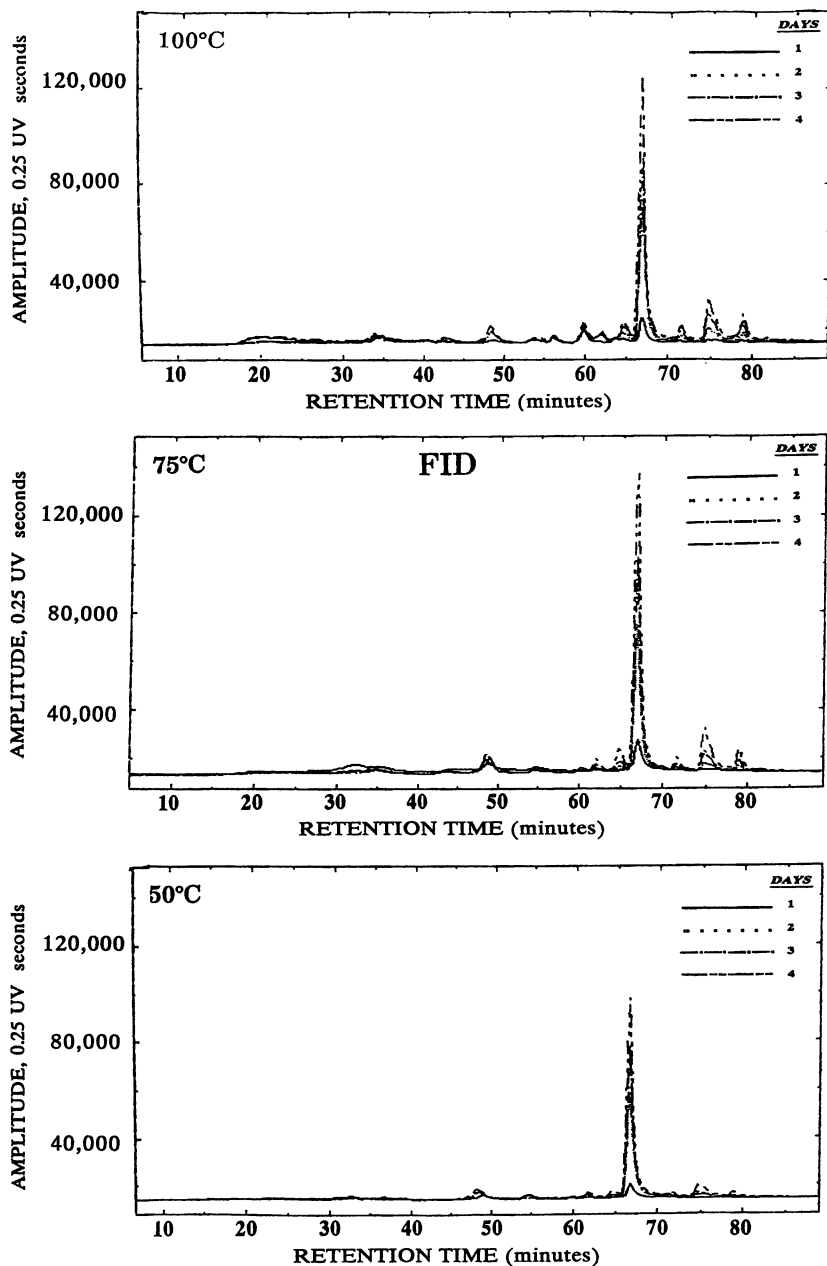


Figure 6: Packed column gas chromatographic profile with flame ionization detection (FID) of cooked ground beef stored for 0, 1, 2, and 4 days at 40°F/4°C (as described in Figure 4). The samples were otherwise identical except for heating and sparging the sample (see Figure 5) at 50°C, 75°C and 100°C.

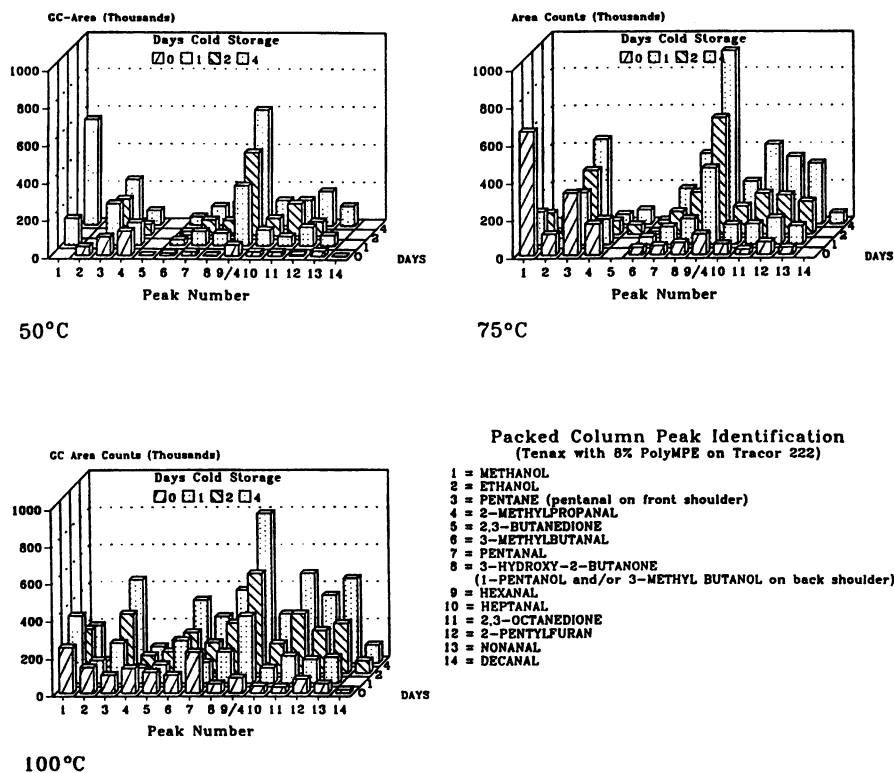


Figure 7: Effect of Purge Temperature on the concentration and resolvability of meat alcohols and carbonyls. The area under the peaks of the samples shown in Figure 6 are presented here. Fourteen (14) peaks were identified and are tabulated on the figure. Peak 9, written as '9/4', is plotted as 1/4th the actual value so that all peaks would appear on the diagram.

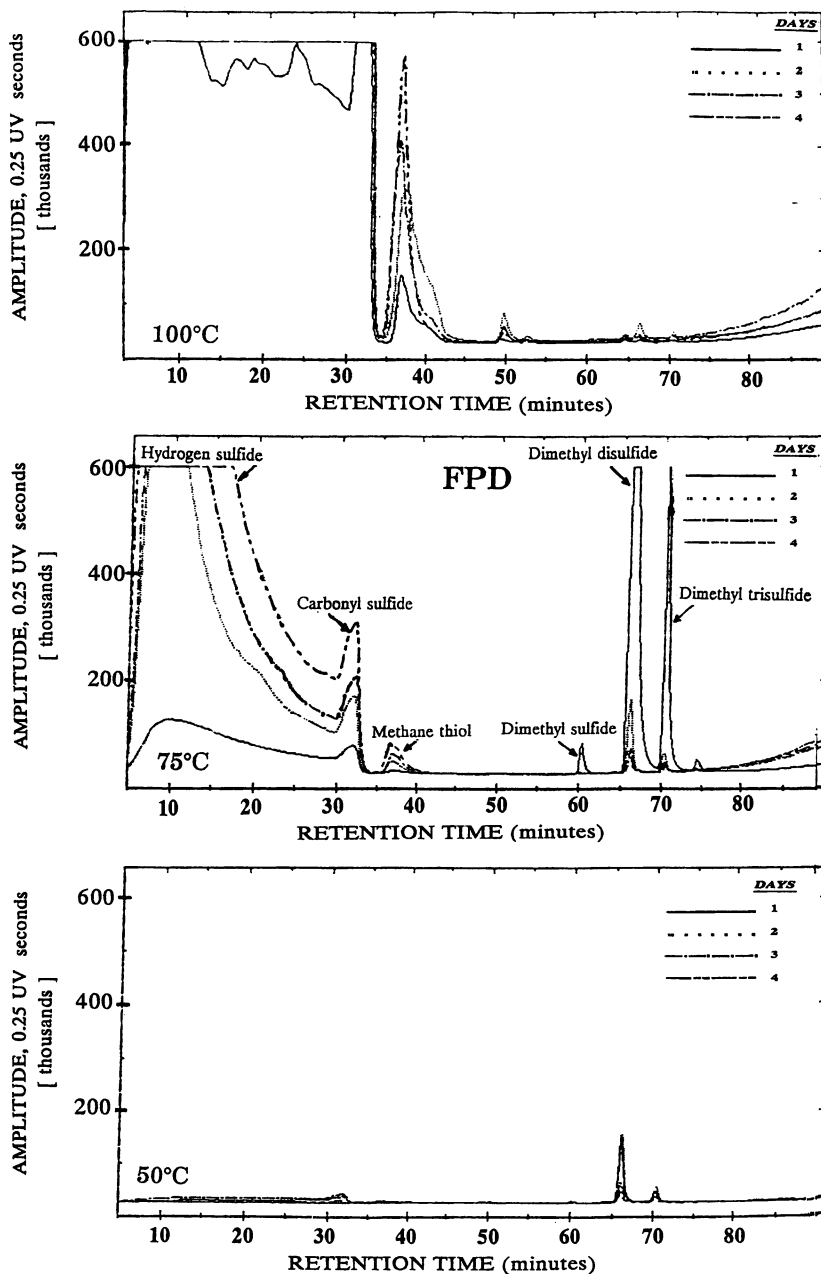


Figure 8: Packed column gas chromatographic profile with flame photometric detection (FPD) of cooked ground beef stored for 0, 1, 2, and 4 days at 40°F/4°C (as described in Figures 4 and 6). The samples were otherwise identical except for heating and sparging the sample (see Figure 5) at 50°C, 75°C and 100°C.

the production of flavor in meat (23). A significant proportion of the organic compounds that are known to produce meat-like flavor contain sulfur and a significant number of these are aldehydes. The specific aldehydes produced during the Maillard reaction and the specific amounts of aldehyde produced are controlled by the specific amino acid and sugars used in the reaction, respectively (22). It would be reasonable to expect that the production of MRPs can be controlled through control of reactants and reaction conditions such as temperature. There is an ever growing list of MRPs found in synthetic mixtures and in meat, but published quantitative correlations between the levels of MRPs and the sensory response of these compounds in meat are not to be found. Although much is known about the formation of MRPs *in vitro*, little is known of their formation and influence on the overall sensory perception and flavor of meat *in vivo*.

The relative impact of sulfur-containing compounds to beef flavor cannot be understated (24). For that reason it is important to have methods available that will yield accurate assessment of the true composition of the flavor components of foods. Analysis of the sulfur compounds found in cooked ground beef has indicated that several of the heterocyclic compounds such as 4-methylthiazole, 2-acetylthiazole, benzothiazole, and 2-furylmethanethiol are fairly stable (6). These latter compounds were identified in ground beef patties that had undergone a fairly strenuous 4 hour steam distillation-extraction protocol. On the other hand, use of milder methods having efficiency of recovery greater than 95% indicated that precooked ground beef did not contain any thiazoles at least at levels greater than 2.5 parts per billion (14).

Other sulfur-containing compounds in meat are thought to be formed by Strecker degradation of cysteine, methionine and alanine and from hydrogen sulfide. Hydrogen sulfide is produced via several mechanisms including free-radical reactions (25). Hydrogen sulfide, which has been shown to be a product of the degradation of dimethyl trisulfide (26), can also react with several components of meat to give ethanedithiol, (methylthio)ethanethiol, and dimethyltrithiolane; the three latter have been shown to increase in meat during storage (6). The data in Figure 9 demonstrate clearly the increase in the tissue level of hydrogen sulfide during prolonged storage of precooked meat particularly at sparge temperatures above 75 °C. This correlates well with the decline in dimethyl trisulfide. The abundance of free radicals during storage and the susceptibility of the sulfur amino acids to radical damage (26) would contribute to an increase in hydrogen sulfide content with storage.

The content of dimethyl sulfide, dimethyl disulfide, and dimethyl trisulfide decreases with increased storage no matter what the sparging temperature (Figure 8 and Figure 9). Similar results were observed for dimethyl trisulfide in more harshly extracted samples (6). Dimethyl disulfide, an oxidation product of methanethiol, can react to form dimethyl trisulfide and dimethyl sulfide. Subsequently, dimethyl trisulfide may be degraded to hydrogen sulfide, carbonyl sulfide and methanethiol (25; 27).

July 15, 2012 | <http://pubs.acs.org>
Publication Date: July 7, 1994 | doi: 10.1021/bk-1994-0558.ch006

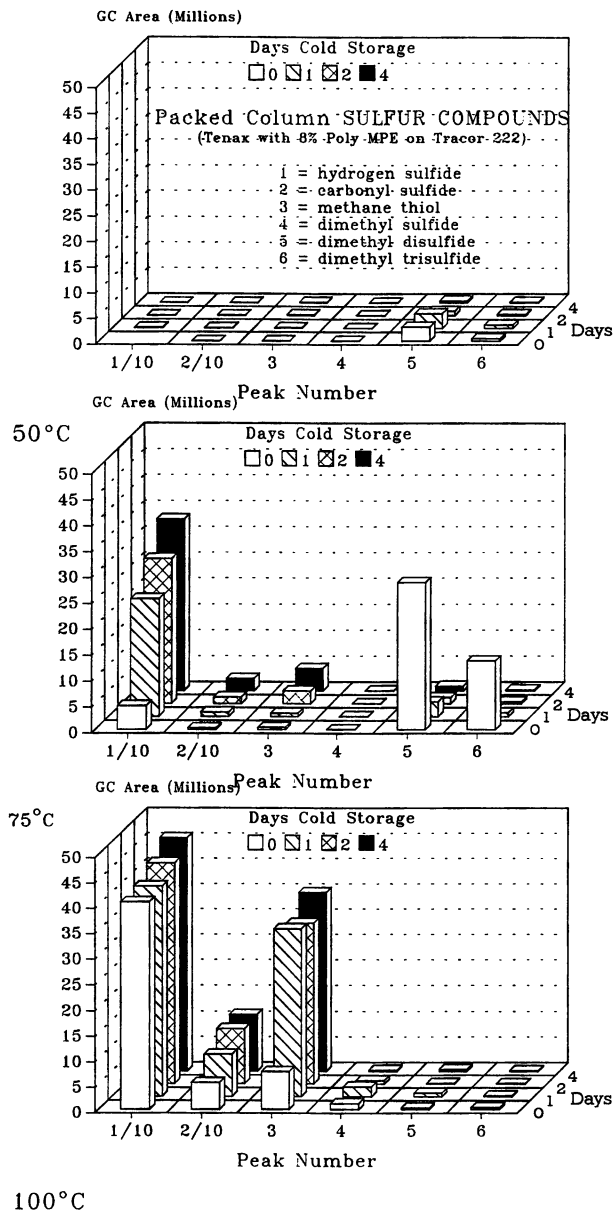


Figure 9: Effect of Purge Temperature on the concentration and resolvability of meat sulfur-containing compounds. The area under the peaks of the samples shown in Figure 8 are presented here. Six (6) peaks were identified and are tabulated on the figure. Peaks 1 and 2, written as '1/10' and '2/10', are plotted as 1/10th the actual value so that all peaks would appear on the diagram. All 3 graphs are presented in the same dimensions for comparison.

While the general trend of the data at most sparging temperatures is to show a decrease in some sulfur-containing compounds (dimethyl sulfide, dimethyl disulfide, and dimethyl trisulfide) and an increase in other sulfur-containing compounds (hydrogen sulfide, carbonyl sulfide, and methane thiol), it is important to note that temperatures that are either too low (50 °C) or too high (100 °C) can lead to misinterpretation of the data. The latter is due either to inefficient extraction of the volatile components from the products (low temperatures) or due to the conversion of one form of a sulfur-containing compound to another (high temperatures, Figures 8 & 9). The data suggest that a sparging temperature of 75 °C, which is the final temperature of most cooked beef products, is probably the optimum temperature to analyze meat for the level of sulfur-containing (Figure 9) and carbonyl containing (Figure 7) products. Independent studies of enzyme activity (hydrolases) and volatile production (3) also point to 75 °C as an optimum temperature for investigation of flavor volatiles.

Effect of end-point cooking temperature (Megabore Columns): In an effort to enhance the number of resolvable volatiles and to minimize the distance the volatiles would need to travel between the sample and the column, a Short Path Thermal Desorber™, SPTD (Scientific Instruments Services (SIS) of New Jersey), was used. This system injected the volatiles directly onto a megabore column. A megabore column was used in lieu of a capillary column since this column, like the packed column, has the capacity to handle much of the water associated with muscle foods. Diatomaceous earth was used between the sample and the column to absorb much of the water. Sample injection, purge, and cryofixation was for 10 minutes at -25 °C through a SPTD. The sample was eluted from a 60 meter, DB-5 0.75 mm ID column placed in a Hewlett-Packard 5890™ gas chromatograph, with a ramp of -25 °C to +100 °C at 5 °C/min followed by a ramp of 100 °C to 250 °C at 10 °C/min. Benzothiophene was used as the external standard. Because several experimental groups were to be compared, all figures were drawn with the abscissa at the same maximum value, i.e., 2000. Presentation of the data in this manner cuts the major peak (hexanal) short on the graph. Therefore, the peak height and peak area of hexanal were inserted on the figures. Analysis of beef volatiles using the megabore column with the SPTD showed the familiar pattern of increase in volatiles that occurs with progressive storage (Figure 10). Sparging is considered consistent between samples since the level of the benzothiophene external standard is constant. In addition to the typical pattern of increase in volatiles with storage, the megabore column has permitted the resolution of several additional volatile components.

Effect of purging temperature (Megabore Column): The inherent ability of megabore columns (connected to the SPTD) to resolve more components than the packed column is utilized to examine the effect of sparge temperature on the extraction and resolution of volatiles from beef. When precooked beef stored for 2 days is examined by sparging at different temperatures, one readily observes an increase in the concentration of resolved volatiles with increasing sparging temperatures (50 °C, 70 °C, and 90 °C). This is clearly seen in Figure 11, where representative volatiles, pentanal, hexanal and heptanal are identified.

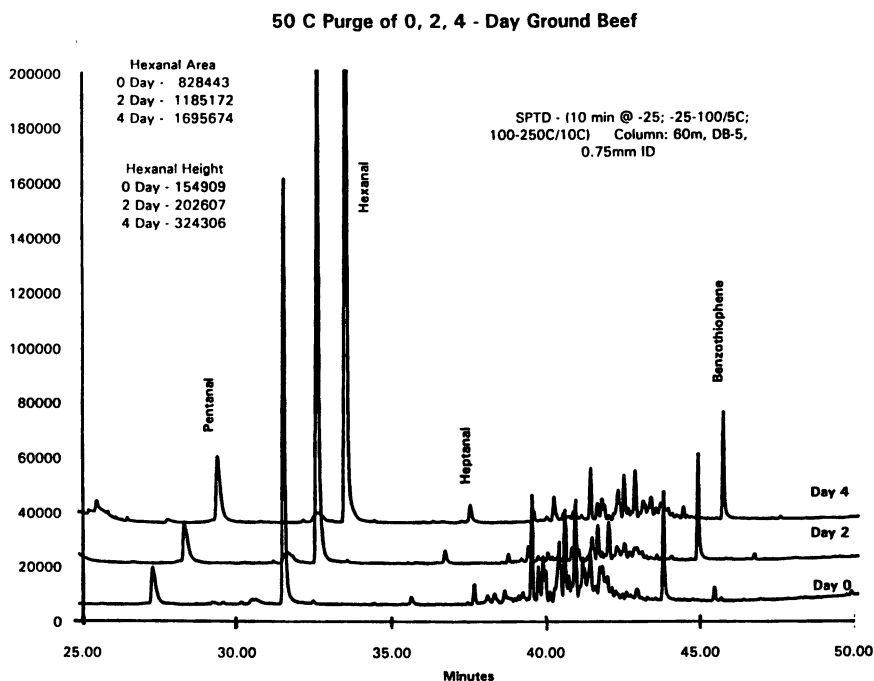


Figure 10: Effect of storage on the development of carbonyl compounds in cooked ground beef. A megabore column was used for concentration, separating, and resolving the beef components. The column was connected directly to the SPTD (short path thermal desorber of SIS) and the volatiles isolated as indicated in the insert. Benzothiophene was used as an external standard.

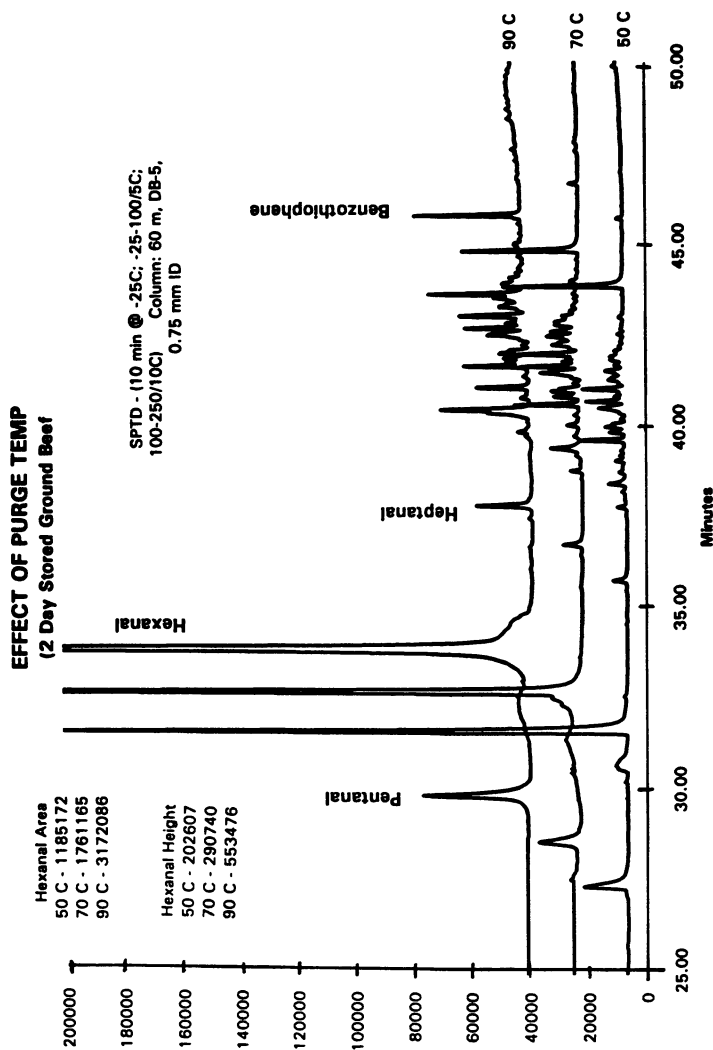


Figure 11: Effect of sparge temperature on the development, concentration, and resolvability of beef carbonyls. The system was similar to that described in Figure 10. Chromatographic conditions are listed in the figure. Sparging temperatures were 50 °C, 70 °C, and 90 °C.

July 15, 2012 | <http://pubs.acs.org>
 Publication Date: July 7, 1994 | doi: 10.1021/bk-1994-0558.ch006

As in Figure 10, sparging is considered consistent between samples since the level of the benzothiophene external standard is constant. It is important to note that, as with the packed column, it is not yet known whether the data from the megabore column examining the effect of purging temperature on the production of new volatiles is a result of actual production of new volatiles not initially present or whether it is a result of carryover with vaporized water or with the enhanced vapor pressures at the higher temperature.

SUMMARY and CONCLUSIONS

Meat flavor deterioration has been shown in numerous documents to be a dynamic process in which free-radical reactions are involved in the deterioration of meat flavor observed during storage. These reactions affect the stability of the lipids and sulfur-containing compounds and lead to the formation of undesirable flavors. The compounds that are formed as a result of these degradation reactions have unique flavor characteristics and ranges of formation and contribute to the overall loss in desirable meaty flavor. While future research will need to determine the full effect of storage time and meat flavor deterioration on the content and composition of the volatile compounds that contribute to desirable and undesirable meat flavors, this study clearly demonstrates that the analytical temperature (sparge temperature) needs to be more cautiously examined and better understood if we are to have a 'true' picture of the flavor components of food.

LITERATURE CITED

- 1 Spanier, A.M.; Miller, J.A. In *Food Flavor & Safety: Molecular analysis and design*. Spanier, A.M., Okai, H. and Tamura, M., Eds.; ACS Symposium Series No. 528; American Chemical Society: Washington, DC; 1993, pp 78-99.
- 2 Spanier, A.M.; Edwards, J.V.; Dupuy, H.P. *Food Tech.* 1988 42(6):110-118.
- 3 Spanier, A.M.; McMillin, K.W.; Miller, J.A. *J. Food Sci.* 1990 55:318-326.
- 4 Spanier, A.M.; Vercellotti, J.R.; James, C., Jr. *J. Food Sci.* 1992 57:10-15.
- 5 St. Angelo, A.J.; Vercellotti, J.R.; Dupuy, H.P.; Spanier, A.M. *Food Tech.* 1988 42(6):133-138.
- 6 Drumm, T.D.; Spanier, A.M. *J. Agric. Food Chem.* 1991 39:336-343.
- 7 Spanier, A.M.; Miller, J.A.; Bland, J.M. In *Lipid Oxidation in Food*. St. Angelo, A.J., Ed.; ACS Symposium Series No. 500; American Chemical Society: Washington, DC; 1992, pp 104-119.
- 8 Timms, M.J.; Watts, B.M. *Food Technol.* 1958, 12(2):240-243.
- 9 Bailey, M.E. *Food Technol.* 1988, 42(6):123-126.
- 10 St. Angelo, A.J.; Spanier, A.M.; Bett, K.L. In *Lipid Oxidation in Food*. St. Angelo, A.J., Ed.; ACS Symposium Series No. 500; American Chemical Society: Washington, DC; 1992, pp 140-160.
- 11 *Lipid Oxidation in Food*. St. Angelo, A.J., Ed.; ACS Symposium Series No. 500; American Chemical Society: Washington, DC; 1992.
- 12 Slover, H.T.; Lanza, E.; Thompson, R.H., Jr.; Davis, C.S.; Merola, G.V. *J. Food Comp. Anal.* 1987 1:26-37.
- 13 Spanier, A.M.; St. Angelo, A.J.; Shaffer, G.P. *J. Agric. Food Chem.* 1992 40:1656-1662.

- 14 Spanier, A.M. In *Developments in Food Science. 29. Food Science and Human Nutrition*. Charalambous, G., Ed.; Elsevier Science Publishers B.V.: Amsterdam; **1992**, pp 695-709.
- 15 Asghar, A.; Gray, J.I.; Buckley, D.J.; Pearson, A.M.; Booren, A.M. *Food Tech.* **1988** 42(6):102-109.
- 16 Hornstein, I.; Crowe, P.F. *J. Agric. Food Chem.* **1960** 8:494-498.
- 17 Sink, J.D. *J. Food Sci.* **1979** 44:1-5.
- 18 Wasserman, A.E. *J. Food Sci.* **1979** 44:6-11.
- 19 Wasserman, A.E.; Talley, F. *J. Food Sci.* **1968** 33:219-223.
- 20 Brown, M.L.; Holbrook, M.; Hoerning, E.F.; Legendre, M.G.; St. Angelo, A.J. Volatile indicators of deterioration in liquid egg products. *Poultry Sci* **1986** 65,1925.
- 21 Vercellotti, J.R.; Crippen, K.L.; Lovegren, N.V.; Sanders, T.H. Defining roasted peanut flavor quality. Part 1. Correlation of GC volatiles with roast color as an estimate of quality. In *Food Science and Human Nutrition*. Vol. 29. Charalambous, G. (Ed.); Elsevier Science Publishers: Amsterdam; **1992**, pp 183-209.
- 22 Danehy, J.P.; In *Advances in Food Research*; Vol. 30; C.O. Chichester, E.M. Mrak and B.S. Schweigert (Eds.); Academic Press, NY; **1986**, pp 77-138.
- 23 Dwivedi, B.K.; In *CRC Critical Reviews in Food Technology* **1975**, 5:487-535.
- 24 Chang, S.S.; Peterson, R.J. Recent developments in the flavour of meat. *J Food Sci.* **1977** 42:298-305.
- 25 Schutte, L.; In *CRC Critical Reviews in Food Technology* **1974**, 4:457-505.
- 26 Gardner, H.W. Effects of lipid hydroperoxides on food components. In, *Xenobiotics in Foods and Feeds*; Finley, J.W., Schwass, D.E., Eds.; ACS Symposium 234; American Chemical Society: Washington, DC; **1983**, pp 63-84.
- 27 Baines, D.A.; Mlotkiewicz, J.A. The chemistry of meat flavour. In *Recent Advances in the Chemistry of Meat*; Bailey, A.J., Ed.; The Royal Society of Chemistry: London; **1984**, pp 119-164.

RECEIVED November 4, 1993

Chapter 7

Changes of Headspace Volatile Compounds Due to Oxidation of Milk Fat During Storage of Dried Dairy Products

Y. B. Lee and C. V. Morr

Department of Food Science and Technology, Ohio State University,
Columbus, OH 43210

Flavor quality is one of the most important factors for determining consumer acceptance of dairy and other food products. Lipid oxidation and Maillard browning reactions that occur during storage of spray dried dairy products result in the formation of volatile off-flavors that greatly impair their flavor quality, thereby limiting their value as ingredients in formulated food products.

Analytical methodologies previously available for isolation, fractionation and identification of volatile compounds formed during storage of dried dairy products are time consuming, tedious and are sometimes subject to error due to artifact formation and incomplete compound recovery. The recently developed dynamic headspace analysis (DHA) technique is suitable for identifying and monitoring changes in concentrations of volatile compounds produced in dried dairy products during storage.

The major objective of this research was to study the changes of headspace volatile compounds produced by oxidation of milkfat in selected, commercial, spray dried dairy products by DHA. A secondary objective was to design a miniaturized water activity (a_w) control chamber for conducting accelerated storage studies on dried dairy products, and that can be sampled for headspace volatile compounds.

Although considerable research has been done on the development of off-flavors in milk and fermented dairy products over the past 10-15 years, relatively little attention has been given to research on off-flavors in dried milk and dairy products (1-4). Two major mechanisms are usually involved in flavor deterioration of dried milk and dairy products, i.e., lipid oxidation and Maillard browning reaction (3,5,6). Ferretti and Flanagan (2) identified 24 volatile compounds in the steam distillate from

0097-6156/94/0558-0098\$08.00/0
© 1994 American Chemical Society

commercial spray dried whey powder that included alkylpyrazines, furans, pyrroles, α -methyl- γ -butyrolactone, isobutyramide, N-methyl-2-pyrrolidinone, 3-hydroxy-2-butanone, benzaldehyde, phenol, benzyl alcohol, maltol, dimethylsulfone, and several organic acids. No attempt was made to distinguish those compounds produced by lipid oxidation from those produced by Maillard browning reaction. Although storage temperature is very important for preventing or inhibiting these chemical reactions (7), moisture and lipid concentration of the dried products is also extremely important in this regard (8). Atomization of milk or whey concentrates into $\geq 150^\circ\text{C}$ air during spray drying would likely promote initiation of the lipid oxidation mechanism. Once initiated, reaction of unsaturated lipids with peroxides, hydroperoxides and free radicals to form the undesirable volatile compounds responsible for the "oxidized" off-flavor would be accelerated.

Oxidized milkfat is commonly described as having an "oxidized" or "cardboard" flavor (9), which differs from the "stale" flavor formed in dried whey and whey protein concentrate (WPC) products. Oxidation of milkfat results in the formation of undesirable volatile compounds in dried milk and whey products (10) and is one of the most important factors limiting the manufacture and utilization of these products. Linoleic, linolenic and several unsaturated fatty acids in milk are especially susceptible to oxidation (9). Aldehydes and other lipid oxidation products from these unsaturated fatty acids are chemically reactive and are believed to promote the Maillard browning reaction (10). Phospholipids are more readily oxidized than the neutral glyceride lipid fraction and is therefore considered as one of the off-flavor precursors in milk (11).

Experimental

Dried milk products. Commercial dried dairy products obtained for this study included: whole milk powder (WMP) that contained 26% milkfat from Maple Island, Inc., Stillwater, MN; nonfat milk powder (NFMP) that contained 0.8 % milkfat, potassium caseinate (PC) that contained $\leq 1\%$ milkfat, and whey protein concentrate (WPC) that contained 7% milkfat from DMV Ridgeview, La Crosse, WI; whey protein isolate (WPI) that contained $< 1\%$ milkfat from Le Sueur Isolates, Le Sueur, MN; and sweet whey powder (WP) that contained 1% milkfat from Holmes Cheese Company, Millersburg, OH.

Miniaturized a_w control chamber. The a_w control chamber design is shown in Figure 1. A saturated slurry of sodium nitrite (NaNO_2) crystals in water was layered on the bottom of a 125 ml serum bottle (Wheaton, Millville, NJ). This arrangement provided a theoretical a_w of 0.59 (12). Six 0.5g aliquots of each dried dairy product were weighed into separate 10 x 75 mm, borosilicate glass, disposable culture tubes (VWR Scientific, Cleveland, OH) and sealed with a teflon-coated septum (Supelco, Bellefonte, PA) and an aluminum cap (Supelco).

Accelerated storage of dried dairy products. The serum bottle was held 2 days in the dark at room temperature to equilibrate a_w of the samples to 0.59. It was

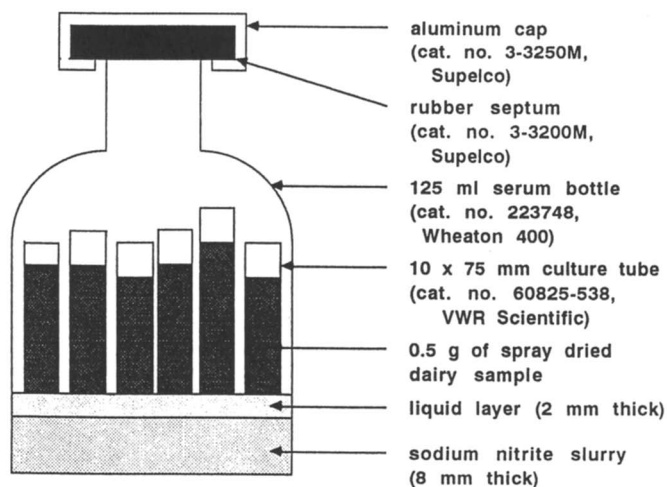


Figure 1. Design of humidity (a_w) control device consisting of a saturated aqueous NaNO_2 slurry in a sealed 125 ml serum bottle.

subsequently exposed to accelerated storage for 6 days at 60°C in the dark and stored in a -20°C freezer until analyzed by DHA.

Dynamic headspace analysis (DHA) of volatile compounds. Serum bottles were held at room temperature, i.e., $20\text{--}24^\circ\text{C}$, for 30 min and attached to the dynamic headspace analyzer (DHA) as shown in Figure 2. Headspace volatile compounds were purged 10 min with ultrapure helium carrier gas at a flow rate of 40 ml/min and trapped onto a Tenax TA chemical column (Tekmar, Cincinnati, OH). Trapped volatiles were desorbed by heating the trap 4 minutes at 160°C and cryogenically focussed at a -150°C capillary interface that was cooled with liquid nitrogen. Focussed compounds were thermally injected onto the capillary gas chromatographic column by heating to 180°C within 1 minute. Compounds were separated with a $30\text{ m} \times 0.25\text{ mm} \times 0.25\text{ }\mu\text{m}$ film thickness DB-WAX column (J&W Scientific, Folsom, CA) in a gas chromatograph (model 5890 II, Hewlett Packard). The column was heated from an initial temperatures of 32°C to 160°C at a rate of $2^\circ\text{C}/\text{min}$. A total ion chromatograph (TIC) of eluted compounds was obtained with a mass-selective detector (model 5971A, Hewlett Packard) and compounds were identified by comparison of their retention index values with those of reference compounds (13) and by computer-matching their mass spectra with that in reference NBS49K.L (Hewlett Packard).

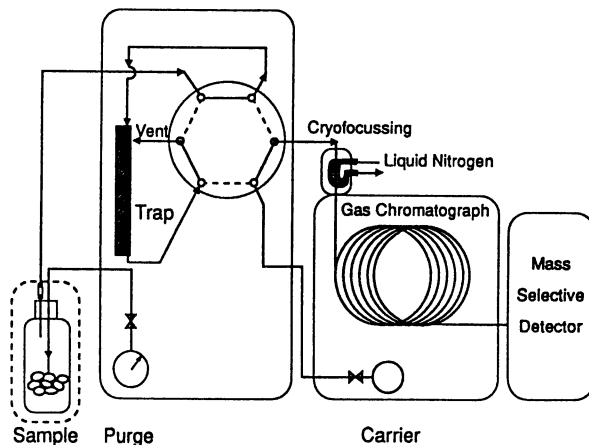


Figure 2. Diagram of the dynamic headspace analysis (DHA) equipment with the 125 ml sealed serum bottle used as a a_w control and external sample device.

Results and Discussion

Volatile compounds in control, dried dairy products. The six spray dried dairy products prior to accelerated storage exhibited a highly acceptable bland and clean odor. The mass spectrum of the unidentified volatile compound exhibiting the largest TIC peak fractionated from all dried milk products except WPC prior to accelerated storage, with a retention time of approximately 6 min, has a base peak at m/z 41. The largest TIC peak eluted from the headspace of WPC with a retention time of 6.8 minutes, was identified as trichloromethane. None of these compounds are believed to be responsible for the off-flavors observed in stored, dried dairy products.

Volatile compounds in stored, dried dairy products. Identification of volatile compounds from the spray dried dairy products that have been stored 6 days at 60°C and a_w 0.59 are provided in Table I. Uniquely different TIC profiles were obtained for each spray dried dairy product after accelerated storage.

Contrary to expectations, NFMP exhibited a TIC profile corresponding to higher concentrations of most volatile compounds than in the TIC from WMP. Since NFMP contains a much lower concentration of milkfat than WMP, this phenomenon may likely be due to differences in lipid composition of the two products. For example, NFMP lipids might be expected to contain predominantly smaller sized milkfat globules with higher proportions of phospholipids and lipoprotein-containing milkfat globule membranes and proportionately less neutral triglycerides than WMP

Table I. Total Ion Chromatogram Peak Areas for Headspace Volatile Compounds Isolated from Spray Dried Dairy Products Stored 6 Days at 60°C and 0.59 a_w

Compounds	Peak area (x 10 ⁶) ^a				
	RI ^b	WMP ^c	NFMP ^c	WP ^c	WPC ^c
Aldehydes					
2-methyl propanal	816	476	533	644	517
Butanal	873	22	27	21	21
2-methyl butanal	914	131	267	436	84
3-methyl butanal	918	192	468	386	63
Hexanal	1086	59	99	121	346
Heptanal	1182	ND	50	30	ND
Octanal	1286	ND	24	ND	34
Nonanal	1390	ND	60	33	67
Ketones					
2-Butanone	900	563	587	460	647
2-Heptanone	1177	93	16	13	108
6-methyl-2-Heptanone ^d	1233	ND	13	3	13
2-Octanone	1281	ND	ND	2	5
2-Nonanone	1386	ND	3	ND	4
Alcohols					
2-Butanol	1032	7	17	12	18
1-Pentanol	1259	ND	21	ND	28
2-Furanmethanol	1644	142	223	28	62
Esters					
Ethyl acetate	890	4	14	7	ND
S-methyl ethanethioate ^d	1048	17	61	24	29
Methyl thiocyanate ^d	1262	4	14	12	47
Hydrocarbons					
Pentane	500	38	75	39	291
Hexane	600	11	29	12	61
Heptane	700	ND	ND	49	38

Continued on next page.

Table I Continued

Compounds	RT ^b	Peak area (x 10 ⁶) ^a				
		WMP ^c	NFMP ^c	WP ^c	WPC ^c	
Aromatic hydrocarbons						
2-methyl furan	870	106	259	152	129	
3-methyl furan	898	61	ND	70	39	
2-ethyl furan ^d	954	12	25	34	44	
2-pentyl furan ^d	1225	ND	5	8	ND	
2-Furancarboxaldehyde ^d	1457	41	74	30	16	
Benzene	938	ND	ND	18	651	
Toluene	1037	9	52	61	51	
Styrene ^d	1250	ND	ND	2	5	
Sulfur-containing compounds						
Carbon disulfide ^d	689	ND	29	47	ND	
Thiobis methane ^a	730	25	42	46	6	
Ethanethiol ^d	751	118	237	244	15	
Dimethyl disulfide	1067	91	158	70	1792	
Dimethyl trisulfide	1363	ND	11	ND	31	
Benzothiazole	1964	ND	25	129	129	
Miscellaneous compounds						
Dichloro methane	927	39	20	20	21	
Trichloro methane	1023	18	ND	32	803	
N ₁ N-dimethyl formamide ^d	1324	ND	31	41	31	

^a Average values of three replicate determinations. ND means less than 1 x 10⁶.

^b Experimentally determined retention index.

^c Product identification: WMP = whole milk powder, NFMP = nonfat milk powder, WP = whey powder, and WPC = whey protein concentrate.

^d Tentatively identified by mass spectrometry.

lipids. Concentrations of 18:1, 18:2; and 18:3 fatty acids in bovine milk phospholipids are 46.7, 12.4, and 3.4 mole %, respectively (14). Differences in phospholipid concentrations in milk and milk products are given by Kurtz (15). Milkfat in whole milk and skim milk contains 0.87 and 17.29% phospholipid, respectively. These lipid compositional differences could well explain the the greater production of volatile compounds in NFMP than in WMP. In addition, differences in the design and operation of the spray dryer as well as in compositional and processing-related factors, i.e., antioxidants, prooxidants, heat treatment, pH, and homogenization, affect the oxidation reaction in milk and dairy products (16).

Major differences were observed in the TIC profiles for WP, WPI and WPC, which were more complicated than for WMP or NFMP. The reasons for these differences are not known, but may be due to the major variations in processing technologies used to manufacture them. For example, WP is made from Swiss cheese whey, a by-product of the cheese manufacturing industry. Milk is subjected to biochemical action of casein coagulating enzymes and lactic acid bacteria during cheese curd formation which may alter the susceptibility of the residual whey lipids to oxidation after spray drying. Membrane fractionation and other processing steps in the manufacture of WPC and WPI may also alter the susceptibility of residual whey lipids after drying. Differences in protein-milkfat ratios in the various whey and milk powders may also result in an altered microstructure of their particles such that the milkfat is more or less protected against oxidation. Differences in polar lipids, lipoproteins and other milkfat globule membrane components may also have a bearing on the formation of volatile compounds in dried dairy products during accelerated storage. The highly unsaturated phospholipid fraction associated with the milkfat globule membrane material is believed to be the site of oxidative deterioration in fluid milk an cream (17).

PC and WPI, the two dried dairy products with the lowest milkfat concentrations, exhibited considerably less production of headspace volatile compounds than the other products during accelerated storage. The best spray dried dairy products from a flavor stability standpoint should be PC and WPI, which contain lowest lipid concentrations. However, additional and more costly processing steps, i.e., ion exchange adsorption for making WPI, are required for manufacturing these latter products (18).

Mechanisms for volatile compound formation in dried dairy products. The two main mechanisms for volatile compound formation in dried dairy products during storage are Maillard browning reaction and lipid oxidation (3,5,6). The substantial concentration of protein and lactose in all of these dried dairy products makes it virtually impossible to rule out the Maillard browning reaction in these studies. In fact, it is possible that these two reactions may be synergistic in nature in dried dairy products. Intermediate and final products from one reaction may actually catalyze the other reaction. Thus, many of the volatile compounds identified in TIC from stored, dried dairy products undoubtedly result from both of these chemical reactions.

Volatile compounds expected to arise from milkfat oxidation, were recovered from the headspace of dried dairy products by DHA after being subjected to

accelerated storage for 6 days at 60°C by DHA (Table I). These included: aldehydes i.e., 2-methyl propanal, butanal, 2-methyl butanal, 3-methyl butanal, hexanal, heptanal, octanal and nonanal; and ketones, i.e., 2-butanone, 2-heptanone, 6-methyl-2-heptanone, 2-octanone, and 2-nonanone. Esters that were identified included: acetic acid, ethyl ester; ethanethioic acid, S-methyl ester; and thiocyanic acid, methyl ester. Acids and lactones were not detected in the headspace of stored, dried dairy products, due likely to their low volatility at the temperature employed for purging.

Milkfat contains roughly 30% oleic acid and approximately 3% linoleic acid (19). Oleic acid is oxidized to form octanal, nonanal, 2-undecenal and 2-decenal, and oxidation of linoleic acid results in the formation of hexanal, 2-octenal and 2,4-decadienal via the hydroperoxide mechanism (20). Aldehydes, which possess a common ion fragment with a mass/charge (m/z) ratio of 44 (21), were among the most abundant compounds formed by lipid oxidation of dried dairy products in the present study. Chromatograms of all volatile compounds isolated from NFMP, WP, and WPC that exhibited a m/z ratio of 44 are shown in Figure 3. Comparison of results for these three spray dried dairy products indicates that the three most abundant aldehydes were in decreasing order hexanal (RT 8.3 min), heptanal (RT 12.8 min), nonanal (RT 25.2 min), and octanal (RT 18.7 min). These results would not be expected on the basis of the percentages of oleic and linoleic acids

WPC stored at 60°C over saturated NaNO_2 solution to maintain an a_w of 0.59 for 6 days exhibited the highest concentrations of the four aldehydes, hexanal, nonanal, heptanal, and octanal, of all dried dairy products studied. WPC also showed the poorest flavor stability among the dried dairy products studied. The reasons for the high susceptibility of WPC to lipid oxidation is not known. WPC contained 7% milkfat and the powder was quite fluffy, thus allowing a more intimate contact of substrate with the residual oxygen from the headspace atmosphere in the serum bottle. It is also likely that WPC had a smaller particle size distribution than the other dried products. Information on the fatty acid composition of the residual lipids in WPC is lacking but it is possible that these lipids may contain a higher percentage of fatty acids that are more susceptible to oxidation than the other dried dairy products. It is for this reason that researchers are attempting to remove the residual lipids from cheese whey prior to manufacturing WPC (18).

Water activity is an important factor that affects the off-flavor formation of spray dried dairy products during storage. The minimum rate of lipid oxidation in milk-based infant foods is at or near an a_w of 0.24 (22). However, Maillard reaction products, which are simultaneously formed during storage of dried dairy products, functioned as antioxidants (23), thus making it difficult to evaluate the relationship between a_w and lipid oxidation rate.

It was shown previously that the use of NaNO_2 for controlling a_w during accelerated storage resulted in a greater degree of lipid oxidation in dried dairy products than was obtained with other a_w -controlling chemicals (24). The release of N_2O by NaNO_2 into the headspace is believed to catalyze the lipid oxidation reaction (24,25).

Conclusions

Although all of the dried dairy products, i.e., whole milk powder, nonfat dry milk, potassium caseinate, whey protein concentrate, whey protein isolate and whey

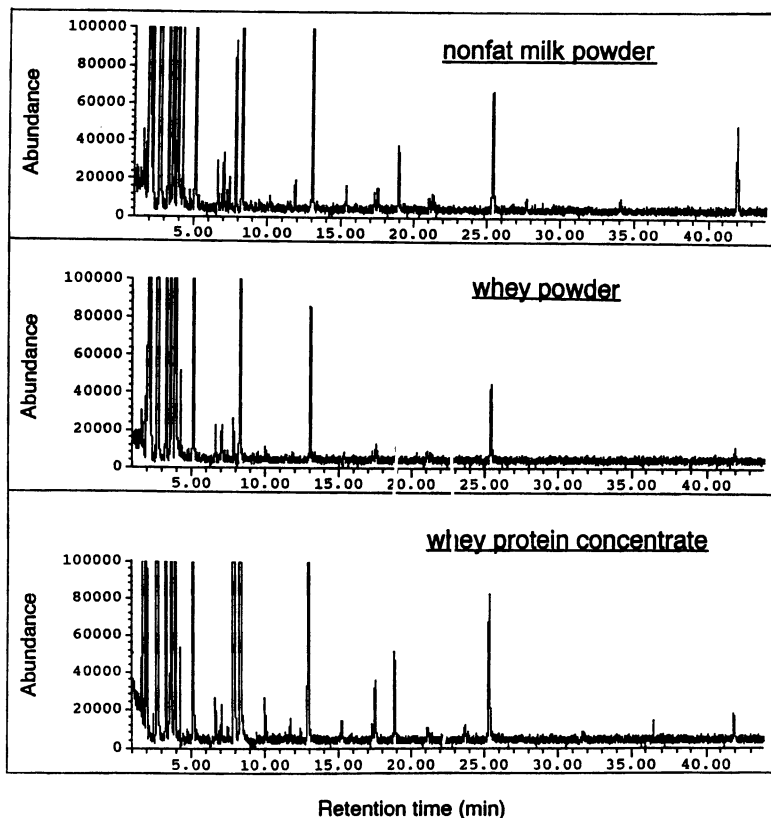


Figure 3. Forty four ion chromatogram (44 IC) of volatile compounds from whole milk powder (WMP), whey powder (WP), and whey protein concentrate (WPC) after accelerated storage.

powder, exhibited a clean and bland flavor before accelerated storage, DHA results revealed different susceptibilities to lipid oxidation during 6 days of accelerated storage at 60°C and a_w of 0.59. DHA 44 ion chromatograms were used as a reliable indicator of the degree of oxidation experienced by these dried dairy products during storage. The humidity (a_w)-controlling device effectively controlled a_w during accelerated storage and also allowed direct sampling of headspace volatiles for DHA of the stored products. The order of decreasing lipid oxidation rates for dried dairy products was: WPI and PC < WMP < WP, NFMP and WPC.

Literature cited

1. Ferretti, A.; Flanagan, V.P. *J. Dairy Sci.* **1971a**, *54*, 1764.
2. Ferretti, A.; Flanagan, V.P. *J. Dairy Sci.* **1971b**, *54*, 1769.
3. Ferretti, A.; Flanagan, V.P. *J. Agric. Food Chem.* **1972**, *20*, 695.

4. McGugan, W.A., Larmond, E.; Emmons, D.B. *J. Inst. Can. Sci. Technol.* **1979**, *12*, 32.
5. Ramshaw, E.H.; Dunstone, E.A. *J. Dairy Res.* **1969**, *36*, 215.
6. Min, D.B.; Tickner, D.B.; Lee, S.H.; Reineccius, G.A. *J. Food Sci.* **1990**, *55*, 401.
7. Driscoll, N.R.; Brennand, C.P.; Handricks, D.G. *J. Dairy Sci.* **1985**, *68*, 1931.
8. Morr, C.V.; Ha, E.Y.W. *Int. Dairy J.* **1991**, *1*, 1.
9. Hammond, E.G. In *Flavor Chemistry of Lipid Foods*; Min, D.B.; Smouse, T.H., Eds.; American Oil Chemist's Society: Champaign, IL, 1989; pp 222-236.
10. Hill, L.M.; Hammond, E.G.; Carlin, A.F.; Seals, R.G. *J. Dairy Sci.* **1969**, *52*, 1917.
11. Sattar, A.; deMan, J.M. *CRC Crit. Rev. Food Sci. Nutr.* **1975**, *7*, 13.
12. Dean, J. A.; *Lange's Handbook of Chemistry*; McGraw-Hill: New York, NY, 1985; pp 10-84.
13. Jennings, W.; Shibamoto, T. *Qualitative Analysis of Flavor and Fragrance Volatiles by Glass Capillary Gas Chromatography*; Academic Press: New York, NY, 1980; pp 30-113.
14. Morrison, W.I. In *Topics in Lipid Chemistry*; Gunstone, F.D., Ed.; Logos Press Ltd.: London, England, 1970, Vol. 1; pp 51-106.
15. Kurtz, F.E., In *Fundamentals of Dairy Chemistry*; Webb, B.H.; Johnson, A.H.; Alford, J.A., Eds.; AVI Publishing Co.: Westport, CT, 1974 2nd Edition; pp 125-219.
16. Weihrauch, J.L. In *Fundamentals of Dairy Chemistry*; Wong, N.P.; Jenness, R.; Keeney, M.; Marth, E.H., Eds.; Van Nostrand Reinhold Co.: New York, NY, 1988, 3rd Edition; pp 215-278.
17. Badings, H.T. Cold-storage Defects in Butter and their Relation to the Autoxidation of Unsaturated Fatty Acids; Ph.D. Thesis, Agricultural University, Wageningen, The Netherlands, 1970.
18. Morr, C.V. *Food Technol.* **1992**, *46*, 110.
19. *Dairy Handbook*; Alfa-Laval: Lund, Sweden, pp 13.
20. Kinsella, J. E. *Chem. Ind.* **1969**, 36-42.
21. Silverstein, R. M.; Bassler, G. C.; Morrill, T. C. *Spectrometric Identification of Organic Compounds*; John Wiley & Sons, Inc.: New York, NY, 1991.
22. Roozen, J. P.; Linssen, J. P. H. In *Lipid Oxidation in Food*; St. Angelo, A. J., Ed.; ACS Symp. Ser. 500; American Chemical Society: Washington, D. C., 1992, pp 302-309.
23. Waller, G. R.; Beckel, R. W.; Adeleye, B. O. In *The Maillard Reaction in Foods and Nutrition*; Waller, G. R.; Feather, M. S., Eds.; ACS Symp. Ser. 215; American Chemical Society: Washington, D. C., 1983, pp 125-140.
24. Lee, Y. B. Ph.D. Dissertation, Ohio State University, Columbus, OH, 1993.
25. Labuza, T. P. In *Moisture Sorption: Practical Aspects of Isotherm Measurement and Use*; American Association of Cereal Chemists: St. Paul, MN, 1984; p 65.

RECEIVED November 4, 1993

Chapter 8

Lipid-Derived Aroma Compounds in Cooked Potatoes and Reconstituted Dehydrated Potato Granules

Juan P. Salinas, Thomas G. Hartman, Karl Karmas, Joseph Lech,
and Robert T. Rosen

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

Volatile compounds that contribute to flavor of steam cooked mashed potatoes and reconstituted dehydrated potato granules were characterized. Aroma components were isolated and concentrated using continuous steam distillation - extraction procedures and/or by dynamic headspace concentration using porous polymer adsorbent trapping media. The steam distillation extracts and adsorbent traps were subsequently analyzed by gas chromatography - mass spectrometry (GC-MS), the latter using short path thermal desorption methodology. Quantification was achieved using matrix-spiked internal standard methodology. Comparison of the two isolation techniques revealed both qualitative and quantitative differences in the extracted profiles which were highly reproducible. The purge and trap - thermal desorption methodology produced GC-MS chromatograms with enhanced recoveries of light end components while the steam distillation extracts contained greater amounts of higher boiling semivolatile species.

In the United States, potatoes are mainly used for processing and as a fresh product. Most of the processed potatoes are frozen, made into chips, dehydrated, or canned. Because of the current increasing demand for easy to cook yet nutritional products, dehydrated mashed potatoes have become a very popular product over many other convenience foods on the market. However, aside from their convenience and nutritional benefits, dehydrated potato products lack fresh potato flavor and they may contain off-flavors (1-7). The lack of fresh flavor has been attributed to both the loss of volatiles during the drying process (8) and the off-flavors generated during the processing and storage as a result of nonenzymatic browning (5-10) and lipid oxidation reactions (1-3,8,10).

Sapers et al. (3) were the first to investigate a hay-like off-flavor developed

0097-6156/94/0558-0108\$08.36/0
© 1994 American Chemical Society

in flakes during storage. This off-flavor was associated with the development of increasing amounts of *n*-hexanal and other lipid oxidation products such as: *n*-pentanal, *n*-heptanal, 2-heptanone, 2-pentylfuran, 2-hexenal, benzaldehyde and three unknowns. A direct correlation between hexanal concentrations and the off-flavor development was found (11,12). The use of antioxidants, oxygen scavengers, and nitrogen have been found effective in the reduction of lipid oxidation during storage. Konstance et al. (12) demonstrated that using the antioxidant BHA alone or with BHT, or using oxygen scavenger laminated pouches could increase the shelf-life of dehydrated potatoes (explosion puffed) to at least one year.

Other off-flavors are also produced as a result of nonenzymatic browning reaction products. For instance, Sapers (5) studied a "puffing" off-flavor described as scorched, wet fur or laundry-like which was developed during the processing of explosion puffed dehydrated potatoes. He was able to reproduce the off-flavor when 2-methylpropanal, 2-methylbutanal and 3-methylbutanal were added to conventionally dehydrated potatoes as a mixture. These methyl substituted aldehydes are the Strecker degradation products of valine, isoleucine, and leucine, respectively. A different off-flavor described as "toasted" was also detected in explosion puffed potatoes. Sapers et al. (6) associated the combination of several nonenzymatic browning reaction products with the intensities of the "toasted" off-flavor. The compounds 2,5-dimethylpyrazine, 2,3- and/or 2,5-methyl ethylpyrazine, trimethylpyrazine and two unknowns were found to have pyrazine-like aromas (burnt, toasted, roasted, nutty, popcorny and bready). The compound, 5-methylfurfural contributed to the aroma characteristic of the thermal degradation of the proline-glucose mixture. Meanwhile, benzaldehyde, a Strecker aldehyde of phenylglycine, imparted a "burnt" aroma.

In dehydrated potato products, the susceptibility to chemical changes responsible for the off-flavor generation during processing will directly depend, among other factors, on the cultural, environmental and processing conditions. The effect of certain raw material and processing variables on the flavor quality and stability of the potato flakes was investigated by Sapers et al. (4). They concluded that the presence of natural pro-oxidants in the peel and of sprouts and rots present in defective potatoes used in the manufacturing of flakes will increase their susceptibility to lipid oxidation during storage. In addition, their stability will further be affected by the removal of natural antioxidants (e.g., phenolic acids, quercetin, and caffeic acid) from the potato during cooking and cooling with maximum process water.

The objective of this study was to characterize the volatile flavor compounds from conventionally air dried reconstituted potato granules and to identify offending species in off-flavored samples. In addition, the effect of aging of the raw potatoes on the flavor of mashed potatoes prepared from partially peeled (95%) potatoes and cooked under steam was investigated. The development of a rapid, sensitive, and highly reproducible analysis methodology using a short path thermal desorber accessory for assessing flavor quality of potato products is also described herein.

Experimental

Materials. Samples of conventionally dehydrated potato granules (8% moisture) and unpeeled Russet Burbank potatoes were obtained from Basic American Foods (Blackfoot, ID). Methylene chloride solvent was capillary-analyzed grade and was acquired from J. T. Baker (Phillipsburg, NJ). 2-Phenoxyethanol, d-8 toluene and d-8 naphthalene internal standards were purchased from Aldrich Chemical Co. (Milwaukee, WI). Tenax-TA adsorbent, 60/80 mesh was obtained from Alltech Associates, Inc. (Deerfield, IL). Carbotrap 20/40 mesh was from Supelco, Inc. (Belefonte, PA). The model TD-1 Short Path Thermal Desorber and all desorption accessories were obtained from Scientific Instrument Services Inc. (Ringoes, NJ). The glass components for the simultaneous steam distillation-extraction apparatus and the Snyder distillation column were purchased from Kontes (Vineland, NJ).

Potato Cooking Procedure. A 12 quart stainless steel pot was filled to a depth of approximately 5 inches with high purity distilled deionized water (Milli-Q purified) and heated to a boil on a stove. Meanwhile, approximately 6 lbs of washed potatoes were peeled using a hand held peeler in such a way that about 5% of the peel remained on the potato. This was done because potatoes used in the manufacturing of dehydrated potatoes are not peeled completely (95%) because they use an economical but less effective method of peeling. The peeled potatoes were then cut cross-wise into sections approximately 1.25 inches thick using a stainless steel knife on a clean cutting surface. The potato slices (1600 grams) were then placed inside a stainless steel mesh wire basket which was placed inside the cooking pot and suspended about 4 inches above the boiling water. Steam cooking minimizes loss of water soluble antioxidants and/or pro-oxidants. The pot was then covered with a lid and the product cooked for 35 minutes or until the cooked potatoes were easily mashed with a fork. Cooked potatoes were then mashed in a stainless steel mixing bowl using a household hand held masher until a creamy consistency was obtained. The mashed potatoes were then rapidly transferred into the simultaneous steam distillation-extraction apparatus or the headspace purge and trap apparatus (Figure 1) as described in the flavor extraction protocol. Dehydrated potato granules were reconstituted in the isolation apparatus using the conditions described in the following section.

Flavor Extraction and Isolation

Simultaneous Steam Distillation-Extraction. All samples were prepared for gas chromatography (GC) and GC-MS analysis using the technique of simultaneous steam distillation-extraction in the apparatus originally described by Likens and Nikerson (13) (the technique is often referred to as Likens-Nikerson

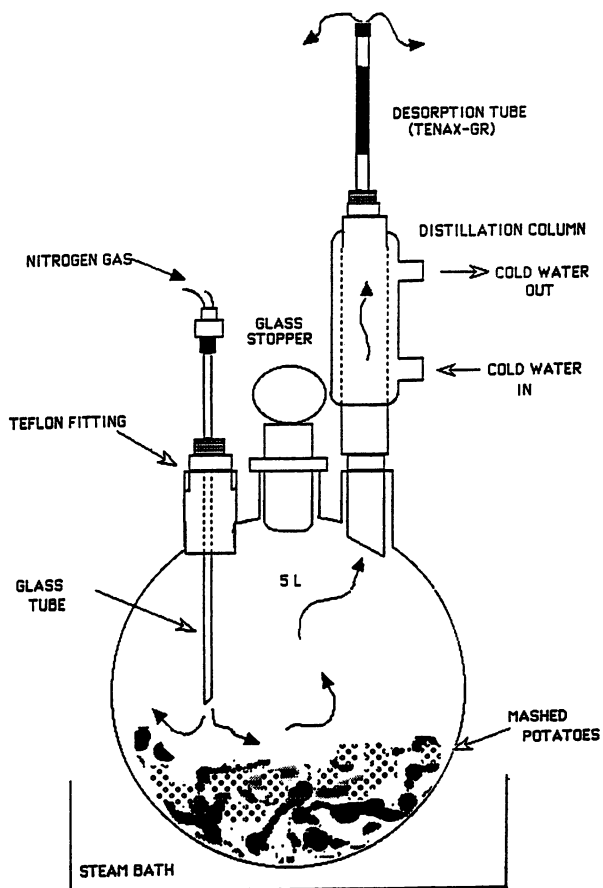


Figure 1. Headspace purge and trap apparatus.

extraction). This technique provides for simultaneous steam distillation and solvent extraction of the distillate on a continuous basis. The following procedure was used.

To 2500 ml of boiling water (ultra high purity glass distilled) in a 5.0 liter round bottom flask were added 2.5 grams of salt, 250 grams of dehydrated potatoes and some teflon boiling chips. When the isolation was performed using freshly prepared mashed potatoes, the following modified protocol was employed. To a 5 liter beaker was added 1150 grams of fresh mashed potatoes, 2.5 grams of salt and 1600 ml of boiling water. The sample was then thoroughly mixed to form a thin paste or slurry which was rapidly transferred into the 5.0 liter flask along with some teflon boiling chips. The flask was then fitted with an inverted distillation trap to act as a foam breaker (in the event of excessive foaming) and the assembly was attached to the Likens-Nikerson apparatus. The potato slurry was then spiked with 2-phenoxyethanol internal standard (100 ppb w/w based on solids content of approximately 250 grams). The apparatus was charged with 200 ml of methylene chloride, and the system was distilled and extracted for 3 hours at 100°C.

Once the extraction was complete the methylene chloride extract was removed from the apparatus and transferred quantitatively into a 250 ml size Kuderna-Danish concentrator fitted with a three ball Snyder column. The extracts were then concentrated over a steam bath to a volume of approximately 5.0 ml. The sample extracts were then finally concentrated to a volume of 1.0 ml using a gentle stream of nitrogen. Aliquots of these extracts (1.0 μ l) were then injected directly into the GC and GC-MS for analysis. The extracts were also subjected to sensory analysis by sniffing. The extracts were stored in glass vials with teflon lined screw capped lids, protected from light. The headspace in the vials was flushed with nitrogen and they were stored at -80°C prior to and following analysis.

Blank runs were routinely conducted using the Likens-Nikerson apparatus described but without the potato product. This served as a control so that any artifacts originating from the isolation apparatus itself (impurities in the water, methylene chloride, internal standard, glassware, etc.) would be readily identified and disregarded.

Purge and Trap Thermal Desorption. In this technique volatile aroma compounds were purged from the samples using a high purity inert gas such as nitrogen. The flavor compounds were then trapped on a porous polymer trap containing Tenax and Carbotrap (50:50). The adsorbent traps were then thermally desorbed directly into the GC-MS system for analysis.

For all the potato samples, 250 grams of dehydrated potato product (or equivalent peeled and sliced cooked potatoes) and 2.5 grams of salt were mixed with water (ultra high purity glass distilled) and mashed into a paste. The potato mash was then spiked with 1 ppm of d-8 toluene and 100 ppb of d-8 naphthalene internal standards, and quickly transferred into a 5 liter 3-neck round bottom flask.

The flask was then sealed at one end with a fitting assembly containing the purging gas rod, and at the other end with a reflux condenser column containing the adsorbent trap. The third opening was sealed with a glass stopper and teflon sleeve. The wet potato mash was heated at 100°C for 1 hour on a steam table during which time the volatile compounds were purged from the product into the Tenax-Carbotrap adsorbent traps using nitrogen as a sparge gas at a rate of 40 ml/min. The charged traps were then dried with high purity nitrogen at a rate of 40 ml/min for 60 min at 30°C. The dried charged traps were then thermally desorbed directly into the GC-MS system using a SIS model TD-1 short path thermal desorption system operated at 220°C for 5 minutes. The GC injection port was maintained at 250°C and 10:1 split ratio was employed. The column was temperature programmed from -20°C to 40°C at a rate of 10°C/min and 40°C to 280°C at a rate of 4°C/min. The mass spectrometer was operated using electron ionization scanning masses 35-350 once each second throughout the GC run.

Instruments and Conditions. Gas chromatographic analyses were conducted using a Varian 3400 gas chromatograph (GC) equipped with a flame ionization detector (FID). Data was acquired and processed using a Varian 4290 integrator and a VG Multichrom computerized data system. The GC was equipped with a 60 meter x 0.32 mm i.d. DB-1 capillary column containing a 0.25 µm film thickness. Helium was used as a carrier gas with a flow rate of 1.0 ml/min which converts to a linear carrier velocity of 20 cm per second. For the analysis of the flavor extracts, the GC injector temperature was 250°C and 1.0 µl aliquots of flavor extracts were made in the splitless injection mode. At 0.5 minutes after injection, the injector split ratio valve was activated to initiate a 100:1 split ratio to serve as a septum purge throughout the remainder of the run. The column oven was temperature programmed from 35°C (held 5 min) to 280°C at a rate of 2°C per minute, then a 60 minute hold at the upper limit.

Combined GC-MS analyses were conducted using a Varian 3400 gas chromatograph directly interfaced to a Finnigan MAT 8230 high resolution magnetic sector mass spectrometer. All chromatographic conditions were identical to those described previously. The mass spectrometer was operated in electron ionization mode scanning masses 35-350 continuously at a rate of 1.0 seconds per decade. Data were acquired and processed using Finnigan MAT SS300 data system. Computerized library searches of mass spectra were conducted using the National Institute of Standards and Technology (NIST, formally the U.S. National Bureau of Standards) mass spectral data base. Any mass spectra which were not identified by the computer library were manually searched against the EPA-NIH and Wiley NBS registries of mass spectra which contain approximately 120,000 entries. Spectra which were not found in any library were manually interpreted to the best of our ability and this often resulted in structural characterization of the unknown species although absolute structure assignments were not always possible.

Quantification. Quantification of the flavor components in the extract samples was accomplished by using 2-phenoxyethanol as a surrogate internal standard. This compound is not found naturally in potato flavor but it contains chemical functionality similar to many potato flavor compounds (aromaticity, alcoholic, ethereal, etc.) so that its extraction efficiency is a good overall measure of flavor yield. On the other hand, for the headspace purge and trap analysis, d-8 toluene and d-8 naphthalene internal standards were utilized. Both are synthetic chemicals and are easily retained by the adsorbent resins. Because of their difference in boiling points (d-8 toluene = 110°C, d-8 naphthalene = 217.7°C), they were able to cover the wide range of volatile chemicals present in the flavor of potato. Concentration levels of the individual flavor compounds in all the samples were calculated by dividing the integrated peak area of the internal standard into that obtained for the flavor components using the GC-FID and/or GC-MS data. The data treatment assumes a response factor of 1.0 thus simplifying the calculations and providing for semi-quantitative estimates of concentrations. This is a reasonably accurate method of quantification since analytical reference standards for all of the hundreds of flavor compounds observed are not readily available for purposes of calculating individual response factors.

Results and Discussion

Core Volatile Compounds. In the analysis of all steam cooked and reconstituted dehydrated potato samples, more than 200 different volatile compounds were identified. However, only 79 compounds were commonly present in all potato samples (core volatile flavor compounds). Many of the volatiles identified have never been reported before as volatile flavor components of fresh cooked potatoes or mashed potatoes. Table I lists the core volatile flavor components of mashed potatoes.

By far the majority of the core volatile compounds found were those resulting from lipid peroxidation. The early part of the chromatograms was dominated by aldehydes, ketones and alcohols resulting from lipid peroxidation. Midway through the GC run the chromatograms contained aliphatic and olefinic hydrocarbons as well as alcoholic, ketonic, and aldehydic derivatives which were also peroxidation products. The end of the runs contained mostly free fatty acids and oxidized analogues as well as esters and waxes. Throughout the runs Strecker degradation and Maillard reaction products were also present as well as natural flavor compounds and breakdown products of tocopherols. Most of the natural flavor compounds identified consisted of terpenoid compounds such as α -thujene, α -pinene, β -pinene, α -terpinene, p-cymene, γ -terpinene, dimethylstyrene, thujyl alcohol and limonene. These terpenes were not listed as core volatiles because they were not consistently found in all samples. However, they could have been present at levels lower than the instrument's limit of detection.

Table I. Core Volatile Components of Fresh Cooked and Reconstituted Dehydrated Potatoes

Sulfur compounds	
* Dimethyl disulfide	Diethyl sulfide
* Dimethyl trisulfide	* Dimethyl tetrasulfide
Methional	2-thiophene carboxaldehyde
Aldehydes	
Acetaldehyde	* 2-Butenal
3-Methylbutanal	2-Methylbutanal
* Pentanal	Hexanal
2-Hexenal	* 2-heptenal
4-Heptenal	2-Octenal
Heptanal	Benzaldehyde
<i>cis</i> -2-heptenal	* Octanal
Phenylacetaldehyde	Nonanal
* 2,4-Octadienal	2,6-Nonadienal
Ethyl benzaldehyde	2-Nonenal
Decanal	2,4-Nonadienal
2,4-Decadienal	* 2-Undecenal
Ketones	
* 2-Butanone	* 2,3-Butanedione
* 1-Penten-3-one	2-Heptanone
* 1,2-Cyclohexanedione	* 2-Methyl-3-octanone
3-Octen-2-one	* 3,5-Octadien-2-one
* 2-Nonen-4-one	* Geranylacetone
* Farnesyl acetone	
Alcohols	
* Furfuryl alcohol	1-Octen-3-ol
* 1-Octanol	2-Octen-3-ol
Phenylethyl alcohol	* <i>p</i> -Menthadienol
* <i>cis</i> -Farnesol	* Nerolidol
Furans	
Tetrahydrofuran	2,3-Dihydro-4-methylfuran
* 2-Ethylfuran	2-pentylfuran
* 5-Methylfurfural	Furfural
Pyridines and Pyrazines	
* Pyrazine	Pyridine
Methylpyrazine	2,5-Dimethylpyrazine
* 2-Pyridine methanol	2-Ethyl-6-methylpyrazine
2-Acetylpyridine	

Continued on next page

Table I. Continued

Miscellaneous Compounds			
	Toluene	*	Styrene
*	α -Copaene	*	Tetradecane
	BHT (antioxidant)	*	Pentadecane
*	Hexadecane		Chlorpropham (sprout regulator)
*	Eicosane		Palmitic acid
	Methyl palmitate		Methyl linoleate
	Linoleic acid		Polyunsaturated long chain alcohol
	Heptadecanoic acid		Propyl octanoate
*	Tricosane		

* indicates newly identified compound of fresh cooked potatoes.

