

ACS SYMPOSIUM SERIES **674**

# Flavor and Lipid Chemistry of Seafoods

**Fereidoon Shahidi**, EDITOR  
*Memorial University of Newfoundland*

**Keith R. Cadwallader**, EDITOR  
*Mississippi State University*

Developed from a symposium sponsored  
by the Division of Agricultural and Food Chemistry



American Chemical Society, Washington, DC

In Flavor and Lipid Chemistry of Seafoods; Shahidi, F., et al.;  
ACS Symposium Series; American Chemical Society: Washington, DC, 1997.



## Flavor and lipid chemistry of seafoods

### Library of Congress Cataloging-in-Publication Data

Flavor and lipid chemistry of seafoods / [edited by] Fereidoon Shahidi, Keith R. Cadwallader.

p. cm.—(ACS symposium series, ISSN 0097-6156; 674)

“Developed from a symposium sponsored by the Division of Agricultural and Food Chemistry at the 212th National Meeting of the American Chemical Society, Orlando, Florida, August 25–29, 1996.”

Includes bibliographical references and indexes.

ISBN 0-8412-3526-0

1. Seafood—Composition—Congresses. 2. Flavor—Congresses.  
3. Lipids—Congresses.

I. Shahidi, Fereidoon, 1951– . II. Cadwallader, Keith R., 1963–  
III. American Chemical Society. Division of Agricultural and Food Chemistry.  
IV. American Chemical Society. Meeting (212th: 1996: Orlando, Fla.)  
V. Series.

TX556.5.F54 1997  
664'.94—dc21

97-20541  
CIP

This book is printed on acid-free, recycled paper.



Copyright © 1997 American Chemical Society

All Rights Reserved. Reprographic copying beyond that permitted by Sections 107 or 108 of the U.S. Copyright Act is allowed for internal use only, provided that a per-chapter fee of \$17.00 plus \$0.25 per page is paid to the Copyright Clearance Center, Inc., 222 Rosewood Drive, Danvers, MA 01923, USA. Republication or reproduction for sale of pages in this book is permitted only under license from ACS. Direct these and other permissions requests to ACS Copyright Office, Publications Division, 1155 16th Street, N.W., Washington, DC 20036.

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**

In Flavor and Lipid Chemistry of Seafoods; Shahidi, F., et al.;  
ACS Symposium Series; American Chemical Society: Washington, DC, 1997.

# Advisory Board

## ACS Symposium Series

**Mary E. Castellion**

ChemEdit Company

**Arthur B. Ellis**

University of Wisconsin at Madison

**Jeffrey S. Gaffney**

Argonne National Laboratory

**Gunda I. Georg**

University of Kansas

**Lawrence P. Klemann**

Nabisco Foods Group

**Richard N. Loepky**

University of Missouri

**Cynthia A. Maryanoff**

R. W. Johnson Pharmaceutical  
Research Institute

**Roger A. Minear**

University of Illinois  
at Urbana-Champaign

**Omkaram Nalamasu**

AT&T Bell Laboratories

**Kinam Park**

Purdue University

**Katherine R. Porter**

Duke University

**Douglas A. Smith**

The DAS Group, Inc.

**Martin R. Tant**

Eastman Chemical Co.

**Michael D. Taylor**

Parke-Davis Pharmaceutical  
Research

**Leroy B. Townsend**

University of Michigan

**William C. Walker**

DuPont Company

# Foreword

**T**HE 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.

ACS BOOKS DEPARTMENT

# Preface

**SEAFOODS** are appreciated worldwide for their distinct and delicate flavors as well as the nutritional benefits they provide, particularly those related to their long-chain omega-3 fatty acid constituents. The flavor of seafoods has been a topic of numerous studies. Historically, reports on seafood flavor have primarily focused on the unpleasant fishy and rancid odors associated with spoiled or deteriorated products. This area continues to be of importance; however, more researchers are beginning to recognize the importance of understanding the basic chemistry and biochemistry involved in desirable flavor attributes of seafoods. To enhance this knowledge, this book's content provides in-depth coverage of the theoretical and applied chemistry and biochemistry of seafood flavor.

This volume was developed from a symposium which took place at the 212th National Meeting of the American Chemical Society, titled "Flavor and Lipid Chemistry of Seafoods", sponsored by the ACS Division of Agricultural and Food Chemistry, in Orlando, Florida, August 25–29, 1996. Its focus was the lipid chemistry of seafoods. Because lipids have a great impact on seafood flavor, their nutritional effects and quality preservation are thoroughly discussed in this volume. Since seafoods are considered to be nutritious, healthy foods, consumer demand for this product is high. As a result of the potential health benefits of seafood lipids, increased attention is being paid to the analysis and chemistry of these substances. This book provides an important resource for those interested in the lipid chemistry of seafoods. Nutritionists, biochemists, chemists, food scientists, and biologists working in academia, government laboratories, and industry will benefit from this publication. Although this volume is intended to serve as a reference book, it may also be used as a text of lipid chemistry for senior undergraduate and graduate students.

We extend our thanks and gratitude to all authors for providing the state-of-the-art contributions that made this publication possible.

**FEREIDOON SHAHIDI**  
Department of Biochemistry  
Memorial University of Newfoundland  
St. John's, Newfoundland A1B 3X9  
Canada

**KEITH R. CADWALLADER**  
Department of Food Science and Technology  
Mississippi Agricultural and Forestry Experiment Station  
Mississippi State University, Box 9805  
Mississippi State, MS 39762–5953

## Chapter 1

# Flavor and Lipid Chemistry of Seafoods: An Overview

Fereidoon Shahidi<sup>1</sup> and Keith R. Cadwallader<sup>2</sup>

<sup>1</sup>Department of Biochemistry, Memorial University of Newfoundland, St. John's,  
Newfoundland A1B 3X9, Canada

<sup>2</sup>Department of Food Science and Technology, Mississippi Agricultural  
and Forestry Experiment Station, Mississippi State University, Box 9805,  
Mississippi State, MS 39762-5953

The beneficial health effects of seafoods are attributed to their lipid components which are rich in long-chain polyunsaturated fatty acids of the omega-3 family. These lipids are responsible for the development of both desirable and undesirable flavors in marine-based food products. However, non-volatile constituents of seafoods, such as free amino acids, nucleotides, sugars and minerals, also contribute to the characteristic taste and flavor of seafoods. An overview of the evaluation of seafood flavors, studies related to their lipid constituents and important recent developments in the field is provided.

The consumption and popularity of seafoods has consistently increased during recent years. Importance of seafoods in health and nutrition was revealed when Bang and Dyerberg (1-5) reported that Eskimos had low rates of coronary heart disease (CHD) and cancer despite their high-fat diet. It was further noted that fat in the Eskimos diet originated from fish and seal. Other epidemiological studies have also confirmed that the incidence of cardiovascular disease (CVD) in fish eating, non-Eskimo, populations was less prevalent than those who did not eat or consumed little fish (6-10). The potential beneficial health effects of seafoods have been ascribed to their lipids which contain a relatively large amount of long-chain omega-3 fatty acids. These omega-3 fatty acids are known to be effective in curing and treatment of certain CVD by lowering serum triacylglycerol and cholesterol and play an important role in the prevention and treatment of hypertension, arthritis and other inflammatory and autoimmune disorders. In addition, omega-3 fatty acids, namely eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA) are essential for normal growth. DHA is a major component of membrane phospholipids in retinal receptors, cerebral grey matter and sperm. Selective incorporation mechanisms appear to exist to supply the fetal brain and retina with the relatively high levels of DHA needed for normal brain development and eye sight. Meanwhile, EPA serves as a precursor of eicosanoids which are hormone-like substances such as prostaglandins, thromboxanes and leucotrienes. These eicosanoids act on cellular messengers and metabolite regulators and are produced by different cell

types in response to both physiological and non-physiological stimuli. The EPA has also been recognized as having therapeutic benefits in human CVD (11).

The content and composition of omega-3 fatty acids in seafoods vary considerably from one species to another (12). Furthermore, it is possible to tailor-make the lipid composition of cultured fish and other aquatic species by manipulating their dietary lipids (e.g. 13). Obviously, these interventions would have a marked impact on the significance of lipids in cultured species as compared with their wild counterparts.

This overview provides a cursory account of seafood flavor and recent developments in their analysis. The importance of quality preservation of fish and other aquatic species as well as marine lipids in order to take full advantage of their beneficial effects is also presented. The application of the available technologies, such as microencapsulation and use of antioxidants are reviewed. Analytical procedures to evaluate the oxidative state of such products and methods of preparing omega-3 fatty acids are also provided.

### Flavor of Seafoods

Seafoods have complex flavor systems comprised of equally important taste- and aroma-active components. The taste active constituents, which are generally non-volatile compounds, such as free amino acids, nucleotides, sugars, mineral salts, etc., have attracted considerable attention and their importance to seafood flavor is well documented (14-21). Several excellent reviews have also been reported on the subject of seafood flavor (17, 19, 22-34).

The aroma characteristics of seafoods may be subdivided into those components contributing to fresh and/or cooked seafood flavor. Study of fresh seafood flavor has attracted much attention due to the importance of aroma to consumer acceptability of fresh seafood (15, 18). The flavor of fresh seafoods, including both fish and shellfish, are primarily impacted by lipoyxygenase-derived lipid-based volatiles (14, 15, 34, 39). However, environmentally-induced flavors as well as amines, primarily trimethylamine, may also influence the flavor of fresh seafood (14-16). More thorough reviews of fresh fish flavor may be found elsewhere (14, 15, 20).

Upon cooking, the flavor of seafoods change dramatically. The thermally-induced changes results in cooked meaty aromas which are often species-specific. Maillard and Strecker degradation reactions play predominant roles in developing the meaty aromas of cooked seafoods. Other reactions such as retro-aldol condensation of dienals (14, 15, 40) and lipid oxidation (41) give rise to many important aroma compounds. The combination of the above reactions are necessary for the evolution of the character-impact aroma compounds in cooked crustaceans (42-61; see Chapters 7-9), fish (41, 62-65; see Chapters 3-6 and 11-13), shellfish (66-69) and other seafoods (70, 71; see Chapters 2 and 8).

### Recent Developments in the Analysis of Seafood Flavors

As previously mentioned, the flavor of seafoods is comprised of both volatile aroma-active and non-volatile taste-active components. Isolation and Identification of these compounds has been an important area of research.

Early investigations on the flavor of seafood mainly focused on the taste-active components. The first study of this type was conducted during the early 1900s (72). Since that time, most investigations in this area have involved the quantitative analysis of extractive components (amino acids, sugars, nucleotides, inorganic salts, etc.) by wet-chemical and/or liquid chromatographic (e.g., amino acid analyzer) methods. Species studied included shrimp (22), crab(23-24), lobster (28), fish (29, 30), shellfish (31) and others (32-34). Some of these studies also employed sensory evaluation to indicate the relative importance of each compound to the overall taste of the seafood (24-26, 28).

It wasn't until the mid 1960s, with the evolution and wider availability of gas chromatography (GC) and GC/mass spectrometry, (GC/MS) that the volatile constituents of seafoods were given much attention. The methods employed for the analysis of volatiles in seafoods were, and are still, essentially the same as those for other muscle foods. Basic strategies and techniques for the isolation and analysis of food volatiles are covered in great depth elsewhere (73, 74). The following discussion will, therefore, focus primarily on recent developments in methodology for the isolation and analysis of volatile (aroma) constituents of seafoods only.

Identification of characteristic and important aroma compounds in seafoods has been challenging due the presence of these compounds at extremely low levels, often at sub parts-per-billion concentrations. Isolation or sampling of volatiles prior to gas chromatographic analysis can be conducted in a number of ways, including equilibrium (65, 75-77) and dynamic (35, 39, 46, 50, 51, 59, 78) headspace sampling; distillation under atmospheric (62) or vacuum (71, 79, 80) conditions with subsequent solvent extraction; simultaneous steam distillation-solvent extraction under atmospheric (21, 42-45, 48, 49, 51, 54-57) or vacuum (57, 59, 60) conditions; direct solvent extraction (58), sometimes with an extract cleanup step such as distillation (66-68) or sublimation in vacuo (64); and direct sample injection (66-68). Each isolation technique has its strengths and weaknesses and each will give somewhat biased results since it selects for certain groups of compounds over others. Furthermore, some methods are more prone to artifact formation than others.

A good approach to account for some of the bias has been to rely on two or more of the above techniques for isolation of volatiles. The chosen techniques should preferably differ on the basis of compound isolation. For example, a headspace sampling method used in conjunction with a direct solvent extraction technique would be superior to the use of two types of headspace techniques or two types of extraction techniques. This strategy has been employed in seafood flavor research (52, 59). Further discussion and demonstration of this approach as applied to seafoods can be found in Chapters 7, 11 and 13.

While various techniques are often used for the isolation of volatiles, analysis of volatile extract is most often accomplished by GC and GC/MS. In addition to these methods, the use of GC/olfactometry has proven useful for the identification of character-impact aroma compounds in a variety of seafoods (46, 50, 51, 57-62, 64, 65, 69, 71), Reviews on GC/O can be found elsewhere (82, 83). Examples of the use of GC/O in the study of seafood aroma can be found in Chapters 4, 11 and 13.

In addition to general methods discussed above, specific techniques can be used when the target substance is known, such as in the case of the analysis of the environmental-derived off-flavors such as geosmin (84, 85) and bromophenols (86)



as well as aldehydes, amides, etc as indicators of flavor quality (41, 76). Use of an electronic aroma sensor for the analysis of fish oil odor is a recent development (87).

### Seafood Lipids and Their Quality Deterioration

As mentioned earlier, lipids from aquatic species are important to both desirable and undesirable flavor quality of such products. Lipids in aquatic species affect the flavor of freshly harvested products as they might undergo lipoxygenase-assisted oxidation (14, 15, 34, 39). However, prolonged storage of seafoods and marine lipids results in flavor deterioration and off-flavor development (88-90). Therefore, inhibition of autoxidation is a major criterion when the products are used as a source of food or food component.

Stabilization of seafoods against oxidative deterioration and aroma reversion may be achieved by employing adequate packaging techniques, low storage temperatures, use of antioxidants (91-93; see Chapters 14-17) and, in case of marine oils, microencapsulation (94-96; see Chapter 22). The latter process, in addition to extending the shelf-life of products, affords a free-flowing powder which can be easily incorporated into foods (97).

For fortification of foods and for pharmaceutical/medicinal applications, preparation of omega-3 fatty acid concentrates might be desirable (see Chapters 20 and 21). This would also reduce the unnecessary intake of saturated fatty acids. Physical, chemical and enzymatic methods might be employed for this purpose. Therefore, low temperature fractional crystallization (98), urea complexation (99) and enzymatic processes such as hydrolysis, alcoholysis and acidolysis, among others, might be employed (100).

### Analysis and Evaluation of the Oxidative Status of Seafood Lipids

Methods of extraction, quantitation, classification and further analysis of lipids have been reviewed elsewhere (101). The type of lipids involved, the proportion and the nature of their constituents and their detailed structural characteristics are important factors to be considered. The nutritional value and oxidative stability of seafoods and marine lipids are affected by these factors. Due to rapid deterioration of lipids in seafoods and marine oils, knowledge about methods of determining oxidative stability of fats and oils must be considered (102-104). Among the frequently used methods, changes in the reactants such as fatty acid composition, oxygen pressure, weight-gain, iodine value and refractive index might be examined (104, 105; see Chapter 17). In addition, formation of primary products of oxidation namely hydroperoxides and conjugated dienes may be studied (104; see Chapter 18). However, primary products of oxidation have no color and flavor of their own and might be readily decomposed to secondary products such as aldehydes, ketones, alcohols and hydrocarbon, among others (106). These secondary oxidation products are generally flavor-active and contribute to the deteriorated flavor of seafoods and marine oils. To determine such products, measurement of 2-thiobarbituric acid reactive substances (TBARS), oxirane value, para-anisidine value (p-AV), Totox value, Totox TBA value, total carbonyls, individual carbonyl components, hydrocarbons, and fluorescent products might be performed (104, 105). Furthermore, instrumental methods of analysis employing electron spin resonance (ESR) (107, 108),

infrared (IR) (109), chemiluminescence (110, 111) and nuclear magnetic resonance (NMR) (112; see Chapter 19) and high performance liquid chromatography (113) are often used. These methodologies have recently been reviewed (102).

### Literature Cited

1. Bang, H.O.; Bang, J. *Lancet* **1956**, *1*, 381-383.
2. Dyerberg, J.; Bang, H.O.; Hjørne, N. *Am. J. Clin. Nutr.* **1975**, *28*, 958-966.
3. Bang, H.O.; Dyerberg, J.; Hjørne, N. *Acta Med. Scand.* **1976**, *200*, 69-73.
4. Dyerberg, J.; Bang, H.O.; Stofferson, E. *Lancet* **1978**, *2*, 117-119.
5. Dyerberg, J.; Bang, H.O. *Lancet* **1979**, *2* 433-435.
6. Nordoy, A.; Goodnight, S. *Arteriosclerosis* **1990**, *10*, 149-163.
7. Kromhout, D.; Bosschieter, E.B.; de L'Coulander, C. *N. Engl. J. Med.* **1985**, *312*, 1205-1209.
8. Shekelle, R.B.; Missell, L., Paul, O., Shryock, A.M.; Stanler, J. *N. Engl. J. Med.* **1985**, *313*, 820.
9. Norell, S.E.; Ahlbom, A.; Feychting, M.; Pedersen, N.L. *Br. Med. J.* **1986**, *293*, 426.
10. Dolecek, T.A.; Grandits, G. *World Rev. Nutr. Diet.* **1991**, *66*, 205-216.
11. Simopoulos, A.P. *Am. J. Clin. Nutr.* **1991**, *54*, 438-463.
12. Ackman, R.G. *Progress Food Nutr. Sci.* **1989**, *13*, 161-241.
13. Watanabe, T. *Comp. Biochem. Physiol.* **1982**, *73B*, 3-15.
14. Kawai, T. *Crit. Rev. Food Sci. Nutr.* **1996**, *36*, 257-298.
15. Lindsay, R.C. *Food Rev. Int.* **1990**, *6*, 437-455.
16. Whitfield, F.B. *Food Rev. Int.* **1990**, *6*, 505-519.
17. Konosu, S.; Yamaguchi, K. In *Chemistry & Biochemistry of Marine Food Products*; Martin, R.E.; Flick, G.; Hebard, C.E.; Ward, D.R., Eds.; AVI Publishing Company: Westport, CN, 1982, Ch. 17, pp. 367-404.
18. Piggot, G.M. *Food Rev. Int.* **1990**, *6*, 661-679.
19. Konosu, S.; Yamaguchi, K. In *Umami: A Basic Taste*; Marcel Dekker, Inc.: New York, 1987, pp. 235-253.
20. Josephson, D.B. *Seafood. Food Sci. Technol.* **1991**, *41*, 179-187.
21. Kubota, K.; Uchida, C.; Kurosawa, K.; Komuro, A.; Kobayashi, A. In *Thermal Generation of Flavors*; Parliment, T.H.; McGorin, R.J.; Ho, C.-T., Eds.; ACS Symposium Series 409; American Chemical Society: Washington, DC, 1989, pp. 376-385.
22. Tarr, H.L.A.; Comer, A.G. *J. Fish. Res. Bd. Can.* **1965**, *22*, 307-311.
23. Porter, R.W. *J. Food Sci.* **1968**, *33*, 311-314.
24. Konosu, S.; Yamaguchi, K.; Hayashi, T. *Bull. Jap. Soc. Sci. Fish.* **1978**, *44*, 505-510.
25. Hayashi, T.; Asakawa, A.; Yamaguchi, K.; Konosu, S. *Bull. Jap. Soc. Sci. Fish* **1979**, *45*, 1325-1329.
26. Hayashi, T., Yamaguchi, K., Konosu, S. *J. Food Sci.* **1981**, *46*, 479-493.
27. Hayashi, T.; Ishii, H.; Shinohara, A. *Food Rev. Int.* **1990**, *6*, 521-536.
28. Shirai, T.; Hirakawa, Y.; Koshikawa, Y.; Toraishi, H.; Terayama, M.; Suzuki, T.; Hirano, T. *Fisheries Science* **1996**, *62*, 283-287.
29. Jones, N.R. *J. Agric. Food Chem.* **1969**, *17*, 712-716.

30. Greene, D.H.; Byrne-Bernatt, E.I. *J. Food Sci.* **1990**, *55*, 257-258.
31. Lee, E.-H.; Heo, W.-D. The taste compounds of *Corbicula elatior*. *Bull. Nat. Fish. Univ. Busan* **1980**, *20*, 31-46.
32. Cha, Y.-J.; Lee, E.-H. *Bull. Korean Fish. Soc.* **1985**, *18(4)*, 325-332.
33. Yamanaka, H.; Matsumoto, M.; Hatae, K.; Nakaya, H. *Nippon Suisan Gakkaishi* **1995**, *61*, 612-618.
34. Ijong, F.G.; Ohta, Y. *Lebensm.-Wiss. u.-Technol.* **1995**, *28*, 236-237.
35. Joshephson, D.B.; Lindsay, R.C.; Stuibler, D.A. *J. Agric. Food Chem.* **1983**, *31*, 326-330.
36. Josephson, D.B.; Lindsay, R.C.; Stuibler, D.A. *J. Agric. Food Chem.* **1984**, *32*, 1344-1347.
37. Josephson, D.B.; Lindsay, R.C.; Stuibler, D.A. *J. Agric. Food Chem.* **1984**, *32*, 1347-1352.
38. Josephson, D.B.; Lindsay, R.C., Stuibler, D.A. *J. Food Sci.* **1985**, *50*, 5-9.
39. Josephson, D.B.; Lindsay, R.C., Stuibler, D.A. *J. Food Sci.* **1987**, *52*, 10-14.
40. Josephson, D.B.; Lindsay, R.C. *JAOCs* **1987**, *64*, 132-138.
41. Yasuhara, A.; Shibamoto, T. *J. Agric. Food Chem.* **1995**, *43*, 94-97.
42. Kubota, K.; Kobayashi, A.; Yamanishi, T. *Agric. Biol. Chem.* **1980**, *44*, 2677-2682.
43. Kubota, K.; Kobayashi, A.; Yamanishi, T. *Agric. Biol. Chem.* **1980**, *44*, 2753-2754.
44. Kubota, K.; Kobayashi, A.; Yamanishi, T. *Agric. Biol. Chem.* **1982**, *46*, 2835-2839.
45. Choi, S.H.; Kobayashi, A.; Yamanishi, T. *Agric. Biol. Chem.* **1983**, *47*, 337-342.
46. Choi, S.H.; Kato, H. *Agric. Biol. Chem.* **1984**, *48*, 1479-1486.
47. Kubota, K.; Shijimaya, H.; Yamanishi, T. *Agric. Biol. Chem.* **1986**, *50*, 2867-2873.
48. Kubota, K.; Watanabe, K.; Kobayashi, A. *Agric. Biol. Chem.* **1988**, *52*, 1537-1540.
49. Kubota, K.; Kobayashi, A. *J. Agric. Food Chem.* **1988**, *36*, 121-123.
50. Vejaphan, W.; Hsieh, T.C.-Y.; Williams, S.S. *J. Food Sci.* **1988**, *53*, 1666-1670.
51. Tanchotikul, U.; Hsieh, T.C.-Y. *J. Food Sci.* **1989**, *54*, 1515-1520.
52. Hsieh, T.C.-Y.; Vejaphan, W.; Williams, S.S.; Matiella, J.E. In *Thermal Generation of Aromas*; Parliment, T.H.; McGorrrin, R.J.; Ho, C.-T., Eds.; ACS Symposium Series 409; American Chemical Society: Washington, DC, 1989, pp. 386-395.
53. Matiella, J.E.; Hsieh, T. C.-Y. *J. Food Sci.* **1990**, *55*, 962-966.
54. Cha, Y.-J.; Baek, H.-H.; Hsieh, T.C.-Y. *J. Sci. Food Agric.* **1992**, *58*, 239-248.
55. Chung, H.Y.; Cadwallader, K.R. *J. Food Sci.* **1993**, *58*, 1203-1207, 1211.
56. Cha, Y.-J.; Cadwallader, K.R.; Baek, H.H. *J. Food Sci.* **1993**, 525-530.
57. Chung, H.Y.; Cadwallader, K.R. *J. Agric. Food Chem.* **1994**, *42*, 2867-2870.
58. Chung, H.Y.; Chen, F.; Cadwallader, K.R. *J. Food Sci.* **1995**, *60*, 289-299.
59. Cadwallader, K.R.; Tan, Q.; Chen, F.; Meyers, S.P. *J. Agric. Food Chem.* **1995**, *43*, 2432-2437.
60. Baek, H.H.; Cadwallader, K.R. *J. Agric. Food Chem.* **1996**, *44*, 3262-3267.
61. Whitfield, F.B.; Freeman, D.J.; Last, J.H.; Bannister, P.A.; Kennett, B.H. *Aust. J. Chem.* **1982**, *35*, 373-383.
62. Withycombe, D.A.; Mussinan, C.J. *J. Food Sci.* **1988**, *53*, 658-660.
63. Cha, Y.J.; Cadwallader, K.R. *J. Food Sci.* **1995**, *1*, 19-24.
64. Milo, C.; Grosch, W. *J. Agric. Food Chem.* **1993**, *41*, 2076-2081.
65. Milo, C.; Grosch, W. *J. Agric. Food Chem.* **1995**, *43*, 459-462.

66. Flick, G.J.; Hubbard, J.A.; Ory, R.L.; Legendre, M.G.; St. Angelo, A.J. In *Chemistry & Biochemistry of Marine Food Products*; Martin, R.E.; Flick, G.; Hebard, C.E.; Ward, D.R., Eds.; AVI Publishing Company: Westport, CN, 1982, pp. 333-346.
67. Burnette, J.A.; Flick, G.J.; Miles, J.R.; Ory, R.L.; St. Angelo, A.J.; Dupuy, H.P. *J. Food Sci.*, **1983**, *48*, 353-359.
68. Reddy, N.R.; Flick, G.J.; Dupuy, H.P.; Boardman, G.D. *J. Food Sci.* **1989**, *54*, 55-59, 182.
69. Tanchotikul, U.; Hsieh, T.C.-Y. *J. Food Sci.* **1991**, *56*, 327-331.
70. Kajiwaru, T.; Hatanake, A.; Kawai, T.; Ishihara, M.; Tsuneya, T. *J. Food Sci.* **1988**, *53*, 960-962.
71. Tamura, H.; Nakamoto, H.; Yang, R.-H.; Sugisawa, H. *Nippon Shokuhin Kagaku Kogaku Kaishi* **1995**, *11*, 887-891.
72. Kato, H.; Rhue, M.R.; Nishimura, T. In *Flavor Chemistry: Trends and Developments*; Taranishi, R.; Buttery, R.; Shahidi, F., Eds.; ACS Symposium Series 388; American Chemical Society: Washington, DC, 1989, pp. 158-174.
73. Marsili, R., Ed.; *Techniques for Analyzing Food Aroma*; Marcel Dekker, Inc.: New York, 1997.
74. Ho, C.-T.; Manley, C.H., Eds.; *Flavor Measurement*; Marcel Dekker, Inc.: New York, 1993.
75. Girard, B.; Nakai, S. Grade classification of canned pink salmon with static headspace volatile patterns. *J. Food Sci.* **1994**, *59*, 507-512.
76. Kryzmiem, M.E.; Elias, L. *J. Food Sci.* **1990**, *55*, 1228-1232.
77. Sugiyama, K.; Onzuka, H.; Takada, K.; Oba, K. *Nippon Nogeikagaku Kaishi* **1990**, *9*, 1461-1465.
78. Yoshiwa, T.; Morimoto, K.; Sakamoto, K.; Ishikawa, Y. *Nippon Suisan Gakkaishi* **1992**, *58*, 2105-2110.
79. Triqui, R.; Reineccius, G.A. *J. Agric. Food Chem.* **1995**, *43*, 453-458.
80. Triqui, R.; Reineccius, G.A. *J. Agric. Food Chem.* **1995**, *43*, 1883-1889.
81. Flament, I. *Lebensm.-Wiss. u.-Technol.*, **1990**, *23*, 274-275.
82. Acree, T.A. In *Flavor Measurement*; Ho, C.-T.; Manley, C.H., Eds.; Marcel Dekker, Inc.; New York, 1993, pp. 77-94.
83. Blank, I. In *Techniques for Analyzing Food Aroma*; Marsili, R., Ed.; Marcel Dekker, Inc.: New York, 1997, pp. 293-329.
84. Tanchotikul, U.; Hsieh, T.C.-Y. *J. Food Sci.*, **1990**, *5*, 1233-1235, 1238.
85. Chung, S.-Y.; Vercellotti, J.R.; Johnson, P.B.; Klesius, P.H. *J. Agric. Food Chem.* **1991**, *4*, 764-769.
86. Whitfield, F.B.; Last, J.H.; Shaw, K.J.; Tindale, C.R. *J. Sci. Food Agric.* **1988**, *46*, 29-42.
87. Xi, Y.; Yamaguchi, T.; Takeuchi, M.; Iida, H. *Nippon Shokuhin Kagaku Kogaku Kaishi* **1995**, *42*, 677-681.
88. Ke, P.J.; Ackman, R.G.; Linke, B.A. *J. Am. Oil Chem. Soc.* **1975**, *52*, 349-353.
89. Shahidi, F.; Synowiecki, J.; Amarowicz, R.; Wamasundara, U.N. In *Lipids in Food Flavors*; Ho, C.-T.; Hartman, T.G., Eds. *ACS Symposium Series 558*, American Chemical Society: Washington, D.C., **1994**, pp. 233-243.
90. Shahidi, E.; Wanasundara, U.N.; Brunet, N. *Food Res. Int.* **1994**, *27*, 555-562.
91. Sherwin, E.A. In *Food Additives*; Brannen, R., Ed.; Marcel Dekker, Inc.: New York, NY, 1990, pp. 139-193.

92. Dziezak, J.D. *Food Technol.* **1986**, *40*(9), 94-102.
93. Wanasundara, U.N.; Shahidi, F. *J.Am. Oil Chem. Soc.* **1996**, *73*, 1183-1190.
94. Taguchi, K.; Iwani, K.; Ibuki, F.; Kawabata, M., *Biosci., Biotech., Biochem.* **1992**, *56*, 560-563.
95. Lin, C.-C.; Lin, S.-Y; Hwan, L.S. *J. Food Sci.* **1995**, *60*, 36-39.
96. Wanasundara, U.N.; Shahidi, F. *J. Food Lipids*, **1995**, *2*, 73-86.
97. Shahidi, F.; Han, X.-Q. *CRC Crit. Rev. Food Sci. Nutr.* **1993**, *33*, 501-547.
98. Haraldsson, G.G. *J. Am. Oil Chem. Soc.* **1984**, *61*, 219-222.
99. Haagsma, N.; Gent, C.M.; Luten, J.B.; Jong, R.W.; Doorn, E. *J. Am. Oil Chem. Soc.* **1982**, *59*, 117-118.
100. Mukherjee, K.D. *Biocatalysis* **1990**, *3*, 277-293.
101. Shahidi, F.; Wanasundara, P.K. J.P.D. In *Food Lipids*, Akoh, C.C.; Min. D., Eds., Marcel Dekker, Inc.: New York, NY, 1997, in press.
102. Shahidi, F.; Wanasundara, U.N. *J. Food Sci. Technol. Inter.* **1996**, *2*, 73-81.
103. Shahidi, F.; Wanasundara, U.N. In *Natural Antioxidants: Chemistry, Health Effects and Applications*, Shahidi, F., Ed.; AOCS Press: Champaign, IL, 1997, pp. 379-396.
104. Shahidi, F.; Wanasundara, U.N. In *Food Lipids*, Akoh, C.C.; Min. D.B., Eds., Marcel Dekker, Inc.: New York, NY, 1997, in press.
105. Olcot, H.S.; Einset, E. *J.Am. Oil Chem. Soc.* **1958**, *35*, 161-162.
106. Shahidi, F. In *Natural Antioxidants: Chemistry, Health Effects and Applications*, Shahidi, F., Ed.; AOCS Press: Champaign, IL, 1997, pp. 1-17.
107. Bors, W.; Michel, C.; Stettmaier, K.; Heller, W. In *Natural Antioxidants: Chemistry, Health Effects and Applications*, Shahidi, F., Ed.; AOCS Press: Champaign, IL, 1997, pp. 346-357.
108. Davies, M.J. *Chem. Phys. Lipids* **1987**, *44*, 149-173.
109. van de Voort, F.R.; Ismail, A.A.; Sedman, J.; Emo, G. *J.Am. Oil Chem. Soc.* **1994**, *74*, 243-253.
110. Burkow, I.C.; Moen, P.; Oreboik, *J.Am. Oil Chem. Soc.*, **1992**, *69*, 1108-1111.
111. Neeman, I.; Joseph, D., Biggeley, W.H.; Seliger, H.H. *Lipids*, **1985**, *20*, 729-734.
112. Wanasundara, U.N.; Shahidi, F. *J. Food Lipids* **1993**, *1*, 15-24.
113. Ebeler, S.E.; Shibamoto, T. In *Lipid Chromatographic Analysis*. Shibamoto, T., Ed.; Marcel Dekker, Inc.: New York, N.Y., 1994, pp. 223-249.

## Chapter 2

# Influence of Processing on the Flavor of Seafoods

Leon C. Boyd

Department of Food Science, North Carolina State University, Box 7624,  
Raleigh, NC 27695-7624

Traditional methods of processing fishery and seafood products have included cooking, pickling, and smoking. However, consumer desire for convenience foods has created the demand for fresh and frozen products and other ready-to-serve products. Since aquaculture products continue to command an increasing market share of processed seafood products, differences in their physical and chemical properties which will affect flavor must be considered during processing. Critical processing parameters affecting the flavor and quality of processed seafoods include quality of raw materials, addition of processing aids, storage, packaging, and handling conditions. The ability to monitor and control key flavor and aroma components associated with desirable and undesirable flavors will assist in developing high quality value-added seafood products.

Consumer acceptance of seafoods depends on a number of factors affecting the quality of the finished product. While safety and wholesomeness are the background of all consumer concerns, the flavor, aroma, and palatability are the major attributes consumers can readily pass judgment on in their acceptance or rejection of the fishery product. Seafoods are unique in that they have the capacity to change very rapidly from a sweet delicate seaweedy aroma and flavor to a stale rancid flavor. Unlike many other meats, seafoods are characterized by diverse flavors within species and certainly across the varied types of seafoods. The extremely delicate flavor of seafoods and their ability to change dramatically over short periods of time continue to be major concerns of all segments of the food processing industry.

### I. Role of Food Processing in Seafood Flavor

Maintenance of the delicate balance of desirable and undesirable flavor compounds of fresh seafoods requires a knowledge of marker compounds affecting quality. As

biochemical markers of quality, such compounds as trimethylamine (TMA), total volatile bases (TVB), and oxidized lipids provide points of quality assessment to determine the loss of flavor and quality and the progression of spoilage (1).

At the point of harvest, fresh fish contain a delicate balance of protein, free amino acids, carbohydrates, nucleotides, organic acids, lipid and lipid derived compounds which give seafoods a unique and characterizing flavor, different from that of other meats. As biochemical changes occur due to the action of spoilage microorganisms and inherent enzymatic activity on proteins, nucleic acids, and lipids, desirable flavors and aromas are replaced by undesirable flavors and aromas. The deamination of adenine nucleotides to inosine monophosphate and the accumulation of hypoxanthine and diamines are good indicators of flavor loss and ensuing spoilage. Even though auto-oxidation of lipids and the accumulation of oxidized flavors is secondary to the formation of fishy flavors and aromas, they too provide an index of the loss of flavor and quality. Thus, the development and measurement of biochemical markers prior to processing is key to maximizing the natural flavor of seafoods (1,2).

Traditional processing of seafoods has involved preparation of raw fish and shellfish for distribution and display at the retail market. However, increased consumer desire for more convenient, ready-to-serve items has placed new demands on the seafood industry to develop novel processing technologies. These technologies must not only maintain the delicate flavor of seafoods, but increase the functional use of seafoods. The quality and flavor of raw and processed seafoods are influenced by a combination of intrinsic and extrinsic factors. A number of studies (2,3) show that the intrinsic qualities of fish and shellfish impart unique and distinguishing flavors to seafoods. These factors include species, size, age, sex, degree of sexual maturation, season of the year, body composition, feed source, and environmental factors. Since many of these factors are beyond the control of food processors, most research studies have been directed toward extending the shelf life of fresh and frozen raw seafoods to reduce spoilage and off-flavors from occurring during distribution and storage. More recently, efforts have also been directed toward developing and marketing fully or partially processed value-added seafood products. Traditional processing technologies focused primarily on maintaining and extending the shelf life of raw seafoods to avoid the typical "fishy" aroma and flavor associated with aging seafoods. Typical processing techniques include temperature control, irradiation, addition of processing aids, and packaging. Novel processing technologies designed to improve the functional value of seafoods as a meat include modified atmospheric packaging (MAP), and more recently the application of hydrostatic pressure. Processing techniques for value-added seafood products include flavored seafood entrees, *sous vide*, surimi, and vacuum injection of processing aids, such as stabilizers and flavor enhancers.

### A. Effects of Chilling, Freezing, and Frozen Storage

Fresh seafoods are highly perishable, and once removed from their natural environment lose their delicate flavor and aroma very rapidly. Since the fresh retail market still represents a significant portion of total seafood sales, numerous studies have focused on extending the shelf life of fresh seafoods by varying the temperature

of storage and packaging conditions. For example, one such study (4) compared the storage stability of channel catfish to hybrid catfish packaged in an overwrap and in a vacuum-skin package, both stored at 4°C. Vacuum-skin packaging increased the shelf life to 13 days with less free fatty acids, less psychrophilic counts, and less flavor losses than observed in overwrapped packages.

Storage studies (5-8) of aquaculture species show a similar trend to that observed for wild species in that as the storage temperature is lowered, the delicate flavor of seafoods is retained. Storage studies (5) of pond-raised hybrid striped bass under chillpack (-2°C) and refrigerated temperatures (3°C) found major improvements in sensory and chemical quality as the temperature of storage was decreased. Fillets were stored with and without the presence of the belly flap in highly oxygen permeable Cryovac E bags (Duncan, SC) with aerobic plate count, hypoxanthine values, and sensory measurements used to assess quality losses. A trained sensory panel found that 21 day chillpack samples were comparable in flavor and aroma to 8 day refrigerated samples with no differences in sensory quality or storage stability due to the presence of the belly flap. Hypoxanthine values appeared to be a good indicator of quality loss.

Since aquaculture fish quite often do not develop the fishy aroma associated with trimethylamine oxide decomposition observed in wild fish, hypoxanthine values and the ratio of inosine to hypoxanthine value have been used to monitor flavor deterioration in aquaculture species. A comparison of the storage stability of wild and hybrid striped bass confirmed that hypoxanthine values and the ratio of inosine to hypoxanthine could be used to assess freshness of both marine and fresh water fish. Cultured striped bass maintained freshness for 10 days at refrigerated temperature compared to 5 days for wild-captured striped bass (6).

Freezing is one of the most frequently used techniques to store and distribute seafoods. During frozen storage, changes in texture and flavor continue to occur, though at a slower rate. Lean fish and shellfish will increase in toughness due to protein aggregation and subsequent water loss. By contrast, fattier species will develop off-flavors due to auto-oxidation of membrane lipids. Studies (6,7) show that improvements in texture and flavor can be affected by adequate temperature control and method of thawing. A frozen storage study (7) of rainbow trout at -18°C, coupled with slow thawing at 5°C, showed that these conditions produced greater biochemical changes ( $\beta$  N-acetylglucosaminidase activity), larger drip volumes, and greater sensory changes than the corresponding -40°C storage and fast thawing in 25°C water.

Similar temperature effects have been demonstrated in hake and mackerel. These are two of the more difficult species to store in that mackerel has a relatively high lipid content and undergoes lipid oxidation and flavor changes during fresh or frozen storage, whereas hake undergoes rapid odor and texture changes due to enzymatic activity. The effects of frozen temperature were demonstrated in a study (8) which compared the effects of -20°C, -30°C and -40°C on the quality of fillets and minces over a 0 to 24 month storage period. Both sensory and textural deteriorations increased over the 24 month storage period. Samples stored at the lower temperatures developed less fishy taste and aroma and retained more of the original sweetness and fresh fish taste. The shelf life for acceptable sensory qualities of hake was extended from 9 to 18 months, whereas for mackerel it was extended from 3 months to 24 months when stored at -40°C.



## B. Effects of Food Additives

As there are limits to which temperature alone can slow the natural deteriorative processes in fresh and frozen seafoods, a number of food additives have been applied to fresh and cooked seafoods in an effort to delay flavor and texture changes. These additives are generally in the areas of antimicrobial agents to extend shelf life of fresh products; antioxidants to delay oxidation during fresh and frozen storage; flavor enhancers to mask off-flavors and intensify natural flavors; and cryoprotectants to reduce texture and flavor changes occurring during frozen storage. However in many instances, processors tend to combine these additives to maximize their effectiveness with least cost and product application foremost in the decision making process (9-11). For example, one such study (10) examined the effects of 2% solutions of sodium lactate, sodium acetate, and propyl gallate in conjunction with an overwrap or vacuum-skin package on quality changes of shrimp and catfish fillets stored at 4°C over a period of 12 days. Sodium acetate and vacuum-skin packaging were the most effective methods of reducing microbial counts and ammonia formation without noticeable changes in pH or color. The 2% propyl gallate was effective in retarding psychrophilic growth but noticeable changes in color were observed.

Application of sodium lactate to shrimp showed an effect similar to that observed with catfish in that reduced microbial counts were obtained and product shelf life was extended from 4 to 12 days. However, "fishy" and "muddy" aftertastes were observed in samples treated with 1% lactate, with 10% of the panelists detecting an aftertaste in the 2% solution described as 'metallic', 'sodium' and or 'chemical'. The sodium content of the fillets was tripled by the addition of the sodium lactate (11).

One of the most innovative processing techniques for seafoods has been the addition of glucose oxidase/catalase in glucose solutions. In this method, the enzyme system generates gluconic acid which lowers the surface pH of the product to delay the outgrowth of the natural flora. This system has been used to effectively extend the shelf life of shrimp by more than 5 days without noticeable changes in flavor, texture or color. The system was most effective when used as a holding solution within 4 hr of catching and was least effective when applied as a dip or after 82 h of holding in ice (12).

The blue crab and salmon industries share the distinction of having "market gluts" that often exceed the fresh market demand resulting in lowered prices to fishermen and processors alike. Therefore, there is often the need to further process large quantities of raw material for later use. Traditionally, this has been accomplished by developing value-added products to handle both excesses during peak production as well as normal processing. These processes involve canning, pasteurization, and freezing. All of these processing methods produce changes in flavor and texture, resulting in reduced product quality and reduced consumer acceptance. Processors have responded by combining cryoprotectants and phosphates to retain moisture and reduce flavor and texture deterioration. For example, sodium tripolyphosphate (STP), sodium glutamate, high pH, and an antioxidant system were applied under high pressure to burbot fillets and whitefish fillets prior to storage under frozen conditions. Samples dipped in STP solutions retained more moisture, better textural qualities, and were preferred overall to controls stored at -60°C, or -12°C (13). Other uses for STP solutions have included the prevention of struvites in canned fish and curd formation

in canned salmon previously frozen. Dips of 5% STP for 2 to 120 seconds effectively reduced curd formation, improved color stabilization, and improved moisture retention as noted by both sensory and objective evaluations (9,14).

Fresh ready-to-eat blue crabmeat is a highly perishable product with a shelf life of only 5 to 7 days. In an effort to extend the harvesting season for the blue crab industry, and to avoid market gluts, many blue crab processors will often pasteurize crabmeat and store it at refrigerated temperatures for up to 90 days. However, noticeable changes in texture and flavor have been observed and market prices generally reflect a poorer quality of product. An alternative processing technique currently being used by the crab industry involves cryogenically freezing crabs following atmospheric cooking.

Cryogenic freezing has also been used in conjunction with cryoprotectants in an effort to protect the delicate flavor and texture of processed crabmeat and as an alternative to pasteurization. A study by Henry et al, (15), examined the effects of cryoprotectants and cryogenic freezing with liquid CO<sub>2</sub> on the quality and flavor of fully cooked crab meat in comparison to pasteurized samples and controls containing no additives. Fully processed crabmeat was dipped in solutions of cryoprotectants that included polydextrose (15%); sucrose/sorbitol (7.5% each) plus 0.5% STP, and water. Samples were frozen and evaluated over 24 months for changes in texture, flavor, and aroma as measured by sensory and objective methods. A trained taste panel found that the addition of the cryoprotectants (i.e. polydextrose alone or as a solution with sucrose/sorbitol/phosphate) improved fresh crab flavor and aroma, sweetness, and texture with less ammonia and sour aroma notes detected. Pasteurized samples contained more sour and rancid flavor notes and less fresh crab flavor and aroma when compared to controls cryogenically frozen and stored at -20°C. Both trained and consumer panels rated polydextrose-treated samples highest in sweetness and retention of natural fresh crab flavor and aroma.

Of the processing aids listed above, phosphates have been used most often in processed seafoods. Their multifunctional use as moisture, texture, and flavor protectors, antioxidants, and cryoprotectants have favored their use in raw and cooked seafood products. The GRAS (generally regarded as safe) status afforded phosphates and their general acceptance by consumers has encouraged the seafood industry to explore their use in a number of functional applications for raw and processed foods (9).

### C. Effects of Modified Atmospheric Packaging

Even though modified atmospheric packaging (MAP) has not been fully accepted as a major processing procedure for seafoods in the United States, primarily due to regulatory concerns, its potential as a method to retain much of the fresh flavor and aroma of minimally processed seafood products appears to be great. The major advantages of MAP are its ability to limit the growth of spoilage microorganisms and inhibit or reduce biochemical degradation by enzymes. Since seafoods are subject to rapid flavor deterioration due to spoilage by microorganisms and oxidation, MAP holds the potential of offering seafood processors a more economical method to extend the shelf life and quality of products while reducing the use of additives.