Although, fresh potatoes contain only an average of 0.1% fat (fresh weight basis) (14), the polyunsaturated linoleic acid and linolenic acid constitute 39.1% and 32.2%, respectively, of the fatty acid composition in air-dried potatoes (15). Therefore, it is expected that the potato fat will be relatively susceptible to oxidative deterioration. Indeed the entire chromatograms presented chemical products of lipid oxidation and sometimes several oxidized high boiling lipids. The lipid oxidation compounds identified included 18 aldehydes, six of which have never been reported as flavor compounds of fresh cooked potatoes. These compounds are 2-butenal, pentanal, octanal, 2,4-octadienal, and 2-undecenal. In addition, *cis*-2-heptenal was tentatively identified. Most of these aldehydes are the oxidative products of oleic acid (e.g., 2-undecenal), linoleic (e.g., pentanal, octanal) and linolenic acid (e.g., 2-butenal, 2-heptenal). The compound 2-heptenal has a strong cardboard-like aroma and is probably derived from higher homologues than oleic, linoleic, and linolenic acids, or by oxidation of smaller C18 unsaturated acids. Although pentanal and octanal were previously identified in autoxidized dehydrated potatoes granules (2), they have not been reported in fresh cooked potato products to date. Other important lipid oxidation aldehydes identified included the aliphatic aldehydes hexanal, heptanal, nonanal, and decanal. Amongst these aldehydes, the generation of hexanal in potato products has been the subject of most investigations (3,11,12).

Hexanal is a major product of autoxidized linoleic acid and is commonly present in autoxidized oils and fats. It has long been used as an index of oxidative deterioration in potato products. Sapers (3) related the presence of a hay-like off-flavor in potato flakes during storage to the development of increasing amounts of hexanal and other lipid oxidation products such as pentanal, heptanal, 2-heptanone, 2-pentylfuran, 2-hexenal, benzaldehyde, and three unknowns. The development of hexanal arrives from the intermediate 2-octenal which is a degradation product of 2,4-decadienal (16). As expected, both intermediate

compounds, 2-octenal and 2,4-decadienal, were found at concentrations lower than the product hexanal. The mechanism of formation follows the classic Farmer autoxidation (17,18,19) or an alpha/beta double bond hydration followed by a retro-aldol condensation mechanism (20).

Another compound resulting from the degradation of 2,4-decadienal (from linoleic acid) is aromatic benzaldehyde (21). The latter is naturally found in essential oils and is responsible for the almond-like flavor of almonds. Other important lipid derived aldehydes are 2,6-nonadienal and *cis*-4-heptenal. Both were identified as flavor components of mashed potatoes. However, they were first recognized as part of the boiled potato flavor by Josephson and Lindsay in 1987 (16). The compound 2,6-nonadienal is the oxidation product of linolenic acid, and imparts a characteristic "green" flavor for example, in cucumbers. The retro-aldol condensation product of 2,6-nonadienal is *cis*-4-heptenal. The latter has been found to exhibit boiled potato-like aroma in diluted concentrations (0.1-0.4 ppb) but, at concentrations greater than 0.7 ppb, *cis*-4-heptenal contributes to a staling-type off-flavor (16). In this investigation, *cis*-4-heptenal was found at concentrations greater than 5.0 ppb, thus probably contributed to stale-type flavors in the product. Newly identified ketone species included 2-butanone, 2,3-butanedione (diacetyl), 1-penten-3-one, 2-methyl-3-octanone and 3,5-octadien-2-one. Cyclohexanedione was tentatively identified.

The diketone, diacetyl, is one of the most important ingredients used for imitation butter flavors. Most of the unsaturated ketones detected are the result of the decomposition of secondary lipid oxidation products. For instance, 3,5-octadiene-2-one is the decomposition product of the five-membered cyclic peroxide form in autoxidized linolenic acid (22). The methyl ketone is probably formed by beta-oxidation of fatty acids. In addition to the ketones mentioned, geranylacetone and farnesylacetone, which are lycopene pigment oxidation products, were observed in fresh mashed potatoes for the first time. Lycopene can exist in the free state in plant tissue, and is the pigment responsible for the color of tomatoes. Although the lipid system tends to oxidize more easily, lycopene with its high unsaturation (8 isoprenoid units) will be susceptible to oxidation by free radical formation. Alcoholic derivatives of carotenoids such as *cis*-farnesol and nerolidol were also identified. Another compound worth mentioning is the formation of 2-pentylfuran from the autoxidation of linoleic acid (23). This cyclic compound forms from the oxidation of the C-10 vinyl radical that forms as a result of the hydroperoxidation and cleavage of the C-9-OOH of linoleic acid. The oxidized vinyl radical will then cyclize to C-13 to form pentyl furaldehyde which then decomposes into 2-pentylfuran (22). This compound has been reported in the flavor volatiles of baked, raw, and boiled potatoes (24,25,26). It contributes to the green, beany, grassy notes of the mashed potato flavor.

In addition to the large number of lipid derived aroma compounds, nonenzymatic browning reaction products also contributed to the overall flavor of mashed potatoes. One of the most important nonenzymatic browning compounds identified in the flavor of mashed potatoes was methional, a Strecker aldehyde of

methionine. It has an odor threshold of 0.2 ppb in water, and contributes to the boiled potato-like aroma of mashed potatoes. This compound has been identified previously in boiled and baked potato volatiles (24,25). Methional can also further decompose to methyl mercaptan and dimethyl disulfide which have low odor thresholds, thus contributing to the overall flavor. In addition to methional, five other sulfur containing chemicals were identified including dimethyl disulfide, diethyl sulfide, and newly identified dimethyl trisulfide, dimethyl tetrasulfide, and 2-thiophenecarboxaldehyde.

Dimethyl disulfide is the product from the oxidation and dehydration of two molecules of methyl mercaptan. Further decomposition of dimethyl disulfide will yield dimethyl trisulfide and dimethyltetrasulfide which are formed by disproportionation. All these chemicals have odors characteristic of onions at very low odor threshold values (0.16-12 ppb/water). Other Strecker aldehydes which were consistently found in the flavor of mashed potatoes were 2-methylbutanal and 3-methylbutanal. Both have been associated with the puffing off-flavor of explosion puffed dehydrated potatoes at large quantities (5,6,8,9). However, when diluted to low concentrations (<20 ppm) they have a sweet, chocolate, sometimes roasted or toasted flavor. In this investigation, both compounds were detected at concentrations less than 20 ppm, thus, probably contributing to the toasted, sweet notes of the potatoes.

A few pyrazines and pyridines associated with nonenzymatic browning reaction in potatoes were also identified. Newly identified pyrazines and pyridines include pyrazine itself and 2-pyridinemethanol. These compounds are formed during heating when an alpha amino acid reacts with an alpha dicarbonyl (Strecker degradation). The resulting Strecker aldehydes and α -aminoketones further react to yield these important heterocyclic aroma compounds. Both these compounds impart characteristic roasted, maybe sweet notes to the aroma of the product. Other heterocyclic compounds include pyridine, methylpyrazine, 2,5-dimethylpyrazine, 2-ethyl-6-methylpyrazine, and 2-acetylpyridine. They all have been previously identified in the flavor profiles of baked potato (27).

The presence of several furans such as furfural, 5-methylfurfural, 2-ethylfuran, 2,3-dihydro-4-methylfuran, and 2-pentylfuran were also observed. Furfural, which forms from glucose pyrolysis and the Maillard reaction, contributes to the generation of a toasted flavor in dehydrated potatoes (6). 2,3-Dihydro-4-methylfuran is being reported here for the first time in potatoes. However, its contribution to the overall mashed potato aroma was not established. In general, furans are formed from the decomposition of Amadori and Heyns products in the Maillard reaction. Although they can also form from the cyclization of oxidized fatty acids fragments such as in the case of 2-ethylfuran and 2-pentylfuran. In contrast to the beany, grassy aroma of 2-pentylfuran, the newly identified 2-ethylfuran imparts sweet and burnt aromas.

Two synthetic materials are presented as core volatile compounds of mashed potatoes, butylated hydroxytoluene (BHT) and chlorpropham. Although they are synthetic materials, BHT and chlorpropham are frequently used in raw

and processed potatoes, and can effect to the overall flavor of the mashed potatoes. Chlorpropham, commonly known as CIPC or under the trade name Sprout-Nip, is a plant growth regulator used to inhibit sprouting in potatoes during storage. Sprouts contain pro-oxidants and phenolic compounds which impart an undesirable bitter and astringent taste to potatoes (28) therefore their growth must be inhibited. The potential for CIPC to cause flavor/off-flavor to the mashed potatoes was not investigated here. However, studies by Mazza and Pietrak (29) showed that aromatic based solvents used to apply CIPC can impart a musty, earthy, potato bin-like aroma and taste to the potatoes. In our investigation, one of the potato samples that had been treated with CIPC contained an off-flavor described also as bin-like. The chromatogram showed a region consisting mainly of petroleum type distillates including several alkyl substituted benzenes. These substituted benzenes were identical to those found in the investigation by Mazza and Pietrak, and may be responsible for the off-odor. The other synthetic chemical that was added was BHT. This chemical is one of the most widely used antioxidants in foods, animal feed, petrol products, synthetic rubbers, plastics, animal and vegetable oils, soaps, etc. In potatoes, BHT is added to the dehydrated product in order to reduce or avoid the destruction of fatty acids by oxidation, thus extending the shelf-life. The use of BHT to minimize lipid peroxidation was only partially successful owing to the fact that highly polyunsaturated species such as linoleic acid (18:3) preferentially autooxidize even in the presence of BHT. Low levels of lipid peroxidation are important in the flavor of potatoes. However, if allowed to proceed unchecked it may often be the cause of objectionable odors and/or flavors in food products.

Effect of Storage Time on Raw Potatoes. The effect of the raw potato age on the flavor of cooked mashed potatoes was observed. Three different age raw potatoes 1-2 months old, 4 months old, and 13-14 months old potatoes were cooked and mashed to obtain the volatile profiles. The raw potatoes were held under cellar storage conditions of 6.1°C and the relative humidity was maintained just under the point of condensation. After Processing the raw potatoes, results indicated that the 1-2 months old potatoes contained the lowest level of overall volatiles. Both lipid oxidation type aroma compounds and those resulting from the Maillard reaction were low in this sample. In contrast, the 13-14 months old potatoes produced a volatile profile which was dominated by Maillard type aroma compounds. The 4 months old potatoes had lower amounts of Maillard type compounds than the 13-14 months old potatoes but higher levels of lipid oxidation derived flavor than any of the other raw potato samples. The chromatograms of the 1-2 months old (Figure 2), 13-14 months old (Figure 3), and 4-months old (Figure 4) potatoes are shown.

The flavor and aroma characteristics from the 4-months old potatoes were dominated by lipid oxidation notes which imparted an undesirable rancid, potato-chip like aroma to the product. This sample contained concentrations of lipid oxidized compounds almost 8000 times higher than the 1-2 months old product,

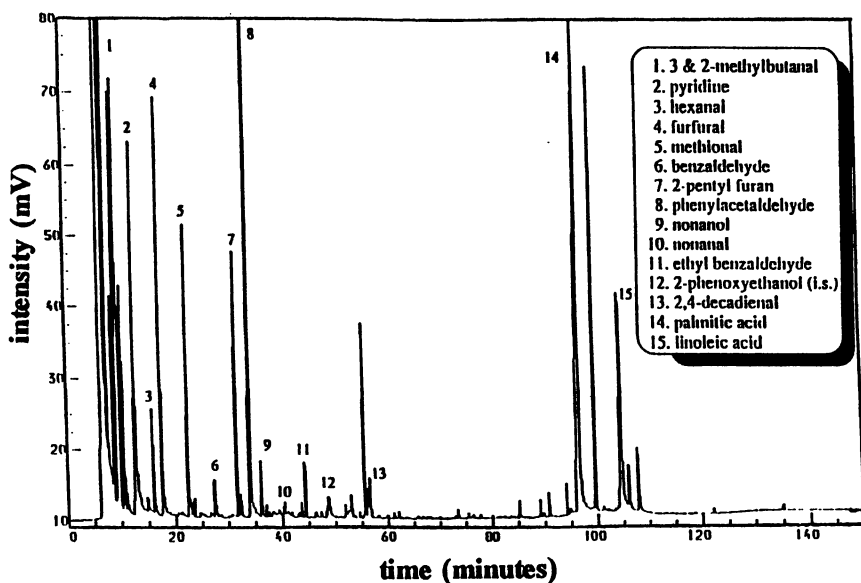


Figure 2. Volatile profile of fresh cooked 1-2 months old potatoes

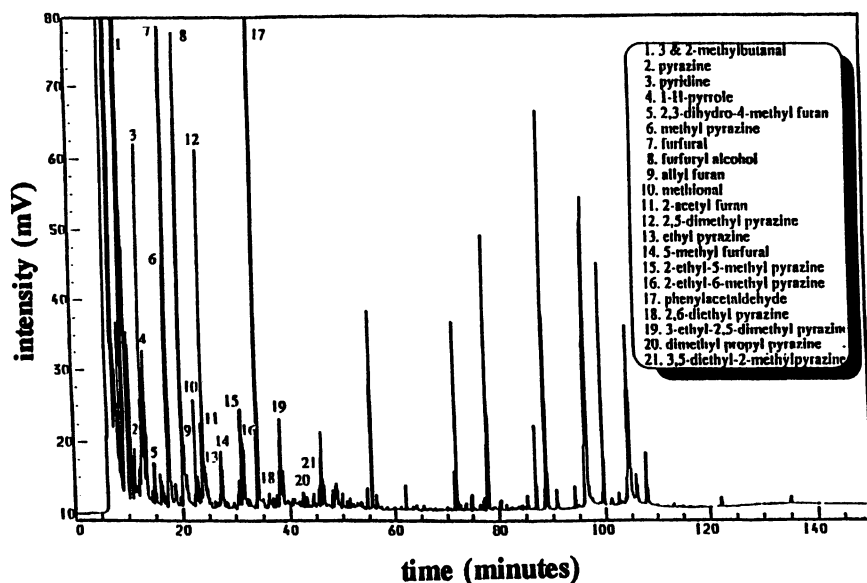


Figure 3. 13-14 Months old potatoes exhibiting high levels of nonenzymatic browning compounds

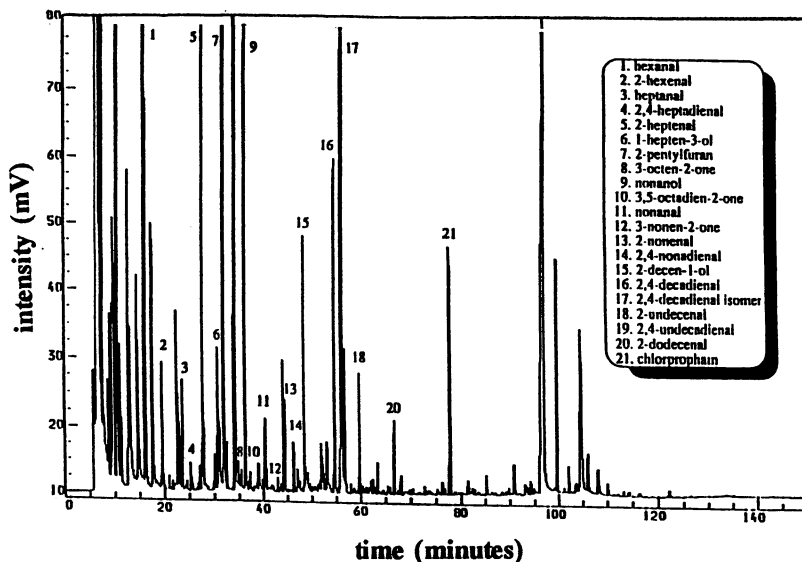


Figure 4. Four months old potatoes exhibiting high levels of lipid oxidation compounds

as calculated by the levels of hexanal. Several lipid oxidation-derived products that were not found in the 1-2 months old potatoes were detected in the 4 months old sample. They included 5 unsaturated ketones, 6 aldehydes, 3 hydrocarbons, and 4 alcohols (Table II). These compounds were not found in the 1-2 months old sample because there was not enough time to permit significant lipid oxidation. In the 13-14 months sample, however, these compounds were masked by the excessive concentrations of Maillard reaction products.

Fatty acids become more available as time progresses during storage. Cherif (30) noticed that the total fatty acid content of potato tubers increase during storage at 10°C and 28°C. Moreover, at 0 and 4°C the total fatty acid content not only increased but it was also accompanied by an increase in the degree of unsaturation. Therefore, it is believed that the 4 months old potatoes had a higher concentration of free unsaturated fatty acids which made the product more susceptible to oxidation. In addition, the presence of sprouts, blackspots and other defects in the raw potatoes can be transferred into the mashed product during processing when the peel is not completely removed. These defects occur during storage at temperatures of 4.4°C or above. Sprouts, blackspots, and other defects contain pro-oxidants that can accelerate the oxidation of fatty acids. Commercially, about 95% of the peel is successfully removed, and potatoes are stored in cellars at temperatures greater than 4.4°C. Therefore, these defects become an important factor in the formation of lipid oxidation volatiles.

Table II. Lipid Oxidation Volatiles in Oxidized 4 Months Old Potatoes

Component	Concentration (ppb)
2-Ethyl furan	484.90
3-Penten-2-one*	138.66
Hexanal	8007.30
3-Hexen-2-one*	5.80
2-Hexenal	204.90
6-Methyl-3,5-heptadien-2-one*	25.10
2-Heptanone	159.40
4-Heptenal	52.57
Heptanal	164.60
2,4-Heptadienal*	69.29
Nonane*	12.30
Benzaldehyde	107.25
2-Heptenal	1213.30
Unknown unsaturated ketone*	29.26
1-Hepten-3-ol*	258.00
2,4-Heptadienal isomer	178.80
2-Pentyl furan	1694.20
3-Octen-2-one	42.49
Octanol	978.80
3,5-Octadiene-2-one	38.30
2-Octen-1-ol*	6.60
3,5-Octadien-2-one isomer	56.17
2,4-Octadienal	30.75
Nonanal	106.60
2-Nonen-4-one	27.29
3-Nonen-2-one*	29.17
2,6-Nonadienal	20.76
2-Nonenal*	139.58
Nonanol*	10.13
2,4-Nonadienal	54.10
2-Decenal*	5.90
2-decen-1-ol*	20.25
<i>cis</i> -2,4-decadienal	716.60
2,4-Decadienal isomer	3005.60
2-Undecenal	206.80
Dodecanal*	9.05
2,4-Undecadienal*	51.99
3-Nonyl-2-one	13.84

Continued on next page

Table II. Continued

Component	Concentration (ppb)
2-Dodecenal*	121.90
Hexadecane*	13.77
Heptadecane*	11.10

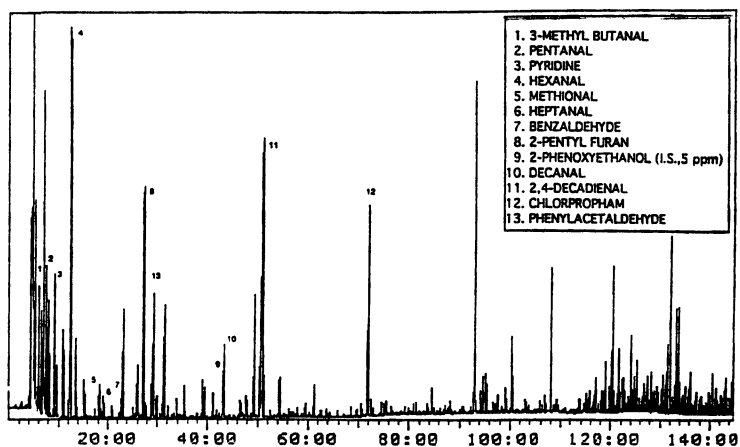
* Indicates lipid-derived compounds not found in 1-2 months old potatoes.

The 13-14 months old potatoes had a volatile profile which was dominated by Maillard type reaction products. They included several furans, pyrans, furanones, furaneols, pyrazines, pyridinones, pyridines, pyrroles, oxazoles, imidazoles, thiophenes and other sulfur containing chemicals (Table III). These compounds arise mainly from reactions of reducing sugars (such as glucose) with the free amino groups of amino acids, peptides and protein components. These potatoes were slightly spongy to the touch due to their age. It is known that the sugar content of potatoes increases when they are stored at low temperatures. Therefore, it is likely that a higher percentage of starch in the matrix of these potatoes had been broken down by amyolytic enzymes to simple sugars such as glucose which was then available for the Maillard reaction. Although not illustrated in the data, many lipid oxidation products were determined to be important contributors to the flavor. However, sensory evaluation of the 13-14 months old potato extract indicated that Maillard-type flavors dominated.

The 1-2 months old potatoes contained most of the flavor impact compounds designated in Table I. These potatoes demonstrated the lowest levels of overall volatiles because many Maillard and lipid oxidation type compounds were not completely generated. This should be expected since lipid oxidation and the breakdown of starch into sugars progresses with age. Therefore, in this sample these two important groups of flavor compounds were found at reduced levels.

Continuous Steam Distillation/Solvent Extraction Vs. Headspace - Purge & Trap - Thermal Desorption for Dehydrated and Raw Potatoes. Head-space vapor analysis and several time consuming extraction and isolation procedures involving distillation/solvent extraction have been described for the analysis of dehydrated potatoes (6,9). In this study a continuous steam distillation/solvent extraction procedure (13) and a rapid analysis protocol involving dynamic head-space concentration / thermal desorption were compared. Both methodologies proved to be quantitative and reproducible. The headspace - purge & trap - thermal desorption (HS-P&T-TD) technique demonstrated to be more reliable for the earlier part of the chromatograms in the analysis of some furans, aldehydes, ketones, alcohols, aliphatic and olefinic hydrocarbons. On the other hand, the continuous steam distillation was more efficient for the extraction of the high boiling compounds including the free fatty acids for both dehydrated and fresh cooked potatoes. A fresh cooked potato sample was chosen to compare the flavor chromatograms obtained by both techniques in Figure 5. Sulfur containing

FLAVOR PROFILE OF POTATOES BY STEAM DISTILLATION - EXTRACTION



FLAVOR PROFILE OF POTATOES BY PURGE & TRAP - THERMAL DESORPTION

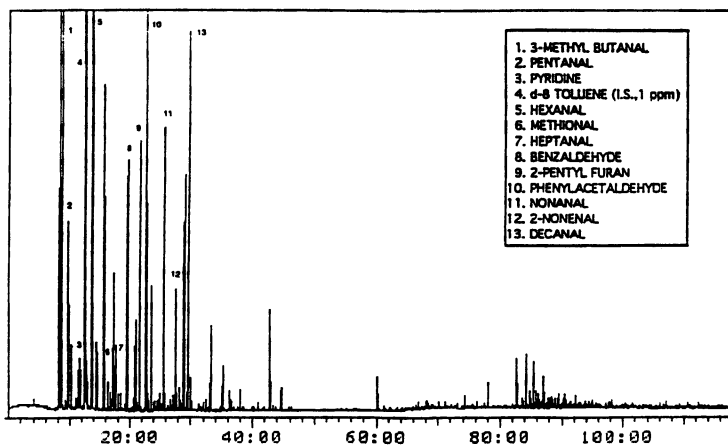


Figure 5. Comparison between the volatile profile obtained by steam distillation-extraction and by headspace purge and trap thermal desorption techniques.

Table III. Non-enzymatic Browning Reaction Products Present in 13-14 Months Old Potatoes

Component	Concentration (ppb)
3-Methylbutanal	750.00
2-Methylbutanal	1200.00
2,5-Dimethylfuran	37.10
Pyrazine	92.38
Pyridine	834.60
Dimethyl disulfide	20.26
Propyl methyl sulfide	17.71
1-H-pyrrole	318.07
4,5-Dimethylloxazole	4.52
2,3-Dihydro-4-methylfuran	69.89
3,4-Dihydro-6-methyl-2H-pyran	4.65
2-Methyl-3(2H)-dihydrofuranone	76.10
Tetrahydrothiophene	19.82
1-Ethyl-1H-pyrrole	19.24
2-Methylpyridine	39.47
1-Methyl-1H-imidazole	42.96
Methylpyrazine	427.54
Furfural	833.15
Methylfurfural	4.67
2-(Methoxymethyl)-furan	12.92
2-Methyl-1H-pyrrole	62.42
Dimethylpyrazine	13.10
3-Methyl-1H-pyrrole	20.98
Trimethylloxazole	3.88
5-Methyl-2(3H)-furanone	88.82
Furfuryl alcohol	1061.99
Allylfuran	9.74
2H-pyran-2-one	85.00
Methional	174.81
2-Acetylfuran	143.26
Dimethylpyrazine isomer	8.15
2,5-Dimethylpyrazine	503.30
Ethylpyrazine	80.02
2,3-Dimethylpyrazine	56.51
C-3 substituted pyrazine	2.19
C-3 substituted pyrazine	4.02
Ethylpyridine	8.92

Continued on next page

Table III. Continued

Component	Concentration (ppb)
5-Methylfurfural	73.95
N-methyl-2-pyridineamine	1.82
Furan carboxylic acid, methyl ester	5.35
1-(2-Methyl-1-butenyl)-pyrrolidine	9.88
Trimethylfuran	34.45
2-Furan methanol acetate	39.00
2-Ethyl-5-methylpyrazine	137.86
2-Ethyl-3-methylpyrazine	49.37
2-Ethyl-6-methylpyrazine	78.78
Pyridine carboxaldehyde	12.84
2-Ethenyl-6-methylpyrazine	10.99
2-Ethenyl-5-methylpyrazine	6.90
2-Acetylpyridine	7.01
Phenylacetaldehyde	2479.54
Methoxy methyl pyrazine	3.04
2,2-Bisfuran	26.82
2-Acetyl ethyl furan	37.41
Furaneol	20.73
Dimethyltetrahydro-2-furaneol	3.96
1-Pentyl-1H-pyrrole	13.70
Pentylpyrrole	3.87
2,3-Dihydrobenzofuran	31.96
2,6-Diethylpyrazine	28.42
3-Ethyl-2,5-dimethylpyrazine	121.94
2,2-Methylene bisfuran	12.80
2,5-Diethylpyrazine	6.37
2-(2-Methyl vinyl)pyrazine	9.61
2-Allyl-5-methylpyrazine	2.95
Dimethyl propyl pyrazine	18.54
1-Ethenyl-2(1H)-pyridione	2.73
Dimethyl propyl pyrazine	12.24
2,3-Diethyl-5-methylpyrazine	7.10
3,5-Diethyl-2-methylpyrazine	26.01
Diethyl methyl pyrazine isomer	7.18
1H-pyrrole-1-(2-furanmethyl)	28.83
Diformylthiophene	5.17
2-(2-Furanmethyl)-5-methylfuran	96.16
2,5-Dimethyl-3-(2-methylpropyl)pyrazine	30.45

Continued on next page

Table III. Continued

Component	Concentration (ppb)
2-Ethenyl benzofuran	11.04
Unknown pyrazine	20.13
2,2-[oxy bis(methylene)] bisfuran	28.88
1-Acetyl-2-hydroxy-5-methylbenzene	289.47
2,5-Dimethyl-3-isopropylpyrazine	5.10
Dimethyl butyl pyrazine isomer	20.33
2,2-(1,2-Ethenediyl)bis furan	2.44
Dimethyl butyl pyrazine isomer	2.01
Dimethyl butyl pyrazine isomer	1.70
Dipyrrole	7.10

volatile compounds which have low odor thresholds such as dimethyl disulfide, dimethyl trisulfide, and dimethyl tetrasulfide were detected using the thermal desorption technique only. The HS-P&T-TD technique also provided for a very rapid analysis of the volatiles and semivolatiles that comprise the potato flavor. This technique contrasts with the extremely time consuming and laborious solvent extraction and isolation protocols. For example, it would normally take over eight hours to prepare a sample using the solvent extraction technique, while it took approximately only 1.5 hrs using the HS-P&T-TD technique. In addition to the simple and rapid analysis, the HS-P&T-TD technique did not require any organic solvent extraction step thus reducing the workers' exposure to potential hazardous vapors and eliminating disposal cost associated with these solvents. Since the HS-P&T-TD technique was only a two step analysis trapping and desorbing the flavor volatiles, the artifact contamination associated with complex solvent extraction and isolation protocols were minimized.

Conclusion

The core flavor of mashed potatoes consists of naturally occurring and thermally generated volatile compounds. These compounds arise mainly from the oxidation of fatty acids, especially highly unsaturated fatty acids, and from the degradation and interaction of amino acids and sugar-amino acids. The extent to which these reactions affect the flavor of the final product will depend on the age of the raw potatoes, storage conditions and processing techniques. Both lipid oxidation and nonenzymatic browning reactions will increase with raw potato age. The thermal desorption technique was preferred over the simultaneous steam distillation technique for its simple and rapid flavor profiling.

Acknowledgments

We acknowledge the Center for Advanced Food Technology (CAFT) Mass Spectrometry Lab facility for providing instrumentation support for this project. CAFT is an initiative of the New Jersey Commission of Science and Technology. We also thank Basic American Foods for providing with samples and financial support to undertake this work. We wish to recognize Lyle Parks, James J. Mathias and especially William H. McArthur from Basic American Foods for their technical assistance and informative discussions. This is NJAES publication No. F-10569-2-93.

Literature Cited

1. Buttery, R. G.; Hendel, C. E.; Boggs, M. M. *J. Agric. Food Chem.* **1961**, *9*, 245-248.
2. Buttery, R. G. *J. Agric. Food Chem.* **1961**, *36*, 248-252.
3. Sapers, G. M.; Panasiuk, O.; Talley, F. B.; Osman, S.; Shawn, R. L. *J. Food Sci.* **1972**, *37*, 579-583.
4. Sapers, G. M.; Panasiuk, O.; Talley, F. B.; Shawn, R. L. *J. Food Sci.* **1973**, *38*, 586-589.
5. Sapers, G. M. *J. Food Sci.* **1970**, *35*, 731-733.
6. Sapers, G. M.; Osman, S.; Doodley, C. J.; Panasiuk, O. *J. Food Sci.* **1971**, *36*, 93-95.
7. Sullivan, J. F.; Konstance, R. P.; Calhoun, F. B.; Cording, J.; Panasiuk, O. *J. Food Sci.* **1974**, *39*, 58-60.
8. Sapers, G. M. *J. Agric. Food Chem.* **1975**, *23*, 1027-1032.
9. Sapers, G. M.; Sullivan, J. F.; Talley, F. B. *J. Food Sci.* **1970**, *35*, 728-730.
10. Sapers, G. M.; Panasiuk, O.; Talley, F. B.; Shawn, R. L. *J. Food Sci.* **1974**, *39*, 555-558.
11. Boggs, M. M.; Buttery, R. G.; Venstrom, D. W.; Belote, M. L. *J. Food Sci.* **1963**, *29*, 487-489.
12. Konstance, R. P.; Sullivan, J. F.; Talley, F. B.; Calhoun, M. J.; Craig, J. C. *J. Food Sci.* **1978**, *43*, 411-414.
13. Likens, S. T.; Nikerson, G. B. *Amer. Soc. Brewing Chem. Proc.* **1964**, 5-9.
14. Talburt, W. F.; Schwimmer, S.; Burr, H. K. *Potato Processing, 4th edition*, 1987, pp. 11-46.
15. Highlands, M. E.; Licciardello, J. J.; Herb, S. F. *Amer. Potato J.* **1954**, *31*, 353-357.
16. Josephson, D. B.; Lindsay, R. C. *J. Food Sci.* **1987**, *52*, 328-331.
17. Schieberle, P.; Grosch, W. *J. Amer. Oil Chem. Soc.* **1981**, *58*, 602-607.
18. Matthews, R. F.; Scanlan, R. A.; Libbey, L. M. *J. Amer. Oil Chem. Soc.* **1971**, *48*, 745-747.

19. Michalski, S. T.; Hammond, E. G. *J. Amer. Oil Chem. Soc.* **1972**, *49*, 563-566.
20. Josephson, D. B.; Lindsay, R. C. *J. Food. Sci.* **1987**, *52*, 1186-1190.
21. Bruechert, L. J.; Zhang, Y.; Huang, T. C.; Hartman, T. G.; Rosen, R. T.; Ho, C.- T. *J. Food. Sci.* **1988**, *53*, 1444-1447.
22. Frankel, E. N. *Prog. Lipid Res.* **1982**, *22*, 1-33.
23. Chang, S. S.; Smouse, T. H.; Krishnamurthy, R. G.; Mookherjee, B. D.; Reddy, B. R. *Chem. Ind.* **1966**, 1926.
24. Buttery, R. G.; Seifert, R. M.; Ling, L. C. *J. Agric. Food. Chem.* **1970**, *18*, 538-539.
25. Buttery, R. G.; Guadagni, D. G.; Ling, L. C. *J. Sci. Food Agric.* **1973**, *24*, 1125-1131.
26. Coleman, E. C.; Ho, C.- T. *J. Agric. Food. Chem.* **1980**, *28*, 66-68.
27. Coleman E. C.; Ho, C.- T.; Chang, S. S. *J. Agric. Food Chem.* **1981**, *29*, 42-48.
28. Mondy, N. I.; Metcalf, C.; Plaisted, R. *J. Food. Sci.* **1971**, *36*, 459-461.
29. Mazza, G.; Pietrak, E. M. *Food. Chem.* **1990**, *36*, 97-112.
30. Cherif, A. *Potato Res.* **1973**, *16*, 126-147.

RECEIVED December 16, 1993

July 15, 2012 | <http://pubs.acs.org>
Publication Date: July 7, 1994 | doi: 10.1021/bk-1994-0558.ch008

Chapter 9

Lipid-Derived Flavor Compounds in Fresh and Dehydrated Tomato Products

Karl Karmas, Thomas G. Hartman, Juan P. Salinas, Joseph Lech,
and Robert T. Rosen

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

Lipid derived flavors are produced in tomatoes during ripening via the action of endogenous enzymes such as lipoxygenase. They may be further influenced by nonenzymatic oxidative decomposition reactions which occur during processing and storage. This investigation focused on the determination of lipid oxidation derived flavors in the fresh and dehydrated tomato products using dynamic headspace concentration (purge and trap - thermal desorption) gas chromatography-mass spectroscopy (GC-MS) procedures. The effects of ripening and the lipoxygenase activity upon the generation of lipid oxidation compounds in fresh tomatoes were studied. Comparisons were made to dehydrated sun-dried tomatoes. The lipid oxidation profiles detected in these systems and their role in flavor development are discussed.

Tomato is the world's third most popular fruit. The world wide production is approximately 49,202,000 metric tons (1). The knowledge of the volatile and semivolatile compounds present is important in understanding which ones contribute most to its flavor, overall quality and biochemical changes. These changes result from ripening and processing such as heating, drying, canning and storage. Many investigations have been carried out to determine the volatile and semivolatile composition of tomatoes and tomato products and over 400 compounds have been previously identified (2). Many of these are products of lipid oxidation and Maillard browning reactions. Lycopene and β -carotene breakdown products are common oxidative decomposition products as are the appearance of Strecker aldehydes from thermal processing. Other typical compounds are the C6 aldehydes and alcohols as a result of lipoxygenase activity. Of the over 400 compounds that have been found in tomatoes none have the character impact of tomato (2). However, the major contributors to fresh tomato

0097-6156/94/0558-0130\$08.00/0
© 1994 American Chemical Society

aroma have been determined to be *cis*-3-hexenal, β -ionone, hexanal, β -damascenone, 1-penten-3-one, 3-methylbutanal, *trans*-2-hexenal, 2-isobutylthiazole, 1-nitrophenylethane and *trans*-2-heptenal (3).

Some of the methods used for tomato analysis are simultaneous steam distillation and solvent extraction, headspace analysis and dynamic headspace concentration (4,5). Of these methods the headspace concentration (purge and trap) methods are most similar to the methods used in this paper. In some investigations tomato flavor volatiles were purged and trapped on to Tenax adsorbent and then eluted with diethyl ether (6,7,8,9). In our experiments the organics are desorbed off of Tenax and Carbotrap or Carbosieve S-III with heat (short path thermal desorption) (10,11).

It was the objective of this study to develop a rapid analytical method (combined purge and trap and short path thermal desorption) for assessing the volatiles of fresh and dehydrated tomato products. The differences between sun dried and fresh tomatoes were profiled. This investigation is the first report on the volatile flavor profile of sun-dried tomatoes. It is hoped that this work can eventually lead to better understanding of the quality of tomatoes as the result of changes in ripening and processing.

Materials

Samples of sun-dried tomatoes were purchased from a local food store. Samples of fresh tomatoes of the variety "Mountain Pride" was purchased from a local farmer. D-8 toluene (internal standard) was obtained from Aldrich Chemical Company (Milwaukee, WI). Tenax TA was obtained from Alltech Associates (Deerfield, IL). Silanized glass wool, Carbotrap 20/40 mesh, and Carbosieve S-III 60/80 mesh was from Supelco Inc. (Belefonte, PA). The short path thermal desorption supplies and accessories were from Scientific Instrument Services (SIS) Inc. (Ringoos, NJ).

Experimental

Sun dried tomatoes were cooled with liquid nitrogen and then ground to a powder with a Bell Art Micromill which was cooled with dry ice. The powdered sun-dried tomatoes (5 g) were placed into a 1/2 inch x 14 inch glass tube. Both ends of the glass tube were plugged with silanized glass wool. An internal standard was matrix spiked (200 ppb based on the total weight of the powdered sun-dried tomatoes) into the sample. The glass sample tube was attached to the SIS Purge and Trap System. In two separated experiments, the tube was purged and trapped for 30 min at 80°C and 50°C with nitrogen at a flow rate of 40 ml/min onto a Tenax-Carbotrap trap. Each trap was dried 45 min using 40 ml/min flow of dry nitrogen to remove water. The Tenax/Carbotrap trap was then thermally desorbed into the GC-MS at 220°C for 5 minutes.

Fresh tomatoes, unblanched and blanched, (10 min in boiling water) were

blended to a slurry with a Cuisinart model DL8 food processor. The slurried tomatoes (100 g) were added into a 100 ml Wheaton Purge & Trap Apparatus. An internal standard of d-8 toluene was matrix spiked at the 165.3 ppb level (dry basis) (12) into the slurried tomatoes. The sample was purged and trapped for 30 min at 53°C with nitrogen at a flow rate of 40 ml/min onto a Tenax-Carbotrap trap. The trap was dried 45 min using 40 ml/min flow of dry nitrogen at room temperature to remove water. The trap was then thermally desorbed into the GC-MS.

The traps from the purge and trap step were thermally desorbed into the GC-MS using an SIS model TD-1 short path thermal desorption system. The desorption conditions were 220°C for five minutes. The gas chromatograph was a Varian 3400 installed with a 60 m x 0.32 mm DB-1 capillary column (J&W Scientific Co.) with a 0.25 µm film thickness. The injector temperature was 250°C; the split ratio was 10:1. The column was temperature programmed from -20°C (hold time of 5 min during the thermal desorption step to achieve cryofocusing) to 40°C (0 min hold) at a rate of 10°C/min; then to 150°C (0 min hold) at a rate of 2°C/min; and finally to 280°C at a rate of 4°C/min with a 20 min hold at the upper limit. The GC column was inserted directly into the ion source of the mass spectrometer via a heated transfer line maintained at 280°C. The mass spectrometer was a Finnigan Mat 8230 high resolution double focusing magnetic sector instrument. The mass spectrometer was operated in electron ionization mode (70 eV) scanning masses 35-350 once each second with a interscan time of 0.8 seconds. The mass spectrometer data was acquired and processed using a Finnigan MAT SS300 Data system. All mass spectra obtained were background subtracted and library searched against the National Institute of Standards and Technology mass spectral reference collection (10). The Wiley/NBS Registry of Mass Spectra and DB-1 Kovats retention time indices were used to help identify compounds (9,13,14).

Results & Discussion

Figures 1 and 2 show the chromatograms obtained for sun-dried and fresh tomatoes using purge and trap - short path thermal desorption GC-MS. The major compounds are also listed in the chromatograms. Table I show all the compounds identified and their relative concentrations (based on comparisons to the internal standard d-8 toluene). Most of the compounds detected have already been reported in the literature as tomato volatiles. Additionally, Figure 3 shows some of the compounds trapped by Carbosieve S-III and Tenax which were not retained by Carbotrap and Tenax. Four additional low molecular weight compounds could be detected with this method. Since no internal standard was injected with these four compounds, the level could not be ascertained.

In this analysis the major volatile compounds in both sun-dried and fresh tomatoes are lipid derived. In sun-dried tomatoes the lycopene and other carotenoid breakdown products (15-19) are the major compounds present. These

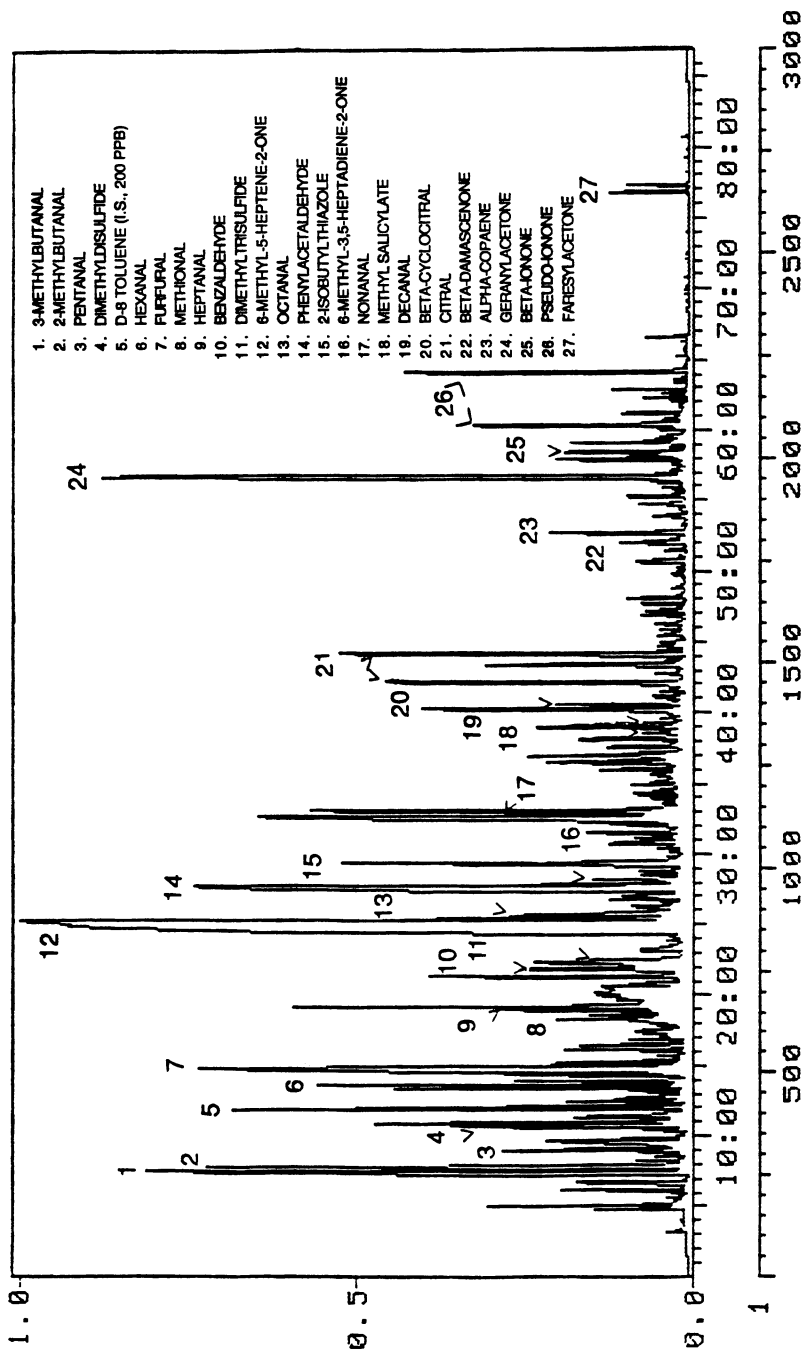


Figure 1. Volatile profile of sun-dried tomatoes (P&T at 80°C for 30 min).

July 15, 2012 | <http://pubs.acs.org>
 Publication Date: July 7, 1994 | doi: 10.1021/bk-1994-0558.ch009

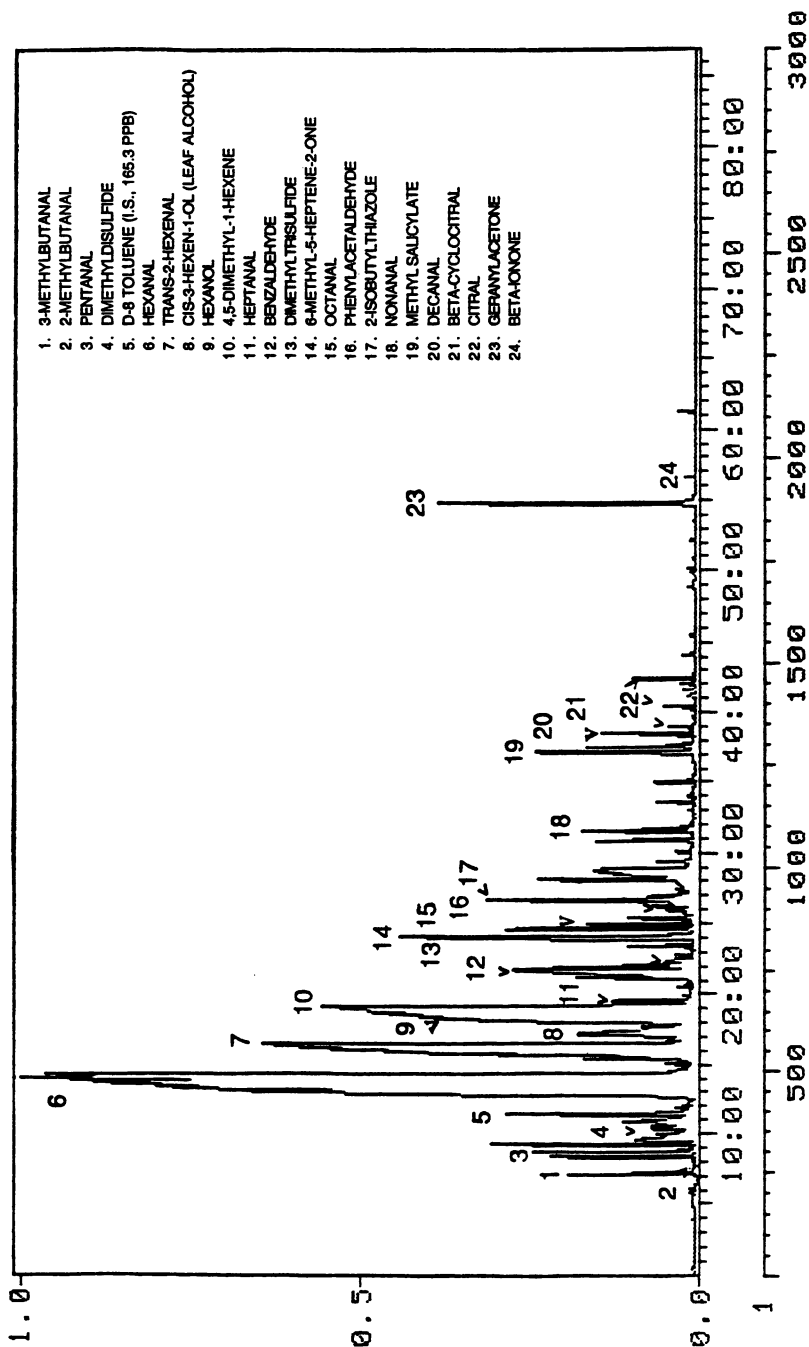


Figure 2. Volatile profile of unblanched red tomatoes (P&T at 53°C for 30 min).

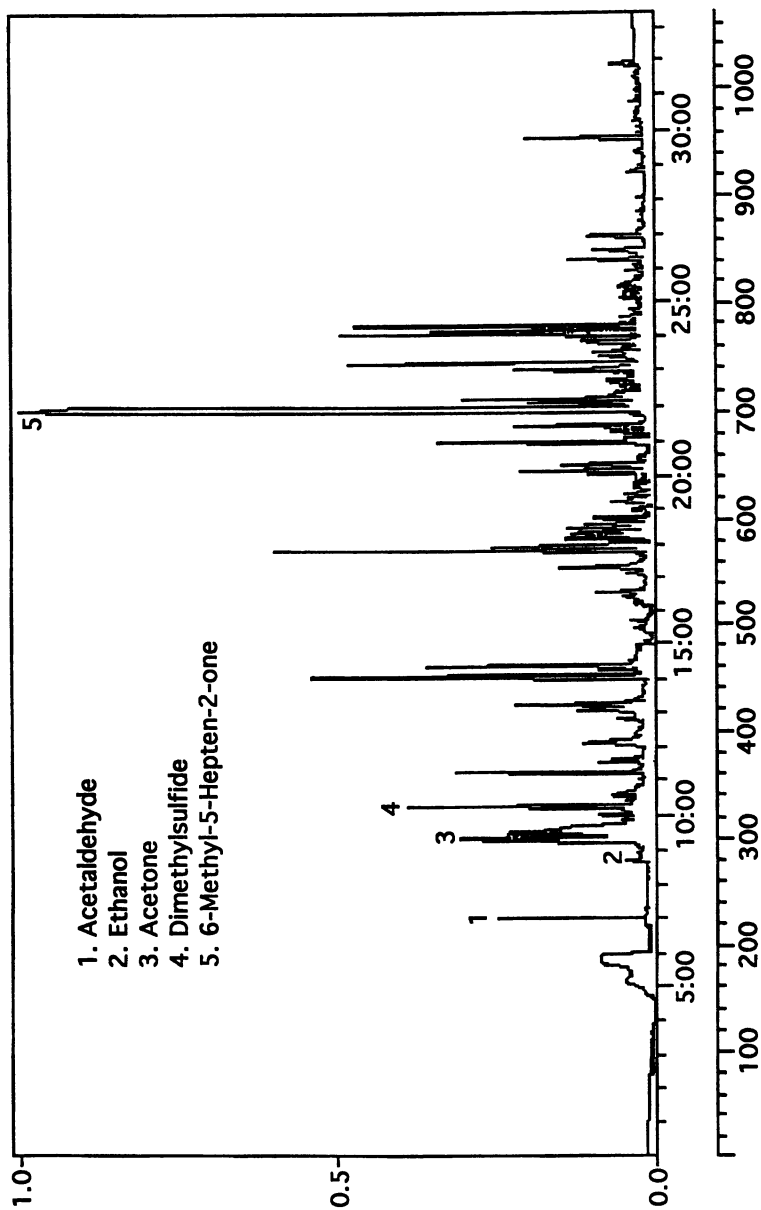


Figure 3. Volatile profile of sun-dried tomatoes with Tenax and Carbosieve S-III (P&T at 80°C for 30 min).

Table I. Volatile Compounds Identified in Sun-dried and Fresh Tomatoes

Compound	Estimated concentration (ppb)	
	Sun-dried	Fresh
2-Methylpropanal	15.9	-
3-Methyl-2-butanone	57.2	-
2-Methylfuran	10.3	-
2-Methyl-3-buten-2-ol	78.7	-
3-Methylbutanal	674.4	68.3
2-Methylbutanal	396.2	4.0
1-Penten-3-one	17.0	-
3-Methyl-2-butanone	5.9	-
Pentanal	37.6	75.5
Acetic acid	168.1	13.4
Heptane	11.4	-
2-Ethylfuran	-	71.0
2,5-Dihydro-3,4-dimethylfuran	2.4	-
3-Penten-2-one	72.2	-
Dimethyldisulfide	145.4	1.5
2-Ethoxypropane	158.8	-
1-Methylthiopropane	117.5	-
2-Pentenal	17.9	-
2-Methyl-2-butenal	-	25.2
3-Methyl-1-butanol	58.0	-
2,3-Dihydro-4-methylfuran	59.4	3.5
3-Methyl-1-butanol	40.1	-
2-Methyl-1-penten-3-ol	7.6	-
3-Hepten-2-one	-	7.7
Hexanal	195.8	4442.2
Octane	6.7	-
2-Furfural	826.7	-
<i>trans</i> -2-Hexenal	2.0	1718.5
<i>cis</i> -3-Hexen-1-ol	-	291.7
2-Methyl-3-penten-1-ol	3.6	-
Xylene	71.8	-
Pentyl acetate	-	34.7
1-Hexanol	20.8	40.0
Methional	157.5	-
2-Heptanone	8.9	-
Heptanal	11.3	-

Continued on next page

Table I. Continued

Compound	Estimated concentration (ppb)	
	Sun-dried	Fresh
2-Acetylfuran	58.6	-
2,6-Dimethylpyrazine	73.9	-
Pentanoic acid	130.2	-
Heptanal	-	17.5
5-Ethyl-2(5H)-furanone	-	129.3
Dimethylphenol	128.1	-
Benzaldehyde	177.9	8.8
2-Heptenal	47.0	101.4
5-Methyl-2-furfural	222.0	-
Dimethyltrisulfide	47.8	54.6
Butanoic acid	-	18.1
6-Methyl-5-hepten-2-one	1988.9	370.5
2-Pentylfuran	119.6	138.2
6-Methyl-5-hepten-2-ol	158.1	-
Octanal	3.2	59.2
γ -Hydroxyhexanoic acid	-	64.4
Phenylacetaldehyde	908.8	7.1
2-Isobutylthiazole	7.8	216.9
β -Thujene	5.8	-
β -Terpinene	-	36.3
D-Limonene	46.5	17.1
2,6-Dimethyl-5-heptenal (Melonal)	335.9	8.7
4-Methylbenzaldehyde	42.1	-
3,4-Dimethylstyrene	2.2	-
6-Methyl-3,5-heptadien-2-one	216.3	-
Nonanal	92.9	66.5
3-(4-Methyl-3-pentenyl) furan (Perillen)	249.4	32.9
1-Isocyano-2-methylbenzene	14.1	-
Fenchol	7.7	-
2-Nonenal	82.1	24.3
2,4-Dimethylbenzaldehyde	98.1	-
Methylacetophenone	34.4	-
Methyl salicylate	36.7	139.9
Decanal	4.8	49.4
4,6,6-trimethyl-bicyclo-[3.1.1.]-hept-3-en-2-one (Berbenone)	215.8	-

Continued on next page

Table I. Continued

Compound	Estimated concentration (ppb)	
	Sun-dried	Fresh
2-Vinylbenzofuran	11.3	-
β -Cyclocitral	82.0	17.1
Dodecane	17.0	-
β -Citral (Neral)	205.5	18.5
3-Methyl-1-naphthalenol	2.9	-
2-Decenal	-	11.0
α -Ethylidene benzeneacetaldehyde	139.6	-
α -Citral (Geranial)	274.9	37.8
Decahydro-1,6-dimethylnaphthalene	1.9	-
2,4-Decadienal	28.6	11.6
2-Undecenal	-	5.0
Tridecane	25.4	-
Eugenol acetate	0.7	-
1,2-Dihydro-4,6,8-trimethylnaphthalene	1.7	-
α -Ionene	2.3	-
1,4,4a,5,6,8,8a-Octahydro-2,5,5,8a-tetramethyl-1-naphthalenemethanol	26.2	-
β -Damascenone	36.5	-
α -Copaene	10.7	-
Tetradecane	23.9	-
α -Ionone	9.9	-
4-(2,2,3,3-Tetrabutyl) phenol ^a	10.6	-
6,10-Dimethyl-5,9-undecadien-2-one	712.3	246.7
Epoxy- β -ionone	17.4	-
β -Ionone	37.6	4.6
Butylated hydroxytoluene (BHT) ^a	107.7	-
2,6-Di-tert-butylphenol ^a	126.5	-
Faresene	17.2	-
Pseudo-Ionone isomer	42.7	-
Farnesal	33.5	-
Nerolidol	6.5	-
Pseudo-Ionone isomer	155.6	-
2-Methylpropanoic acid	2.2	-
Farnesylacetone	28.7	-

^amigrants from packaging material

compounds are 6-methyl-5-hepten-2-one, 6-methyl-3,5-hepten-2-one, 6-methyl-5-hepten-2-ol, melonal, farnesylacetone, geranylacetone (6,10-dimethyl-5,9-undecadien-2-one), farnesal, farnesene, β -cyclocitral, α -citral, β -citral, β -damascenone, α -ionone, β -ionone, pseudo-ionone, epoxy- β -ionone, α -ionene and xylene. Strecker aldehydes (16) are also present in sun-dried tomatoes. Some of these compounds are 3-methylbutanal, 2-methylbutanal, acetaldehyde, methional, 2-methylpropanal, and phenylacetaldehyde. This indicates that free amino acids and reducing sugars are key precursors to browning flavor in tomatoes. Other Maillard browning products such as furfural were also found. In the literature 3-methylbutanal and furfural have been used as processing indicators (20-21). The sun-dried tomatoes were noticeably "browned" so the appearance of Maillard reaction compounds including Strecker aldehydes should definitely be expected.

In fresh unblanched tomatoes the linoleic and linolenic fatty acid breakdown (via the lipoxygenase enzyme) lead to the C6 aldehydes and C6 alcohols formation (2,5,22). Thus the major compounds are hexanal, hexanol, *cis*-3-hexenol and *trans*-2-hexenal. The major portion of *trans*-2-hexenal was a GC artifact caused by isomerization of the *cis*-3-hexenal (Chapter 19, this volume). At high concentrations hexanal can give rancid flavor while at lower concentrations it contributes to the green flavor note of tomato (2). Some of the breakdown products of lycopene, β -carotene as well as Strecker aldehydes, and other Maillard browning products were present in the fresh unblanched tomatoes but at lower levels in comparison to sun-dried tomatoes.

Figure 4 shows the concentration of hexanal versus tomato type and processing technique. In all blanched samples, hexanal levels were relatively low. In fresh unblanched tomatoes the concentration of hexanal is the greatest. Hexanal levels increase as a function of ripening; this can be explained by the enzymic breakdown of linoleic acid by lipoxygenase (2,5,23). During the sample preparation steps used in our work, the tomatoes were blended to a slurry leading to cellular breakdown and the elevated activity of lipoxygenase. Blanching tomatoes decreased the amount of hexanal produced due to the denaturation of lipoxygenase enzyme. In sun-dried tomatoes the level of hexanal is lower than in fresh unblanched tomatoes. This is due to deactivation of the enzyme due to the low water activity in the dehydrated product.

Figure 5 shows the concentration of *trans*-2-hexenal for various tomato samples. The *cis*-3-hexenal has a stronger green note than the *trans*-2-hexenal. Both compounds contribute to tomato aroma (2) and low concentrations of *cis*-3-hexenal and *trans*-2-hexenal contribute to desirable flavor notes. Little or no *trans*-2-hexenal was detected in the sun-dried tomatoes.

Figure 6 shows the concentration of *cis*-3-hexenol for various tomato samples. The *cis*-3-hexenol concentration levels can be explained similarly to the hexanal description previously given. The *cis*-3-hexenol has a green note flavor which contributes to tomato aroma (2). No *cis*-3-hexenol was detected in the sun dried or blanched tomatoes.

Figure 7 shows the concentration of 6-methyl-5-hepten-2-one versus tomato type and processing technique. As pointed out earlier, this is the major compound identified in sun-dried tomatoes. This compound is reported to have fruity flavor

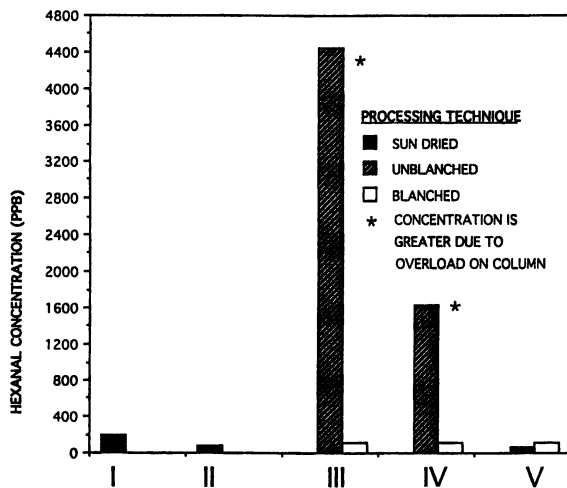


Figure 4. Concentration of hexanal versus tomato type and processing technique. I: sun-dried tomatoes, P&T at 80°C; II: sun-dried tomatoes, P&T at 50°C; III: red tomatoes, P&T at 53°C; IV: medium ripe tomatoes, P&T at 53°C; V: green tomatoes, P&T at 53°C.

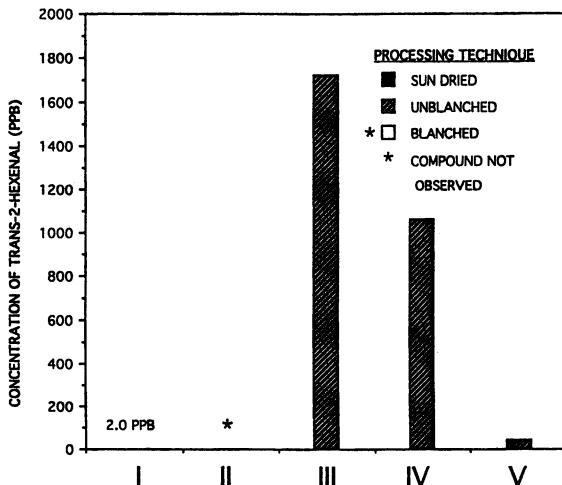


Figure 5. Concentration of *trans*-2-hexenal versus tomato type and processing technique. I: sun-dried tomatoes, P&T at 80°C; II: sun-dried tomatoes, P&T at 50°C; III: red tomatoes, P&T at 53°C; IV: medium ripe tomatoes, P&T at 53°C; V: green tomatoes, P&T at 53°C.

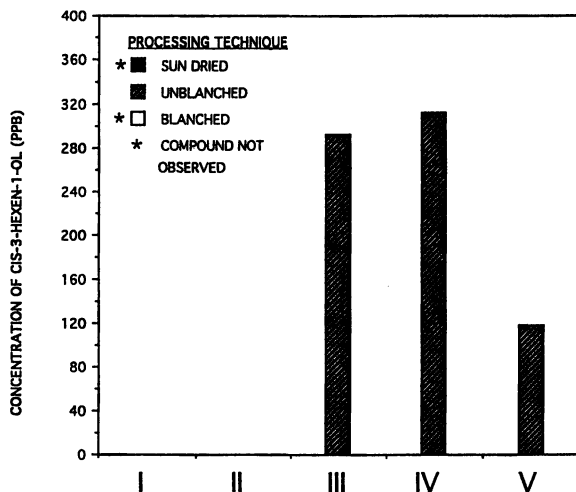


Figure 6. Concentration of *cis*-3-hexen-1-ol versus tomato type and processing technique. I: sun-dried tomatoes, P&T at 80°C; II: sun-dried tomatoes, P&T at 50°C; III: red tomatoes, P&T at 53°C; IV: medium ripe tomatoes, P&T at 53°C; V: green tomatoes, P&T at 53°C.

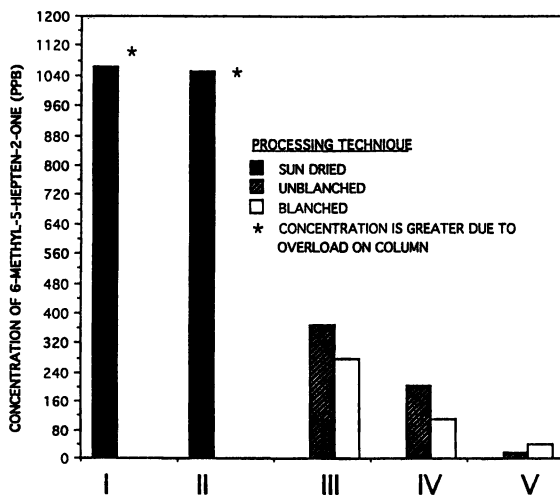


Figure 7. Concentration of 6-methyl-5-hepten-2-one versus tomato type and processing technique. I: sun-dried tomatoes, P&T at 80°C; II: sun-dried tomatoes, P&T at 50°C; III: red tomatoes, P&T at 53°C; IV: medium ripe tomatoes, P&T at 53°C; V: green tomatoes, P&T at 53°C.

July 15, 2012 | http://pubs.acs.org
 Publication Date: July 7, 1994 | doi: 10.1021/bk-1994-0558.ch009

quality at low concentration while at higher concentrations it may impart off-flavor (15). In sun-dried tomatoes the concentration of 6-methyl-5-hepten-2-one is greater than in fresh products. During sun drying, conditions are optimal for the photooxidation and or nonenzymatic oxidation degradation of lycopene. In the fresh tomatoes it appears that the level of 6-methyl-5-hepten-2-one increases as a function of ripeness. In the blanched tomatoes the level of 6-methyl-5-hepten-2-one is less than in the unblanched, and this may indicate that there is a combination of both thermal and enzymic breakdown occurring.

Thus chemical changes in tomatoes can be used to reflect different levels of processing and ripening. The relevant pathways include lipid breakdown as well as Maillard browning. Indicator compounds can be selected specifically to represent age and degree of processing.

Acknowledgements

We acknowledge the Center for Advanced Food Technology (CAFT) mass spectrometry facility for providing instrumentation support. This is New Jersey Agricultural Experiment Station (NJAES) publication #F-10569-3-93.

Literature Cited

1. Davies, J. N.; Hobson, G. E. *CRC Crit. Rev. Food Sci. Nutr.* **1981**, *20*, 205-279.
2. Petro-Turza, M. *Food Rev. Internat.* **1987**, *2*, 309-351.
3. Buttery, R. G.; Teranishi, R.; Flath, R. A.; Ling, L. C. In *Flavor Chemistry: Trends and Developments*; Teranishi, R.; Buttery, R. G.; Shahidi, F. Eds.; ACS Symp. Ser. 388; American Chemical Society: Washington D.C., 1989; pp 213-222.
4. Hayase, F.; Chung, T.-Y.; Kato, H. *Food Chem.* **1984**, *14*, 113-124.
5. Maarse, H. *Volatile Compounds in Foods and Beverages*, Marcel Dekker, Inc.: New York, 1991; pp 247-281.
6. Buttery, R. G.; Teranishi, R.; Ling, L. C. *J. Agric. Food Chem.* **1987**, *35*, 540-544.
7. Buttery, R. G.; Teranishi, R.; Ling, L. C.; Flath, R. A.; Stern, D. J. *J. Agric. Food Chem.* **1988**, *36*, 1247-1250.
8. Buttery, R. G.; Teranishi, R.; Flath, R. A.; Ling, L. C. *J. Agric. Food Chem.* **1990**, *38*, 729-795.
9. Buttery, R. G.; Teranishi, R.; Ling, L. C.; Turnbaugh, J. G. *J. Agric. Food Chem.* **1990**, *38*, 336-340.
10. Hartman, T. G.; Karmas, K.; Chen, J.; Shevade, A.; Deagro, M.; Hwang, H.-I. In *Phenolic Compounds in Food and Their Effects on Health I: Analysis, Occurrence, and Chemistry*; Ho, C.-T.; Lee, C. Y.; Huang, M. T. Eds.; ACS Symp. Ser. 506; American Chemical Society: Washington, D.C., 1992; pp 60-76.

11. Hartman, T. G.; Lech, J.; Karmas, K.; Salinas, J.; Rosen, R. T.; Ho, C.-T. 1993. In *Flavor Measurement*; Ho, C.-T.; Manley, C. H. Eds.; Marcel Dekker, Inc.: New York, 1993, pp 37-60.
12. Haytowitz, D. B.; Matthews R. H. *Composition of Foods: Vegetables and Vegetable Products*, Agriculture Handbook # 8-11, 1984; pp 449-458.
13. The Restek Advantage, Industrial Solvent Analysis Simplified, January 1992, Vol.3, No.1.
14. McLafferty, F. W.; Stauffer, D. B. *The Wiley/NBS Registry of Mass Spectral Data*; John Wiley & Sons: New York, 1989.
15. Cole, E. R.; Kapur, N. S. *J. Sci. Food Agric.* 1957, 8, 360-365.
16. Berlitz, H.-D. *Food Chemistry*; Spinger-Verlag: Berlin-Heidelberg, 1987; pp 189-20, 267.
17. Kanaseawud, P.; Crouzet, J. C. *J. Agric. Food Chem.* 1990, 38, 237-243.
18. Onyewu, P. N.; Daun, H.; Ho, C.-T. In *Thermal Generation of Aromas*, Parilment, T. H.; McGorin, R. J.; Ho, C.-T. Eds.; ACS Symp. Ser. 409, American Chemical Society: Washington, D.C., 1989, pp 247-256.
19. Schrier, P.; Drawert, F.; Bhiwapurkar S. *Chem. Mikrobiol. Technol. Lebensm.* 1979, 6, 90-91.
20. Eichner, K.; Ciner-Doruk, M. *Prog. Food Nutr. Sci.* 1981, 5, 115-135.
21. Kanner, J.; Harel, S.; Fishbein, Y.; Shalom, P. *J. Agric. Food Chem.* 1981, 29, 948-949.
22. Frankel, E.N. *Prog. Lipid Res.* 1982, 1-33.
23. Kazeniak, S. J.; Hall, R. M. *J. Food Sci.* 1970, 35, 519-530.

RECEIVED April 4, 1994

Chapter 10

Aroma Generation in Extruded and Heated Wheat Flour

Hui-Ing Hwang¹, Thomas G. Hartman², Mukund V. Karwe^{1,2},
Henry V. Izzo¹, and Chi-Tang Ho^{1,2}

Department of Food Science¹ and Center for Advanced Food
Technology², New Jersey Agricultural Experiment Station, Cook College,
Rutgers, The State University of New Jersey, New Brunswick, NJ 08903

Aroma generation in wheat flour processed by heating and extrusion cooking at different temperatures and moisture contents was investigated. Volatile components of the heated and extruded wheat flour were analyzed by gas chromatography (GC) using a short-path thermal desorption technique and identified by mass spectrometry (MS). Lipid oxidation products, Maillard-type flavors, and some sulfur-containing compounds were identified in both processes. The quantitative analysis showed that Maillard-type flavors were predominant in the heating process. Lipid oxidation products were the major compounds in the extrudates prepared at high-moisture content and low-die temperature. By lowering water content and increasing die temperature during extrusion processing, lipid degradation compounds decreased and the Maillard products dominated the flavor profile. The lipid oxidation products significantly increased in the sample extruded at low-moisture content and high-die temperature during storage.

Lipid oxidation is one of the most important reactions in fat-containing food. During cooking or processing of food, various lipid degradation products are generated and cause either pleasant or undesirable odors. In food, lipids can be oxidized by both enzymatic and nonenzymatic mechanisms (1). Basically, the lipid oxidation reaction involves a free radical mechanism and the different decomposition pathways may lead to a large number of volatile products including alkanes, alkenes, aldehydes, ketones, alcohols, esters, and acids. The polyunsaturated fatty acids such as linoleic, linolenic, and arachidonic are the most susceptible to lipid oxidation. However, random oxidation may also occur in saturated fatty acids at relatively high temperatures (2).

Since food is a complex system, those compounds produced from lipid

0097-6156/94/0558-0144\$08.00/0
© 1994 American Chemical Society

oxidation may easily react with other food components to generate more complicated products. It has been reported that lipid degradation products such as 2,4-decadienal and hexanal can interact with Maillard reaction intermediates to form long-chain alkylpyrazines as well as other heterocyclic compounds (3-5).

Extrusion has been used in the food industry for over 50 years. Extrusion provides the most efficient means of converting electrical and mechanical energy into thermal energy for cooking feed and food formulations (6). Therefore, the extrusion process has been applied to a wide variety of food including textured vegetable protein (TVP), ready-to-eat breakfast cereals, animal feed, and snack foods (7). In most of the flavor studies relating to extrusion processing, efforts have been concentrated on the effect of the pre- or post-extrusion application to the retention of volatile compounds (8-10). Since the extrusion process is considered a high-temperature short-time process, many reactions, especially lipid degradation, could occur. In addition, the interaction between lipid degradation and other food reactions, such as the Maillard reaction, might also become possible. The goals of this study were to investigate the effect of the extrusion process on lipid degradation as well as on the relationship between the Maillard reaction and lipid degradation. The stability of extrudates during the storage was also evaluated. Furthermore, a comparison in the volatile generation between the extrusion process and the heating process was studied.

Experimental

Materials. High-gluten wheat flour [14.0% protein (d.b.), 0.52% ash, and 14% moisture] was purchased from the Bay State Milling Co. (Clifton, NJ) and is sold under the trade name Bouncer. Deuterated toluene (d-8 Toluene) was obtained from Aldrich Chemical Co. (Milwaukee, WI). Tenax-TA (2,6-diphenyl-*p*-phenylene oxide) adsorbent 60-80 mesh was obtained from Alltech Associates (Deerfield, IL). Carbotrap (activated graphitized carbon) adsorbent 20-40 mesh, C₅-C₂₅ *n*-paraffin standard, and silanized glass wool were purchased from Supelco Inc. (Belefonte, PA).