The basic principle of MAP involves the removal and/or partial replacement of oxygen with primarily carbon dioxide in conjunction with other inert gases, such as nitrogen and carbon monoxide. Because of the high solubility of CO<sub>2</sub> in water where it forms carbonic acid, reductions in surface pH occur resulting in less growth of aerobic spoilage microorganism. MAP has also been shown to exhibit bacteriocidal effects similar to that observed for anti-microbial agents. The net effect on flavor and aroma is that less trimethylamine is formed, therefore, less off-odors. Numerous studies (16-20) have demonstrated that MAP can extend the shelf life of seafoods with only minor changes in flavor. One of the major flavor changes most frequently cited is the presence of an "acid-sour" flavor due to the growth of lactic acid producing bacteria. However, this has been shown to dissipate upon opening the package. For example, in a study (17) which compared the effects of 0%, 20%, and 40% CO<sub>2</sub> atmospheres on rockfish and salmon steaks, both 20% and 40% levels of CO<sub>2</sub> reduced the formation of trimethylamine and ammonia with the 40% level being the most effective.

In a study (18) which combined laminated high/low density semi-permeable packages in a CO<sub>2</sub> enriched environment, MAP extended the shelf life of trout without noticeable changes in flavor. An 80:20 mixture of CO<sub>2</sub> and nitrogen, with and without the addition of 2.3% potassium sorbate was used as a dip prior to packaging. Shelf life was doubled from 10 to 20 days with a noticeable drop in the "grassy" or "seaweed-like" odor to a predominant stale odor. No rancidity was observed. Objective assessment of oxidation by thiobarbituric acid reactive substances (TBARS) measurements indicated that neither MAP nor potassium sorbate suppressed oxidation. Other studies with cultured fish, such as tilapia, showed a similar trend in that shelf life of fillets were extended from 9 days in air to 25 days in 75% CO<sub>2</sub>:25% N<sub>2</sub> atmosphere using high barrier bags (19).

The *hurdle-barrier concept* (3), which involves the application of several or multiple barriers to prevent the outgrowth of microorganisms, has also been used in conjunction with MAP. Chlorine when applied as a dip was compared to distilled water, followed by packaging in 60% CO<sub>2</sub>, 6% O<sub>2</sub> and 34% N<sub>2</sub> using hybrid striped bass strips. Even though chlorine additions had no effect on aerobic plate counts, or taste, modified K values (i.e. excluding inosine values) showed that chlorine addition to MAP increased shelf-life from 4 to more than 7 days. Taste panel scores indicated that hybrid striped bass strips packaged in MAP retained more flavor than samples stored in air after 7 days, and were rated average after 13 days of refrigerated storage (20).

Currently, there appears to be very little application of MAP in the processing of seafood products in the United States due to concerns for safety. The distribution and retail purchasing patterns in the US and the lack of consumer education are cited as the two major drawbacks to the expansion of MAP in the US seafood industry. Reports (3, 18, 19) indicate, however, that MAP is being applied to meats, poultry, and seafoods in many parts of Europe and Japan. Both retailers and consumers have readily accepted MAP as an "innovative technology" that improves the quality and flavor of seafoods. In Japan, the use of oxygen absorbers in conjunction with CO<sub>2</sub> generators and ethanol generators are widely used to extend the shelf life of seafoods (3). The absorbers are marketed as 'Ageless' (Mitsubishi Gas Chemical Co) and are contained as sachets in packaged meats and seafood for extended shelf life. The

major flavor disadvantage of using absorbers occurs in high-fat or high moisture foods where some off-flavors may be produced due to dissolution of CO<sub>2</sub> into the product.

#### D. Effects of Irradiation

As a traditional process, the use of low doses of irradiation has been shown to be highly effective in reducing microbial spoilage of seafoods. Recent US legislation allowing the use of 3 kGy for processed chicken is an indication that use of low doses of irradiation is likely to be accepted in other meat products as more data become available on its safety and its effectiveness in inhibiting the growth of pathogenic microorganisms. Optimal irradiation doses have been shown to vary from 0.75 to 2.5 kGy (3) and can extend the shelf life of seafoods from 2 weeks to as much as 6 weeks when stored at 0 to 5°C. In an Austrian study (21), the effects of different levels of irradiation (i.e. 0, 1, 3, and 5 kGy) on the microbiological and sensory quality of various seafoods were examined. The seafoods differed widely in their fat content and included such products as mackerel, mullet, red emperor, whiting, sand crab, and two species of prawns, (i.e. king and Moreton Bay). With the exception of king and Moreton Bay prawns, doses higher than 3 kGy caused adverse odor and flavor changes. The off-flavors produced by the irradiation were described as "strong, dirty/off/unclean", and increased in intensity with increasing doses of irradiation. The flavor of the prawns was described as "very salty/sulfur dioxide/chemical/off" and were positively impacted by increasing doses of irradiation (i.e. up to 3 kGy).

Irradiation of frozen grass prawns stored at -10°C showed that the threshold level for detecting an irradiation flavor was 4.5 kGy. However, doses as low as 2.5 kGy were also effective in reducing microbial counts, whereas all doses decreased the levels of C<sub>20:5</sub>, C<sub>22:6</sub> and thiamine levels (22).

#### E. Effects of High Hydrostatic Pressure

The application of hydrostatic pressure is one of the most innovative processing techniques and is of great interest to the meat, poultry, and seafood industries. The potential benefits of high pressure include the following:

1. Less damage to heat-labile compounds than observed with traditional heat-processing;
2. Inactivation of natural enzymes that lead to product deterioration during prolonged frozen storage;
3. Inactivation of pathogenic and spoilage microorganisms that cause deterioration of foods;
4. Increased gelation of proteins needed in comminuted products as well as tenderization of pre-rigor meats often used in comminuted products.

The effectiveness of high pressure in decreasing enzymatic activity is of major concern to the seafood industry in that seafoods deteriorate during short-term and long-term storage leading to texture and flavor changes. Seafood lipids are characterized by triglycerides containing high amounts of long chain highly unsaturated fatty acids as well as phospholipids of even higher unsaturation. During

frozen storage, phospholipid hydrolysis is increased leading to increases in free fatty acids and loss of protein quality as oxidized lipids form complexes with protein.

Several studies (3,21,23-26) have shown the potential of high pressure as a technique to enhance the storage stability of seafood products. Application of pressures of 1 kPa to 10 kPa to minced sardine caused decreases in bacterial counts, and improvements in lipid quality. When compared to non-treated samples, pressure treated samples contained higher K-values and lower free fatty acids, while phospholipid hydrolysis, generally a signal of flavor deterioration, was reduced (23, 24).

Other studies (25,26) on the application of high pressure to cod and mackerel muscle found mixed results. Pressures of up to 6 kPa decreased phospholipid hydrolysis. However, increases in peroxide values of pressure treated samples were observed. Application of high pressure to oysters has also shown mixed results in that flavor was enhanced with no indication of oxidation, though color and texture were impacted negatively. The investigators (21) postulated that the improved flavor might have been due to greater impregnation of the salty liquor into the flesh of the oysters. These very limited studies indicate that additional research is needed to determine whether positive analytical findings on decreases in phospholipid hydrolysis, free fatty acids, and enzymatic activity can be translated into meaningful reductions in oxidized flavors and improved texture of processed seafood products.

#### F. Effects of Cooking, Fermentation, and Thermal Processing

Many studies (2,26-31) have well documented that thermal processing of meats and seafoods produce unique flavor and aromas. Many of these flavors and aromas are products of the composition of the plant or animal and the action of the processing procedure(s) used in preparing the food. Typical processing procedures used in the seafood industry include pickling, conventional heat processing, pasteurization, boiling, and most recently, *sous vide* processing. Full expression of seafood flavors appears to be a composite of the natural composition of the raw seafood product, the environment, and the effects of the processing procedure (2,26-28). For example, pickled fish have a distinct flavor that is due to a blend of alcohols and carbonyls with each species of fish and seafood being uniquely different. Even though only trace quantities of eight and nine carbon volatile carbonyls may be present in pickled fish, the low threshold of these compounds allow them to be major players in seafood flavors (26). In a study (32) which compared the volatile components of several salt-fermented fish to fermented shrimp pastes, the flavor causing components appeared to be different. Aldehydes, ketones, alcohols, and esters, products of lipid degradation through fermentation, were present in all the fish examined. However, lipid-derived components were lower in the shrimp paste, whereas nitrogen containing compounds, such as the pyrazines, predominated in the fermented shrimp and are believed to be the characterizing flavor compounds.

The characterizing compounds of crabmeat volatiles produced by boiling and pasteurization have been shown to be affected by the method of cooking (33). Fifty-three compounds were identified in boiled crabmeat yielding the greatest number of volatiles which corresponded to greater intensity of flavor and aroma when compared to pasteurized crabmeat.

*Sous vide* processing is currently regarded as the most novel technique for preserving the delicate and unique flavor of cooked seafoods. Raw products are typically placed in a flexible oxygen impermeable package, with or without the addition of spices, vacuum sealed, and pasteurized to an appropriate temperature. Pasteurized products are rapidly cooled and stored between 0°C and 5°C until used by the consumer. Since *sous vide* processed foods are generally pasteurized to the minimum temperature necessary to kill vegetative cells of spoilage microorganisms, the process is generally regarded as a very mild heat process which maintains the cellular integrity of products; and therefore, both the flavor and texture of products is improved. Other major advantages of *sous vide* processed foods include minimum flavor and nutrient losses, and extended shelf life of products. As high quality raw ingredients and tightly controlled temperature conditions are required throughout the processing and distribution of *sous vide* processed foods, concerns for safety and the lack of adequate controls are the major stumbling blocks preventing the adoption of the technique by the seafood industry (2,31)

## II. Processing of Aquaculture Products and Flavor Changes

The processing of cultured seafood products demands special attention in that results from controlled and antidotal studies (2,34-39) show that compositional differences exist between cultured and wild-caught fish within the same species, and that these differences may influence flavor and textural quality as well as consumer acceptance of cultured seafoods. Since compositional and environmental conditions have been shown to influence the flavor and quality of cultured seafoods, it is important for seafood processors to define and maximize conditions which can enhance the flavor and quality while minimizing conditions which negatively impact flavor and stability. Studies (35-37) show that farmed-raised fish differ in color, texture, flavor, and quality from free-living fish. Farm-raised fish have softer texture, less hue, and have a milder, less robust flavor than wild-caught fish. Differences in flavor can often be accounted for due to differences in diet composition, native differences in lipoxxygenase activity, and the impact of the environment.

Storage stability studies of farm-raised hybrid striped bass compared to wild striped bass show that farm-raised bass developed less off-flavors described as fishy, oxidized, and cod-liver. Objective assessment of freshness as indicated by inosine to hypoxanthine ratio and hypoxanthine values confirmed sensory data showing that farmed-raised fish reached critical spoilage levels in 10 day compared to 5 day for wild striped bass (34). Similar results have been demonstrated in catfish (34) and in trout (37) in which increased dietary supplementation of tocopherol and astaxanthin, respectively, increased storage stability resulting in reduced oxidation and decreased rate of discoloration.

Farm-raised fish typically possess very mild flavor; however, under poor growing conditions, rapid uptake of off-flavors may occur resulting in strong "earthy-musty" flavors. Farm-raised catfish are notorious for their uptake of off-flavors and it is believed this is due to the rapid absorption of odorants through the gills in contrast to marine fish absorption of compounds through the blood. Controlled studies of the uptake of dimethyl sulfide and 2-pentanone by live channel catfish show that the two

flavorants are absorbed within minutes (i.e. 30 min) and that the uptake of selected flavorants can be translated into increased flavor scores (39).

Thus, these studies (33-39) demonstrate that while aquaculture products may not contain the hue, flavor, and texture of wild species, opportunities exist to enhance flavor and storage stability through dietary supplementation. Studies of saltwater Pacific salmon show that the sea-, brine-, and iodine-like flavors present in many saltwater salmon are due to bromophenols and that these compounds, though absent from fresh water salmon, are easily absorbed by fresh water fish (38). Similarly, studies of the rainbow trout and of farm-raised catfish show that both color and oxidative stability of aquaculture raw materials can be improved by the additions of astaxanthin and tocopherols (39). The addition of astaxanthin at 70 mg/kg of feed was effective in delaying oxidative deterioration in trout for up to 29 wk compared to 17 wk in untreated frozen trout.

### Conclusions

Traditional processing techniques for seafoods have been designed primarily to extend the shelf life of fresh and frozen seafoods. However, since many of today's consumers desire convenient, easy-to-prepare meals, seafood processors are using a combination of traditional and novel techniques to extend the shelf life of seafoods and to protect and improve the delicate flavor of seafoods during storage and distribution. Even though traditional techniques, such as adequate temperature control techniques, irradiation, and the use of additives can effectively extend shelf life of products, the application of modified atmospheric packaging, high pressure, and *sous vide* processing hold the potential to improve the functional use of seafoods while maintaining and improving flavor.

### Literature Cited

1. Ashie, I.N.A.; Smith, J.P.; Simpson, B.K. *Crit. Rev. Food Sci. Nutr.* **1996**, *36*, 121.
2. Love, R.M. *The Food Fishes: Their Intrinsic Variation and Practical Implications*; Farrand Press: London, 1988; pp. 149-159.
3. Haard, N.F. *Food Res. Int.* **1992**, *25*, 289-307.
4. Huang, Y.W.; Lovell, R.T.; Dunham, R.A. *J. Food Sci.* **1994**, *59*, 64-66.
5. Boyd, L.C.; Green, D.P.; LePors, L.A. *J. Food Sci.* **1992**, *57*, 59-62.
6. Karahadian, C.; Fowler, K.P.; Brannan, R.G. *J. Aquatic Sci.* **1995**, *4(1)*, 7-29.
7. Nilsson, K.; Ekstrand, B. *J. Food Sci.* **1995**, *60*, 627-635.
8. Wanderer C.K.; Sagi, I.; Hwang, K.T.; Regenstein, J.M. *J. Food Sci.* **1993**, *58*, 1208-1211.
9. Dziezak, J.D. *Food Tech.* **1990**, *44(4)*, 79-92.
10. Zhuang, R.Y.; Huang, Y.A.; Beuchat, L.R. *J. Food Sci.* **1995**, *61*, 241-261.
11. Williams, S.K.; Rodrick, C.R.; West, R.L. *J. Food Sci.* **1995**, *60*, 636-639.
12. Donero, M.; Egana, W.; Tarky, W.; Cifuentes, A.; Torres, J.A. *J. Food Sci.* **1993**, *58*, 774-779.
13. Krivchenia, M.; Fennema, O. *J. Food Sci.* **1988**, *53*, 1004-1008.
14. Wekell, J.C.; Teeny, F.M. *J. Food Sci.* **1988**, *53*, 1009-1013.

15. Henry, L.K.; Boyd, L.C.; Green, D.P. *J. Sci. Food Agric.* **1995**, *69*, 21-26.
16. Stammen, K.; Gerdes, D.; Caparaso, F. *Crit. Rev. Food Sci. Nutr.* **1990**, *29*, 301-347.
17. Brown, W.D.; Albright, M.; Watts, D.A.; Heyer, B.; Spruce, B.; Price, R.J. *J. Food Sci.* **1980**, *45*, 93-97.
18. Barnett, H.J.; Conrad, J.W.; Nelson, R.W. *J. Food Prot.* **1987**, *50*, 645-651.
19. Przybylski, L.A.; Finerty, M.W.; Grodner, R.M.; Gerdes, D.L. *J. Food Sci.* **1989**, *54*, 269-273.
20. Handumrongkul, C.; Silva, J.L. *J. Food Sci.* **1994**, *59*, 67-69.
21. Poole, S.E.; Mitchell, G.E.; Mayze, J.L. *J. Food Sci.* **1994**, *59*, 85-105.
22. Hau, L.-B.; Liew, M.; Yeh, L. *J. Food Prot.* **1992**, *55*(3), 198-202.
23. Hoover, D.G.; Metrick, C.; Papineau, A.M.; Farkas, D.F.; Knorr, D. *Food Tech.* **1989**, *43*(3), 99-107.
24. Wada, S. In *High Pressure and Biotechnology*; Balny, C.; Hayashi, R.; Heremans, K.; Masson, P., Eds.; Colloque INSERM/Libbey Eurotext Ltd: London, 1992; Vol 224, pp 235-238.
25. Murakami, T.; Kimura, I.; Yamagishi, T.; Yamashita, M.; Sugimoto, M.; Satake, M., In *High Pressure and Biotechnology*; Balny, C.; Hayashi, R.; Heremans, K.; Masson, P., Eds.; Colloque INSERM/Libbey Eurotext Ltd: London, 1992; Vol 224, pp 329-331.
26. Ohshima, T.; Nakagawa, T.; and Koizumi, C. In *Seafood Science and Technology*; Bligh, E.G., Ed.; Fishing News Books: Oxford, England, 1992; pp 64-75.
27. Josephson, D.B.; Lindsay, R.C.; and Stuiber, D.A. *J. Food Sci.* **1987**, *52*, 596-600.
28. Josephson, D.B.; Lindsay, R.C. In *Biogenesis of Aromas*; Parliment, T.H.; Croteau, R., Eds.; ACS Symposium Series No. 317; American Chemical Society: Washington, DC, 1986; pp 201-219.
29. Kawai, T. *Crit. Rev. Food Sci. Nutr.* **1986**, *36*(3), 257-298.
30. Kubota, K.; Uchida, C.; Kurosawa, K.; Komuro, A.; Kobayashi, 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 376-385.
31. Beauchemin, M. In *Advances in Fisheries Technologies and Biotechnology for Increased Profitability*. Voigt, M.N.; Botta, J.R., Eds.; Technomics Publishing Co.: Lancaster, PA, 1989, pp 169-188.
32. Cha, Y.J.; Cadwallader, K.R. *J. Food Sci.* **1995**, *60*, 19-24.
33. Matiella, J.E.; Hsieh, T.C.-Y. *J. Food Sci.* **1990**, *55*, 962-966
34. Erickson, M.C. *J. Food Sci.* **1991**, *56*, 1113-1115.
35. Mohr, V. In *Seafood Quality Determination*; Kramer, D.E.; Liston J., Eds.; Elsevier Science Publishers: Amsterdam, 1986; pp 87-96.
36. Fowler, K.P.; Karahadian, C.; Greenberg, N.J.; Harrell, R.M. *J. Food Sci.* **1994**, *59*, 70-90.
37. Bjerkeng, B.; Johnsen, G. *J. Food Sci.* **1995**, *60*, 284-288.
38. Boyle, J.L.; Lindsay, R.C.; Stuiber, D.A. *J. Food Sci.* **1992**, *57*, 918-92.
39. Maligalig, L.L.; Caudle, L. *J. Food Sci.* **1975**, *41*, 1242-1245.



## Chapter 3

# Role of Aldehydes in Cooked Fish Flavors

Takayuki Shibamoto and Masahiro Horiuchi

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

n-Saturated aldehydes (formaldehyde, acetaldehyde, propanal, butanal, pentanal, hexanal, heptanal, octanal, and nonanal) formed in the headspace of heated fish flesh (herring, mackerel, sardine, and squid) were derivatized to their corresponding thiazolidine derivatives and then analyzed by a capillary gas chromatography (GC) with nitrogen-phosphorus detection. Formaldehyde and acetaldehyde generally made up the largest quantities of aldehydes recovered. Volatile compounds formed in headspace of heated menhaden fish oil with or without trimethylamine oxide (TMAO) were also analyzed by GC and GC/mass spectrometry. Major compounds formed in headspace of heated fish oil without TMAO were aldehydes including n-C<sub>1</sub>-C<sub>10</sub> saturated aldehydes and some branched aldehydes. Only two nitrogen-containing compounds (N,N-dimethylformamide, N-methylpyrrole) were found in volatiles formed in headspace of heated fish oil with TMAO. These two nitrogen-containing compounds made a strong contribution to the cooked flavor of fish oil.

Cooked fish has a unique characteristic flavor that is evolved from a mixture of low molecular weight aldehydes and browning reaction products. The browning reaction, which is an interaction between carbonyl compounds and amine compounds, produces many volatiles including cooked flavor compounds (1). Lipids have been reported as precursors of carbonyl compounds in the formation of browning-flavor compounds in lipid-rich foods such as fish (2). Fish flesh contains high levels of lipids ranging from 0.5% to 25% (3). Figure 1 shows the fatty acid content of fish oils from menhaden and herring. Polyunsaturated fatty acids (PUFAs) render fish flesh extremely susceptible to oxidation and rapid degradation. These processes result in the production of volatile aldehydes such as formaldehyde, acetaldehyde, and propanal (4). These carbonyl compounds undergo secondary reactions with amine compounds to produce a cooked flavor.

Trimethylamine oxide (TMAO) is reported to be present, often at high levels, in fish, particularly in fish living in cold water. Table I shows serum TMAO concentrations in some marine fish (5). TMAO has been predicted to form several amine compounds upon thermal degradation (6). Therefore, aldehydes formed from lipid oxidation and amines produced from TMAO by a heat treatment may react to form a cooked flavor through the browning reaction (Figure 2).

In the present study, volatile aldehydes formed in the headspace of various heated fish flesh were determined. Also compounds formed from the interaction of menhaden fish oil and TMAO were analyzed to investigate the formation of cooked fish flavor.

**Table I. Serum trimethyl amine oxide (TMAO) concentrations in marine fishes**

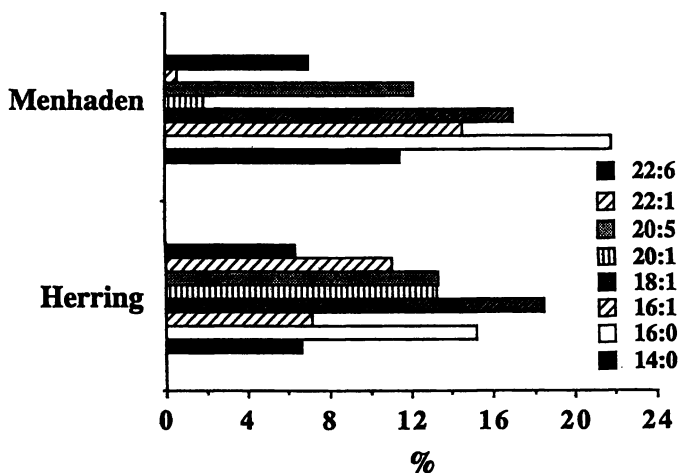
Species	TMAO (mmol/L)	
	Northern Japan	Alaska
Pacific herring	26.4	46.6
Surf smelt	24.6	31.8
Whitespotted greenling	11.9	12.0
Saffron cod	6.8	4.3
Walleye pollock	0.7	2.9
Sculpin	0.2	1.3

Adapted from ref. 5.

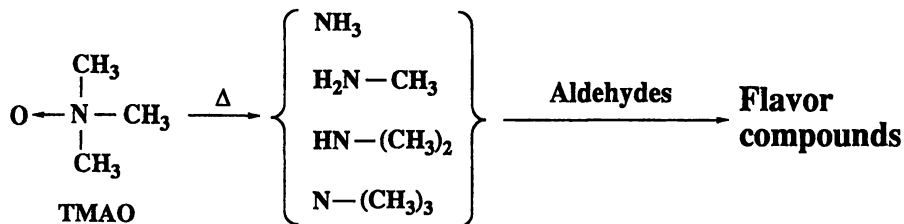
## Experimental

**Collection of Aldehydes Formed in a Headspace of Heated Fish Flesh.** Figure 3 shows the apparatus used to collect volatile aldehydes from heated fish flesh. A fish sample (100 g) was placed in a 3-L three-neck round-bottom separable flask. When the flask temperature reached 200 °C, 7 L of vapor in the headspace of the flask was drawn into the two impingers connected in a series at 300 mL/min and then the solutions in the impingers were combined. Each impinger contained 50 mL of an aqueous cysteamine solution (20 mg/mL). After the solution was allowed to stand for 30 min at room temperature, it was extracted with 10 mL of dichloromethane three times. The extracts were combined and then poured onto a column packed with anhydrous sodium sulfate to remove the water. The extract was condensed to 2 mL by distillation through a Vigreux column. A 10 mL benzene solution of N-methylacetamide (10 mg/mL) was added as an internal standard for GC analysis.

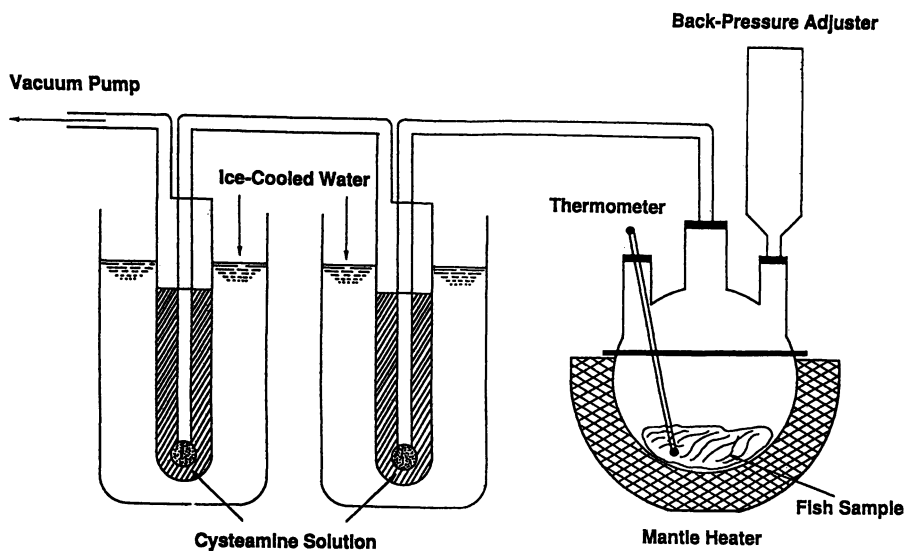
**Collection of Headspace Volatiles Formed from a Fish Oil Heated with or without TMAO.** Menhaden fish oil (10 g, Sigma Chemical Co., St. Louis, MO) was placed in a 50 mL two-neck, round-bottom flask with or without 0.5 g of TMAO (Aldrich Chemical Co., Milwaukee, WI). The flask was connected to a simultaneous purging and solvent extraction apparatus (SPE) prepared previously (7). The fish oil was heated at 200 °C and the headspace volatiles were purged into 250 mL of deionized water by a steam of purified air (flow rate 7.2 mL/min) for 6 h. The volatiles dissolved by the water were simultaneously and continuously extracted with 50 mL of dichloromethane. After the extract was dried over anhydrous sodium sulfate, it was condensed using a



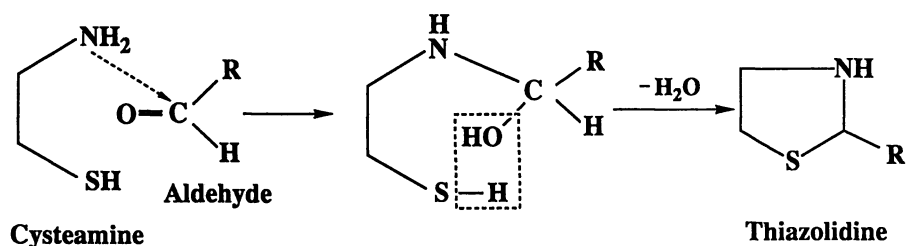
**Figure 1.** Fatty acid composition of fish oils from menhaden and herring.



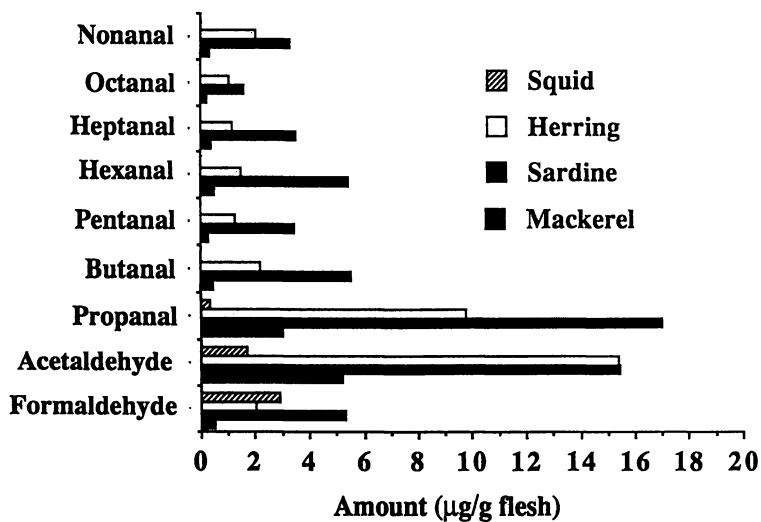
**Figure 2.** Formation of flavor compounds from TMAO and aldehydes upon heat treatment.



**Figure 3.** Apparatus used to collect headspace volatiles formed from heated fish flesh.



**Figure 4.** Reaction mechanism of cysteamine and aldehydes.



**Figure 5.** Results of aldehydes analysis in headspace volatiles formed from the heated fish flesh.

rotary flash evaporator and then further condensed under purified nitrogen stream to 0.3 mL.

For specific analysis of aldehydes, aqueous cysteamine solution was placed in a SPE instead of deionized water. Thiazolidines derived from aldehydes with cysteamine were extracted simultaneously and continuously with 50 mL of dichloromethane for 6 h. After the extract was dried over anhydrous sodium sulfate, it was condensed using a rotary flash evaporator and then further condensed under purified nitrogen stream to 0.3 mL.

**Instrumental Analysis.** A Hewlett-Packard (HP) Model 5890 gas chromatograph (GC) equipped with a 30 m X 0.25 mm i.d. ( $d_f = 0.25$  mm) DBWAX fused silica capillary column and a flame ionization detector (FID) were used for comprehensive analysis, 30 m X 0.25 mm i.d. ( $d_f = 0.25$  mm) DB-1 fused silica capillary column and nitrogen-phosphorus detector (NPD) were used for specific analysis of aldehydes.

A Varian 3500 GC interfaced to a Finnigan Mat Model 800 ion trap detector was used for MS identification of the GC components. The column and oven conditions were identical to the ones used for the HP GC.

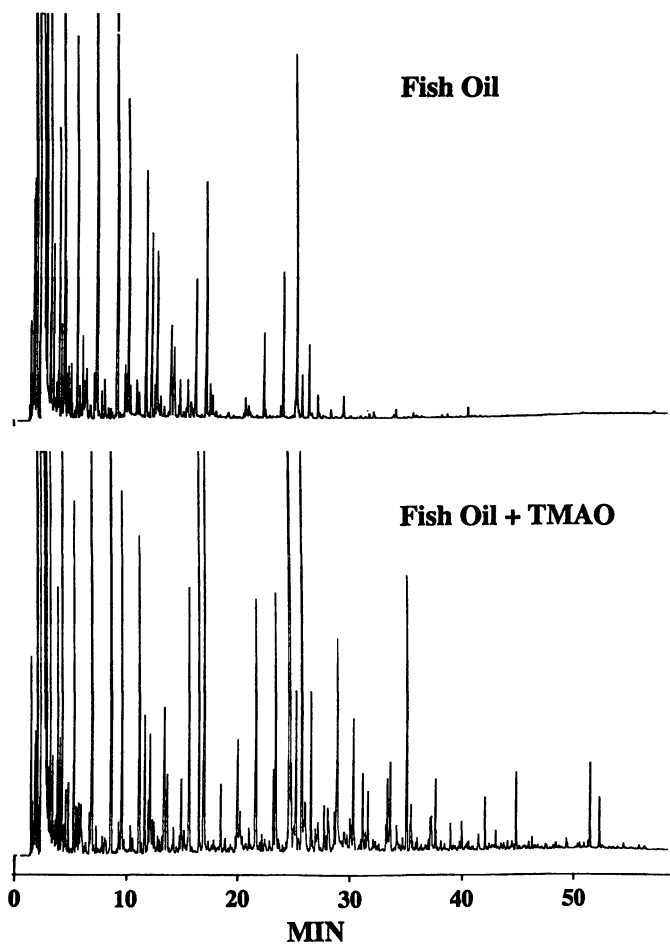
## Results and Discussion

It is extremely difficult to analyze highly volatile and reactive aldehydes such as formaldehyde and acetaldehyde. Most commonly used methods for these aldehydes involve derivatization with 2,4-dinitrophenylhydrazine (8). However, this derivatization requires a strong acidic condition which may alter the chemicals of interest.

The cysteamine method for aldehyde analysis is simple and specific. Volatile aldehydes react with cysteamine readily to form thiazolidines under mild conditions of neutral pH and room temperature. Moreover, yields of thiazolidine derivatives are over 95% (9). The mechanism of this reaction is shown in Figure 4.

Figure 5 shows results of aldehydes analysis in headspace of heated fish flesh of various species. Determination of formaldehyde is not possible without derivatization due to its high volatility and reactivity. The amounts of formaldehyde recovered ranged from 0.48 (marckerel) to 5.31 mg/g (sardine). The amounts of acetaldehyde found ranged from 1.70 (squid) to 15.47 mg/g (sardine). Formation of propanal in relatively large amounts was observed in herring and sardine, suggesting that these fish contain high level of  $\omega$ -3 fatty acids (10). The total amounts of aldehydes formed from marckerel and squid were relatively low. Herring and sardine produced over 3 mg of total aldehydes in a headspace from 100 g of flesh. Generally, fish flesh containing high levels of lipids such as sardine and herring produced more aldehydes than those containing low levels of lipids such as squid. Therefore, it was speculated that fish oil plays an important role in formation of aldehydes which would undergo secondary reactions with amine compounds to form cooked flavors.

Figure 6 shows typical gas chromatograms of volatiles recovered from heated fish oil with or without TMAO. It is obvious that addition of TMAO produced greater numbers of volatiles. Figure 7 shows relative amounts of n-saturated aldehydes formed in the headspace of heated fish oil with or without TMAO. It is difficult to

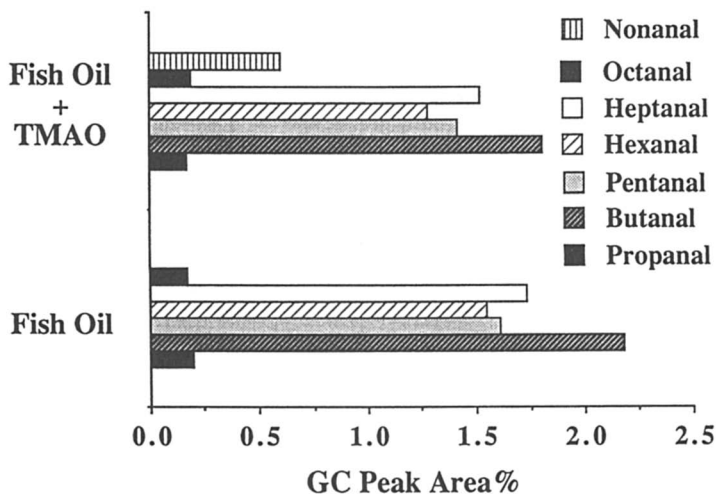


**Figure 6.** A typical gas chromatogram of volatiles formed from the heated menhaden fish oil with or without TMAO.

discuss quantitative variations among volatile aldehydes because some highly volatile aldehydes such as formaldehyde and acetaldehyde were not recovered in this experiment. Propanal was expected to be generated at the highest level because of the presence of  $\omega$ -3 fatty acids in fish oil. However, the values of propanal were lower than expected values because certain amounts of propanal escaped from the system during the experiment. Figure 8 shows the unsaturated aldehydes recovered. Acrolein was recovered in a relatively large amount. Acrolein is known to be formed from oxidative dehydration of glycerol formed from lipids (11). In fact, 120 g of various cooking oils produced acrolein ranged from 30 mg (soybean oil) to 72 mg (olive oil) (12).

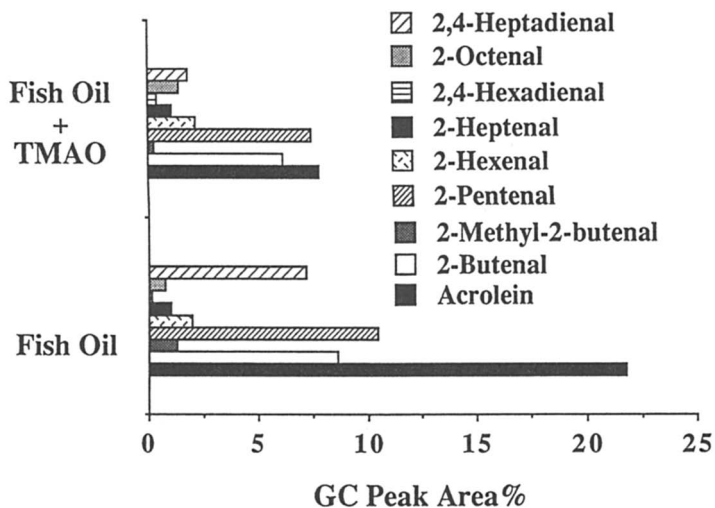
Figure 9 shows compounds identified only in the volatiles obtained from fish oil heated with TMAO. Unexpectedly, only two nitrogen containing compounds, *N,N*-dimethylformamide and *N*-methylpyrrole, were recovered. Also there were some unsaturated aldehydes and dienals. Therefore, TMAO may work as an oxidative agent rather than as a precursor of flavor compounds (13). However, presence of *N,N*-dimethylformamide and *N*-methylpyrrole gave a flavor similar to cooked fish, suggesting that these two compounds play a dominant role in cooked fish flavor.

Figure 10 shows a typical gas chromatogram of thiazolidine derivatives obtained from reaction between cysteamine and aldehydes formed in the headspace of heated fish oil without TMAO. Figure 11 shows results of quantitative analysis of aldehydes. The values are average of an experiment with three replicates. As mentioned above, quantitative analysis of highly volatile aldehydes, particularly formaldehyde and acetaldehyde, in the vapor phase is extremely difficult. Formaldehyde tends to be adsorbed onto a glass surface (14). Moreover, dichloromethane contains a small amount of formaldehyde (15). Therefore, the results of formaldehyde analysis are not reported here. In contrast to the results from the

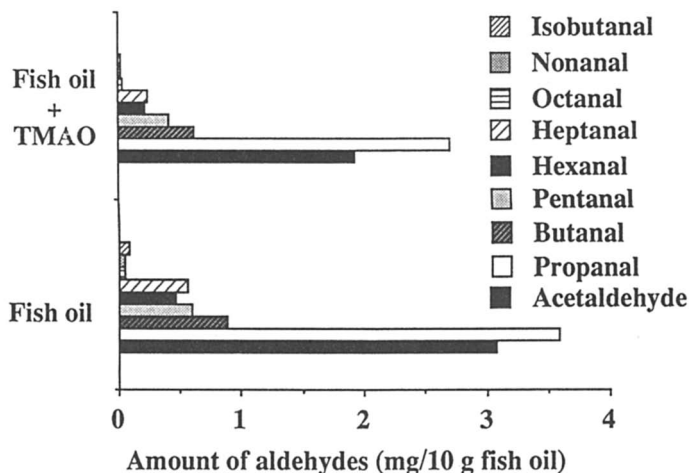


**Figure 7.** Relative amount of n-saturated aldehydes formed in the headspace of heated manhaden fish oil with or without TMAO.

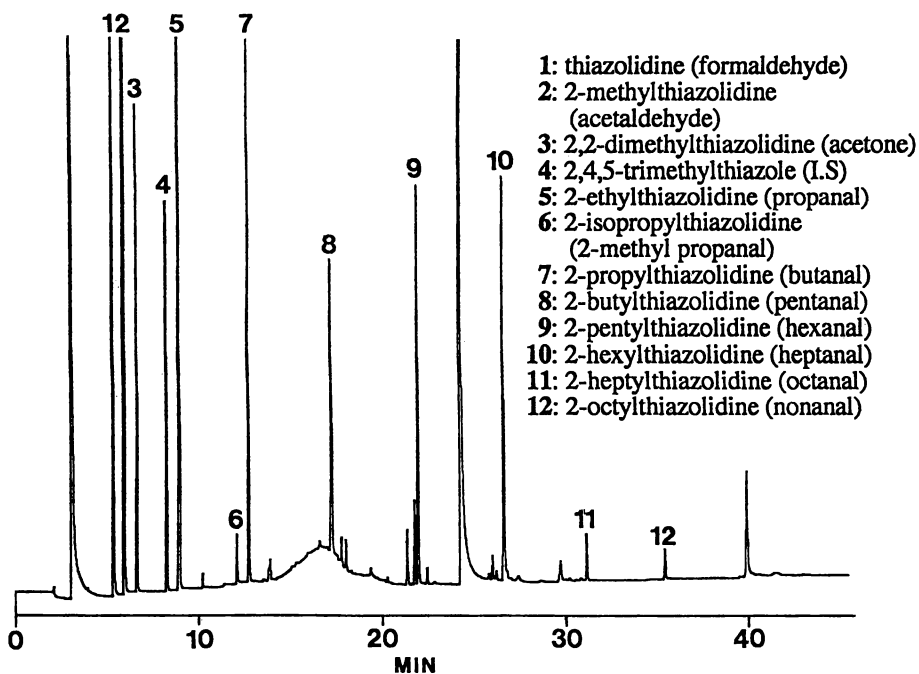




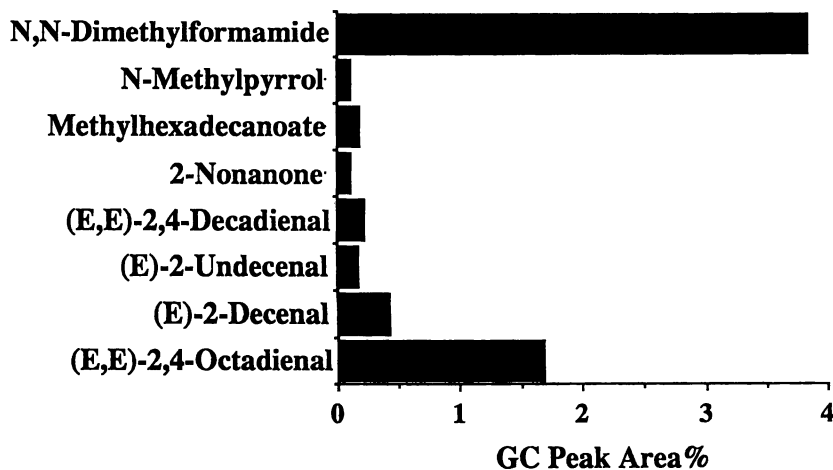
**Figure 8.** Relative amount of unsaturated aldehydes formed in the headspace of heated manhaden fish oil with or without TMAO.



**Figure 9.** Relative amount of compounds recovered only from the headspace of heated manhaden fish oil with TMAO.



**Figure 10.** Gas chromatogram of thiazolidines derived from aldehydes formed in the headspace of heated manhaden fish oil without TMAO.



**Figure 11.** Results of quantitative analysis of aldehydes formed in the headspace of heated manhaden fish oil with or without TMAO.

experiment without cysteamine, acetaldehyde and propanal were recovered at higher levels. Levels of aldehydes, in particular acetaldehyde and propanal, were reduced by addition of TMAO, suggesting that these aldehydes underwent secondary reaction by the action of TMAO.

In the present study, TMAO was used as a source of amines for the browning reaction. Because many reactive carbonyl compounds such as dienals and unsaturated aldehydes were formed from heated fish oil, selection of an amine precursor such as an amino acid may be important to obtain cooked fish flavor. Investigation using a model system consisting of fish oil and cysteine is currently under way.

### Literature Cited

1. Shibamoto, T. In *Instrumental analysis of foods, recent progress*; Charalambous, G., Inglett, G., Eds.; Academic Press: New York, 1983, Vol. 1; pp. 229–278.
2. Ohnishi, S.; Shibamoto, T. *J. Agric. Food Chem.* **1984**, *32*, 987–992.
3. Kinsella, J. E. *Seafoods and Fish Oil in Human Health and Disease*. Marcel Dekker: New York, 1987.
4. Esterbauer, H. In *Free Radicals, Lipid Peroxidation, and Cancer*; McBrien, D.C.H., Slater, T. F., Eds.; Academic Press: New York, 1982, pp. 101–128.
5. Raymond, J. A. *Fish Physio. Biochem.* **1994**, *13*, 13–22.
6. Reece, P. *J. Sci. Food Agric.* **1983**, *34*, 1108–12.
7. Umamo, K.; Shibamoto, T. *J. Agric. Food Chem.* **1987**, *35*, 14–18.
8. Papa, L. J.; Turner, L. P. *J. Chromatogr. Sci.* **1972**, *10*, 747–50.
9. Yasuhara, A. and T. Shibamoto. *Agric. Biol. Chem.* **1989**, *53*, 2273–2274.
10. Boyd, L.C.; King, M.F.; Sheldon, B. *J. Am. Oil Chem. Soc.* **1992**, *69*, 325–330.
11. Adkins, H.; Hartung, W. H. *Synthesis Organiques*; Masson: Paris, 1935.
12. Umamo, K. and T. Shibamoto. *J. Agric. Food Chem.* **1987**, *35*, 909–912.
13. Knolker, H. J. *J. Prakt. Chem.-Chem. Zei.* **1996**, *338*, 190–192.
14. Yasuhara, A.; Shibamoto, T. *J. Assoc. Off. Anal. Chem.* **1989**, *72*, 899–902.
15. Hayashi, T.; Reece, C.; Shibamoto, T. *J. Assoc. Off. Anal. Chem.* **1986**, *69*, 101–105.