Extrusion Process of Wheat Flour. The extruded samples were prepared on a Werner Pfliederer ZSK-30 co-rotating twin-screw extruder (Werner Pfliederer, Ramsey, NJ) with a barrel diameter of 30.85 mm and an L/D ratio of 29:1. The extruder barrel was electrically heated and contained five independently controlled heating zones. Die temperatures were recorded by a thermocouple insert at the die plate and the die had two holes of 3.0 mm diameter each. Wheat flour was fed into the extruder with a K-Tron Series 7100 loss-in-weight feeding system (K-Tron Corp., Pitman, NJ). Water was fed into the first extruder section, immediately after the feed section, by a metering pump (U.S. Electric Motors, Milford, CT). Total feed moisture was varied by changing the amount of water delivered by the metering pump and feed rate of the wheat flour while maintaining a constant total mass flow rate. Two sets of samples were extruded. In the first set, the sample

was extruded at 185°C, with a 16% moisture feed (w/w), screw speed of 500 rpm, and a mass flow rate of 225 g/min. The other sample was extruded at 160°C, with a 20% moisture feed (w/w), screw speed of 500 rpm, and a mass flow rate of 225 g/min. These extrudates were stored in closed glass jars at 4°C before analysis.

Volatile Isolation from Extrudates. Thirty grams of extrudate were ground in a benchtop grinder (Glen Mills, Maywood, NJ) with dry ice to prevent heat-labile volatiles' destruction. The ground sample (10 g) was packed in the center of a glass tube and the silanized glass wool was placed on the two ends of the tube. One microliter of 1.001 mg/ml deuterated toluene was spiked into the tube as the internal standard. The tube was further sealed in a Scientific Instrument Services, Inc. (SIS) solid sample purge-and-trap apparatus (Ringoes, NJ). Nitrogen was used at a flow rate of 40 mL/min to purge the volatiles into a silanized glass-lined stainless steel desorption tube (4.0 mm i.d. x 10 cm length) from Scientific Instrument Services, Inc. (Ringoes, NJ). It consisted of 3 cm bed volume of Tenax-TA adsorbent and 3 cm bed volume of Carbotrap adsorbent. This volatile isolation was carried out at 80°C for one hour.

Volatile Generation and Isolation from the Heating Process. Two different temperatures (160°C and 180°C) were chosen for the heating process. For each sample, fifty grams of wheat flour, which contained 14% of moisture, were placed in a reaction vessel and heated at a desired temperature for one hour. The heated sample was transferred into a glass tube and the silanized glass wool was placed on the two ends of the tube. One microliter of 1.001 mg/ml deuterated toluene was spiked into the tube as the internal standard. The volatile isolation of the heated samples was carried out by an SIS solid sample purge-and-trap apparatus and the condition was exactly the same as mentioned above.

Volatile Analysis by Gas Chromatography-Mass Spectrometry (GC-MS). The desorption tube which was prepared from the isolation procedure was connected to an SIS model TD-1 Short Path Thermal Desorption unit. This desorption unit was interfaced with a Varian 3400 gas chromatograph coupled with a Finnigan MAT 8320 high-resolution double focusing magnetic sector mass spectrometer (TD-GC-MS). The desorption tube was then injected into the GC and thermally desorbed the volatiles to the injection port of the GC at 220°C for 5 min. However, the temperature of the GC chamber was maintained at -20°C by dry ice to cryofocus the volatiles as a narrow band at the head of the capillary column during the desorption process. After the desorption tube was removed from the injection port of the GC, the volatiles were analyzed by GC-MS. The GC was operated with an injector temperature of 250°C, with a split ratio of 10:1, and a helium carrier flow rate of 1.0 ml/min. The GC column was a nonpolar fused silica capillary column [60 m x 0.32 mm (i.d.), 0.25 μ m thickness, DB-1; J&W Scientific Co.] and was temperature programmed from -20°C to 280°C at a rate

of 10°C per minute with a 20 minute hold at the upper limit. Volatiles were semi-quantified via peak area ratio calculation to that of the internal standard, deuterated toluene. Linear retention indices for the volatiles were determined through the use of a C₅-C₂₅ n-paraffin standard according to the method of Majlat et al. (11). The mass spectrometer electron ionization was set at 70 eV and the source temperature was 250°C with a filament emission current of 1 mA, scanning masses 35-350, and a 0.8 second interscan time. All mass spectra obtained were identified by utilizing an on-line computer library (NIST).

Results and Discussion

A total eight-one compounds has identified in Table I. The lipid degradation products identified included aldehydes, ketones, alcohols, hydrocarbons, and 2-pentylfuran. The Strecker aldehydes as well as Maillard reaction-derived heterocyclic compounds such as furans, pyrroles, and pyrazines were also identified. In addition, sulfur-containing compounds such as dimethyl disulfide, thiazoles, and thiophenes were found.

Although the lipid content of wheat flour is low (approximately 2-3% w/w), wheat lipids are rich in linoleic, linolenic, and oleic acids (12). The oxidative products of linoleic acid, which is the most abundant fatty acid in wheat flour (13), included pentanal, hexanal, 2-*t*-octenal, 4-*t*-nonenal, 2-*t*-nonenal, 2,4-nonadienal, 2-*t*,4-*t*-decadienal, 1-pentanol, 1-hexanol, and 2-pentylfuran. 2-*t*,4-*t*-decadienal, which had a pleasant deep-fat-fried flavor could further convert to a number of components such as 2-octenal, hexanal and acetaldehyde through further oxidation or retro-aldolization reaction (14). Benzaldehyde has also been considered as a thermal degradation product of 2-*t*,4-*t*-decadienal and its formation pathway has been suggested by Bruechert et al. (15).

In addition to linoleic acid, oleic acid was also the major fatty acid of the lipids occurring in wheat flour (13). Compounds such as heptanal, nonanal, decanal, and 2-*t*-decanal were produced from autoxidation of oleic acid. The compounds generated from the autoxidation of linolenic acid included 2-hexenal, 2-*t*-heptenal, 2-*t*,4-*t*-heptadienal, 2-*t*-penten-1-ol, and 3-*t*,5-*t*-octadien-2-one.

The heterocyclics included furans, pyrroles, and pyrazines. These were typical Maillard reaction products. Alkylpyrazines are generally recognized as important contributors to the flavors of all roasted, toasted, or similarly heated foods (16). The most direct route to their formation resulted from the interaction of α -dicarbonyl compounds with amino acids through the Strecker degradation reaction.

Sulfur-containing compounds were investigated in the present study and were significant contributors to the thermal production of flavors. Those sulfur-containing flavors were generated from the thermal degradation of cysteine and methionine. The formations of dimethyldisulfide, dimethyltrisulfide, and methylpentylsulfide resulted from the interaction of two methylthio radicals, that were produced from methionine degradation, or from the reaction between

Table I. Volatile Aroma Compounds Identified from Extrudates

Compounds	I _k ^a	Quantitation (ng/g of extrudates)			
		E-1 ^b	EA-1 ^b	E-2 ^b	EA-2 ^b
Carbonyls					
butanal	583	- ^c	-	-	17.59
pentanal	695	-	214.62	182.50	864.10
hexanal	807	236.60	778.98	1183.50	830.03
heptanal	914	56.60	21.50	129.00	200.36
nonanal	1130	-	34.25	158.40	114.54
decanal	1238	-	23.56	120.90	-
dodecanal	1437	-	27.84	-	-
3-methylbutanal	662	192.90	122.06	117.50	1.93
2-methylbutanal	671	123.10	78.03	67.50	7.97
2-hexenal	790	-	-	-	59.79
2- <i>t</i> -heptenal	959	-	-	-	13.68
2- <i>t</i> -octenal	1077	-	-	165.50	24.25
4- <i>t</i> -nonenal	1122	-	-	-	34.08
2- <i>t</i> -nonenal	1179	-	-	72.33	47.45
2- <i>t</i> -decanal	1320	-	-	-	13.87
2-butyl-2-octenal	1093	-	-	-	57.58
2- <i>t</i> ,4- <i>t</i> -heptadienal	1010	-	4.40	-	180.48
2,4-nonadienal	1275	-	7.54	-	65.76
2- <i>t</i> ,4- <i>t</i> -decadienal	1350	-	73.16	74.20	-
benzaldehyde	970	121.40	75.62	335.10	215.18
phenylacetaldehyde	1055	61.30	33.65	-	24.25
4-hydroxy-2- <i>t</i> -octenal	1094	-	-	-	5.73
2-butanone	598	44.30	-	28.30	-
2-hexanone	798	34.80	-	-	-
2-heptanone	904	92.60	28.84	113.80	132.65
6-undecanone	1333	-	-	-	13.25
5-methyl-2-isopropyl- cyclohexanone	1089	-	19.21	-	-
3-methyl-3-buten-2-one	700	-	26.25	-	23.74
3- <i>t</i> -octen-2-one	973	-	91.18	-	393.14
3,4,5-trimethyl-2-cyclo- penten-1-one	1066	-	-	-	24.25
3- <i>t</i> ,5- <i>t</i> -octadien-2-one	1088	-	62.15	126.60	498.59
2-methyl-5-isopropyl- 2-cylcohexen-1-one	1276	-	-	35.80	-

Continued on next page

Table I. continued

Compounds	I _k ^a	Quantitation (ng/g of extrudates)			
		E-1 ^b	EA-1 ^b	E-2 ^b	EA-2 ^b
Carbonyls - continued					
1-hydroxy-2-propanone	717	80.10	12.05	-	-
3-hydroxy-2-butanone	754	-	67.73	43.80	-
1-hydroxy-2-heptanone	1078	-	-	-	314.73
2,3-butanedione	594	55.10	17.94	-	-
2,3-pentanedione	699	114.60	-	-	-
2,3-octanedione	1002	-	-	-	33.81
acetic acid	711	178.40	12.05	-	15.67
Alcohols					
1-butanol	689	54.90	200.61	122.70	13.22
1-pentanol	760	-	-	78.30	180.74
1-hexanol	890	86.70	-	390.40	35.85
2- <i>t</i> -penten-1-ol	700	-	-	71.10	-
1-octen-3-ol	1004	-	-	141.33	126.92
7-octen-4-ol	1024	-	-	-	5.53
3-methyl-1-butanol	788	84.90	23.88	281.30	4.02
4-(1-methylpropyl)-phenol	1353	-	-	90.13	-
Hydrocarbons					
heptane	723	-	-	60.64	-
nonane	936	-	-	39.08	35.85
undecane	1147	-	-	53.74	-
dodecane	1253	-	6.63	-	-
tetradecane	1460	-	62.06	156.82	52.03
pentadecane	1569	-	343.69	261.45	-
hexadecane	1673	-	366.78	133.23	-
toluene	778	-	21.50	71.23	-
xylene	867	-	-	-	17.05
naphthalene	1223	-	7.54	85.35	34.08
1-methylnaphthalene	1341	-	-	69.64	-
1,8-dimethylnaphthalene	1474	-	28.01	113.65	34.74
trimethylnaphthalene	1599	-	-	99.77	-

Continued on next page

Table I. continued

Compounds	I _k ^a	Quantitation (ng/g of extrudates)			
		E-1 ^b	EA-1 ^b	E-2 ^b	EA-2 ^b
Furans					
2-ethylfuran	711	-	-	75.40	53.42
2-acetylfuran	925	-	11.04	-	-
2-furfural	832	-	28.84	243.00	165.66
2-furfuryl alcohol	876	184.50	19.90	-	-
2-pentylfuran	1020	-	64.49	409.50	180.48
Nitrogen-containing compounds					
1H-pyrrole	768	66.00	-	31.40	-
2-methyl-1H-pyrrole	850	-	-	24.90	-
pyrazine	739	348.80	216.18	35.80	-
methylpyrazine	838	843.50	381.57	-	-
2,5-dimethylpyrazine	931	398.70	430.97	126.60	135.34
2,3-dimethylpyrazine	937	78.00	21.50	-	-
vinylpyrazine	945	70.60	-	-	-
2-ethyl-5-methylpyrazine	1017	112.30	28.84	31.00	-
trimethylpyrazine	1021	-	64.49	-	-
2-vinyl-5-methylpyrazine	1039	-	16.08	-	-
3-ethyl-2,5-dimethylpyrazine	1103	-	102.85	56.70	77.36
Sulfur-containing compounds					
dimethyldisulfide	749	62.80	-	46.95	17.08
dimethyltrisulfide	987	-	-	51.20	-
thiazole	739	-	-	35.80	-
2-methylthiophene	781	52.30	-	-	-
2-formylthiophene	980	119.10	3.03	-	-
Total		3954.90	4283.09	6442.84	5397.82

^aI_k = Calculated retention indices with n-paraffins (C₅-C₂₅) as references; ^bE-1 = Extruded at 185°C with a moisture feed of 16% without storage; EA-1 = extruded at 185°C with a moisture feed of 16% after one year storage; E-2 = extruded at 160°C with a moisture feed of 20% without storage; EA-2 = extruded at 160°C with a moisture feed of 20% after one year storage; ^cNot detected

methylthio radicals and other radicals such as alkyl radicals formed from lipid degradation. Some thiazoles and thiophenes have been reported as volatile compounds derived from the thermal interaction of cysteine and reducing sugars. These thiazoles and thiophenes were involved in the development of "meaty" flavors (17).

The data from Tables I and II shows that the total amounts of volatiles produced from the heating process were 4 to 20 times higher than those from the extrusion process. During the extrusion process, pressure builds up within the extruder barrel as the plasticized material progresses towards the die. As the food exits at the die, steam is flashed off due to the pressure differential between the extrudate and the surrounding environment. Flavors and odors may be lost/removed during expansion at the extruder die (7). Therefore, most of the volatiles were lost at the die. Volatile generation under heating conditions produced a stronger flavor due to less loss. The yield of volatile formation from heating at 180°C was larger than at 160°C. This indicated that temperature enhanced the production of volatile compounds during the heating process.

The volatile compounds were further classified into three different categories that included lipid degradation, Maillard reaction, and others. The relative percentages for these three different types of classifications are shown in Figure 1. Sample E-2, which was extruded at a die temperature of 160°C with a 20% moisture feed, contained about 80% of lipid degradation products, whereas sample E-1, which was extruded at a die temperature of 185°C with a 16% moisture feed, contained larger amounts of Maillard-type of compounds than the lipid degradation products. It has been known that the Maillard reaction has a maximum reaction rate at intermediate moisture levels. It was also reported that the rate of Maillard reactions was enhanced by increasing the extrusion temperature and was offset by increasing the moisture content (18-19). Therefore, it is reasonable that the sample E-1 had dominant Maillard-type flavors. In addition, the results indicated that the volatile compounds from the Maillard reaction were predominant in both of the heated samples that contained 14% moisture. It also appears that there were more Maillard-type flavors produced when the sample was heated at a higher temperature. The flavor profile of the sample E-1, which was extruded at a higher die temperature, was similar to that of the products formed by the heating process.

Few studies mentioned flavor as a positive attribute of a cooking extruder probably because the volatile compounds are lost as the material exits the extruder at the die. Many extrusion processors have relied on the post-extrusion flavor application or the introduction of thermally resistant flavors prior to the extrusion process (20). Based on the present study, it is suggested that by carefully controlling the extrusion parameters, a desirable flavor profile, which is similar to that of the heating process, could be obtained.

Sample E-1, which contained a large amount of unoxidized fatty acids, was very susceptible to lipid oxidation during the storage process. Therefore, the total yield of the lipid degradation products in sample EA-1, which is sample E-1 stored

Table II. Volatile Aroma Compounds Identified from the Heated Samples

Compounds	I _k ^b	Quantitation ^a	
		W-160 ^c	W-180 ^c
Carbonyls			
pentanal	695	0.27	0.73
hexanal	807	0.96	1.34
nonanal	1130	0.24	- ^d
decanal	1238	0.13	-
3-methylbutanal	662	0.37	1.65
2-methylbutanal	671	0.39	2.00
2-methyl-2- <i>t</i> -butenal	737	0.21	-
2- <i>t</i> ,4- <i>t</i> -octadienal	893	0.23	0.16
benzaldehyde	970	0.96	-
2-butanone	598	0.92	-
2-hexanone	798	0.17	-
2-heptanone	904	0.56	0.69
2-pentadecanone	1701	0.13	-
3-methyl-2-butanone	685	0.11	-
2-methyl-3-pentanone	692	0.14	-
2-methyl-5-isopropyl-2-cylcohexen-1-one	1276	0.04	-
2-hydroxy-3-pentanone	799	-	0.26
1-(acetyloxy)-2-propanone	850	-	0.18
Alcohols			
1-butanol	689	-	0.95
1-hexanol	890	0.48	-
1-octen-3-ol	1004	0.23	-
3-methyl-1-butanol	788	0.85	0.79
Hydrocarbons			
undecane	1147	0.03	-
tetradecane	1460	0.02	-
pentadecane	1569	0.07	-
1-tridecene	1505	0.11	-
naphthalene	1223	0.06	-

Continued on next page

Table II. Continued

Compounds	I_k^b	Quantitation ^a	
		W-160 ^c	W-180 ^c
Furans			
2-furfuryl alcohol	876	1.64	11.19
2-pentylfuran	1020	-	3.08
Nitrogen-containing compounds			
pyrazine	739	0.09	3.91
methylpyrazine	838	5.38	24.24
ethylpyrazine	912	4.22	-
2,5-dimethylpyrazine	931	-	21.99
2,3-dimethylpyrazine	937	0.30	-
2-ethyl-5-methylpyrazine	1017	3.07	11.31
isopropenylpyrazine	1060	-	0.28
2-ethyl-3,5-dimethylpyrazine	1075	-	1.26
3-ethyl-2,5-dimethylpyrazine	1103	0.96	2.78
3,5-diethyl-2-methylpyrazine	1153	0.16	0.85
3-isopentyl-2,5-dimethylpyrazine	1311	0.08	-
Sulfur-containing compounds			
dimethyl disulfide	749	0.21	1.25
dimethyl trisulfide	987	0.10	-
methylpentyl disulfide	1135	0.05	-
thiazole	739	0.09	-
4-methylthiazole	803	-	0.27
2-formylthiophene	980	-	0.37
2-pentylthiophene	1163	0.08	-
Total		24.11	91.53

^aValues in micrograms per gram of wheat flour; ^b I_k = Calculated retention indices with n-paraffins (C₅-C₂₅) as references; ^cW-160 = Heated at 160°C; W-180 = heated at 180°C; ^dNot detected.

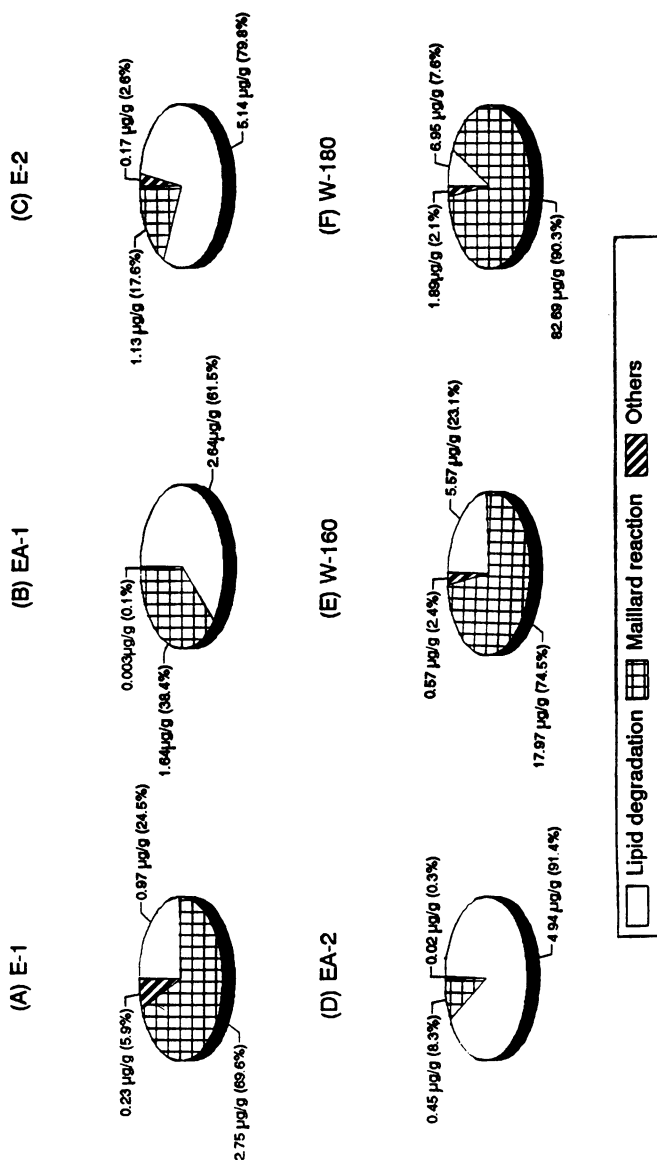


Figure 1. The relative percentages of volatiles generated from lipid degradation, Maillard reaction, and other reaction during extrusion and heating processes. E-1 = extruded at 185°C with a moisture feed of 16% and without storage; EA-1 = extruded at 185°C with a moisture feed of 16% and after one year storage; E-2 = extruded at 160°C with a moisture feed of 20% and without storage; EA-2 = extruded at 160°C with a moisture feed of 20% and after one year storage; W-160 = heated at 160°C; W-180 = heated at 180°C.

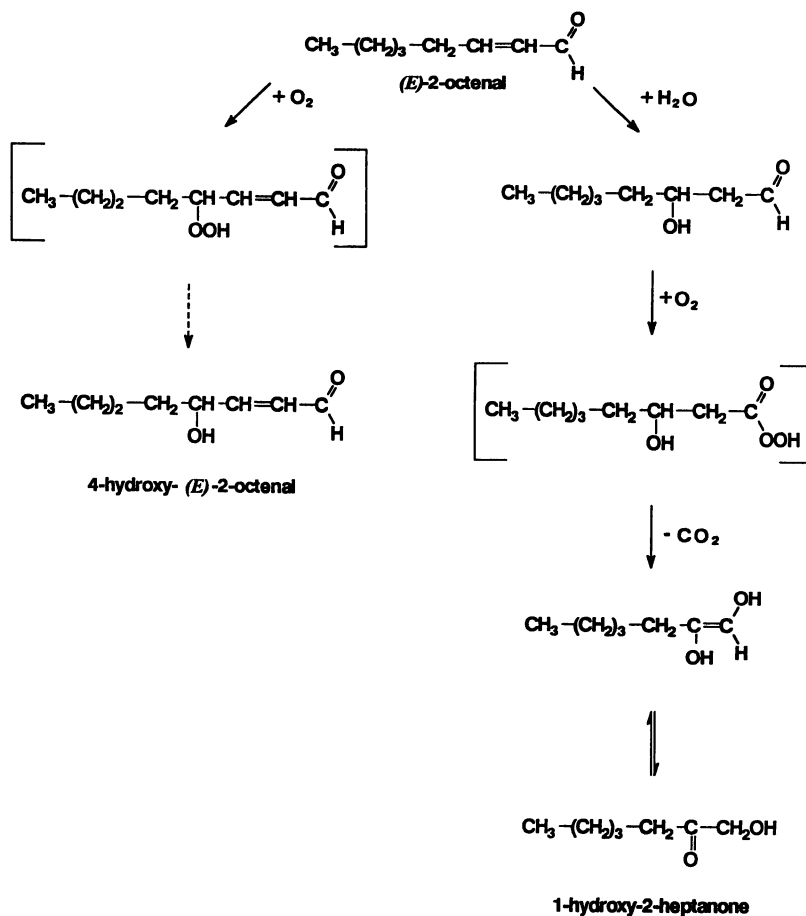


Figure 2. Postulated formation pathways of 4-hydroxy-*(E)*-2-octenal and 1-hydroxy-2-heptanone from 2-octenal (SOURCE: Adapted from ref. 21. Copyright 1993, American Chemical Society).

for one year, significantly increased (Figure 1). However, the amount of the lipid degradation compounds in sample EA-1 was still lower than that in sample E-2, which was extruded at die temperature of 160°C with a 20% moisture feed. It shows that the rate of lipid degradation was much slower in the storage process than in the extrusion process. Although the total amounts of lipid degradation products did not significantly change between sample E-2 and sample EA-2, which is sample E-2 stored for one year, some of the new lipid degradation products were found in sample EA-2 such as 2-hexenal, 2-*t*-heptenal, 2-*t*-decenal, 2-butyl-2-octenal, 1-hydroxy-2-heptanone, and 4-hydroxy-2-*t*-octenal. It implies that the lipid degradation still occurred in sample EA-2; however, it was not shown in the total yield of the lipid degradation products. This was because some lipids gradually degraded to oxidative compounds and some of the oxidative compounds volatilized to air or underwent further reactions. For example, 2-butyl-2-octenal can be formed from the dimerization of hexanals *via* the aldol addition reaction. These results indicated that the yield of hexanal in sample EA-2 was lower than in sample E-2. In addition, 1-hydroxy-2-heptanone and 4-hydroxy-2-*t*-octenal identified in sample EA-2 were the degradation products of 2-*t*-octenal. The formation mechanisms of 1-hydroxy-2-heptanone and 4-hydroxy-2-*t*-octenal shown in Figure 2 have been proposed by Grein et al. (21). The yields of the Maillard-type flavors decreased in both extrudates during the storage process. Even though the Maillard reaction could occur during storage, its rate was very slow in these studies.

Acknowledgements

New Jersey Agricultural Experiment Station Publication No. F-10544-1-94 supported by State Funds and the Center for Advanced Food Technology. We thank the Center for Advanced Food Technology Mass Spectrometry Laboratory for providing instrumentation support and the Scientific Instrument Services Inc. of Ringoes, New Jersey for donation of the Short Path Thermal Desorption apparatus. The Center for Advanced Food Technology is a member of the New Jersey Commission for Science and Technology. We also acknowledge Dr. Robert T. Rosen, Mr. J. Lech, and Mrs. Joan B. Shumsky for their assistance.

Literature Cited

1. Nawar, W. W. In *Food Chemistry*; Fennema, O. R., Ed.; Marcel Dekker: New York, NY, 1985; pp 139-244.
2. Whitfield, F. B. *CRC Crit. Rev. Food Sci. Nutr.* **1992**, *31*, 1-58.
3. Ho., C.-T.; Bruechert, L. J.; Zhang, Y.; Chiu, E.-M. In *Thermal Generation of Aromas*, Parliment, T. H.; McGorin, R. J.; Ho, C.-T., Eds.; ACS Symposium Series No. 409; American Chemical Society: Washington, D.C., 1989; pp 105-113.

4. Huang, T. C.; Bruechert, L. J.; Rosen, R. T.; Hartman, T. G.; Ho, C.-T. *J. Agric. Food Chem.* **1987**, *35*, 985-990.
5. Zhang, Y.; Ho, C.-T. *J. Agric. Food Chem.* **1989**, *37*, 1016-1020.
6. Huber, G. R. *Food Technol.* **1991**, *45*, 160-162.
7. Camire, M. E. *J. Amer. Oil Chem. Soc.* **1991**, *68*, 200-205.
8. Chen, J.; Reineccius, G. A.; Labuza, T. P. *J. Food Technol.* **1986**, *21*, 365-383.
9. Lane, R. P. *Cereal Foods World* **1983**, *28*, 181-183.
10. Palkert, P. E.; Fagerson, I. S. *J. Food Sci.* **1980**, *45*, 526-528.
11. Majlat, P.; Erdos, Z.; Takacs, J. *J. Chromatogr.* **1974**, *91*, 89-103.
12. Barnes, P. J. In *Recent Advances in Chemistry and Technology of Fats and Oils*, Hamilton, R. J.; Bhati, A., Eds.; Elsevier Applied Science: London, UK, **1987**; pp 79-107.
13. Morrison, W. R. *Cereal Chem.* **1978**, *55*, 548-558.
14. Josephson, D. B.; Lindsay, R. C. *J. Food Sci.* **1987**, *52*, 1186-1190.
15. Bruechert, L. J.; Zhang, Y.; Huang, T. C.; Hartman, T. G.; Rosen, R. T.; Ho, C.-T. *J. Food Sci.* **1988**, *53*, 1444-1447.
16. Maga, J. A. *Food Rev. Int.* **1992**, *8*, 479-558.
17. Tannenbaum, S. R.; Young, V. R.; Archer, M. C. In *Food Chemistry*; Fennema, O. R., Ed.; Marcel Dekker: New York, NY, **1985**; pp 477-544.
18. Bjorck, I.; Noguchi, A.; Asp, N.-G.; Cheftel, J. C.; Dahlqvist, A. *J. Agric. Food Chem.* **1983**, *31*, 488-492.
19. Fors, S. M.; Eriksson, C. E. *J. Sci. Food Agric.* **1986**, *37*, 991-1000.
20. Maga, J. A. In *Extrusion Cooking*, Mercier, C.; Linko, P.; Harper, J. M., Eds.; American Association of Cereal Chemists: St. Paul, MN, **1989**; pp 387-398.
21. Grein, B.; Huffer, M.; Scheller, G.; Schreier, P. *J. Agric. Food Chem.* **1993**, *41*, 2385-2390.

RECEIVED February 3, 1994

Chapter 11

Effect of Packaging on the Lipid Oxidation Storage Stability of Dehydrated Pinto Beans

Thomas G. Hartman, Karl Karmas, Juan P. Salinas, Reginald Ruiz,
Joseph Lech, and Robert T. Rosen

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

This investigation studied the development of lipid oxidation flavors in precooked dehydrated pinto beans stored in different packaging environments. The total lipid content and the fatty acid composition of the beans were determined. The volatile flavor constituents of a commercial product were also characterized. To simulate various packaging environments the precooked, dehydrated beans were stored for up to six months under the following conditions: 1) exposed to air, 2) exposed to air with added butylated hydroxytoluene (BHT), 3) under nitrogen headspace and 4) under nitrogen headspace with added BHT. Throughout the storage stability study, samples were periodically monitored for the presence of hexanal and other oxidative decomposition products. This was accomplished using purge & trap - thermal desorption - gas chromatography - mass spectrometry methodology. The oxidative stability of the product as influenced by the various storage conditions (in decreasing order of oxidation) was found to be air \geq air with BHT > nitrogen \geq nitrogen with BHT.

Pinto beans are a grain legume cultivar of the family *Fabaceae*, commonly called the pea or bean family, formerly the Leguminosae. *Phaseolus vulgaris* is the genus and specie. They are a nutritious food source which contains high quality protein, complex carbohydrates, vitamins, minerals, high fiber and a relatively low lipid content that is rich in polyunsaturates. They are the primary ingredient in "refried beans", an ethnic food preparation of Mexican origin that has become increasingly popular in the United States and abroad. Refried beans are typically prepared by soaking pinto beans in water for an extended period (~24 hours) to promote moisture uptake and softening. The soaked beans are then mashed, mixed with additional lipid, salt and/or spices and are cooked by various methods

0097-6156/94/0558-0158\$08.00/0
© 1994 American Chemical Society

such as frying or pressure cooking. As a convenience to the consumer, instant refried beans are produced commercially by canning or in a precooked dehydrated form. The dehydrated product is then simply reconstituted with boiling water to produce an instant preparation. The highly polyunsaturated nature of the lipid in the beans limits the oxidative stability of the product, especially after processing. The storage stability and flavor attributes of the precooked dehydrated product are the focus of this investigation.

Numerous studies have been conducted on the chemical composition, processing, cultivation, usage and nutritional aspects of pinto beans. A comprehensive review of the early literature has been compiled by Sathe, Deshpande and Saunkhe (1). They report the proximate composition of dry pinto beans as moisture (9%), protein (25%), fat (1.3%), fiber (5%) and carbohydrates (69%). However, it is noted that considerable variation is encountered when analyzing different lots of beans. Presumably, different cultivars and agricultural variation account for these observations. An earlier investigation on the proximate composition of raw dry pinto beans by Meiners et. al. (2) reported moisture content (15%), protein (19%), fat (1.2%), fiber (6%) and ash (4%). The protein quality and anti-nutritional properties of pinto beans were the basis for a study conducted by Hove, King and Hill (3). Based on rat feeding trials they reported a protein efficiency ratio of zero for raw pinto beans. However, after cooking the efficiency ratio increased dramatically indicating that cooked pinto beans offer a source of high quality dietary protein. The poor efficiency observed in the raw beans is attributed to the presence of protease inhibitors and other anti-nutritional factors common to pinto beans and other legume seeds. Although pinto beans offer an excellent source of protein, vitamins, minerals, lipid and carbohydrates consumer acceptability has been limited by its tendency to cause flatulence. This is due to the relatively high content of flatulence-causing sugars such as raffinose and stachyose that are present in the beans. In a study conducted by Borejszo and Khan they reported a reduction in these flatulence-causing sugars following high temperature extrusion of pinto bean high starch fractions (4). Presumably, the high temperature, short time extrusion conditions favored consumption of these sugars via Maillard and/or thermal degradation reactions.

Several early investigations focussed on the lipid composition of pinto beans. Korytnyk and Metzler determined the total lipid content to be 1.85% (5). They reported the fatty acid percentages to be myristic (trace), pentadecanoic (trace), palmitic (32.5), stearic (4.6), oleic (7.2), linoleic (31.2), linolenic (22.0) and behenic (2.5). A study conducted on pinto beans by Takayama, Muneta and Wiese found the total crude lipid and the ratio of triglycerides to phospholipid (6). The crude lipid content of pinto beans obtained from several northeastern U.S. growing areas averaged 3%. The ratio of triglycerides to phospholipid was found to be nearly 1:1. The investigators determined the fatty acid composition on a mole % basis for both forms of conjugated lipid. The triglycerides were found composed of myristic (0.1), palmitic (16.0), stearic (0.5), oleic (8.7), linoleic (26.7) and linolenic (47.9). The fatty acid composition of the phosphatides were palmitic

(40.0), stearic (0.6), oleic (49.5) and linoleic (10.0). From this data it appears that the neutral lipid fraction in pinto beans harbors most of the highly unsaturated fatty acids. A more recent review compiled by Pattee et. al., reports the total lipid content of pinto beans to be 1.85% with a 1.6:1 molar ratio of triglycerides to phosphatides (7). The reported fatty acid composition of the beans was found similar to previous investigations. This review also discusses the effects of enzymatic (lipoxygenase) and nonenzymatic oxidizing lipid in legumes with respect to flavor, storage stability, nutrition and processing. However, little information is devoted specifically to pinto beans. Pinto beans like other legume seeds contain lipoxygenase enzyme activity that normally requires deactivation during processing. The effects of blanching methods and post blanch treatments on the quality attributes of canned dry pinto beans was the focus of a study conducted by Davis, Twogood and Black (8). They determined that hot water at 96°C for 4 minutes or 4-8 minutes of steam produced adequate blanching with minimal deterioration of product quality.

Many flavor investigations have been conducted on legume seeds but relatively few have focussed specifically on pinto beans. A study by Lovegren et. al., profiled the volatile compounds in dry pinto beans and other legume seeds using gas chromatography - mass spectrometry (GC-MS) methodology (9). They reported the presence of over 23 volatile compounds detected in dry bean samples and list a range of observed concentrations. However, the authors analyzed many dry bean samples (pinto, red kidney, black, navy, mung, lima and soybeans) and did not distinguish which compounds if any were specific to pinto beans. Most of the compounds described were typical lipid oxidation products and/or Strecker aldehydes from the Maillard reaction. Furthermore, nearly all samples analyzed were reported to contain chlorinated compounds such as chloroform, dichlorobenzene and trichlorobenzene. Aromatic compounds such as benzene, toluene, naphthalene and methylnaphthalene were also consistently observed. Despite the claims by the authors that these compounds are natural products it is highly suspect that many of them (especially the chlorinated compounds) are artifacts in origin.

This investigation represents the first report of volatile compounds in a commercial instant refried bean food preparation of which the major constituent is pinto bean. It also details the lipid oxidation storage stability of the precooked dehydrated product in different packaging environments. Purge & trap - thermal desorption - GC-MS analytical methodology that has been described previously was employed for this study (10,11). For the sake of completeness the raw pinto beans used to produce the product were analyzed for total lipid and fatty acid composition.

Experimental

Materials and Methods. Samples of raw pinto beans and a commercial instant refried bean (pre-cooked and dehydrated) preparations were supplied by Basic

American Foods (Plover, WI). The raw dry pinto beans were grown in South Dakota. The instant refried bean product is produced in a proprietary process that involves washing, soaking, cooking and dehydration. A freshly manufactured product was provided for testing. Methanol solvent was capillary-analyzed grade and was acquired from J.T. Baker (Phillipsburg, NJ). Whatman # 1 filter paper was from Fisher Scientific Company (Springfield, NJ). The internal standard d-8 toluene and antioxidant butylated hydroxytoluene (BHT) were obtained from Aldrich Chemical Company (Milwaukee, WI). Glass 1-quart mason jars were from Ball Corporation (Muncie, IN). Carbotrap 20/40 mesh adsorbent and acid washed, silane treated glass wool were obtained from Supelco, Inc. (Belefonte, PA). Tenax-TA 60/80 mesh adsorbent, custom designed stainless steel mason jar lids with valves, SIS model TD-1 Short Path Thermal Desorber, SIS Solid Sample Purge and Trap Apparatus and all thermal desorption accessories were generously donated by Scientific Instrument Services (SIS) Inc. (Ringoos, NJ). The GC capillary column, a DB-1, 60 meter x 0.32 mm i.d. x 0.25 μm film was from J&W Scientific Company (Folsom, CA).

Volatiles Analysis of Instant Refried Bean Product. The volatiles in the instant refried bean product were profiled using purge & trap - thermal desorption - GC-MS methodology that has been described previously (10,11). The precooked dehydrated refried bean product was reconstituted with boiling water according to the manufacturers instructions (1347 g of product + 2739 g boiling water). The product was mixed by stirring and then allowed to sit for several minutes covered. An aliquot (10 g) of reconstituted product was then transferred into an SIS Solid Sample Purge & Trap Apparatus. The sample was then matrix-spiked with d-8 toluene internal standard at a level of 1.0 ppm w/w by the addition of 1.0 μl of a 10.0 mg/ml solution in methanol. The apparatus was maintained at 80°C for 30 minutes during which time the volatiles were sparged with nitrogen at a flow rate of 40 ml/min. The volatiles were trapped on SIS glass lined stainless steel adsorbent tubes (10 cm L x 3.0 mm i.d.) packed with 50 mg of 60/80 mesh Tenax-TA and 50 mg of 20/40 mesh Carbotrap. After sample collection excess moisture was removed from the adsorbent trap by a flow of nitrogen (40 ml/min.) for 15 minutes. The adsorbent trap was then thermally desorbed directly into the GC using an SIS model TD-1 Short Path Thermal Desorption module operated at 220°C for 5 minutes at a carrier gas (helium) flow rate of 10 ml/minute. The GC was a Varian 3400 containing a 60 meter x 0.32 mm i.d. x 0.25 μm film thickness DB-1 capillary column with a carrier gas (helium) flow rate of 1.0 ml/min. The injector temperature was 220°C and a 10:1 split ratio was employed. The column temperature program was -20°C (held 5 minutes during thermal desorption to achieve cryofocussing) to 280°C at a rate of 10°C per minute. The GC column was directly interfaced to the mass spectrometer ion source via a heated transfer line maintained at 280°C. The mass spectrometer was a Finnigan MAT 8230 high resolution, double focussing, magnetic sector instrument operated in standard (70 eV) electron ionization mode. The source temperature was 250°C and the

July 15, 2012 | <http://pubs.acs.org>
Publication Date: July 7, 1994 | doi: 10.1021/bk-1994-0558.ch011

instrument was operated at a resolution of 1000. The instrument was scanned continuously between masses 35-350 once each second with an interscan time of 0.8 seconds. Mass spectra were acquired and processed using a Finnigan Mat SS300 computerized data system. Individual mass spectra were all background subtracted and library searched against the NIST mass spectral reference collection. Additional libraries such as the Wiley, EPA-NIH, Eight Peak Index and our own custom collection of mass spectra were also consulted if necessary. Structural assignments were further confirmed on the basis of GC retention time order and manual interpretation. Semi-quantitative analysis was achieved by integration of the GC-MS total ion chromatograms. The peak area integration values for all compounds were divided by that obtained from the d-8 toluene matrix-spiked internal standard to obtain semi-quantitative estimates of concentration. No response factor corrections or calibrations curves were utilized.

Lipid Oxidation Storage Stability. The precooked dehydrated pinto beans (200 g) were placed in 1-quart glass mason jars fitted with custom designed stainless steel lids. The lids contained sparging tube inlet and exhaust valves equipped with Teflon-faced silicone septa seals. To simulate various packaging environments the product containing mason jars were conditioned as follows:

- 1) product alone with air headspace
- 2) product with air headspace and added BHT (180 ppm w/w)
- 3) product alone with nitrogen headspace
- 4) product with nitrogen headspace and added BHT (180 ppm w/w)

The BHT antioxidant was added to the product by applying 3.6 ml of a 10 mg/ml stock solution in methanol to a 4 inch diameter sheet of Whatman # 1 filter paper. The methanol was allowed to evaporate and then the paper was cut into sections approximately 1 cm². The cut up filter paper was then placed into the mason jars containing the product and shaken. This was intended to simulate the use of commercially available packaging that is supplied impregnated with antioxidant. Migration of BHT into the product was subsequently verified by the presence of a large peak in the GC-MS chromatograms. The nitrogen atmosphere was created by purging the product through the sparge inlet in the jars for approximately 10 minutes with the vent valve in the open position. Both valves were then closed, thus providing a leak-tight seal. The jars were then stored at room temperature in the dark for the duration of the study. At various time intervals (1, 12, 57 & 187 days) 10 g aliquots of product were removed and tested for hexanal concentration. The analysis methodology was exactly the same as described for the volatiles profiling except that the dehydrated beans were analyzed in the dry form without reconstitution with boiling water.

Lipid Analysis and Fatty Acid Composition. Samples of raw dry pinto beans were analyzed for total crude lipid and fatty acid composition using standard AOCS methodology. The analyses were performed by Bittner Laboratories (Blackfoot, ID).

Results and Discussion

The total crude lipid content of the raw dry pinto beans was found to be 1.86% w/w. The individual fatty acids present in the lipid and their percentages are summarized in Table I. The observed values are in reasonable agreement with those obtained by previous investigators (6-8).

Table I. Fatty Acid Profile of Raw Dry Pinto Beans

Fatty Acid	% of Total Lipid
myristic (14:0)	0.16
palmitic (16:0)	19.08
palmitoleic (16:1)	0.16
stearic (18:0)	1.96
oleic (18:1)	9.31
linoleic (18:2)	27.84
linolenic (18:3)	40.51
arachidic (20:0)	0.08
behenic (22:0)	0.36
lignoceric (24:0)	0.55

Although the total lipid content is low, the relatively high percentage of polyunsaturates such as linolenic and linoleic acid predispose the product to the development of oxidative off-flavors unless strict precautions are taken. In the intact beans the lipid is in a conjugated highly compartmentalized form (7). The shielding properties of the waxy cuticle and cell structure of the bean combined with the presence of natural antioxidants offers a high degree of protection. However, once the cellular structure of the beans have been ruptured during processing the lipid becomes highly susceptible to oxidation via lipoxygenase and/or autoxidation. The lipoxygenase activity is generally eliminated by the effects of thermal processing but non-enzymatic oxidation remains problematic. Prudence must be applied to minimize the effects of prooxidants during processing and storage of the product. Since the beans contain chlorophyll and other potential photosensitizing agents the effects of singlet oxygen catalyzed photooxidation must be considered. Product exposure to metals such as copper must also be minimized due to their well-known prooxidant activity. Furthermore, the water activity of the final dehydrated product should be controlled such that it falls within a range (~0.3 - 0.5) to minimize both lipid oxidation and Maillard reactions during storage. It

is well known that over drying of products often leads to higher levels of lipid oxidation. This is presumably because hydroperoxides are partially stabilized by the hydrogen bonding effect of boundary layer water in intermediate moisture content systems.

The volatile compounds detected in the reconstituted instant refried beans are presented in Table II. Compounds are listed in the order of elution from the GC. Approximately 70% of the peaks on the GC-MS chromatogram were positively identified. Many of the remaining 30% were branched aliphatic hydrocarbons with isoprenoid-type character and firm identification was not possible. Many of these hydrocarbons are presumed to be degradates of isoprenoid-type pigments and/or from the waxy cuticle of the bean.

An analysis of the volatile compounds shown in Table II indicates that at least three systems are contributing to the overall flavor attributes of the refried bean product. The homologous series of aldehydes and ketones are well known lipid oxidation products and are present at levels above sensory threshold. They undoubtedly contribute a grassy, beany, oxidized lipid note to the product. Many of the compounds detected are derived from the Maillard reaction indicating that reducing sugars and amino acids are important flavor precursors in this system. For instance, 2 & 3-methylbutanal are the Strecker degradation aldehydes of isoleucine and leucine respectively. Similarly the pyrroles, pyrazines and other compounds such as furfural are all well known Maillard reaction products. These compounds are indicators of high temperature processing. They are expected to impart nutty, roasted, burnt and cooked notes to the product. Also important to flavor are the sulfur containing compounds observed. The sulfuryl notes in the refried bean product are very prominent. Compounds such as these presumably arise from the thermal decomposition of sulfur-containing amino acids such as methionine and cysteine. All of these classes of compounds blend together to form the characteristic aroma of the refried beans.

The data pertaining to the storage stability study is summarized in Table III. The data is also illustrated in graphic form in Figure 1.

The storage stability data clearly indicates that a nitrogen headspace provides the best inhibition of lipid oxidation. It appears that no increased protection was afforded by the combined use of nitrogen and BHT antioxidant. Similarly, no significant differences were observed between product packaged with an air headspace or containing added BHT except for a marginal improvement at 57 days storage. This is probably because the conjugated triene system of highly polyunsaturated fatty acids such as linolenic acid can preferentially oxidize even in the presence of antioxidants such as BHT. In addition, it must be noted that despite the slightly higher hexanal levels observed in the air packaged samples the products at the end of the study were not at all rancid or otherwise unsuitable for consumption.

July 15, 2012 | <http://pubs.acs.org>
Publication Date: July 7, 1994 | doi: 10.1021/bk-1994-0558.ch011

Table II. Volatile Compounds Detected in Instant Refried Beans

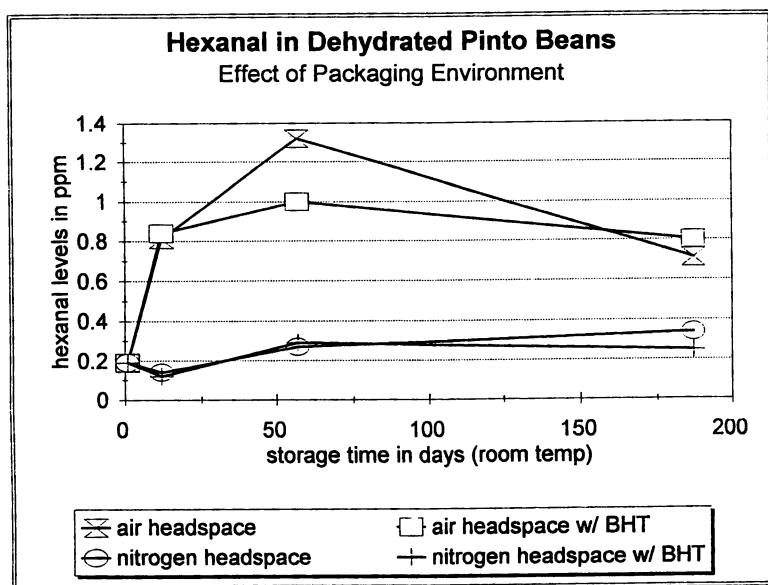
Compound	Estimated Concentration (ppb) ¹
<i>n</i> -pentane	42
3-methylbutanal	268
2-methylbutanal	82
1-butanol	112
pentanal	264
1-methyl-1H-pyrrole	173
dimethyl disulfide	675
methyl propyl sulfide	408
dimethylsulfone	38
1H-pyrrole	595
toluene	450
hexanal	584
furfural	306
methylpyrazine	459
ethylbenzene	133
2-heptanone	186
heptanal	369
styrene	103
ethylpyrazine	681
<i>n</i> -nonane	293
benzaldehyde	886
dimethyl trisulfide	422
1-heptanol	177
6-methyl-5-hepten-2-one	254
2-ethyl-6-methylpyrazine	108
2-ethyl-3-methylpyrazine	992
2-vinyl-6-methylpyrazine	140
C-4 alkyl-substituted pyrazine isomer	434
3,5-octadien-2-one	452
nonanal	805
dipropyl disulfide	220
3,5-diethyl-2-methylpyrazine	198
decanal	391

¹Estimated concentration based on peak area comparison to that of a matrix-spiked internal standard (d-8 toluene)

July 15, 2012 | <http://pubs.acs.org>
Publication Date: July 7, 1994 | doi: 10.1021/bk-1994-0558.ch011

Table III. Effect of Packaging Environment on Hexanal Levels in Precooked Dehydrated Pinto Beans

Storage Time (days)	Hexanal Concentration (ppm)			
	Air	Air w/BHT	Nitrogen	Nitrogen w/BHT
1	0.19	0.19	0.19	0.19
12	0.81	0.84	0.14	0.12
57	1.32	1.00	0.27	0.29
187	0.71	0.80	0.34	0.25

**Figure 1.** Hexanal concentration in precooked, dehydrated Pinto beans stored at room temperature in various simulated packaging environments.

Conclusion

Although the total lipid content of pinto beans is low a relatively high percentage is composed of polyunsaturated fatty acids such as linoleic and linolenic. These fatty acids limit the long term oxidative stability of the product unless care is taken during processing and storage. Unless subjected to an oxidative stress a properly manufactured product can be expected to easily attain a six month shelf life without the expense of added antioxidant and/or modified atmosphere barrier packaging. Packaging of product in a vapor barrier film with a nitrogen flushed atmosphere significantly inhibits lipid oxidation and can extend shelf life of the product. However, these advantages must be weighed against the increased cost of such packaging systems.

Acknowledgements

We thank Basic American Foods for providing samples, financial and technical support. We acknowledge the Center for Advanced Food Technology (CAFT) Mass Spectrometry Lab facility for providing instrumentation support for this project. CAFT is an initiative of the New Jersey Commission on Science and Technology. This is NJAES publication No. F-10569-4-93.

Literature Cited

1. Sathe, S. K.; Deshpande, S. S.; Salunkhe, D. K. *CRC Crit. Rev. Food Sci. Nutr.* **1981**, *20*, 1-45.
2. Meiners, C. R.; Derise, N. L.; Lau, H. C.; Ritchey, S. J.; Murphy E. W. *J. Agric. Food Chem.* **1976**, *24*, 1122-1126.
3. Hove, E. L.; King, S.; Hill, G. D. *N.Z. J. Agric. Res.* **1978**, *21*, 457-462.
4. Borejszo, Z.; Khan, K. *J. Food Sci.* **1992**, *57*, 771-777.
5. Korytnyk, W.; Metzler, E. A. *J. Sci. Food Agric.* **1963**, *14*, 841-844.
6. Takayama, K. K.; Muneta, P.; Wiese, A. C. *J. Agric. Food Chem.* **1965**, *13*, 269-271.
7. Pattee, H. E.; Salunkhe, D. K.; Sathe, S. K.; Reddy, N. R. *CRC Crit. Rev. Food Sci. Nutr.* **1982**, *17*, 97-139.
8. Davis, D. R.; Twogood, M. L.; Black, K. R. *J. Food Sci.* **1980**, *45*, 817-820.
9. Lovegren, N. U.; Fisher, G. S.; Legendre, M. G.; Schuller, W. H. *J. Agric. Food Chem.* **1979**, *27*, 851-853.
10. Manura, J. J.; Hartman, T. G. *Amer Lab.* **1992**, May 46-52.
11. Hartman, T. G.; Lech, J.; Karmas, K.; Salinas, J.; Rosen, R. T.; Ho, C-T. In *Flavor Measurement*, Ho, C-T.; Manley, C. H., Eds.; Marcel Decker: New York, 1993; pp 37-60.

RECEIVED February 23, 1994

Chapter 12

Influence of Finishing Diets on Lamb Flavor

M. E. Bailey¹, J. Suzuki², L. N. Fernando¹, H. A. Swartz³,
and R. W. Purchas⁴

¹Department of Food Science and Human Nutrition, University of Missouri, 21 Agriculture Building, Columbia, MO 65211

²Soda Aromatic Company, 1573-4 Fumakata Noda, Chiba 270-02, Japan

³Department of Cooperative Research, U.S. Department of Agriculture, Lincoln University, 900 Moreau Drive, Jefferson City, MO 65101-3594

⁴Department of Sheep Husbandry, Massey University, Palmerston North, New Zealand

Headspace gas liquid chromatography-mass spectrometry (GLC-MS) was used to analyze volatiles from fat of lambs finished on various diets in New Zealand and Missouri. Sensory analysis of ground lamb was used to quantitate "strong", "grassy" and "lamby" flavors. Sensory scores for all these attributes were lower in samples from corn-finished animals than in those from forage-finished animals. Radish top-fed lamb had lower flavor intensity than lamb finished on ryegrass and clover, and lamb from animals finished on blackeye (cowpea) forage had less grassy flavor than lamb finished on alfalfa or grass. Strong flavor, which was a combination of grassy flavor and lamby flavor, was statistically related to diterpenoid and aldehyde contents; grassy flavor was related to diterpenoid and acid contents; and lamby flavor was related to aldehyde, ketone and acid contents. Animals finished on the various diets could be separated into classes by discriminant and canonical statistical analyses of the volatile compound data from GLC-MS analyses.

Thirty years ago, Hornstein and Crowe (1) concluded that the characteristic flavor of meat from different species resulted from constituent volatile lipids. They later reported that the oxidation of unsaturated fatty acids contributed to these unique flavors (2).

Volatile lipids constitute over 90% of the chemical ingredients identified as contributing to meat flavor (3). The acceptance of these volatiles from individual species of animals as desirable meat flavors is somewhat dependant upon the training preference and the psychology of the individual evaluating the flavor (4).

0097-6156/94/0558-0170\$08.00/0
© 1994 American Chemical Society

Two major sources of lipids contribute to the undesirable flavor associated with lamb flavor: the unique volatile compounds resulting from the metabolism of lipids by the animal and their oxidation; and lipids resulting from precursors in the diet consumed by the animals.

Characteristic Odor of Lamb

This topic was thoroughly reviewed by Young et al. (5), who concluded that the characteristic odor of lamb is a result of volatiles in the lipid fraction. The most convincing data relating lipid content and flavor was that published by Wong et al. (6), who identified 40 short and medium class fatty acids in an acid extract from heated mutton fat. They found that the primary acids associated with mutton odor were the 4-methyl C₉ and C₁₀ fatty acids and identified 4-methyl octanoic acid (Hircinoic acid) and 4-methyl nonanoic acid as the most important even though they are present in lamb fat at very low concentrations (7).

Garton et al. (8) suggested that the branched acids with methyl substituents at even-numbered carbon atoms result from the incorporation of methyl malonyl Co. A (from propionate metabolism) instead of malonyl Co. A during chain lengthening.

Purchas et al. (9) could not substantiate that individual fatty acids were related to sheep flavor; and other lipids, such as oxidized products from unsaturated fatty acids, have been identified as contributing to lamb flavor. There is sufficient evidence in the literature to show that lamb odor is present in non-oxidative systems (1, 10, 11).

Sulfur compounds have also received attention as contributors to sheep flavor. It is assumed that H₂S is evolved from the degradation of sulfur amino acids, but there is no evidence that the amino acid profile of lamb is different from that of other animals (12); and large quantities of H₂S are evolved during cookery of all meat.

The best evidence supports the theory that intermediate chain fatty acids are responsible for the characteristic flavor of lamb.

Effect of Diet on Lamb Flavor

The effect of diet on lamb flavor has been a thoroughly studied problem. Barbella et al. (13) first reported that the diet of lamb influenced the flavor of meat. Most research on the influence of diet on lamb flavor has been directed to the study of undesirable flavor resulting from feeding forage.

Forage from grazing lands (supplemented with hay and silage) provides 63% of the feed for dairy cows, 73% of the feed for beef cattle and 90% of the feed for sheep. Meat animals are fed 100% forage in many countries, and it is anticipated that long-term population growth, high costs of growing feed grains and consumer desire for lean beef may increase demand for forage feeding. However, a major problem with forage feeding and finishing diets is the resulting grassy flavor of the meat products.

Grassy flavor is different from oxidized flavor and characteristic flavors from meat of animal species. Berry et al. (14) may have been the first to coin the term "grassy". Melton et al. (15, 16) found that the terms "dairy", "fishy", "milky-oily", "off-flavor" and "putrid" were descriptive of beef patties from grass-fed steers. Greater detail on descriptors used for describing off-flavors in meat were published by Melton et al. (17, 18).

Stronger flavors from sheep finished on forage have been recognized for many years. A summary of some of this work is given in Table I. It is obvious from the above discussion that two factors are involved in the less desirable flavor of lamb in selected consumer societies. Lamb tissue metabolically forms volatile compounds that are recognized as the characteristic flavor of lamb that is not universally accepted; and ingredients in forage feed of these animals can result in low acceptance due to grassy flavor. Either or both of these attributes can be compounded by oxidation of tissue occurring post mortem.

Table I. Influence of Forage Pasture on Lamb Flavor

<i>Pasture Type</i>	<i>Authors</i>	<i>Meat Flavor Results</i>
White clover versus Ryegrass	Cramer et al. (19) Shorland et al. (20) Czochanska et al. (21)	Clover fed stronger than ryegrass
Lucerne (alfalfa)	Park et al. (22)	Intense, sharp
Rape	Park et al. (22)	Sickly
Vitch	Park et al. (22)	Intense flavor
Alfalfa versus Other pasture	Nicol and Jagusch (23) Park et al. (24)	Alfalfa most intense odor
Weeds (panthenium and wild turnip)	Ford and Park (25)	Taints

Studies were designed and carried out at our respective institutions to improve the acceptability of lamb by examining the influence of various forage types on its flavor. During this endeavor, volatile compounds thought to be related to undesirable flavor of lamb were separated and identified, and their relationship to lamb flavor and the feeding regimen was determined.

Experimental Methods

Lambs were grown on a variety of forages at three institutions. Lambs were finished to consumer weight on the forages by the Sheep Husbandry Department of Massey University in New Zealand; the Animal Science Department at the University of Missouri-Columbia (MU); and the Department of Cooperative Research, Lincoln University. The flavor of the meat was determined by sensory analysis, and the character of the volatile compounds was determined by the headspace analysis method of Suzuki and Bailey (26).

Lamb Samples Studied. Separate studies were carried out on the loins of two groups of animals. One group was raised and finished on forage in New Zealand at Massey University and compared with loins from animals finished on corn grain by the Animal Science Department at MU. This study consisted of two trials of animals raised during two consecutive years. The other study was of loin samples from animals raised by the Department of Cooperative Research at Lincoln University. The latter study consisted of four separate trials during a five year period.

Study I, Trial 1, was on loins of animals finished on pasture of four separate forages: white clover (*Trifolium repens*), lucerne (*Medicago sativa*), lotus (*Lotus corniculatus*) and perennial ryegrass (*Lolium perenne*). Data from loin samples of these animals were compared with that from samples of animals finished at MU on corn fed *ad libitum* or on fescue *ad libitum* supplemented with two pounds of corn per day (fescue/corn).

Study I, Trial 2 was on animals raised in New Zealand on clover, lucerne, lotus, radish and ryegrass compared to animals finished at MU on corn grain.

Study II consisted of four trials, but only the last three will be discussed in detail because of the similarities to the trials in Study I.

Trials 2 and 3 of Study II consisted of 48 lambs fed in three groups of 12 each on a rotational paddock system of California blackeye cowpea (*Vigna unguiculata* L. walp), Cultivar Vita 3 cowpea (an indeterminate type), alfalfa and corn/soybean/oat hay meal. All four groups were fed *ad libitum* to an average weight of 100 pounds. Ten animals from each group were selected for study.

Trial 4 in Study II consisted of 24 lambs assigned to one of four groups finished on California blackeye cowpea, sudan grass (*sorghum vulgura sudanensis*), a cottonseed diet supplemented with corn meal and molasses to give 16% protein, and grain/oat hay. All were fed *ad libitum* to a final weight of 100 pounds.

Volatile Lipid Analyses. The method of Suzuki and Bailey (26) was used for the headspace analysis of volatiles from lamb fat samples.

Sensory Analyses. Sensory analyses of the grassy and lamby flavors was carried out by the quantitative descriptive analysis method of Stone et al. (27) as modified by Suzuki (28) for the analyses of lamb. The sensory panel members

used a non-structured quantitative scale from mild to strong to characterize the flavor of samples in the first study and similar scales to quantify grassy flavor and lamby flavor in the second study.

Statistical Analyses. Two-way analysis of variance described by Snedecor and Cochran (29) was used to evaluate changes among variables. Stepwise discriminant and cononical analyses were applied to GLC data to classify animal samples into groups based on diet. In these methods the order of variables was selected according to their F values, their multiple correlation with other groups and their ratios to total variations (30). Analyses were performed using SAS/STAT (31).

Results and Discussion

Sensory Analyses of Flavor Intensity. Study I of lambs raised in New Zealand consisted of 72 animals that were finished on clover, ryegrass, lucerne and lotus compared to lambs finished on fescue/corn and corn at MU. The flavor was judged for intensity only. Meat samples in Trial 1 from animals finished on clover, ryegrass and lucerne were all more intense in flavor than those from animals finished on lotus, fescue/corn and corn *ad libitum* (Table II). The corn-finished samples were significantly ($P < 0.05$) milder in flavor than those from animals finished on lotus. Samples from animals finished on corn grain were the mildest in flavor, although not significantly different from animals finished on fescue/corn.

Table II. Mean^a Flavor Scores^b of Meat from Animals Fed on Different Diets in New Zealand Study

<i>Rations</i>	<i>Flavor Intensity</i>	
	<i>Trial 1</i>	<i>Trial 2</i>
Corn	33.6 ^c	27.3 ^c
Fescue/corn	42.4 ^c	N/A
Radish	N/A	45.1 ^d
Lotus	68.6 ^d	56.7 ^{de}
Lucerne	77.7 ^{de}	56.5 ^{de}
Ryegrass	86.0 ^e	65.6 ^e
Clover	87.1 ^e	63.8 ^e

^a N = 60

^b Range of scoring: 0 = very mild; 100 = very intense

^{c,d,e} Means followed by same letter do not differ significantly ($P < 0.05$)

Sensory results from animal samples studied in Trial 2 are also presented in Table II. Samples from animals finished on ryegrass, clover, lotus, radish and lucerne were more intense in flavor than those from animals finished on corn. The flavor scores for samples from animals fed radish were significantly ($P < 0.05$) milder than those from animals finished on ryegrass and clover, but were stronger in flavor than samples from animals finished on corn.

From the results of these studies, meat from animals finished on corn grain was milder than that of animals finished on forage. It was an advantage to feed two pounds of corn grain to animals feeding on fescue even though fescue contributes to grassy flavor (32). Radish tops produced animals with relatively mild flavor compared to other forages. This may be a good alternative diet for finishing lambs compared to other forages.

Sensory data on grassy and lamby flavors of samples from animals grown in Study II, Trials 3 and 4 are summarized in Table III. The grassy flavor intensity of grain-fed lambs in Trial 3 was significantly ($P < 0.05$) less than that of alfalfa and California blackeye-fed lambs, and was also less ($P < 0.056$) than lambs fed Vita 3. Lamb from animals finished on alfalfa was significantly ($P < 0.01$) more grassy than that from animals fed grain/oat hay. Fat from animals finished on grain/oat hay was significantly ($P < 0.01$) less lamby in flavor than fat from animals finished on the three forages, and there was no difference in the lamby flavor of samples from animals finished on the forages.

Table III. Mean Sensory Scores for Flavor of Lamb Fat in Study II

Type of Feed	Grassy Flavor		Lamby Flavor	
	Trial 3 ^a	Trial 4 ^b	Trial 3 ^a	Trial 4 ^b
Grain/oat hay	33.7 ^c	30.5 ^c	39.4 ^c	49.0 ^c
California blackeye	44.3 ^d	37.4 ^{cd}	55.3 ^e	52.5 ^c
Vita 3	43.6 ^d		55.7 ^e	
Alfalfa	51.1 ^{de}		53.0 ^e	
Cotton seed		42.6 ^{cd}		56.2 ^c
Sudan grass		46.9 ^d		44.9 ^c

^a N = 70

^b N = 48

^{c,d,e} Values with different letters within the same column are significantly different (^{cd} = $P < 0.05$; ^{de} = $P < 0.01$; grain:Vita 3 at $P < 0.056$)

Samples from animals in Trial 4 finished on sudan grass had significantly ($P < 0.05$) greater grassy flavor than those from animals finished on grain/oat hay. There were no differences in grassy flavor among samples from animals

finished with the other forages. There also were no differences in lamby flavor among the various samples.

Considering all the data analyzed for grassy and lamby flavors of meat from animals finished on cowpeas, alfalfa, sudan grass and grain, it appears that grain is the best diet for finishing lambs to reduce these flavors. California blackeye is a better forage for reducing grassy flavor than Vita 3 blackeye, and both are better finishing diets than sudan grass and alfalfa in reducing grassy flavor of lamb. Lamby flavor may also be reduced by feeding grain, but in general this flavor was not influenced by the feeding regimen.

Volatile Organic Compounds from Lamb Fat. In the study of volatile compounds from animals finished on forage in New Zealand and on corn grain in Missouri, 47 volatile compounds were identified by mass spectrometry and quantified by gas liquid chromatography (GLC). These compounds consisted of 9 acids, 8 hydrocarbons, 7 terpenoids, 9 aldehydes, 7 ketones and 7 lactones.

The highest concentration of total volatiles was found in samples from animals finished on ryegrass (Figure 1). The total volatiles from fat of animals finished on lotus, lucerne and clover were about the same, and the total volatiles from fat of animals finished on corn were the least in concentration.

Among the important volatiles analyzed were intermediate chain (C_6 - C_9) free fatty acids (Table IV). Decanoic acid was significantly ($P < 0.05$) more concentrated in ryegrass, lotus, lucerne and clover than in the samples from animals receiving corn grain. In general, the free fatty acids were higher in concentration in the samples from animals finished on clover, lucerne, lotus and ryegrass compared to those from animals finished on radish and corn grain, but most of these differences were not significant. These observations are important because of the close relationship between these acids and hircinoic acid identified by Wong et al. (6) as important in lamb flavor.

Wong et al. (7) reported that 4-methyl octanoic acid (hircinoic acid) was a major contributor to lamb flavor, but this acid was not detected in samples analyzed by the procedures used. However, the fatty acids analyzed were all in the molecular weight range of hircinoic acid. All except nonanoic acid were highly ($P < 0.001$) correlated with flavor strength evaluated by the taste panel.

Other volatile compounds correlating with flavor strength at this level ($P < 0.001$) of significance were pentanal, -pinene, 2,3-octanedione, octanal, 2,4-heptadienal, 2,3-nonanedione, 2-hydroxy-2-octanone, nonanal, 2-nonenal, dodecane, 2-undecenal, phytane and phyt-2-ene. Most of the lactones were negatively correlated with lamby flavor. Hexanal, 2,4-hexadienal, 2-decenal, 2-undecenal, 2-tridecane, 2-pentadecanone, phyt-1-ene, neophytadiene, phytadiene, dihydrophytol and nonanoic acid were all significantly related to lamby flavor at P 0.05 level or better.

Table IV. Concentration of Volatile Fatty Acids from Fat of Lambs^a Finished on Different Diets (Study I, Trial 2)

<i>Acids</i>	<i>Concentration, ppm (Standard Deviation)</i>					
	<i>Corn</i>	<i>Radish</i>	<i>Clover</i>	<i>Lucerne</i>	<i>Lotus</i>	<i>Ryegrass</i>
Hexanoic acid	7.78 ^b (5.69)	9.11 ^{bc} (7.46)	13.50 ^{bc} (5.80)	16.94 ^c (6.69)	17.28 ^c (15.02)	19.56 ^c (5.60)
Heptanoic acid	2.94 ^b (1.84)	2.89 ^b (2.27)	3.17 ^b (0.89)	2.83 ^b (0.78)	4.22 ^{bc} (1.88)	5.61 ^c (2.00)
Octanoic acid	6.33 ^{bc} (3.21)	5.89 ^{bc} (3.93)	6.03 ^{bc} (1.74)	5.33 ^b (1.35)	9.83 ^{cd} (3.50)	9.22 ^d (2.74)
Nonanoic acid	8.00 ^{bc} (6.51)	7.61 ^{bc} (7.78)	3.11 ^b (2.44)	1.72 ^b (0.85)	12.61 ^c (17.91)	11.39 ^c (5.98)
Decanoic acid	9.17 ^b (2.72)	9.39 ^b (3.86)	15.11 ^c (4.10)	15.28 ^c (2.66)	24.33 ^c (8.39)	15.72 ^d (6.34)

^a Average of six animals^{b,c,d} Means followed by same letter do not differ significantly ($P > 0.05$)

Despite the high significant correlation levels, no one compound was correlated highly with sensory lamby flavor. The highest correlation (0.652) was found between phyt-2-ene and sensory flavor, which accounted for 43% of the variation. A multiple regression equation relating some of these variables and sensory lamb flavor was:

Lamb flavor strength =

$$29.50 + 42.05X_4 - 55.24X_{11} + 3.84X_{12} - 32.86X_{21} + 0.84X_{36} - 7.06X_{37} + 116.08X_{41} + 88.86X_{43}$$

[X_4 = heptanal; X_{11} = 2,3-octanedione; X_{12} = 3-hydroxy-2-octanone; X_{21} = tetradecane; X_{36} = neophytadiene; X_{37} = phyt-2-ene; X_{41} = δ -pentadecalactone; X_{43} = phytol]

These variables accounted for 76.5% of the variation in lamb flavor.

Seventeen components analyzed by GLC were selected by stepwise discriminant analyses as predictor variables for separating the various samples analyzed into full groups relative to finishing diets received by the animals. These compounds selected in order of partial R^2 were: phyt-1-ene, α -pinene, hexanal, phyt-2-ene, 2,3-octanedione, octadecane, 2-hexadecane, δ -pentadecalactone, pentanal, phytol, dodecane, 2-tridecanone, 2-heptadecanone, 2-nonanal,

3-hydroxy-2-octanone, γ -dodecalactone and 2-pentadecanone. Samples were classified correctly into their treatment groups by stepwise discriminant and canonical analyses.

Two-dimensional canonical scattergrams revealed expected cluster formation by the seven finishing diets relative to the concentration of the 20 volatile compounds chosen by discriminant analysis (see Figure 2). Fat samples analyzed were from animals fed on corn, fescue/corn, clover, lucerne, lotus, ryegrass and radish (Figure 2, Samples 1 through 7, respectively). Lamb samples having mild flavor scores (corn, fescue/corn and radish; Figure 2, Samples 1, 2 and 7) were separated from the other forage-fed groups. Samples from animals finished on clover, lucerne and lotus (Figure 2, Samples 3, 4 and 5) were closely assembled as if they were in one group. Fat samples from ryegrass-fed lambs (Figure 2, Sample 6) formed a separate group.

Although the taste panel could not discriminate samples by flavor intensity of lambs finished on different forage rations, multivariate statistical analyses of flavor volatiles from lamb fat were successfully used to classify samples according to their finishing diets.

Volatile organic compounds from lamb fat were also analyzed in Study II, Trials 2, 3 and 4. Volatile compounds from the fat of these animals were similar to those reported for Study I. Fifty compounds were quantitated by the GLC method of Suzuki and Bailey (26). In Trial 2, Vita 3 blackeye samples contained more terpenes than California blackeye or corn grain (Figure 3). In Trial 3 (Figure 4), Vita 3 samples had more neophytadiene, phytadiene and phytol, but alfalfa samples had more phyt-2-ene. In Trial 4 (Figure 5), sudan grass had more terpenes than samples from animals receiving the other treatments. Phyt-2-ene was the most concentrated terpenoid in all samples, particularly so in the samples from sudan grass and alfalfa.

Overall results from these studies indicate that the amount of diterpenoids in fat from grain-fed animals was lower than that from forage-fed animals; and that fat from animals finished on cottonseed and California blackeye had diterpene values similar to those of animals finished on corn. The animals receiving cottonseed also had corn in their rations.

γ -Lactones were found to be significantly higher in concentration in grain-finished animals (Figures 6 and 7), and δ -lactones were more highly concentrated in the forage-finished animals. Five ketones were identified in fat from lamb in these studies.

Although certain trends are apparent from the above data, no single compound analyzed in these samples was highly correlated with grassy or lamby flavor. The highest correlation between volatiles and grassy flavor was for heptanoic acid, which accounted for 13% of the variation in grassy flavor, and for 2-pentadecanone, which accounted for 25% of the variation in lamby flavor.