## Chapter 4

# Determination of Potent Odorants in Ripened Anchovy (*Engraulis encrasicolus* L.) by Aroma Extract Dilution Analysis and by Gas Chromatography–Olfactometry of Headspace Samples

Réda Triqui<sup>1</sup> and Helmut Guth<sup>2</sup>

<sup>1</sup>Institut Agronomique et Vétérinaire Hassan II, B.P. 6202–Instituts 10101, Rabat, Morocco

<sup>2</sup>Deutsche Forschungsanstalt für Lebensmittelchemie, Lichtenbergstrasse 4, D–85748 Garching, Germany

The potent odorants of ripened anchovy were evaluated by aroma extract dilution analysis (AEDA) and gas chromatography-olfactometry of headspace samples (GCO-H). For AEDA, volatiles were isolated by direct solvent extraction followed by sublimation in vacuo to recover solvent plus volatile constituents. Methional and (Z)-1,5-octadien-3-one were the most potent odorants among eighteen odorants in the flavor dilution range of 10-1000. Eleven odorants were detected by GCO-H of 20 mL headspace samples of ripened anchovy equilibrated at 35°C. Results of GCO-H were in good agreement with those of AEDA in that methional and (Z)-1,5-octadien-3-one were perceived at the small headspace samples of ripened anchovy (1 mL). Based on results of GCO-H, acetaldehyde, 3-methyl-butanal and 3-methyl-ropanal were the most intense odorants among the highly volatile constituents of ripened anchovy.

Recently, we reported on volatile compounds of importance to the aroma of anchovy and on changes in flavor profiles associated with the ripening process (1-2). In these investigations, the volatiles were isolated from anchovy by distillation of an aqueous slurry under vacuum at temperatures of 57-58°C and by subsequent solvent extraction of the distillate. Due to the relatively high temperatures used for isolation, the potential for generating thermally induced artifacts was not dismissed. Therefore, in the present study the volatiles were isolated by sublimation in vacuo (3).

Aroma extract dilution analysis is limited to odorants boiling higher than the solvent used for the extraction and dilution steps. Furthermore, odorants boiling in the same range as the extraction solvent are partially lost during the concentration of the extract by distilling off the solvent (4). To evaluate the contribution of highly volatile compounds to the flavor of ripened anchovy, AEDA was complimented by gas chromatography-olfactometry of headspace samples (GCO-H) (5-6).

Table I. Volatile compounds identified in ripened anchovy

No <sup>a</sup>	Compound	Odor Description	RI <sup>b</sup>		ID <sup>c</sup>
			DB-5	FFAP	
6	2,3-pentanedione	butter-like		1053	(1)
7	hexanal	green, fresh grass		1076	(1)
8	(E)-2-hexenal	green	853	1207	(1)
9	methional	potato-like	907	1445	(1)
10	2-acetyl-1-pyrroline	roasty, popcorn-like	923	1323	(2)
11	1-octen-3-one	mushroom-like	980	1289	(2)
12	(Z)-1,5-octadien-3-one	geranium-like	985	1365	(2)
13	2,4-heptadienal <sup>e</sup>	fatty	997		(2)
14	(E,E)-2,4-heptadienal	fatty	1012	1457	(1)
15	5-ethyl(5H)-dihydro-furan-2-one <sup>d</sup> (agelicalactone)		1037	1588	(4)
16	phenylacetaldehyde	floral	1050	1619	(1)
17	(E)-2-octenal	fatty	1060	1415	(1)
18	(E,Z)-3,5-octadien-2-one	fatty-fruity	1095		(1)
19	(E,E)-2,4-octadienal	fatty	1110	1576	(1)
20	(E,Z)-2,6-nonadienal	cucumber-like	1152	1571	(1)
21	(E)-2-nonenal	cucumber-like, green	1160	1521	(1)
22	(E,E)-2,4-nonadienal	fried fat-like	1212	1683	(1)
23	2,4,6-nonatrienal <sup>e</sup>	sweat, anise-like	1270	1913	(3)
24	2,4,6-nonatrienal <sup>e</sup>	sweat, anise-like	1278		(3)
25	(E,Z)-2,4-decadienal	fatty, green	1296	1734	(1)
26	(E,E)-2,4-decadienal	fried fat-like	1316	1790	(1)
27	4,5-epoxy-(E)-2-decenal	metallic	1387	1989	(2)

<sup>a</sup>Compound number in Figure 1. <sup>b</sup>Calculated retention index on capillaries DB-5 and FFAP. <sup>c</sup>Compound identified on the basis of the following criteria: (1) Comparison with the reference compound on the basis of odor quality perceived at the sniffing port, the RI values on capillary DB-5 and/or FFAP and the mass spectra obtained by MS (EI) and MS (CI); (2) The MS signals were too weak for interpretation. The compound was identified on the basis of the remaining criteria listed in footnote (1).; (3) Identified from Ulrich (15) and by comparing the RI values on both capillaries and the mass spectra of the synthesized compound.; (4) Compound tentatively identified by comparison with data from the library of mass spectra. <sup>d</sup>Odorless at the sniffing port. <sup>e</sup>Configuration of isomer not determined.

The aim of the present study was to employ AEDA and GCO-H procedures to evaluate potent odorants of ripened anchovy and to recommend indicator odorants for the objective assessment of ripening and of the quality of the final product.

### Materials and Methods

**Chemicals.** Pure samples of standard compounds (Table I, footnote 1) were provided by the Deutsche Forschungsanstalt für Lebensmittelchemie, Garching, Germany. Synthesis of 2,4,6-nonatrienal was done by the aldol condensation of (E,E)-2,4-heptadienal and acetaldehyde (7). Acetaldehyde (1.3 g) was added to a test tube containing (E,E)-2,4-heptadienal (1.1 g in 1 ml ether). After addition of 2 drops of a 50% aqueous solution of potassium hydroxide, the mixture was allowed to react under stirring for 24 hr at room temperature. The yield by this procedure was low (about 5%).

**Anchovy.** Two samples of whole (not filleted) fully ripened anchovy (12 weeks of ripening) were obtained from a local processor in the Rabat area, Morocco. Anchovy were immersed in a salt-brine and stored in closed cans at 4°C for later analysis.

**Isolation of the volatiles.** Fish (100 g) were cut in small pieces, soaked in methylene chloride (100 ml) and then homogenized using an ultra-turrax for two min. To the suspension, 100 ml of methylene chloride was added and the solvent was filtered off under low vacuum. The remaining material was further washed with 50 ml of methylene chloride and filtered. The extract was then dried over anhydrous sodium sulfate and concentrated to about 100 ml by distilling off the methylene chloride on a Vigreux column (50 x 1 cm) at 40°C.

The volatile compounds were distilled off from the nonvolatile material under high vacuum in the apparatus previously described (3), but with the following modifications: two traps cooled with liquid nitrogen were used and the water jacketed tube was held at a temperature of 35°C. The sample was poured into a 250 ml distillation flask and frozen for 30 min in liquid nitrogen. Following sublimation of the volatiles and of the solvent in vacuo ( $10^{-4}$  mbar), the temperature of the water bath was increased to 35°C and the sublimation was continued for a further 2 h. The condensate of the first cooling trap was dried over anhydrous sodium sulfate and then concentrated to about 2 ml by distilling off the solvent on a Vigreux column (50 x 1 cm) and then by microdistillation (8).

**High Resolution Gas Chromatography (HRGC).** HRGC was performed by means of a Carlo Erba GC (Carlo Erba, Hofheim, Germany) using DB-5 and DB-FFAP fused silica capillaries (each 30 m x 0.32 mm, film thickness 0.25  $\mu$ m; J & W Scientific, Folsom, USA). The samples were applied by the on-column injection technique at 35°C. After 1 min, the temperature of the GC oven was raised by 40°C/min to 60°C, held 5 min isothermally, then raised at 4°C/min to 250°C (230°C for DB-FFAP). The flow rate of the carrier gas (helium) was 2 ml/min. At the end of the capillary, the effluent was split 1:1 (by volume) into an FID detector and a sniffing port using deactivated uncoated fused silica capillaries (40 x 0.3 mm). The FID and sniffing port were held at a temperature of 200°C. The splitter was flushed with helium to accelerate the split flow to 10 ml/min. Nitrogen (20 ml/min) was used as makeup gas

for the FID. Retention data of the compounds are presented as retention indices (RI) (9).

**HRGC-Eluate Sniffing.** The aroma concentrates were analyzed by HRGC and by eluate sniffing of the following dilution series: The original extract (100 ml) was stepwise diluted (1+9, v/v) by addition of methylene chloride. The potent odorants were located in the capillary gas chromatograms by AEDA (10).

**Mass Spectrometry (MS).** MS analyses were performed with a Finnigan MS 8230 (Bremen, Germany) mass spectrometer using the aforementioned DB-5 and DB-FFAP capillary columns. Mass spectra in the electronic impact mode MS (EI) were generated at 70 eV and in the chemical ionization mode MS (CI) at 90 eV with isobutane as reagent gas.

**Gas chromatography-Olfactometry of Headspace Samples (GCO-H).** GCO-H was performed with a CP-9001 GC connected to a purge and trap system TCT/PTI 4001 (Chrompack, Frankfurt, Germany) as recently reported (6-11), but with the following modifications: The GC was equipped with a DB-5 fused silica capillary as previously described. The sample of ripened anchovy (10 g) was put into the vessel (volume: 250 ml), sealed with a septum, and then held for 30 min in a 35°C water-bath. The headspace volumes detailed in Table I were drawn into a gastight syringe and then injected at a flow rate 10 ml/min into the purge system operating in the desorption mode for 10 min at a temperature of 250°C. The conditions used for headspace analysis were the same as previously reported (6), except the temperature program for the capillary DB-5 was modified as follows: Before the start of each run the capillary was cooled to 0°C. After injection of the sample the temperature was held at 0°C for 1 min, then raised by 8°C/min to 250°C and held for 10 min.

## Results

The volatile fraction of ripened anchovy was isolated from 100 g of fish by solvent extraction at room temperature followed by sublimation in high vacuo. The resulting concentrated isolate had an odor similar to the characteristic smell of ripened anchovy. The volatile compounds which were identified in the samples investigated are reported in Table I. With the exception of compound no. 10 and isomers of 2,4,6-nonatrienal (no. 18 and 19), all the remaining volatiles were identified earlier (1). Two potent volatiles previously reported were not detected in the present study: (Z)-4-heptenal and 3-methyl-nonan-2,4-dione.

The screening of the volatiles by AEDA indicated 18 potent odorants in the flavor dilution range of 10-1000 (Figure 1). Methional and (Z)-1,5-octadien-3-one showed the highest FD-factors. The aromagram (Figure 1) also shows that 7 additional odorants contribute with relatively high FD factors to the flavor of ripened anchovy. It is worth mentioning that compound no. 19, identified as 2,4,6-nonatrienal, had a low FD factor in the present study, while previously it showed the highest FD value (1).

The results of GCO-H are shown in Table II. Direct sniffing of the air drawn by a gas tight syringe showed that the characteristic odor of ripened anchovy was

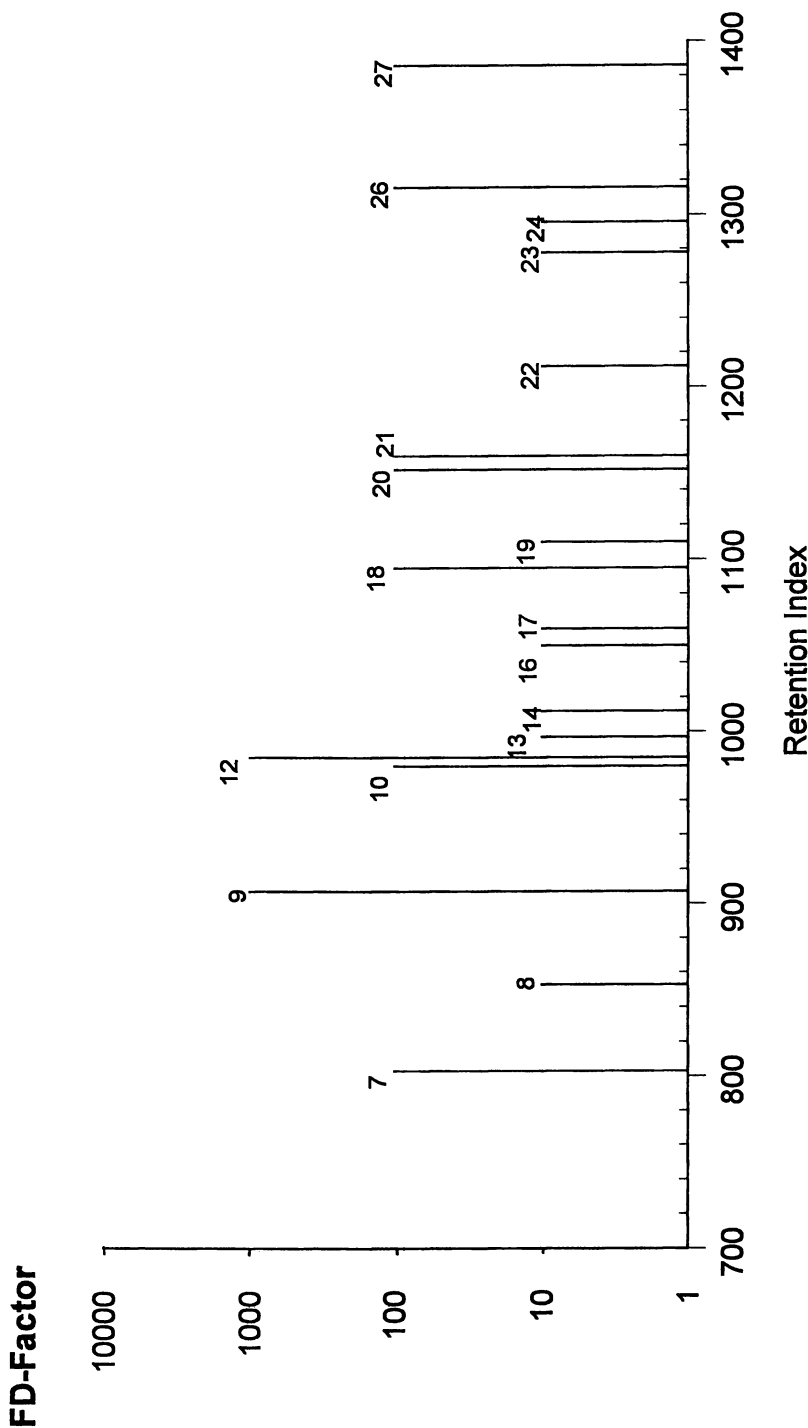


Figure 1. Flavor dilution chromatogram of volatile compounds isolated from ripened anchovy. (X axis is in retention indices on DB-5 capillary column; peak numbers correspond to those in Table I.)



perceived in a headspace volume of 20 ml. This volume was used for starting the GCO-H. Eleven odorants were detected in the 20 ml samples of the air above ripened anchovy. In addition to the compounds identified by AEDA, some highly volatile odorants (no. 1, 2, 3, and 5) were identified by comparing their RI values and odor qualities with those of the corresponding reference compounds. Compounds no. 6, 9, 11, 12, 20 and 26 were readily identified on the basis of the results of the AEDA. The results of GCO-H are in good agreement with those of AEDA since methional and (Z)-1,5-octadien-3-one were perceived in the low headspace volume of 1 ml. On the basis of their low headspace volume, acetaldehyde, 3-methylbutanal and 2-methylpropanal are the most potent of the highly volatile odorants of ripened anchovy.

**Table II. Volatile compounds identified in ripened anchovy by static headspace gas chromatography-olfactometry<sup>a</sup>**

No <sup>a</sup>	Compound	RI <sup>b</sup>	Odor Description	Vol(mL) <sup>c</sup>
1	acetaldehyde	<400	sweet	1
2	dimethyl sulfide	500	sulfurous, cabbage-like	20
3	2-methylpropanal	550	malty	10
4	unknown	n.d.	sulfurous	20
5	3-methylbutanal	651	malty	1
6	2,3-pentanedione	702	butter-like	10
9	methional	904	potato-like	1
11	1-octen-3-one	977	mushroom-like	10
12	(Z)-1,5-octadien-3-one	984	geranium-like	1
20	(E,Z)-2,6-nonadienal	1147	cucumber-like	10
26	(E,E)-2,4-decadienal	1314	fried fat-like	10

<sup>a</sup>The compounds were identified by comparison with the reference substances on the basis of retention index (RI) on capillary DB-5 and odor description assigned during GCO-H. <sup>b</sup>Retention index on DB-5 capillary. <sup>c</sup>Lowest headspace volume in which the odorant was perceived at the sniffing port during GCO-H. The lowest volume injected during GCO-H was 1 mL.

## Discussion

The characteristic flavor of ripened anchovy is obtained by ripening the fish at room temperature. Therefore, it was necessary to apply flavor isolation methods not involving heating of sample. The flavor isolates obtained in this study had an aroma quality similar to that of the fish samples while those obtained previously (1) had more green background notes.

The results of AEDA show that methional and (Z)-1,5-octadien-3-one belong to the potent volatiles of ripened anchovy. We found that the dilutions in which only

these two odorants were perceived by HRGC-eluate sniffing had still much of the characteristic aroma and the fishiness character of ripened anchovy. This is further confirmed by the results of GCO-H, since these two volatiles were perceived in the low headspace volume of 1 ml. On the basis of calculation of odor activity values (OAVs, ratio of concentration to odor threshold), (Z)-1,5-octadien-3-one and methional were found to be the most potent odorants of freshly boiled trout (*Salmo fario*) (12).

On the basis of their low headspace volume of 1 ml, acetaldehyde, 2-methylpropanal and 3-methylbutanal were the key, highly volatile components of ripened anchovy flavor. We have previously discussed the occurrence of strecker aldehydes in flavor isolates from anchovy as possibly being artifacts of aroma isolation (1). The present study shows that these aldehydes are actually important to anchovy flavor. Methional and phenylacetaldehyde were also found in anchovy which were ripened in the presence of microbial inhibitors. Therefore we can assume that strecker aldehydes are most likely formed from the breakdown of free amino acids by carbonyl compounds or fatty acid hydroperoxides. This may also explain the observation of some manufacturers that some extent of proteolysis is necessary before the flavor can develop during ripening.

The results of AEDA (Figure 1) indicate that the remaining odorants showing relatively high FD factors are either lipoxygenase-derived volatiles, e.g. 1-octen-3-one and (E,Z)-2,6-nonadienal, or products of lipid oxidation, e.g. (E,Z)-3,5-octadien-3-one and (E,E)-2,4-decadienal. Their importance to anchovy flavor was previously emphasized but the current investigation has shown that strecker aldehydes are of particular significance to this flavor. The combination of these aldehydes with products from lipid oxidation is likely responsible for the specific flavor obtained after ripening.

The unknown volatile which previously showed the highest FD factor in the FD chromatograms of anchovy (1) was identified as 2,4,6-nonatrienal. While there are eight possible cis-trans isomers of this compound, only two odor-active isomers were detected in anchovy with an odor somewhat reminiscent of anise. Owing to their low FD factor, they are only components of background flavor. 2,4,6-Nonatrienal was first characterized in the vacuum volatile oil of blended dry red and white beans (13). It has been postulated that this compound arises from an unusual breakdown of the linolenic acid in the bean (13).

## Conclusion

The salting and ripening of anchovy is a process that is still carried out empirically. We have previously emphasized the need to develop methods for the objective assessment of both the progress of ripening and the quality of the final product. An objective analytical approach would be to accurately quantify, at different stages of ripening, a few potent volatiles of anchovy, e.g. methional and (Z)-1,5-octadien-3-one by a stable isotope dilution assay (14).

## Literature Cited

1. Triqui, R.; Reineccius, G.A. *J. Agr. Food Chem.* **1995**, *43*, 453-458.
2. Triqui, R.; Reineccius, G.A. *J. Agr. Food Chem.* **1995**, *43*, 1883-1889.
3. Sen, A.; Laskawy, G.; Schieberle, P.; Grosch, W. *J. Agr. Food Chem.* **1991**, *9*, 757-759.
4. Semmelroch, P.; Grosch, W. *Lebensm. Wiss. Technol.* **1995**, *28*, 310-313.
5. Guth, H.; Grosch, W. *Flavour and Fragrance J.* **1993**, *8*, 173-178.
6. Milo, C.; Grosch, W. *J. Agr. Food Chem.* **1995**, *43*, 459-462.
7. Bohlmann, F.; Jastrow, H. *Chem. Ber.* **1962**, *95*, 2939.
8. Bemelmans, J.M.H. In *Progress in Flavor Research*; Land, D.G.; Nursten, H. E., Eds; Applied Sciences: London, 1979: pp 79-98.
9. Halang, W.A.; Langlais, R.; Kugler, E. *Anal. Chem.* **1978**, *50*, 1829-1832.
10. Guth, H.; Grosch, W. *Lebensm. Wiss. Technol.* **1990**, *23*, 59-65.
11. Guth, H.; Grosch, W. *J. Agr. Food Chem.* **1994**, *42*, 2862-2866.
12. Milo, C.; Grosch, W. *J. Agr. Food Chem.* **1993**, *41*, 2076-2081.
13. Buttery, R.G. *J. Agr. Food Chem.* **1975**, *23*, 1003-1004.
14. Schieberle, P.; Grosch, W. *J. Agr. Food Chem.* **1987**, *35*, 252-257.
15. Ullrich, F. Ph.D. Thesis, Technical University of Munich, Garching, Germany, 1989.

## Chapter 5

# Aroma Compounds of Fresh and Stored Mackerel (*Scomber scombrus*)

C. Alasalvar, P. C. Quantick, and J. M. Grigor

School of Applied Science and Technology, Food Research Center,  
University of Lincolnshire and Humberside, 61 Bargate, Grimsby,  
North East Lincolnshire, DN34 5AA, United Kingdom

Volatile aroma compounds of raw and cooked mackerel samples (fresh, and ice stored for 12 days inside a polystyrene box at  $2\pm 2^\circ\text{C}$ ) were analyzed by static headspace analysis/gas chromatography/ mass spectrometry (SHA/GC/MS). Compounds tentatively identified by MS were confirmed by comparing their mass spectra and GC retention times to those of standard compounds. Out of 32 peaks, 16 volatile compounds were identified in both raw and cooked mackerel samples. Among the identified compounds were 8 aldehydes, 2 ketones, 3 alcohols, 1 furan, 1 sulfur-containing compound and 1 alkene. Sensory and instrumental measurements of raw and cooked mackerel aroma were conducted throughout storage to establish relationships. Volatile aroma compounds of fresh and deteriorated mackerel samples were also analyzed by dynamic headspace analysis/gas chromatography/mass spectrometry (DHA/ GC/MS). Sixty five compounds were identified. Among these components, 4 aldehydes, 3 ketones, 10 alcohols, 2 esters, 3 alkenes, 3 sulfur-containing compounds and 1 acid were observed. The advantages and disadvantages of both SHA and DHA techniques are discussed.

Aroma is one of the most important quality determinants of fresh fish. Freshness is a primary concern of consumers. Consumers require quality and convenience, with safety, aroma and freshness as priorities, and price ranking only fifth in consideration (43). Subjective grading by sensory assessments is desirable because it provides immediate quality information, but it is difficult to detect sub-threshold concentrations of staling aroma compounds. This is also important in cooked fish since cooking would mask any undesirable or staling aroma changes in fish provided that these changes are not extreme (2). As a result, there is a demand for methods to objectively measure fish aroma quality. The ultimate goal being to

instrumentally detect and quantify the influential aroma compounds in fish at a rate that is compatible with contemporary industrial practices (22).