In order to obtain more quantitative relationships between sensory data and volatile data in Study II, multiple correlation statistics were used. The multiple correlation data relating grassy flavor and volatiles in this study was as follows:

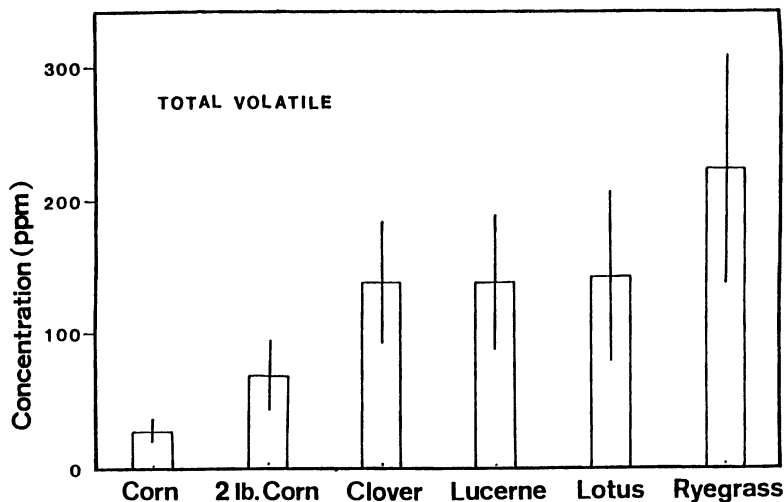


Figure 1. Total volatiles of fat from lambs on different diets (Study I, Trial 2).

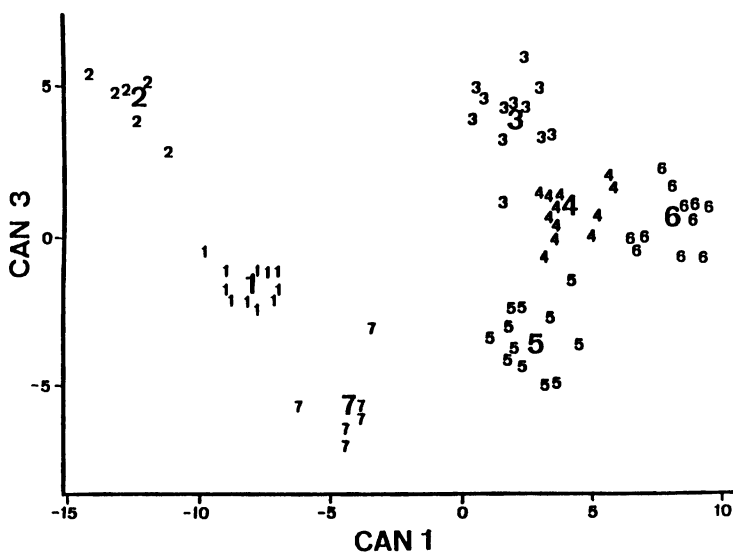


Figure 2. Scattergram (Can 1 versus Can 3) of fat from Study I, Trial 2 lambs based on quantitation of volatile compounds analyzed by GLC-MS.

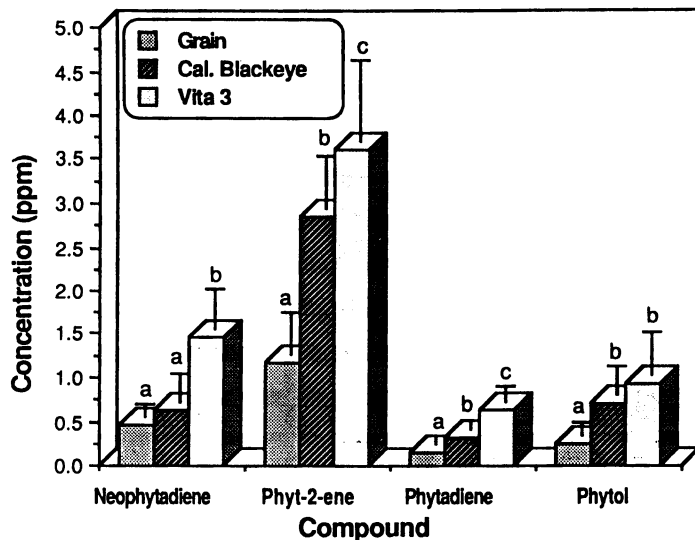


Figure 3. Concentrations of terpenoids in fat from lambs having significant differences among feeding treatments in Study II, Trial 2. Bars with different letters within a group are significantly different at $P < 0.05$. Error bars indicate standard deviation.

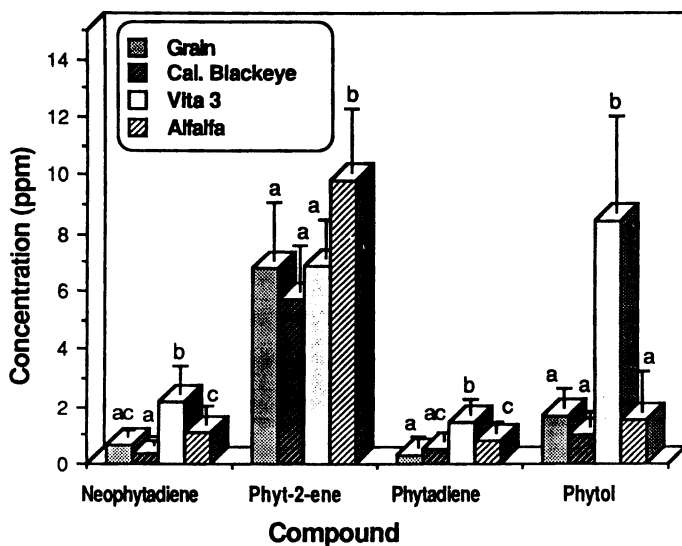


Figure 4. Concentrations of terpenoids in fat from lambs having significant differences among feeding treatments in Study II, Trial 3. Bars with different letters within a group are significantly different at $P < 0.05$. Error bars indicate standard deviations.

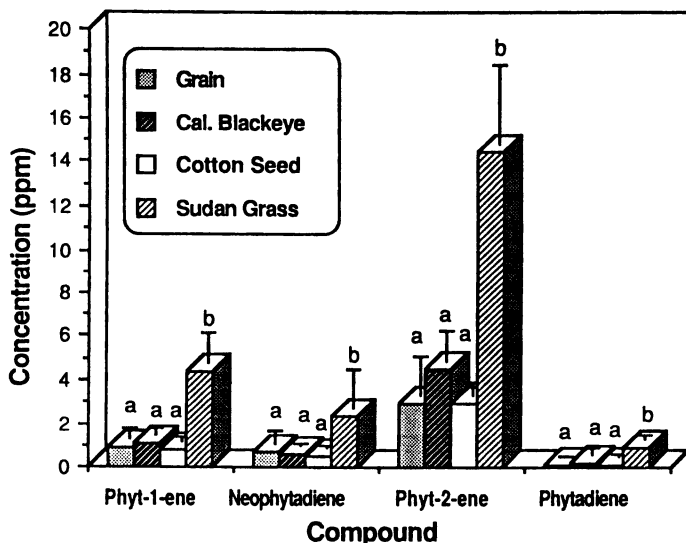


Figure 5. Concentrations of terpenoids in fat from lambs having significant differences among feeding treatments in Study II, Trial 4. Bars with different letters within a group are significantly different at $P < 0.05$. Error bars indicate standard deviations.

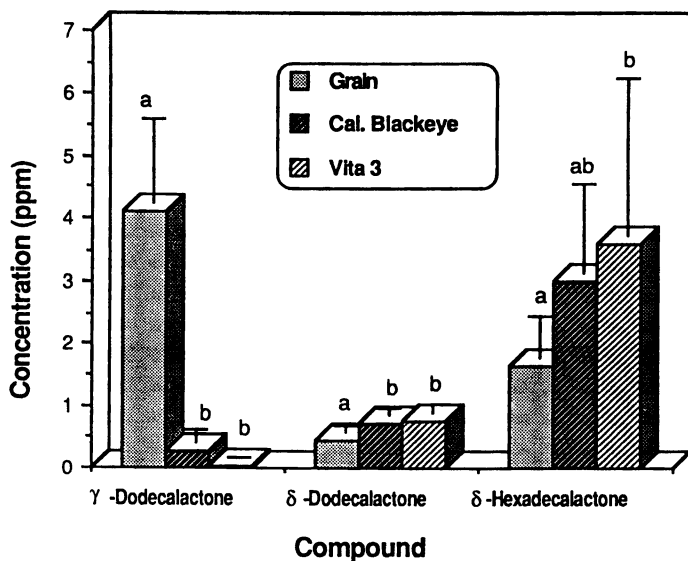


Figure 6. Concentrations of lactones in fat from lambs having significant differences among feeding treatments in Study II, Trial 2. Bars with different letters within a group are significantly different at $P < 0.05$. Error bars indicate standard deviations.

Grassy flavor intensity =

$$11.17 + 21.68X_{13} + 15.90X_{14} - 1.00X_{24} + 14.57X_{28} - 7.07X_{42} + 1.65X_{43} + 15.90X_{44}$$

[$R^2 = 0.551$; X_{13} = heptanoic acid; X_{14} = 2,3-nonanedione; X_{24} = 2-undecenal + decanoic acid; X_{28} = δ -decalactone; X_{42} = neophytadiene; X_{43} = phyt-2-ene; X_{44} = phytadiene]

The equation for lamby flavor was:

Lamby flavor intensity =

$$22.55 + 39.00X_7 + 15.86X_{10} - 6.50X_{13} - 16.03X_{17} - 29.28X_{21} + 26.34X_{31} + 3.41X_{33} + 5.28X_{35} - 3.53X_{38} - 3.78X_{50} + 1.75X_{52}$$

[$R^2 = 0.915$; X_7 = 2-heptenal; X_{10} = octanal; X_{13} = heptanoic acid; X_{17} = 2-nonenal; X_{21} = 2-undecanone; X_{31} = bovolide (2,3-dimethyl-2,4-nonadiene-4-olide); X_{33} = hexadecane; X_{35} = 2-pentadecanone; X_{38} = phyt-1-ene; X_{50} = dihydrophytol; X_{52} = δ -hexadecalactone]

Intermediate molecular weight fatty acids, aldehydes, ketones and diterpenoids were associated with grassy flavor, while aldehydes, ketones and bovolide were the only compounds identified to have a strong positive relationship with lamby flavor.

Stepwise discriminant analysis was applied to volatile data to classify animals into groups based on diet in Trials 3 and 4. Volatile compounds selected in Trial 4 are listed in Table V according to their strongest predictor values. Unknown 3 was present in samples from animals fed cottonseed meal and was very similar in structure and molecular weight to terpenoids. Therefore we have two diterpenoid-like compounds, three ketones, two acids and two lactones strongly associated with fat samples from animals relative to diets.

These data were used to classify samples based on treatment by discriminant analysis and the data were evaluated by canonical analysis. The samples were completely and accurately separated by discriminant and canonical analyses into the appropriate feeding groups.

Figure 8 is a scattergram of Can 1 versus Can 2 relating volatile compounds analyzed by GLC and samples from animals fed the various rations. These results indicate that multivariate statistical analysis of data from flavor volatiles from lamb fat can be successfully used to classify the lambs according to their finishing diets.

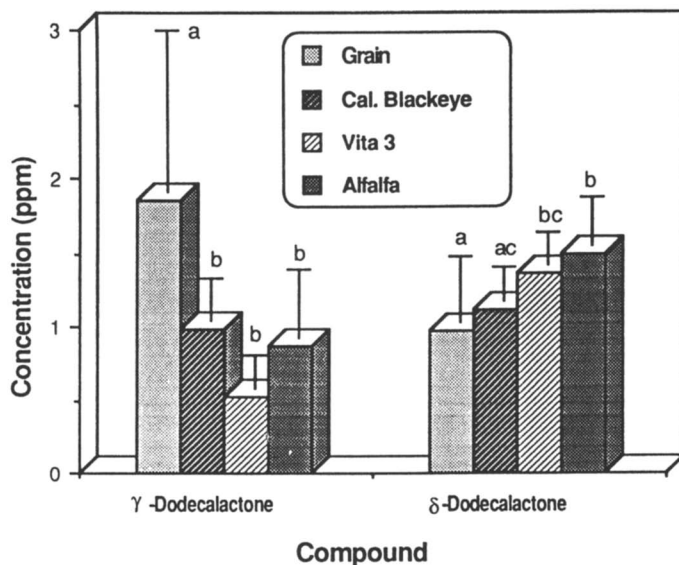


Figure 7. Concentrations of lactones in fat from lambs having significant differences among feeding treatments in Study II, Trial 3. Bars with different letters within a group are significantly different at $P < 0.05$. Error bars indicate standard deviations.

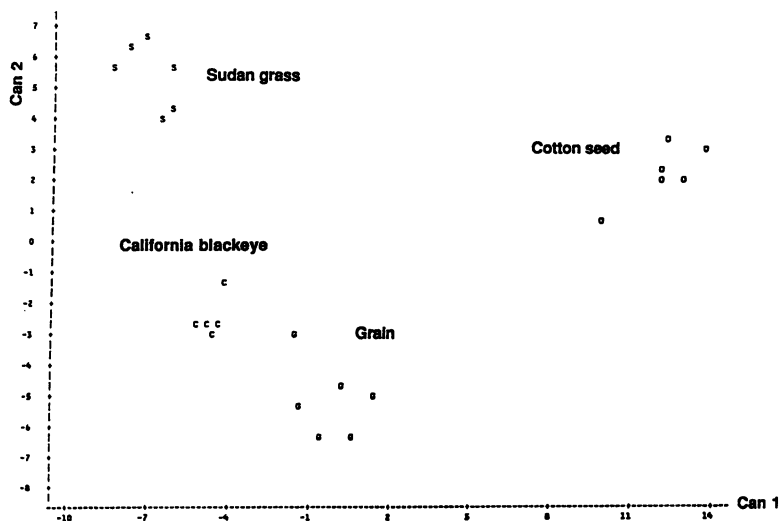


Figure 8. Scattergram (Can 1 versus Can 2) of fat from Study II, Trial 4, lambs based on quantities of volatile compounds analyzed by GLC-MS.

Table V. Volatile Components from Lamb Fat Selected in Stepwise Discriminant Analysis as Treatment Predictor Variables in Study II, Trial 4

Step	Variable		Partial R ²
	Entered	Removed	
1	Unknown 3		0.910
2	Phyt-2-ene		0.845
3	2-Pentadecanone		0.595
4	γ-Dodecalactone		0.542
5	Bovolide (2,3-dimethyl-2,4-nonadiene-4-olide)		0.521
6	Acetic acid		0.553
7	2-Tridecanone		0.645
8	Hexanoic acid		0.398
9	Tetradecanone		0.348

Acknowledgments

Contribution from the Missouri Agricultural Experiment Station. Journal Series Number 11,990

Literature Cited

1. Hornstein, I.; Crowe, P.F. *J. Agric. Food Chem.* **1963**, *11*, 147-149.
2. Hornstein, I.; Crowe, P.F. *J. Gas Chromatogr.* **1964**, *2*, 128-133.
3. Bailey, M.E. In *The Maillard Reaction in Foods And Nutrition*; Waller, G.; Feather, M.S., Eds.; ACS Symposium Series No. 215, American Chemical Society: Washington, DC, 1983; pp 169-184.
4. Field, R.A.; Williams, J.C.; Miller, G.J. *Food Technol.* **1983**, *37*, 258-263.
5. Young, O.A.; Reid, D.H.; Smith, M.E.; Braggins, T.J. In *Meat Flavor*; Shahidi, F., Ed.; Chapman & Hall: New York, NY..
6. Wong, E.; Nixon, L.N.; Johnson, C.B. *J. Agric. Food Chem.* **1975**, *23*, 495-498.
7. Wong, E.; Johnson, C.B.; Nixon, L.N. *New Zealand J. Agric. Res.* **1975**, *18*, 261-266.
8. Garton, G.A.; Hovell, F.D.D.; Duncan, W.R.H. *Br. J. Nutr.* **1972**, *28*, 409-416.
9. Purchas, R.W.; Johnson, C.B.; Birch, E.J.; Winger, R.J.; Haggard, C.J.; Keogh, R.G. Personal communication, **1986**, Palmerston North, NZ.
10. Locker, R.H.; Moore, V.J. *Food Technol. NZ* **1977**, *12*, 27-31.

11. Berry, B.W.; Cross, H.R.; Smith, G.C. *J. Musc. Foods* 1989, 1, 45-57.
12. Macy, R.L.; Naumann, H.D.; Bailey, M.E. *J. Food Sci.* 1964, 29, 142-148.
13. Barbella, N.G.; Hankins, O.G.; Alexander, L.M. *Am. Soc. Anim. Prod., Proc. Ann. Rept.* 1936, 29, 289.
14. Berry, B.W.; Mega, J.A.; Calkins, C.R.; Wells, L.H.; Carpenter, Z.L.; Cross, H.R. *J. Food Sci.* 1980, 45, 1113.
15. Melton, S.L.; Amiri, M.; Davis, G.W.; Backus, W.R. *J. Anim. Sci.* 1982, 55, 77.
16. Melton, S.L.; Amiri, M.; Davis, G.W.; Backus, W.R. *J. Food Sci.* 1982, 47, 699.
17. Melton, S.L.; Davidson, P.M.; Mount, J.R. In *Warmed-Over Flavor of Meat*; St. Angelo, A.J.; Bailey, M.E., Eds.; Academic Press: Orlando, FL, 1987; pp 141-164.
18. Melton, S.L. *J. Anim. Sci.*, 1990, 68, 4421-4435.
19. Cramer, D.A.; Barton, R.A.; Shorland, F.B.; Czochanska, Z. *J. Agric. Sci.* 1967, 69, 367-373.
20. Shorland, F.B.; Czochanska, Z.; Moy, M.; Barton, R.A.; Rae, A.L. *J. Sci. Food Agric.* 1970, 21, 1-4.
21. Czochanska, Z.; Shorland, F.B.; Barton, R.A.; Rae, A.L. *J. Agric. Res.* 1970, 13, 662-663.
22. Park, R.J.; Corbett, J.L.; Furnival, E.P. *J. Agric. Sci. (Camb.)* 1972, 78, 47-52.
23. Nicol, A.M.; Jagusch, K.T. *J. Sci. Food Agric.* 1971, 22, 464-466.
24. Park, R.J.; Ford, A.L.; Minson, D.J.; Baxler, R.I. *J. Agric. Sci. (Camb.)* 1975, 84, 209-213.
25. Ford, A.L.; Park, R.J. In *Developments in Meat Science*; Lawrie, R.A., Ed.; Applied Science: London, 1980; pp 219-249.
26. Suzuki, J.; Bailey, M.E. *J. Agric. Food Chem.* 1985, 33, 343-347.
27. Stone, H.; Sidel, J.L.; Bloomquist, J. *Cereal Foods World* 1980, 642-644.
28. Suzuki, J. Ph.D. Thesis, University of Missouri, Columbia, 1985, 1-261.
29. Snedecor, G.W.; Cochran, W.G. *Statistical Methods*, Eighth edition, Iowa State University Press, Ames IA. 1989.
30. Powers, J.J.; Keith, E.S. *J. Food Sci.* 1968, 33, 207-213.
31. SAS/STAT. User's Guide, Version 6, Fourth edition, Volume 2. SAS Institute, NC. 1989.
32. Larick, D.K.; Hedrick, H.B.; Bailey, M.E.; Williams, J.E.; Hancock, D.L.; Garner, G.B.; Morrow, R.E. *J. Food Sci.* 1987, 52, 245-251.

RECEIVED November 4, 1993

Chapter 13

Contribution of Lipid-Derived Components to the Flavor of Alligator Meat

K. R. Cadwallader¹, H. H. Baek¹, H. Y. Chung¹, and M. W. Moody²

¹Department of Food Science, Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, Baton Rouge, LA 70803

²Louisiana Cooperative Extension Service, P.O. Box 25100, Baton Rouge, LA 70894-5100

Volatile compounds were isolated from tail meat of both wild (ca. 4-, 7-, and 10-ft in length) and farm-raised (ca. 4-ft in length) alligators by vacuum simultaneous distillation-solvent extraction (SDE) and dynamic headspace sampling (DHS). Analysis of SDE and DHS extracts by aroma extract dilution analysis (AEDA) revealed that six lipid-derived compounds were predominant among the odor-active compounds isolated. A seventh unknown compound having a pungent, metallic aroma was also predominant. Hexanal and (Z)-4-heptenal were found to contribute undesirable green, cut-grass and fishy, rancid aromas, respectively, and are thought to be primarily responsible for the off-flavor in alligator meat. Other predominant odor-active compounds included 1-octen-3-one, (E)-2-nonenal, (E)-2-octenal, and (E,Z)-2,6-nonadienal.

Alligator production has become an important industry in Louisiana and Florida. Traditionally, hides were considered the only marketable product, but in recent years, the sale of meat has accounted for over 25% of the total revenue generated. The value of farm-raised alligator meat in Louisiana was over \$2.2 million in 1992. An additional \$1.0 million was generated from the sale of wild alligator meat during the September harvest. Alligator tail meat is high in protein (29.1%), low in fat (2.9%), and low in cholesterol (64.8 mg/100 g) (1). Wild alligator meat is considered inferior to farm-raised due to a fishy or rancid off-flavor (2), which may be particularly strong in meat from older or larger animals

Qualitative and quantitative information on the volatile composition of fat from both wild and farm-raised alligators has been reported previously (3). It was believed that certain volatile lipid-derived compounds, e.g. hexanal, play important roles in the off-flavor associated with alligator fat due to their predominance and low aroma thresholds. However, the relative impact of volatile lipid-derived compounds on alligator meat flavor, especially in comparison with other odor-active compounds present, is unknown.

0097-6156/94/0558-0186\$08.00/0
© 1994 American Chemical Society

Aroma extract dilution analysis (AEDA) has been successfully employed for the evaluation of odorants in cucumbers (4) and dill seed and herb (5) as well as for the determination of relative intensities of volatile flavor compounds during the autoxidation of linoleic acid (6). AEDA involves the analysis of a serially diluted flavor extract by gas chromatography/olfactometry (GC/O) to obtain a flavor dilution factor (FD-factor) for each odor-active substance present in the original extract. An FD-factor for a particular component can be defined as the highest dilution at which it is detected by GC/O. Results from AEDA help provide a better understanding of the role each compound plays in the overall flavor.

The objective of the present study was to determine the importance of volatile lipid-derived components to the flavor of alligator tail meat by identifying predominant odor-active compounds by AEDA. To further validate AEDA results, two different extraction techniques were employed for isolation of volatile compounds.

Materials & Methods

Sample Preparation. Tail meat from wild alligators (ca. 4-, 7-, and 10-ft in length) was obtained from a processor in St. Martinville, LA. Tail meat from farm-raised alligators (ca. 4-ft in length) was obtained from a processor in Scott, LA. Three animals were sampled for each treatment. All meat was obtained immediately after processing. The external fat layer was removed with just lean meat used for this study. Meat was vacuum packaged in FreshPak 500™ plastic bags (Koch Supplies, Inc., Kansas City, MO) and stored at -20°C until analyzed. Preparation of meat prior to extraction of volatile compounds was done as follows: meat was thawed at 4°C, sliced into small pieces (ca. 1 cm³), and cooked in a convection oven at 350°F (176.7°C) for 30 min in a covered baking dish.

Simultaneous Steam Distillation-Solvent Extraction (SDE). SDE was carried out under vacuum (ca. 24-26 in.Hg; b.p. 45-60°C) to minimize artifact formation during extraction. It was necessary to modify the standard SDE apparatus (Cat. No. K-5230101-0000, Kontes, Vineland, NJ) as follows: 1) standard valve at U-joint was replaced with vacuum valve; 2) liquid nitrogen cold trap was installed between the SDE apparatus and vacuum pump to minimize solvent entrainment into pump; 3) vacuum valve was installed between SDE apparatus and vacuum pump to prevent back-flush of vacuum oil or other contaminants during venting of sample flask; and 4) sample flask consisted of a 4-L, 3-neck round bottom flask; one neck for vacuum valve and one for thermometer. Cooked alligator meat (300 g) plus 1 L distilled water and 80 µg of 2,4,6-trimethylpyridine (TMP) was extracted for 2 h with 75 mL of redistilled dichloromethane. Air was evacuated for 30 min prior to heating of sample flask. SDE extracts were kept at -20°C overnight to facilitate water removal. Volume of SDE extract was reduced to ca. 10 mL under a gentle stream of nitrogen, dried over 3 g anhydrous sodium sulfate, and then further reduced to 100 µL prior to analysis.

Dynamic Headspace Sampling (DHS). Cooked alligator meat (300 g) plus 1 mg TMP was placed into a purge and trap vessel (1 L, Cat No. 991780, Wheaton, Millerville, NJ). Vessel was oriented in the DHS mode. DHS conditions were as follows: sample temperature was maintained at 60°C; helium was swept over sample at 30 mL/min; purge time was 2 h; and volatiles were trapped onto a Tenax-TA trap (600 mg). Volatiles were eluted from trap with 10 mL redistilled diethyl ether. Ether extract was dried and concentrated to 100 μ L prior to analysis as described above.

Gas Chromatography/Mass Spectrometry/Olfactometry (GC/MS/O). In order to achieve more timely and accurate correlations between spectral data and olfactory or sniffing port responses we have equipped our GC systems with sniffing ports, allowing simultaneous spectral scanning and sniffing of GC effluents. GC/MS system consisted of an HP 5790A GC equipped with a flame ionization detector (FID) and connected to an HP 5970B mass selective detector (MSD) (Hewlett-Packard Co., Palo Alto, CA). Five μ L of each extract was injected (splitless mode; 155°C injector temperature; 30 sec valve delay) simultaneously into dual (closely matched) fused silica open tubular (FSOT) columns (Supelcowax 10, 60 m length x 0.25 mm i.d. x 0.25 μ m film thickness; Supelco, Inc., Bellefonte, PA). Injector effluent, after passing through a FSOT precolumn (1 m length x 0.25 mm i.d.), was split 1:1 to each column using a glass Y-splitter. Column 1 was connected to the MSD, while the end of the column 2 was split 1:1 to an FID and sniffing port supplied with humidified air. FID and sniffing port transfer line temperatures were 250 and 200°C, respectively. Helium was used as carrier gas at a linear velocity of 25 cm/s. It was necessary to partially restrict the flow between column 1 and MSD (using ca. 1 m length x 0.1 mm i.d. FSOT column) in order to achieve the same linear velocity for both columns. Oven temperature was programmed from 40 to 175°C at a rate of 2°C/min with initial and final hold time of 5 and 30 min, respectively; temperature was then further increased at 6°C/min to 200°C and held for 30 min. MSD conditions were as follows: capillary direct MS interface temperature, 200°C; ion source temperature, 200°C; ionization voltage, 70 eV; mass range, 33-300 a.m.u.; and electron multiplier voltage, 2200 V.

Gas Chromatography/Fourier Transform Infrared Spectrometry/Olfactometry (GC/FTIR/O). GC/FTIR system consisted of an HP 5890 GC/HP 5965B infrared detector (IRD) (Hewlett-Packard Co.). Effluent from the end of GC column was split 1:1 between IRD and sniffing port. GC conditions were the same as above except that a 0.32 mm i.d. Supelcowax 10 column was used. IRD conditions were as follows: light pipe and transfer lines temperature, 200°C; narrow band mercury cadmium telluride (MCT) IR detector; optical resolution, 8 cm^{-1} ; and coadd factor, 2 scans/spectrum.

Compound Identification and Quantification. Compound identifications were based on comparison of GC retention indices (RI)(7), mass and infrared spectra, and odor properties of unknowns with those of authentic standard compounds analyzed under identical experimental conditions. Tentative identifications were based either

on matching mass spectra of unknowns with those in the Wiley/NBS mass spectral database (Hewlett-Packard, Co., 1988) or on matching RI values and odor properties of unknowns with those of authentic standards.

Concentrations of positively identified compounds were determined using MS response factors for each compound relative to the internal standard. Response factors were determined by analyzing standard compounds at three levels .

Aroma Extract Dilution Analysis (AEDA). GC/O system consisted of an HP 5790A GC (Hewlett-Packard Co., Palo Alto, CA) equipped with a flame ionization detector (FID) and a sniffing port. Effluent from the end of the GC column was split 1:1 between FID and sniffing port. Serial dilutions of SDE and DHS extracts were prepared using dichloromethane as diluent. GC conditions were the same as for the GC/IRD system except that oven temperature was programmed from 40°C to 200°C at a rate of 6°/min with initial and final hold times of 5 and 20 min, respectively.

GC/O was performed by three panelists familiar with alligator meat flavor. Panelists were asked to assign aroma properties to each compound detected in the sample dilutions. The highest dilution at which an individual component was detected was defined as its FD-factor. FD-chromatograms presented herein are those of only one panelist, since all responses were essentially the same.

Results & Discussion

Predominant odor-active compounds in alligator tail meat having FD-values higher than 8 for DHS extracts and 128 for SDE extracts are listed in Table I. A combined total of 17 odor-active compounds were detected in DHS extracts. FD-chromatograms of volatiles isolated by DHS were similar for all alligators (Figure 1). Among these, compounds 5, 10, and 18 had the highest FD-factors. Compounds 5 and 10 were identified as hexanal and (Z)-4-heptenal, respectively. Compound 18 was unidentified and possessed a pungent, metallic aroma. Other compounds having relatively high FD-factors included 1-octen-3-one (No.13), (E)-2-octenal (No.21), (E)-2-nonenal (No.28), (E,Z)-2,6-nonadienal (No.29), and an unknown compound (No. 3) having a sour, rotten onion aroma. Identification and description of other detected odor-active compounds are presented in Table I.

All compounds detected in DHS extracts also were detected in SDE extracts with the exception of the internal standard (2,4,6-trimethylpyridine)--detected only in DHS extracts. FD-chromatograms of volatiles isolated by SDE show that 37 odor-active compounds were detected (Figure 2). FD-chromatograms for farm-raised and 10-ft wild alligators had a greater number of odor-active compounds compared with 4- and 7-ft wild alligators. Compounds exhibiting highest FD-factors in SDE extracts included hexanal (No.5), (Z)-4-heptenal (No.10), 1-octen-3-one (No.13), (E)-2-octenal (No. 21), (E)-2-nonenal (No. 28), (E,Z)-2,6-nonadienal (No. 29), and three unknown compounds having pungent, metallic (No.18), nutty, baked potato (No.25) and stale, nutty (No.26) aromas. The main difference between FD-chromatograms of DHS and SDE extracts was the appearance of odor-active compounds with RI values over 1600 in SDE extracts . Most of these compounds

Table I. Odor-active compounds in alligator tail meat

No. ^a	Compound	Methods of Identification	RI ^b	Odor Description ^c
1	Pentanal	IR,MS,RI,odor	976	pungent, green
2	2,3-Butanedione	RI,odor	979	creamy, buttery
3	unknown		1000	sour, rotten onion
4	2,3-Pentanedione	IR,MS,RI,odor	1053	creamy, buttery
5	Hexanal	IR,MS,RI,odor	1078	green, cut-grass
6	unknown		1082	skunky
7	unknown		1095	sour, rotten onion
8	unknown		1160	nutty, chocolate
9	Heptanal	IR,MS,RI,odor	1177	green, sweet
10	(Z)-4-Heptenal	MS,RI,odor	1233	fishy, rancid
11	unknown		1272	musty, mushroom
12	Octanal/Cyclohexanone	IR,MS,RI,odor	1280	sweet, wine-like
13	1-Octen-3-one	MS,RI,odor	1392	metallic, mushroom
14	unknown		1319	nutty
15	unknown		1328	nutty, popcorn
16	unknown		1347	sweet, floral
17	TMP (I.S.) ^d		1357	pungent, catty
18	unknown		1374	pungent, metallic
19	Nonanal	IR,MS,RI,odor	1388	sweet, floral
20	unknown		1400	mushroom
21	(E)-2-Octenal	IR,MS,RI,odor	1423	peanut skin
22	unknown		1430	sweet, floral
23	unknown		1435	nutty, potato chip
24	1-Octen-3-ol	IR,MS,RI,odor	1438	mushroom
25	unknown		1445	nutty, baked potato
26	unknown		1499	stale, nutty
27	Benzaldehyde	IR,MS,RI,odor	1516	sweet, honeysuckle
28	(E)-2-Nonenal	IR,MS,RI,odor	1528	stale, sour
29	(E,Z)-2,6-Nonadienal	IR,MS,RI,odor	1583	cucumber-like
30	unknown		1639	sweet, burnt sugar
31	Dimethylbenzaldehyde	IR,MS,RI,odor	1702	spicy, floral
32	unknown		1713	sweet, melon-like
33	unknown		1745	sweet, fatty
34	2,4-Decadienal	IR,MS	1758	sweet, fatty
35	unknown		1767	nutty, popcorn
36	unknown (dienal?)	IR	1823	sweet, fatty
37	unknown		1914	sweet, burnt sugar
38	unknown (dienal?)	IR	2024	sour, cardboard

^a Numbers correspond to those in figures 1 and 2

^b Retention Index on Supelcowax 10 OTGC column

^c Aroma description as perceived at GC-sniffer port

^d I.S. = internal standard

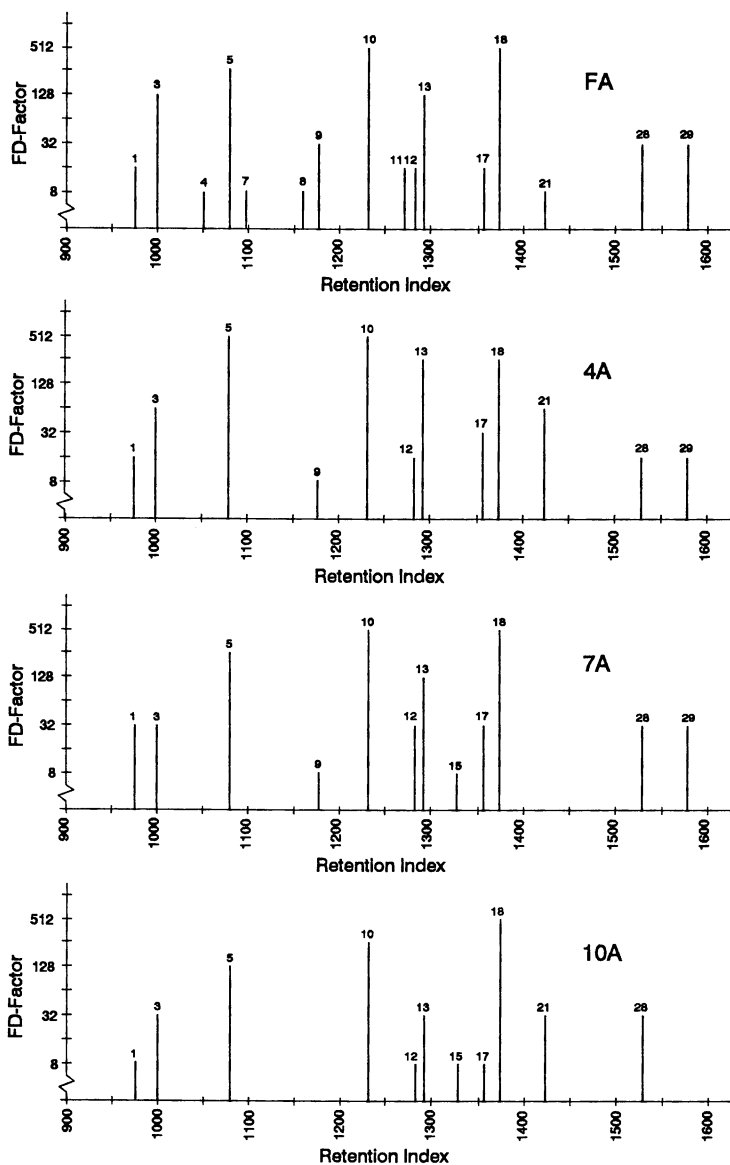


Figure 1. Flavor dilution chromatograms of volatiles isolated from alligator tail meat by DHS. Peak numbers correspond to those in Table I. FA: farm-raised alligator; 4A: 4-ft wild alligator; 7A: 7-ft wild alligator; 10A: 10-ft wild alligator.

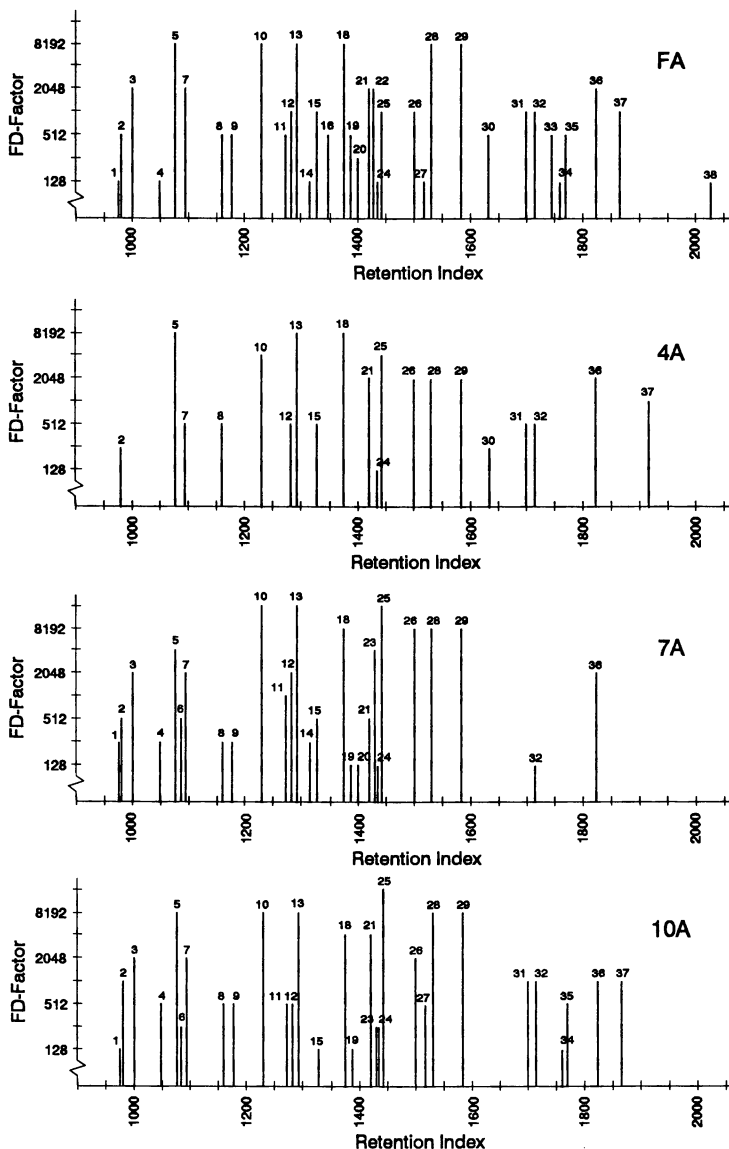


Figure 2. Flavor Dilution Chromatograms of volatiles isolated from alligator tail meat by SDE. Peak numbers correspond to those in Table I. FA: farm-raised alligator; 4A: 4-ft wild alligator; 7A: 7-ft wild alligator; 10A: 10-ft wild alligator.

had sweet, fatty and sweet, tallowy aromas reminiscent of cooked alligator meat. The additive contribution of these compounds to alligator seems important despite their lower FD-factors.

Based on the results of AEDA of both DHS and SDE extracts, it is apparent that hexanal, (Z)-4-heptenal, (E)-2-octenal, (E)-2-nonenal, (E,Z)-2,6-nonadienal, 1-octen-3-one, and one unidentified compound having a pungent, metallic aroma (No. 18), are the predominant odor-active compounds in alligator tail meat flavor. Hexanal had an undesirable green, cut-grass aroma, whereas the aroma of (Z)-4-heptenal was perceived as fishy and rancid. Both compounds, as well as (E,Z)-2,6-nonadienal, (E)-2-octenal, (E)-2-nonenal, and 1-octen-3-one, are lipid-derived compounds (6,8-13). Hexanal has been shown to be an important element in the aroma of fresh whitefish (11), but its contribution to the aroma of alligator meat flavor is thought to be undesirable since consumers prefer alligator to have chicken or pork-like flavor (2). Accumulation of (Z)-4-heptenal in certain foods, e.g., cold stored cod and oxidized soybean oil, has been reported as undesirable (14,15); however, (Z)-4-heptenal imparted a desirable earthy/potato-like aroma in boiled potatoes (16). (Z)-4-Heptenal is believed to negatively impact alligator meat flavor. (E,Z)-2,6-Nonadienal was regarded as having a desirable cucumber-like aroma; however, this compound is readily converted to (Z)-4-heptenal through a water mediated retro-aldol condensation process (8). This conversion may have occurred during cooking of the samples. (E,Z)-2,6-Nonadienal may have been derived from omega-3 polyunsaturated fatty acids (PUFA) (10). Eicosapentaenoic and docosahexaenoic acids comprise about 3.0 and 6.0%, respectively, of the total fatty acids in alligator tail meat (1). Hexanal is readily formed during the oxidation of PUFA. The omega-3 PUFA are most likely the principal source of hexanal due to their higher susceptibility to autoxidation. 1-Octen-3-one and (E)-2-nonenal have been reported to contribute heavy, plant-like and strong, cucumber-like notes, respectively, to the aroma of fresh whitefish (11). (E)-2-Octenal, peanut skin-like aroma, was probably derived from 2,4-decadienal by a water-mediated alpha/beta double bond hydration, retro-aldol condensation (13). The above three compounds (No.13, 21, and 28) are believed to have a positive impact on the aroma of alligator meat. Based on these results it is apparent that the combined aromas of hexanal and (Z)-4-heptenal mask the desirable aromas of 1-octen-3-one, (E)-2-nonenal, and (E)-2-octenal. This is believed to be especially true for (Z)-4-heptenal because of its strong and distinctive fishy, rancid aroma.

The relationship between concentration and odor threshold values obtained from the literature for predominant lipid-derived compounds in alligator tail meat is presented in Table II. All compounds were present in concentrations exceeding their threshold values. Odor units for individual compounds correlated well with their FD-factors with the exception of (Z)-4-heptenal, which had an odor unit range lower than expected. This also was true for the comparison of hexanal versus (Z)-4-heptenal in DHS extracts (Compounds 13, 28, and 29 were below GC/MS detection limits in DHS extracts.). However, the odor of (Z)-4-heptenal was very intense in all extracts. It is possible that the odor threshold reported for this compound is too high, since its flavor (or taste) threshold (0.04 $\mu\text{g/L}$, 13) is much lower.

Through use of AEDA it was possible to identify important character-impact volatile flavor compounds in alligator tail meat. Lipid-derived compounds were found to play important roles in alligator flavor and off-flavor. Results of this study may be helpful in designing production schemes and antioxidant systems leading to the improvement of alligator flavor.

Table II. Concentrations and odor threshold values for predominant lipid derived odor-active volatile compounds in alligator tail meat

No. ^a	Compound	Conc Range ^b ($\mu\text{g}/\text{kg}$)	Odor Threshold ($\mu\text{g}/\text{L}$)	Odor Unit Range ^c
5	Hexanal	1506-3815	5 ^d	301-763
10	(Z)-4-Heptenal	30-67	0.8 ^e	38-84
13	1-Octen-3-one	20-40	0.09 ^f	220-440
21	(E)-2-Octenal	87-211	3 ^d	29-70
28	(E)-2-Nonenal	139-422	1 ^g	139-422
29	(E,Z)-2,6-Nonadienal	35-54	0.1 ^g	350-540

^a Numbers correspond to those in Table I and in figures 1 and 2

^b Concentration range for all alligator groups based on SDE data

^c Odor unit = concentration of compound divided by odor threshold in water

^d Threshold ($\mu\text{L}/\text{L}$) in water (17)

^e Threshold in water (14)

^f Threshold in water (18)

^g Threshold in water (19)

Acknowledgments

Approved for publication by the Louisiana Agricultural Experiment Station as manuscript No. 93-21-7193. Support for this study was provided by the Louisiana Agricultural Experiment Station.

Literature Cited

1. Leak, F.W.; Lamkey, J.W.; Johnson, D.D.; Balaban, M.O. A Further Analysis of Florida Alligator Meat as a Wholesome Food Product; Aquaculture Report Series. Florida Department of Agriculture and Consumer Services: Tallahassee, FL, 1988.
2. Tippy, J. *Alligator farmers hope to tempt the doubting tastebud*. The Daily Reveille, Baton Rouge, LA, Feb. 13, 1990. pp 4,13.
3. Baek, H.H.; Pike, M.M.; Bidner, T.D.; Moody, M.W.; Cadwallader, K.R. *Evaluation of the off-odor associated with wild alligator fat*. Abstract No. 359. Presented at the Annual Meeting of the Institute of Food Technologists. New Orleans, LA. 1992.

4. Schieberle, P.; Ofner, S.; Grosch, W. *J. Food Sci.* **1990**, *55*, 193.
5. Blank, I.; Grosch, W. *J. Food Sci.* **1991**, *56*, 63.
6. Ullrich, F.; Grosch, W. *Z. Lebensm. Unters. Forsch.* **1987**, *184*, 277
7. van den Dool, H.; Kratz, P.D. 1963. *J. Chromatogr.* **1963**, *II*, 463.
8. Josephson, D.B.; Lindsay, R.C. *J. Am. Oil Chem. Soc.* **1987**, *64*, 132.
9. Lamikanra, V.T.; Dupuy, H. *J. Food Sci.* **1990**, *55*, 861.

10. Josephson, D.B.; Lindsay, R.C.; Stuiber, D.A. *J. Agric. Food Chem.* **1984**, *32*, 1347.
11. Josephson, D.B.; Lindsay, R.C.; Stuiber, D.A. *J. Agric. Food Chem.* **1983**, *31*, 326.
12. Buttery, R.G.; Seifer, R.M.; Ling, L.C. *J. Agric. Food Chem.* **1970**, *18*, 538.
13. Josephson, D.B.; Lindsay, R.C. *J. Food Sci.* **1987**, *52*, 1186.
14. McGill, A.S.; Hardy, R.; Burt, J.R.; Gunstone, F.D. *J. Sci. Food Agric.* **1974**, *25*, 1477.
15. Seals, R.G.; Hammond, E.G. *J. Am. Oil Chem. Soc.* **1970**, *47*, 278.
16. Josephson, D.B.; Lindsay, R.C. *J. Food Sci.* **1987**, *52*, 328.
17. Buttery, R.G.; Turnbaugh, J.G.; Ling, L.C. *J. Agric. Food Chem.* **1988**, *36*, 1006.
18. Swoboda, P.A.T.; Peers, K.E.J. *J. Sci. Food Agric.* **1977**, *28*, 1019.
19. Forss, D.A.; Dunstone, E.A.; Ramshaw, E.H.; Stark, W. *J. Food Sci.* **1962**, *27*, 90.

RECEIVED November 4, 1993

Chapter 14

Flavor Chemistry of Dairy Lipids

Review of Free Fatty Acids

I. J. Jeon

Department of Animal Sciences and Industry, Kansas State University,
Manhattan, KS 66506

Free fatty acids in dairy products, particularly in ultrahigh-temperature (UHT) processed milk and Cheddar cheese, are discussed. Although a fairly large amount of total free fatty acids is present in UHT milk, their influence on milk flavor is insignificant. This is because most fatty acids released from milk fat are long-chain fatty acids. Two different types of lipase apparently are involved in the lipolysis of UHT milk. Because patterns of releasing fatty acids are different from one lipase source to another, the source of lipase utilized is extremely important in cheese with reference to balancing flavor characteristics exerted by individual free fatty acids. Ratios and concentrations of these fatty acids could be significant parameters for determining cheese flavor characteristics.

Many excellent book chapters and review articles on the flavor chemistry of dairy lipids have appeared during the last three decades. Most works in the 1960's dealt extensively with lipids and their oxidation products (1-4). A number of excellent works have considered the mechanisms of the formation of chemical compounds from milk lipids, as well as their implications for the flavor of dairy products (2, 5). In addition, general reviews on off-flavors related to dairy lipids also have been published for milk (6, 7) and other dairy products (8). The purpose of this paper is to present some recent findings on free fatty acids in milk and dairy products, particularly in ultrahigh-temperature (UHT) processed milk and Cheddar cheese.

Influence of Free Fatty Acids on the Flavor of Dairy Products

A unique feature of dairy lipids is that milk fat contains fairly large amounts of butyric, caproic, caprylic, capric, and lauric acids. Butyric acid residues alone comprise 8.5% of total fatty acid residues in milk fat triglycerides, and the total of

0097-6156/94/0558-0196\$08.00/0
© 1994 American Chemical Society

these short-chain fatty acids amounts to 21% (9). Milk fat also contains small amounts of β -keto acids and hydroxy acids, which are known to be precursors of methyl ketones and lactones, respectively. It may be worthwhile to note that the distribution of these fatty acids in milk fat triglycerides is not random. For example, at position 3, the distribution of butyric acid is about 97% and that of caproic acid is 84%. These are strikingly high preference, and this difference in distribution is significant in regard to the hydrolysis of milk fat by the enzyme lipase.

Normal milk contains a small amount of free fatty acids, and the significance of their contribution to the normal flavor of milk is unclear because their concentrations are well below their flavor threshold levels. However, it is well known that, in milk and other bland flavored dairy products, they cause lipolyzed flavor when their concentrations reach above usual levels (8). The literature suggests that no single fatty acid is a predominant contributor to the lipolyzed flavor in milk, although it is associated with short-chain fatty acids and, rarely, long-chain (C14 to C18) fatty acids (10, 11). On the other hand, Cheddar cheese and other aged cheese varieties contain a fairly large amount of free fatty acids, and these are considered to be essential flavor components (12, 13, 14). However, excess free fatty acids cause the lipolyzed off-flavor in cheese (15, 16, 17). In addition, concentrations of specific free fatty acids have an important influence on the flavor of aged cheese (13).

Free Fatty Acids and UHT Milk Flavor

Concentrations of total free fatty acids in UHT milk increase during aseptic storage. Research suggested that these concentrations as measured by acid degree value might exceed the lipolyzed flavor threshold levels recognized for raw and pasteurized milk (18, 19). However, no lipolyzed off-flavor was detected in these UHT milk samples. Recently, Choi and Jeon (20) found that the high concentrations of total free fatty acids observed were mostly due to the increase in long-chain fatty acids. The UHT milk samples that they utilized were indirectly processed in a commercial processing plant at 138°C for 10 s and aseptically packaged in 236 ml Brick Pak cartons. The samples were stored at 23 and 35°C and analyzed for free fatty acids by a solid phase extraction and gas chromatographic technique. As illustrated in Figure 1, few changes occurred in the concentrations of short-chain free fatty acids at 23°C storage, although lauric acid (C12) showed a moderate increase (14%) after 12 wk. However, during storage at 35°C, concentrations of all short-chain free fatty acids showed small but steady increases ranging from 9% for caprylic (C8) to 45% for lauric (C12) acid after 12 wk (Figure 2). However, actual concentrations of these fatty acids, except capric acid, in the UHT milk samples were well below the reported flavor threshold concentrations of 25.0, 14.0, 7.0, and 8.0 ppm for butyric (C4), caproic (C6), capric (C10), and lauric (C12) acids, respectively (2). Apparently, levels of individual short-chain free fatty acids at least four times higher than those found in these UHT milk samples are needed to produce moderately lipolyzed off-flavor in pasteurized milk. In contrast, long-chain free fatty acids, except oleic acid (C18:1), showed

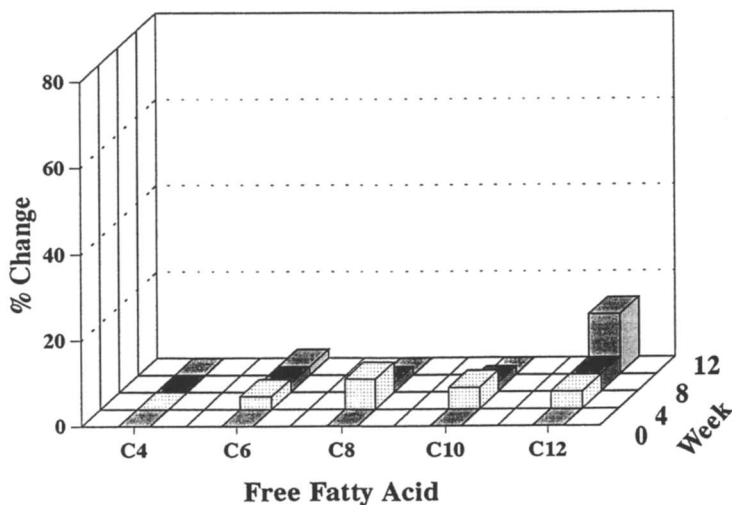


Figure 1. Percent changes in concentrations of individual short-chain free fatty acids in UHT milk during storage at 23°C. The free fatty acids C4, C6, C8, C10, and C12 represent butyric, caproic, caprylic, capric, and lauric acids, respectively.

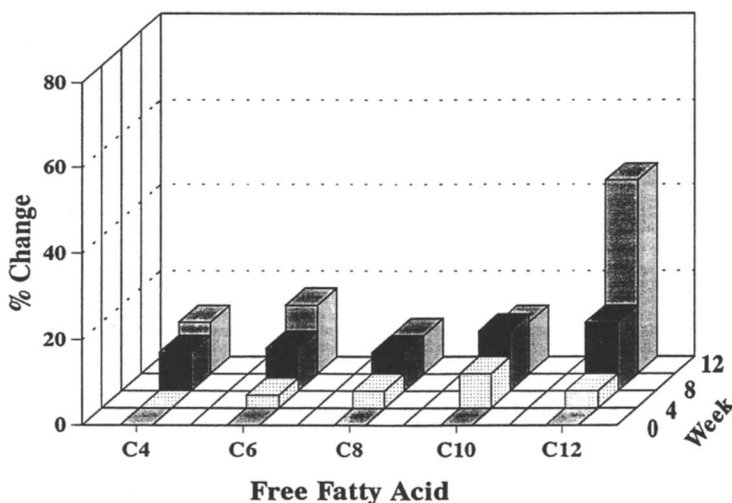


Figure 2. Percent changes in concentrations of individual short-chain free fatty acids in UHT milk during storage at 35°C. The free fatty acids C4, C6, C8, C10, and C12 represent butyric, caproic, caprylic, capric, and lauric acids, respectively.

moderate but significant increases ($P < 0.05$) at 23°C, ranging from 7 to 20% after 12 wk (Figure 3). At 35°C, the increases of these free fatty acids were much more rapid (Figure 4). For example, stearic acid showed the most increase (63%) after 12 wk, followed by myristic (50%), linoleic (40%), palmitic (38%), and oleic acids (26%).

Cause for Increase of Free Fatty Acids in UHT milk. The increase of free fatty acids in UHT milk during storage is believed to be caused by heat-resistant lipase (21, 22, 23). This is either an indigenous milk lipase (milk lipoprotein lipase) or a bacterial lipase produced by psychrotrophic bacteria during cold storage of raw milk (24). Most lipoprotein lipase can be inactivated at 77°C for 16 s (25) or 98°C for 1 s (9). However, bacterial lipases are much more heat-resistant. Several reports have indicated that some bacterial lipases of *Pseudomonas fluorescens* have survived in milk during UHT processing (22, 26, 27). Recently, Choi and Jeon (20) presented some evidence of residual lipase activities in commercial UHT milks, as well as in their centrifugal fractions (12,000 × g for 30 min at 4°C). The cream fraction showed the highest lipase activities according to an agar diffusion method used, followed by the aqueous supernatant and casein precipitates. In addition, the cream fraction preferentially hydrolyzed long-chain fatty acids from milk fat, whereas the aqueous supernatant fraction hydrolyzed both short- and long-chain fatty acids, indicating that more than one kind of lipase might be involved in the lipolysis of UHT milk during storage. Results of DEAE-cellulose chromatography indicated that protein isolates of the aqueous supernatant contained three lipase-active fractions, whereas milk fat globule membrane proteins (from the cream) exhibited only one lipase-active fraction. SDS-PAGE analysis revealed that the lipase-active fractions from the aqueous supernatant contained a major or minor κ -casein component as well as other casein and whey protein moieties. However, the lipase-active fraction of the milk fat globule membrane proteins was composed mainly of α -casein.

Types of Lipase Involved in UHT Milk. Lipases associated with milk are known to be specific or nonspecific with regard to the liberation of fatty acids from milk fat (8). Generally, ratios of individual fatty acids released are used to determine the types of lipase involved in the lipolysis of milk fat (20, 28, 29). Choi and Jeon (20) reported no significant differences in the ratios of free fatty acids between commercial UHT milks stored at 23 and 35°C, suggesting that the same kind of mechanisms was involved in releasing fatty acids from milk fat at the two different storage temperatures. However, the ratios of the long-chain free fatty acids released were significantly higher than those of the fatty acids in milk fat, indicating the involvement of a rather specific lipase. Further examination of the milk samples revealed some evidence for the involvement of two different types of lipase in the lipolysis of UHT milk fat. According to the lipase-active fractions obtained by DEAE-cellulose chromatography, the lipase associated with the aqueous supernatant (centrifugal fraction) produced ratios of fatty acids similar to those in milk fat. However, lipase associated with the milk fat globule membrane proteins yielded ratios of stearic and oleic acids significantly higher than those in

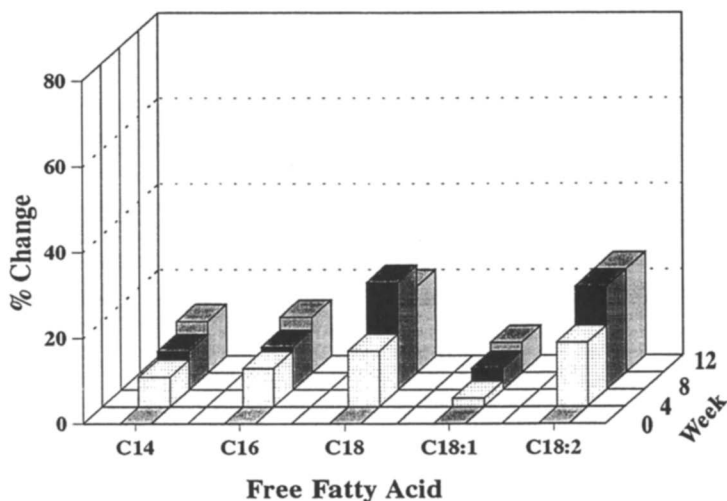


Figure 3. Percent changes in concentrations of individual long-chain free fatty acids in UHT milk during storage at 23°C. The free fatty acids C14, C16, C18:0, C18:1, and C18:2 represent myristic, palmitic, stearic, oleic, and linoleic acids, respectively.

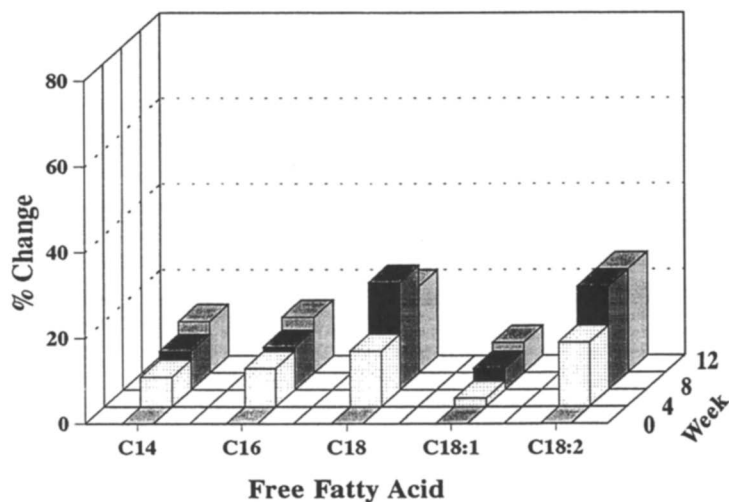


Figure 4. Percent changes in concentrations of individual long-chain free fatty acids in UHT milk during storage at 35°C. The free fatty acids C14, C16, C18:0, C18:1, and C18:2 represent myristic, palmitic, stearic, oleic, and linoleic acids, respectively.

milk fat. Therefore, these results suggested that the lipase activity associated with protein isolates of the aqueous supernatant is of a nonspecific lipase, perhaps milk lipoprotein lipase, whereas that associated with the milk fat globule membrane proteins is of a microbial origin, because it is more preferential toward some long-chain fatty acids.

Free Fatty Acids and Cheddar Cheese Flavor

As indicated previously, short-chain free fatty acids play a significant role in the flavor of Cheddar cheese (12, 13, 30), whereas long-chain fatty acids may contribute little to the direct cheese flavor but influence an overall cheese background flavor (15, 31). Arbige et al. (32) suggested that the total concentration of C4, C6, and C8 free fatty acids is an important factor for flavor development during cheese ripening. Lin and Jeon (33) suggested the total concentration of C4 and C6 free fatty acids as a good indicator of this flavor development. On the other hand, Marsili (34) reported that the best indicator of lipolytic age of Cheddar cheese was the combination of the C10, C12, C14, and C16 free fatty acids.

Concentrations of free fatty acids in Cheddar cheese are quite high, although considerable variations have been observed by different investigators (Table I). For example, in 3- to 4-month aged cheese, butyric acid content ranges from 13 to 76 ppm and caproic acid content from 8 to 29 ppm. Medium and sharp cheeses also show quite a variation in concentrations. This large variability is quite understandable because many factors, such as source of milk, manufacturing practices, and curing conditions, will affect the degree of lipolysis. In addition, the method of analysis used also would affect the quantitative results. For example, Bills and Day (15) used a silicic acid column to isolate free fatty acids from Cheddar cheese. The results of Woo and Lindsay (16) were obtained using a silicic acid-KOH arrestant column. Lin and Jeon (33) and Marsili (34) used neutral alumina for the extraction of free fatty acids (35).

Table I. The free fatty acid content of Cheddar cheese at various ages

Age (Mo)	Free Fatty Acids (ppm)									Reference
	C4	C6	C8	C10	C12	C14	C16	C18:0	C18:1	
3-4	76	29	36	55	87	191	516	104	319	6
4	69	31	37	69	88	211	542	232	1027	11
3	20	12	8	30	46	139	371	116	292	28
3	13	8	<5	16	31	112	274	140	360	29
6	8	8	<5	23	46	152	373	161	404	29
6	15	2	6	25	37	103	285	524	--	43
7-12	127	33	43	53	83	236	510	192	458	6
10	23	18	19	50	48	226	452	160	416	29
12	46	50	11	64	63	265	500	179	486	29
12	111	33	38	67	68	183	397	131	--	43

It is generally believed that young Cheddar cheese contains low levels of free fatty acids, whereas aged, desirably flavored, Cheddar cheese has intermediate concentrations (16). This is usually true, if comparisons are made within the same investigator's data in Table I (particularly ref. 34). However, if comparisons are made among similar age groups (3, 6, 10 months, etc.) across all investigators, no clear trend can be seen for free fatty acids. Nevertheless, results of free fatty acid analysis of a total of 39 commercial Cheddar cheese samples (good, normal flavored) of various ages indicated that free fatty acid contents are generally higher with increased age (Figures 5 and 6). This study utilized 11 mild, seven medium, 15 sharp, and six extra-sharp samples representing several different manufacturers and were collected randomly at local supermarkets. Generally, the concentrations of short-chain fatty acids increased as the age of the cheese increased (Figure 5). This is most evident for butyric and caproic acids, followed by caprylic and capric acids. The increasing trend is somewhat obscure in sharp cheese because of lower concentrations of capric acid. Also note that this trend is less apparent in the long-chain free fatty acids (Figure 6). The mild cheese samples shows the lowest concentrations of the long-chain fatty acids, whereas the extra-sharp samples have the highest concentrations. The sharp cheese samples show consistently lower concentrations of all long-chain free fatty acids than the medium cheese. According to a canonical function derived using canonical discriminant analysis, the concentrations of C6, C16, and C18 free fatty acids are the best parameters for indicating the age of cheese. The relationship between canonical scores and free fatty acids is:

$$\text{Canonical score} = -0.156 (\text{C6}) - 0.023 (\text{C16}) + 0.150 (\text{C18})$$

Table II displays the percent of cheese samples classified into age categories using the canonical function equation. It shows that the canonical equation correctly classified 83.3% of both mild and medium cheeses and 80.0% of extra-sharp cheese (Table II). However, only 54.5% of sharp cheese was correctly classified. This low percent is probably due to lower concentration of free fatty acids in sharp cheese samples than in medium cheese. Nevertheless, free fatty acids possibly could be used to identify the age of cheese (or degree of aging) more accurately if a large pool of commercial samples were analyzed and the canonical functions computed.

Table II. Percentage of Cheddar cheese classified into particular age groups on the basis of computed canonical scores

Labeled Age	Age Categories (percent)			
	Mild	Medium	Sharp	Extra-sharp
Mild	83.3	0.0	16.7	0.0
Medium	16.7	83.3	0.0	0.0
Sharp	18.2	0.0	54.5	27.3
Extra-Sharp	20.0	0.0	0.0	80.0

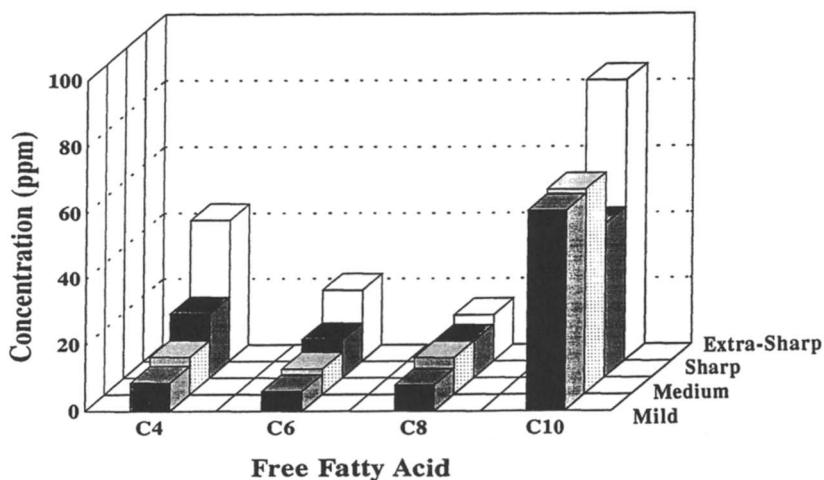


Figure 5. Concentration of short-chain free fatty acids in commercial Cheddar cheese samples by age. The free fatty acids C4, C6, C8, C10, and C12 represent butyric, caproic, caprylic, capric, and lauric acids, respectively.

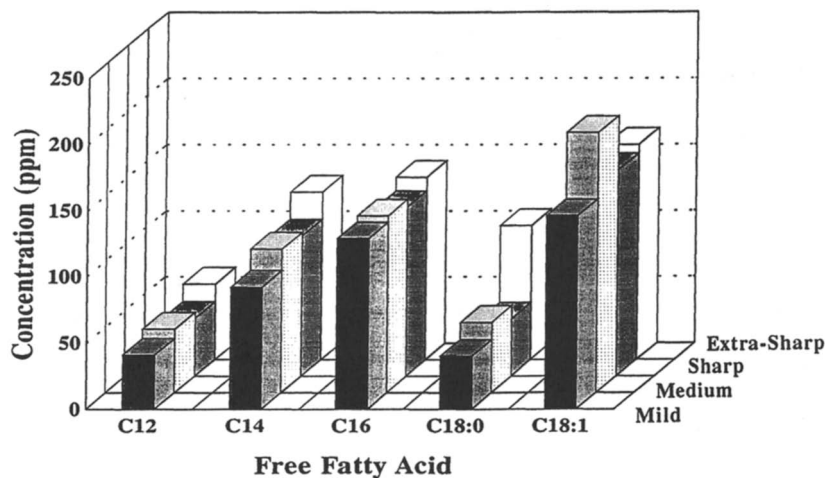


Figure 6. Concentration of long-chain free fatty acids in commercial Cheddar cheese samples by age. The free fatty acids C14, C16, C18, and C18:1 represent myristic, palmitic, stearic, oleic, and linoleic acids, respectively.

Source of Free Fatty Acids in Cheddar Cheese. The free fatty acids in cheese are derived from two major sources: (a) breakdown of milk fat by lipolysis and (b) metabolism of carbohydrates and amino acids by bacteria (36). Nakae and Elliott (37) demonstrated that the free fatty acids from acetic to caproic can be produced from casein hydrolysates, but the bulk of evidence indicates that lipolysis is the principal contributor of free fatty acids with chain lengths of C4 and greater (38, 30). The origins of lipase that causes lipolysis in cheese can include indigenous milk lipase (lipoprotein lipase), contaminant bacteria in the cheese milk, culture bacteria, and contaminant bacteria from the cheese-making process. The source of lipase is extremely important in terms of its specificity toward milk fat triglycerides (8). For example, lipoprotein lipase is nonspecific; it releases fatty acids in nearly the same proportions that are found in milk. On the other hand, microbial lipases may or may not be specific, depending on their sources. Kwak *et al.* (29) demonstrated patterns of the preferential release of short-chain fatty acids from milk fat by various sources of lipase. According to their results, lipases of ruminant-animal origins showed an extremely high ratio of butyric acid but low ratios of caprylic and capric acids. *Pseudomonas fluorescens* and all porcine lipases showed no extremes in ratios of the fatty acids liberated. Lipases from molds and *Chromobacterium viscosum* produced lower ratios of butyric acid than other groups but a higher ratio of capric acid.

Indigenous Milk Lipase. Lipoprotein lipase is relatively unstable and can be mostly inactivated by normal pasteurization (72°C for 16 s) of cheese milk. However, pasteurization does not completely inactivate the enzyme. Shipe and Senyk (25) suggested that heating milks to 76.6°C for 16 s would be needed to prevent lipolyzed flavor problems during storage at 5°C for 7 days. Driessen (39) investigated the heat resistance of milk lipoprotein lipase over the temperature range of 50 to 70°C and reported a D value of 12 s at 72°C. These results suggest that residual lipoprotein lipase can contribute to the lipolysis of milk fat in cheese during ripening.

Lipase from Contaminant Bacteria in Cheese Milk. Many of the microorganisms that contaminate dairy products are lipolytic, *i.e.*, they produce lipase. The most common sources of lipase are psychrotrophic bacteria, predominantly *Pseudomonas* species. The number of these organisms in milk increases during storage and may produce significant amounts of lipase after about 3 days. Many of these bacterial lipases are heat-resistant and may not be inactivated by the pasteurization of milk for cheese making (8). These lipase not only will be active against milk fat triglycerides during cheese ripening, but also may cause serious lipolyzed off-flavor in cheese. This is because microbial lipases may produce unusually high levels of free fatty acids as well as release different ratios of fatty acids that found in milk fat as discussed previously. Law *et al.* (40) reported that strong lipolytic off-flavor was observed in cheese when butyric acid and medium-chain fatty acids were released from milk fat by *Pseudomonas fluorescens* lipase.

Lipase from Culture Organisms. Lactic culture bacteria are capable of weakly hydrolyzing milk fat during cheese making and the subsequent ripening period. Reiter *et al.* (41) reported that a slight increase of butyric acid and longer fatty acids occurred during cheese making in every cheese with starter culture and further small increases occurred during ripening. However, no increase occurred in the content of these acids in cheese made with δ -gluconolactone, which was used as an acid-producing agent. Similar results were observed by Fryer *et al.* (42). In addition, some starter streptococci appeared to be more lipolytic than others.

Lipase from Contaminant Bacteria during Cheese Making. In dairy products, microbial growth is frequently associated with post-manufacture lipolysis (43). Fryer *et al.* (42) speculated that an important part of the cheese microflora is derived from the air or from dairy equipment during cheese making. This is supported by the fact that cheese made under aseptic conditions yielded lower concentrations of free fatty acids (40)

Conclusions

Although concentrations of free fatty acids in UHT milk increase as a function of storage time and temperature, their influence on milk flavor appears to be relatively small. This is because most fatty acids released are long-chain fatty acids, and the concentrations of short-chain free fatty acids are well below their reported threshold values. However, if nonspecific bacterial lipase were present, the concentrations of short-chain free fatty acids would be increased much more than usual and would adversely affect milk flavor. Apparently, two different types of lipase are involved in liberating fatty acids from milk fat triglycerides. One is associated with an aqueous centrifugal fraction, which is nonspecific, perhaps lipoprotein lipase, whereas the other is associated with a cream fraction and is preferential toward long-chain fatty acids, suggesting a microbial origin.

The concentrations of free fatty acids reported by different investigators for Cheddar cheese vary considerably. However, analysis of commercial cheese samples from different manufacturers indicated a general trend of increasing concentrations of free fatty acids as the age of the cheese increases. It is possible that free fatty acid concentrations could be used for identifying the age of cheese if a large pool of samples were analyzed and canonical functions were computed. Because patterns of releasing fatty acids are different from one lipase source to another, the source of lipase is extremely important in cheese for balancing flavor characteristics exerted by individual free fatty acids. Ratios as well as concentrations of free fatty acids could be important parameters for determining cheese flavor characteristics.

Acknowledgments

The author is indebted to his former graduate students, particularly I. W. Choi and G. S. Zink, for part of the data presented in this manuscript. This work has been supported by Kansas Agricultural Experiment Station, Kansas State University, contribution no. 93-450-B.