Volatile aroma compounds can be measured by several methods, but static headspace analysis (SHA) and dynamic headspace analysis (DHA) coupled with gas chromatography/mass spectrometry (GC/MS) have gained in popularity as effective, simple and rapid techniques for the analysis of volatile compounds in fish and other foods, since with these methods volatiles can be analyzed with minimum sample preparation and contamination. Moreover, these techniques also incur low risk of artifact formation as compared with steam distillation, vacuum distillation, solvent extraction and others (46).

Numerous applications of SHA and DHA techniques have been recently developed for the analysis of volatiles in fish and other foods. Volatiles of many seafood commodities have been analyzed by these techniques including determination of dimethyl sulphide in cod (42), amines in hake, sole, cod, rockfish, perch and lingcod (38), volatile halocarbons in eel, carp, striped bass and spot fish (9), ethanol in canned salmon (16), volatiles in canned salmon (13) and volatile aroma compounds in mackerel (1).

Very fresh fish flavors and aromas are characterized by mild, green and planty notes that are easily recognized (22), where the chemical basis is centered around the polyunsaturated fatty acids of fish lipids. The major flavor-impact compounds are several: 6-, 8-, and 9-carbon aldehydes, ketones and alcohols which are derived from fatty acids via specific lipoxygenase activity (21). By contrast, spoiled fish flavors and aromas are characterized by rancid/fishy, strong sulphurous and off-flavors (40, 41, 49). Sulphur compounds, phenols, certain fatty acids and (Z)-4-heptenal give rise to spoiled or putrid aromas in fish (15, 30, 31, 36). Chemical alterations of volatile compounds during storage of fish also contribute to various fish flavors (30).

SHA and DHA are widely used in volatile analysis and it is appropriate here to review generally the advantages and disadvantages of both techniques. The operation of SHA is simple and rapid and involves the chromatographic separation of a predetermined volume of vapor headspace above a sample held in a closed vial. In this technique, the sample is sealed in a vial and incubated. The volatiles are allowed to equilibrate between the sample and the gas phase, an aliquot of which is then injected into the GC-column for analysis (48). SHA is limited to the detection of low boiling point compounds in high abundance (13). SHA is insensitive to intermediate and high molecular weight aroma compounds and the water vapor in headspace gas samples can damage some gas chromatographic columns. By contrast, SHA is often the method of choice for highly volatile compounds in seafoods which are difficult to trap on adsorbents, e.g., hydrogen sulfide, dimethyl sulfide, methyl mercaptan, the short-chain alkylamines and short-chain neutral volatiles (22).

DHA is a technique which concentrates aroma volatiles on an adsorbent bed and in this technique, the sample is purged with an inert gas. The volatiles are swept out of the sample and adsorbed on a tubular trap filled with Tenax, charcoal or other suitable sorbent. The adsorbed volatile compounds in the Tenax trap are thermally desorbed at higher temperature. Desorbed compounds are cryofocused in the capillary interface at low temperature (e.g., -120°C) using liquid N<sub>2</sub> and the

condensed volatile compounds in the capillary interface are injected into the GC-column for analysis.

During the early stages of rancidity, some of the key volatile compounds are present only in very low concentrations and SHA is not sensitive enough to detect them. Better sensitivity, as compared with SHA, can be obtained by purging the sample (47). One of the main advantages of DHA is that equilibrium between gas phase and sample is not necessary and the desorption time can be chosen in such a way that all the volatiles are released from the sample. However, the human nose samples food aroma in an equilibrium state, therefore the volatiles trapped by the DHA method may not necessarily relate to the true aroma of the food (33) and SHA may be a more appropriate technique for predicting the sensory response. A further problem associated with DHA is that a large amount of water vapor from a sample can be simultaneously purged along with other volatiles. This problem is especially serious for DHA experiments carried out at high sample purging temperatures as water vapor decreases the trapping efficiency of the sorbent cartridge, such as Tenax-TA. Both dynamic and static methods may be used, but in the case of extremely low concentration levels where large sample volumes are necessary, the dynamic system is more convenient because of the possible enrichment of the components (12).

The objectives of this study were to: (a) investigate the volatile aroma changes in both fresh and ice stored raw and cooked mackerel samples and to identify the most important volatile compounds contributing to the stale aroma by SHA/GC/MS; (b) compare the sensory techniques and instrumental measurements of raw and cooked mackerel aroma throughout storage; and (c) investigate the volatile aroma compounds in fresh and deteriorated mackerel samples by DHA/GC/MS.

### Materials and Methods

In this section, SHA of volatile aroma compounds of mackerel samples will be reviewed. The experimental procedure of DHA/GC/MS were described in a previous study (1).

**Materials.** Atlantic mackerel (*Scomber scombrus*) was used which had been stored in ice for at least two days. Standard compounds for the determination of GC retention times and MS were purchased from Aldrich Chemical Co., and Sigma Chemical Co. Expanded polystyrene boxes were supplied from Grimsby Fish Dock, UK.

**Sample Preparation.** On arrival at the laboratory, the fish were placed in a polystyrene box with flake ice (3:1 ratio; 24 kg fish: 8 kg ice) and stored at  $2\pm 2^{\circ}\text{C}$  for 12 days. A 0.5 ml aliquot of internal standard (50 ppm 3-hexanol in deionized distilled water) plus 10 g of mackerel white muscle was homogenized for 2 min using a Waring blender. Six fish per day were randomly sampled on day 0, 3, 6, 9 and 12. Three fish were used for the volatile analysis of raw fish. The remaining three fish were used for sensory assessment of raw fish prior to cooking and filleting. One fillet of each cooked fish was used for volatile analysis, while the

other fillet was used for sensory evaluation. To prepare the cooked fish sample, approximately 30 g white muscle of mackerel were sealed in plastic bags and then cooked in steam for 15 min in a covered aluminum pan. The cooked samples were served hot to the panelists in bags for immediate opening and analysis.

**SHA/GC/MS.** Mass spectra of volatiles were obtained by a combination of a Varian Genesis Headspace Autosampler, Star 3400 CX GC and a Saturn GC/MS/MS 4D (Varian Associates Inc. CA). A 10 g minced mackerel sample in 22 ml headspace vial was placed in a Genesis Headspace Autosampler. The SHA conditions (incubation temperature of the vial, time of vial incubation, column headpressure, vent/fill loop time and injection time) were carried out as already described (13). A Varian Star 3400 CX GC with a high resolution gas chromatography column (DB-5MS; 30 m x 0.25 mm i.d x 0.25  $\mu$ m film thickness; J & W Scientific, Folsom, CA) operated with ultrahigh purity helium as carrier gas at a flow rate of 1 ml/min was used. Each sample was injected in the splitless (model 1077) mode (240°C injection temperature; 70 sec valve delay). The GC oven temperature was programmed from 35 to 175°C at 3°C/min with an initial hold time of 5 min. The GC peak areas were calculated by an electronic integrator. MS conditions were ion source temperature: 180°C, ionization voltage: 70 eV, mass scan range: 33-350 a.m.u., electron multiplier voltage: 1750 V and scan rate: 1000 msec. The obtained mass spectra were tentatively identified by comparison to reference spectra of NIST92/EPA/OG1 Mass Spectral Database (Varian Associates Inc. CA. and Ogava & Co., Japan). Compound identities were confirmed by comparing their mass spectra and GC retention times to those of standard compounds. Electron ionization (EI) was used. Further, chemical ionization (CI) was also used to aid identification and to confirm molecular weights. Triplicate analyses were performed for each sample.

**Sensory Assessment.** Fish quality was evaluated by 10 trained panelists. Shelf-life determinations were based on quality of the raw and cooked fish. The organoleptic assessment of raw fish were assessed (3) according to the Tasmania Food Research Unit (FTRU) Scheme. Each panelist was given up to 4 simple descriptors, which scored from 0 to a maximum of 3, where 0 represented good quality and any higher score indicated poor quality. The score for the separate characteristics were summed to give an overall sensory score (maximum score 40). This scheme gave a score of 0 for very fresh fish with increasingly larger totals as the fish deteriorated. The measurement of freshness of cooked fish (odor and flavor) were assessed (17) according to the Torry Scheme. A hedonic scale from 10 to 0 was used, 10 denoting absolutely fresh and 0 completely putrid or spoiled.

## Results and Discussion

**Sensory Analysis of Raw and Cooked Mackerel During Storage.** The organoleptic mean score of raw mackerel were analyzed during 12 days of storage in ice (Figure 1). In this scheme, 0 denoted absolutely fresh fish and 40 completely spoiled, a score of  $\approx$ 20 coincided with the level at which the fish were considered unacceptable by the members of the panel. Scores increased as deterioration increased (3). Mackerel can be stored fresh in ice up to 9 days, after which the

bacteriological counts (in excess of  $10^6$  cfu/g), trimethylamine and histamine formation are rapid (19).

Figure 2 shows the changes in sensory quality (odor and flavor) of cooked mackerel through 12 days of storage. The characteristic odor of mackerel gradually decreased in intensity during storage. In this scale, 10 donated absolutely fresh fish (strong seaweedy odors; fresh, sweet flavors characteristic of the species) and 0 completely putrid. A score of 4 (lactic acid and sour milk, or byre-like odors; some off flavors and some bitterness) was considered unacceptable by the members of the panel. Although mackerel were unacceptable on day 9 by the organoleptic mean score (Figure 1), cooked mackerel was considered of good quality, characterized by caramel or toffee-like odors; neutral flavor, but no off flavors. The reason for this could be explained in that cooking clearly would appear to mask any undesirable changes observed in fish provided these changes are not extreme.

Cooking can also mask quite significant differences in pre-cooked duckling with taste panelists having difficulty in distinguishing relatively fresh from obviously deteriorated duckling post-cooking (Hanna, J., University of Lincolnshire & Humberside, personal communication, 1993.).

**SHA of Volatile Aroma Compounds in Raw Mackerel During Storage.** Volatile aroma compounds of raw mackerel were analyzed. Out of 32 peaks, 16 volatile compounds were tentatively identified by SHA/GC/MS during 12 days of storage in ice (Table I). The identified compounds (8 aldehydes, 2 ketones, 3 alcohols, 1 furan, 1 sulfur-containing compound and 1 alkene) will be discussed in detail.

Eight aldehydes were identified in raw mackerel. The total peak area proportion of aldehydes decreased (Table I) during storage (66.51, 64.94 and 47.38%; day 0, 6, and 12, respectively), showing that these compounds were initially dominant in the fresh mackerel and contribute sweet floral, fruity aroma in seafood products. The majority of aldehydes are considered lipid autoxidation products (8). Hexanal, heptanal, octanal and dodecanal were detected in higher concentrations than 2-pentenal, 2-methyl-2-pentenal, (E)-2-hexenal and (Z)-4-heptenal during storage. These compounds have been previously reported in seafood products (8, 25, 45). The most abundant volatile compound, comprising about 34.9% of the total aldehydes, was hexanal which contributes to the distinct coarse, green plant-like, grassy and apple-like aromas of most fresh fish (23). Hexanal is formed by a 15-lipoxygenase system acting on n-3 or n-6 polyunsaturated fatty acids (30). (Z)-4-Heptenal, considered responsible for cold-stored off-flavor in frozen cod (35, 36, 40), was found in low concentrations in fresh and stored mackerel. Although (Z)-4-heptenal does not appear to possess a fishy flavor, it causes a pronounced potentiation of the fishiness contributed by the 2,4,7-decatrinalens (26).

Two ketones (cyclohexanone and 2-nananone), which have been previously reported in snow crab and boiled crayfish tail meats (5, 46), were found at higher proportions in fresh mackerel compared to stored mackerel. 2-Nananone has also been found in canned salmon (13), blue crab meat (8) and refrigerated (2°C) whitefish (22). Ketones, in general, contribute to the sweet floral, fruity odors of fish (4, 45).



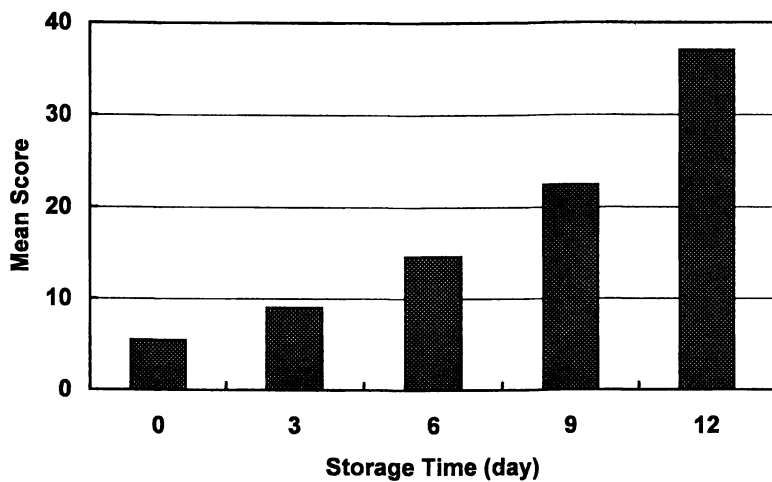


Figure 1. Organoleptic mean scores of raw mackerel

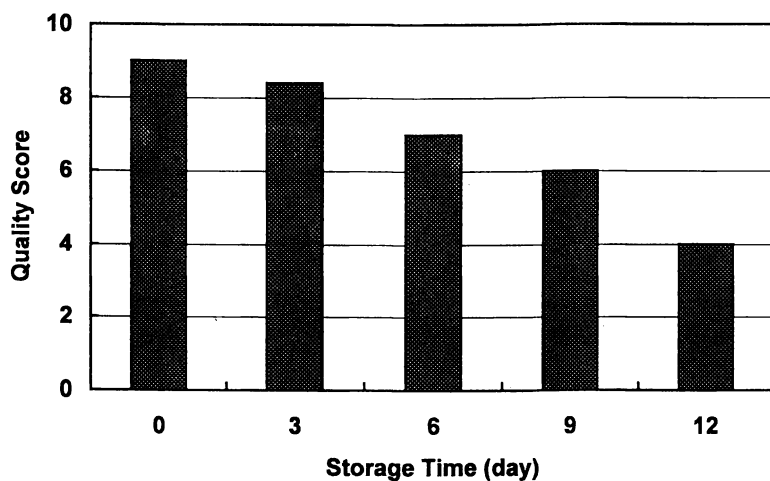


Figure 2. Changes in sensory quality of cooked mackerel

Table I. Results of Static Headspace Analysis of Volatile Aroma Compounds in Raw Mackerel

Storage Time (Day)	0			6			12		
	Retention Time (min)	Peak Area Ratio <sup>a</sup>	Peak Area Proportion (%) <sup>b</sup>	Peak Area Ratio	Peak Area Proportion (%)	Peak Area Ratio	Peak Area Proportion (%)		
Aldehydes	2-Pentenal	2.88	0.551	0.286	0.194				
	Hexanal	3.85	2.792	1.599	0.623				
	2-Methyl-2-pentenal	4.83	0.104	0.041	0.001				
	(E)-2-Hexenal	5.79	0.177	66.51	0.022	64.94	47.38		
	(Z)-4-Heptenal	7.90	0.237		0.205				
	Heptanal	7.98	1.290		0.597				
	Octanal	13.71	1.089		0.404				
	Dodecanal	20.66	1.759		0.388				
	Ketones	Cyclohexanone	10.24	0.762	8.96	0.178	5.74	3.10	
		2-Nonanone	19.85	0.316		0.135			
Alcohols	3-Methyl-1-butanol	2.63	0.430	0.538	1.020				
	2-Penten-1-ol	3.23	0.364	9.64	0.372	19.30	41.27		
	1-Octen-3-ol	12.34	0.364		0.143				
Furan	2-Pentylfuran	12.66	1.596	13.27	0.413	7.58	3.07		
S. cont. comp.	Dimethyl trisulfide	10.84	0.036	0.30	0.063	1.16	4.32		
Alkene	(Z)-1,3,6-Octatriene	6.88	0.158	1.32	0.070	1.28	0.86		

<sup>a</sup> Peak area of component/peak area of internal standard.<sup>b</sup> Peak area proportion represents data for the group of compounds.

Various identified alcohols may be formed by the decomposition of the secondary hydroperoxides of fatty acids (45). The peak area proportion of alcohols increased during storage period, except 1-octen-3-ol which decreased. 1-Octen-3-ol imparts a desirable mushroom-like odor as well as green and plant-like aromas to fish (21). 1-Octen-3-ol, an enzymatic reaction product derived from lipids, is one of the volatile components widely distributed in fresh and salt-water fish (23). Eight carbon alcohols appear to be present in all species of fish (24).

2-Pentylfuran, which was reported to contribute a sweet, burnt, bitter and cooked meat flavor to foods (32), was found at higher proportion in fresh mackerel (13.27%) compared to stored mackerel (3.07%, on day 12). However, it has also been reported as having a fruity odor (10). This compound was described as sweet, spicy and green in crayfish processing waste (44). 2-Pentylfuran was also found in boiled and pasteurized blue crab meat samples (34) and in canned salmon (13).

Dimethyl trisulfide was the only sulfur containing compounds detected in mackerel. It was found in very low concentrations (0.30%) and increased during the storage period. The aroma of dimethyl trisulfide has been described as green vegetable-like in crayfish waste (44). More information about sulfur-containing compounds will be discussed in DHA of volatile aroma compounds in fresh and deteriorated mackerel.

One alkene ((Z)-1,3,6-octatriene), which was found in mackerel, decreased during the storage period. Although this compound was not detected in all seafood products, 1,3,5-octatriene has been found in Pacific oyster (20), in spawning-condition freshwater Pacific salmon (25) and fresh and salt-water fish (23).

**SHA of Volatile Aroma Compounds in Cooked Mackerel During Storage.** The same numbers of volatile compounds identified in raw mackerel (Table I) were also identified in cooked mackerel during 12 days of storage (Table II). The peak area proportions of aldehydes, ketones, the furan and the alkene decreased, whereas alcohols and sulfur-containing compounds increased during storage. Higher proportions of aldehydes and alcohols were observed in fresh cooked mackerel compared to raw mackerel (Table I), which could be due to cooking facilitating an increase in volatility of these compounds during incubation of the sample in the headspace vial. On day 12, aldehydes were in higher proportion in cooked mackerel than in raw mackerel, but other volatiles were lower. A high proportion of aldehydes in cooked stored mackerel (day 12) might have crucial effects on overall aroma quality by masking undesirable aroma changes in fish. This could be significant where fish are purchased by processors or restaurants and then cooked/processed in the same way since undesirable changes may be masked but some amines, especially histamine, and other toxicants may be at potentially dangerous levels.

**DHA of Volatile Aroma Compounds in Fresh and Deteriorated Mackerel.** In a previous study (1), the volatile aroma compounds of mackerel samples (fresh, and frozen then stored 5 days at 15±2°C (FR mackerel)) were analyzed with a fused silica capillary column (DB-Wax: 60 m x 0.25 mm i.d. x 0.25 µm film thickness) by DHA/GC/MS. Out of 65 peaks observed in the total ion chromatograms of fresh and FR mackerel samples, 26 volatile compounds were identified (Table III).

Table II. Results of Static Headspace Analysis of Volatile Aroma Compounds in Cooked Mackerel

Storage Time (Day)	Compounds	0			6			12		
		Retention Time (min)	Peak Area Ratio <sup>a</sup>	Peak Area Proportion (%) <sup>b</sup>	Peak Area Ratio	Peak Area Proportion (%)	Peak Area Ratio	Peak Area Proportion (%)		
Aldehydes	2-Pentenal	2.87	0.824		0.739		0.255			
	Hexanal	3.83	3.383		3.587		1.091			
	2-Methyl-2-pentenal	4.81	0.113		0.090		0.001			
	(E)-2-Hexenal	5.79	0.149	71.67	0.072	65.96	0.035	61.26		
	(Z)-4-Heptenal	7.89	0.398		0.466		0.359			
	Heptanal	7.97	1.460		1.470		0.724			
	Octanal	13.69	0.906		0.964		0.470			
Ketones	Dodecanal	20.64	1.160		1.217		0.379			
	Cyclohexanone	10.23	0.846	8.61	0.450	7.18	0.050	2.52		
Alcohols	2-Nananone	19.84	0.163		0.486		0.086			
	3-Methyl-1-butanol	2.63	0.596		0.853		1.244			
	2-Penten-1-ol	3.21	0.535	11.77	1.069	18.83	0.335	30.71		
	1-Octen-3-ol	12.33	0.248		0.536		0.082			
Furan	2-Pentyfuran	12.66	0.675	5.76	0.720	5.52	0.075	1.40		
S. cont. comp.	Dimethyl trisulfide	10.83	0.101	0.86	0.195	1.49	0.191	3.52		
Alkene	(Z)-1,3,6-Octatriene	6.86	0.155	1.33	0.133	1.02	0.032	0.60		

<sup>a</sup> Peak area of component/peak area of internal standard.<sup>b</sup> Peak area proportion represents data for the group of compounds.

Table III. Results of Dynamic Headspace Analysis of Volatile Aroma Compounds in Fresh and FR(\*) Mackerel

Compounds	Retention Time (min)	Fresh Mackerel		FR Mackerel	
		Peak Area Ratio <sup>a</sup>	Peak Area Proportion (%) <sup>b</sup>	Peak Area Ratio	Peak Area Proportion (%)
Aldehydes					
2-Methylpropanal	5.46	0.190		1.149	
Butanal	6.00	0.083	8.66	-	0.24
2,2-Dimethylpropanal	7.78	0.159		-	
Nonanal	22.78	0.132		-	
Ketones					
2-Butanone	6.42	0.444		4.064	
3-Methyl-2-butanone	7.63	0.211	13.65	-	0.84
2,3-Pentanedione	9.58	0.238		-	
Alcohols					
Ethanol	6.96	1.178		1.600	
1-Propanol	9.34	-		8.800	
1-Penten-3-ol	13.19	1.364		10.238	
iso-Amyl alcohol	15.30	-		51.904	
(Z)-2-Pentenol	19.75	0.185	46.37	0.677	16.57
1-Hexanol	21.13	-		0.351	
1-Octen-3-ol	25.25	0.158		-	
2-Ethylhexanol	26.82	0.147		-	
1,3-Butanediol	29.61	-		2.257	
2,3-Butanediol	31.10	-		3.967	
Ester					
Ethyl acetate	6.24	0.541	8.27	1.574	0.76
S-Methyl ethanethioate	9.53	-		1.927	

Continued on next page

Table III. Continued

Compounds	Retention Time (min)	Fresh Mackerel		FR Mackerel	
		Peak Area Ratio <sup>a</sup>	Peak Area Proportion (%) <sup>b</sup>	Peak Area Ratio	Peak Area Proportion (%)
Alkanes					
1-Propane	6.05	-	-	1.501	-
Hexane	7.85	-	23.04	0.996	1.58
n-Pentadecane	27.26	1.507	-	5.081	-
S. cont. comp.					
Dimethyl sulfide	5.00	-	-	0.493	-
Dimethyl disulfide	10.37	-	-	354.836	73.90
Dimethyl trisulfide	22.46	-	-	0.657	-
Acid					
Acetic acid	25.34	-	-	29.429	6.11

\* Frozen then stored 5 days at 15±2°C.