Literature Cited

- (1) Day, E. A. In *Flavor Chemistry*; Gould, R. F., Ed.; Advances in Chemistry Series 56; American Chemical Society: Washington, DC, 1966, pp 94-120.
- (2) Kinsella, J. E. *Chem. & Ind. (London)* **1969**, January 11, 36-42.
- (3) Kinsella, J. E.; Patton, S.; Dimick, P. S. *J. Amer. Oil Chem. Soc.* **1967**, *44*, 449-454.
- (4) Patton, S. In *Symposium on Foods: Lipids and Their Oxidation*; Schultz, H. D.; Day, E. A.; Sinnhuber, R. O., Ed.; AVI Publishing Co., Inc.: Westport, CT, 1962, pp 190-201.
- (5) Korycka-Dahl, M.; Richardson, T. *J. Dairy Sci.* **1980**, *63*, 1181-1198.
- (6) Bassette, R.; Fung, D. Y. C.; Mantha, V. R. *CRC Critical Reviews in Food Sci. & Nutr.* **1986**, *24*, 1-51.
- (7) Shipe, W. F. In *The Analysis and Control of Less Desirable Flavors in Foods and Beverages*; Charalambous, G., Ed.; Academic Press: New York, NY, 1980, pp 201-239.
- (8) Jeon, I. J. In *Food Taints and Off-Flavours*; Saxby, M. J., Ed.; Blackie Academic & Professional: Glasgow, UK, 1993, pp 122-149.
- (9) Walstra, P.; Jenness, R. *Dairy Chemistry and Physics*; John Wiley & Sons: New York, NY, 1984; pp. 63-68.
- (10) Bills, D. D.; Scanlan, R. A.; Lindsay, R. C.; Sather, L. *J. Dairy Sci.* **1969**, *52*, 1340-1345.
- (11) Scanlan, R. A.; Sather, L. A.; and Day, E. A. *J. Dairy Sci.* **1965**, *48*, 1582-1584.
- (12) Kristofferson, T.; Gould, I. A. *J. Dairy Sci.* **1958**, *41*, 717. (Abstract)
- (13) Ohren, J. A.; Tuckey, S. L. *J. Dairy Sci.* **1965**, *48*, 765. (Abstract)
- (14) Patton, S. *J. Dairy Sci.* **1963**, *46*, 856-858.
- (15) Bills, D. D.; Day, E. A. *J. Dairy Sci.* **1964**, *47*, 733-738.
- (16) Woo, A. H.; Lindsay, R. C. *J. Dairy Sci.* **1982**, *65*, 1102-1109.
- (17) Woo, A. H.; Kollodge, S.; Lindsay, R. C. *J. Dairy Sci.* **1984**, *67*, 874-878.
- (18) Earley, R. R.; Hansen, A. P. *J. Dairy Sci.* **1982**, *65*, 11-16.
- (19) Rerkrai, S.; Jeon, I. J.; Bassette, R. *J. Dairy Sci.* **1987**, *70*, 2046-2054.
- (20) Choi, I. W.; Jeon, I. J. *J. Dairy Sci.* **1993**, *76*, 78-85.
- (21) Adams, D. M.; Brawley, T. *J. Dairy Sci.* **1981**, *64*, 1951-1957.
- (22) Anderson, R. E.; Danielsson, G.; Hedlund, C. B.; Svensson, G. *J. Dairy Sci.* **1981**, *64*, 375-379.
- (23) Driessen, F. M.; Stadhouders, J. *Neth. Milk Dairy J.* **1974**, *28*, 10-22.
- (24) Burton, H. *Ultra-High-Temperature Processing of Milk and Milk Products*. Elsevier Appl. Sci.: London, UK, 1988; pp. 268.
- (25) Shipe, W. P.; Senyk, G. F. *J. Dairy Sci.* **1981**, *64*, 2146-2149.
- (26) Griffiths, M. W.; Phillips, J. D.; Muir, D. D. *J. Appl. Bact.* **1981**, *50*, 289-303.
- (27) Fox, P. F.; Stepaniak, L. *J. Dairy Res.* **1983**, *50*, 77-80.
- (28) Nelson, J. H. *J. Amer. Oil Chem. Soc.* **1972**, *49*, 559-562.

- (29) Kwak, H. S.; Jeon, I. J.; Perng, S. K. *J. Food Sci.* **1989**, *54*, 1559-1564.
- (30) Ohren, J. A.; Tuckey, S. L. *J. Dairy Sci.* **1969**, *52*, 598-607.
- (31) Forss, D. A. *J. Dairy Sci.* **1969**, *52*, 832-840.
- (32) Arbige, M. V.; Freund, P. R.; Silver, S. C.; Zelko, J. T. *Food Technol.* **1986**, *40(4)*, 91-98.
- (33) Lin, J. C. C.; Jeon, I. J. *J. Food Sci.* **1987**, *52*, 78-83 & 87.
- (34) Marsili, R. *J. Dairy Sci.* **1985**, *68*, 3155-3161.
- (35) Deeth, H. C.; Fitz-Gerald, C. H.; Snow, A. J. *N. Z. J. Dairy Sci. Technol.* **1983**, *18*, 13-20.
- (36) Aston, J. W.; Dullely, J. R. *Aust. J. Dairy Tech.* **1982**, *37*, 59-64.
- (37) Nakae, T.; Elliot, J. A. *J. Dairy Sci.* **1965**, *48*, 287-292.
- (38) Foda, E. A.; Hammond, E. G.; Reinbold, G. W.; Hotchkiss, D. K. *J. Dairy Sci.* **1974**, *57*, 1137-1142.
- (39) Driessen, F. M. *Int. Dairy Fed. Bull.* **1989**, *238*, 71-93.
- (40) Law, B. A.; Sharp, M. E.; Chapman, H. R. *J. Dairy Res.* **1976**, *43*, 459-468.
- (41) Reiter, B.; Fryer, T. F.; Pickering, A.; Chapman, H. R.; Lawrence, R. C.; Sharpe, E. M. *J. Dairy Res.* **1967**, *34*, 257-272.
- (42) Fryer, T. F.; Reiter, B.; Lawrence, R. C. *J. Dairy Sci.* **1967**, *50*, 388-389.
- (43) Downey, W. K. *J. Dairy Res.* **1980**, *47*, 237-252.

RECEIVED November 4, 1993

Chapter 15

Flavor Chemistry of Fish Oil

Charles F. Lin

Missouri Development Center, Westreco Inc., Nestle S. A.,
3916 Pettis Road, St. Joseph, MO 65403

Application of n-3 fatty acids as a health food supplement has been recognized for many years. Unfortunately, the odor of fish oil is the major factor limiting its food application. Trimethylamine which characterizes the fishy odor in most marine fish is the product of endogenous and bacteria enzyme catalyses. Products of fish oil oxidation i.e. (*E,E,Z*)-2,4,7-decatrinal, (*E,Z,Z*)-2,4,7-decatrinal and (*Z*)-4-heptenal also impart a fishy odor to oils. Aldehydes have a green or plant-like note; ketones (1-octen-3-one) have a metallic off-flavor. Short-chain unsaturated alcohol (1-penten-3-ol) has a medicinal odor and others have a green unpleasant odor. A deodorization process can remove almost all compounds related to a fishy odor, or off-flavor in fish oils. However, reversion flavor of fish oils during storage can generate pentylfuran and aldehydes that have green and beany odors.

The existence of highly unstable characteristics in nature and lack of antioxidants suitable for fish oil application have encouraged both the food industry and the fish oil industry to adopt new technologies that may provide us a new vision to utilize fish oil in foods. This type of techniques will be discussed.

Since the use of sardine oil as a food ingredient was discontinued in the 1950s, the FDA has determined that fish oils were totally new ingredients for human foods. As a result, 90% of the fish oil produced in the United States was exported to Europe as food oils and 10% was used domestically as nonfood ingredients (1,2).

In June 1977, the United States Menhaden Industry and representatives of the United States National Marine Fisheries Service (NMFS) formed a task force to develop the strategy necessary to obtain approval of menhaden oil as an edible

0097-6156/94/0558-0208\$08.90/0
© 1994 American Chemical Society

product. This petition took twelve years to find the final solution because a comprehensive series of toxicological tests were required for the approval of fish oils as food ingredients. Finally, on September 15, 1989, the FDA affirmed the GRAS status of partially hydrogenated (PHMO) and hydrogenated menhaden oils (HMO) for direct use as human food ingredients.

After solving the legal issue, the remaining question of using menhaden oil as a food ingredient is its highly unstable tendency toward oxidative deterioration. Lipid peroxidation in fish oils is a major degradative process responsible for the deterioration of fish, fish products and fish oils during storage. Due to its high polyunsaturated fatty acid content, fish oils are more susceptible than other fats or oils to the development of rancidity during storage, either in pure form or with food ingredients. This deficit, in turn, limits the direct use of fish oils as food ingredients. At the present time in the U.S. market, lack of antioxidants which has implicated the situation of fish oil application.

This disadvantage, however, may be overshadowed by another discovery that dietary oils contain eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which correlate to the reduction of human cardiovascular risks. Menhaden oil, with a content of 14.16% EPA and 10.26% DHA (Table I), has recently become a much more valuable ingredient than any other product of the fish oil industry. This, in turn, has directly affected economic growth in the United States since menhaden oils have contributed 99% of total fish oil production. In 1986, the United States exported unrefined menhaden oil to Europe at \$0.01-0.015/lb. After European refining, we then imported EPA and DHA capsules at \$170/lb. The United States spent a total of 150 million dollars on EPA and DHA to satisfy the health-conscious consumers market (2). This expenditure has increased about 7% annually, with an estimated \$240 million for 1993.

As a food industry manufacturer, it will always be our job to give consumers what they want. Therefore, new concept must be continually evaluated and new market strategies must be carefully developed. Implementation of the use of EPA and DHA, with a convenient entrees into the market, will increase attractiveness of existing product lines and this, in turn, may ensure successful penetration of new "value added" products. In a highly competitive market, manufacturers should be able to utilize EPA and DHA to differentiate products between a specific brand name and their competitors.

Unfortunately, at the present time, the lack of a breakthrough has caused a technological lag using EPA/DHA as a full commercial operation in the food industry. Techniques such as the isolation of EPA and DHA from fish oils, encapsulation and application methods are needed at this time. In this chapter, technologies relating to industry implementation, as well as mechanism of oxidative deterioration in fish oils, will be discussed.

Fish Oil Production in the United States

According to Stansby (3), whale oil was produced in the United States as early as

Table I. Fatty Acids Composition of Menhaden Oil (% by weight)

Fatty acids	% by weight	Fatty acids	% by weight
C14:0	6.85	C20:0	0.17
C15:0	0.46	C20:1	1.48
C16:0	14.83	C20:2	0.18
C16:1	9.74	C20:3	0.37
C16:2	1.62	C20:4	2.09
C16:3	1.51	C20:5*	14.16
C16:4	1.53	C21:5	0.76
C17:0	0.38	C22:0	0.10
C18:0	2.55	C22:1	0.33
C18:1	9.58	C22:4	0.24
C18:2	1.93	C22:5	2.82
C18:3	1.48	C22:6**	10.26
C18:4	3.09	C24:0	0.60
C19:0	0.00	C24:1	0.22

* Eicosapentaenoic acid (EPH)

** Docosahexaenoic acid (DHA)

Source: Zapata Haynie Co., P.O. Box 175, Reedville, VA 22539, Reproduced with permission.

in 1640; however, it was not until 1811 that the American fish oil industry actually began in Rhode Island.

In 1950s, production of fish oil from Atlantic fisheries reached 130 to 180 million pounds annually, but in the 1960s, less than 0.5 million pounds a year were produced. On the west coast of the United States, Pacific Ocean fishing depended on sardines for fish oil and production reached peak levels of 45,000 Mt/year in the 1930s and 1940s (3). Most of the sardine oil was processed into food oils for margarine and shortening. Since shortening prepared by sardine oil permitted a higher sugar:flour ratio without altering the final texture of the product, this type of shortening was in great demand in the baking industry during that period. Unexpectedly, in the early 1950s, the disappearance of the Pacific sardine ceased the use of fish oil as an edible oil (4). Since then, about 90% of fish oil produced in the United States has exported to Europe as food oils, and 10% has remained in the domestic market as nonfood oils (1,2). This situation continued until September 15, 1989 when the FDA affirmed the GRAS status of partially hydrogenated and hydrogenated menhaden oils which could be directly

used as human food ingredients. This approval represents a milestone for the fish oil industry and reopens the door to using fish oil in the human food industry.

Fish Oil Processing in the United States

Traditionally, in the United States fish industry, there are three steps to obtain fish oil. After arriving at the processing facility, the fish is cooked in a steam jacketed vessel for 1 to 6 hours. The second step is the mechanical pressing of the cooked fish to separate the liquid phase from the solid phase. The liquid phase is further processed under centrifugation to obtain crude fish oil. This oil contains some types of impurities for which physical, chemical and thermal methods are necessary for purification (Table II). Several methods have been reported to remove these impurities (5-7).

After physical purification such as storage and degumming, off-flavor compounds at ppb levels are still found in fish oils. These off-flavor compounds are further removed by alkali refining, clay bleaching and steam deodorization.

Past and Present Food/Medicine Uses of Fish Oils

Past Food/Medicine Uses of Fish Oil. Fish oils have been used as food ingredients in Iceland, Greenland, Norway and Scotland for thousands of years. Due to its therapeutic value, cod liver oil was used as a curative medicine to help alleviate the cause of night blindness as early as 1657. From 1752 to 1784, this oil was effective in treating arthritis in England. In 1890, cod liver oil was used to treat children suffering from rickets in the United States.

Food uses of fish oils in the United States started in 1920s with annual consumption of sardine oil about 50 tons. At the end of 1920s, total consumption of sardine oil was 8,000 tons/year. In 1936, annual consumption jumped to 20,000 tons and in 1940, 50,000 tons. During peak consumption years (1940s), 75,000 tons of fish oil had been processed for use in margarine and shortening in the United States (2).

In 1951, shortly after the disappearance of the California sardine, the FDA issued a regulation that fish oil was not to be included in the list of acceptable ingredients for foods (2).

Present Food Uses of Fish Oils. Most of the world's fish oil production, 1.5 million metric tons, is used in Europe, South America and Japan for the manufacture of salad oils, frying fats, table margarines, low-calorie spreads, baking fats and emulsifiers. In food systems, the β -prime form of fish oils will remain stable and experience no crystal growth during storage. Therefore, margarine and shortening made with PHMO will retain their smoothness and plasticity. PHMOs also have steep melting curves and impart a nice cool feeling on the palate (8).

Table II. Impurities in Crude Fish Oil and Methods Associated to Purification in Fish Oil Industry

Methods Used	Impurities to be Removed
1. Storage	Insoluble impurities
2. Degumming	Phospholipids Sugars Resins Proteinaceous compounds Trace metals
3. Alkali refining	Free fatty acids Pigments Phospholipids Oil insolubles Water solubles Trace metals
4. Washing	Soaps
5. Drying	Water
6. Bleaching	Pigments Oxidation products Trace metals Sulfur-containing compounds Trace soaps
7. Steam deodorization	Free fatty acids Mono- and diglycerides Aldehydes Ketones Chlorinated hydrocarbons Pigment derived products Cholesterol Environmental contamination
8. Fractionation	Higher-melting triglycerides
9. Hydrogenation	Reduce the level of polyunsaturates
10. Interesterification	Rearrangement of the triglycerides
11. Vacuum stripping	Cholesterol Environmental contamination
12. Low-temperature	Cholesterol crystallization Environmental contamination
13. Supercritical fluid	Cholesterol extraction Environmental contamination

Reproduced with permission from Reference 5. Copyright 1990.
Chapman & Hall.

Informative Fish Oil Chemistry

In order to explain the reason that fish oils are much more labile than common vegetable oils, basic oil chemistry such as oxidation rate, induction period and oxygen uptake were determined and reported by several researchers (9-10). The relative oxidation rates of fatty esters at 36.5°C were found to be highly correlated to its molecular unsaturation i.e. oleate (1.0), Linoleate (8.0), Linolenate (21.7) and EPA+DHA (39.1). Relative oxygen uptake (first two days in air) of oleate, linoleate, linolenate, EPA and DHA were < 1, 1, 99, 743 and 948, respectively. Induction periods (90 lux at 5°C) were also related to degree of unsaturation of fatty esters. Oleate was found more than 100 days (estimated) and linoleate, linolenate and EPA+DHA, 50, 20 and 4 days, respectively (11).

The high oxidation rates of EPA and DHA and the instability of their hydroperoxides caused the rapid formation of secondary products such as volatile aldehydes and other compounds which, in turn, impart flavor reversion in fish oils (11).

Mechanism of Lipid Oxidation in Fish Membranes

Membrane Lipid Oxidation. It is generally accepted that lipid peroxidation in meat is initiated at the membrane level, and that the phospholipids in the membranes are the primary centers for the initiation of peroxidation (12). The localization of relatively large amounts of polyunsaturated fatty acids in the membranes of mitochondria, microsomes and lipoproteins make them highly vulnerable to peroxidative changes (13). Prooxidants such as oxygen, peroxidase enzymes (14), heme and nonheme iron (15), hydrogen peroxide, and superoxide radicals (16) are normally present in muscle cells. These endogenous compounds play an important role in the formation of the primary pool of biological catalysts in muscle tissues.

Molenaar et al. (17) proposed that α -tocopherol is located adjacent to membrane-bound enzymes such as reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. In membranes, the arachidonyl residue of the phospholipids forms a complex with the phytol chain of the tocopherol molecule. Thus, the chromanol ring of the α -tocopherol is in contact with the NADPH oxidase.

The unsaturated fatty acids surrounding the oxidases are exposed to free radical attack, but are protected by the redox function of the chromanol ring of the α -tocopherol molecule. Because the range of action of a free radical is about 3 nm, α -tocopherol molecules are only needed directly adjacent to the oxidases that produce free radicals (17).

In the cytosol of a cell, the superoxide anion, being produced by NADPH oxidase, interacts with hydrogen ions to produce hydrogen peroxide, which is then distributed in both the aqueous and membrane phases of the cell (18). In the aqueous phase, glutathione peroxidase and catalase destroy most of the hydrogen

peroxide formed. The remaining hydrogen peroxide may move into the membranes and react with superoxide anion to form the hydroxy radical (OH). Membrane-bound α -tocopherol can trap hydroxy radicals as quickly as they are formed. Otherwise, this extremely reactive radical may initiate peroxidation of membrane lipids (18).

Based on high polyunsaturated fatty acids content in the fish membrane, a proposed mechanism for lipid peroxidation in the fish tissue is initiated in the membrane-bound lipids. This mechanism has been proven in other species such as chicken and swine (19) while in fish, solid data have not been generated yet.

Enzyme Mediation of Fish Oil Deterioration. It is believed that three endogenous enzymes of fish tissue i.e. lipoxygenases (20-21), NADH-dependent microsomal oxidase (22) and myeloperoxidase (23) are able to initiate deterioration of unsaturated fish lipids. Hydroperoxide is the primary product following degradation products.

Lipoxygenases. Of particular interest among the three endogenous enzymes is lipoxygenases which mediate long-chain n-3 fatty acids to produce specific compounds such as 2,4,7-decatrienal isomers, and cause a characteristic fishy odor. This enzyme also mediate polyunsaturated fatty acids to form volatile alcohols and carbonyls in freshly harvested fish (Table III) (21).

End products of lipoxygenases are hexanal, unsaturated C₈ or C₉ alcohols, unsaturated C₉ aldehydes, such as 2-nonenal, 2,6-nonadienal and 3,6-nonadienal. Some of these compounds may pass into oils from rendering and are responsible for the green, melon-like and green-cucumber note in fish oils. Some of unsaturated C₆ and C₈ carbonyls and alcohols impart a plant-like note to fish oils.

Therefore, lipoxygenase-mediated reaction makes the fish oils unacceptable as edible oils. At the same time, this reaction reduces the nutritional benefit of EPA and DHA as a human dietary supplement, along with their market values.

Myeloperoxidase. This enzyme initiates the degradation process of fish tissue to form superoxide anions and hydrogen peroxide which, in turn, randomly attack the olefin bonds of fish lipids (23).

NADH-dependent oxidase. In the microsome membrane, this enzyme requires ADP and iron ions to initiate hydroperoxidation. Machlin (18) reported that the initiation of membrane lipid oxidation included superoxide anion and hydroxyl free radical production. The process of initiation requires other ingredients to participate such as oxygen, hydrogen peroxide, redox glutathione, NADP/NADPH and iron ions. Of course, in the live cell membranes, different redox enzymes such as glutathione peroxidase, xanthine oxidase and superoxide dismutase are presented and also involved in the initiation of membrane lipid oxidation. Products of this type of oxidation are listed in Table IV.

Table III. Carbonyls and Alcohols Produced by Lipoxygenase-mediated Reaction in Freshly Harvested Fish

Compounds	Amount (ppb)
Alcohols	
1-penten-3-ol	3-30
(<i>Z</i>)-3-hexen-1-ol	1-10
1-octen-3-ol	10-1000
1,5-octadien-3-ol	10-100
2-octen-1-ol	1-20
2,5-octadien-1-ol	1-20
6-nonen-1-ol	0-15
3,6-nonadien-1-ol	0-15
Carbonyls	
Hexanal	10-100
(<i>E</i>)-2-hexenal	1-10
1-octen-3-one	0.1-10
1,5-octadien-3-one	0.1-5
2-octenal	0.1-5
(<i>E</i>)-2-nonenal	0-25
(<i>E,Z</i>)-2,6-nonadienal	0-35
6-nonenal	trace
3,6-nonadienal	trace

SOURCE: Adapted from ref. 21. Copyright 1986, American Chemical Society.

Table IV. Carbonyls Identified in Ice Stored Fresh Fish Generated by Enzyme-mediate Oxidative Degradation

Carbonyls	Amount (ppb)
Hexanal	2
(<i>E,Z</i>)-2,4-heptadienal	1-10
(<i>E,E</i>)-2,4-heptadienal	1-10
(<i>E,Z</i>)-3,5-octadien-2-one	0.1-2
(<i>E,E</i>)-3,5-octadien-2-one	0.1-2
(<i>E,Z</i>)-2,4-decadienal	1-5
(<i>E,E</i>)-2,4-decadienal	1-5

SOURCE: Adapted from ref. 21. Copyright 1986, American Chemical Society.

Determination of Fish Freshness by Trimethylamine. As in their digestive process for osmoregulation, most species of marine fish produce trimethylamine oxide (TMO). After harvesting, in fresh or iced fish, bacteria enzymes mediate the reaction of TMO to trimethylamine (TMA). On the other hand, in frozen fish, endogenous enzymes and lipoxygenases catalyze the reduction process of TMO to dimethylamine (DMA) (24-27). TMA has fishy odor at 1:1,500 and 1:8,000 dilution (28) and is related to the acceptance/quality/market value of the fish. Concentration of TMA in fish depends on the time and temperature of storage as does the microbes load.

The concentration of TMA in fish tissue can be determined either through chemical analysis or gas chromatography (GC). Dyer and Monsey (29) demonstrated a colorimetric method in 1945 which was the earliest and most widely used form of chemical analysis. Many variations are available in the literature, but still retain the same concept of chemical reaction and color development. Recently, Hiatt (30), Lundstrom and Racicot (27) and Krzymien and Elias (31) described another method for quick determination of TMA in fish tissue by GC. By adding *n*-propylamine (100 ng) as the internal standard, sampling and analysis are completed in less 5 min (31).

After rendering and steam deodorization (200-208°C, 6% w/w steam, 3 hr), almost all DMA and TMA in fish tissue will be either destroyed or removed.

Mechanism of Lipid Autoxidation in Fish Oils

Singlet Oxygen. Singlet oxygen exerts a nonselective interaction of any olefin bonds in unsaturated systems. This initiation involves photosensitizers that allow spin conversion to occur. Spin inversion increases the energy state of molecular oxygen to form the higher energy singlet state that allows direct olefinic interaction at random processes during initiation (32).

Natural photosensitizers in fish tissue include porphyrin ring structures, such as heme and chlorophyll, which pass into oil through rendering. The menhaden fish is primarily a phytoplankton feeder which can easily contaminate residual chlorophyll in oils from its stomach contents.

Farmer-type autoxidation. Autoxidation of fish oil initiated by Farmer-type hydrogenation-abstraction can generate free radicals and start oxidative degradation of fatty acids. This nonenzymic oxidation accounts for a large portion of the off-flavor after fish oil processing and following storage. Primary products of this reaction are hydroperoxides and, in turn, these compounds lead to forming carbonyl compounds, such as ketones and aldehydes, alcohols and cyclic compounds. The typical secondary products of these hydroperoxides are 2,4,7-decatrienal isomers which introduce a fishy odor into oil at low concentrations (33). Products of this type of autoxidation are listed in Table V.

Table V. Volatile Compounds in the Dynamic Headspace of Gulf Menhaden Oil Samples from Refining and Deodorization

Compounds	Ret. time	Crude Area %	Step I	Step II	Step III
			% of crude	% of crude	% of crude
Alkanes					
Octane	6.5	1.31	23	53	13
Nonane	8.5	0.71	46	63	0
Decane	13.3	1.02	42	75	0
Undecane	22.4	1.33	34	75	0
Dodecane	35.2	2.12	28	72	0
Tridecane	49.1	2.22	85	170	0
Teradecane	62.5	1.55	127	127	t
Pentadecane	76.1	8.34	160	112	2
Hexadecane	88.3	0.65	97	41	2
Heptadecane	101.1	12.71	40	2	0
Octadecane	112.5	0.12	0	0	0
Nonadecane	123.6	0	0	0	0
Alcohols					
2-Propanol	9.8	0.45	21	70	t
2-Pentanol	24.5	0.09	11	29	0
1-Butanol	28.9	0.22	12	28	t
1-Pentanol	41.8	0.13	22	0	0
1-Hexen-3-ol	42.6	0.91	8	37	0
(<i>E</i>)-2-Penten-1-ol	50.8	0.02	0	0	0
1-Hexanol	56.3	0.94	17	119	0
2-Hexen-1-ol	63.5	0.62	10	4	0
1-Octen-3-ol	69.4	0.22	119	40	0
2-Nonanol	80.1	0.13	52	25	0
1-Octanol	83.4	0.04	0	0	0
1,2-Propanediol	86.2	0.29	-	-	*
3,5-Octadien-2-ol	90.6	0.12	61	34	0
2-Undecanol	103.5	0.22	54	0	0
1-Decanol	106.2	0.31	22	0	0
1-Dodecanol	129.6	0	-	-	-
Phenol	135.4	0	-	-	-

Continued on next page.

Table V. Continued

Compounds	Ret. time	Crude Area %	Step I	Step II	Step III
			% of crude	% of crude	% of crude
Aldehydes					
<i>n</i> -Butanal	8.2	0.97	12	47	1
<i>n</i> -Pentanal	12.3	0.82	28	79	t
(<i>Z</i>)-2-Butenal	17.1	0.55	12	63	0
<i>n</i> -Hexanal	21.2	1.56	22	58	2
(<i>Z</i>)-2-Pentenal	26.8	1.48	11	42	0
<i>n</i> -Heptanal	33.6	1.42	15	38	t
(<i>E</i>)-2-Hexenal	37.9	0.97	13	43	0
(<i>E</i>)-4-Heptenal	41.3	0.24	38	160	0
<i>n</i> -Octanal	47.5	0.61	17	58	t
(<i>E</i>)-2-Heptenal	52.1	0.31	20	54	0
<i>n</i> -Nonanal	61.7	0.51	51	74	0
2,4-Hexadienal	63.1	0	-	-	-
(<i>Z</i>)-2-Octenal	63.9	0.13	96	115	0
(<i>E</i>)-2-Octenal	66.3	0.43	5	33	0
2,4-Heptadienal ^a	71.2	0.69	51	29	0
<i>n</i> -Decanal	74.1	0.15	26	38	0
2,4-Heptadienal ^a	74.8	1.39	54	17	0
Benzaldehyde	78.5	0.34	27	21	5
(<i>E</i>)-2-Nonenal	80.5	0.14	74	46	0
(<i>Z</i>)-4-Decenal	81.5	0.14	62	110	0
2,4-Octadienal	83.5	0.11	43	12	0
2,6-Nonadienal ^a	86.6	0.06	133	0	0
2,4-Octadienal	87.1	0.34	24	24	0
(<i>E</i>)-4-Decenal	94.3	0.02	0	0	0
2,4-Nonadienal	99.3	0	-	-	-
2,4-Nonadienal	103.1	0	-	-	-
2,4-Decadienal	107.4	0.04	0	0	0
2,4-Decadienal	112.1	0.12	0	0	0
2,4-Undecadienal ^a	113.8	0	-	-	-
Nonatrienal ^a	121.2	0	-	-	-
Nonatrienal ^a	123.2	0.03	0	0	0
Decatrienal ^a	130.4	0	0	0	0
Decatrienal ^a	133.5	0	0	0	0

Continued on next page

July 15, 2012 | http://pubs.acs.org
 Publication Date: July 7, 1994 | doi: 10.1021/bk-1994-0558.ch015

Table V. Continued

Compounds	Ret. time	Crude Area %	Step I	Step II	Step III
			% of crude	% of crude	% of crude
Ketones					
2-Propanone	5.3	18.11	-	-	-
2-Butanone	6.9	0.64	9	51	0
1-Penten-3-one	15.3	0.95	21	51	0
3-Hexanone	17.6	0.38	11	80	0
2,3-Pentanedione	19.1	0.25	11	37	0
2,3-Hexanedione	27.3	0.05	95	415	0
3-Heptanone	30.8	2.77	18	37	0
3-Nonanone	52.9	2.35	-	-	-
3-Methyl-2,4-pentanedione	61.1	0.23	14	41	0
3-Nonen-2-one	77.9	0.22	62	124	1
Octadien-2-one	84.5	0.08	21	74	0
Acetophenone	95.2	0.21	56	31	0
2-Ethylpentanone	105.6	0.08	0	0	0
Aromatic Hydrocarbons					
Benzene	10.4	0.46	21	34	3
Toluene	16.8	0.28	18	62	4
Ethylbenzene	25.8	0.06	34	191	0
<i>p</i> -Xylene	26.1	0.01	16	288	0
<i>m</i> -Xylene	27.8	0.21	27	40	0
<i>o</i> -Xylene	33.2	0.11	28	174	0
Propylbenzene	36.1	0.15	49	45	0
<i>c</i> 3-Alkylbenzene	38.2	0.18	0	0	0
4-Ethyltoluene	38.9	0.22	118	63	0
1,3,5-Trimethylbenzene	41.1	0.21	0	0	0
2-Ethyltoluene	44.1	0.89	36	79	0
4-isoPropyltoluene	45.6	0.31	33	92	0
1,2,4-Trimethylbenzene	46.1	0.24	10	21	0
3-isoPropyltoluene	49.5	2.36	22	50	0
1,4-Diethylbenzene	50.1	0	-	-	-
<i>c</i> 3-Alkylbenzene	52.7	0.07	72	244	0
1,2-Diethylbenzene	53.3	0.04	125	402	0
1,2,3-Trimethylbenzene	53.9	0.23	22	20	0

Continued on next page

Table V. Continued

Compounds	Ret. time	Crude Area %	Step I	Step II	Step III
			% of crude	% of crude	% of crude
Aromatic Hydrocarbons - continued					
2-Ethyl-1,4-dimethylbenzene	57.9	0	-	-	-
c5-Alkylbenzene	59.1	0.11	0	0	0
c4-Alkylbenzene	60.2	0.11	0	0	0
1,2,4-Trimethylbenzene	66.1	0.01	119	68	0
1,4-Dichlorobenzene	68.1	0.34	66	64	68
c4-Alkylbenzene	73.7	0.09	0	0	0
1,2,3,4-Tetramethylbenzene	75.3	0.04	86	9	0
Miscellaneous					
2-Ethylfuran	10.9	3.95	23	34	t
Chloroform	12.9	0.43	-	-	*
Dimethyldisulfide	20.2	0.23	14	0	0
Limonene	34.5	0.18	0	0	0

^a Configuration of geometric isomers were not determined.

* : Compounds present only in the Step III samples.

% : Area/Area of crude oil x 100

- : Compounds were not detected in the samples.

t : Trace amount present (< 1%)

Step I : Alkali-refined.

Step II : Alkali-refined/clay-bleached.

Step III : Alkali-refined/clay-bleached/steam-deodorized with 0.1% Tenox 20A.

SOURCE: Adapted from ref. 39. Copyright 1990, Institute of Food Technologists.

Purification of Menhaden Oils

Materials. Crude menhaden oils were recovered by a centrifugal separator from the oil phase of a pressed liquor which was obtained after mechanical pressing of steam-cooked menhaden fish. The crude oils were processed in sequence as follows:

<u>Refining Steps</u>	<u>Method used</u>
1. Alkali refining	AOCS Method Ca-9a-52 (34)
2. Clay bleaching	Tonsil Optimum FF(S) activated leaching clay
3. Steam deodorization	200-208°C steam, 6% w/w, 3 hr

Monitor of purification by Dynamic Headspace Sampling (DHS). Volatile components in fish oils were collected with a dynamic headspace sampler consisting of a Tekmar 4200/4000 (Cincinnati, OH) system. Fish oils (0.1g) were purged at ambient temperature with helium gas (99.999%) at a flow rate of 40 ml/min for 2 min to remove any oxygen inside the sample tube. Then, samples were heated and maintained at 65°C for 30 min to allow the volatile compounds to be purged and absorbed into a trap cartridge containing Tenax TA (0.3g, 60/80 mesh, Chrompack Inc., Raritan, NJ). Volatiles trapped in the Tenax TA were then flash-desorbed at 185°C with a helium flow rate of 40 ml/min, and cryogenically (liquid nitrogen) focused in a fused silica capillary column prior to chromatography.

Determination of Volatile Compounds by Gas Chromatography/Mass Spectrometry (GC/MS). Separation and determination of volatile components in fish oils were performed on a fused silica capillary column (Supelcowax 10, 60 m x 0.25 mm i.d. x 0.25 μ m film thickness) installed in a Hewlett-Packard 5792A GC (Palo Alto, CA). Helium gas at a linear velocity of 25 cm/sec was used as the carrier gas. The column temperature was programmed from 40°C, held for 5 min, increased to 175°C at 1°C/min, further increased to 195°C at 5°C/min, and then maintained at 195°C until the end of the GC run.

A mass selective detector (HP 5970B MSD) was used for mass spectrometry. The MS ion source temperature was 200°C; electron ionization energy was 70 eV; and the GC/MSD capillary direct interface temperature was 195°C.

Efficiency of removing volatile compounds from fish oils. A series of alkanals, alkenals, alkadienals and alkatrienals were determined by DHS/GC/MS in crude menhaden oils (Table V). Most of these aldehydes contributed to the characteristic oxidized oily odors, such as green grassy, waxy, and rancid in the crude oils (Table VI). Alkatrienals i.e. nonatrienals and decatrienals, were also found at ppb levels in the dynamic headspace of the crude oils. Deca-2,4,7-trienal

Table VI. Odor Characteristics of Individual Compound from Crude Menhaden Oil

Odor Characteristics		Compound Names
Major note	Minor note	
Green	Cut grass	Hexanal
	Greasy	(<i>E</i>)-2-penenal
	Greasy	(<i>E</i>)-2-heptenal
	Waxy	Heptanal
	Oily	(<i>E</i>)-2-hexenal
	Vegetable	2,4-Heptadienal
	Fruity	Decanal
Pesticidelike		Benzene
		1,3,5-Trimethylbenzene
		1,2,4-Trimethylbenzene
		1,2,3-Trimethylbenzene
Fatty	Floral	Nonanal
	Citrus	Decanal
	Waxy	Nonenal
Fruity	Cherry	Benzaldehyde
	Waxy	(<i>E</i>)-2-octenal
	Citrus	2-Nonanone
Acid	Vinegarlike	Acetic acid
	Astringent	Propanoic acid
Oxidized	Oily	2,4-Decadienal
	Oily	Nonatrienal isomers
	Fishy	Decatrienal isomers
Others	Painty	(<i>E</i>)-2-butenal
	Cooling	3-Heptanone
	Dirty socks	Isobutanoic acid
	Dirty socks	Butanoic acid
	Parmesan cheese	Pentanoic acid
	Sweaty socks	Hexanoic acid
	Medicinal	Phenol
	Metallic	1-Octene-3-one

SOURCE: Adapted from ref. 37. Copyright 1989, American Oil Chemists' Society.

isomers were the key compounds responsible for a strong fishy odor in the crude oil. This was consistent with the findings of Badings (35), Meijboom and Stroink (33) and Hsieh et al. (36-37). Steam-deodorization (Step 3) can effectively remove a total of 99% of most aldehydes in the oils (Table V).

Several alcohols, with a saturated chain from C₃ to C₁₂ and an unsaturated chain such as 1-hexen-3-ol, 2-penten-1-ol, 2-hexen-1-ol, 1-octen-3-ol and 3,5-octadien-2-ol, were determined in the dynamic headspace of the crude oils (Table V). Refining Step 1 and 2 removed 96% of volatile alcohols; Step 3, steam-deodorization, took care of the rest (Figure 1).

A total of eleven ketones with different chain lengths were detected in crude menhaden oils (Table V). Refining steps 1 and 2 had mixed rates of removing ketones, and step 3 removed all ketones from crude oils (Figure 1).

Twenty benzene-containing compounds, suspected of environmental origin, were detected in crude oils (Table V). These series of compounds were strong, odor-contributing components and had to be eliminated from oils by a steam-deodorization process (step 3). Benzene was the only compound in this family determined by DHS/GC/MS in the deodorized oil. Since alkanes (C₈-C₁₈), benzaldehyde, 1,4-dimethylbenzene, 1,4-dichlorobenzene, *o*-xylene, 1-ethyl-2-methylbenzene, and phenol were found in the dynamic headspace of the plastic container material (data not shown), it was determined that the benzene in the oil most probably originated from plastic container.

Volatile acids from menhaden oils also can be determined by DHS/GC/MS. Acetic, acrylic, propionic, crotonic, butyric and valeric acids were found in menhaden oil samples by paper chromatography (38). In this experiment, C₂ to C₆ acids were found in crude menhaden oils (data not shown).

Total ion chromatograms shown in Figure 1 indicates that monitoring odorous volatiles by DHS/GC/MS can be very informative for the fish oil industry and will aid in producing food-grade fish oils with minimum odor (39).

Antioxidant Systems

Antioxidant System in Fish Oils. Tenox 20A (0.1% w/w; E. Kodak, Kingsport, TN) and citric acid (0.1%) were added to the deodorized menhaden oils as antioxidants. Tenox 20A contains 20% t-butyl hydroquinone, 3% citric acid, 32% glycerol monooleate, 15% propylene glycol, and 30% corn oil.

Other systems contained α -tocopherol. Trolox C and α -tocopherol are quite successful in soybean oils but not in fish oils. Both α -tocopherol and Trolox C are capable of donating a hydrogen atom radical from the hydroxyl group of the chroman ring to quench free radicals in oxidizing lipid systems (40). In the initial stage of free radical quenching, α -tocopherol readily donates its hydrogen atom resulting in chroman-free radicals and is an effective competitor for abstraction of hydrogen atoms from highly unsaturated fatty acids in fish oils. Formation of 1,5-octadien-3-ol and 1,5-octadien-3-one in menhaden oil under accelerated conditions was higher in the oil with α -tocopherol than the one without this antioxidant (41).

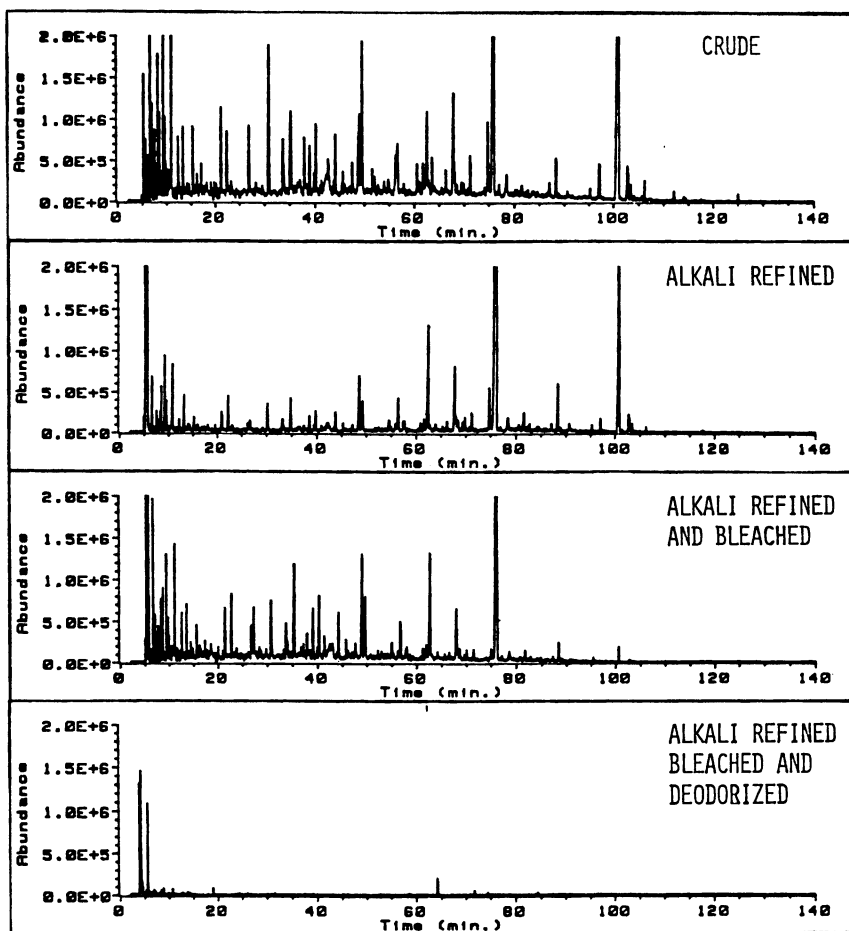


Figure 1. Dynamic headspace gas chromatographic profiles of volatile compounds in (1) crude, (2) alkali refined, (3) alkali refined and bleached, and (4) alkali refined, bleached and deodorized menhaden oils.

These authors reported that the H-donating character of the tocopherol provided the direct precursors of the (*E,Z,Z*)-2,4,7-decatrinal, causing fishy flavors in fish oils. On the other hand, fish oils without α -tocopherol produce (*E,E,Z*)-2,4,7-decatrinal. These results, again, indicated that α -tocopherol may be not the right antioxidant regarding oxidative degradation in fish oils. Therefore, creation of a completely new antioxidant system is necessary in order to apply fish oil to foods.

Antioxidant Systems in Fish. Protection of minced menhaden fish from rancidity during frozen storage was tested by Hwang and Regenstein in 1988 (42). Results of their experiment had shown that ascorbic acid (0.5%) and erythorbic acid (0.5%) had the best antioxidant activity compared to EDTA or citrate. The same experiment also demonstrated that vacuum packing had outpaced Tenox (0.08% TBHQ) while the samples without antioxidant were the least stable, but not as significant as those samples with Tenox (0.02% TBHQ).

In the fish industry, eviscerated Atlantic cod was stored on ice containing 0.2% citric acid and 0.05% potassium sorbate for the purpose of extending shelf life during transportation, up to 10 days beyond harvesting. Due to low pH and mold growth inhibition, this system seemed quite successful for keeping fish fresh during transportation (43).

Stability of Menhaden Oil

Storage of Menhaden Oil. In this experiment, deodorized menhaden oil, without antioxidants, was kept under room temperature (21.1°C) and in a hot box (37.8°C) for 20 days. Ten sensory panelists were recruited from different backgrounds (dietary habits) who had prior experience in sensory evaluation of fishy odors from seafoods. Fish oils were scored on a 5-point intensity scale as follows:

<u>Description of fishy odor</u>	<u>Scores</u>
Very pronounced fishy	5
Pronounced fishy	4
Moderate fishy	3
Mild fishy*	2
Very mild fishy	1
Undetectable	0
* Rejection level	

Without any antioxidants, the induction period of the deodorized menhaden oils was 3 days at 21.1°C and less than 1 day at 37.8°C. At 19 days of storage, menhaden oils at 21.1°C reached the mild-fishy level, causing rejection by our panelists. On the other hand, rejection by the panelists occurred at only 5 days for the samples stored at 37.8°C.

At eight days, menhaden oils at 37.8°C emitted a very strong fishy odor

and the intensity was maintained about four days. Then, the strong fishy odor became weaker and weaker, day after day. At the same time, the oxidized off-flavor in the 37.8°C stored sample became stronger, day after day. Degradation of compounds contributing to fishy odor, such as 2,4,7-decatrienal isomers occurred at 12 days' storage at 37.8°C, and carbonyl compounds formed accordingly.

Since shelf life of the deodorized menhaden oil was not long enough to meet the criteria set by the food industry, creation of a desirable antioxidant system for menhaden oils is necessary.

Storage of Cookies Containing Menhaden Oil. Deodorized menhaden oils, without antioxidants, were incorporated into a molasses cookie recipe as listed in Table VII. This mixture was baked at 190.6°C for 8 min. Cookies were packed in tinted glass bottles and kept at room temperature (21.1°C), and in a hot box (37.8°C) for 20 days.

Results indicate that the cookies stored at 21.1°C had mild-fishy odor and became unacceptable at ten days, while 37.8°C stored samples, at seven days. The combination of food ingredients and an oven-baking process caused menhaden oils to be more vulnerable toward oxidative degradation.

Table VII. Recipe of Molasses Sugar Cookies

Ingredients	Volume measurement
All purpose flour	2 1/4 cup
Cinnamon sugar	1 teaspoon
Egg	1 whole
Ground cloves	1/2 teaspoon
Ground ginger	1 teaspoon
Molasses	1/4 cup
Salt	1/2 teaspoon
Soda	2 teaspoons
Sugar	1 cup
Deodorized menhaden oil ^a	3/4 cup

Add 1 cup sugar, molasses and egg into deodorized menhaden oil; mix well. Combine flour, soda, salt and spices; add to sugar mixture, mixing until blended. Shape dough into 1 inch balls and roll in sugar. Place 2 inches apart on greased cookie sheet. Bake at 190.6°C for 8 minutes.

^a Deodorized menhaden oil with no antioxidant.

Source: Zapata Haynie Co., P.O. Box 175, Reedville, VA 22539, Reproduced with permission.

Another experiment was designed to use 0.5% citric acid and 0.5% ascorbic acid as antioxidants in deodorized menhaden oil and the rest of the parameters remained the same as mentioned above. No fishy odor has been determined by the panelists. Instead, they smelled an oxidized off-flavor from the cookies. Induction period for the cookies stored at 21.1°C was eleven days, six days longer than cookies without antioxidants. The cookies stored at 21.1°C was detected to have a mild-oxidized off-flavor at seventeen days, seven days longer than that of the cookies with menhaden oils containing no antioxidants. Cookies at 37.8°C had an induction period of one day, the same as cookies without citric acid and ascorbic acid. At this temperature, cookies with or without ascorbic acid and citric acid reached the rejection level of off-flavor intensity (mild oxidized off-flavor), and were the same for eight days. Considering the existence of such unstable characteristic in nature, creation of a desirable antioxidant system for menhaden oil is a must.

Industrial Prospective of Fish Oils

Recent increases in the public's awareness of possible health benefits in reducing cardiovascular risks by supplementing dietary intake with n-3 polyunsaturated fatty acids has created a large demand for EPA and DHA capsules. The majority of purified fish oil, with a variety of EPA and DHA, has been imported from Japan and Europe at approximately 150 million dollars at the consumer level in 1987, and has increased 7% annually, with an estimated \$240 million for 1993.

In the United States, the application of EPA and DHA to food items is a totally new concept and has not been tested in the market yet. This is due to the lack of commercially available EPA and DHA in the United States, and the lack of technology to deal with extraction, protection and methods of utilization. The following new technologies may provide us a new vision to utilize fish oil in food systems.

Supercritical Fluids Extraction. According to Spinelli et al. (44), traditional methods such as chemical and thermal processes for purifying fish oils are detrimental to the relatively labile polyunsaturated fatty acids unique to fish oils. Instead, Supercritical Fluid Extraction (SFE) is a superior alternative method because it can effectively remove odor bodies, pigments, and off-flavor compounds from fish oils with little changes in the fatty acid profile.

Under high pressure (1070 psi) and relatively low temperature (31°C), SFE employs supercritical carbon dioxide to elute fatty acids based on their chain length. Therefore, EPA and DHA in a fish oil matrix can be extracted and concentrated by SFE processing and the rest of the oils, with low market value, can be hydrogenated to increase its stability. Thus, the nutritional benefit of using EPA and DHA in special foods can be maximized, and off-flavor caused by low value fish oil in foods can be minimized.

Carbon dioxide is a nontoxic, nonflammable, and environmental acceptable

solvent. Moreover, carbon dioxide has normal boiling points that are well below room temperature. Therefore, the target fatty acids, i.e. EPA and DHA extracted by the SFE method are virtually free of solvent upon complete depressurization. The most practical importance of carbon dioxide is its mild critical temperature at 31°C by which thermally labile EPA and DHA can be fractionated without having further deodorization (205°C, 3 hr).

Krukonis et al. (45) and Krukonis (46) described a prototype design for a continuous countercurrent SFE process of EPA and DHA. Stout et al. (47) published a simplified flow diagram of a plant at production scale of the continuous-countercurrent SFE process. There are two columns with the first one used to fractionate a light extract, C₁₆ and C₁₈. The second was employed to separate products of C₂₀ and C₂₂. In general terms, esters are continuously introduced into the side of the first column where the ester contacts supercritical carbon dioxide. The carbon dioxide stream, being introduced from the bottom of the first column, would flow upward while the ester stream would flow downward due to heavier density, that is, countercurrently.

The supercritical carbon dioxide strips the shorter-chain esters as soon as they contact each other. At the same time, the down-flowing esters are concentrated at the bottom of the first column with longer-chain esters, such as C₂₀ and C₂₂. These components, in turn, are then fed into the side of the second column from where they are countercurrently stripped and separated into C₂₀-rich and C₂₂-rich components. The more soluble C₂₀ esters will be isolated as the overhead product while C₂₂ esters will be retained in the bottom of the second column.

At the present time, technology has not been available to fractionate a single n-3 ester of the same chain length within the same family, i.e. C₂₀ or C₂₂ from the mixture in the SFE system. Fortunately, the C₂₀ or C₂₂ mixture at the same ratio as in fish oil or 90% concentrated products, would be acceptable for human foods enhancement. This may be not too difficult to accomplish by using SFE system at a reasonable cost level.

Encapsulation of EPA and DHA. Both EPA and DHA obtained from the fractionation of SFE methods are very sensitive to oxidative deterioration and must be encapsulated within a functional covering that provides protection, convenience and controlled release at a desired temperature and time.

When EPA and DHA are released at the outlet of the second column of the SFE, they must be kept under vacuum condition and maintained at low temperature. Next, they are mixed and surrounded by a water soluble coating material (wall material) such as corn syrup solid, a hydrocolloid and maltodextrin or whey. Because the two phases are likely to be immiscible, an emulsifier, such as egg or soy lecithin, is added to the suspensive solution. This solution is homogenized and atomized into the heated nitrogen gas flow of a spray-dry chamber. In the hot nitrogen gas, the EPA and DHA take on a spherical shape as core-active-ingredients while the soluble material forms a capsule around the

core portion by mass attraction and hardens as evaporation takes place. Small emulsion droplets and the gentle physical condition of the spray drier are two factors for successful encapsulation (48). The process parameters which have been reported to affect the retention of core material during spray drying were described by Reineccius (49).

Next, encapsulates (EPA and DHA) undergo another step of coating such as Bed Fluidization. This method is useful for manufacturing and protecting EPA or DHA from moisture and releasing at a specific temperature, i.e. microwave heating right before serving. According to this method, the encapsulates are placed in a chamber with an upward nitrogen gas current. As they travel frequently to the spray head at the top of the chamber, they are coated repeatedly. The successive thin layers get thicker as the particles cycle through the chamber several times. At this juncture, the coating or wall materials are either a wax or a fat which protect these active ingredients (EPA or DHA) from severe conditions such as moisture, oxygen and metal ions of foods (48).

In an effort to meet food industry demands, production of EPA and DHA is becoming more complex and high technology is required which includes the discovery of a new wall material which functions as an uninterrupted film.

Aseptic Implementation. Since encapsulated EPA and DHA are not retort temperature stable, aseptic implementation of these encapsulates into frozen entrees has become a real challenge to food engineers. Fortunately, the food industry does not require the encapsulated EPA and DHA to be evenly distributed within the food matrix. Therefore, it is highly possible that encapsulated EPA and DHA can be applied by a spotting method under frozen temperature.

At the present time, there is no technology available in the literature that is ready for the food industry to start a full-time operation. Therefore, the fish oil industry and the food industry must work together to design a cutting-edge research to solve technical problems and to meet market demands.

Biotransaction of EPA/DHA to Meat System.

The consumption of fish or fish oils is believed to have much benefit to human health, increasing the efficiency of the immune system and reducing the risk of atherosclerosis. Unfortunately, there are many people who do not like fish, and the objectionable odor of fish oils. This situation has put the fish oil industry into a dilemma. To solve this problem, food scientists must find a way to fuse EPA and DHA into a highly acceptable food system and obtain approval from the FDA. This plan will utilize the techniques called "biological transaction".

In the animal industry, alteration of lipid composition by dietary means has already been developed, especially for poultry (27). Fatty acids from different sources, i.e. coconut, soybean and linseed were successfully introduced into chicken mitochondria, microsome and lipoprotein membranes by feed regimen (27). Similarly, a proposed dietary supplement of good-quality of EPA and DHA

in the feeds, also can be successfully transferred into chicken membrane lipids. These type of meat would be beneficial to human health, minus any image of fish or fish oils. Thus, the food industry could expect no resistance in launching these products in the near future, nor be under any FDA restraints or regulations.

The last task we need to deal with is to create a protection system at the subcellular membrane level, adjacent to EPA and DHA, in poultry meat. This is because poultry meat tends to be more sensitive toward oxidative deterioration during storage, from a feeding regimen of high polyunsaturated fatty acids (27). Stabilization of membrane lipids can be obtained by incorporating α -tocopherol into subcellular levels, adjacent to EPA and DHA, through dietary supplement. This technique has been successfully accomplished by mixing 100 ppm α -tocopherol acetate in feed 3 weeks before marketing, at a cost 3-5 cents per chicken at a body weight 5 lbs (27).

Benefits should outweigh the costs since the food industry can market this type of product as "Mighty Chicken", at a reasonable price. Overall, this operation appears to be of particular value among consumers, the fish oil industry, the animal industry and the food industry.

Conclusion

In the near future, there are two methods that may efficiently utilize fish oil in food systems: High Key and Low Key. The "High Key" method should employ high technology, such as Supercritical Fluid Extraction to isolate EPA and DHA from fish oils, following encapsulation and aseptic implementation in the "value added" convenient entrees. The rest of the fish oils, with low market value, will be suitable for hydrogenation to increase their stability. The "Low Key" method is the transfer of EPA and DHA into subcellular membranes of meat animals (chicken, turkey or swine) by dietary means. Consumers would have benefit from EPA and DHA by eating this type of meat and, simultaneously, maintain a clear image of ingesting fish oil as a food ingredient.

Literature Cited

1. AOCS, *Symposium on Marine oil*. 1987, New Orleans, LA.
2. Bimbo, A. P. *J. Amer. Oil Chem. Soc.* **1987**, *64*, 706-715.
3. Stansby, M. E. *IAFMM News Summary* **1978**, *42*, 117.
4. Bimbo, A. P. Paper presented at the National Fish Meal and Oil Association Congressional Breakfast, 1983.
5. Bimbo, A. P. In *Fish Oils in Nutrition*; Stansby, M. E., Ed.; Van Nostrand Reinhold Co: New York, 1990, pp. 181-222.
6. FAO, *FAO Food and Nutrition Paper* **1977**, *3*, 94.
7. Young, F. V. K. *Chem. Ind.* **1978**, 692-703.
8. Bimbo, A. P. *J. Amer. Oil Chem. Soc.* **1989**, *66*, 1717-1726.

9. Ikeda, N.; Fukuzumi, K. *J. Japan Oil Chem. Soc.* **1978**, *27*, 21-26.
10. Cho, S.-Y.; Miyashita, K.; Miyazawa, T.; Fujimoto, K.; Kaneda, T. *J. Amer. Oil Chem. Soc.* **1987**, *64*, 876-879.
11. Fujimoto, K. In *Flavor Chemistry of Lipid Foods*; Min, D. B.; Smouse, T. H., Eds.; The American Oil Chemists' Society: Champaign, IL, 1988, pp. 190-195.
12. Gray, J. I.; Pearson, A. M. In *Advances in Meat Research*; Pearson, A. M.; Dutson, T. R., Eds.; Van Nostrand Reinhold Co.: New York, 1987, pp. 221-269.
13. Buege, J. A.; Aust, S. D. In *Methods in Enzymology*; Fleischer, S.; Packer, L., Eds.; Academic Press: New York; 1978, Vol. 52; pp. 302-310.
14. Vladimirov, Y. A.; Olenov, V. I.; Suslova, T. B.; Cheremisina, I. P. *Adv. Lipid Res.* **1980**, *17*, 173-249.
15. Igene, J. O.; Pearson, A. M. *J. Food Sci.* **1979**, *44*, 1285-1290.
16. Kanner, J.; Harel, S. *Arch. Biochem. Biophys.* **1985**, *237*, 314-321.
17. Molenaar, I.; Hulstaert, C. E.; Hardonk, M. J. In *A Comprehensive Treatise*; Machlin, L. J., Ed.; Marcel Dekker, Inc.: New York, 1980, 372-382.
18. Machlin, L. J. In *Handbook of Vitamins: Nutritional, Biochemical, and Clinical Aspects*; Machlin, L. J., Ed.; Marcel Dekker, Inc.: New York, 1984, pp. 99-145.
19. Lin, C. F. Ph. D. Dissertation; Michigan State University, 1988.
20. German, J. B.; Kinsella, J. E. *J. Agric. Food Chem.* **1985**, *33*, 680.
21. Josephson, D. B.; Lindsay, R. C. In *Biogenesis of Aromas*; Parment, T.H.; Croteau, R. Eds.; ACS Symp. Ser. No. 201, American Chemical Society: Washington, D.C., 1986, pp. 201-219.
22. Slabyj, B.M.; Hultin, H. O. *J. Food Sci.* **1984**, *49*, 1392-1393.
23. Kanner, J.; Harel, S.; Hazan, B. *J. Agric. Food Chem.* **1986**, *34*, 506-510.
24. Castell, C. H.; Smith, B.; Neal, W. *J. Fish Res. Board Canada* **1971**, *28*, 1-5.
25. Regenstein, J. M.; Schlosser, M. A.; Samson, A.; Fey, M. In *Chemistry and Biochemistry of Marine Food Products*; Martin, R. E.; Flick, G. J.; Hebard, C. E.; Ward, D. R., Eds.; AVI Publishing Co.: Westport, CT, 1982, pp. 137-148.
26. Hebard, C. E.; Flick, G. F.; Martin, R. E. In *Chemistry and Biochemistry of Marine Food Products*; Martin, R. E.; Flick, G. F.; Hebard, C. E.; Ward, D. R., Eds.; AVI Publishing Co.: Westport, CT, 1982, pp. 149-304.
27. Lundstrom, R. C.; Racicot, L. D. *J. Assoc. Off. Anal. Chem.* **1983**, *66*, 1158-1163.
28. Stansby, M. E. In *Fish Oils in Nutrition*; Stansby, M.E., Ed.; Van Nostrand Reinhold Co.: New York, 1993, pp. 120-140.
29. Dyer, W. J.; Monsey, Y. A. *J. Fish Research Board Canada* **1945**, *6*, 359.
30. Hiatt, M. H. *Anal. Chem.* **1983**, *55*, 506-516.
31. Krzymien, M. E.; Elias, L. *J. Food Sci.* **1990**, *55*, 1228-1235.

32. Nawar, W. W. In *Food Chemistry*; Fennema, O. R., Ed. Marcel Dekker, Inc.: New York, 1985, 139-244.
33. Meijboom, P. W.; Stroink, J. B. A. *J. Amer. Oil Chem. Soc.* **1972**, *49*, 555-558.
34. AOCS, *Official Methods*. American Oil Chemists' Society: Champaign, IL, 1980.
35. Badings, H. T. *Netherland Milk Dairy J.* **1970**, *24*, 216-222.
36. Hsieh, T.C.-Y.; Lin, C. F.; Crowther, J. B.; Bimbo, A. P. *Tropical and Subtropical Fisheries Technological Society of the Americas* **1988**, 154-161.
37. Hsieh, T. C.-Y.; Williams, S. S.; Vejaphan, W.; Meyers, S. P. *Amer. Oil Chem. Soc.* **1989**, *66*, 114-117.
38. Chipault, J. R.; McMeans, E. J. *J. Agric. Food Chem.* **1965**, *13*, 15-17.
39. Lin, C. F.; Hsieh, T. C.-Y.; Crowther, J. B.; Bimbo, A. P. *J. Food Sci.* **1990**, *55*, 1669-1672.
40. Terao, J.; Matsushita, S. *Lipids* **1986**, *21*, 255-260.
41. Karahadian, C.; Lindsay, R. C. In *Flavor Chemistry, Trend and Developments*; Tranishi, R.; Buttery, R. G.; Shahidi, F., Eds.; ACS Symp. Ser. No. 388; American Chemical Society: Washington, DC., 1989, pp. 60-75.
42. Hwang, K. T.; Regenstein, J. M. *J. Food Sci.* **1988**, *54*, 1120-1124.
43. Eilters, J. *Food Processing* **1992**, Dec., 53.
44. Spinelli, J.; Stout, V. F.; Nilsson, W. B. U. S. Patent No. 4,692,280, 1987.
45. Krukoni, V. J.; Bambara, C. J.; Jivian, J. E.; Nilsson, W. B.; Martin, R. M. 194th ACS Meeting, New Orleans, LA.
46. Kurkonis, V. J. In *Supercritical Fluid Extraction and Chromatography*; ACS Symp. Ser. 366; 1988, pp. 26-43.
47. Stout, V. F.; Nilsson, W. B.; Krzynowek, J.; Schlenk, H. In *Fish Oils in Nutrition*; Stansby, M. E., Ed.; Van Nostrand Reinhold Co.: New York, 1990, pp. 73-119.
48. Hegenbart, S. *Food Product Design* **1993**, April, 29.
49. Reineccius, G. A. In *Flavor Encapsulation*; Risch, S. J.; Reineccius, G. A., Eds; ACS Symp. Ser. No. 370; American Chemical Society: Washington, D.C., 1988, pp. 55-66.

RECEIVED February 23, 1994

Chapter 16

Omega-3 Fatty Acid Composition and Stability of Seal Lipids

Fereidoon Shahidi, J. Synowiecki, R. Amarowicz,
and Udaya Wanasundara

Department of Biochemistry, Memorial University of Newfoundland,
St. John's, Newfoundland A1B 3X9, Canada

Blubber and intramuscular lipids of seal meat contain a large proportion of highly unsaturated fatty acids (HUFA) of omega-3 type. The content of eicosapentaenoic acid (EPA, 20:5), docosapentaenoic acid (DPA, 22:5) and docosahexaenoic acid (DHA, 22:6) in mechanically separated seal meat (MSSM) was 6.9, 5.6 and 10.1%, respectively. Corresponding values for seal blubber prepared by a low-temperature rendering process were approximately 6.5, 4.3 and 8.8%, respectively. While the content of polar lipids in seal blubber was 1.1%, its content in MSSM was 21.2%. The ratio of omega-3 to omega-6 fatty acids was approximately 9.8 for blubber and 7.4 for meat lipids. Prolonged heat processing of seal blubber brought about degradation and/or isomerization of the sensitive HUFA. However, seal blubber oil was more stable to oxidative changes than fish oil as evidenced by weight gain data, conjugated dien and peroxide values. Preservation of omega-3 fatty acids by either a low-temperature processing or controlled rendering may be required to prevent their quality deterioration and formation of undesirable flavors.

Seafood products play an important role in nutrition and health status of humans. Polyunsaturated fatty acids (PUFA) have long been recognized as desirable dietary components. The interest in long-chain omega-3 fatty acids namely eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are found abundantly in seafoods, began nearly 3 decades ago (1). These fatty acids are considered essential because they cannot be synthesized by humans and must be ingested in the diet (2). Greenland Eskimos consumed a considerable amount

of seal meat and blubber and long-chain PUFA from these sources. The latter compounds were found to be responsible for health-pro biochemical and physiological changes in the body. The beneficial effects of PUFA have been ascribed to their ability to lower serum triacylglycerols and cholesterol. While DHA is essential for proper functioning of eye and may have a structural role in the brain, EPA serves as a precursor to eicosanoid compounds. The eicosanoids are hormone-like substances including prostaglandins, thromboxanes and leukotrienes, which act on cellular messengers and metabolic regulators and are produced by different cell types in response to physiological and non-physiological stimuli. Eicosanoids are involved in such diverse physiopathologic and physiologic processes as thrombosis, arteriosclerosis, inflammation, immune response, among others. EPA has also been recognized as having therapeutic benefits in human cardiovascular diseases (3). Docosapentaenoic acid (DPA) is an intermediary species between EPA and DHA (4) which is found in high proportions in seal blubber and intramuscular lipids and is also present in human milk (5). However, little information is available on its epidemiological properties. Nonetheless, long chain omega-3 fatty acids have been shown to possess beneficial effects in the prevention, or possible treatment, of coronary heart diseases, diabetes, high blood pressure, and autoimmune diseases (6). Beneficial physiological effects have also been observed in the inflammatory area, related to treatment of asthma, arthritis, migraine, psoriasis and nephritis. Potential applications have also been proposed in treatment of cancer of breast, prostate and colon (7).

Long-chain omega-3 fatty acids of seal blubber are present mainly in 1- and 3-positions of the triacylglycerol molecules. The omega-3 fatty acids of fish oils are known to be randomly distributed and are more abundant in the 2-position of the triacylglycerols. Therefore, seal blubber lipids may be assimilated more effectively than fish oils.

Based on the current trends in hunting of harp seal (*Phoca groenlandica*), approximately 1.2 million metric tons of blubber in annually available for processing (8). However, the potential production of blubber oil, based on the current 186,000 allowable catch is approximately 4 million metric tons. Hence, it is prudent to fully utilize this readily available source of omega-3 PUFA.

Oxidative quality of marine oils is known to have significant effects on their storage stability and nutritional value. Because of the presence of HUFA, these oils easily undergo autoxidation during processing and storage, thus producing a wide array of undesirable compounds such as hydroperoxides and their breakdown products namely hydrocarbons, ketones, aldehydes, alcohols, epoxides, etc. The relative rate of autoxidation of oleate, linoleate and linolenate is reported to be in the order of 1:40-50:100 on the basis of oxygen uptake and in the order of 1:12:25 on the basis of peroxide formation (9). Polyunsaturated fatty acids, such as arachidonic acid, EPA and DHA, containing 4, 5, and 6 double bonds, respectively, are much less stable than linoleic and linolenic acids. Arachidonic acid was reported to be oxidized 2.9 times faster than linoleic acid (10). Ethyl esters of EPA and DHA were oxidized rapidly even at 5°C in the dark after an induction period of 3-4 days, whereas the induction period of linoleate and linolenate were 20 and 60 days, respectively. Similarly, oxygen

uptake of EPA and DHA esters after induction period was 5.2 and 8.5 times faster than that of ethyl linolenate (11). Therefore, inhibition of oxidation is a major criterion when marine oils are incorporated into food products. The toxicity studies of oxidized oil has been of considerable interest for the last few decades. Oxidation of oil results in the development of off-flavors which can be evaluated by sensory tests. However, the routine evaluation of flavor by taste panels is tedious, sometimes subjective, expensive and difficult to compare results from different laboratories (12). Therefore, different chemical methods have been suggested for assessing oxidative quality of oils either by measuring their primary or secondary oxidation products (12,13). However, there is no information available on the oxidative stability of seal oil with respect to other marine oils.

The present study was undertaken to examine the fatty acid composition of seal blubber and intramuscular lipids. Since lipids are the major precursors of aroma in muscle foods, the composition of lipids in meat and subcutaneous fat play an important role in their oxidative deterioration and off-flavor development. Hence, oxidative stability of blubber lipids was also studied.

Materials and Methods

Extraction of Seal Blubber and Intramuscular Lipid. Seal oil was prepared from raw blubber of harp seal (*Phoca groenlandica*). In the laboratory, protein residues were separated and after repeated washing, the resultant oil was treated with a bicarbonate solution and washed again with water. The final product was dried over anhydrous sodium sulfate. In the industrial scale, the blubber was rendered using a steam injection process with subsequent removal of residues from the bottom of the tanks and removal of oil by phase separation. The rendering process took approximately 2.5 h at a temperature of 100°C. Intramuscular lipids of seal meat were extracted into a chloroform-methanol-water mixture as described by Bligh and Dyer (14). Isolated lipids were quantified (15) and used for experimentation.

Seal blubber and intramuscular lipids of meat, extracted according to Bligh and Dyer (14), were subjected to transmethylation in acidified methanol and methyl esters so prepared were subsequently quantified as described elsewhere (16). Separation of lipid classes was done according to Christie (16).

Sample Preparation. For weight gain studies, two grams of oil samples (in triplicates) were placed in a petri dish, traces of water were removed in a vacuum oven at 35°C, reweighed and stored in a forced air oven at 65°C. The weight gain of sample was recorded at 24 h intervals. Twenty five millilitres of oil were stored separately under the same conditions in small open glass containers for other chemical analyses.

Official methods (17) were used for determination of peroxide value (PV), 2-thiobarbituric acid reactive substances (TBARS) and iodine value (IV). The conjugated dienes (CD) and conjugated trienes (CT) were determined using

IUPAC (18) methods of analyses. The cholesterol content of samples was determined as described previously (15).

Results and Discussion

The contents of neutral and polar lipids as well as cholesterol in blubber and intramuscular fat of harp seal (*Phoca groenlandica*) are summarized in Table I. Approximately 98.9% of blubber lipids were composed of triacylglycerols. However, presence of 21.2% polar components in seal intramuscular lipids indicated the very lean nature of the meat; polar lipids are derived mainly from structural components of cells. This corresponds well with low lipid content of seal meat which averages between 1.8 and 2.7%. The total lipid content of raw blubber was $95.5 \pm 0.7\%$, of which $84.2 \pm 3.1\%$ were recovered after aqueous washing. Upon caustic refining and bleaching $71.1 \pm 3.7\%$ refined, bleached seal oil (RBSO) was obtained.

Table I. Content of neutral and polar lipids, cholesterol and iodine value seal blubber and intramuscular lipids

Component in 100 g sample	Blubber	Intramuscular Lipids
Neutral Lipids (g)	98.9 ± 0.2	78.8 ± 1.1
Polar Lipids (g)	1.1 ± 0.2	21.2 ± 0.5
Cholesterol (mg)	105.7 ± 22.0	-
Iodine Value (g)	146.3 ± 1.0	-

Table II summarizes different classes of lipid fatty acids from mechanically separated seal meat (MSSM). Corresponding results for seal blubber lipids are shown in Table III. A close scrutiny of the data presented in Tables II and III indicates that a) the content of HUFA in both subcutaneous and intramuscular lipids of seal is in the order of $DHA > EPA > DPA$; b) more than 50% of lipids in seal are monounsaturates; c) the ratio of the $\omega 3$ to $\omega 6$ polyunsaturates in the neutral fraction is higher than that in the polar lipid; d) the ratio of saturates to unsaturates is much higher in the polar fraction as compared with the neutral fraction. Furthermore, the polar lipids of seal blubber and intramuscular lipids, contrary to the general expectation, possessed less HUFA than the neutral triacylglycerol components.

Figure 1 shows the effect of steam rendering over a period of 2.5 h, in an industrial-scale operation (Carino Company, Dildo, NF), on the fatty acid composition of seal blubber oil. The content of long chain omega-3 fatty acids in the oil decrease progressively as the duration of heat processing increased. Isomerization and/or oxidation of these highly sensitive fatty acids is contemplated. Incorporation of a food-grade antioxidant/chelator system and/or low-temperature processing of blubber is expected to minimize undesirable structural changes in

the oil. In contrast to heat processing, caustic or bicarbonate refining of the oil enhanced its content of long-chain omega-3 fatty acids. Possible removal of saturated fatty acids by precipitation/crystallization might be involved (results not shown).