<sup>a</sup> Peak area of component/peak area of internal standard.<sup>b</sup> Peak area proportion represents data for the group of compounds.

SOURCE: Adapted from ref.1.

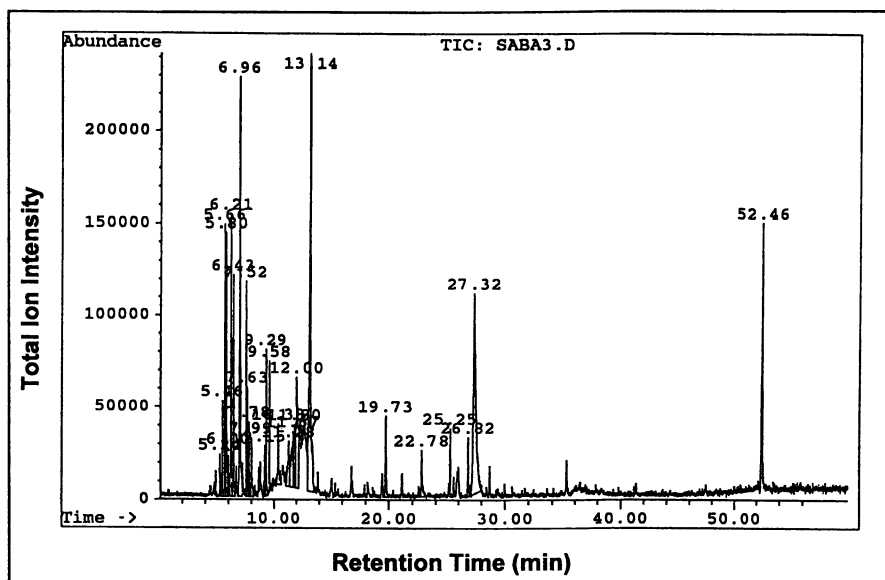


Figure 3. Total ion chromatography of volatile aroma compounds in fresh mackerel

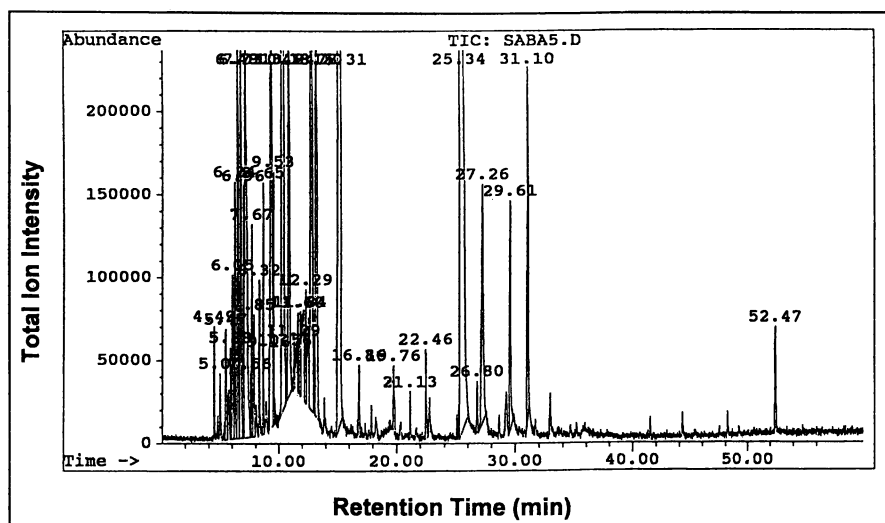


Figure 4. Total ion chromatography of volatile aroma compounds in FR mackerel

Typical total ion chromatographs for fresh and FR mackerel samples are shown in Figures 3 and 4, respectively. Retention times correspond to compounds listed in Table III. Some compounds found in DHA were not detected in SHA, which could be due to the different columns, techniques and storage conditions of fish used for each study.

Aldehydes were found at higher concentration in fresh mackerel compared to FR mackerel (8.66% and 0.24%, respectively) (Table III). Three ketones in the fresh and one ketone in the FR mackerel were detected in proportions of 13.65% and 0.84%, respectively. As compared to SHA results (Table I), aldehydes and ketones were also higher in fresh mackerel compared to stored mackerel. The level of 1-penten-3-ol was higher in FR mackerel. Short-chain alcohols generally possess relatively high aroma thresholds and, as a group, these might contribute to sweet aroma (22). Ethanol was the most abundant short-chain alcohol measured in both fresh and FR mackerel. As the chain length of alcohols increases, the flavor becomes more intense (27).

Esters were found at higher proportion in fresh compared to FR mackerel (8.27% and 0.76%, respectively). Two esters (ethyl acetate and S-methyl ethanethioate) identified in FR mackerel may be products of the esterification of corresponding alcohols and carboxylic acids (39). Ethyl acetate was the most abundant short-chain ester formed, but because of its high aroma threshold it may not contribute as significantly to the sweet stage of flavor development as other, less abundant esters (22). This compound has been found in crayfish waste (44) and snow crab (5). In general, esters give fruity flavors (44).

Alkanes, which contribute very little to the overall flavor of foods (14), were identified in fresh (n-pentadecane) and FR (1-propane, hexane and n-pentadecane) mackerel. n-Pentadecane has previously been observed in blue crab meat (8) and hexane in canned salmon (13).

Three sulfur-containing compounds such as dimethyl sulfide, dimethyl disulfide and dimethyl trisulfide were found in FR mackerel, comprising about 74% of total peak area proportions. These compounds have been found in fish and other marine products (18, 46, 49). The most abundant volatile component, comprising about 99.7% of the total sulfur-containing compounds, was dimethyl disulfide which was responsible for onion-or cabbage-like odors (11, 46). Dimethyl disulfide may be an oxidation product of methanethiol or a bacterial degradation product of methionine (7). Dimethyl sulfide was found in haddock after 8 days storage at 0°C, but was not found at the beginning of the storage period (37). Dimethyl trisulfide imparts an onion off-flavor in prawn and cooked cabbage and a spoiled odor in crayfish meat (49). Sulfur-containing compounds generally give strong sulphurous, cooked cabbage odors in marine products (41) and are considered important volatile aroma components in marine crustaceans (6, 29). Some sulfur-containing compounds are responsible for the rancid-nutty odor of cooked Antarctic krill (28).

Acetic acid, which could be formed by decomposition of either lipid autoxidation or secondary hydroperoxides of fatty acids, was the only acidic compound in FR mackerel. Volatile acids give cheesy or ammoniacal odors depending on concentrations (5). This compound has been previously found in fish (13, 25).



## Conclusion

The organoleptic mean scores of raw mackerel showed good agreement with deterioration throughout the storage period. However, up to day 9, the taste panel scored the cooked fish as being generally of good quality. Possibly, the aldehydes masked the undesirable changes in the cooked mackerel. Instrumental measurement of volatile aroma compounds provide a way to objectively assess flavors of fish. Combined SHA & DHA/GC/MS techniques applied to identify important volatile aroma compounds in fish showed benefits and could be used to provide data on the improvement of aroma quality. Both techniques have potential to be used for quality control due to their simple and rapid operations.

In terms of identifying all the volatiles available in fish, DHA is more advantageous than SHA, since during the early stage of rancidity, some volatile aroma compounds are only present in very low concentrations and SHA is not sensitive enough to detect them. Moreover, SHA is limited to the detection of low boiling point compounds, e.g., ethanol. Fewer volatile compounds were detected in SHA compared to DHA, which may be due to the different columns and techniques used. For instance, no alkanes, esters or acids were detected by the SHA method.

In general, the identified aldehydes, ketones, alcohols and esters gave fresh fish aroma, whereas sulfur-containing compounds and acid gave off/spoiled-odors in mackerel. Sulfur-containing compounds, especially dimethyl disulfide, might be used as a spoilage index of fish. Aroma quality of fish may be severely affected due to the high concentration of this compound. The formation of the compounds responsible for off-odors in deteriorated mackerel arise primarily from lipid oxidation. However, volatile formation by microbial action may also be implicated.

## Literature Cited

1. Alasalvar, C.; Aishima, T.; Quantick, P.C. *Food Sci. Technol., Int.* **1995**, *1*, 125.
2. Alasalvar, C.; Quantick, P.C. *Abstract of Foodborne Disease: Consequences & Prevention*; Oxford Brookes University, Oxford, March 30-31, 1995.
3. Branch, A.C.; Vail, A.M.A. *Food Technol. Australia.* **1985**, *37*, 352.
4. Cha, Y.J.; Baek, H.H.; Hsieh, T.C.-Y. *J. Sci. Food Agric.* **1992**, *58*, 239.
5. Cha, Y.J.; Cadwallader, K.R.; Baek, H.H. *J. Food Sci.* **1993**, *58*, 525.
6. Choi, S.H.; Kobayashi, A.; Yamanishi, T. *Agric. Biol. Chem.* **1983**, *47*, 337.
7. Christensen, B.W.; Kjaer, A.; Madsen, J.O. *J. Amer. Oil Chem. Soc.* **1981**, *58*, 1053.
8. Chung, H.Y.; Cadwallader, K.R. *J. Food Sci.* **1993**, *58*, 1203.
9. Entz, R.C.; Hollifield, H.C. *J. Agric Food Chem.* **1982**, *30*, 84.
10. *Fenaroli's Handbook of Flavor Ingredients*; Furia, T.E.; Bellanca, N., Eds.; CRC Press: Cleveland, OH., 1981; Vol.2.
11. Fors, S. In *The Maillard Reaction in Foods and Nutrition*; Waller, G.R.; Feather, M.S., Eds.; ACS Symposium Series 215; American Chemical Society: Washington, DC., 1983; pp 185-286.
12. Frasse, P.; Richard-Molard, D. *J. High Resolution Chromatogr. Chromatogr. Communic.* **1989**, *12*, 631.

13. Girard, B.; Nakai, S. *J. Food Sci.* **1991**, *56*, 1271.
14. Grosch, W. In *Developments in Food Science 3A*; Morton, I.D.; MacLeod, A.J., Eds.; Elsevier Science Publishing: New York, 1982; pp 325-398.
15. Herbert, R.A.; Ellis, J.R.; Shewan, J.M. *J. Sci. Food Agric.* **1975**, *26*, 1187.
16. Hollingworth, T.A.Jr.; Throm, H.R.; Wekell, M.; Trager, W.F.; O'Donnell, M.W.Jr. *J. Assoc. Off. Anal. Chem.* **1986**, *69*, 524.
17. Howgate, P.F. In *Fish Handling & Processing*; Aitken, A.; Mackie, I.M.; Merritt, J.H.; Windsor, M.L., Eds.; ISBN 0 11 491741 8; HMSO Press: Edinburgh, 1982; pp 177-186.
18. Hsieh, T.C.-Y.; Vejaphan, W.; Williams, S.S.; Matiella, J.E. In *Thermal Generation of Aromas*; Parliment, T.H.; McGorin, R.J.; Ho, G.T., Eds.; ACS Symposium Series 409; American Chemical Society: Washington, DC., 1989; pp 386-395.
19. Jhaveri, S.N.; Leu, S.S.; Constantinides, S.M. *J. Food Sci.* **1982**, *47*, 1808.
20. Josephson, D.; Lindsay, R.C.; Stuiber, D.A. *J. Food Sci.* **1985**, *50*, 5.
21. Josephson, D.B.; Lindsay, R.C. In *Biogenesis of Aromas*; Parliment, T.H.; Croteau, R., Eds.; ACS Symposium Series 317; American Chemical Society: Washington, DC., 1986; pp 201-226.
22. Josephson, D.B.; Lindsay, R.C.; Olafsdottir, G. In *Seafood Quality Determination*; Kramer, D.A.; Liston, J., Eds.; Elsevier Science Publishers: Amsterdam, 1987; pp 27-47.
23. Josephson, D.B.; Lindsay, R.C.; Stuiber, D.A. *J. Agric. Food Chem.* **1984**, *32*, 1344.
24. Josephson, D.B.; Lindsay, R.C.; Stuiber, D.A. *J. Agric. Food Chem.* **1984**, *32*, 1347.
25. Josephson, D.B.; Lindsay, R.C.; Stuiber, D.A. *J. Food Sci.* **1991**, *56*, 1576.
26. Karahadian, C.; Lindsay, R.C. *J. Amer. Oil Chem. Soc.* **1989**, *66*, 953.
27. Kochhar, S.P. In *Food Taints and Off-Flavours*; Saxby, M.J., Ed.; Blackie Academic and Professional: Glasgow, 1993; pp 150-202.
28. Kubota, K.; Kobayashi, A.; Yamanishi, T. *Agric. Biol. Chem.* **1980**, *44*, 2677.
29. Kubota, K.; Shijimaya, H.; Kobayashi, A. *Agric. Biol. Chem.* **1986**, *50*, 2867.
30. Lindsay, R.C. In *Seafoods: Chemistry, Processing, Technology and Quality*; Shahidi, F.; Botta, J.R., Eds.; Blackie Academic and Professional: Glasgow, 1994; pp 75-84.
31. Lindsay, R.C.; Josephson, D.B.; Olafsdottir, G. In *Seafood Quality Determination*; Kramer, D.A.; Liston, J., Eds.; Elsevier Science Publishers: Amsterdam, 1987; pp 221-234.
32. Maga, J.A. *CRC Crit. Rev. Food Sci. Nutr.* **1979**, *11*, 355.
33. Maga, J.A. In *Principles and Applications of Gas Chromatography in Food Analysis*; Gordon, M.H., Ed.; Ellis Horwood: Chichester, 1990; pp 86-110.
34. Matiella, J.E.; Hsieh, T.C.-Y. *J. Food Sci.* **1990**, *55*, 962.
35. McGill, A.S.; Hardy, R.; Burt, R.J.; Gunstone, F.D. *J. Sci. Food Agric.* **1974**, *25*, 1477.
36. McGill, A.S.; Hardy, R.; Gunstone, F.D. *J. Sci. Food Agric.* **1977**, *28*, 200.

37. Merritt, C.; Mendelsohn, J.M. *Abstract of American Chemical Society National Meeting 142*; American Chemical Society: 1962; pp 14A.
38. Miller, A.; Scanlan, R.A.; Lee, J.S.; Libbey, L.M. *J. Agric Food Chem.* **1972**, *20*, 709.
39. Peterson, R.J.; Chang, S.S. *J. Food Sci.* **1982**, *47*, 1444.
40. Ross, D.A.; Love, R.M. *J. Food Technol.* **1979**, *14*, 115.
41. Shankaranarayana, M.L.; Raghavan, B.; Abraham, K.O.; Natarajan, C.P. In *Food Flavours*; Morton, I.D.; McLeod, A.J., Eds.; Elsevier Science Publishers: New York, 1989; pp 169-281.
42. Sipos, J.; Ackman, R.G. *J. Fish. Res. Bd. Canada.* **1964**, *21*, 423.
43. Sloan, A.E.; McNutt, K.W.; Powers, M. *The Shelflife of Foods and Beverages*; Charalambous, G., Ed.; Elsevier Science Publishers: Amsterdam, 1986; pp 63-72.
44. Tanchotikul, U.; Hsieh, T.C.-Y. *J. Food Sci.* **1989**, *54*, 1515.
45. Tanchotikul, U.; Hsieh, T.C.-Y. *J. Food Sci.* **1991**, *56*, 327.
46. Vejaphan, W.; Hsieh, T.C.-Y.; Williams, S.S. *J. Food Sci.* **1988**, *53*, 1666.
47. Venema, A. *J. High Resolution Chromatogr. Chromatogr. Communic.* **1986**, *9*, 637.
48. Westendorf, R.G. *J. Chromatogr. Sci.* **1985**, *23*, 521.
49. Whitfield, F.B.; Freeman, D.J.; Bannister, P.A. *Chem. Ind.* **1981**, *10*, 692.

## Chapter 6

# Gas Chromatography–Mass Spectrometry Analysis of Volatile Flavor Compounds in Mackerel for Assessment of Fish Quality

Hui-Zhen Zhang and Tung-Ching Lee<sup>1</sup>

Department of Food Science, Center for Advanced Technology, and the Institute of Marine and Coastal Sciences, Cook College, Rutgers, The State University of New Jersey, P.O. Box 231, New Brunswick, NJ 08903

Volatile flavor compounds of mackerel stored on ice at different times were extracted by simultaneous steam distillation/solvent extraction (SDE) technique, and analyzed by gas chromatography/mass spectrometry (GC/MS). Sixty-two volatile compounds were identified, including aldehydes, ketones, alcohols, hydrocarbons, etc. Many compounds were derived from lipid oxidation. Also, some compounds were derived from microbial degradation. Among these identified compounds, the quantitative data show that the amount of some volatile compounds, such as 3-pentanone, 2-heptanone and indole, etc., increases with increasing storage times of mackerel. Correspondingly, the flavor quality of mackerel decreases with increasing storage time based on our overall sensory smell test and hypoxanthine freshness test.

Volatile aroma compounds are generally considered to be important parameters for determining the flavor quality and spoilage index of fish and other seafoods. This is because the aroma of fresh seafood products is strongly related to consumer perceptions about immediate quality. Both industry and regulatory agencies are continuously seeking methods to directly measure volatile aroma compounds. These measurements could augment sensory assessments. Although sensory assessment is desirable, its rate limits its use in modern fish processing and handling sites. In addition, sensory panels are subject to fatigue, resulting in erroneous assessments. Therefore, there is a demand for methods to objectively measure fish and other seafood aroma quality, and finally to instrumentally detect and quantify influential volatile aroma compounds in fish and other seafood to assess fish quality.

Volatile aroma compounds of fish derived from microbial degradation and lipid oxidation are closely related to fish quality (1-3). Pleasant planty, green, and melony aromas and flavors are easily recognized and readily associated with fresh fish, and

<sup>1</sup>Corresponding author

generally are highly valued by consumers. These aromas and flavors are provided by aldehydes, ketones, and alcohols, which are derived from long-chain polyunsaturated fatty acid via specific oxygenases (4-6). During the storage of fish, lipid oxidation generates a large number of volatile compounds, including aldehydes, ketones, alcohols, hydrocarbons and other products. Accumulation of these compounds caused changes in flavor, color, and texture of fish and other seafood products (7). Furthermore, the production of trimethylamine, dimethylamine and ammonia from trimethylamine oxide via microbial metabolism and enzymatic degradation also contributes to the fish volatile profile. Identification of the aroma compounds responsible for the odor of fresh and spoiling fish to assess fish quality has been the subject of many investigations. Josephson et al. (8) determined that the compounds responsible for the odor of whitefish were (E)-2-nonenal, (E,Z)-2,6-nonadienal, and 6-nonen-1-ol, which were responsible for the heavy, plant-like aromas. Lerke and Huck (9) reported that ethanol was the only GC peak among many identified volatile compounds which increased in magnitude between good, questionable, and decomposed canned tuna. Rayner et al. (10) analyzed the volatile components of trout, crab, shrimp, and oysters by GC/MS and reported that the indoles characterized the putrid odor. A study by Miler et al. (11) noted that hexanal and trimethylamine contribute to the off-odors emitted during the spoilage of fish. McGill et al. (12,13) investigated the contribution of (Z)-4-heptenal, (E)-2-heptenal, and (E,Z)-2,4-heptadienal to the development of off-flavors during frozen storage of cod.

The volatile aroma compounds of the fish varies with fish species, specific enzymes, climate during the period after catching and transportation, storage temperature, microbial population, and concentration of microconstituents, such as mineral salts (14,15). Although some fish volatile aroma compounds have been studied (8,16-18), limited information has been reported about the changes in aroma compounds during the storage of mackerel. Alasalvar et al. (19) studied volatile aroma compounds of fresh and deteriorated mackerel using dynamic headspace concentration and GC/MS analysis. Their study only analyzed two kinds of samples and less than 20 volatile compounds were identified. Therefore, it is essential to further study the changes of volatile compounds at different storage times and provide indepth information for quality assessment.

The objectives of our study were to extract volatile flavor compounds in mackerel at different storage times by simultaneous steam distillation/solvent extraction (SDE) technique, to separate and identify the flavor compounds by capillary column GC/MS, and to screen the flavor compounds that are associated with quality deterioration in mackerel to be used as indicators for fish quality assessment.

## Materials and Methods

**Fish sample preparation.** Mackerel were obtained from a local seafood store (Captain C's, New Brunswick, NJ). The edible flesh with attached skin was ground using a Cuisinart Food Processor (Dlc-118B, Cuisinarts, Inc., Norwich, CT). The ground fish sample was mixed well and kept on ice for different storage times of 0, 2, 4, 5 and 7 days. Day 0 was the same day that the mackerel was bought from

fish store. The fish store obtained the fresh fish from the boat the same day they were purchased.

**Hypoxanthine analysis.** Hypoxanthine (Hx) measurement was conducted according to a procedure described by Burns et al. (20). The HPLC system used for this determination was a Waters 501 System (Marlborough, MA) with a Waters 484 tunable absorbance detector set at 254 nm. A reversed phase column (DELTA PAK C18, 300A, 3.9 mm x 15 cm, Waters, Marlborough, MA) was employed for separations.

**Simultaneous distillation and extraction (SDE) technique for obtaining the volatile compounds from mackerel sample.** A Likens-Nickerson type SDE head was used for preparation of the SDE extract. A 2.5-L round bottom flask was the sample flask containing 200 g of fish meat sample, plus 2 mL aqueous solution of 2.5 µg/mL of internal standard (2,4,6-trimethylpyridine). A 2.0-L round bottom flask containing distilled water as a water vapor generator was connected to the sample flask. A 250-mL flask containing 70 mL of methylene chloride was attached to the solvent arm of the SDE head. The flask with the water vapor generator and solvent was heated to a boil and simultaneous distillation with extraction was continued for 2 h. The small amount of water in the extract was removed as ice after storing the extract at -20°C for 6 h. The volume of the extract was reduced to 2 mL by evaporating the solvent. The residual moisture in the extract was removed by drying the extract over 0.5 g of anhydrous sodium sulfate. Finally, the volume of the extract was reduced to approximate 0.5 mL under a nitrogen stream. This sample was subsequently analyzed by GC or GC/MS.

**Gas chromatography/mass spectrometry (GC/MS) analyses.** First an HP 5890 GC (Hewlett Packard, Avondale, PA) equipped with a flame ionization detector (FID) was used to find the optimal separation conditions for SDE extracts. Chromatograms were recorded with an HP 3396A integrator. The separation of volatile compounds was accomplished with a DB 1701 capillary column (0.32 mm x 50 m, J&W Scientific Co. Rancho Cordova, CA), with helium as the carrier gas. The test conditions were: injector 210°C, detector 230°C, carrier gas 1.5 ml/min, and split ratio 25:1. The GC oven was programmed to hold for 4 min at the initial temperature of 50°C and increased at 1.0°C/min to 100°C, and then to hold for 1.0 min at temperature 100°C and increased at 5°C/min to 200°C. 2,4,6-Trimethyl pyridine was the internal standard to quantify the volatile compounds from mackerel.