Table II. Classes of lipids of mechanically separated seal meat

Fatty Acids, Area %	Total	Neutral	Polar
Total Saturates (TS)	20.1	18.2	32.2
Total Unsaturation (TU)	79.9	81.8	67.8
Total Monounsaturates	52.3	54.1	43.1
Total Polyunsaturates	27.6	27.7	24.7
EPA	6.9	6.5	8.8
DPA	5.6	6.3	1.6
DHA	10.1	10.6	5.6
Total ω 3	24.4	25.4	16.8
Total ω 6	3.3	2.4	7.9
ω 3/ ω 6	7.39	10.58	2.13
TS/TU	0.25	0.22	0.48

Figure 2 shows the weight gain data of RBSO under Schaal oven test over a 16-day period. The weight gain of the oil was minimal over a 24 h storage. Possible presence of natural antioxidants in the oil might be responsible for better stability of seal oil compared to cod liver oil. Cod liver oil showed weight gain of 2.0% (w/w) over the same period. The weight gain of the oil continued rapidly from day 1 to day 3, after which much smaller oxygen uptake was noticed. The increase in the weight gain may be due to the addition of oxygen to lipid molecules to form hydroperoxides during primary stages of oxidation. Further storage of the oil for up to 16 days resulted in relatively small loss of weight, perhaps due to the breakdown of heat-labile hydroperoxides and volatilization of some secondary oxidation products under experimental conditions. Privett and Nickell (19) have reported that addition of oxygen to lipid to form hydroperoxides is reasonably quantitative during initial stages of oxidation. Olcott and Einset (20) have reported that the weight gain serves as a useful technique to evaluate the oxidative stability of edible oils. Ke and Ackman (22) reported that the methods is simple, has a satisfactory reproducibility and can be used to compare oxidation of lipids obtained from different parts of fish. However, surface exposure of the sample is very important in carrying out the experiments (22).

As expected, the weight gain data correlated well ($r = 0.912$) with corresponding peroxide values for RBSO (Figure 3). The weight gain of the oil arises from the uptake of oxygen and formation of lipid hydroperoxides. Similar to the weight gain data, PV of seal blubber oil were generally smaller in magnitude than those for cod liver oil.

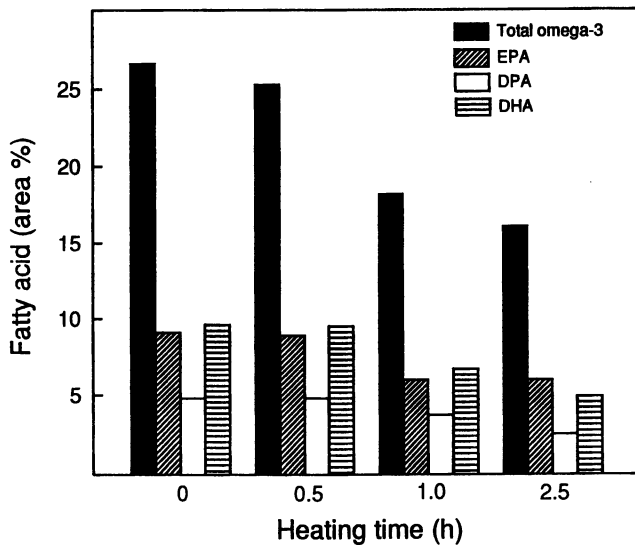


Figure 1. Effect of steam rendering (at 100°C) on the long-chain omega-3 fatty acids of seal blubber oil (sample used in this study was from a different batch than that used in other studies).

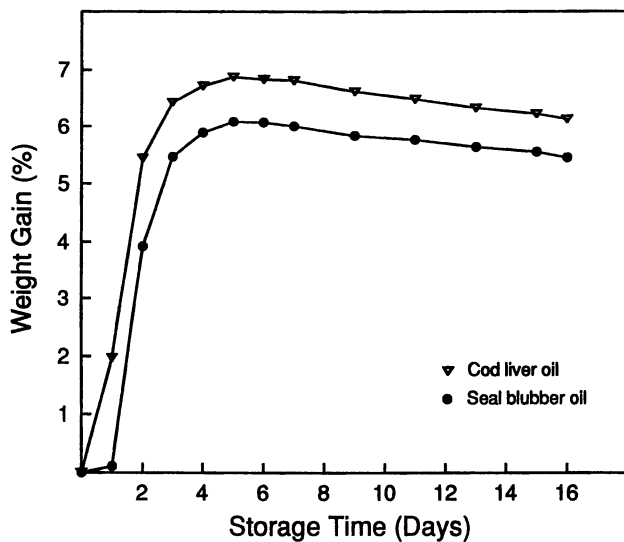


Figure 2. Weight gain of seal blubber and cod liver oils stored at 65°C.

July 15, 2012 | http://pubs.acs.org
 Publication Date: July 7, 1994 | doi: 10.1021/bk-1994-0558.ch016

Table III. Classes of lipids and seal blubber

Fatty Acid, Area %	Total	Neutral	Polar
Total Saturates (TS)	13.0	13.2	24.1
Total Unsaturates (TU)	87.0	86.8	75.9
Total Monounsaturates	63.3	63.1	47.7
Total Polyunsaturates	23.6	23.7	28.2
EPA	6.5	7.0	6.1
DPA	4.3	4.1	3.2
DHA	8.9	8.7	5.0
Total ω 3	21.4	21.9	17.1
Total ω 6	2.2	2.0	3.4
ω 3/ ω 6	11.09	10.95	5.03
TS/TU	0.15	0.15	0.32

Lipid radicals formed during the initiation step may undergo rearrangement. Thus, the methylene-interrupted feature of HUFA of seal blubber is lost in favor of formation of conjugated dienes. Figure 4 represents the variation of CD in RBSO over a 16-day storage and Figure 5 represents the correlation ($r = 0.966$) of conjugated dienes, expressed as the absorbance at 234 nm, with corresponding peroxide values. Meanwhile, formation of CT (absorbance at 268 nm) followed a trend similar to that observed for CD (Figure 4).

As hydroperoxides are the primary products of lipid oxidation (23), PV provides a clear indication concerning the initial oxidation potential of different lipids. Conjugated diene value may also be used to determine the initial rate of oxidation (9). Jackson (24) indicated that formation of hydroperoxides normally coincides with CD formation in oils upon oxidation. The CD assay is faster than PV determination and does not depend on chemical reactions such as color development for its determination. Therefore, CD content may be used as a measure of primary oxidation products for both seal blubber and cod liver oils. According to both of these methods, seal blubber oil is more stable than cod liver oil. Structural differences between the two oils as well as possible presence of stabilizers in seal blubber may be responsible for this observation.

Figure 6 summarizes changes in the content of TBARS of seal blubber oil over a 16-day storage period at 65°C. It is interesting to note that the content of TBARS remained relatively unchanged between days 3 and 12 during the storage period. Therefore, it is concluded that the rate of formation of TBARS is equivalent to that of their disproportionation and/or further reaction. In contrast, TBARS of cod liver oil increased progressively over the entire storage period. These results are in agreement with sensory characteristics of seal blubber and fish oils. While cod liver oil attained an intense off-flavor after one day of storage, the blubber oil showed a delayed response (2 days) to off-flavor development.

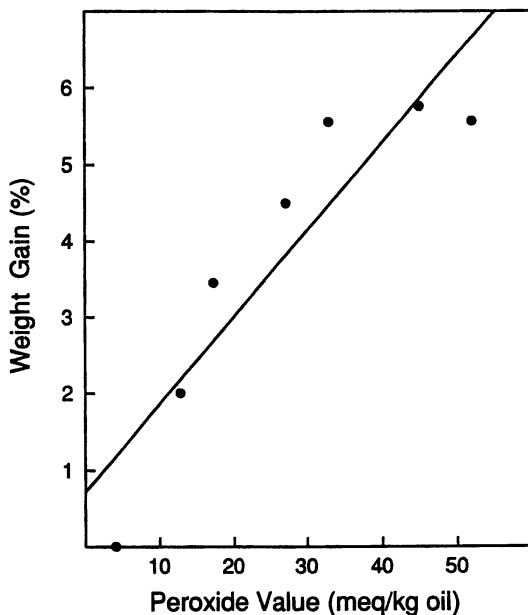


Figure 3. Relationship between peroxide value and weight gain data (corr. coeff. $r = 0.912$) of seal blubber oil during accelerated oxidation at 65°C .

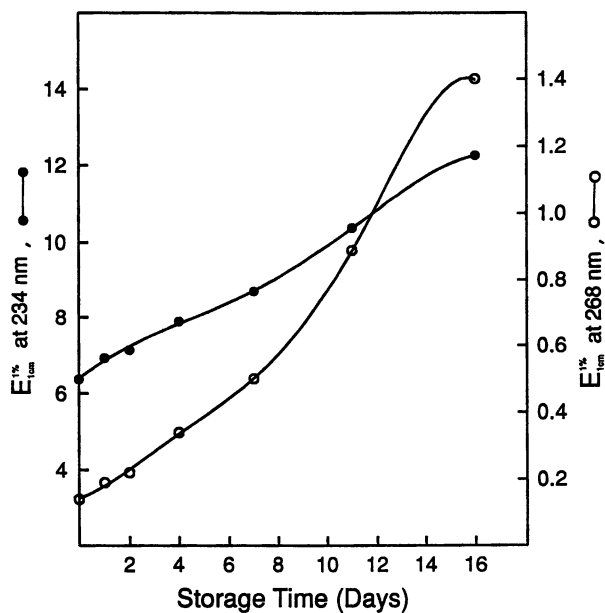


Figure 4. Conjugated diene and triene values of seal blubber oil during accelerated oxidation at 65°C .

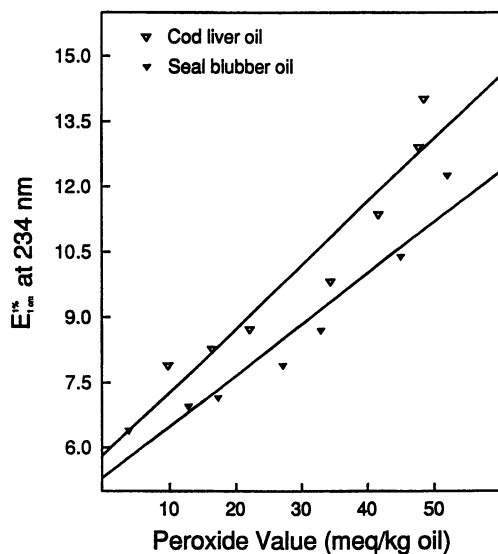


Figure 5. Relationship between peroxide value and conjugated diene value of seal blubber oil (corr. coeff. $r = 0.966$) and cod liver oil (corr. coeff. $r = 0.953$) during accelerated oxidation at 65°C .

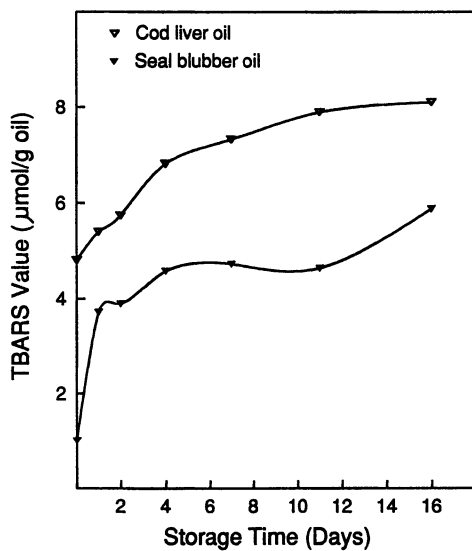


Figure 6. The 2-thiobarbituric acid reactive substances (TBARS) values of seal blubber and cod liver oils stored at 65°C .

Elucidation of the chemical identity of major contributors to off-flavor development in seal blubber oil is currently under investigation.

Polyunsaturated fatty acids are among the most easily oxidizable components of foods and cells and many of the oxidized products including peroxides, free radicals and aldehydes are highly toxic and mutagenic (25). The ease of non-enzymatic free radical oxidation of fatty acids is proportional to the number of methylene groups between double bonds, thus DHA, DPA and EPA are highly prone to oxidation. Therefore, protection of HUFA of the omega-3 type is essential in order to counterbalance any harmful effects and to take full advantage of their nutritional and health-related benefits.

Conclusions and Further Research Needs

Seal blubber lipids consist of approximately 22% long-chain omega-3 fatty acids, namely EPA, DPA and DHA. These fatty acids are dominant in positions of 1 and 3 of the triacylglycerol and thus are susceptible to hydrolysis by pancreatic lipase. In fish oils DHA is mostly in the 2-position but the EPA may be less specifically located. Seal blubber lipids also contains considerable amounts of monoenes and DPA. The oxidative stability of seal blubber lipids is better than other marine oils. Further research is needed to explore possibilities for value-added utilization of this readily available source of omega-3 fatty acids for food and pharmaceutical applications.

Literature Cited

1. Bang, H.O.; Byerberg, J. *Acta. Med. Scand.* 1976, 200, 69-73.
2. Mueller, B.A.; Talbert, R.L. *Clin. Pharm.* 1988, 7, 795-807.
3. Dyerberg, J.; Bang, H.O.; Stofferson, E.; Moncada, S.; Vane, J.R. *Lanset* 1978, 15(July), 117-119.
4. Von Schacky, C.; Weber, P.C. *Clin. Invest.* 1985, 76, 2446-2450.
5. Koletzko, B.; Thiel, I.; Springer, S. *European J. Vlin. Nutr.* 1992, 46, 545-555.
6. Gordon, D.T.; Ratiff, V. In: *Advances in Seafood Biochemistry: Composition and Quality*. Flick, Jr, G.J.; Martin, R.E. Eds.; Technomic Publishing Company, Inc., Lancaster and Basel, 1992.
7. Braden, L.M.; Carroll, K.K. *Lipids* 1986, 21, 285-291.
8. Shahidi, F.; Naczki, M.; Dunajski, E.; Walsh, K. 1992. Presented at the 37th Atlantic Fisheries Technological Conference, Percé, Quebec. August 23-26.
9. Hsieh, R.J.; Kinsella, J.E. *Adv. Food Nutr. Res.* 1989, 33, 233-242.
10. Porter, N.A.; Lehman, L.S.; Weber, B.A.; Smith, K.J. *J. Am. Oil Chem. Soc.* 1981, 103, 6447-6452.
11. Cho, S-Y.; Miyashita, K.; Miyazawa, T.; Fujimoto, K.; Kaneda, T. *J. Am. Oil Chem. Soc.* 1987, 64, 876-880.

12. Gray, J.I. *J. Amer. Oil Chem. Soc.* **1978**, *55*, 539-546.
13. Dugan, L. Jr. *J. Amer. Oil Chem. Soc.* **1955**, *32*, 605-609.
14. Bligh, E.G.; Dyer, W.J. *Can. J. Biochem. Physiol.* **1959**, *37*, 911-917.
15. Shahidi, F.; Synowiecki, J. *Can. Inst. Food Sci. Technol. J.* **1991**, *24*, 269-272.
16. Christie, W.W. In: *Lipid Analysis*. 2nd Ed. Pergamon Press, New York, **1982**; pp 107-134.
17. AOCS. *Official Methods and Recommended Practices of American Oil Chemists' Society*. **1990**; 4th Ed. Amer. Oil Chem. Soc. Champaign, Illinois.
18. IUPAC, *Standard Methods for the Analysis of Oils and Fats and Derivatives*. **1987**; 7th Ed. Blackwell Scientific Pub. Ltd., Oxford.
19. Privett, O.S.; Nickell, C. *J. Amer. Oil Chem. Soc.* **1956**, *33*, 156-163.
20. Olcott, H.S.; Einset, E. *J. Amer. Oil Chem. Soc.* **1958**, *35*, 161-162.
21. Ke, P.J.; Ackman, R.G. *J. Amer. Oil Chem. Soc.* **1976**, *53*, 636-640.
22. Know, T.W.; Snyder, H.E.; Brown, H.G. *J. Amer. Oil Chem. Soc.* **1984**, *61*, 1843-1846.
23. Labuza, T.P. *CRC Crit. Rev. Food Technol.* **1971**, *2*, 355-405.
24. Jackson, H.W. *J. Amer. Oil Chem. Soc.* **1981**, *58*, 227-231.
25. Sanders, T. *Food Sci. Technol.* **1987**, *1*, 162-164.

RECEIVED December 16, 1993

Chapter 17

Volatile Compounds of Lards from Different Treatments

Lucy Sun Hwang and Chung-Wen Chen

Graduate Institute of Food Science and Technology, National Taiwan University, Taipei, Taiwan, Republic of China

Foods cooked in lard usually have better flavor than when cooked in vegetable oils. The objective of this study was to investigate the origin of the lard flavor. Pork fat was dry-rendered or wet-rendered to obtain crude lard. These two different lards were then refined resulting in dry-rendered lard and wet-rendered lard. Results of sensory evaluation showed that crude dry-rendered lard had the strongest flavor intensity among the four lards. Crude dry-rendered lard also contained the highest amounts of trace components (phospholipids and amino acids) and flavor contributing volatiles after heating at 190°C. There were 50 volatile compounds (23 acidic and 27 nonacidic) identified. Volatile compounds of the four different lards were similar qualitatively, but different quantitatively. These results seemed to indicate that wet-rendering and refining of lard could reduce the trace components which might be important in producing the lard flavor.

Lard is a traditional edible fat for Chinese people. Foods cooked or fried in lard usually have better flavor than in vegetable oils (1). However, lard is rich in saturated fatty acids and cholesterol which contribute adverse effects resulting in cardiovascular diseases (2). It is, therefore, useful to investigate the origin of the lard flavor during cooking in order to develop an imitation lard flavor.

The flavor of heated pork fat has been studied considerably (3-9). Aldehydes, ketones, alcohols, hydrocarbons, acids and lactones were identified in the volatiles. The lactones, mainly saturated γ - and δ - lactones, were postulated to be derived from the thermal oxidation of linoleic acid (7) or oleic acid (10). They might also be converted from the oxidation products of fatty acids such as aldehydes and alcohols (11).

0097-6156/94/0558-0244\$08.00/0
© 1994 American Chemical Society

Lard is generally prepared either by dry-rendering or by wet-rendering. The dry-rendered lard with pork back fat as the raw material usually has better flavor than the wet-rendered lard and it is used as cooking fat or shortening. The wet-rendered lard with pork belly fat as the raw material usually has an undesirable flavor and must be refined before further use.

In this study, the effects of the two rendering methods and refining process on the flavor of lard were investigated by chemical analysis and sensory evaluation. The minor constituents of the different lards were also analyzed and correlated with lard flavor.

Materials and Methods

Materials. Fresh adipose tissue from porcine backs was purchased from a local butcher. Blood and meat were carefully removed from the adipose tissue prior to use.

Preparation of Lard

1. Dry Rendering. Porcine adipose tissue was macerated before being heated in a stainless steel vessel at 100°C with constant stirring for one hour. Oil was pressed out of the tissue and filtered to obtain the crude dry-rendered lard (CDL).

2. Wet Rendering. Porcine adipose tissue was macerated first and then mixed with an equal weight of distilled water. The mixture was heated at 100°C in a stainless steel vessel for one hour followed by filtration. The filtrate was centrifuged and the upper layer was collected as the crude wet-rendered lard (CWL).

3. Solvent Extraction. The macerated porcine adipose tissue was repeatedly extracted with a chloroform: methanol (2:1, v/v) solvent mixture three times. The solvent was removed from the extract by rotary evaporation to obtain the solvent-extracted lard (SEL).

Refining of Lard. Crude lard was refined with 3N sodium hydroxide at 90°C to remove free fatty acids followed by bleaching with activated clay at 95-110°C. The bleached lard was then deodorized by vacuum steam distillation at 235-260°C for 2 hrs to yield the refined lard.

Sensory Evaluation. Sensory evaluations of the different lards were conducted with melted lard kept at 45°C. Both the odor intensity and the preference tests were performed. For odor intensity, lards were evaluated on a 7-point scale by a panel of 13 members. A triangle test was first conducted to screen out the unqualified panelists. For the preference test, lards were evaluated on a 9-point scale by a panel of 18 members. Duncan's New Multiple Range Test was used for the statistical analysis.

Chemical Analyses. Fatty acid composition of lards was determined according to the method of Christie (12). Phospholipid composition of lards was determined according to the methods of Yokochi and Suzuki (13), Yamagishi et al. (14), and Yamanaka and Fujita (15). Free amino acid composition of lards was determined according to the method of Piez and Morris (16).

Heating Experiment. Lard was heated in a stainless steel vessel (B in Figure 1) on a hot plate (A) at 190°C for 2 hrs. The volatiles formed during heating were collected in a cold-water-cooled hood (C) and a series of cold traps (H, I, J, K) cooled in dry ice and isopropanol (H, I) or liquid nitrogen (J, K). The volatiles were driven from the heated lard with the aid of a pump (N), and the air flow in the system was controlled at 7 liters/min by adjusting the control valve (M) and monitored by a flow meter (L). Flask (F) was used to trap water and other less volatile components which were condensed in a cold water condenser (E). After the two-hour heating period, the volatile compounds collected in C ~ K, called smoke condensates, were washed down with diethyl ether.

Analysis of Smoke Condensates. The ether extract of the smoke condensate was fractionated into acidic and nonacidic fractions with a 10% sodium carbonate solution followed by concentration in an Oldershaw column. The acidic fraction was methylated before injection into a gas chromatograph (GC). A Hewlett-Packard Model 5890 GC equipped with a flame ionization detector (FID) was employed. The GC column was a 50 m x 0.32 mm i.d. CP-WAX 52 CB fused silica capillary column (Chrompack, Holland). The temperatures of the injector and detector were 240°C and 250°C, respectively. The column oven temperature was held at 50°C for 5 min and then programmed to 200°C at 1.5°C/min and kept at 200°C for 55 min. The hydrogen carrier gas flow rate was 2.4 ml/min. The split ratio was 58:1. The identification of the GC peaks was accomplished by GC-MS, comparing the retention indices and co-chromatography with the authentic compounds. Mass spectra were obtained by using a Hewlett-Packard Model 5982B gas chromatography-mass spectrometer which was set at 70eV and a source temperature of 200°C.

Results and Discussion

Composition of Lards from Different Treatments. Crude lards from dry-rendering and wet-rendering were refined to obtain refined dry-rendered lard (RDL) and refined wet-rendered lard (RWL). The fatty acid composition, phospholipid composition and free amino acid composition of the four different lards were determined in order to understand their differences. Results are listed in Tables I to III. The fatty acid compositions of the four lards (Table I) were quite similar. Oleic acid was the major fatty acid (~ 40%) followed by palmitic acid (~ 25%) and linoleic acid (~ 16%).

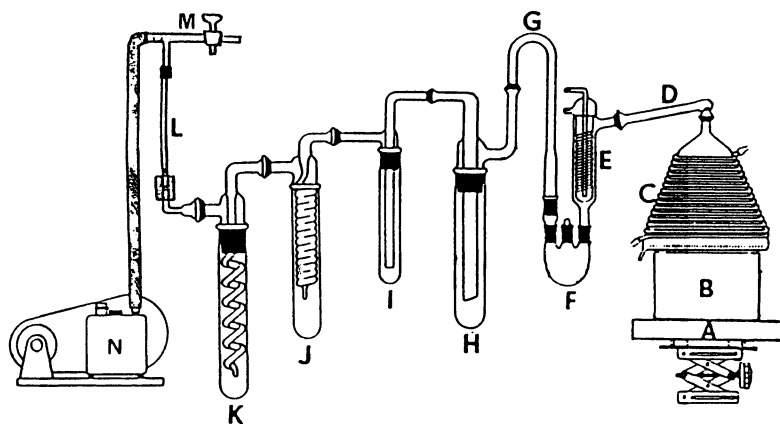


Figure 1. Apparatus for heating lard and cold trapping volatiles.

Table I. The Fatty Acid Compositions of Lards from Different Treatments

Lard	Fatty Acid Composition (% Weight) ^a									
	12:0	14:0	16:0	16:1	17:0	18:0	18:1	18:2	18:3	20:1
CDL	0.14	1.58	25.39	2.13	0.24	12.03	40.83	16.40	0.68	0.58
RDL	0.15	1.62	25.89	2.17	0.27	12.12	41.10	15.59	0.60	0.49
CWL	0.10	1.57	25.85	1.96	0.28	11.98	40.91	16.23	0.65	0.47
RWL	0.10	1.54	25.36	1.96	0.28	11.75	41.79	16.26	0.48	0.48

a: The weight of each fatty acid was determined with the addition of 19:0 triglyceride as an internal standard.

Table II. Phospholipid Composition of Lards from Different Treatments

Phospholipid	SEL	CDL	RDL ($\mu\text{g/g}$ lard)	CWL	RWL
Phosphatidylserine (PS)	0.353	0.330	-	0.261	-
Phosphatidylethanolamine (PE)	3.872	3.341	-	0.064	-
Phosphatidylcholine (PC)	7.661	2.155	-	2.551	-
Phosphatidylcholine plasmalogen (PCP)	0.581	0.202	-	0.131	-
Sphingomyeline (S)	1.135	0.285	-	0.588	-
Total	13.602	6.370	-	3.595	-

Table III. Free Amino Acid Composition of Lards from Different Treatments

Amino acid	SEL	CDL	RDL (n mole/g lard)	CWL	RWL
Thr	0.590	0.304	-	trace	-
Ala	1.143	0.456	0.287	0.331	0.229
Leu	0.312	-	-	-	-
Ser	1.714	1.326	0.386	1.012	0.324
Gly	0.884	0.640	0.447	0.497	0.296
Ilu	0.314	-	-	-	-
Val	0.785	-	-	-	-
Total	5.742	2.726	1.120	1.840	0.849

The phospholipid compositions of the four lards and lard obtained from chloroform/methanol (Folch) solvent extraction are listed in Table II. The solvent extracted lard (SEL) contained the highest amounts of phospholipids since it was directly extracted from the adipose tissue. There were five phospholipids detected in SEL by HPLC. Phosphatidyl choline (PC) was the major phospholipid, followed by phosphatidyl ethanolamine (PE) and sphingomyeline (S). The phosphatidyl choline plasmalogen (PSP) and phosphatidyl serine (PS) contents were less than 1 $\mu\text{g/g}$. Both Grigor et al. (17) and Christie and Moore (18) reported that PC was the major phospholipid in lard, similar to our findings. The second major phospholipid was different, however. It was phosphatidyl ethanolamine plasmalogen (PEP) and S as reported by Grigor et al. (17) and Christie and Moore (18), respectively. Among the four lards, crude dry-rendered lard (CDL) had the highest amount of total phospholipids followed by crude wet-rendered lard (CWL). The difference in phospholipid composition of the two crude lards lies in the contents of PE. Having a primary amine, PE can form hydrogen-bonding with water and thus it is more hydrophilic. During wet-rendering, PE was lost in the water, therefore CWL had less PE than CDL. Both of the two refined lards showed no detectable amount of phospholipids by HPLC analysis indicating that the phospholipids in the crude lards were completely removed during the refining processes.

Only seven free amino acids were found in SEL (Table III). After rendering and refining, only three major amino acids (alanine, serine and glycine) were left in the lard. The dry-rendered lards contained more free amino acids than the wet-rendered lards. This may also be due to the hydrophilicity of amino acids.

Sensory Evaluation. Results of the sensory evaluation of the different lards are shown in Table IV. For odor intensity, CDL was found to be significantly stronger than the other three lards by the 13 panelists. As for the acceptability of the four lards, the 18 panelists showed no preference among the four.

Volatile Compounds in the Smoke Condensates. The smoke condensates collected from heating the four lards at 190°C for 2 hrs were analyzed by GC, GC/MS and co-chromatography with standard compounds. The nonacidic volatile compounds identified are listed in Table V. There were a total of 27 compounds identified, including 4 hydrocarbons, 4 alcohols, 14 aldehydes, 2 ketones, 1 γ -lactone and 2 other compounds. 2,4-Decadienal, *trans*-2-decenal, *trans*-2-undecenal, *trans*-2-heptenal, nonanal and hexanal were the major ones contributing to 70% of the total volatiles. Among them, 2,4-decadienal, *trans*-2-heptenal and hexanal were the thermal oxidation products of linoleic acid (19), while *trans*-2-decenal, *trans*-2-undecenal and nonanal were formed from oleic acid (20). The shorter chain aldehydes and ketones, such as formaldehyde, acetaldehyde, propanal, 2-pentanone and butanal were not found in this study, possibly due to the volatile collecting system and the analytical conditions. From Table V, it was also noted that the four lards were similar in volatile composition, qualitatively, and the difference

Table IV. Sensory Scores of the Odor Intensity and Preference Test of Lard

	CDL	RDL	CWL (Score)	RWL
Odor intensity ^c	5.23±0.63 ^a	2.54±0.69 ^b	2.27±0.99 ^b	2.35±0.92 ^b
Preference test ^d	5.33±2.33 ^a	4.44±1.42 ^a	5.44±1.34 ^a	4.78±1.63 ^a

a-b: The different letters indicate a significant difference between treatments. (P < 0.01)

c: Evaluated on a 7-point scale (7 = strongest; 1 = weakest) by a trained panel of 13 members.

d: Evaluated on a 9-point scale (9 = like extremely; 1 = dislike extremely) by a panel of 18 members.

lies in the quantity of the volatiles. Again, CDL contained the highest total amount of volatiles, followed by CWL, RDL and RWL. The trend is similar to that of phospholipids and free amino acids.

The acidic fraction of the volatile compounds contained 23 compounds as listed in Table VI. Among the volatiles identified, hexanal dimethyl acetal and nonanal dimethyl acetal were identified by GC-MS and the GC retention index. 4-Decenoic acid was tentatively identified by GC-MS only. The two major acidic volatiles in the smoke condensate were found to be hexadecanoic (stearic) acid and *cis*-9-octadecenoic (oleic) acid. They are the two most abundant fatty acids in lard (Table I). Nonanedioic acid was found, for the first time, in heated lard flavor. It has been reported as one of the autoxidation products of oleic acid (21). The postulated mechanism of formation is shown in Figure 2. Nonanedioic acid could also be formed from linoleic acid or linolenic acid by a similar mechanism. It is evident from Table VI that the compounds identified were the same for all four lards. Similar to the situation of the nonacidic fraction, the difference among the four lards was in the quantity of the volatiles, and CDL contained the highest amount of total volatiles.

Relationship between Lard Flavor and Its Minor Components. Table VII summarizes the amounts of some flavor-contributing volatiles in the four lards. CDL showed the highest content of these compounds followed by CWL, RDL and RWL. Apparently, dry-rendering can yield lard with a stronger flavor. This is also in agreement with the sensory scores (Table II).

Since the volatile compounds collected from lard during heating are the fatty acid thermal decomposition products mainly, the similarity between the four different lards in their fatty acid composition (Table I) results in the same volatile

Table V. Identity and Quantity of Nonacidic Volatiles in the Smoke Condensates of Lard during Heating

Compound identified	I _k ^a (CW-20M)	CDL	RDL (mg/100g lard) ^b	CWL	RWL
Hydrocarbons					
<i>n</i> -Octane	800	9.12	2.21	8.62	4.57
<i>n</i> -Decane	1000	1.00	0.68	1.06	0.44
<i>n</i> -Undecane	1100	t	0.57	t	0.31
<i>n</i> -Pentadecane	1500	1.04	0.83	0.07	0.40
Subtotal		11.16	4.29	9.75	5.72
Alcohols					
1-Penten-3-ol	1159	1.04	0.74	0.99	0.46
<i>n</i> -Pentanol	1251	4.08	3.89	3.94	3.08
1-Hexanol	1355	t	0.36	t	0.25
1-Octen-3-ol	1453	2.21	1.83	1.89	1.40
Subtotal		7.33	6.82	6.82	5.19
Aldehydes					
<i>n</i> -Pentanal	983	4.63	3.31	4.60	2.82
<i>n</i> -Hexanal	1087	10.87	8.13	10.38	7.60
<i>tr</i> -2-Hexenal	1223	1.38	1.16	1.40	0.74
<i>n</i> -Octanal	1296	2.67	2.12	2.07	1.86
<i>tr</i> -2-Heptenal	1331	11.04	8.45	10.44	7.23
<i>n</i> -Nonanal	1401	10.92	8.15	8.70	6.99
<i>tr</i> -2-Octenal	1437	3.24	2.66	2.77	2.40
<i>tr,cis</i> -2,4-Heptadienal	1470	1.18	1.00	1.07	0.60
<i>tr, tr</i> -2,4-Heptadienal	1499	3.77	3.05	3.32	1.97
<i>tr</i> -2-Nonenal	1544	3.14	2.46	2.59	1.89
<i>tr</i> -2-Decenal	1653	11.82	10.24	9.37	8.15
<i>tr</i> -2-Undecenal	1762	11.08	9.83	8.91	7.85
<i>tr,cis</i> -2,4-Decadienal	1773	3.53	3.00	3.11	2.51
<i>tr, tr</i> -2,4-Decadienal	1820	13.48	11.34	11.95	9.59
Subtotal		92.75	74.90	80.68	62.20
Miscellaneous compounds					
Ethyl acetate	890	0.65	t	0.44	0.38
2-Pentylfuran	1238	0.80	0.43	0.88	0.38
γ-Octalactone	1927	t	0.38	t	t
2-Pentadecanone	2033	0.62	0.23	0.54	t
2-Heptadecanone ^c	2296	1.46	0.79	1.33	0.71
Subtotal		3.53	1.83	3.19	1.47
Total		114.77	87.84	100.44	74.58

a: Retention index on carbowax 20M; b: Quantified with internal standard (hexadecane); c: Tentatively identified by GC-MS and GC retention index; t: Less than 0.15 mg/100g lard.

Table VI. Identity and Quantity of Acidic Fraction Volatiles in the Smoke Condensates of Lard during Heating

Compound identified	I _k ^a (CW-20M)	CDL	RDL (mg/100g lard) ^b	CWL	RWL
Methyl pentanoate	1093	0.10	0.10	0.07	0.07
Hexanal dimethyl acetal ^c	1169	1.77	1.06	1.89	0.82
Methyl hexanoate	1194	1.00	0.89	0.59	0.48
Methyl heptanoate	1295	0.40	0.34	0.26	0.16
Methyl octanoate	1398	0.45	0.41	0.35	0.26
Methyl 2-heptenoate	1404	0.10	0.12	0.09	0.06
Nonanal dimethyl acetal ^c	1474	2.36	1.53	2.55	1.05
Methyl nonanoate	1501	1.20	0.81	0.51	0.38
Methyl decanoate	1605	1.56	0.96	1.70	1.43
Methyl 4-decenoate ^d	1656	0.68	0.35	0.47	0.10
Methyl 2-decenoate	1720	0.08	0.15	0.08	0.10
Methyl dodecanoate	1813	1.44	0.72	1.32	0.36
Methyl tetradecanoate	2021	1.68	0.54	1.32	0.39
Methyl pentadecanoate	2125	0.12	0.09	0.06	0.07
Nonanedioic acid dimethyl ester	2146	0.08	0.04	t	t
Methyl hexadecanoate	2228	8.66	3.56	8.17	3.06
Methyl 9-hexadecenoate	2254	1.27	0.44	1.01	0.36
Methyl heptadecanoate	2329	0.08	0.05	0.07	0.04
Methyl octadecanoate	2433	2.25	1.51	2.32	1.37
Methyl <i>cis</i> -9-octadecenoate	2455	11.95	4.81	10.67	4.62
Methyl <i>tr</i> -9-octadecenoate	2457	0.77	0.34	0.68	0.33
Methyl 9,12-octadecadienoate	2511	3.87	1.28	3.40	1.27
Methyl 9,12,15-octadecatrienoate		0.15	0.04	0.13	t
Total		42.02	20.14	37.71	16.78

a: Retention index on carbowax 20M; b: Quantified with internal standard (tridecanoic acid); c: Tentatively identified by GC-MS; d: Tentatively identified by GC-MS and GC retention index; t : Less than 0.04 mg/100g lard

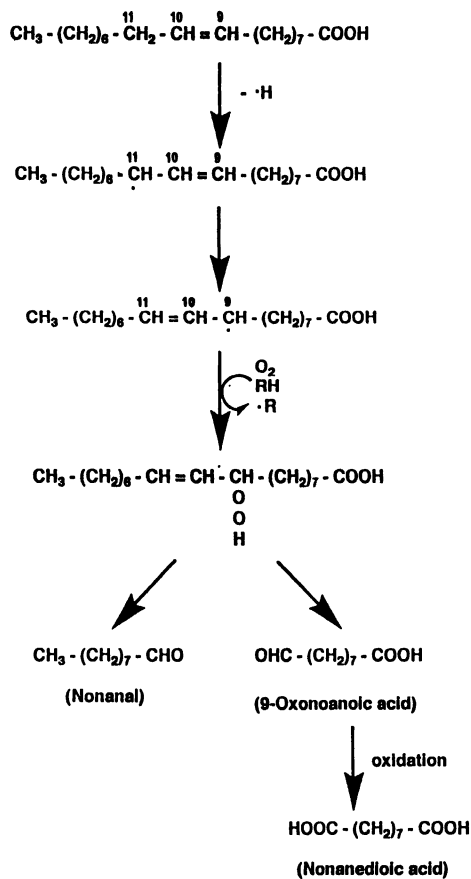


Figure 2. Proposed mechanism for the formation of nonanedioic acid.

Table VII. The amounts of volatile flavor contributing compounds from lards of different treatments

Compounds	CDL	RDL	CWL	RWL
	(mg/100g lard)			
1-Octen-3-ol	2.21	1.83	1.89	1.40
<i>n</i> -Hexanal	10.87	8.13	10.38	7.60
<i>n</i> -Octanal	2.67	2.12	2.07	1.86
<i>tr</i> -2-Heptenal	11.04	8.45	10.44	7.23
<i>n</i> -Nonanal	10.92	8.15	8.70	6.99
<i>tr</i> -2-Octenal	3.24	2.66	2.77	2.40
<i>tr</i> -2-Nonenal	3.14	2.46	2.59	1.89
2,4-Decadienal	17.01	14.34	15.06	12.10
2-Pentylfuran	0.80	0.43	0.88	0.38
γ - Octalactone	trace	0.38	trace	trace
Total	61.90	48.95	54.78	41.85

a: The amount of volatile flavor contributing compounds was calculated from Table V.

compounds qualitatively. Their quantitative difference may be related to the composition of minor constituents. As shown in Table II, CDL has the highest amount of phospholipids, especially PE. The phospholipids from pig adipose tissue contains highly unsaturated fatty acids such as C_{20:3}, C_{20:4}, and C_{20:5} (18). They are more susceptible to oxidation. Besides this quantitative effect of phospholipids, the high PE content of CDL might also contribute to its stronger flavor. Dawson et al. (22) reported that addition of PE to linoleic acid resulted in more volatile production than the addition of PC. The prooxidant effect of PE may be due to the accelerated decomposition of peroxides (23).

Although amino acids were known to be antioxidative toward linoleic acid oxidation (24), their effects on lard flavor were probably overshadowed by phospholipids.

Conclusion

Crude dry-rendered lard has the strongest flavor according to sensory evaluation. It also contains the highest amount of volatile flavor compounds, followed by crude wet-rendered lard, refined dry-rendered lard and refined wet-rendered lard. However, the volatile flavor compounds formed from heating of the four lards are

similar qualitatively. The same trend was observed in the analyses of phospholipid and free fatty acid compositions. It was, therefore, concluded that wet-rendering and refining of lard could reduce the trace components which might be important in producing the lard flavor.

Literature Cited

1. Pokorny, J. In *Flavor Chemistry of Lipid Foods*; Min, D. B.; Smouse, T. H., Eds.; American Oil Chemists' Society: Champaign, IL, 1989; pp. 113-155.
2. Grundy, S. M. In *Nutrition, Lipid, and Coronary Heart Disease*; Levy, R. I.; Rifkind, B. M.; Dennis, B. H.; Ernst, N., Eds.; Raven Press: New York, 1979, pp. 89-118.
3. Gaddis, A. M.; Ellis, R. *Science* **1957**, *126*, 745.
4. Gaddis, A. M.; Ellis, R.; Currie, G. T. *Food Res.* **1959**, *24*, 283-297.
5. Wishner, L. A.; Keeney, M. J. *Amer. Oil Chem. Soc.* **1965**, *42*, 776-778.
6. Watanabe, K.; Sato, Y. *Agric. Biol. Chem.* **1969**, *33*, 242-249.
7. Watanabe, K.; Sato, Y. *Agric. Biol. Chem.* **1969**, *33*, 1411-1418.
8. Watanabe, K. and Sato, Y. *Agric. Biol. Chem.* **1970**, *34*, 1710-1715.
9. Yasuhara, A. and Shibamoto, T. *J. Food Sci.* **1989**, *54*, 1471-1472 & 1484.
10. Watanabe, K. and Sato, Y. *Agric. Biol. Chem.* **1971**, *35*, 278-281.
11. Watanabe, K. and Sato, Y. *Agric. Biol. Chem.* **1970**, *34*, 464-472.
12. Christie, W. W. *Lipid Analysis*; Pergamon Press: Oxford, UK, 1982; pp. 53-54.
13. Yokochi, T.; Suzuki, O. *Yukagaku*, **1982**, *31*, 580-585.
14. Yamagishi, T.; Akiyama, H.; Kimura, S.; Toyoda, M. *J. Amer. Oil Chem. Soc.*, **1989**, *66*, 1801-1808.
15. Yamanaka, K. and Fujita, N. *Yukagaku*, **1990**, *39*, 393-397.
16. Piez, K.A.; Morris, L. *Anal. Biochem.* **1960**, *1*, 187-190.
17. Grigor, M. R.; Moehl, A. and Snyder, F. *Lipids*, **1972**, *7*, 766-768.
18. Christie, W. W.; Moore, J. H. *Comp. Biochem. Physiol.* **1974**, *47B*, 409-415.
19. Selke, E.; Rohwedder, W. K.; Dutton, H. J. *J. Amer. Oil Chem. Soc.*, **1980**, *57*, 25-30.
20. Selke, E.; Rohwedder, W. K.; Dutton, H. J. *J. Amer. Oil Chem. Soc.*, **1977**, *54*, 62-67.
21. King, G. *J. Chem. Soc.* **1956**, 587-593.
22. Dawson, P. L.; Sheldon, B. W.; Larick, D. K.; Turner, B. E. *J. Agric. Food Chem.* **1991**, *39*, 12-16.
23. Tsai, L. S.; Smith, L. M. *Lipids*, **1971**, *6*, 196-202.
24. Marcuse, R. *J. Amer. Oil Chem. Soc.*, **1962**, *39*, 97-103.

RECEIVED December 16, 1993

Chapter 18

Hexanal as an Indicator of the Flavor Deterioration of Meat and Meat Products

Fereidoon Shahidi and Ronald B. Pegg

Department of Biochemistry, Memorial University of Newfoundland,
St. John's, Newfoundland A1B 3X9, Canada

Hexanal is a dominant oxidation product of linoleic acid. In cooked meats, during the early stages of storage, the level of hexanal increased faster than other aldehydes. Therefore, hexanal may serve as a useful index of lipid oxidation and meat flavor deterioration (MFD) during this period. However, upon extended storage of meat, the content of hexanal declined markedly. Reactions of hexanal with meat components or its further oxidation to hexanoic acid may be responsible for this observation. Hence, caution should be exercised when using hexanal as an indicator of lipid oxidation and MFD. This article reports on the occurrence of hexanal in various cooked muscle foods as well as its suppression by nitrite curing or by treatment with nitrite-free composite systems. The use of hexanal as an indicator of lipid oxidation for cooked meats was compared to the classical 2-thiobarbituric acid test and sensory evaluations.

The oxidation of unsaturated lipids has been extensively studied since it relates to deterioration of muscle foods, production of both desirable and undesirable breakdown products and numerous reactions associated with other food constituents (1). In 1958, Tims and Watts (2) observed that lipid oxidation of cooked meats stored under refrigerated conditions was more pronounced than that of raw or frozen meat. To describe this rapid development of lipid-derived oxidized flavor, they coined the term "warmed-over flavor" (WOF). In recent years, demand has grown for pre-cooked, ready-to-eat meat products in the marketplace and in fast food franchises, thereby providing expanding potential for consumer exposure to WOF (3). Since WOF development is a dynamic process of flavor change, due principally to a cascade of oxidative events (4), an understanding of the mechanism and prevention of its occurrence in meat and meat products is important to scientists.

0097-6156/94/0558-0256\$08.72/0
© 1994 American Chemical Society

Oxidative changes in meat lipids primarily involve autoxidation processes, which are accompanied by various oxidative and non-oxidative secondary reactions (5). While hydroperoxides, the primary products of lipid autoxidation, are odorless and tasteless, their degradation leads to the formation of complex mixtures of low-molecular-weight compounds with distinctive aromas. Principally, these include alkanes, alkenes, aldehydes, ketones, alcohols, esters, epoxy compounds, polymers and acids (6,7). Aldehyde breakdown products play a significant role in the flavor of cooked meat as they possess low odor threshold values and are responsible for the development of WOF and rancidity. The degree of unsaturation of the fatty acid constituents of meat lipids primarily dictates the rate of WOF development. In the late 1980s, various researchers showed that WOF was not solely a consequence of lipid oxidation (8-11). These authors suggested that there was strong evidence that protein degradation reactions were involved and that heteroatomic compounds formed from these reactions may be implicated with the phenomenon of WOF, particularly with the deterioration of desirable meaty flavor notes. It was therefore proposed that meat flavor deterioration (MFD) was the more accurate term to use.

The 2-Thiobarbituric Acid (TBA) Test

Although there are many assays available for assessing the oxidative status of meat and meat products, the TBA test is widely used for this purpose (12-14). Malonaldehyde is a relatively minor product of autoxidation of polyunsaturated fatty acids. It reacts with the TBA reagent to produce a pink-colored complex with a distinctive absorption maximum at 532 nm. The concentration of this chromogen is then used as an objective measure for evaluation of the oxidative state of cooked meats. The TBA test was once believed to be specific for malonaldehyde (12-15), but this is not so. Because the TBA reagent reacts with other products of lipid oxidation, forming red complexes with an identical absorption maximum as the 2-thiobarbituric acid-malonaldehyde complex (16-19), the term 2-thiobarbituric acid reactive substances (TBARS) is now commonly used in place of TBA number or value (20,21). Ironically, malonaldehyde has very little or no odor of its own and, in this respect, may have no effect on the flavor of meat and meat products (7).

In most cases, the concentration of TBARS of meat tend to increase over the storage period, reach a maximum value and then start to decline. This decline may be due to further reactions of malonaldehyde with meat constituents such as amino groups of amino acids and DNA molecules (22-24). Thus, one may not know with which side of the "hill" one is dealing, but during the early stages of MFD, significant correlations have been reported to exist between TBARS of meat volatiles and sensory scores (25-27). Ward (28) suggested that without knowledge of the exact nature of the TBARS, what TBARS-adduct(s) are formed, the fatty acid profile of the lipids in question, the oxidative pathways taken by components

of the lipid system leading to the formation of TBARS, and the relationship of the TBARS to flavor producing molecules, the TBA assay is only of limited value for assessing the extent of oxidation or relating it to the sensory response. Shahidi and Hong (29,30) suggested that the relative, rather than the absolute, values of TBARS should be compared against one another in such determinations.

Hexanal Analysis and its Role in MFD

An alternative approach for assessing lipid oxidation in meat products is to measure the carbonyl compounds formed upon degradation of fatty acid hydroperoxides. Carbonyl compounds have been identified as significant contributors to the flavor of uncured meats (31,32). Some have exceptionally strong aromas and can be detected during autoxidation of fatty acids, even if they are present at low concentrations. The concentration of some of these aldehydes has been shown to correlate with MFD. In particular, the concentration of hexanal has been suggested to be a useful primary marker of MFD (26,33,34).

Hexanal is a seemingly ubiquitous component of food, both fresh and stored. This stems from the fact that practically all foods have some linoleate (18:2 ω 6), the fatty acid from which hexanal is derived. A profile of the fatty acids found in muscle tissue of various animals is presented in Table I.

Table I. Unsaturated fatty acid content of lipids in various muscle foods

Fatty Acid	Content (%)				
	Beef ^a	Chicken ^b	Fish ^c	Lamb ^d	Pork ^e
18:1 ω 9	33.44	20.25	19.59	19.51	12.78
18:2 ω 6	10.52	14.20	5.88	18.79	35.08
18:3 ω 3	1.66	0.90	8.07	0.44	0.33
20:2 ω 6	0.69	---	0.20	0.35	---
20:3 ω 6	2.77	1.30	0.36	0.62	1.31
20:4 ω 6	8.51	11.60	3.75	13.01	9.51
20:5 ω 3	0.76	1.55	7.16	---	1.31
22:4 ω 6	0.88	2.10	0.65	---	0.98
22:5 ω 3	0.92	1.90	2.39	---	2.30
22:6 ω 3	---	---	2.39	---	2.30
Total	60.15	53.80	50.44	52.72	65.90

^a Adapted from Ref. 35; ^b Adapted from Ref. 36; ^c Adapted from Ref. 37.

^d Adapted from Ref. 38.; ^e Adapted from Ref. 39.

Linoleate plays a significant role in the oxidized flavor of all meats, especially pork. Initial products of autoxidized linoleate consist predominately of the 9- and 13- hydroperoxides (46.5 and 49.5%, respectively) because the reactivity of the diallylic system favors attack of oxygen at carbon positions 9 and

13 (40). The 9-, 10-, 12- and 13-hydroperoxides at 32, 17, 17 and 34%, respectively, are products of photosensitized oxidation of linoleate (40). These hydroperoxides are unstable, and fragmentation occurs by homolytic and heterolytic cleavage mechanisms (41). Homolytic β -scission of 13-hydroperoxyoctadeca-9,11-dienoic acid produces an alkoxy radical intermediate. This undergoes carbon-carbon splitting forming either pentane and 13-oxo-9,11-tridecadienoic acid, or hexanal and an unsaturated C₁₂ fatty acid (42). Products of homolytic β -scission of 9-hydroperoxyoctadeca-10,12-dienoic acid include octanoic acid and 2,4-decadienal, or 9-oxo-nonanoic acid and a C₉ unsaturated hydrocarbon (Figure 1). Autoxidation of methyl linoleate in model systems has been reported to produce many aldehydes as shown in Table II.

Table II. Dominant volatile aldehydes derived from autoxidation of methyl linoleate^a

Aldehyde	Quantity ^b μg/g	Odor Threshold Value (ppb)	
		in Water	in Oil
Pentanal	55	10	100
Hexanal	5100	4.5	150
Heptanal	50	30	45
<i>trans</i> -2-Heptenal	450	50	14000
Octanal	45	40	50
<i>cis</i> -2-Octenal	990	--	--
<i>trans</i> -2-Octenal	420	4	7000
<i>cis</i> -3-Nonenal	30	--	--
<i>trans</i> -3-Nonenal	30	--	--
<i>cis</i> -2-Decenal	20	--	--
<i>trans</i> -2, <i>trans</i> -4-Nonadienal	30	90	460
<i>trans</i> -2, <i>cis</i> -4-Decadienal	250	--	20
<i>trans</i> -2, <i>trans</i> -4-Decadienal	150	0.1	200

^a Adapted from Ref. 40.

^b One gram of linoleate was autoxidized at 20°C by an uptake of 0.5 mole oxygen per mole linoleate.

By far, hexanal predominates among these volatile aldehydes, but this is not surprising. Hexanal is the only aldehyde that arises from both the 9 and 13 hydroperoxides of linoleate, and from other unsaturated aldehydes formed during oxidation of linoleate (43). The production of 2,4-decadienal is always less than that of hexanal because this dienal can only arise through β -scission of 9-hydroperoxyoctadeca-10,12-dienoic acid. In the autoxidized linoleate model system containing both saturated and unsaturated aldehydes, 2,4-decadienal oxidized faster forming hexanal than the saturated aldehydes. Schieberle and

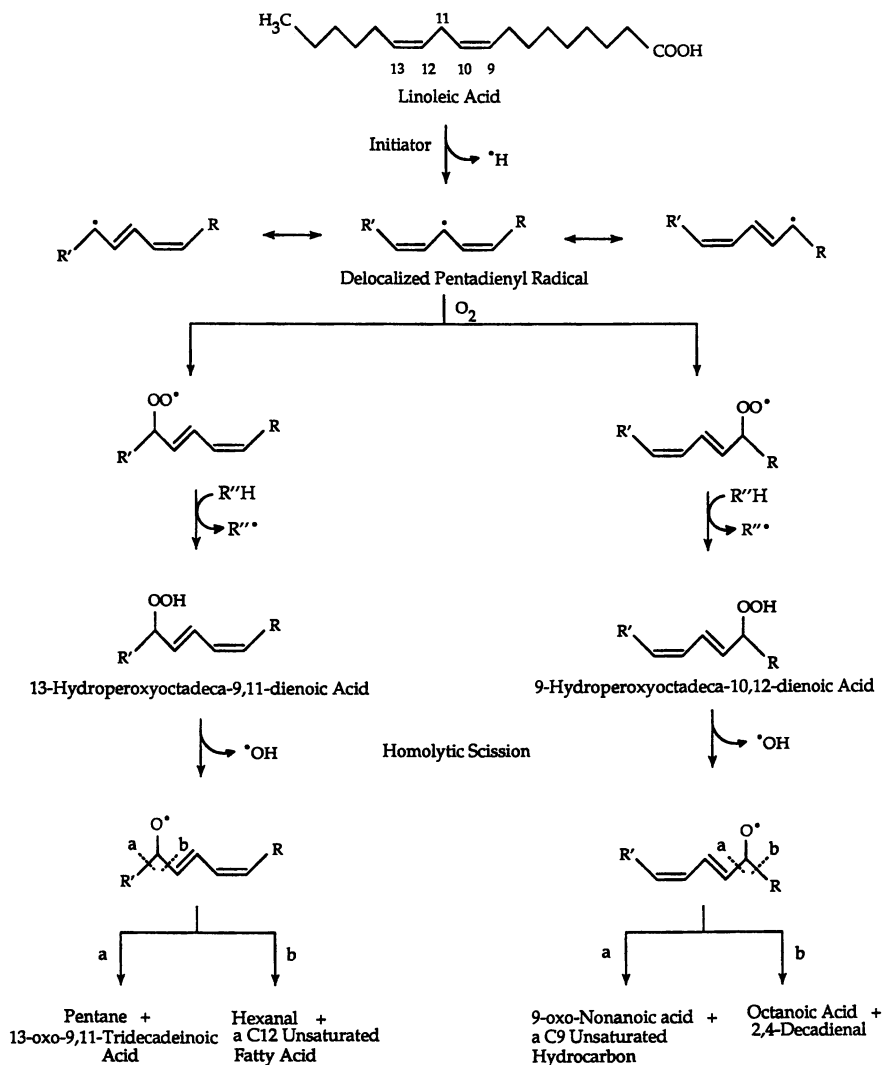


Figure 1. Autoxidation of linoleic acid and the production of hexanal. Adapted from Ref. 41.

Grosch (43) suggested that attack of free peroxy radicals (RO_2^{\cdot}) on the unsaturated moieties of 2,4-decadienal produces peroxy peroxides which are more labile than the primary hydroperoxides themselves. They decompose readily to hexanal, 2-butene-1,4-dial and other organic compounds (Figure 2). Matthews *et al.* (44) identified pentane, furan, ethanal, hexanal, acrolein, butenal, 2-heptenal, 2-octenal, benzaldehyde, glyoxal, *trans*-2-butene-1,4-dial, acetic acid, hexanoic acid, 2-octenoic acid, 2,4-decadienoic acid and benzene as the oxidation products of 2,4-decadienal in model systems.

Usefulness of Hexanal as a Primary Indicator of MFD

In the late 1970s and early 80s, reports appeared which noted the presence of hexanal in cooked muscle foods and its possible role as an indicator of lipid oxidation. Occurrence of hexanal and other aldehydic degradation products from autoxidation of edible oils had already been known for some time (44-46). Bailey *et al.* (33) reported the formation of low-molecular-weight aldehydes in cooked roast beef upon storage, and commented that hexanal and 2-pentylfuran were good indicators of lipid autoxidation. They also found that there were little, if any, qualitative differences in the volatiles produced during storage of meat at 4°C over 3 days, but there were quantitative differences.

Dupuy *et al.* (34) noted that in cooked ground roast beef, pentanal, hexanal, 2,3-octanedione, nonanal and the total volatiles increased appreciably during the storage period at 4°C as did the sensory scores and TBA numbers. Of all of these compounds, hexanal content increased most, from 0.05 to 35 ppm, after 5 days of storage. A similar trend was observed in cooked chicken and turkey meats. In the white and dark muscles of chicken, hexanal levels increased from 0.1 to 15 ppm and from 0.9 to 11 ppm, respectively, during the same period. Similar data were acquired for the white and dark muscles of turkey. The level of hexanal and total volatiles was approximately 3 times greater for cooked beef compared to chicken or turkey after 5 days of storage. It was concluded that since the concentration of hexanal increased more rapidly than any other aldehyde, it should be a useful primary marker of WOF development.

Dupuy *et al.* (34) also noted that addition of sodium chloride to meats, prior to thermal processing, stimulated the formation of carbonyl compounds during storage, whereas, the addition of sodium tripolyphosphate (STPP) in the presence of sodium chloride inhibited its formation at the levels tested. Love and Pearson (47) had previously reported that the addition of STPP, which retards oxidation in meats by its chelating ability, caused a 50% decrease in hexanal production in a model system. Stoick *et al.* (3) who examined the hexanal levels of cooked, restructured beef steaks reported that STPP reduced hexanal levels to 50% of a sodium chloride control, whereas, addition of the antioxidant, *t*-butylhydroquinone (TBHQ), provided more complete protection by keeping hexanal levels at 3% of the salt-containing control.

The addition of antioxidants to meat systems retards autoxidation and limits production of overtone carbonyl compounds. Barbut *et al.* (48) showed that addition of rosemary oleoresin or a butylated hydroxyanisole/butylated

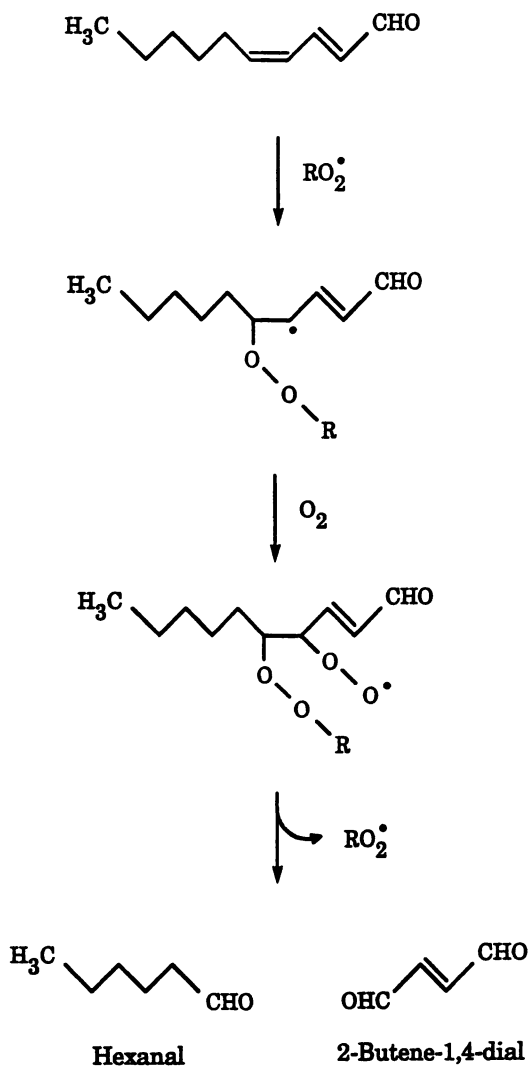


Figure 2. Proposed pathway for the breakdown of 2,4-decadienal to hexanal. Adapted from Ref. 43.

hydroxytoluene (BHA/BHT) antioxidant mixture to a cooked turkey sausage substantially reduced measurable TBARS as well as the content of oxidatively-derived carbonyls such as pentanal, hexanal, heptanal and 2,3-octanedione. Their results were in agreement with those of Shahidi *et al.* (26) who demonstrated that hexanal levels in cooked ground pork could be controlled by the addition of various antioxidants and chelating agents. Shahidi *et al.* (26) further showed that the hexanal content of meats treated with different antioxidants and chelating agents were linearly interrelated with their corresponding TBA values and sensory scores. These authors noted that after storing the cooked pork control sample for 2 days, the TBA numbers were practically identical. It was suggested that the hexanal content would be a better indicator of the oxidative state of cooked meats than TBA values in the early stages of storage. These values were linearly correlated with the TBA numbers at day 35 as shown in Figure 3 (correlation coefficient 0.995).

Morrissey and Apte (49) examined the volatile constituents of cooked beef, pork and fish after 2 days of refrigerated storage and the role of heme and non-heme iron in hexanal production. The carbonyl compounds isolated were derivatized with 2,4-dinitrophenylhydrazine, and the resulting hydrazones were separated using reversed-phase high performance liquid chromatography and identified at 360 nm by a UV-VIS detector. Because preliminary studies had indicated that hexanal production continually increased during the early stages of storage of muscle foods, while other volatiles did not show a consistent pattern of increase during the same period, Morrissey and Apte (49) focused their attention solely on hexanal generation. They ascribed the inconsistencies in the other volatiles to further oxidation or degradation resulting in new compounds. The hexanal concentration in fish muscle after 2 days of storage at 4°C was more than 2 times that of beef and 3 times that of pork. Noteworthy is the fact that these values correlated highly with TBA values. The influence of heme and non-heme iron in the systems showed that their stimulating effect on hexanal production was in the order of $\text{Fe}^{+2} > \text{hemoglobin} > \text{ferritin}$. Hexanal formation is obviously a function of the lipid profile and the presence of prooxidants, antioxidants and chelators in the system.

Ang and Young (50) investigated the flavor volatiles of cooked chicken during storage by a static headspace-gas chromatography (HS-GC) methodology. They reported that TBA values and hexanal levels increased in cooked chicken patties during a 5-day storage period at 4°C (correlation coefficient 0.95). These authors also observed that addition of STPP depressed TBARS and hexanal values accordingly. Su *et al.* (51) showed that in cooked chicken breast patties during 3 days of refrigerated storage significant correlations existed between values of TBARS, hexanal, and other HS volatiles, namely pentanal, heptanal and the total volatiles. These studies suggested that the rapid HS-GC technique may substitute for the TBA test.

Spanier *et al.* (52) went a step further and reported relationships among GC volatiles, TBARS markers, and descriptive sensory attributes of cooked beef patties. They showed that during a 4 day storage period significant correlations (correlation coefficients > 0.7) existed between pentanal, hexanal and TBARS

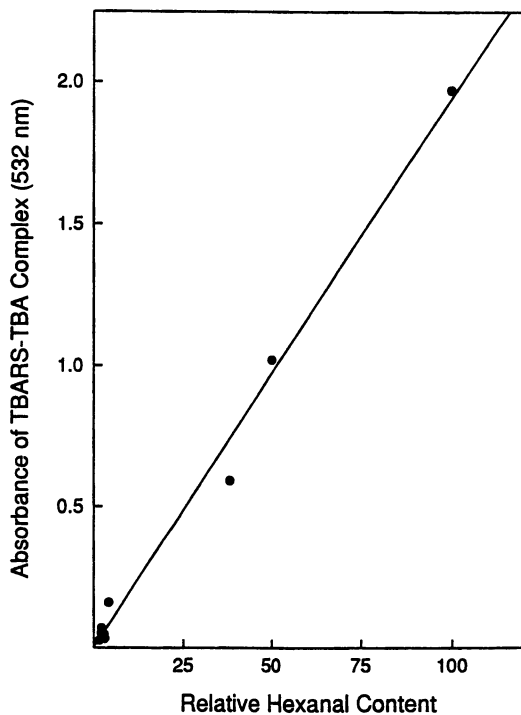


Figure 3. Relationship of TBARS values (35 days of storage) with relative hexanal content (2 days of storage). Adapted from Ref. 26.

values and desirable sensory descriptors (such as cooked beefy/brothy) and WOF descriptors (such as painty and cardboardy). Similar correlations have been previously reported (9,53,54).

Potential drawbacks of using hexanal as an indicator of MFD.

Shahidi and Pegg (55) examined the volatiles of cooked ground pork using the static HS-GC methodology outlined by Ang and Young (50). The rapid method used in this investigation did not allow for analysis of all possible compounds related to MFD, but most of the HS volatiles determined were low-molecular-weight aldehydes. A typical chromatogram of the HS volatiles of cooked pork after 5 days of storage is presented in Figure 4. The dominant aldehydes detected were pentanal (peak #6) and hexanal (peak #7). Uncooked pork samples contained negligible amounts of these aldehydes, as determined in preliminary tests. Other aldehydes detected included acetaldehyde (peak #1), propanal (peak #2), isobutanal (peak #3), butanal (peak #4), isopentanal (peak #5), heptanal (peak #8) and octanal (peak #9). These aldehydes were separated by capillary GC and were tentatively identified by retention time matching of the GC peaks with those of commercially available standards.

The HS volatile profiles detected during the study period were qualitatively similar, but were quantitatively different. The peak areas of several volatile compounds increased substantially during the early stages of storage. Pentanal (peak #6) and hexanal (peak #7) levels increased by 350 and 650%, respectively, by day 6, reached a maximum and then declined. Many studies have illustrated the increase in hexanal content during the first few days of storage (0 to 5) of cooked muscle foods and its correlation with TBA values or sensory scores, but after this period, the content of hexanal is not reported. Because aldehydes are quite reactive, they continuously oxidize. Wu and Chen (56) examined changes in the volatile compounds generated from various edible oils during storage at 55°C for 26 weeks. They observed that as aldehyde levels decreased from 62-87% to 47-67%, the volatile acid content, especially hexanoic acid, increased from 1-6% to 12-33%. Furthermore, the concentrations of both hexanal and hexanoic acid were relatively high (100-300 ppm) in the stored oils after 26 weeks. Wu and Chen (56) suggested that hexanoic acid was chiefly responsible for the "rancid" note in oxidized oils. Hexanal has been characterized as having a powerful, penetrating, fatty-green, grassy odor whereas hexanoic acid has a heavy, acrid-acid, fatty rancid odor, often described as "sweat-like" aroma (57).

Palamand and Dieckmann (58) subjected hexanal to autoxidation by passing a slow stream of air through it at 70°C. These authors reported that hexanal underwent oxidation, polymerization, and degradation resulting in the production of a large number of flavor-active compounds, most notably hexanoic acid. A list of those compounds identifiable in the acid (represented as ethyl esters) and non-acid fractions from the autoxidation of hexanal at 70°C after 48 h is presented in Table III. The rate of degradation and generation of hexanal and hexanoic acid, respectively, is shown in Figure 5.

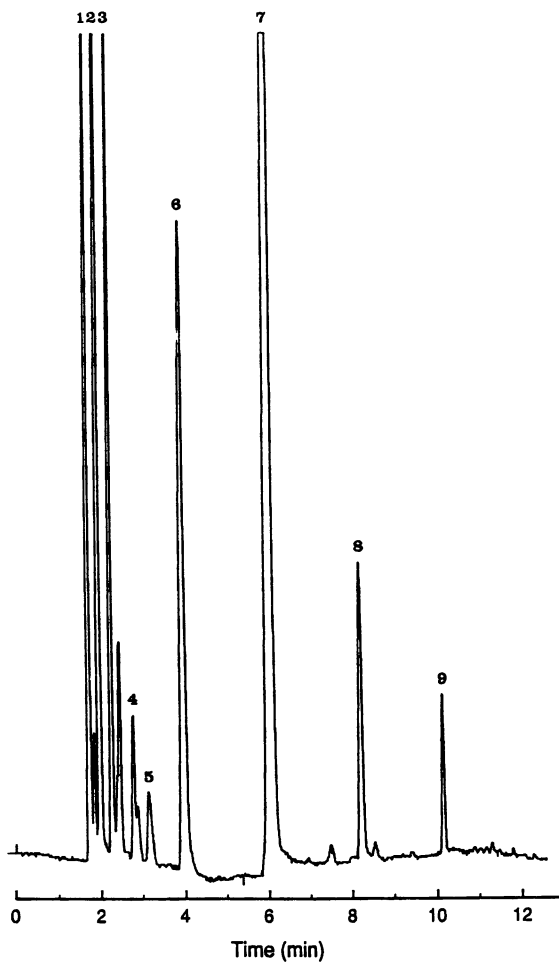


Figure 4. A HS-GC chromatogram of the flavor volatiles of cooked ground pork stored for 5 days at 4°C.

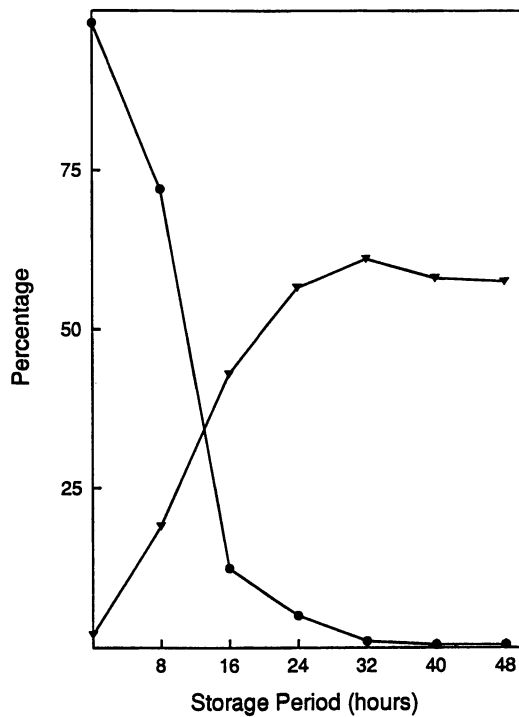


Figure 5. Rate of oxidation of hexanal, ●, and generation of hexanoic acid, ▼. Adapted from Ref. 58.

Table III. Some compounds present in the non-acid and acid fractions of autoxidized hexanal^a

Acid Fraction ^b	Non-acid Fraction
Diethyl ether	Diethyl ether
Acetone	Acetone
Ethyl acetate	Ethyl acetate
Ethanol	2-Methyl-5-ethylheptane
Ethyl butanoate	2,2-Dimethylpentane
Ethyl pentanoate	Hexanal
Ethyl hexanoate	Ethyl pentanoate
Ethyl heptanoate	2-Methylpentanal
Ethyl octanoate	1-Pentanol
2,6-Dimethoxyheptanone	δ -Pentalactone
Propyl hexyl ketone	γ -Hexalactone
Ethyl decanoate	δ -Hexalactone
Ethyl levulinate	4-Ethyl-2-octene
Pentanoic acid	Di- <i>n</i> -amyl ketone
γ -Hexalactone	Cyclohexyl propyl ketone
Ethyl undecanoate	2-Dodecenal
Ethyl dodecanoate	Ethyl dodecanoate
Hexanoic acid	hexanoic acid
Heptanoic acid	<i>trans</i> -Hexenyl decanoate
Ethyl tetradecanoate	γ - and δ -Nonalactone
Octanoic acid	γ - and ϵ -Decalactone
	γ - and δ -Undecalactone
	γ - and δ -Dodecalactone

^aAdapted from Ref. 58.

^bAcids were identified as their ethyl ester derivatives.

Brodnitz (59) and Wantanbe and Sato (60) suggested that autoxidation of saturated fatty acids and aldehydes proceeds *via* a free-radical mechanism. For example, the formation of lactones, identified as oxidation products of hexanal, proceeds by such a mechanism (Figure 6). However, a decrease in the concentration of aldehydes is not solely a consequence of oxidation as cross-linking reactions of the aldehydes with various components in the meat matrix may also be involved.

Shahidi and Pegg (55) reported that the concentration of pentanal and hexanal in the pork volatiles reached a maximum of 8.0 and 29 ppm, respectively, on day 6 (Figure 7). The increase in pentanal and hexanal concentrations was linear over this period (*i.e.*, days 0 to 6), after which, a decreasing trend was observed. A given hexanal level may correspond with two points during the storage period of cooked meats. Caution should therefore be exercised when using hexanal as an indicator of lipid oxidation and MFD. A similar trend is generally

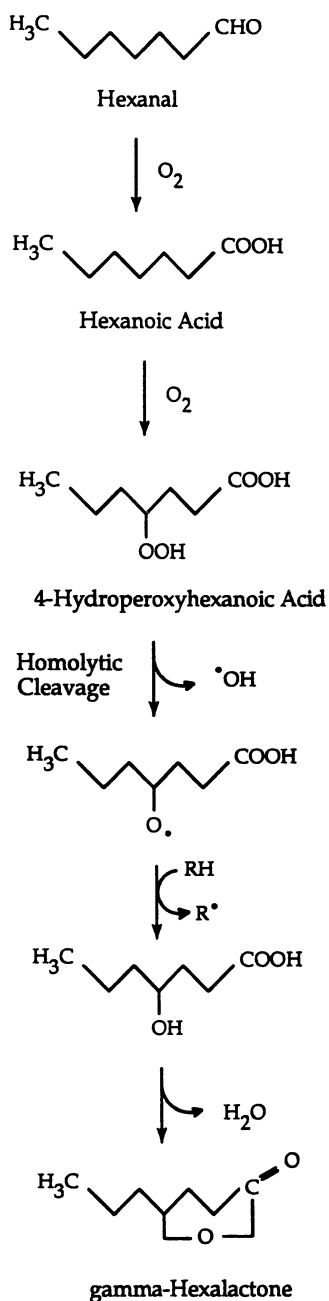


Figure 6. Formation of lactones from the oxidation of hexanal. Adapted from Ref. 58.

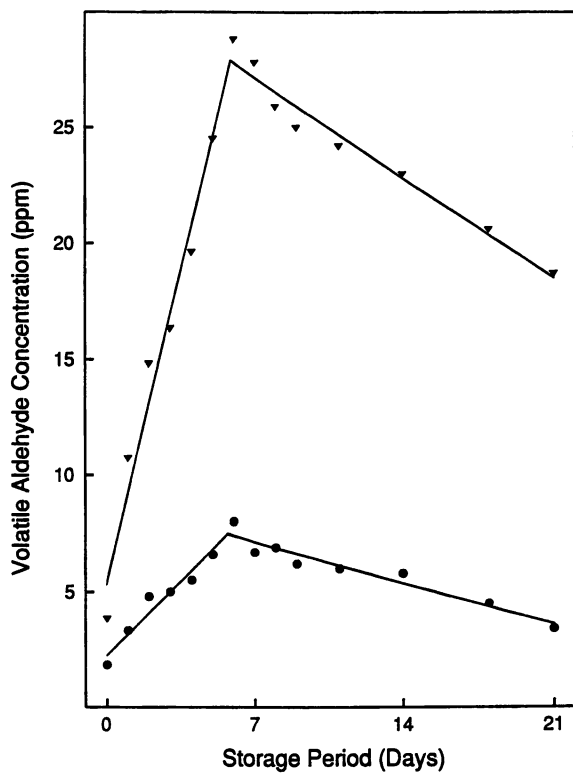


Figure 7. The concentration of pentanal, ●, and hexanal, ▼, in cooked pork volatiles during storage at 4°C.

observed for TBA values as mentioned earlier. Nonetheless, hexanal levels do correspond well with a single point during the early stages of storage, during which WOF and MFD occurs.

Role of Nitrite in Prevention of MFD and Hexanal Generation

The role of nitrite in cured meat flavor is complex and the chemical changes that are responsible for this unique flavor brought about in meat are not entirely understood. The relationship of nitrite to cured meat flavor was first described by Brooks *et al.* (61) who concluded that the characteristic flavor of bacon was primarily due to the action of nitrite. They further suggested that a satisfactory bacon product could be produced using only sodium chloride and sodium nitrite and that an adequate cured flavor could be obtained with a nitrite concentration as low as 10 ppm. Generally, a nitrite concentration of 50 ppm is considered as being necessary for adequate cured-meat flavor development, but Simon *et al.* (62) and MacDougall *et al.* (63) reported that higher taste panel scores were consistently obtained as nitrite addition levels increased.

Nitrite's role in cured-meat flavor development involves its antioxidative efficacy by retarding the breakdown of unsaturated fatty acids and the formation of secondary oxidation products. Sato and Hegarty (64) reported that while nitrite at a concentration of 2000 ppm eliminated lipid oxidation in cooked beef, at 50 ppm, it was capable of suppressing its development. Results obtained by Younathan and Watts (65) and Hadden *et al.* (66) indicated lower TBA values for cured as compared to uncured meats. Fooladi *et al.* (67), who also compared TBA values of cured and uncured beef, pork and chicken, reached a similar conclusion. Meat flavor deterioration does not occur in cured meat products.

Ockerman *et al.* (68) extracted volatile compounds from dry-cured hams by vacuum distillation and cold trap collection. They identified the constituents as being aldehydes, ketones, bases, and sulfur compounds, all of which are known contributors to the aroma of uncured cooked meat. Thereafter, Cross and Ziegler (69) examined the volatile constituents isolated from uncured and cured hams by a gas chromatography methodology. They reported that hexanal and pentanal were present in appreciable amounts in the volatiles of uncured, but were barely detectable in the volatiles of cured ham. They also reported that the volatiles, after passage through a solution of 2,4-dinitrophenylhydrazine, had the characteristic cured-ham aroma, regardless of whether cured or uncured hams were used. Cured and uncured chicken and beef volatiles, after stripping their carbonyl compounds by passage through 2,4-dinitrophenylhydrazine solutions, also possessed an aroma similar to that of cured ham. Cross and Ziegler (69) concluded that treating meat with nitrite does not seem to contribute any new volatile compounds to the flavor of cured meats, with the exception of nitrogen oxides that are not present in cooked uncured meat. Therefore, they postulated that cured-ham aroma represents the basic flavor of meat derived from precursors other than triacylglycerols, and that the aromas of various types of cooked meat depend on the spectrum of carbonyl compounds derived by lipid oxidation.

Shahidi (7) reported that elimination of lipid oxidation, either by curing or by stripping of carbonyl compounds from volatiles of untreated cooked meats, caused a major effect on the flavor perception of meats. This author noted that qualitative differences due to the possible presence of less active flavor components can not be ruled out. Nonetheless, gas chromatographic analyses of the volatiles of cured meat revealed a much simpler spectrum than their uncured counterparts, with drastic suppression in the content of major aldehydes, such as hexanal and pentanal (Table IV) which are known to be responsible for MFD.

Table IV. Effect of curing on the relative concentration of major aldehydes in pork flavor volatiles*

Aldehyde	Relative Concentration	
	Uncured	Cured
Hexanal	100	7.0
Pentanal	31.3	0.5
Heptanal	3.8	<0.5
Octanal	3.6	<0.5
2-Octanal	2.6	—
Nonanal	8.8	0.5
2-Nonanal	1.0	—
Decanal	1.1	—
2-Undecenal	1.4	0.5
2,4-Decadienal	1.1	—

*Adapted from Ref. 7.

Shahidi (7) proposed that any agent, or combination of agents that could prevent lipid oxidation, with the exception of nitrite precursors, would in principal, duplicate the antioxidant role of nitrite in the curing process, thereby preventing hexanal generation and MFD. According to Shahidi (14), this is in line with findings of other researchers and its validity was confirmed by preliminary sensory evaluations, but mutton was not included in these studies.

A simplistic view, attempting to present a unifying theory of the origin of the basic flavor of meat, species differentiation, and MFD is provided in Figure 8. It postulates that meat when cooked acquires its characteristic species flavor which is caused by volatile carbonyl compounds, such as hexanal and pentanal, formed by oxidation of its lipid components (*i.e.*, primarily phospholipids). Further oxidation during storage of cooked meat results in the deterioration of its flavor. Curing with nitrite suppresses the formation of oxidation products. It may be assumed that the flavor of nitrite-cured meats is actually the basic natural flavor of meat from different species without being influenced by overtone carbonyls

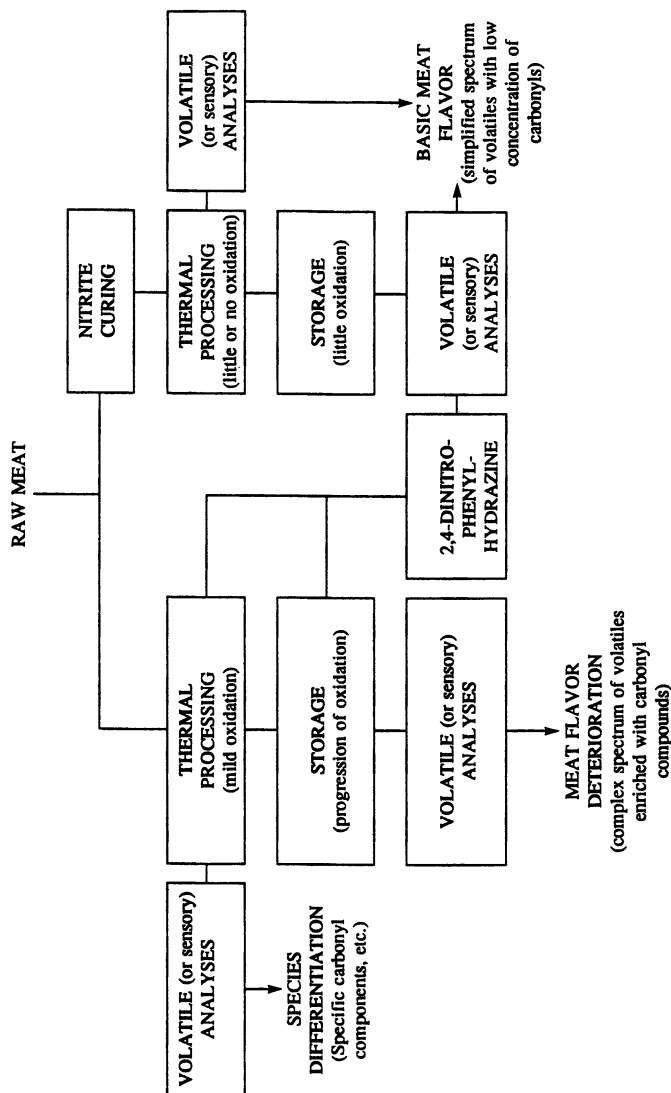


Figure 8. Consequence of cooking, curing, and storage on flavor of cooked meats and development of meat flavor deterioration (MFD). Reproduced from Ref. 14. Copyright 1992. American Chemical Society.

July 15, 2012 | http://pubs.acs.org
 Publication Date: July 7, 1994 | doi: 10.1021/bk-1994-0558.ch018

derived from oxidation of their lipid components. Further support for this view has recently been provided by Ramarathnam *et al.* (31,32), but the postulate does not easily explain the fact that intensity of cured meat flavor is proportional to the logarithm of nitrite concentrations as reported by MacDougall *et al.* (63), or the apparent persistence of the characteristic "mutton" flavor after nitrite curing of sheep meat (70).

The volatiles of cooked uncured, nitrite-cured and nitrite-free treated pork systems, stored for a 4 week period at 4°C, were examined using the static HS-GC methodology outline above (50). For nitrite-cured samples, sodium nitrite and sodium ascorbate were added directly to comminuted pork. For nitrite-free treated meats, the composite system consisted of a colorant, namely the preformed cooked cured-meat pigment (CCMP), and an antioxidant/sequestrant mixture of butylated hydroxyanisole/sodium ascorbate/sodium tripolyphosphate. Nitrite-free composite curing systems containing the CCMP have mimicked the characteristics of their nitrite-cured counterparts (71-76). The preformed CCMP, added to meat model systems, has been shown to have a weak antioxidative effect (72), but its suppression of hexanal generation has not been reported. As indicated earlier, hexanal concentrations in cooked uncured pork systems increased by 650% after 6 days of refrigerated storage and then declined. For nitrite-cured samples, hexanal levels were depressed indicating that nitrite successfully retarded hexanal generation and MFD (Figure 9). During the 4 week storage period, the hexanal level in nitrite-cured pork increased slowly and by day 28 was 2.0 ppm which represents only 9% of the level reached on day 6 by the uncured sample. For nitrite-free cured samples, hexanal concentrations followed a similar trend to their nitrite-cured analogs; by day 28, the hexanal content was 3.5 ppm which is only 16% of the level of the uncured sample after 6 days of refrigerated storage. Use of TBHQ instead of BHA as an antioxidant in the nitrite-free curing mixture at a 30 ppm level has been reported to provide better protection to pork samples against lipid oxidation and hexanal generation (results not shown), but its use in Canada is prohibited. After 28 days of storage at 4°C, hexanal levels in the CCMP-treated and nitrite-cured systems continued to increase. For the nitrite-free systems, a maximum hexanal level was eventually reached and, in some cases, a moderate decline was noted, again suggesting that caution should be exercised when evaluating the hexanal content of cooked treated meat systems. Further research on control of hexanal generation in nitrite-free and nitrite-cured meat systems with various formulations and under different storage conditions is in progress.

Summary

Although hexanal has been used as an index of lipid oxidation and MFD, it is not intended to imply that it is mainly responsible for the characteristic off-flavor of stored meat. The relationship between hexanal concentration and off-flavour notes, perceived by sensory means, is statistical and does not offer any physiological explanation of changes that occur in meat upon storage (77). Nonetheless, hexanal detection by the HS-GC method has potential for use as an

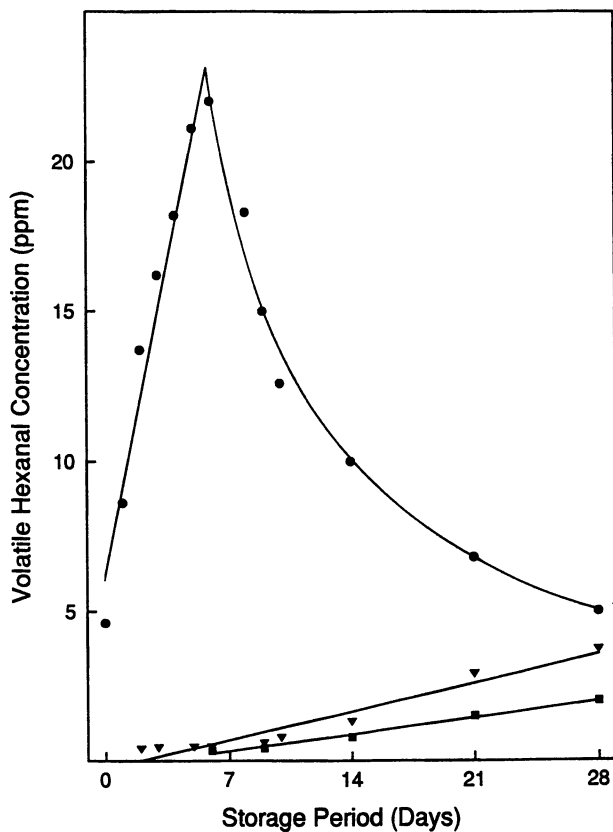


Figure 9. The relative content of hexanal in uncured, ●, nitrite-free treated, ▼, and nitrite-cured, ■, cooked ground pork during storage at 4°C.

indicator for quality control during processing and storage of meat products. Hexanal concentrations may also be used for evaluating frozen and cured-meat products where oxidation proceeds slowly, or when the TBA methodology may lead to erroneous results.

Acknowledgements

Financial support from the Natural Sciences and Engineering Research Council (NSERC) of Canada is gratefully acknowledged.

Literature Cited

1. Wong, D.W.S. In: *Mechanism and Theory in Food Chemistry*, Van Nostrand Reinhold, New York, NY, 1989, pp. 1-47.
2. Tims, M.J. and Watts, B.M. *Food Technol.* 1958, 12, 240-243.
3. Stoick, S.M., Gray, J.I., Booren, A.M. and Buckley, D.J. *J. Food Sci.* 1991, 56, 597-600.
4. Asghar, A., Gray, J.I., Buckley, D.J., Pearson, A.M. and Booren, A.M. *Food Technol.* 1988, 42(6), 102-108.
5. Gray, J.I. and Monahan, F.J. *Trends Food Sci. Technol.* 1992, 3, 315-319.
6. Shahidi, F., Rubin, L.J. and D'Souza, L.A. *CRC Crit. Rev. Food Sci. Nutr.* 1986, 24, 141-243.
7. Shahidi, F. In: *Flavor Chemistry. Trends and Developments*, Teranishi, R., Buttery, R.G. and Shahidi, F., Eds., American Chemical Society, Washington, DC, 1988, pp. 188-201.
8. Vercellotti, J.R., Kuan, J.W., Liu, R.H., Legendre, M.G., St. Angelo, A.J. and Dupuy, H.P. *J. Agric. Food Chem.* 1987, 35, 1030-1035.
9. St. Angelo, A.J., Vercellotti, J.R., Dupuy, H.P. and Spanier, A.M. *Food Technol.* 1988, 42(6), 133-138.
10. Spanier, A.M., Edwards, J.V. and Dupuy, H.P. *Food Technol.* 1988, 42(6), 110 & 112-118.
11. Vercellotti, J.R., Kuan, J.W., Spanier, A.M. and St. Angelo, A.J. In: *Thermal General of Aromas*, Parliment, T.H., McGorin, R.J. and Ho, C.-T., Eds., American Chemical Society, Washington, DC, 1987, pp. 452-459.
12. Tarladgis, B.G., Watts, B.M., Younathan, M.T. and Dugan, L.R., Jr. *J. Am. Oil Chem. Soc.* 1960, 37, 44-48.
13. Melton, S.L. *Food Technol.* 1983, 37(7), 105-111 & 116.
14. Shahidi, F. In: *Lipid Oxidation in Food*, St. Angelo, A.J., Ed., American Chemical Society, Washington, DC, 1992, pp. 161-182.
15. Tarladgis, B.G., Pearson, A.M. and Dugan, L.R., Jr. *J. Sci. Food Agric.* 1964, 15, 602-607.
16. Kosugi, H. and Kikugawa, K. *Lipids* 1986, 21, 537-542.

17. Witz, G., Lawrie, N.J., Zaccaria, A., Ferran, H.E., Jr. and Goldstein, B.D. *J. Free Rad. Biol. Med.* **1986**, *2*, 33-39.
18. Kosugi, H., Kato, T. and Kikugawa, K. *Anal. Biochem.* **1987**, *165*, 456-464. 19. Kosugi, H., Kato, T. and Kikugawa, K. *Lipids* **1988**, *23*, 1024-1031.
20. Ke, P.J., Cervantes, E. and Robles-Martinez, C. *J. Sci. Food Agric.* **1984**, *35*, 1248-1254.
21. Gray, J.I. and Pearson, A.M. In *Advances in Meat Research. Vol 3: Restructured Meat and Poultry Products*, Pearson, A.M. and Dutson, T.R., Eds., Van Nostrand Reinhold, New York, NY, **1987**, pp. 221-269.
22. Crawford, D.L, Yu, T.C. and Sinnhuber, R.O. *J. Agric. Food Chem.* **1966**, *14*, 182-184.
23. Chio, K.S. and Tappel, A.L. *Biochem.* **1969**, *8*, 2821-2827.
24. Reiss, U. and Tappel, A.L. *Lipids* **1973**, *8*, 199-202.
25. Igene, J.O., Yamauchi, K., Pearson, A.M. and Gray, J.I. *Food Chem.* **1985**, *18*, 1-18.
26. Shahidi, F., Yun, J., Rubin, L.J. and Wood, D.F. *Can. Inst. Food Sci. Technol. J.* **1987**, *20*, 104-106.
27. Ang, C.Y.W. and Lyon, B.G. *J. Food Sci.* **1990**, *55*, 644-648 & 673.
28. Ward, D.D. *Milchwissenschaft* **1986**, *40*, 583-588.
29. Shahidi, F. and Hong, C. *Food Chem.* **1991**, *42*, 339-346.
30. Shahidi, F. and Hong, C. *J. Food Biochem.* **1991**, *15*, 97-105.
31. Ramarathnam, N., Rubin, L.J. and Diosday, L.L. *J. Agric. Food Chem.* **1991**, *39*, 344-350.
32. Ramarathnam, N., Rubin, L.J. and Diosday, L.L. *J. Agric. Food Chem.* **1991**, *39*, 1839-1847.
33. Bailey, M.E., Dupuy, H.P. and Legendre, M.G. In: *The Analysis and Control of Less Desirable Flavors in Foods and Beverages*, Charalambous, G., Ed., Academic Press, Inc., New York, NY, **1980**, pp. 31-52.
34. Dupuy, H.P., Bailey, M.E., St. Angelo, A.J., Vercellotti, J.R. and Legendre, M.G. In: *Warmed-Over Flavor of Meat*, St. Angelo, A. J. and Bailey, M.E., Eds., Academic Press, Inc., Orlando, FL, **1987**, pp. 165-191.
35. Igene, J.O., Pearson, A.M., Dugan, L.R., Jr. and Price, J.F. *Food Chem.* **1980**, *5*, 263-276.
36. Moerck, K.E. and Ball, H.R., Jr. *J. Food Sci.* **1974**, *39*, 876-879.
37. Mai, J. and Kinsella, J.E. *J. Food Sci.* **1979**, *44*, 1101-1105 & 1109.
38. Lazarus, C.R., Deng, J.C. and Watson, C.M. *J. Food Sci.* **1977**, *42*, 102-107.
39. Yamauchi, K., Nagai, Y. and Ohashi, T. *Agric. Biol. Chem.* **1980**, *44*, 1061-1067.
40. Belitz, H.-D. and Grosch, W. In: *Food Chemistry*, Springer-Verlag, Berlin, Germany, **1987**, pp. 128-200.
41. Frankel, E.N., Neff, W.E. and Selke, E. *Lipids* **1984**, *19*, 790-800.
42. Frankel, E.N. *J. Sci. Food Agric.* **1991**, *54*, 495-511.
43. Schieberle, P. and Grosch, W. *J. Am. Oil Chem. Soc.* **1981**, *58*, 602-607.

44. Matthews, R.F., Scanlan, R.A. and Libbey, L.M. *J. Am. Oil Chem. Soc.* **1971**, *48*, 745-747.
45. Warner, K., Evans, C.D., List, G.R., Dupuy, H.P., Wadsworth, J.I. and Goheen, G.E. *J. Am. Oil Chem. Soc.* **1978**, *55*, 252-256.
46. Henderson, S.K., Witchwoot, A. and Nawar, W.W. *J. Am. Oil Chem. Soc.* **1980**, *57*, 409-413.
47. Love, J.D. and Pearson, A.M. *J. Agric. Food Chem.* **1976**, *24*, 494-498.
48. Barbut, S., Josephson, D.B. and Maurer, A.J. *J. Food Sci.* **1985**, *50*, 1356-1359 & 1363.
49. Morrissey, P.A. and Apte, S. *Sci. Aliments* **1988**, *8*, 3-14.
50. Ang, C.Y.W. and Young, L.L. *J. Assoc. Off. Anal. Chem.* **1989**, *72*, 277-281.
51. Su, Y., Ang, C.Y.W. and Lillard, D.A. *J. Food Sci.* **1991**, *56*, 881-883 & 898.
52. Spanier, A.M., Vercellotti, J.R. and James, C., Jr. *J. Food Sci.* **1992**, *57*, 10-15.
53. St. Angelo, A.J., Vercellotti, J.R., Legendre, M.G., Vinnett, C.H., Kuan, J.W., James, C., Jr. and Dupuy, H.P. *J. Food Sci.* **1987**, *52*, 1163-1168.
54. St. Angelo, A.J., Crippen, K.L. Dupuy, H.P. and James, C., Jr. *J. Food Sci.* **1990**, *55*, 1501-1505 & 1539.
55. Shahidi, F. and Pegg, R.B. *J. Food Lipids* **1993**. In press.
56. Wu, C.-M. and Chen, S.-Y. *J. Am. Oil Chem. Soc.* **1992**, *69*, 858-865.
57. Arctander, S. In: *Perfume and Flavor Chemicals*, Published by Stefen Arctander, Elizabeth, NJ, **1969**, pp. 1592 & 1596.
58. Palamand, S.R. and Dieckmann, R.H. *J. Agric. Food Chem.* **1974**, *22*, 503-506.
59. Brodnitz, M.H. *J. Agric. Food Chem.* **1968**, *16*, 994-999.
60. Watanabe, K. and Sato, Y. *Agric. Biol. Chem.* **1970**, *34*, 464-472.
61. Brooks, J., Hainer, R.B., Moran, T. and Pace, J. Department of Scientific and Industrial Research, Food Investigation Board. Special Report 49, His Majesty's Stationery Office, London, UK, **1940**, pp. 2-4.
62. Simon, S., Ellis, D.E., MacDonald, B.D., Miller, D.G., Waldman, R.C. and Westerberg, D.O. *J. Food Sci.* **1973**, *38*, 919-923.
63. MacDougall, D.B., Mottram, D.S. and Rhodes, D.N. *J. Sci. Food Agric.* **1975**, *26*, 1743-1754.
64. Sato, K. and Hegarty, G.R. *J. Food Sci.* **1971**, *36*, 1098-1102.
65. Younathan, M.T. and Watts, B.M. *Food Res.* **1959**, *24*, 728-734.
66. Hadden, J.P., Ockerman, H.W., Cahill, V.R., Parrett, N.A. and Borton, R.J. *J. Food Sci.* **1975**, *40*, 626-630.
67. Fooladi, M.H., Pearson, A.M., Coleman, T.H. and Merkel, R.A. *Food Chem.* **1979**, *4*, 283-292.
68. Ockerman, H.W., Blumer, T.N. and Craig, H.B. *J. Food Sci.* **1964**, *29*, 123-129.
69. Cross, C.K. and Ziegler, P. *J. Food Sci.* **1965**, *30*, 610-614.
70. Reid, D.H., Young, O.A. and Braggins, T.J. *Meat Sci.* **1993**, *35*, 171-182.

71. Wood, D.S., Collins-Thompson, D.L., Usborne, W.R. and Picard, B. J. *Food Prot.* **1986**, *49*, 691-695.
72. Shahidi, F., Rubin, L.J. and Wood, D.F. *J. Food Sci.* **1987**, *52*, 564-567.
73. Shahidi, F., Rubin, L.J. and Wood, D.F. *Meat Sci.* **1988**, *22*, 73-80.
74. Shahidi, F. and Pegg, R.B. *Food Chem.* **1990**, *38*, 61-68.
75. Shahidi, F. and Pegg, R.B. *J. Muscle Foods* **1991**, *2*, 297-304.
76. Shahidi, F. and Pegg, R.B. *Food Chem.* **1992**, *43*, 185-191.
77. Ihekoronye, A.I. *Die Nahrung* **1990**, *34*, 81-88.

RECEIVED December 16, 1993

Chapter 19

Control of the Production of *cis*-3-Hexenal, Lipid-Derived Flavor Compound, by Plant Cell Culture

Shan-rong Chou and Chee-Kok Chin

Department of Plant Science, Rutgers, The State University of New Jersey, New Brunswick, NJ 08903

The feasibility of using plant cell culture to produce *cis*-3-hexenal, a valuable flavor compound derived from linolenic acid was explored. A green alfalfa cell line capable of producing *cis*-3-hexenal was established. The level of production, however, was relatively low; in the range of 80 to 180 $\mu\text{g/g}$ fresh weight. The level of production could be increased by manipulation of the culture medium and culture condition. The results revealed that *cis*-3-hexenal production was limited by low availability of the precursor linolenic acid and low level of hydroperoxide lyase, a key enzyme in the biosynthetic pathway. Various approaches, including biotransformation to overcome these limiting steps were examined.

C_6 aldehydes including *cis*-3-hexenal, *trans*-2-hexenal, and *n*-hexenal have a characteristic fresh 'green note' flavor. They are present in various fresh fruit and vegetables. These C_6 aldehydes contribute to the flavor of freshness of the produce. Since they are volatile compounds, they are easily lost during processing such as sterilization. To reconstitute the freshness flavor, it is necessary to add back these compounds at a later stage of processing. Also, in food industry C_6 aldehydes are important flavoring agents in many manufactured food products.

Although C_6 aldehydes are ubiquitous in nature, so far no plant has been found to contain high enough quantity to make it economically practical as a source of the compounds. Chemical synthesis of *cis*-3-hexenal has been achieved (1). Nevertheless, consumers in general prefer natural compounds over ones synthesized chemically.

In recent years with the advancement of plant cell culture technology it is possible to grow plant cells in large quantity in controlled environment similar to growing microorganisms (2). Cultured plant cells could be an alternative to whole plant as a source of phytochemicals. There are certain advantages of cultured cells over whole plant in phytochemical production. First, because the cells are cultured

0097-6156/94/0558-0282\$08.00/0
© 1994 American Chemical Society

in controlled conditions, the regulation of production can be more easily monitored. Thus if the production levels are low, the limiting steps are easier to identify. Second, cultured plant cells have been found to be amenable to manipulation to increase the yield of phytochemicals (3). Here we report our attempts to produce C₆ aldehyde with cultured alfalfa cells.

Material and Methods

Establishment of Cell Culture. Alfalfa (*Medicago sativa*) seeds were sterilized in 10% Clorox containing 0.1% Tween 20 for 20 min, and rinsed 4 times in sterile, distilled water. The seeds were kept moist in a sterile container over night, and the next day were re-sterilized, as described above. Five seeds were transferred to a test tube (25 x 100 mm) containing 10 ml of half strength Murashige Skoog (MS) medium (4) containing 3% sucrose, 0.6% agar and no growth regulators. Six-day-old seedlings were cut into pieces of approximately 7 mm in length and placed on MS medium, pH 5.8, containing 12.8 μ M NAA, 12.8 μ M BA, 2% sucrose, and 0.6% agar. The cultures were kept in a culture room at 25 \pm 2 $^{\circ}$ C under Coolwhite fluorescent light (30 μ mol/m²/s) with a 16-hour photoperiod. Large amounts of loose callus tissues formed on these cultures. The callus tissues were teased off and transferred to 60 ml of liquid MS medium containing the same hormone combination in 125 ml flasks. The cultures were maintained at 25 \pm 2 $^{\circ}$ C on a gyrotary shaker at 200 rpm, and unless otherwise indicated, suspension cell cultures were kept in the dark. The cultures were maintained by transferring 10 ml of 7-day-old culture to 50 ml of fresh medium weekly.

Determination of C₆ Aldehyde Production. C₆ aldehyde was determined by a method described by Hatanaka et. al. (5). One gram of cells was homogenized in 10 ml of distilled water with a Virtis blender at maximum speed for 30 sec. The homogenate was filtered with one layer of Miracloth, then transferred to a test tube (25 mm x 100 mm) and 100 mg of heptanol was added as an internal standard. The filtrate was flushed with oxygen for 5 seconds, capped with a serum stopper, and incubated at 37 $^{\circ}$ C for 10 min. One ml of head space vapor was injected into a Hewlett-Packard 5840A Gas Chromatograph equipped with a J&W DBWAX 20 M column and an FID detector. Amounts of *trans*-2-hexenal were determined according to a precalibrated standard curve using heptanol as internal standard.

Lipoxygenase Activity Assay. Lipoxygenase (LOX) activity of cell and leaf homogenate was measured by the amount of oxygen taken up at 25 $^{\circ}$ C with an oxygen analyzer (Hansatech DWI Oxygen Electrode from Decagon Instrument, Pullman, Washington). Two grams of leaf tissues or cultured cells were homogenized in 10 ml of 0.025 M McIlvaine's buffer, pH 6.8, containing 0.4 M sucrose with a Virtis blender at maximum speed for 30 sec. The homogenate was filtered with one layer of Miracloth. The reaction mixture contained 0.9 ml of the extract and 0.1 ml (15 μ moles) of linoleic acid. Enzyme activity was calculated from the initial rate of oxygen uptake, assuming an initial dissolved oxygen

concentration of 0.24 mM. The reaction mixture without substrate or enzyme was used as a control.

Hydroperoxide Lyase Assay. Hydroperoxide lyase (HLA) catalyses the conversion of 13-hydroperoxide of linolenic acid to *cis*-3-hexenal. Hydroperoxide lyase activity was assayed by the disappearance of 13 hydroperoxide as indicated by the decrease in absorbance at 234 nm using a recording spectrophotometer (6). The reaction mixture contained 0.6 ml of the 13-hydroperoxide solution, 0.1 ml of enzyme solution, and 0.1 M potassium phosphate buffer, pH 6.5, in a final volume of 3 ml. The reaction was run at room temperature and was initiated by the addition of the enzyme solution.

Fatty acid Analysis. Fatty acids were extracted and analyzed by the method of Folch et. al. (7). Two grams (fresh Wt) of leaf tissue or cultured cells were homogenized with 40 ml of chloroform-methanol (2:1) in a TenBrock tissue grinder. The homogenate was filtered with Whatman No. 2 paper and the filtrate transferred to a separation funnel. Ten ml of water were added to the filtrate and the chloroform fraction was collected and transferred to a Buchi evaporator and evaporated to dryness in vacuo at 35°C. The residue was redissolved in chloroform and transferred to a reaction vial and dried under a stream of nitrogen gas. Two milligrams of heptadecanoic acid dissolved in 1 ml of toluene was added as internal standard. One ml of methanolic-base reagent was added and the mixture was incubated at 80°C for 30 min. After the reaction mixture cooled, 3 ml of water and 3 ml of methylene chloride was added. The organic fraction was then separated and injected into a GC.

Chlorophyll Analysis. Chlorophyll was analyzed by the method of Mackinney (8).

Protein Determination. Protein was determined by the method of Lowry et al (9).

Preparation of 13-Hydroperoxy Linolenic Acid. 13-Hydroperoxy linolenic acid was prepared by the method described by Hamberg (10) by incubating 1 ml of 8 mM linolenic acid, 32 ml 0.06 M of borate buffer, pH 9.0, and 2 mg soybean lipoxygenase (Sigma Chemical) in an O₂-saturated atmosphere at room temperature for 40 minutes.

Determination of 13- and 9-Hydroperoxy Linolenic Acids. High performance liquid chromatography was performed with a computerized LDC-HPLC system (Milton-Roy) using a 250-mm i.d. x 4.6 mm Econosil C₁₈ reverse-phased column equilibrated with 70% acetonitrile. Derivatized and filtered *p*-bromophenyl bromide sample (1000 µl) was injected. The elution (flow-rate 1 ml/min) was performed with a linear gradient from 70% to 100% acetonitrile.

Results and Discussion

In this study *cis*-3-hexenal isomerized to *trans*-2-hexenal in our assay process.

Therefore the amounts of *trans*-2-hexenal reported here should represent the sum of *cis*-3-hexenal and *trans*-2-hexenal.

Cultured alfalfa cells produced only 20% of the C₆ aldehyde generated by leaf cells. Compared to leaf cells, cultured alfalfa cells also had less lipoxygenase, hydroperoxide lyase, and chlorophyll (Table I). Hatanaka and Harada reported that in plants, C₆ aldehyde was converted from linolenic acid by lipoxygenase and hydroperoxide lyase (11). The much lower lipoxygenase and hydroperoxide lyase activities found in cultured alfalfa could be the cause of low C₆ aldehyde levels. Another possible reason that the cultured cells produced lower leaf aldehyde was that less precursor of C₆ aldehyde, viz. linolenic acid was available. Table II shows that cultured alfalfa cells had much lower linolenic acid as well as other fatty acids.

It is not known what conditions lead to lower levels of fatty acids in cultured cells. However, it is known that chloroplast is an important site for fatty acid

Table I. *Trans*-2-Hexenal Production, Lipoxygenase, Hydroperoxide Lyase, and Chlorophyll Levels of Alfalfa Leaves and Cultured Cells

Tissue	tr-2-hexenal μmol/g/10min	LOX μmol O ₂ /g/min	HLA μmol/g/10min	Chlorophyll μg/g
Leaf	0.6	0.6	1.5	71
Cell	6.8	42	10.6	3400

Table II. Fatty Acids of Alfalfa Leaves and Cells

Tissue	Fatty acid, mg/g				
	Palmitic	Stearic	Oleic	Linoleic	Linolenic
Leaves	1.10	0.17	0.06	0.73	4.94
Cells	0.41	0.03	0.00	0.61	0.51

Data represent averages of three replicates.

synthesis (12). Chloroplast development is normally dependent on light. When the cells were cultured in the dark as in this case, the cells essentially, as indicated by low chlorophyll level, had no developed chloroplast (Table III). This could be an important reason why the levels of fatty acids including linolenic acid were low. Indeed, providing the cells with light increased the chlorophyll (Table III) and linolenic acid (Table IV) levels. At the same time there was a corresponding increase in C₆ aldehyde production (Table III).

Table III. Effects of Light on Chlorophyll, LOX, HLA and *tr*-2-Hexenal in Cultured Alfalfa Cells

Light Intensity μmol/m ² /s	Chorophyll μg/g	LOX μmole O ² /g/hr	HLA μmole/g/ 10min	<i>tr</i> -2-Hexenal μmole/g
0	8 ± 0.7	19.9 ± 0.1	0.40 ± 0.04	0.61 ± 0.05
5	50 ± 4.3	24.4 ± 0.12	0.52 ± 0.03	0.98 ± 0.07
50	122 ± 1.0	26.0 ± 0.13	0.60 ± 0.05	1.34 ± 0.19

Coolwhite fluorescent light was used as light source. Fourteen-day-old cells were analysed. Data represent averages of three experiments, each with four replicates.

Compared to light intensity in the field which can exceed 2000 μmol/m²/s, the light intensity provided to the cells in our study in the laboratory was much lower, 50 μmol/m²/s; approximately one fortieth of that of the light in the field. To raise the light intensity in the laboratory to that found in the field would generate a large amount of heat beyond the cooling capacity of our culture room leading to significant rise in temperature to a level harmful to the cells. Thus it probably is not practical to increase C₆ aldehyde production much further by raising the light intensity for the cells.

In addition to photosynthesis, cultured green plant cells can produce organic compounds including linolenic acid from exogenous carbon source. Experiment was carried out to examine whether the levels of carbon source (sucrose) in the medium could affect the linolenic acid and C₆ aldehyde levels. Increasing sucrose concentration up to 4% led to increase in C₆ aldehyde production. (Table V), but above 4% sucrose, production of C₆ aldehydes declined moderately. Increased

sucrose concentration also increased the levels of linolenic acid, but concentrations beyond 4% reduced levels of linolenic acid (Table VI). Thus, there was a limitation of using higher sucrose to increase the linolenic acid and C₆ aldehyde levels.

Table IV. Effect of Light on Fatty Acid Levels of Cultured Cells

Light Intensity μmol/m ² /s	Fatty acid, mg/g				
	Palmitic	Stearic	Oleic	Linoleic	Linolenic
0	0.70	0.70	0.01	1.44	0.36
5	0.85	0.02	0.01	1.66	0.72
50	0.82	0.04	0.00	1.35	0.97

Data represent averages of two experiments. Fourteen-day-old cells were analyzed. H eptadecanoic acid were used as internal standard.

Table V. Effects of Sucrose Concentrations on LOX, HLA, and *tr*-2-Hexenal in Cultured Alfalfa Cells

Sucrose %	LOX μmole O ² /g/hr	HLA μmole/g/10min	<i>tr</i> -2-Hexenal μmole/g
1	4.43	0.02 ± 0.0	0.3 ± 0.01
2	5.78 ± 0.19	0.43 ± 0.02	0.83 ± 0.04
3	8.95 ± 0.4	0.49 ± 0.01	1.23 ± 0.06
4	17.03 ± 0.77	0.52 ± 0.01	1.83 ± 0.08
5	21.58 ± 0.69	0.50 ± 0.02	1.57 ± 0.08
6	29.87 ± 1.14	0.49 ± 0.02	1.36 ± 0.06
8	36.87 ± 0.81	0.42 ± 0.02	1.32 ± 0.06

Data represent averages of two experiments, each with four replicates. Fourteen-day-old cells were analyzed.

Table VI. Effects of sucrose concentrations on linolenic acid levels in cultured alfalfa cells

Sucrose %	Linolenic Acid μg/g
2	0.28
3	0.42
4	0.43
5	0.39
6	0.34
8	0.32

Fourteen-day-old cells were analyzed. Data represent averages of three experiments.

Both the light intensity and sucrose concentration experiments showed that linolenic acid and C₆ aldehyde levels appeared to be correlated, i.e., low levels of C₆ aldehydes were caused by low levels of linolenic acid. If this is true then one should be able to increase the C₆ aldehyde levels by raising levels of linolenic acid. One approach to increase the linolenic acid of the cells was to directly supplement the culture medium with linolenic acid. However, it was found that linolenic acid supplementation not only did not increase but actually suppressed the C₆ aldehyde production (Table VII). In addition, it was found that linolenic supplementation reduced chlorophyll levels and hydroperoxide lyase activity. The effects of linolenic acid could be observed even in relatively short time; in 12 hours the chlorophyll level was reduced by 50% and after 48 hours, no chlorophyll was detected (Table VIII). Although there was a rapid bleaching of the chlorophyll, production of C₆ aldehydes did increase in this 48-hour period.

The linolenic acid added to the medium disappeared from the medium quite rapidly; from 719 to 371 μmoles in 3 hours (Table VIII). Initially there was a concomitant increase in 13-hydroperoxy and 9-hydroperoxy linolenic acid in both the medium and the cells. Subsequently the 13-hydroperoxy linolenic acid in the medium and cells declined after 6 hours whereas the 9-hydroperoxyl linolenic acid increased throughout the 48-hour period. The results suggest that the lipoxygenase was more active than hydroperoxide lyase. Consequently, hydroperoxides of linolenic acid were quickly produced, but due to less hydroperoxide lyase activity most of the hydroperoxides of linolenic acid were not converted in timely fashion to C₆ aldehyde but accumulated as peroxides. The results also show that alfalfa cells had two types of lipoxygenase; one converting linolenic acid to 13-hydroperoxide

and the other to 9-hydroperoxide. The bleaching of the chlorophyll was probably the consequence of chlorophyll oxidation by elevated levels of hydroperoxides. Indeed the bleaching of chlorophyll by linolenic acid supplementation could be duplicated by the addition of hydrogen peroxide to the culture. Addition of hydrogen peroxide caused the cells to lose chlorophyll and weight (Table IX). Surprisingly higher amounts of hydrogen peroxide, a known strong oxidant, than linolenic acid was needed to cause the bleaching of chlorophyll and loss of cell weight. Under the microscope, cells damaged by linolenic acid and hydrogen peroxide looked very similar (data not shown).

Table VII. Effects of Linolenic Acid Supplementation on Chlorophyll, Hydroperoxide Lyase and *tr*-2-Hexenal in Cultured Alfalfa Cells

Linolenic Acid mM	Chlorophyll $\mu\text{g/g}$	HLA $\mu\text{mol/g/10min}$	<i>tr</i> -2-Hexenal $\mu\text{mole/g}$
0.0	142.0 \pm 5.8	0.58 \pm 0.03	1.31
0.1	136.2 \pm 3.0	0.52 \pm 0.03	1.10
0.4	51.0 \pm 6.0	0.11 \pm 0.01	0.03
0.8	4.6 \pm 3.2	0.17 \pm 0.01	0.00

Seven-day-old cells were analyzed. Data represent averages of two experiments each with 4 replicates.

In this study we identify two limiting factors of C_6 aldehyde production by cultured alfalfa cells, namely the precursor linolenic acid, and hydroperoxide lyase which converts 13-hydroperoxide to *cis*-3-hexenal. The levels of linolenic acid levels could be moderately increased by raising the light intensity for cell culture and sucrose levels in the culture medium. These elevated levels of linolenic acid led to corresponding moderate increase in C_6 aldehyde production. The levels of linolenic acid could be increased sharply by directly supplying the cells with exogenous linolenic acid. The cells rapidly convert the exogenous linolenic acid to 13- and 9-hydroperoxide of linolenic acid. These hydroperoxides, however, were not converted as quickly to C_6 aldehyde due to low hydroperoxide lyase activity. Apparently if production of C_6 aldehyde by cultured cells is to become practical, ways will have to be found to increase the hydroperoxide lyase activity. Several approaches may be possible. Initially, it would be practical to search for plants with high hydroperoxide lyase and use them to initiate cell cultures. Secondly, one may try to raise the hydroperoxide lyase activity. Several strategies have been demonstrated

Table VIII. Effects of Linolenic Acid on Chlorophyll, 13-Hydroperoxide Linolenic Acid, 9-HPOLNA, and *tr*-2-Hexenal Levels in Cultured Alfalfa Cells

Time hr	Chlorophyll $\mu\text{g/g}$	Linolenic Acid		13-HPOLA		9-HPOLA		<i>tr</i> -2- Hexenal
		Medium μmol	Cells	Medium μmol	Cells	Medium μmol	Cells	
0	178	719.4	39.4	0.0	26.8	0.0	52.1	0.63
3	182	371.1	54.7	137.9	34.3	26.4	127.9	1.11
6	182	324.3	94.5	209.5	55.9	37.2	92.0	1.47
12	81	56.6	61.9	41.3	39.5	43.9	133.6	--
24	23	43.3	54.4	25.1	31.6	72.1	138.5	5.39
48	0	41.3	61.7	27.0	31.5	125.4	161.8	6.66

Linolenic acid was added to give an initial concentration of 0.11%. Data represent averages of two experiments.

Table IX. Effects of Hydrogen Peroxide Concentrations on Growth and Chlorophyll Levels in Cultured Alfalfa Cells

H_2O_2 mM	Cell Weight g	Chlorophyll $\mu\text{g/g}$
0	13.03	144
0.6	12.40	136
1.2	11.35	116
2.4	9.99	96
4.8	8.43	77

Hydrogen peroxide was added to 7-day-old cell culture. Analysis was performed 7 days later. Data represent averages of two experiments.

to be effective in raising the activities of certain enzymes in culture plant cell. The strategies include using compounds called elicitors which induce production of enzymes and adjusting cultural conditions to promote the production of certain enzymes (13, 14, 15). A third approach presupposes that the low hydroperoxide lyase activity found in the cells is due to low amount of the enzyme produced. If regulation of expression of the hydroperoxide gene is modified to allow high expression, then more enzyme will be produced and the total activity will then increase. Since gene expression is controlled by regulatory sequences including the promotor sequence (16), recombinant DNA technology could be utilized to increase the expression of hydroperoxide lyase. This approach will require isolating and cloning of the hydroperoxide lyase gene, substituting the native regulatory sequence with one which allows very high expression and reintroducing the modified hydroperoxide gene back into the plant cells (14). This should produce cells with higher hydroperoxide lyase activity. Hopefully, with cells possessing higher hydroperoxide lyase we should be able to increase C₆ aldehyde production by direct linolenic acid supplementation.

Literature Cited

1. Kajiwara, T.; Harada, T.; Hatanaka, A. *Agric. Biol. Chem.*; **1975**; *39*, pp 243-247.
2. Chin, C-K.; Pedersen, H. In *Phenolic Compounds in Food and their Effects on Health I*; Ho, C-T.; Lee, C.Y.; Huang, M-T., Eds.; ACS Symp. Ser. American Chemical Society: Washington, DC, 1992, 506, pp 51-56.
3. Berlin, J. *Endeavour* **1984**; *8*; 5-8.
4. Murashige, T.; Skoog, F. *Physiol. Plant*; **1962**; *15*, 473-497.
5. Hatanaka, A.; Kajiwara, T.; Sekiya, J.; Hirata, H. *Agric. Biol. Chem.*; **1976**; *40*, 2177-2180.
6. Galliard, T.; Mathew, J.A.; Fishwick, M.J.; Wright, A.J. *Phytochem.*; **1976**; *15*, 1647-1650.
7. Folch, J.; Lees, M.; Stanley, G.H.S. *J. Biol. Chem.*; **1957**; *226*, 497-509.
8. Mackinney, G. *J. Biol. Chem.*; **1941**; *140*, 315-322.
9. Lowry, O.H.; Rosebrough, N.J.; Farr, A.L.; Randall, R.J. *J. Biol. Chem.*; **1951**; *193*, 265-275.
10. Hamberg, M. *Anal. Biochem.*; **1971**; *43*, 515.
11. Hatanaka, A.; Kajiwara, T.; Sekiya, J. In *Biogenesis of Aromas*; T.H. Parliment and R. Croteau; Eds. Amer. Chem Soc., Washington, **1986**, 167-175.
12. Harwood, J.L. *Ann. Rev. Plant Physiol. Plant Mol. Biol.*; **1988**; *39*, 101-138.
13. Pedersen, H.; Cho, G.H.; Hamilton, R.; Chin, C-K., *Ann. New York Acad. Sci.*, **1987**; *506*, 163-170.
14. Raskin, I. *Salicylate, Plant Physiology*; **1992**; *99*, 799-803.
15. Staswick, P.E. *Jasmonate, genes, and fragrant signals, Plant Physiology*; **1992**; *99*, 804-807.
16. Darnell, J; Lodish, H.; Baltimore, D.; *Mol. Cell Biol*; **1990**, 391-449.
17. Dittrich, H. *Proc. Nat. Acad. of Sci., USA*, **1991**; *88*, 9969-9973.

RECEIVED November 4, 1993

Chapter 20

Canola Oil Flavor Quality Evaluation by Dynamic Headspace Gas Chromatography

S. K. Raghavan, D. R. Connell, and A. Khayat

Hunt-Wesson, Inc., 1645 West Valencia Drive, Fullerton, CA 92633

A procedure for rapid and automated evaluation of the flavor quality of canola oils was developed based on the quantitation of selected lipid oxidation products. Oil volatiles were stripped for 20 minutes at 150°C into a Tenax adsorbent and transferred to a gas chromatograph fitted with a 60 meter DX-1 capillary column. Typical lipid oxidation products observed include pentanal, hexanal, octanal, nonanal and decadienal. Unlike most other edible oils, the major breakdown products from this oil are the *cis*, *trans*- and *trans*, *trans*-2,4-heptadienals with an odor character generally described as oily, fatty and putty. Flavor equations were developed by correlating selected known volatile compounds to the traditional sensory evaluation flavor score using stepwise multiple regression analysis. Using several three component equations, regression coefficients ranging from 0.8 to 0.92 were obtained. Typical aged oils taken from supermarket shelves showed a significant increase in most volatile degradation compounds over the fresh oils particularly 2-pentenal (44 fold increase), *t,t*-2,4-decadienal (12 fold) and *t,t*-heptadienal (13 fold).

Rapeseed crop varieties, particularly those originating from Asia, are generally high in erucic acid (HEAR) and glucosinolates. The anti-nutritive nature of these constituents render these oils undesirable for general consumption or for use as animal feed. Erucic acid content of HEAR oils generally range from 22 % to 60 %. Low erucic acid oils (LEAR) are genetic modifications of HEAR varieties that result in an overall erucic acid content under 5 %. These oils are also referred to as single-low or single-zero varieties. Canola oil is a LEAR oil with less than 2 % erucic acid content and with low glucosinolates level. This combination is

0097-6156/94/0558-0292\$08.00/0
© 1994 American Chemical Society

often referred to as double-low or double-zero varieties. Triple-low or triple-zero varieties also were cultivated with low levels of erucic acid, glucosinolates and fiber.

Canola oil is a low erucic acid rapeseed oil produced primarily in Canada, China, Japan and the European community. Primarily a polyunsaturated oil, canola oil has a high oleic acid content that typically exceeds 60 % and a total unsaturated content of greater than 94 %. The highly unsaturated nature of this oil along with possibly the relatively high level of chlorophyll makes it susceptible to oxidative degradation. Market demand for canola oil as an edible oil has been strong over the past few years. While mostly used as salad oils, canola oil is rapidly gaining acceptance as a substitute for vegetable oil in general food preparation uses. Much of this demand can be attributed to the nature and amount of its unsaturated fatty acids. Erucic acid content of canola oil is generally at or below 0.5 %. On the average, canola oil consists of less than 6% saturated fat (Table I). It also is unique among edible oils in the relatively high amount of linolenic acid content. It has been suggested that the ratio of linoleic to linolenic acid in canola oil, at approximately 2:1, is nutritionally desirable (1).

Table I. Fatty Acid Composition of Selected Oils

Fatty Acid	Canola	Soybean	Corn	Peanut	Olive
14 : 0	0.04	0.1	-	0.1	-
16 : 0	3.5	10.8	11.4	10	11
18 : 0	1.5	4	1.9	2.3	2.2
20 : 0	0.5	-	-	-	-
22 : 0	0.2	-	-	-	-
Total Sat.	5.74	14.8	13.3	17.8	13.2
16 : 1	0.2	0.2	-	0.1	0.8
18 : 1	61.7	23.8	25.3	47.1	75.8
20 : 1	1.4	0.2	-	1.4	0.3
22 : 1	0.5	-	-	-	-
Total Mono.	63.8	24.2	25.3	48.6	76.9
18 : 2	19.7	53.3	60.7	33.6	8.3
18 : 3	10.7	7.1	0.7	-	0.6
Total Poly.	30.4	60.4	61.4	33.6	8.9

Instrumental approach for the determination of oil flavor quality is a well established technique that has been successfully demonstrated by many researchers (2,3). Most of this work was based on the correlation between one or more of the volatile degradation components of an oil and its sensory flavor score. Many laboratories including ours have obtained excellent correlations for soybean, corn and olive oil volatiles with their sensory scores (4,5). Typically, such studies include compounds such as pentane, hexanal, and decadienal as well as the total volatiles. Our studies have also demonstrated that the major volatile emissions from fresh and aged canola oil are the *cis, trans*- and *trans, trans*-2,4-heptadienals. Originating from the breakdown of linolenic acid, their odors have been generally described as fatty, putty and deep fried. In comparison, the major decomposition products from soybean, corn and olive oils that originate primarily from the decomposition of linoleic acid are hexanal, *trans*-2-heptenal and 2,4-decadienal with olive oil also generating large quantities of nonanal, a decomposition product of oleic acid (Table II). Unique volatile constituents have been reported for canola oil including trace amounts of tetrahydrofuran, ethyl furan and ethyl acetate (6).

Table II. Major Volatile Compounds from Edible Oils

Compound	Origin	Amount (ppb)			
		Canola	Soybean	Corn	Olive
heptanal	C18:1	10.4	12.5	24.7	74.2
octanal	C18:1	20.6	22.3	24.1	32.0
nonanal	C18:1	48.9	83.2	58.9	578.0
hexanal	C18:2	28.7	36.0	146.4	455.9
<i>t</i> -2-heptenal	C18:2	44.4	54.0	164.0	214.3
<i>t</i> -2-octenal	C18:2	99.9	89.7	91.4	239.2
2,4-nonadienal	C18:2	11.0	4.4	7.6	17.7
<i>c, t</i> -2,4-decadienal	C18:2	30.9	28.8	47.3	66.6
<i>t, t</i> -2,4-decadienal	C18:2	49.0	55.8	83.0	178.1
2-pentenal	C18:3	15.3	6.1	1.1	12.1
<i>c, t</i> -2,4-heptadienal	C18:3	99.9	43.7	21.2	16.5
<i>t, t</i> -2,4-heptadienal	C18:3	115.8	32.1	10.4	54.3

Dynamic headspace analysis using capillary gas chromatography has been the method of choice of most recent researchers investigating oil flavor quality based on its excellent sensitivity, selectivity and reproducibility. Many variations of this technique have been published and successfully demonstrated including purge and trap techniques that use polymer adsorbents and/or cryofocussing. Where speed of operation has been a priority, particularly in routine quality audits, headspace enrichment adsorbent traps for sample preconcentration along with auto sampling capability have been extremely effective. Using such a system, good correlations have been demonstrated for determining the flavor attribute of soybean oil using the aldehydes *t*-2-heptenal, *t*-2-octenal and *trans*, *trans*-2,4-decadienal (7). Similar results have also been observed for other edible oils (8). While there have been many published reports on the volatile degradation compounds from fresh and aged canola oils, the emphasis in this study is to develop a procedure for correlating canola oil volatiles to their sensory flavor scores. The method presented here will be a rapid, automated, sample enrichment based purge and trap analysis with capillary gas chromatography. Volatile compounds will be identified and quantified in a number of fresh and aged oils and selected components will be statistically correlated with sensory evaluation data using stepwise regression analysis.

Experimental

Most of the oils used in this study were part of a nationwide quality audit and were selected to cover a wide range of production dates. Fresh oils were obtained directly from the oil refinery. Owing to the difficulty of finding oils of extremely rancid quality, rancidity was induced through a modification of the Schaal oven test procedure. All oils were stored in 100 ml. vials and the headspace volume blanketed with high purity nitrogen gas. The vials were stored in a -10° C freezer and individual vials were thawed to room temperature prior to analysis. Peroxide values were determined for all the oils.

One gram of oil was placed in a clean 10 cc glass tube, blanketed with nitrogen and purged with ultra high purity nitrogen gas at a flow rate of 50 ml/min for 20 minutes at 150° C. Volatiles were trapped in a polymer adsorbent consisting of Tenax, Ambersorb, charcoal and glass beads. Through a series of narrow bore focusing traps, the purged volatiles were desorbed using a Dynatherm Model 910 Multiple Thermal Desorption Unit and analyzed with a Shimadzu 14A gas chromatograph fitted with a 60m x 0.32 mm DX-1 fused silica capillary column (J&W Scientific) and a flame ionization detector. Up to eight samples could be loaded simultaneously on this unit. For gas chromatographic separation, the column was initiated at 50° C and ramped at 5° C/min to a final temperature of 210° C for a total run time of 30 minutes. Run times under 25 minutes were successfully demonstrated by using a ramp of 7° C/min. However, under these conditions, certain volatiles including hexanal are only partially resolved and cannot be used reliably in establishing sensory correlations. Methyl nonanoate

was used as the internal standard for quantitation of volatiles. All identifications were done on a Finnigan ITS-40 Ion Trap Mass Spectrometer.

Sensory analysis was performed by a seven member expert panel using a 10 point hedonic scale with the following descriptors: extreme to strong (1-2), strong (3-5), moderate (6-7), slight (8-9) and bland (10). Statistical analysis was based on stepwise regression analysis which determines combinations of these aldehydes that best match the sensory data. Components are entered into the classification process one at a time until correlation ceases to improve notably.

Results and Discussion

Peroxide value of the oils that were selected for this study ranged from 0.2 meq/kg to 11 meq/kg. Headspace volatiles from decomposition of these lipid hydroperoxides observed in a typical fresh and aged canola oil is seen in Figure 1. The flavor character of fresh canola oil with peroxide value of 0.2 meq/kg is relatively odorless and is generally described as bland. Major compounds in this profile include the *cis*, *trans*- and *trans*, *trans*-2,4-heptadienals (fatty, putty, deep fried), hexanal (green), *trans*-2-heptenal (fatty, soapy, fruity), *trans*-2-octenal (fatty, nutty), nonanal (tallowy) and *trans*, *trans*-2,4-decadienal (deep fried). Most of these compounds, however, appear below their odor detection threshold in freshly processed canola oil and are not perceived. Of the major degradation byproducts, only hexanal and *trans*, *trans*-2,4-decadienal exceed their odor detection threshold and contribute to the aroma of this oil. In many instances, direct correlations between peroxide values and the concentration of individual aldehydes were observed. For instance, a regression coefficient (r^2) of 0.83 was determined for 2,4-decadienal and the peroxide value. Correlation between peroxide values and sensory scores were generally poorer. This may be due to the differences in sensory perception unique to canola oil. While a peroxide value of 2 meq/kg or above is indicative of rancidity in soybean oil, canola oil with a peroxide value of 10 was considered acceptable by taste panelists (9). Furthermore, peroxide values may not indicate the actual extent of oil degradation because the lipid hydroperoxides formed decompose readily during storage and heating. The volatile profile of fresh canola oil is similar in composition to that of soybean oil (Figure 2). However, in canola oil the major components are the *cis*, *trans*- and *trans*, *trans*-2-heptadienals. In comparison the major compounds observed in fresh soybean, corn and olive oils are *trans*-2-heptenal, hexanal and nonanal respectively.

Aged canola oil shows a sharp increase in all known degradation products with all major compounds appearing well above their odor detection thresholds. The aroma is dominated by *cis*, *trans*- and *trans*, *trans*-2,4-heptadienals, hexanal, nonanal and the *cis*, *trans*- and *trans*, *trans*-2,4-decadienals. Octen-3-one, which has a strong metallic flavor which is generally considered an off-odor in oils, occurs at a concentration of 75 ppb in this aged oil which is approximately 750 times above its odor detection threshold. Despite its relatively low concentration,

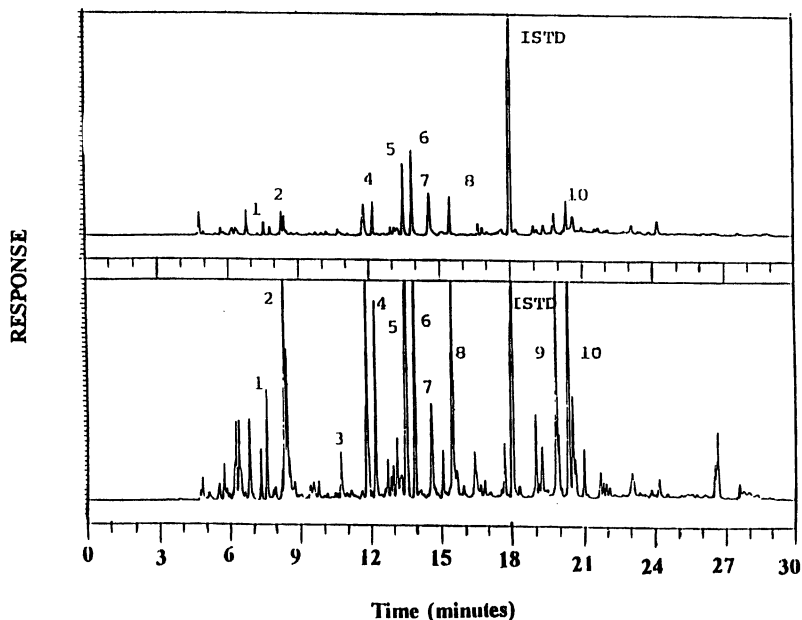


Figure 1. Gas chromatographic profile of fresh (top) and aged canola oils. (1 = 2-pentenal; 2 = hexanal; 3 = heptanal; 4 = 2-heptenal; 5, 6 = *c,t* and *t,t*-2,4-heptadienals; 7 = 2-octenal; 8 = nonanal; 9, 10 = *c,t* and *t,t*-2,4-decadienals; ISTD = internal standard)

metallic note of this compound contributes significantly to the overall flavor character of the aged oil. All these compounds, in combination, contribute to the overall rancidity of aged canola oil.

Response was determined at concentrations ranging from 20 ppb to 1 ppm. Using paraffin oil, sensitivity in the sub-part per trillion level was demonstrated for hexanal. Response of six selected aldehydes representing the major compounds observed at these concentrations was extremely linear with r^2 of 0.99 determined for most compounds. Precision for these six aldehydes was determined from quintuplicate analyses for both fresh and aged oils. Coefficient of variation ranged from 0.9 % to 14.9 % with an average of 6.4 % (Table III). Recoveries, determined at six concentration levels for each of these aldehydes, ranged from an average of 95 % to 104 %. Relatively high variation observed for nonanal and 2,4-decadienal (> 10 %) is a result of contaminating artifacts from previous runs coeluting with these peaks. In high volume, rapid analyses this is not an uncommon occurrence, particularly with the higher molecular weight

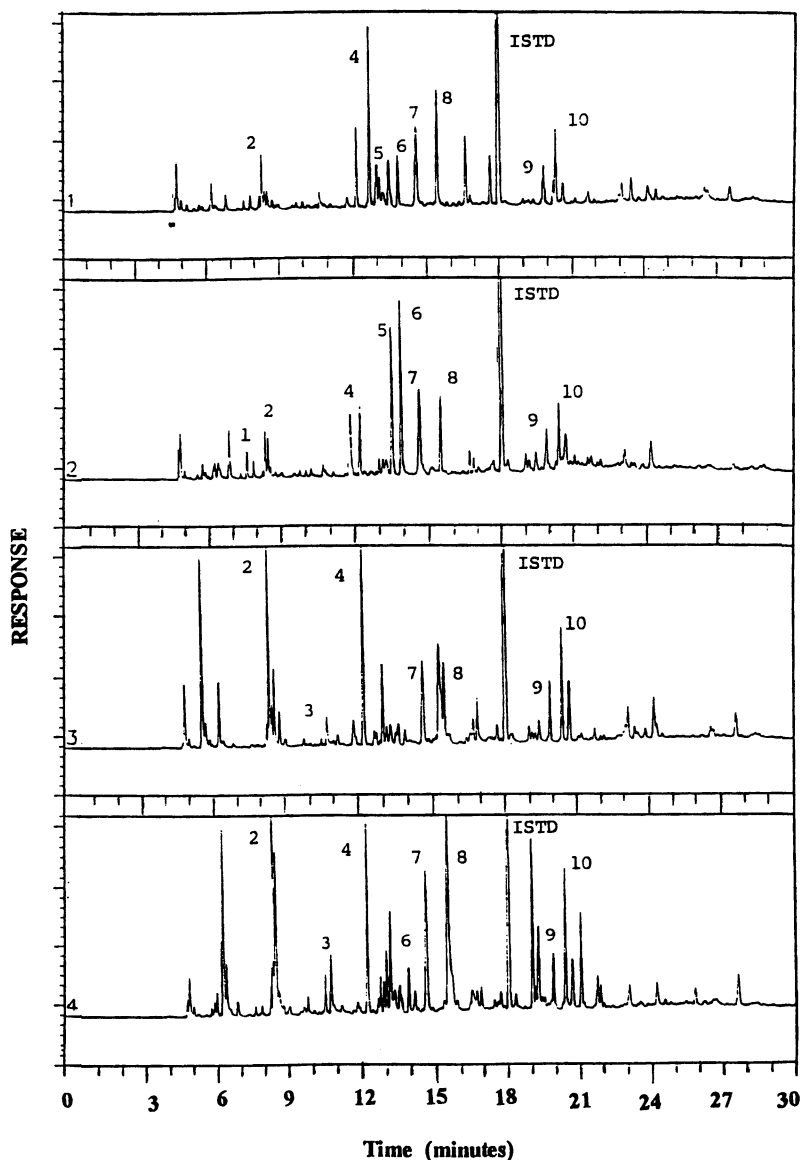


Figure 2. Gas chromatographic profile of soybean, canola, corn and olive oils (top to bottom) of similar production age. (1 = 2-pentenal; 2 = hexanal; 3 = heptanal; 4 = 2-heptenal; 5, 6 = *c,t* and *t,t*-2,4-heptadienals; 7 = 2-octenal; 8 = nonenal; 9, 10 = *c,t* and *t,t*-2,4-decadienals; ISTD = internal standard)

Table III. Replicate Analysis of Fresh and Aged Canola Oils

Sample	Concentration (ppb)					
	2-pentenal	hexanal	heptanal	2,4-hepta-dienal	nonanal	2,4-deca-dienal
Fresh	8.3	113.5	34.3	77.7	113.4	104.2
	10.3	112.1	36.2	88.9	86.7	109.1
	9.2	114.7	31.2	80.8	95.3	102.9
Mean	9.3	113.4	33.9	82.4	98.5	105.4
CV (%)	9.6	2.3	6.6	5.7	14.9	2.0
Aged Oil	388.5	753.1	157.9	1064.3	401.2	1231.5
	422.0	774.2	178.3	1169.4	405.9	1391.6
	388.9	722.3	159.7	997.5	430.0	1103.2
Mean	399.8	749.9	165.3	1077.1	412.4	1242.1
CV (%)	0.9	4.0	6.9	8.4	3.0	12.0

compounds. If desired, cleaning cycles can be implemented for the traps and column to minimize this effect. Careful attention is also needed during setup for accurate measurement of decadienal particularly in establishing proper temperatures in the transfer zones. With adequate attention, variation below 5 % (CV) is consistently observed.

In this study, all the major volatiles that were conclusively identified were considered for correlation with taste panel flavor scores. Using a procedure based on the studies of Min (10) and others, the concentration of the volatile components of these oils were correlated to their sensory flavor scores. With stepwise regression analysis, single components were matched with sensory data followed by two-set components, three-set components, and so on until the entire list of compounds selected was exhausted. Equations were developed for each of these models based on the following relationship:

$$\text{Oil Flavor Score} = b - [(m \times A_1) + (m \times A_2) + \dots (m \times A_n)]$$

where b = y-intercept, m = slope, and A_n = normalized peak area for compound n .

On this basis, instrumental flavor scores developed from these equations were compared to sensory flavor scores. A three component model with pentenal,

heptadienal and decadienal produced a regression coefficient of 0.87. These three compounds, originating from linolenic acid degradation, are the three most abundant volatiles observed in aged canola oils. Regression values improved significantly with a combination of 2,4-hexadienal, 2-heptenal and 2-octenal ($r^2 = 0.92$). Even better correlations were observed when models with more than three components were developed. However, owing to the complexities of incorporating many compounds in the model, such equations are not practical in high volume, routine analyses.

The unique polyunsaturated fatty acid composition of canola oil renders it more prone to lipid oxidation than most other edible oils. Lipid hydroperoxides are readily formed and degrade rapidly to form the volatile oxidation products. The approach outlined here offers an effective and reliable manner to determine the flavor quality of canola oil based on these volatile oxidation products. With excellent sensitivity and precision, the predicted flavor scores for canola oil correlate well with traditional sensory scores in a rapid and continuous operation.

Literature Cited

1. Ackman, R. G. In *Canola and Rapeseed: Production, Chemistry, Nutrition and Processing Technology*; Shahidi, F., Ed.; Van Nostrand Reinhold: New York, NY, 1990, pp 81-98.
2. Fraser, M. S.; Khayat, A. *J. Amer. Oil Chem. Soc.* **1986**, *63*, 410.
3. Williams, J. L.; Applewhite, T.H. *J. Amer. Oil Chem. Soc.* **1977**, *54*, 461-463.
4. Dupuy, H. P.; Rayner, E. T.; Wadsworth, J. I.; Legendre, M. G. *J. Amer. Oil Chem. Soc.* **1976**, *54*, 445-449.
5. Min, D. B. *J. Amer. Oil Chem. Soc.* **1983**, *60*, 544-545.
6. Snyder, J. M.; Frankel, E. N.; Selke, E. *J. Amer. Oil Chem. Soc.* **1985**, *62*, 1675-1679.
7. Raghavan, S.K.; Reeder, S.; Khayat, A. *J. Amer. Oil Chem. Soc.* **1989**, *66*, 942-947.
8. Raghavan, S. K.; Connell, D. R.; Khayat, A. Presented at ACS Annual Meeting, Denver, CO, 1993.
9. Hawrysh, Z.J. In *Canola and Rapeseed: Production, Chemistry, Nutrition and Processing Technology*; Shahidi, F., Ed.; Van Nostrand Reinhold: New York, NY, 1990, pp 99-122.
10. Min, D.B. *J. Food Sci.* **1981**, *46*, 453-456.

RECEIVED December 16, 1993

Chapter 21

Stabilization of Canola Oil by Natural Antioxidants

Fereidoon Shahidi and Udaya Wanasundara

Department of Biochemistry, Memorial University of Newfoundland,
St. John's, Newfoundland A1B 3X9, Canada

Canola is a major source of vegetable oil in the world. Canada is the main producer and exporter of canola products. The content of saturated fatty acids of canola oil is the lowest among all common sources of vegetable oil. However, stabilization of canola oil is required in order to prevent the formation of undesirable flavors during storage and heating. Stability of canola oil as such and as affected by a number of synthetic antioxidants and novel natural ingredients, under Schaal oven test conditions at 65°C, was monitored by determining the weight gain, content of peroxides and thiobarbituric acid reactive substances (TBARS). Novel canola extracts and a number of flavonoids of natural origin were tested. Oxidative stability of the oils treated with natural ingredients was considerably better than that of the control, and in many cases were equivalent or superior to that imparted by commonly used synthetic antioxidants.

Canola is the most important vegetable oil in Canada. In 1991, canola accounted for 79% of salad/cooking oils, and 47% and 59% of vegetable oils used for production of margarine and shortening in Canada, respectively (1). Development of genetically improved, low erucic acid, canola varieties boosted the World demand for canola oil and increased its use in foods. The ratio of linolenic (C18:3) to linoleic (C18:2) acids in the oil is approximately 1:2 and this is considered to be nutritionally favorable (2). However, development of off-flavors, due to the autoxidation of unsaturated fatty acids in canola oil, especially C18:3 is often criticised (3). The oxidative deterioration of canola proceeds via a similar mechanism as for other vegetable oils (4) and involves primarily autoxidative reactions followed by those having oxidative and non-oxidative character.

0097-6156/94/0558-0301\$08.00/0
© 1994 American Chemical Society

Hydroperoxides are the primary products of lipid oxidation, however, they do not contribute to flavor as they are bland in taste and are colorless and odorless (5). Upon decomposition of hydroperoxides, however, secondary products such as hydrocarbons, alcohols, ketones and aldehydes are produced. These compounds influence the flavor of oils.

The degree of unsaturation of a fatty acid has a significant effect on its oxidation rate. The relative rate of oxygen uptake and hydroperoxide decomposition of C18:3 are much faster than those of C18:2 and oleic acid (C18:1) (6). Due to rapid oxidation of linolenic acid, development of off-flavor in canola oil is of concern to processors. Linolenic acid accounts for 10-12% of the total fatty acids in canola oil. In addition, traces of heavy metals, especially those of iron and copper in edible oils are known to have a marked effect on the rate of oil oxidation (7).

Durkee (8) first suggested that C18:3 is an important precursor of the off-flavor compounds. Frankel (5) summarized supporting data for this theory and confirmed that compounds responsible for off-flavor character of soybean oils were derived from linolenic acid. These compounds included acetaldehyde, propanal, pentanal, hexanal, 2,4-heptadienal, 2,4,7-decatrinal and 2-pentenyl furan. Mechanisms for the breakdown of pure linolenate, linoleate and oleate hydroperoxides, as well as some products of further decomposition of secondary oxidation products have been discussed by Frankel (9). Snyder *et al.* (10) have analyzed volatile components of several commercially processed vegetable oils, including canola and soybean oils, both fresh and after storage under Schaal oven test conditions. The volatile compounds found in the stored canola oil were associated with the major fatty acids present in the oil. Unlike other vegetable oils, canola and soybean, which contain large amounts of C18:3, produced measurable quantities of 2,4-heptadienal (11,12). Canola oil formed more nonanal and a much smaller concentration of propane than most of the other oils. Trace amounts of tetrahydrofuran, ethyl furan and ethyl acetate were found only in canola oil. In the oils containing polyunsaturated fatty acids, the substantial increases in the amounts of pentane, hexanal and 2-heptenal during storage were related to the presence of C18:3. Hawrysh (4) has also examined the major volatiles of canola oil and found that large amounts of butane/pentane, hexanal and 2,4-decadienals were formed under accelerated oxidation conditions. Formation of these volatile compounds coincided with oxidative deterioration of oils. Therefore, stabilization of canola oil is required in order to prevent the formation of off-flavors.

Antioxidants are major ingredients which protect the quality of oils by retarding oxidation. In the edible oil industry, synthetic antioxidants and chelating agents are often used because they are effective and inexpensive. Currently BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene) and a mixture of BHA/BHT/MGC (monoglyceride citrate) are used as antioxidants in the canola oil industry. However, increased popularity of natural food additives may prompt more food manufacturers to replace synthetic antioxidants with

ingredients containing natural antioxidative compounds (13). Therefore, research on natural ingredients has gained momentum as they are generally considered to pose no health risk to the consumers. Most naturally-occurring antioxidative compounds are flavonoids, phenolic acids, lignans, terpenes, phospholipids and polyfunctional organic acids (14). Tocopherols (α , β , γ and δ) occur as minor constituents in all vegetable oils, and are among the best known and most widely used natural antioxidants. Their antioxidant activity is in the order of δ -tocopherol (most effective) > γ -tocopherol > β -tocopherol > α -tocopherol (least effective) (15). Canola meal has been reported to have a high content of phenolic compounds (1-2% of defatted meal) (16,17). These compounds include phenolic acids (18,19), flavonoids (20) and condensed tannins (21). However, presence of high levels of phenolic compounds in canola meal is undesirable due to their adverse effects on the nutritional and organoleptic properties of the meal (22). Therefore, removal of phenolic compounds from canola meal and their proper utilization would present new opportunities for the canola meal industry.

Plant flavonoids are recognized as important compounds in conferring stability against autoxidation of vegetable oils (23). The relationship between the chemical structure of flavonoids and their antioxidant activity has been thoroughly investigated (24-26). Effectiveness of flavonoids in retarding lipid oxidation in lipid-containing foods is related to their ability to act as free radical acceptors (25,26), or as chelators of metal ions. Metal chelation by flavonoids is due to ortho-dihydroxy (3',4'-dihydroxy) grouping on the B-ring and to the ketol structure in the C-ring in their chemical structure (27). Lack of at least one of these groups may reduce or even delete the chelating ability of flavonoids.

Application of some natural phenolics from plant extracts and flavonoids to meat model systems has previously been reported from our laboratories (27,28). The present paper summarizes the results of application of canola extract and natural flavonoids on the oxidative stability of refined-bleached (RB) canola oil.

Materials and Methods

Materials. Canola seeds (variety Candle) and refined-bleached (RB) canola oil containing no antioxidants were obtained from CanAmera (Saskatoon, SK). Synthetic antioxidants (BHA, BHT and TBHQ) and commercially available flavonoids were obtained from either Sigma (St. Louis, MO) or Aldrich Chemical Company (Milwaukee, WI). Monoglyceride citrate (MGC) was obtained from Griffiths Laboratories (Scarborough, ON).

Sample Preparation. Dried canola extract (CE) was prepared as given in the flow diagram (Figure 1). The antioxidant activity of these extracts (100, 200, 500 and 1000 ppm) as well as flavonoids and TBHQ (200 ppm) and a mixture of BHA/BHT/MGC (100/100/50 ppm; MGC as citric acid equivalents) in RB canola oil was tested. For weight gain studies, two grams of each sample (in triplicate) were placed in a petri dish, traces of water were removed in a vacuum oven overnight at 35°C, reweighed and stored in a forced air oven at 65°C. The

weight gain of sample was recorded at 24 h intervals. The time required for a 0.5% weight increase of oil was taken as an index of stability. Twenty five millilitres of each sample were stored separately under the same conditions in small open glass containers for performing other chemical analyses.

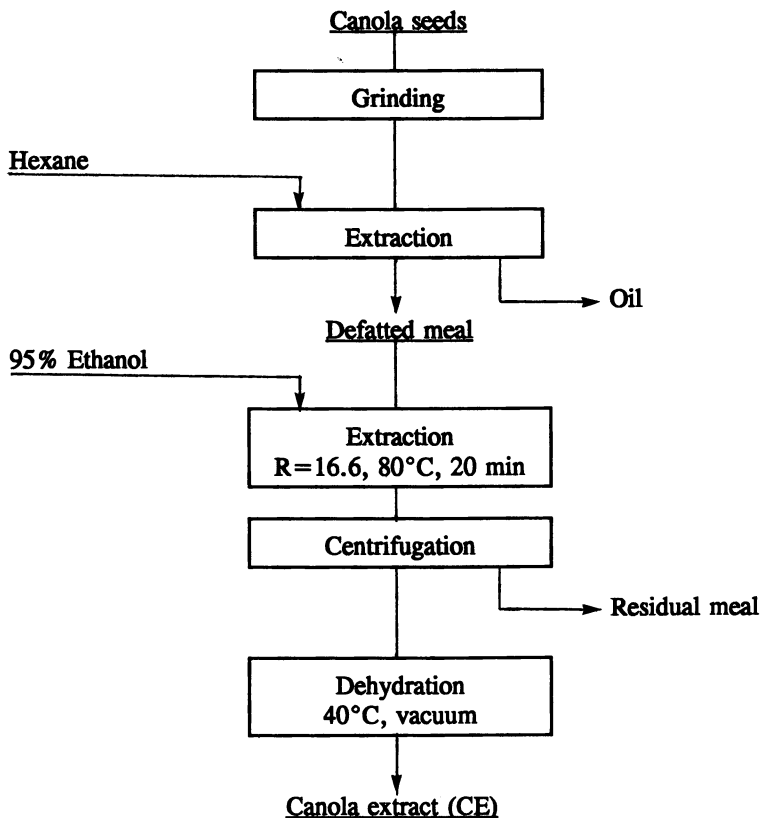


Figure 1. Preparation of dried canola extract.

Other chemical analyses of oils included determination of peroxide value (PV) (29) and the percentage inhibition of TBARS production using the classical 2-thiobarbituric acid (TBA) test (29) as given in the following equation.

Mean percent inhibition of TBARS production =

$$\left(1 - \frac{\text{TBARS content of treated sample}}{\text{TBARS content of control}} \times 100\right)$$

Results and Discussion

Recovery and Composition of Canola Extract (CE). The recovery of combined ethanolic extract of canola meal was $12.9 \pm 0.5\%$ on a dried, deoiled weight basis. The extract contained 18.3% phenolic compounds. Other constituents included crude proteins (18.5%), fat (6.3%), total sugars (32.0%) and minerals (5.6%).

Effect of CE and Flavonoids on Weight Gain. Table I summarizes the time required to obtain 0.5% weight gain in canola oil to which CE, BHA/BHT/MGC and TBHQ were added under accelerated oxidation conditions. Although numerical values varied from one batch to another, the same trends were always observed. The time required to achieve a 0.5% weight gain in canola oil was increased with increasing concentration of CE. Weight gain data of canola oil treated with different flavonoids as compared with BHA/BHT/MGC and TBHQ are shown in Table II. All flavonoid-treated samples exhibited a delayed induction period compared to the control. It is accepted that each day under Schaal oven test at 65°C is equivalent to one month of storage at ambient temperatures (30). The extension of the induction period of oxidation in canola oil by using CE-500, CE-1000 and TBHQ was 2, 2.5 and 2.7 times that of the control, respectively. Samples containing CE-500 and CE-1000 had a delayed induction period comparable to that of BHA/BHT/MGC which is commonly used in canola oil in Canada. Extension of induction period by (-)epicatechin and myricetin was 2 and 5 times that of the control, respectively. Furthermore, (-)epicatechin and myricetin were more effective than BHA/BHT/MGC; however, myricetin was even more effective than TBHQ, the most effective synthetic antioxidant used by the food industry.

Effect of CE and Flavonoids on Peroxides Formation. Addition of CE at levels of 100 to 1000 ppm to canola oil significantly ($P < 0.05$) decreased the PV during accelerated oxidation (Table III). Increase in addition level of CE paralleled a decrease in the formation of peroxides. However, PV of canola oil treated with 200, 500 and 1000 ppm of CE were similar up to day-2. After day-5, PV of samples containing varying concentrations of CE were significantly ($P < 0.05$) different. For up to 13 days, PV of the control sample increased from 0.37 meq/kg (fresh oil) to 159 meq/kg (oxidized oil), however, corresponding values for oils treated with CE-500 and CE-1000 were increased from 0.30 to 87.2 and from 0.32 to 80.0 meq/kg, respectively. CE at 1000 ppm level was most effective and gave much lower PV than the control, BHA/BHT/MGC- and other CE-treated samples.

The scientific literature in the past two decades is replete with reports on antioxidative activity of natural ingredients. Different kinds of seed hulls and meals have been studied for their antioxidative properties (31-33). Most of these antioxidative compounds are phenolic in nature. However, only few reports are available on antioxidative ability of *Brassica* seed meals. Shahidi *et al.* (27) have reported that low pungency mustard flour and its extracts effectively retarded warmed over flavor (WOF) development in meat model systems. They have indicated that the antioxidative efficacy of these extracts was directly proportional to their total content of phenolics.

Table I. Effect of treatment of canola oil with different levels of canola extract (CE) the time required for a 0.5% weight gain

Treatment (ppm)	Days
Control	3.2
CE (100)	5.0
CE (200)	6.0
CE (500)	6.5
CE (1000)	7.8
BHA/BHT/MGC (250)	6.3
TBHQ (200)	8.5

Table II. Effect of treatment of canola oil with different flavonoids on the time required for a 0.5% weight gain¹

Treatment	Days
Control	3.2
Flavones:	
Apigenin	3.5
Chrysin	3.5
Flavonols:	
Kaempferol	3.5
Morin	5.5
Myricetin	15.0
Quercetin	5.8
Rutin	6.0
Flavanones:	
Naringenin	3.5
Naringin	5.5
Flavononols:	
Taxifolin	4.0
Flavan-3-ol:	
(-)Epicatechin	7.0
BHA/BHT/MGC	6.3
TBHQ	8.5

¹All samples were treated with 200 ppm additives, except for BHA/BHT/MGC which was added at 250 ppm.

Table III. Effect of canola extract (CE), BHA/BHT/MGC and TBHQ on peroxide value (meq/kg oil) of refined-bleached canola oil stored at 65°C¹

Treatment (ppm)	Storage period, Days					
	0	2	5	9	13	
Control	0.37±0.01 ^a	22.50±0.70 ^a	83.60±0.50 ^a	125.00±4.00 ^a	159.00±3.00 ^a	
CE (100)	0.32±0.02 ^a	7.86±1.25 ^b	42.80±0.60 ^b	63.30±0.30 ^b	94.60±1.50 ^b	
CE (200)	0.31±0.03 ^a	1.92±0.11 ^d	38.80±0.30 ^c	62.20±0.90 ^b	92.00±0.60 ^c	
CE (500)	0.30±0.00 ^a	1.47±0.15 ^d	32.90±1.00 ^c	53.10±1.10 ^c	87.20±1.20 ^d	
CE (1000)	0.32±0.03 ^a	1.31±0.02 ^d	22.70±0.40 ^c	43.20±0.70 ^c	80.00±0.90 ^c	
BHA/BHT/MGC (250)	0.31±0.05 ^a	3.03±0.12 ^d	36.50±1.40 ^d	55.10±1.00 ^c	62.80±0.70 ^f	
TBHQ (200)	0.32±0.04 ^a	1.13±0.11 ^d	2.02±0.23 ^b	3.77±0.28 ^f	5.37±0.48 ^e	

¹Values in the same column bearing different superscripts are significantly ($P < 0.05$) different.

The changes in PV of flavonoid-treated oils as a function of time are assembled in Table IV. Canola oil treated with quercetin, myricetin, morin, rutin and (-)epicatechin exhibited lower PV (<50%), up to nine days as compared with the control sample. However, samples treated with 250 ppm BHA/BHT/MGC showed relatively higher peroxide formation as compared with some of the flavonoids examined in this study. Among the flavonoids tested, myricetin, quercetin and rutin served best in lowering the formation of peroxides in the samples. Myricetin was superior to all other flavonoids and gave PV of 2.6, 10.2, 12.0 and 27.8 meq/kg on days 2, 5, 9 and 13, respectively; whereas corresponding values of the control samples were 22.5, 83.6, 125.0 and 159.0 meq/kg.

Effect of CE and Flavonoids on TBARS formation. Determination of TBARS as a measure of formation of secondary oxidation products is most frequently made to monitor oxidation of vegetable oils. In this work addition of CE, flavonoids, BHA/BHT/MGC and TBHQ to canola oil showed a significant ($P < 0.05$) effect on reducing TBARS formation as compared with the control sample (Figure 2 and 3). Among these additives, TBHQ was most effective in retarding TBARS formation at 200 ppm level. Ability of TBHQ to lower TBARS values of stored canola oil has been reported in the literature (34). The CE at >200 ppm performed a better inhibitory effect than BHA/BHT/MGC on the formation of TBARS. The general trend of the activity of flavonoids on TBARS formation, indicated that flavonols, flavan-3-ols and flavanones were more effective than other types of flavonoids. Among flavonoids tested, rutin, quercetin, naringenin, naringin, (-)epicatechin and myricetin were able to inhibit the formation of TBARS by >40% over the entire storage period. The overall order of potency of flavonoids on inhibition of TBARS formation was as follows:

Myricetin > (-)epicatechin > naringin > naringenin > quercetin > rutin > morin > kaempferol > taxifolin > apigenin > chrysin

Results of this study indicate that CE and some of the flavonoids tested have a marked effect on prevention of oxidation of canola oil. The effect of CE on suppressing the formation of oxidation products was better than BHA/BHT/MGC but less than that of TBHQ. Effectiveness of myricetin was similar to that of TBHQ. Present findings also lend support to previous reports (34,35) that BHA/BHT/MGC was less effective than TBHQ in retarding the formation of secondary oxidation products of canola oil.

Among the flavonoids tested in this study, flavonols namely myricetin, quercetin and rutin as well as (-)epicatechin (flavan-3-ol; Table V) exhibited superior antioxidant properties in canola oil. The flavanones; naringenin and naringin, (Table V) were also effective but were less potent than flavonols and flavan-3-ol. Flavonoids may act as primary antioxidants by donating a hydrogen atom to the peroxy radicals derived from oxidizing fatty acids (36) and may also function as free radical acceptors or chain breakers and serve as metal chelators.

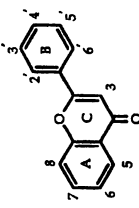
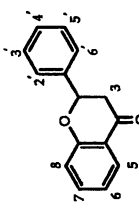
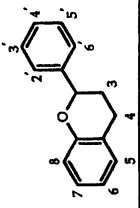
Table IV. Effect of flavonoids, BHA/BHT/MGC and TBHQ on peroxide value (meq/kg oil) of refined-bleached canola oil stored at 65°C^{1,2}

Treatment	Storage period, Days				
	0	2	5	9	13
Control	0.37±0.01 ^a	22.50±0.70 ^a	83.60±0.50 ^a	125.00±4.00 ^a	159.00±3.00 ^a
Flavones:					
Apigenin	0.37±0.01 ^a	20.10±0.10 ^b	63.40±0.10 ^b	107.20±1.50 ^b	153.30±1.20 ^b
Chrysin	0.35±0.01 ^a	22.20±0.20 ^a	65.80±0.30 ^b	93.10±2.30 ^d	145.40±2.70 ^d
Flavonols:					
Kaempferol	0.37±0.01 ^a	12.00±0.40 ^c	47.10±1.40 ^{d,e}	96.90±1.20 ^e	145.20±1.70 ^d
Morin	0.36±0.04 ^a	9.41±0.51 ^f	38.40±1.40 ^{g,h}	64.40±0.70 ^f	130.30±2.50 ^e
Myricetin	0.36±0.01 ^a	2.62±0.11 ⁱ	10.20±0.40 ^j	12.00±0.30 ^j	27.80±0.10 ^j
Quercetin	0.34±0.03 ^a	7.41±0.21 ^k	31.30±0.30 ^k	54.70±0.80 ^h	100.70±1.30 ^k
Rutin	0.37±0.00 ^a	4.63±0.12 ^h	45.20±0.60 ^e	64.20±1.10 ^f	133.30±1.40 ^e
Flavanones:					
Naringenin	0.35±0.01 ^a	14.60±0.10 ^c	40.40±1.20 ^g	52.50±0.70 ^h	85.50±1.00 ^h
Naringin	0.36±0.01 ^a	13.60±0.30 ^d	48.00±0.40 ^d	57.50±0.10 ^g	85.90±0.30 ^h
Flavononols:					
Taxifolin	0.38±0.02 ^a	21.90±0.20 ^a	55.50±0.40 ^e	76.90±0.70 ^e	149.30±1.20 ^e
Flavan-3-ol:					
(-)Epicatechin	0.36±0.01 ^a	8.00±0.10 ^g	41.10±0.30 ^f	46.90±0.50 ⁱ	90.90±1.30 ^g
BHA/BHT/MGC	0.31±0.05 ^a	3.02±0.10 ⁱ	36.50±1.40 ^h	55.10±1.00 ^{g,h}	62.80±0.70 ^h
TBHQ	0.32±0.04 ^a	1.13±0.12 ^j	2.02±0.23 ^k	3.77±0.28 ^k	5.37±0.48 ^k

¹All samples were treated with 200 ppm additives, except for BHA/BHT/MGC which was added at 250 ppm.

²Values in the same column bearing different superscripts are significantly ($P < 0.05$) different.

Table V. Chemical structures of flavonoids

Chemical Structure	Substituents							Compound		
	3	4	5	7	2'	3'	4'		5'	
	H	O	OH	OH	H	H	OH	H	Flavones: Apigenin	
	H	O	OH	OH	H	H	OH	H	Chrysin	
	OH	O	OH	OH	H	H	OH	H	Flavonols: Kaempferol	
	OH	O	OH	OH	H	H	OH	H	Quercetin	
	OH	O	OH	OH	H	H	OH	OH	Myricetin	
	OH	O	OH	OH	OH	H	OH	H	Morin	
	O-rutinose	O	OH	OH	OH	H	OH	H	Rutin	
		H, H	O	OH	OH	H	H	OH	H	Flavanones: Naringenin
		H, O-rhamnoglucose	O	OH	OH	H	H	OH	H	Naringin
		H, OH	O	OH	OH	H	H	OH	H	Flavonols: Taxifolin
	H, OH	H, H	OH	OH	H	H	OH	H	Flavan-3-ol: (-)-Epicatechin	

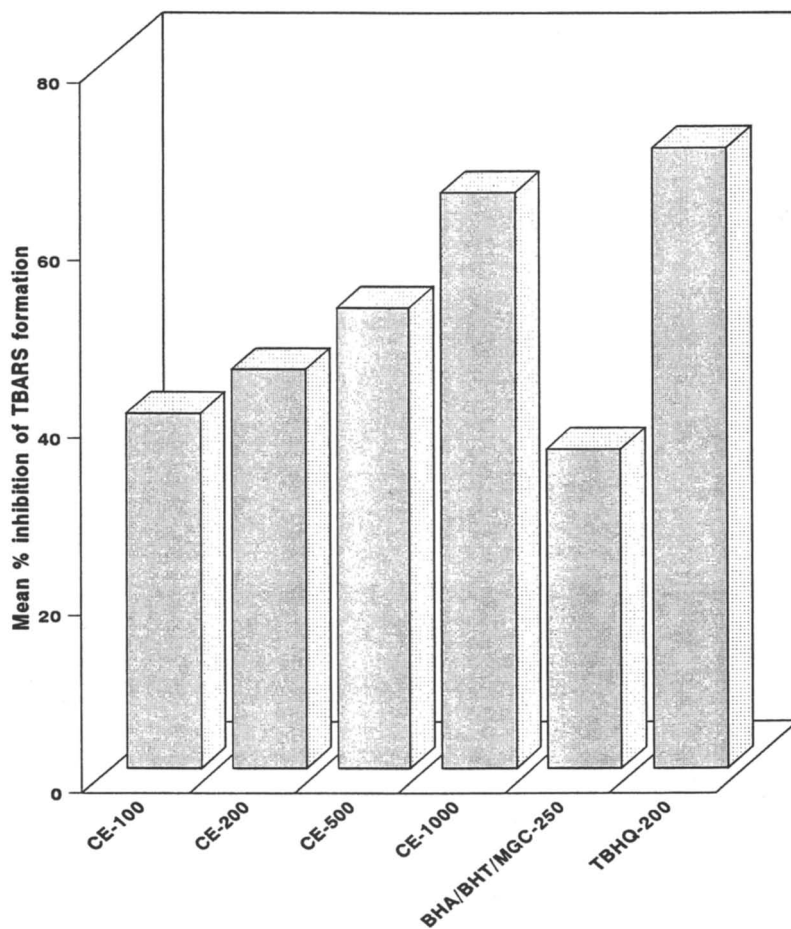


Figure 2. Percent inhibition of TBARS formation by canola extract (CE) and synthetic antioxidants during thermal oxidation of refined-bleached canola oil.

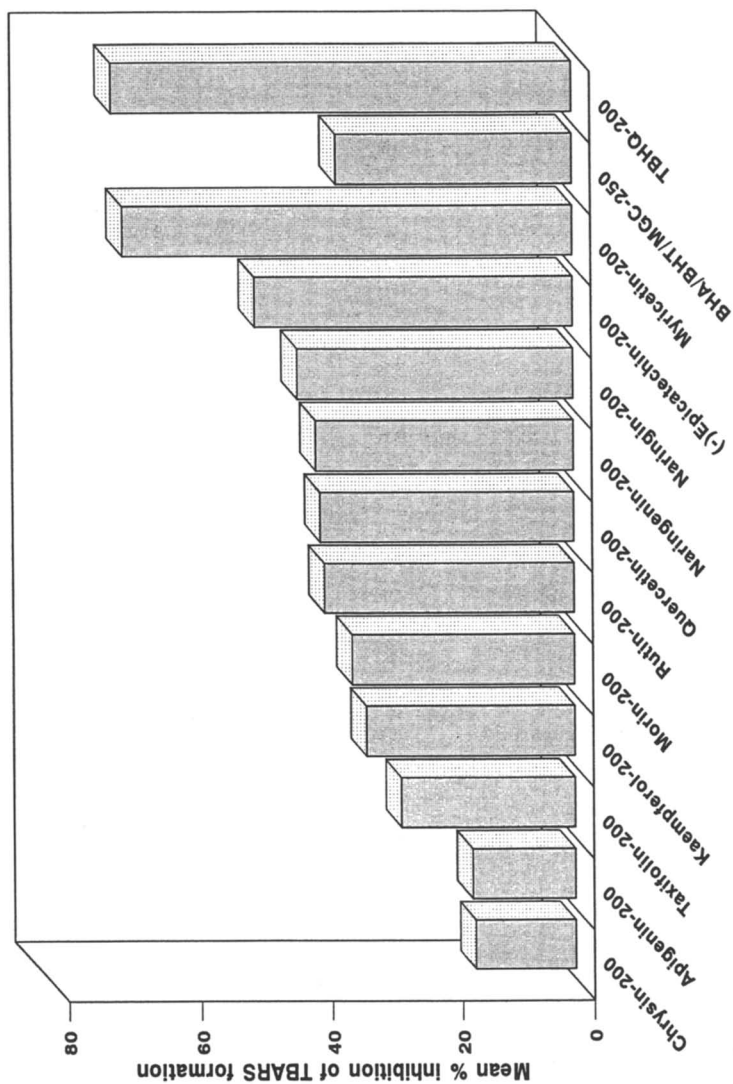


Figure 3. Percent inhibition of TBARS formation by flavonoids and synthetic antioxidants during thermal oxidation of refined-bleached canola oil.

Larson (37) has reported that quercetin and some flavonoids are also potent quenchers of singlet oxygen.

Structure-Activity Relationships. The antioxidant activity of flavonoids is dictated primarily by their chemical structure. All flavonoids possessing a 3', 4'-dihydroxy configuration possess strong antioxidant activity. Myricetin with an additional hydroxy group at the 5' position shows a better antioxidant activity than corresponding flavonols devoid of a 5'-hydroxy group, i.e. quercetin (Table V). Naringin and naringenin with a single hydroxy group on the B-ring exhibit limited antioxidative activity. Therefore, hydroxylation of the B-ring is a major factor when examining the antioxidant activity of flavonoids. Rutin with an etherified sugar moiety at the 3 position of its C-ring showed a lower antioxidant activity than quercetin which has a hydroxy group at the 3 position. Therefore, glycosylation of flavonoids reduces their antioxidant activity, perhaps due to their inability to donate a hydrogen atom to lipid free radicals. In addition, (-)epicatechin which is a flavan-3-ol showed antioxidant activity similar or superior to that of quercetin. Myricetin possessing the largest number of hydroxyl groups in its structure was the most active flavonoid. Similar results were obtained by Das and Pereira (26) for palm oil and by Ramanathan and Das (38) for ground fish samples. Our study also revealed that several flavonoids especially flavonols, flavan-3-ol and flavanones may be considered as potential antioxidants for the stabilization of canola oil.

Literature Cited

1. Statistics Canada. *Oil and Fats Catalogue 1991*, No. 32-006. Vol. 42(12).
2. Ackman, R.G. In: *Canola and Rapeseed, Production, Chemistry, Nutrition and Processing Technology*; Shahidi, F., Ed.; Van Nostrand Reinhold: New York, 1990; pp. 81-98.
3. Tokarska, B.; Hawrysh, Z.J.; Clandinin, M.T. *Can. Inst. Food Sci. Technol. J.* 1986, 19, 130-133.
4. Hawrysh, Z.J. In: *Canola and Rapeseed, Production, Chemistry, Nutrition and Processing Technology*; Shahidi, F., Ed.; Van Nostrand Reinhold: New York 1990; pp. 99-122.
5. Frankel, E.N. *Prog. Lipid Res.* 1980, 19, 1-22.
6. Grosch, W. In: *Autoxidation of Unsaturated Lipids*; Chan, H.W.S., Ed.; Academic Press: London, 1987; pp. 95-139.
7. Benjelloun, B.; Talau, T.; Delmas, M.; Gaset, A. *J. Amer. Oil Chem. Soc.* 1991, 68, 210-211.
8. Durkee, M.M. *Ind. Eng. Chem.* 1936, 28, 898-902.
9. Frankel, E.N. *Prog. Lipid Res.* 1982, 22, 1-33.
10. Snyder, J.M.; Frankel, E.N.; Selke, E. *J. Amer. Oil Chem. Soc.* 1985, 62, 1675-1679.
11. Frankel, E.N.; Neffe, W.E.; Selke, E. *Lipids* 1981, 16, 279-285.

12. Selke, E.; Rohwedder, W.K. *J. Amer. Oil Chem. Soc.* **1983**, *60*, 1853-1858.
13. Marshall, W.E. *Food Technol.* **1974**, *28*, 50-56.
14. Dugan, L.R. In: *Autoxidation in Food and Biological Systems*; Simic, M.G.; Karel, M., Eds.; Plenum Press: New York, **1980**, pp. 261-282.
15. Pokorny, J. *Trends Food Sci. Technol.* **1991**, *9*, 223-227.
16. Dabrowski, K.J.; Sosulski, F.W. *J. Agric. Food Chem.* **1984**, *32*, 128-130.
17. Shahidi, F.; Nazck, M. *J. Amer. Oil Chem. Soc.* **1992**, *32*, 67-103.
18. Kozłowska, H.; Zadernowski, R.; Sosulski, F.W. *Nahrung* **1983**, *27*, 449-453.
19. Zadernowski, R. *Acta Acad. Agricult. Techn. Ols. Technologia Alimentorum* **1987**, *21*, 4-46.
20. Zadernowski, R.; Nowak, H.; Kozłowska, H. **1991**; Abstract No. C-40. Presented at 8th International Rapeseed Congress. Saskatoon, Canada. July 9-11.
21. Shahidi, F.; Nazck, M. *J. Food Sci.* **1989**, *54*, 1082-1083.
22. Clandinin, D.R.; Robblee, A.R. *J. Amer. Oil Chem. Soc.* **1981**, *58*, 682-686.
23. Pratt, D.E.; Watts, B.M. *J. Food Sci.* **1964**, *29*, 27-33.
24. Simpson, T.H.; Uri, N. *Chem. Ind.* **1956**, 956-957.
25. Hudson, B.J.F.; Lewis, J.I. *Food Chem.* **1983**, *10*, 47-55.
26. Das, N.P.; Pereira, T.A. *J. Amer. Oil Chem. Soc.* **1990**, *67*, 255-258.
27. Shahidi, F.; Wanasundara, P.K.J.P.D.; Hong, C. In: *Phenolic Compounds in Food and Their Effect on Health I*; Ho, C-T.; Lee, C. Y.; Huang, M-T. Eds.; ACS Series 506, Washington D.C. **1991**; pp. 214-222.
28. Shahidi, F. *Bull. Liason Groups Polyphenols.* **1988**, *14*, 361-362.
29. AOCS. *Official Methods and Recommended Practices of American Oil Chemists' Society* **1990**; 4th Ed. Amer. Oil Chem. Soc. Champaign, Illinois.
30. Evans, C.D.; List, G.R.; Moser, H.A.; Cowan, J.C. *J. Amer. Oil Chem. Soc.* **1973**, *50*, 218-222.
31. Onyeneho, S.N.; Hettiarachchy, N. *J. Agric. Food Chem.* **1991**, *39*, 1701-1704.
32. Wanasundara, W.M.U.N. *Stabilization of canola oil by naturally-occurring antioxidants* **1993**; M.Sc. thesis, Memorial University of Newfoundland.
33. Whittern, C.C.; Miller, E.E.; Pratt, D.E. *J. Amer. Oil Chem. Soc.* **1984**, *61*, 1075-1078.
34. Hawrysh, Z.J.; Shand, P.J.; Tokarska, B.; Lin, C. *Can. Inst. Food Sci. Technol. J.* **1988**, *21*, 549-554.
35. McMullen, L.M.; Hawrysh, Z.J.; Lin, C.; Tokarska, B. *J. Food Sci.* **1991**, *56*, 1651-1659.
36. Torel, J.; Cillard, J.; Cillard, *Phytochem.* **1986**, *25*, 383-385.
37. Larson, R.A. *Phytochem.* **1988**, *27*, 969-978.
38. Ramanathan, L.; Das, N.P. *J. Agric. Food Chem.* **1992**, *40*, 17-21.

RECEIVED December 16, 1993

Chapter 22

Capillary Gas Chromatography Procedure for Determining Olive Oil Flavor

S. K. Raghavan, D. R. Connell and A. Khayat

Hunt-Wesson, Inc., 1645 West Valencia Drive, Fullerton, CA 92633

A rapid gas chromatographic headspace technique was developed for determination of the flavor quality of processed olive oils. Headspace volatiles were collected at 150° C for 20 minutes over a Tenax trap and the individual constituents were separated using a 60 m x 0.32 mm DX-1 capillary gas chromatographic column. Volatile compounds identified in the headspace gases of olive oil include hexane, ethyl acetate, *t*-2-heptenal, *t*-2-octenal, nonenal, undecenal and *t,t*-2,4-decadienal as well as other aldehydes and alcohols. Based on an internal standard quantitative procedure, many of these volatiles were correlated to the traditional sensory evaluation technique using step-wise multiple regression analysis. Correlation coefficients of 0.98 and better were typically obtained when sensory flavor scores were compared to these lipid decomposition products. Overall, instrumental analysis based on headspace gas analysis is an extremely effective method for rapidly determining the flavor quality of olive oils.

Olive is the fruit produced by several sub-varieties of the genus *Olea Europaea* L., of the Oleaceae family. The oil extracted from the fruit is one of the most important fats in the diet of a large number of people from Mediterranean regions from which more than 95% of the global supplies originate. Currently, worldwide olive cultivation extends over a total area of approximately 13,300 acres with fruit production ranging from 90 to 260 pounds per acre. Production of olives and olive oil has nearly doubled in the course of the last 50 years. Average yearly production has soared from 700,000 tons of oil to 1,600,000 tons (1). Most of the oil produced through pressing is consumed without any refining as long as the acidity and organoleptic characteristics are satisfactory. Otherwise, the oil is refined and blended with virgin oil. Solvent extracted olive oil is also refined and blended with virgin oil before consumption.

0097-6156/94/0558-0315\$08.00/0
© 1994 American Chemical Society

Olive oil is chiefly a mixture of glycerides, including small quantities of free fatty acids, glycerol, phosphatides, pigments, carbohydrates, proteins, flavor compounds, sterols, and resinous substances of uncertain identity. Oil of good quality is characterized by a fragrant and delicate flavor. Olive oil is almost unique among vegetable oils in that it can be consumed without any refining. Its moderate degree of unsaturation is considered nutritionally desirable over the high degree of saturation or unsaturation of many other edible fats and oils. Unfortunately, not all of the olive oil marketed around the world is of very good quality. Large quantities of olive oil must be refined, mainly because they originate from poor quality fruit effectively altering its unique sensory attributes.

Chemical and physical tests have been developed to determine the extent of oxidative rancidity in olive oil with the object of monitoring the development of off-flavors (2). Many procedures have been described in the literature relating volatiles in oils to flavor quality (3,4). Specific studies were directed at understanding the complex odor characteristics of these oils. The result of such investigations to date indicates that gas chromatography volatile procedures appear extremely effective in assessing the flavor attribute of vegetable oils (5).

A wide variety of analytical procedures for predicting the quality of olive oil have been reported in the literature, almost all of which are indirect measures of its flavor (6,7). Since the introduction of gas chromatography to the analysis of foods, considerable progress has been made in the flavor evaluation of vegetable oils by this technique. The high degree of specificity and accuracy offered by gas chromatography has made this approach highly effective for the study of complex flavor systems present in most foods, including the edible oils. Early efforts using direct oil injections in packed gas chromatographic columns demonstrated the true potential of this approach. With improvements in equipment and techniques, excellent correlation with sensory evaluations have been reported by many investigators for various oils (8,9). With advances in oil processing leading to cleaner and extremely bland oils, thermal desorption samplers along with high resolution gas chromatography have enhanced the sensitivity of this technique and increased its usefulness for today's products.

Lipids become rancid as a result of oxidation, and this oxidative rancidity is a major cause of food deterioration. Fats and oils can undergo detrimental flavor changes through oxidative reactions when influenced by factors such as light and heat. The most important lipids in olive oil are the unsaturated fatty acids, particularly oleate, linoleate and linolenate. Of these, the major fatty acid, by far, is oleic acid comprising of greater than 75 % of the total fatty acid content. It has been reported that saturated carbonyl compounds, such as pentanal, hexanal, octanal and nonanal are the major compounds observed in oxidized olive oil (10). Many of these studies have demonstrated the expected inverse relationships between specific gas chromatographic peaks and the flavor quality of olive oil. As the concentration of these components in the headspace increased, the sensory quality of the oil decreased. Excellent correlation with sensory data have been

reported with correlation coefficients of 0.9 or better. Modification of the sampling procedure for concentrating the volatile flavor compounds prior to gas chromatographic analysis resulted in a significant improvement in the sensitivity and extended the usefulness of this technique as an objective instrumental method for olive oil quality evaluation. Many more compounds were reported in this manner including hydrocarbons, alcohols, aldehydes, ketones and esters (11).

Most dynamic headspace samplers fitted with Tenax adsorbent traps are adequate for isolation of a wide range of the lipid decomposition products. Compounds of high volatility, particularly the short chain hydrocarbons, are best quantified under cryofocussing conditions which extends the total analysis time. A more rapid focussing approach available in the thermal desorber used in this study is trap-to-trap transfer with narrow bore adsorbent traps. Since transfer of captured volatiles from this narrow bore trap is executed rapidly, very little peak distortion is encountered even in the early section of the chromatographic profile. This study will focus on the correlation between specific oil oxidation compounds isolated from deodorized olive oil and its sensory evaluation profile using this rapid thermal desorption technique. The important, major volatile components will be quantified and, using regression analysis, those compounds that best match the quality attribute of olive oil will be identified. A predictive model will be developed and tested against random market samples.

Experimental

Oils in this study were highly refined and rebled with a small amount of the virgin oil. Unlike virgin olive oil, a freshly refined sample of this oil is low in total volatiles and has a slight olive odor profile. Most oils were obtained from the market and aged, as needed, under room temperature and light exposure. Freshly deodorized oils were obtained directly from the refinery. All oils were bottled in 100 ml glass vials immediately upon recapping, flushed with high purity inert gas and stored in a -10°C freezer.

Because of the complex nature of the volatiles profile of aged olive oil, special attention had to be given to avoid sample carryover from one analysis to the next. The focus of this study was to compare and rate these complex flavor profiles with the relatively simplified profiles of the freshly deodorized oils. Experimental conditions were optimized for rapid analysis yielding maximum sensitivity. Two microliters of internal standard were added to a one gram sample of the oil in a clean glass vial and the headspace volatiles were purged for 20 minutes at 150°C into a Tenax-Amborsorb polymer adsorbent trap. Entrapped volatiles were desorbed using the Dynatherm Model 910 Multiple Thermal Desorption system coupled to a Shimadzu Model 14A gas chromatograph. All gas chromatographic separations were carried out with a 60 meter by 0.32 mm ID DX-1 capillary column which consists of 10 % polyethylene glycol and 90 % dimethylpolysiloxane. Starting at an oven temperature of 50°C , and held for 1 minute, adequate separation was achieved with a relatively rapid ramp rate of 5°

C per minute to a final temperature of 210° C and held for 5 minutes. All quantitation was done with methyl nonanoate prepared in soybean oil at 1.05 ppm as the internal standard.

A seven-member trained olive oil flavor panel evaluated the olive oil samples using 10-point intensity scales with the following descriptors: 1-2, extreme; 3-5, strong; 6-7, moderate; 8-9, slight; 10, bland. Samples included stressed and store purchased olive oils blended at various levels with freshly deodorized olive oil. A preliminary session was conducted in which a 100% non-stressed olive oil, serving as a control, was presented to the panelists for rating and character description. Other oils, along with their ratings and descriptors, were presented at each session for comparison to the control. Generally, even the most freshly deodorized oils were rated by panelists as moderate in flavor (a 7.0 rating) while the worst oils were rated as strong (2.5 to 3.0 rating). For all the oils in this study, panelists had never rated oils outside of this range.

Results and Discussion

Figure 1 displays chromatograms of freshly refined and moderately aged olive oils. The freshly deodorized olive oil retains very little of the odor compounds present in the headspace of the virgin oil. This profile is dominated by the aldehydes from lipid oxidation such as nonanal, *trans*-2-heptanal and *trans,trans*-2,4-decadienal. Nonanal, the largest component observed under these conditions, is a by-product of oleic acid decomposition and imparts a slight tallowy flavor to the oil. This is not unexpected since olive oil consists in excess of 75 % oleic acid. Other decomposition products of this fatty acid including heptanal and octanal are also observed in this profile and also exceed their odor thresholds. One of the unique features of olive oil among edible oils is the relatively low amount of linoleic acid present. Despite this, the observed levels of decomposition aldehydes originating from linoleic acid is relatively high among the freshly deodorized edible oils. Among this group are hexanal, *trans*-2-heptenal, *trans*-2-octenal and *trans,trans*-2,4-decadienal. Most of the compounds selected for correlation to sensory flavor score data originate from the lipid hydroperoxides of these fatty acids. Aged, deodorized olive oil retains much of its virgin odor character. All the known lipid oxidation products have intensified with hexanal, nonanal and *trans,trans*-2,4-decadienal being the largest. The majority of the compounds that were identified and submitted for sensory correlations were the aldehydes. Ketones such as 1-octen-3-one and alcohols such as methyl propanol and methyl butanol and ethyl acetate were also observed in this profile. In the aged oil many of these constituents coelute with another compound rendering it difficult to quantify accurately in olive oil.

The primary goal of this study is to implement a method for routine determination of the quality attribute of olive oil as an alternative to the traditional sensory evaluation procedure. With speed being a major objective, total baseline separation of all components was not possible. The aldehydes selected for the

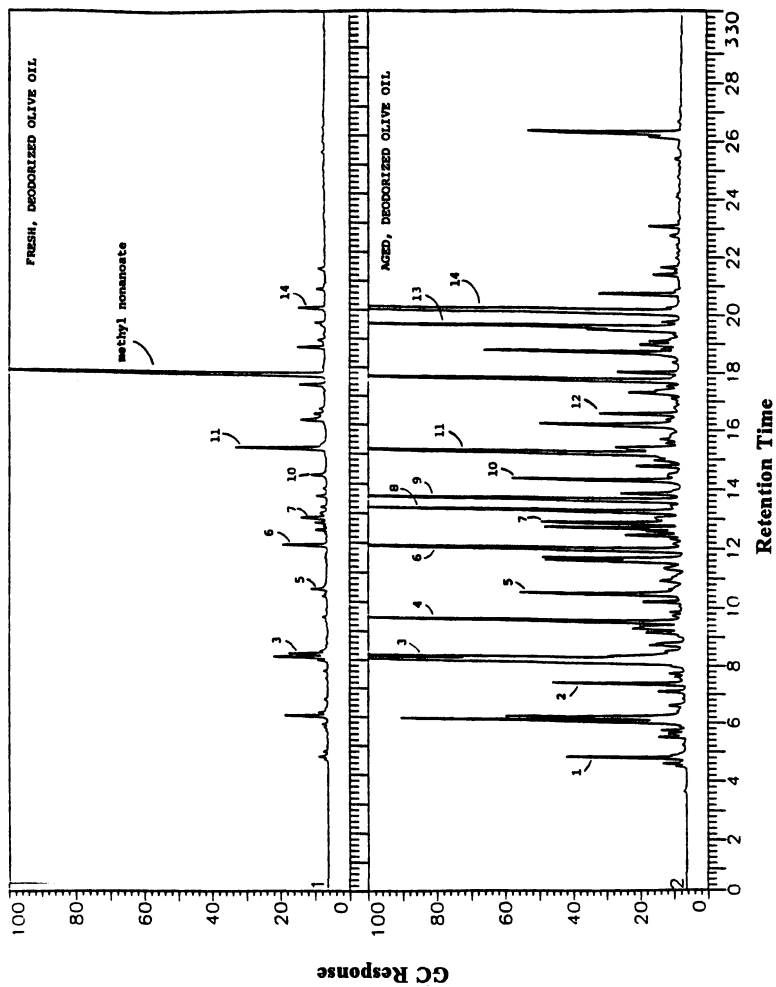


Figure 1. Chromatographic profiles of freshly deodorized and aged olive oils (1 = ethyl acetate, 2 = 2-pentenal, 3 = hexanal, 4 = 2-hexenal, 5 = heptanal, 6 = 2-heptenal, 7 = octanal, 8 = *c,t*-2,4-heptadienal, 9 = *t,t*-2,4-heptadienal, 10 = 2-octenal, 11 = nonanal, 12 = 2-nonenal, 13 = *c,t*-2,4-decadienal, 14 = *t,t*-2,4-decadienal)

July 15, 2012 | <http://pubs.acs.org>
 Publication Date: July 7, 1994 | doi: 10.1021/bk-1994-0558.ch022

correlation study were the ones well resolved on this system. For this group of compounds, response in the concentration range from 140 ppb to 3 ppm was excellent with regression values exceeding 0.9 (Table I). Precision for all compounds used in the correlation study was good with coefficient of variation generally ranging from less than 1 % to approximately 20 %.

Table I. Response of selected aldehydes

Concentration (ppm)	Peak Area				
	ISTD	pentenal	heptenal	octenal	decadialal
0.140	213980	17760	39220	23630	19280
0.270	230690	85940	124800	74990	54910
0.540	175270	139430	177000	142940	209990
1.100	257980	378710	422850	349650	384510
2.700	172140	750490	793670	662950	661176
R ²		0.99	0.98	0.98	0.95
CV %	17.5				

Fifteen olive oils with sensory flavor scores ranging from 7.0 (moderately to slightly rancid) to 3.0 (strongly rancid) were analyzed by this method. From these, fifteen to twenty compounds were selected for correlation determination. With stepwise multiple regression analysis, three compound combinations were identified that best matched the reported sensory flavor score for these oils. In many instances, single and dual components also demonstrated good correlation coefficients. Pentenal, for instance, returned a correlation coefficient of 0.99 (Figure 2). Despite this excellent correlation, single components were not used in developing a predictive flavor scoring system since the possibility exists for any one compound to be incompletely quantified in a high speed, high volume analysis. Hexanal separation, for instance, gets progressively worse as the efficiency of the column declines. In addition, owing to the unpredictability of the oxidation process itself and the fact that more than one mechanism may be involved in the generation of lipid hydroperoxides, individual components may not appear (or are below the detection threshold) in some fresh oils. In freshly deodorized olive oil, 2-pentenal is not reliably quantified for this very reason (Figure 1).

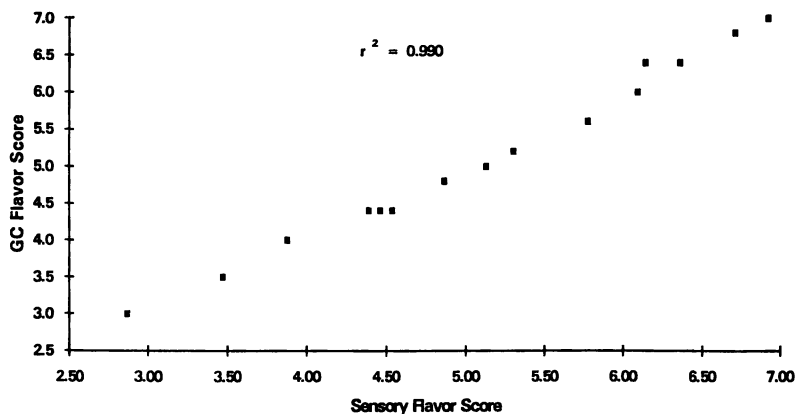


Figure 2. Correlation between actual flavor scores and predicted flavor scores based on 2-pentenal in olive oils.

Predictive flavor scores were developed based on these three component models according to the equation for a straight line :

$$\text{Olive oil flavor score} = b - [(\text{slope} \times A_{\text{heptenal}}) + (m \times A_{\text{octenal}}) + (m \times A_{\text{decadienal}})]$$

where b = y-intercept, m = slope, and A = normalized peak area. Predicted flavor scores, generally, matched well with sensory flavor scores (Table II). Of the three component combinations, the best model for predicting the flavor score of olive oil is the one based on *trans*-2-heptenal, *trans*-2-octenal and *trans*, *trans*-2,4 decadienal which returned a regression value of 0.99 (Figure 3). Instrumental response for these three compounds is excellent even in freshly deodorized oils. Replacing 2-octenal with nonanal, the major lipid oxidation constituent observed in deodorized olive oils, also returned an excellent correlation of 0.98 (Figure 4). The total peak area obtained was also evaluated as a predictor of oil flavor quality and demonstrated good correlation to the sensory ratings. However, in high volume analyses, quantitation of the total peak area is risky as artifacts tend to be included in the computations and yield unreliable results.

Using one of these three component models, excellent predictions are possible for determining the rancidity of olive oils with dynamic headspace gas chromatography. The method outlined here is rapid and fully automated and yet provides the high degree of accuracy demanded from these analyses. Flavor quality prediction also holds well with higher virgin blends despite the complex volatiles nature of these oils.

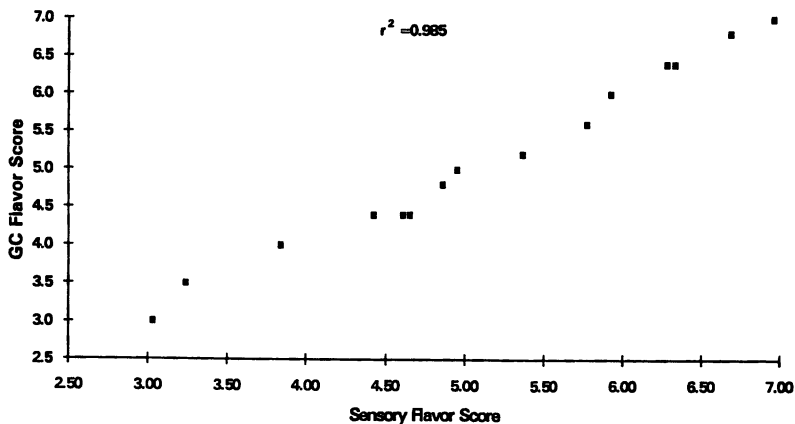


Figure 3. Correlation between actual flavor scores and predicted flavor scores based on 2-pentenal + 2-octenal + 2.4-decadienal in olive oils.

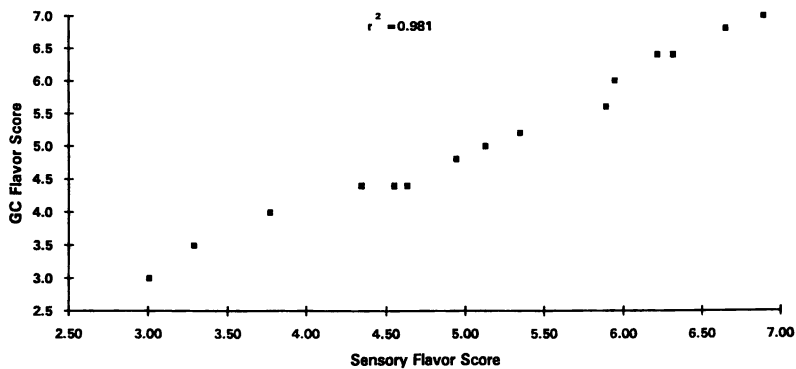


Figure 4. Correlation between actual flavor scores and predicted flavor scores based on 2-heptenal + 2-nonenal + 2.4-decadienal in olive oils.

Table II. Predicted Flavor Scores and Sensory Scores of Olive Oils

Sensory score	Predicted Flavor Score			
	Group 1	Group 2	Group 3	Group 4
7.0	6.92	6.98	6.89	6.96
6.8	6.71	6.75	6.65	6.68
6.4	6.14	6.10	6.22	6.28
6.4	6.36	6.39	6.31	6.33
6.0	6.09	6.10	5.94	5.92
5.6	5.78	5.79	5.89	5.77
5.2	5.30	5.24	5.35	5.36
5.0	5.13	5.12	5.12	4.95
4.8	4.86	4.83	4.94	4.86
4.4	4.54	4.51	4.63	4.65
4.4	4.46	4.43	4.34	4.42
4.4	4.39	4.35	4.55	4.61
4.0	3.88	3.90	3.77	3.84
3.5	3.47	3.48	3.29	3.23
3.0	2.87	2.95	3.01	3.03
R ²	0.99	0.99	0.98	0.99

Group 1 = *t*-2-pentenal; Group 2 = *t*-2-pentenal, *t*-2-heptenal, *t,t*-2,4-decadienal; Group 3 = *t*-2-heptenal, nonanal, *t,t*-2,4-decadienal; Group 4 = *t*-2-heptenal, *t*-2-octenal, *t,t*-2,4-decadienal

Literature Cited

1. Kiritsakis, A. K.; Min, D. B. In *Flavor Chemistry of Lipid Foods*; Min, D. B.; Smouse, T. H., Eds.; American Oil Chemists' Society: Champaign, IL, 1989; pp 196-221.
2. Dupuy, H. P.; Rayner, T.; Wadsworth, J. I.; Legendre, M. G. *J. Amer. Oil Chem. Soc.* **1977**, *54*, 445.
3. Walkling, A. E.; Zmachinski, M. *J. Amer. Oil Chem. Soc.* **1977**, *54*, 454.
4. Jackson, H. W.; Giacherio, D. J.. *J. Amer. Oil Chem. Soc.* **1977**, *54*, 458.
5. Progress Report of the AOCS Flavor Nomenclature and Standards Committee. *J. Amer. Oil Chem. Soc.* **1982**, *59*, 116A.
6. Gray, J. I. In *Flavor Chemistry of Fats and Oils*; Min, D. B.; Smouse, T. H., Eds.; American Oil Chemists' Society: Champaign, IL, 1985; pp 223-239.

7. Dupuy, H. P.; Raymer, E. T.; Wadsworth, J. I. *J. Amer. Oil Chem. Soc.* **1976**, *53*, 628.
8. Raghavan, S. K.; Reeder, S. K.; Khayat, A. *J. Amer. Oil Chem. Soc.* **1989**, *66*, 942.
9. Snyder, J. M.; Frankel, E. N.; Selke, E. *J. Amer. Oil Chem. Soc.* **1985**, *62*, 1675.
10. Fedeli, E. *Prog. Chem. Fats Other Lipids* **1977**, *15*, 57-74.
11. Flath, R. A.; Forrey, R. R.; Guadagni, D. G. *J. Agric. Food Chem.* **1973**, *21*, 948.

RECEIVED February 23, 1994

Author Index

- Amarowicz, R., 233
Baek, H. H., 186
Bailey, M. E., 170
Barker, C. Craig, 49
Cadwallader, K. R., 186
Chen, Chung-Wen, 244
Chen, Qinyun, 2
Chin, Chee-Kok, 282
Chou, Shan-rong, 282
Chung, H. Y., 186
Connell, D. R., 292, 315
Fernando, L. N., 170
Grimm, C. C., 78
Hartman, Thomas G., 108,130,144,158
Ho, Chi-Tang, 2,49,61,144
Hsieh, Rudolf Juuping, 30
Hwang, Hui-Ing, 144
Hwang, Lucy Sun, 244
Izzo, Henry V., 144
Jeon, I. J., 196
Karmas, Karl, 108,130,158
Karwe, Mukund V., 144
Khayat, A., 292,315
Lech, Joseph, 108,130,158
Lee, Min-Hsiung, 61
Lee, Y. B., 98
Lin, Charles F., 208
Miller, J. A., 78
Min, David B., 15
Moody, M. W., 186
Morr, C. V., 98
Pegg, Ronald B., 256
Purchas, R. W., 170
Raghavan, S. K., 292,315
Ritter, William J., 49
Rosen, Robert T., 108,130,158
Ruiz, Reginald, 158
Salinas, Juan P., 108,130,158
Shahidi, Fereidoon, 233,256,301
Spanier, A. M., 78
St. Angelo, A. J., 78
Suzuki, J., 170
Swartz, H. A., 170
Synowiecki, J., 233
Traci, Paul A., 49
Wanasundara, Udaya, 233,301
Wu, Chung-May, 61
Yang, Wesley T., 15
Yu, Tung-Hsi, 61
Zhang, Yuangang, 49

Affiliation Index

- Agricultural Research Service, 78
Campbell Soup Company, 30
Food Industry Research and
Development Institute, 61
Hunt-Wesson, Inc., 292,315
Kansas State University, 196
Lincoln University, 170
Louisiana State University
Agricultural Center, 186
Massey University, 170
Memorial University of Newfoundland,
233,256,307
Missouri Development
Center, 208
National Taiwan University,
61,244
Ohio State University, 15,98
Rutgers University, 2,49,61,
108,130,144,158,282
Soda Aromatic Company, 170
U.S. Department of Agriculture,
78,170
Universal Flavors Cooperation, 49
University of Missouri, 170

Subject Index

A

- Alcohols, formation, 7,9*f*
- Aldehydes
 - C₆, 282–283
 - formation, 4,6*t*,7,8*t*
- Alk(en)ylcysteine sulfoxides, flavor precursors of garlic, 61
- Alligator meat, lipid-derived component, effects on flavor, 186–194
- Alliin and 2,4-decadienal model system, 64,66*t*,70–72*f*
- Animal lipoyxygenase, sources, 32,33*t*
- Animal tissues, lipoyxygenase activity, 39
- Antioxidant(s), 302–303
- Antioxidant system, fish oil, 223,225
- Apple, fresh and off-flavors associated with lipoyxygenase activity, 36
- Aroma compounds in cooked potatoes and reconstituted dehydrated potato granules, *See* Lipid-derived aroma compounds in cooked potatoes and reconstituted dehydrated potato granules
- Aroma extract dilution analysis, 187
- Aroma generation in extruded and heated wheat flour
 - categories and amounts of volatile compounds, 151,154*f*,156
 - compound identification, 147–153
 - experimental procedure, 145–147
 - formation pathways, 155*f*,156
 - heating vs. extrusion, 148–153
- Autoxidation of lipids in fish oil, mechanism, 216–220

B

- Banana, fresh and off-flavors associated with lipoyxygenase activity, 37
- Beans, green and winged, fresh and off-flavors associated with lipoyxygenase activity, 37–38

- Beef, temperature related to lipid volatile production, 78–96
- Biotransaction, eicosapentaenoic and docosahexaenoic acids, 229–230

C

- Canola oil
 - composition, 292–293
 - oxidative deterioration, 301–302
 - stability, 301–313
 - volatile components, 302
- Canola oil flavor quality evaluation
 - by dynamic headspace GC
 - experimental procedure, 295–296
 - headspace volatiles, 296–299
 - oil flavor scores, 299–300
 - peroxide values, 296
- Capillary GC procedure for olive oil determination
 - actual vs. predicted flavor score, 320,321–322*f*,323*t*
 - advantages, 321
 - experimental procedure, 317–318
 - profiles vs. aging, 318,319*f*
 - response of aldehydes, 318,320*t*
- Carbonyl components in beef, temperature dependence, 86,88–89*f*
- Carotenoids, singlet oxygen quencher, 23–24
- Cheddar cheese flavor and free fatty acids, 201–205
- Chemical properties, triplet and singlet oxygen, 16–18
- Chemistry of singlet-oxygen oxidation of foods, 15–28
- Composition, seal lipids, 233–242
- Control of lipoyxygenase activity, stability and mechanisms, 40–41
- Cooked potatoes, lipid-derived aroma compounds, 108–127
- Corn, fresh and off-flavors associated with lipoyxygenase activity, 38

Cucumber, fresh and off-flavors associated with lipoxygenase activity, 37

D

Dairy product flavor, influence of free fatty acids, 196–197

Dairy product storage, dried, headspace volatile compound changes from milk fat oxidation, 98–106

2,4-Decadienal and deoxyalliin model system, 67–68*t*, 71, 73–74*f*

2,4-Decadienal model system, 69*t*, 75

Decomposition of hydroperoxides
alcohols and other compounds, 7,9*f*
aldehyde formation, 4,6*t*, 7,8*t*
furan formation, 7,10*f*
ketone formation, 7,9*f*
reaction, 4,5*f*

Deep-fat frying flavors, role of lipids, 50–51

Dehydrated pinto beans, packaging related to lipid oxidation storage stability, 158–166

Dehydrated potato granules, reconstituted, lipid-derived aroma compounds, 108–127

Dehydrated potato products, off-flavor formation, 108–109

Dehydrated tomato products, lipid-derived flavor compounds, 130–142

Diets, finishing, effect on lamb flavor, 170–184

2,5-Dimethyl-3-pentylpyrazine, 12–13

Docosahexaenoic acid, encapsulation by supercritical fluid extraction, 228–230

Dried dairy product storage, headspace volatile compound changes from milk fat oxidation, 98–106

Dry-rendered lard, flavor, 245

Dynamic headspace GC, canola oil flavor quality evaluation, 295–300

E

Edible oils, volatile compounds, 294

Eicosapentaenoic acid
aseptic implementation of encapsulates, 229
biotransaction to meat system, 229–230
encapsulation by supercritical fluid extraction, 228–229

Extruded wheat flour, aroma generation, 144–156

Extrusion applications, 145

F

Farmer-type autoxidation, lipid autoxidation in fish oils, 216–220

Fatty acid, degree of unsaturation vs. oxidation rate, 302

Finishing diet, effect on lamb flavor
experimental procedure, 173–174
forage pasture, 171–172
sensory analyses of flavor intensity, 174–176
volatile organic compounds from fat, 176–184

Fish

antioxidant systems, 225
fresh and off-flavors associated with lipoxygenase activity, 38–39

Fish lipoxygenase, sources, 33

Fish membranes, lipid oxidation mechanism, 213–216

Fish oil

antioxidant system, 223, 225
applications, 208
flavor chemistry, 208–230
industrial prospective, 227–229
informative chemistry, 213
lipid autoxidation, 216–220
food and medicine uses, 211
processing in U.S., 211, 212*t*
production in U.S., 209–211

Fish oil—*Continued*
purification using supercritical fluid extraction, 227–228

Flavonoids, structure–antioxidant activity relationship, 303

Flavor

- alligator meat, contribution of lipid-derived components, 186–194
- cheddar cheese, and free fatty acid content, 201–205
- dairy products, and free fatty acid content, 196–197
- food, role of lipids, 2
- lamb, influence of finishing diets, 170–184
- olive oil, capillary GC determination procedure, 315–323
- ultra-high-temperature processed milk, and free fatty acids, 197–201

Flavor chemistry

- dairy lipids, related to free fatty acids, 196–206
- fish oil, 208–230

Flavor compounds

- formation from lipoxygenase pathway, 10–11
- fresh and dehydrated tomato products, *See* Lipid-derived flavor compounds in fresh and dehydrated tomato products

Flavor deterioration

- dried milk and dairy products, 98–99
- meat and meat products, 256–275

Flavor precursors of garlic, volatile compounds generated from thermal interaction, 61–75

Flavor quality, canola oil, 292–300

Food flavors

- contribution of lipoxygenase pathway, 30–47
- role of lipids, 2–13

Free radical autoxidation of lipids, steps, 2–3

Free radical mechanism, control of lipoxygenase activity, 40–41

Fresh tomato products, lipid-derived flavor compounds, 130–142

Furans, formation, 7,10*f*

G

Garlic, nonvolatile flavor precursors, 61–75

γ -Glutamylalk(en)ylcysteine dipeptides, flavor precursors of garlic, 61

Grape, fresh and off-flavors associated with lipoxygenase activity, 38

Green beans, fresh and off-flavors associated with lipoxygenase activity, 37–38

Green peas, fresh and off-flavors associated with lipoxygenase activity, 37

H

Headspace volatile compound changes from milk fat oxidation during dried dairy product storage

- experimental procedure, 99–101
- formation mechanisms, 104–105,106*f*
- volatile compounds, 101–104
- water activity control chamber, 99,100*f*

Heat stability, control of lipoxygenase activity, 41

Heated pork fat, flavor, 244

Heated wheat flour, aroma generation, 144–156

Heterocyclic flavor compound formation, and lipid-derived aldehydes, 58

Hexanal

- analysis, 258–262
- drawbacks as indicator of meat flavor deterioration, 265–271
- prevention of generation by nitrite, 271–275

- Hexanal—*Continued*
role in meat flavor deterioration,
258–262
usefulness as primary indicator of meat
flavor deterioration, 261,263–265
cis-3-Hexanal, control of production
by plant cell culture, 282–289
High erucic acid oils, description, 292
Hydrogen peroxide addition,
cis-3-hexanal production, 288–289,290*t*
Hydroperoxides
fatty acids and esters, 3–10
role in flavor of oil, 302
- K
- Ketones, formation, 7,9*f*
- L
- Lamb, characteristic odor, 171
Lamb flavor, influence of finishing
diets, 170–184
Lard, volatile compounds from different
treatments, 244–254
Light, effect on production of *cis*-3-
hexanal by cell culture, 285–287
Likens–Nikerson extraction, 110,112
Linolenic acid
in cultured alfalfa cells, 288–290
precursor of off-flavor
compounds, 302
Lipase, contamination in cheese milk,
204–205
Lipid(s)
dairy products, flavor
chemistry, 196–205
in food flavors, overview, 2–13
Maillard reaction effect, 49–59
sensory quality of foods, 49
singlet oxygen oxidation, 9–10
Lipid autoxidation in fish oils,
farmer-type autoxidation, 216–220
Lipid degradation, interaction with
Maillard reaction for volatile
compound formation, 12–13
Lipid-derived aroma compounds in
cooked potatoes and reconstituted
dehydrated potato granules
continuous steam distillation–solvent
extraction vs. headspace thermal
desorption, 123,124*f*,127
core volatile compounds, 114–119
experimental procedure, 110–113
purge-and-trap thermal desorption
procedure, 112–113
quantification procedure, 114
simultaneous steam distillation–
extraction procedure, 110,112
storage time, 119–123,125–126*t*
Lipid-derived carbonyl compounds, effect
on Maillard reaction, 54–58
Lipid-derived components, contribution
to alligator meat flavor, 186–194
Lipid-derived flavor compound
control of production, 282–291
fresh and dehydrated tomato products
experimental procedure, 131–132
identification, 132,136–139
processing technique, 139–142
tomato-type, 139–142
volatile profiles of sun-dried and fresh
tomatoes, 132–135
Lipids, effect on Maillard reaction as
medium, 50–54
Lipid foods, singlet oxygen reaction
mechanisms, 19–20
Lipid-mediated Maillard reaction and
volatile formation, 49–59
Lipid oxidation
fish membranes, 213–216
food component, 30
process, 144
role in deterioration of muscle
foods, 256
See also Oxidation of lipids in food
Lipid oxidation storage stability of
dehydrated pinto beans, 158–166

Lipid volatile production from beef, temperature, 78–96
 Lipoxygenase(s), role in fish oil deterioration, 214–216
 Lipoxygenase pathway, contribution to food flavors, 30–47
 Low erucic acid oils, description, 292

M

Maillard reaction

interaction with lipid degradation for volatile compound formation, 12–13
 lipid-mediated, and volatile formation, 49–59
 phospholipid, 58,59*t*
 process, 49
 water content, 51–53
 Marine oils, oxidative quality, 234–235
 Meat, definition, 79
 Meat flavor, perception, 78–79
 Meat, flavor deterioration, hexanal as indicator, 256–275
 Menhaden, storage, 225–227
 Menhaden oil
 approval for food use, 208–209
 fatty acid composition, 209,210*t*
 lipid peroxidation, 209
 purification, 221–223,224*f*
 stability, 225–227
 Metal chelation mechanism, control of lipoxygenase activity, 40–41
 Milk fat oxidation, *See* Oxidation of milk fat
 Muscle foods, role of lipid oxidation in deterioration, 256
 Mushroom, fresh and off-flavors associated with lipoxygenase activity, 37
 Myeloperoxidase, role in fish oil deterioration, 214,216

N

Nitrite, role in prevention of meat flavor deterioration and hexanal generation, 271–275
 Nonenzymatic browning reaction, *See* Maillard reaction

O

Odor, lamb, 171
 Oil flavor quality, determination, 294–295
 Oil flavor score, calculation, 299
 Olive, production, 315
 Olive oil
 analytical procedures for quality prediction, 316
 composition, 316
 flavor determination using capillary GC, 315–323
 flavor score, calculation, 321
 GC peaks vs. flavor quality, 316–317
 lipids, 316
 refining, 315
 Omega-3 fatty acid(s), role in nutrition and health, 233–234
 Omega-3 fatty acid composition and stability of seal lipids
 classes of lipids, 236,237*t*,239*t*
 composition vs. storage, 239–242
 content of neutral and polar lipids, 236
 experimental procedure, 235–236
 future research, 242
 steam, 236–237,238*t*
 storage stability, 237–238,240*f*
 Oxidation
 lipids
 fish membranes, mechanism, 213–216
 food, 15
 process, 144
 unsaturated, role in deterioration of muscle foods, 256
 marine oils, 234–235
 milk fat, 98–106

P

- Packaging, effect on lipid oxidation**
storage stability of dehydrated pinto beans
experimental procedure, 160–161
fatty acid composition, 162
hexanal levels vs. packaging environment, 164,166*t,f*
lipid analytical procedure, 162
lipid content of raw dry beans, 163*t*,164
lipid oxidation storage stability analytical procedure, 162
volatile analytical procedure for instant refried bean product, 161–162,164,165*t*
- Peas, green, fresh and off-flavors**
associated with lipoxygenase activity, 37
- Peroxide formation, stability effect of canola oil, 305–309**
Phaseolus vulgaris, *See* Pinto beans
- Phospholipids, Maillard reaction, 58,59*t***
- Photosensitized oxidation**
lipid foods, 20–25
mechanisms, 18–19,22*f*
- Physiological roles of lipoxygenase pathway, animal and plant tissues, 39–40**
- Pinto beans**
dehydrated, effect of packaging on lipid oxidation storage stability, 158–166
description, 158
flavor studies, 160
lipid composition, 159–160
protein quality, 159
use in refried beans, 158–159
- Plant cell culture, control of *cis*-3-hexanal production, 282–291**
- Plant lipoxygenases, sources, 31–32,33*t***
- Plant tissues, physiological roles of lipoxygenase pathway, 39–40**

Potato

- forms, 108
fresh and off-flavors associated with lipoxygenase activity, 38
- Poultry lipoxygenase, sources, 32–33**
- Processed potatoes, off-flavor formation, 108–109**
- Pyrazine formation, lipid-derived carbonyl compound effect, 54–55**

R

- Rapeseed crop varieties, composition, 292**
- Reconstituted dehydrated potato granules, lipid-derived aroma compounds, 108–127**
- Reduced nicotinamide adenine dinucleotide dependent oxidase, role in fish oil deterioration, 214–216**
- Refried beans, preparation procedure, 158–159**

S

- Seafood products, role in nutrition and health, 233**
- Seal blubber, composition, 234**
- Seal lipids, omega-3 fatty acid composition and stability, 233–242**
- Sensory characteristics of Maillard reaction products, 53–54**
- Singlet oxygen**
chemical properties, 16–18
generation, 15
hydroperoxide formation, 15–16
reaction mechanisms with lipid foods, 19–20
- Singlet-oxygen lipid oxidation process, 9–10**
quenching mechanisms and kinetics, 20–23
- Singlet-oxygen oxidation of foods, chemistry, 15–28**

- Singlet-oxygen quenchers in vegetable oil oxidations, 23–25
- Soybean, fresh and off-flavors associated with lipoxygenase activity, 36
- Stability
 canola oil, 305–309
 control of lipoxygenase activity, 40
 menhaden oil, 225–227
 seal lipids, 233–242
- Storage
 dried dairy products, headspace
 volatile compound changes from milk fat oxidation, 98–106
 menhaden oil, 225–227
- Storage stability of dehydrated pinto beans, 158–166
- Sucrose concentration, effect on *cis*-3-hexanal production by plant cell culture, 286–288
- Sulfur-containing components in beef, temperature dependence, 86,90–93
- Sulfur-containing compounds, effect of lipid-derived carbonyl compounds on formation, 55–57
- Supercritical fluid extraction
 eicosapentaenoic and docosahexaenoic acid encapsulation, 228–229
 use for fish oil purification, 227–228
- T**
- Tea, fresh and off-flavors associated with lipoxygenase activity, 38
- Temperature, effect on lipid volatile production from beef
 color, 79–80,81*f*
 enzymatic activity, 80
 membrane alterations, 80,82*f*
 off-flavor volatile production, 83,84*f*
 sparging, 93,95*f*,96
 storage, 83,85–94
- Thermal interaction of 2,4-decadienal and garlic flavor precursors, 61–75
- Thermal processing of food products, 49
- Thiobarbituric acid reactive substances, 308,310–313
- 2-Thiobarbituric acid test, 257–258
- Tocopherols, singlet-oxygen quencher, 24–25
- Tomato
 analytical methods, 131
 aroma compounds, 130–131
 fresh and off-flavors associated with lipoxygenase activity, 36
 importance of understanding volatile and semivolatile compounds, 130
 worldwide production, 130
- Triplet oxygen, chemical properties, 16–18
- U**
- Ultra-high-temperature processed milk flavor, 197–201
- V**
- Vegetable oil oxidations, singlet-oxygen quenchers, 23–25
- Volatile compound(s)
 canola oil, 302
 edible oils, 294
 lards from different treatments
 experimental procedure, 245–247
 fatty acid composition, 247–249
 lard flavor vs. minor components, 250,254*t*
 sensory evaluation, 249,250*t*
 smoke condensates, 249–253
 role in lamb flavor, 170–184
 thermal interaction of 2,4-decadienal and flavor precursors of garlic
 alliin and 2,4-decadienal model system, 64,66*t*,70–72*f*
 2,4-decadienal and deoxyalliin model system, 67–68*t*,71,73–74*f*

- Volatile compound(s)—*Continued*
thermal interaction of 2,4-decadienal
and flavor precursors of garlic—
Continued
2,4-decadienal model system, 69*t*,75
experimental procedure, 62–64
final pH, appearance, and flavor, 64*t*
GC identification, 64,65*f*
- Volatile compound formation
lipid-mediated Maillard reaction
in model systems, 49–59
Maillard reaction–lipid
degradation reaction, 12
oxidation of milk fat, 99
- Volatile lipids, contribution to meat
flavor, 170
- W
- Warmed-over flavor, 256–257
Weight gain, effect of canola extract
and flavonoids, 305,306*t*
Wet-rendered lard, flavor, 245
Wheat flour, extruded and heated,
144–156
Winged beans, fresh and off-flavors
associated with lipoxygenase
activity, 37

Production: Susan F. Antigone

Indexing: Deborah H. Steiner and Janet S. Dodd

Acquisition: Rhonda Bitterli

Cover design: Amy Hayes

Printed and bound by Maple Press, York, PA