Next, the GC-MS was used to tentatively identify the volatile compounds. The GC was a Varian 3400 system equipped with the same capillary column as the above HP 5890 and directly interfaced with a Finnigan MAT 8230 high resolution, magnetic sector mass spectrometer (MS). The MS was operated in electron ionization mode (70 eV), scanning masses 35-350 once each second with a 0.8 interscan time. Data were recorded and processed using a Finnigan MAT spectra 300 data system. Library searches of unknown mass spectra were conducted using the NIST and EPA-NIH mass spectral reference collection databases.

## Results and Discussion

**Volatile compounds from mackerel stored at different times identified by GC/MS.** Volatile compounds identified by GC/MS are listed in Table I. A total 62 volatile compounds were identified including aldehydes, ketones, alcohols, hydrocarbons, etc. A lot of compounds were derived from lipid oxidation. Heptanal, octanal, 1-octanol and nonanone were probably decomposed from oleate hydroperoxides (21). Pentene, pentanal, hexanal, 2-pentyl furan and octanol were possibly decomposed from linoleate hydroperoxides (21). 2-pentyl furan, 2,4-heptadienal, and hexanal could have originated from docosahexaenoate hydroperoxides (22,23). 2-Hexenal probably was generated from eicosapentaenoic hydroperoxides (1). Indole, from protein degradation, was identified (24). Compounds containing chloride are artifacts due to the SDE solvent, methylene chloride.

**The hypoxanthine (Hx) contents of mackerel samples at different storage times.** The use of Hx as an index of fish quality was first done at the Torry Research Station (25,26). After that, researchers, such as Beuchat (27) and Spinelli et al. (28) reported that Hx levels in fish during iced storage had a good correlation with the degree of freshness. Generally, it is recognized that Hx content in fish is an accurate indicator of fish freshness. Hx was, therefore, used as a freshness index to evaluate the fish samples used to screen for volatile compounds as new quality indicators. The Hx content of mackerel samples at different storage times is shown in Table III. For mackerel stored on ice, Hx increased at a moderate rate, reaching 5.14  $\mu\text{mol/g}$  after 7 days storage. The Hx level abruptly increased between days 0 and 2, and 3 and 4. These results were in agreement with those of Jahns et al. (29). Based on an overall sensory smell evaluation of mackerel, after 4 days of storage the mackerel had developed a spoiled odor. These results are consistent with those previously reported by Jhaveri et al. (30). The average maximum Hx contents in seafood were reported at approximately 5  $\mu\text{mol/g}$  (31,32).

**Quantitative changes of volatile compounds from mackerel stored at different times.** Table I also shows the quantitative changes of volatile compounds from mackerel stored at different times. According to an overall sensory smell test, after 4 days of storage on ice the mackerel had developed an off-flavor. The off-flavor increased with the storage times. In other words, the freshness of the fish sample decreased with the storage times from fresh to deterioration. The decrease in freshness is related to the generation of volatile compounds. Even though these compounds were detected at low levels, the compounds having a low threshold will still reflect the quality. Among those identified compounds (Table I), the concentrations of several compounds increased with storage times, such as 3-pentanone, isopentyl alcohol, 2-heptanone, 1-undecene-3-yne, 2-ethyl-1-hexanol, indole, n-heptadecane, etc. Table II shows that volatile compounds identified by GC/MS quantitatively increased with storage times. From Table II, we can see that it is possible to use volatile compounds as quality indicators of mackerel stored on ice to assess its quality. In addition, the volatile compounds in fish, such as diacetyl that decrease with storage times, can also be used to assess fish quality.

**Table I. Volatile compounds in mackerel at different storage times quantified by GC with FID**

Compound	Relative Concentration (ng/g)				
	Day 0	2	4	5	6
Methyl chloride	0.28	0.30	1.42	0.93	6.55
Trimethylamine	2.39	0.38	2.54	3.42	1.40
Pentene	3.70	1.72	3.18	4.29	3.67
Dichloroethylene	2.02	0.75	1.52	1.92	1.15
Carbon disulfide	0.19	0.68	ND*	ND	ND
Diacyl	5.03	4.51	2.18	1.47	ND
Cyclohexene	43.82	34.67	50.43	45.07	67.07
2-Ethyl furan	3.23	3.73	3.21	4.72	3.85
Cyclopentanone	1.67	2.10	2.41	3.43	1.49
Pentanal	2.25	2.33	2.32	2.88	2.33
3-Pentanone	0.24	0.54	4.16	10.38	19.82
1-Penten-3-ol	35.14	27.01	30.39	38.21	41.28
Octane	1.12	1.20	2.32	4.80	ND
1-Hydroxy-2-propanone (acetol)	0.43	0.41	0.77	0.95	ND
2,4-Octadiene	0.073	0.70	0.95	ND	0.47
Tetrachloroethylene	0.38	ND	ND	1.13	2.65
Isopentyl Alcohol	1.23	1.61	6.57	14.72	47.91
Branched C-5 Alcohol (fusel Oil)	ND	ND	1.32	3.88	11.26
3-Hydroxy-2-butanone (acetoin)	2.83	2.70	3.09	3.73	ND
2,3 Dihydro-4-methylfuran	5.81	5.27	5.58	6.71	ND
Hexanal	7.68	7.55	7.60	6.58	0.25
(E)-2-Penten-1-ol	18.92	20.19	12.37	23.74	21.63
2-Methoxypropane	0.53	1.23	0.78	0.88	ND
Z-3-Hexen-1-ol	0.55	0.91	0.96	1.54	1.26
E-2-Hexenal	6.29	5.98	6.30	8.13	ND
1-Hexanol	1.15	4.16	6.06	10.16	7.60
2-Heptanone	0.63	0.85	1.23	1.93	2.60
Heptanal	1.64	2.03	2.21	1.95	ND
4-Heptenal	2.07	2.72	3.15	2.78	1.30
2-Pentylfuran	0.22	0.45	ND	0.43	0.47

*Continued on next page.*



Table I Continued

Dimethyltrisulfide	0.12	ND	ND	ND	3.36
Dimethylcyclohexane	0.68	0.71	ND	1.12	1.91
Dimethylcyclooctadiene	0.47	1.03	ND	ND	0.84
3-Octanone	1.90	4.30	4.24	5.74	4.41
Benzaldehyde	0.65	1.70	2.07	3.70	4.08
1-Undecene-3-yne	0.62	1.72	1.92	2.06	4.63
2,4-Heptadienal	0.40	1.44	1.42	1.14	ND
2-Ethyl-1-hexanol	0.11	0.95	1.99	3.66	7.08
3,3-Dimethyl-1,4-pentadiene	3.03	7.02	6.95	6.98	1.27
1-Octanol	0.55	ND	1.32	1.59	1.50
2-Nonanone	0.11	ND	0.76	ND	1.11
Nonanal	2.70	4.69	6.61	3.44	2.68
Phenol	0.46	1.21	1.02	1.48	1.31
2,2-Dimethylpentadienal	0.50	2.10	2.79	2.57	7.00
2,4-Octadienal	0.46	1.31	2.39	2.03	1.95
Phenylethyl Alcohol	ND	ND	ND	3.58	15.81
2-allylphenol	0.37	ND	ND	ND	ND
Tridecene	ND	ND	ND	ND	0.17
6,6-Dimethylbicyclo[3.1.1]- hept-2-ene-2-methanol (Myrtenol)	1.22	2.36	ND	ND	ND
Decenal	0.55	1.70	2.57	1.83	ND
2-Undecanone	0.29	ND	3.20	7.48	ND
1-Pentadecene	0.39	0.67	ND	ND	ND
1-Pentadecane	34.21	27.7	31.82	37.46	59.35
Indole	0.32	ND	6.46	11.22	23.78
n-Hexadecane	2.81	2.68	3.24	2.78	7.07
2,6-Di-t-butyl- <i>p</i> -cresol (antioxidant)	ND	ND	ND	ND	7.44
1-Heptadecene	4.03	3.65	4.69	4.11	5.51
n-Heptadecane	83.29	90.30	104.0	127.5	170.1
n-Nonadecane	1.56	2.09	3.62	2.94	5.65
Hexadecanal	2.69	4.27	2.35	ND	ND
Methyl Palmitate	0.13	ND	0.88	2.06	ND
2-Hetadecanone	1.83	1.22	1.50	ND	0.94

\*Not detected.

**Table II. Quantitative data for volatile compounds increasing in mackerel during storage**

Compound	Relative Concentration (ng/g)				
	Day 0	2	4	5	6
3-Pentanone	0.24	0.54	4.16	10.38	19.82
Isopentyl alcohol	1.23	1.61	6.57	14.72	47.91
Fusel oil	ND	ND	1.32	3.88	11.26
2-Heptanone	0.63	0.85	1.23	1.93	2.60
1-Undecene-3-yne	0.62	1.72	1.92	2.06	4.63
2-Ethyl-1-hexanol	0.11	0.95	1.99	3.66	7.08
Indole	0.32	ND	6.46	11.22	23.78
n-Heptadecane	83.29	90.30	104.0	127.5	170.1

**Table III. The effect of storage time on the hypoxanthine (Hx) content and sensory smell value in mackerel**

Storage time (day)	Hx Content ( $\mu\text{mol/g}$ )	Sensory smell test
0	1.14	1 (fresh)
2	1.60	2
3	1.62	3
4	2.30	4 (beginning off-flavor)
5	3.18	5 (unacceptable)
7	5.14	6

## Conclusion

The quantities of some volatile flavor compounds of mackerel extracted by SDE and identified by GC/MS increased with storage time, and the fish quality also decreased with storage times confirmed using the levels of hypoxanthine (Hx) content as a freshness index. Therefore, the volatile compounds could be used as quality indicators for the assessment of fish quality. Further research is needed to determine the sensory characteristics of the volatile compounds quantified in the present work.

## Acknowledgments

This is publication number D-10114-6-97 of the New Jersey Agricultural Experiment Station. This work was supported by grants from Rutgers/NOAA Cooperative Marine Education and Research Program (CMER)

## References

1. Josephson, D.B.; Lindsay, R.C.; Stuibler, D.A. *J. Agric. Food Chem.*, **1984**, *32*, 1347-1352.
2. Alur, M.D.; Venugopal, V.; Nerkar, D.P. *J. Food Sci.*, **1989**, *54*, 1111-1115.
3. Lindsay, R.C. *Food Rev. Internat.*, **1990**, *6*, 437-455.
4. German, J.B.; Kinsella, J.E. *J. Agric. Food Chem.*, **1985**, *33*, 680-683.
5. Josephson, D.B.; Lindsay, R.C. In *Biogenesis of Aromas*; Parliment, T.H.; R. Croteau, R., Eds., ACS Symposium Series No. 317; American Chemical Society: Washington, DC, 1986; pp. 201-219.
6. Hsieh, R.J.; Kinsella, J.E. *J. Agric. Food Chem.*, **1989**, *37*, 279-286.
7. Khayat, A.; Schwall, D. *Food Technol.*, **1983**, *7*, 130-140.
8. Josephson, D.B.; Lindsay, R.C.; Stuibler, D.A. *J. Agric. Food Chem.*, **1983**, *31*, 326-330.
9. Lerke, P.A.; Huck, R.W. *J. Food Sci.*, **1977**, *42*, 755-758.
10. Rayner, E.T.; Dupuy, H.P.; Legendre, M.G.; Grodner, R.M.; Cook, J.A.; Novak, A.F.; Toloday, D.J. *J. Food Sci.*, **1981**, *47*, 76-78.
11. Miler, K.B.M.; Kolakowska, A.; Czerniejewska-Surma, B.; Sznajdrowska, W.B. *Nahrung*, **1983**, *27*, 403-406.
12. McGill, A.S.; Hardy, R.; Burt, J.R. *J. Sci. Food Agric.*, **1974**, *25*, 1477-1489.
13. McGill, A.S.; Hardy, R.; Gunstone, F.D. *J. Sci. Food Agric.*, **1977**, *28*, 200-205.
14. Josephson, D.B.; Lindsay, R.C.; Stuibler, D.A. *J. Food Sci.*, **1991**, *56*, 1576-1579.
15. Yasuhara, A.; Shibamoto, T. *J. Agric. Food Chem.*, **1995**, *43*, 94-97
16. Vejaphan, W.; Hsieh, T.C.-Y.; Williams, S.S. *J. Food Sci.*, **1988**, *54*, 1666-1670.
17. Sakakibara, H.; Ide, J.; Yanai, T.; Yajima, I.; Hayashi, K. *Agric. Biol. Chem.*, **1990**, *54*, 9-16.
18. Girard, B.; Nakai, S. *J. Food Sci.*, **1991**, *56*, 1271-1274.

19. Alasalvar, C.; Aishima, T.; Quantick, P.C. *Food Sci. Technol. Int.*, **1995**, *1*, 125-127.
20. Burns, B.G.; Ke, P.J. *J. Assoc. Off. Anal. Chem.*, **1985**, *68*, 444-448.
21. Frankel, E.N.; Neff, W.E.; Selke, E. *Lipids*, **1981**, *16*, 279-285.
22. Noble, A.C.; Nawar, W.W. *J. Assoc. Off. Anal. Chem.*, **1971**, *48*, 800-803.
23. Noble, A.C.; Nawar, W.W. *J. Assoc. Off. Anal. Chem.*, **1975**, *52*, 92-95.
24. Chung, H.Y.; Cadwallader, K.R. *J. Food Sci.*, **1993**, *58*, 1203-1207.
25. Jones, N.R. *Analyst*, **1960**, *85*, 111-115.
26. Jones, N.R.; Murray, J. *J. Sci. Food Agric.*, **1962**, *13*, 475-480.
27. Beuchat, L.R. *J. Food Sci.*, **1973**, *38*, 453-455.
28. Spinelli, J.; Eklund, M.; Miyanchi, D. *J. Food Sci.*, **1964**, *29*, 710-714.
29. Jahns, F.D.; Howe, J.L.; Coduri, R.J.; Rand, A.G. *Food Technol.*, **1976**, *30*, 27-
30. Jhaveri, S.N.; Leu, S.S.; Constantinides, S.M. *J. Food Sci.*, **1982**, *47*, 1808-1810.
31. Burt, J.R.; Gibson, D.M.; Jason, A.G.; Sanders, H.R. *J. Food Technol.*, **1976**, *11*, 73-77.
32. Burt, J.R. *Process. Biochem.*, **1977**, *12*, 32-36.

## Chapter 7

# Lipoxygenase and Sulfur-Containing Amino Acids in Seafood Flavor Formation

B. S. Pan<sup>1</sup>, J. R. Tsai<sup>2</sup>, L. M. Chen<sup>1</sup>, and C. M. Wu<sup>3</sup>

<sup>1</sup>Department of Marine Food Science, National Taiwan Ocean University,  
2 Pei-Ning Road, Keelung, Taiwan, Republic of China

<sup>2</sup>Department of Food Science, Der Yuh Junior College of Nursing  
and Management, 336 Fu Hsing Road, Keelung 202 Taiwan, Republic of China

<sup>3</sup>Food Industry Research and Development Institute, Hsin-Chu,  
Taiwan, Republic of China

Headspace analysis of Neptune rose shrimp yielded 2-(1-propenylthio)-thiophene and 3,4-dihydrothienyl-[3,4,B]-5-carboxythiophene, while dimethyl trisulfide, 2,4,5-trimethyl thiazole, 1-propanesulphinic acid methyl ester, and *cis*- and *trans*-3,5-dimethyl-1,2,4-trithiolane were found in the steam distillate. The steam distillate of cultured tiger prawn contained a greater number of sulfur-containing compounds including dimethyl disulfide, isopropyl thiophene, *cis*- and *trans*-3,5-dimethyl-1,2,4-trithiolane, 2-acetyl- and benzo-thiazole, 3,5,6-trimethyl-1,2,4-dithiazine and 2,6-dimethyl-4-butyl-1,3,5 dihydrodithiazine. Increased distillation time resulted in increased H<sub>2</sub>S formation in a semilogarithmic correlation. Reaction of cysteine and glucose in phosphate buffer produced 1-methylthio-2-propanone and gave an aroma approximating that of canned tuna in oil. Fresh fish aroma was obtained by lipoxygenase-catalyzed dioxygenation of highly unsaturated fatty acids, C<sub>20:5</sub> and C<sub>22:6</sub>, and commercial fish oil. A scheme of reactions responsible for the formation of fresh seafood aroma is proposed.

Volatile components of crustacea and their products reported in literature (1-18) include alkanes of both linear and branched-chain, aldehydes, ketones, acids, esters, aromatic compounds, nitrogen-containing compounds (i.e. pyridines, pyrazines, amides, and amines), sulfur-containing compounds of both acyclic and cyclic structures, and other compounds.

Pyrazines and S-containing compounds were considered important contributors to the odor of fermented and cooked shrimps (1-9). The contents of pyrazines, thialdines and trithiolanes differed between precooked and raw Antarctic krills due to difference in contents of ammonia and free amino acids (7). Dimethyl sulfide and dimethyl propiothetin were higher in frozen raw Antarctic krill than in all other shrimps examined (12). The ethyl substituted sulfur compounds characterized their respective cooked odor (8). While dimethyl trisulfide resulting from microbial

spoilage of raw royal red prawn produced off-flavor (13), it gave a green, vegetable-like odor to crayfish waste (14). The S-containing compounds, therefore, seem to be responsible for either the characteristic crustacean flavor, or potentially off-flavors.

The objectives of our study were to determine the role of S-containing compounds in shrimp flavor and try to enhance the desirable seafood aroma derived from S-containing compounds. Since the difference in the steam distillate of the frozen raw and the precooked Antarctic krills was affected by the amount of pyrazines and carbonyl compounds present (10), and prolonged heating resulted in increased content of pyrazines in shrimp. The mechanism of their formation in seafood of prime freshness also was of interest.

## Experimental

**Shrimp.** Ocean-caught Neptune rose shrimp, *Parapenaeus fissurus*, and cultured tiger prawn, *Penaeus japonicus*, were used in preparing shrimp volatile extracts.

**Preparation of Shrimp Steam-Distillate Concentrate.** Fresh tiger prawn was homogenized with distilled water (1:1.5 w/v) and extracted with 50 ml of glass-distilled pentane/ether (1:1 v/v) for 2 h using a Likens-Nickerson apparatus. The extract was dried over anhydrous sodium sulfate and concentrated using a spinning band distillation apparatus before gas chromatographic analysis.

**Preparation of Headspace Aroma Concentrate.** The shrimp volatiles were purged with high-purity nitrogen onto a porous polymer Tenax-TA (Chrompack, Middleburg, Netherlands) at 26°C for 2 h at a flow rate of 60 ml/min. The trapped volatile compounds were eluted with pentane/ether (1:1 v/v), dried, then concentrated in the same way as the steam distillate.

**Gas Chromatographic (GC) Analysis.** The aroma concentrates were analyzed using a Shimadzu GC-8A (Kyoto, Japan) equipped with a CP-WAX 52 CB fused silica capillary column, 50 m x 0.32 mm (Chrompack, Middleburg, Netherlands) and flame ionization detector (FID). The oven temperature was programmed from 50°C for 5 min and increased to 200°C at 1.5°C/min, then held for 60 min at 200°C. Both the injection and detector were set at 250°C. The carrier gas was hydrogen at a flow rate of 1.4 ml/min. The data were recorded on a Hewlett-Packard 3390 integrator (Palo Alto, Ca). The retention indices (RIs) of the volatile components were calculated using n-paraffin (C7-C25, Alltech Assoc.) as reference compounds (20).

**GC-MS Analyses.** A Hewlett-Packard 5985B system equipped with a fused silica capillary column (CP-WAX 52 CB, 50 m x 0.32 mm) was used for GC/MS. The carrier gas was helium at 1.8 ml/min. The oven temperature was programmed as mentioned above. The ionization voltage was 70 eV, and the ion source temperature was 200°C.

**Browning Intensity.** The absorbance (420 nm) of a 10% trichloroacetic acid (TCA) extract was used as an index of the browning intensity.

**Free Amino Acid Analysis.** Water extract of shrimp was mixed with a mixture 10% sulfosalicylic acid:1.47% trisodium citrate, 1:3 v/v, and filtered. The filtrate was analyzed (Amino Acid Analyzer, LKB 4150, UK).

## Results & Discussion

### Sulfur-Containing Components in Shrimp Volatile

Two sulfur-containing components, 3,4-dihydrothienyl-[3,4,B]-5-carboxythiophene and 2-(1-propenylthio)thiophene, were identified in the headspace of unheated Neptune rose shrimp, while five other sulfur-containing compounds were found in the steam distillate including dimethyl trisulfide, 1-propanesulphonic acid methyl ester, cis- and trans-3,5-dimethyl-1,2,4-trithiolane, and 2,4,5-trimethyl thiazole (Table I). Dimethyl trisulfide was not found in the headspace but was present in the steam distillate of the shrimp (Table I). This compound also was found in the steam distillates of crabmeat and by-product (*I*), indicating heating accelerated its formation in crustacean meat. Microbial action may also lead to its production (*I4*), as shown by the high content in by-products (*8X*) compared with crabmeat (*I*). Numbers and levels of sulfur-containing compounds were higher in the steam distillate than in the unheated shrimp (*I7*).

**Table I. Comparison of volatile sulfur-containing compounds in Neptune rose shrimp (*Parapenaeus fissurus*) isolated by headspace method and Likens-Nickerson method and analyzed by GC/MS**

Compound	RI*	Headspace	Steam Distillate
dimethyl trisulfide	1335		+
2,4,5-trimethyl thiazole	1376		+
2-(1-propenylthio)-thiophene	1517	+	
1-propanesulphonic acid, methyl ester	1590		+
trans-3,5-dimethyl-1,2,4-trithiolane	1617		+
cis-3,5-dimethyl-1,2,4-trithiolane	1630		+
3,4-dihydrothienyl-(3,4,B)-carboxythiophene	1702	+	

\*retention index on CP WAX 52CB column

Volatile sulfur-containing of tiger prawn were obtained using a Likens-Nickerson simultaneous distillation and solvent extraction apparatus, and then analyzed by gas chromatography and GC-MS (Table II). The volatile sulfur-containing compounds included dimethyl disulfide, isopropyl thiophene, cis- and trans-3,5-dimethyl-1,2,4-trithiolane, 2-acetyl thiazole, benzothiazole, 3,5,6-trimethyl-1,2,4-dithiazine, and 2,6-dimethyl-4-butyl-1,3,5-dihydro dithiazine.

2-Acetyl thiazole was found in tiger prawn in addition to the previous finding in Neptune rose shrimp (*I8*). This compound gave a nutty and popcorn aroma and has been formed from thermal degradation of cysteine in model system (*2I*). Heating

**Table II. Volatile sulfur-containing compounds in tiger prawn (*Panaeus japonicus*) extracted for 2 h using Likens-Nickerson method**

Compound	RI*	Conc ( $\mu\text{g}/\text{kg}$ )	Mass Spectral Data
dimethyl disulfide	1058	71.23	94(80), 79(40), 45(28), 96(13)
isopropyl thiophene	1240	36.59	111(100), 105(35), 126(33), 69(22), 41(21)
trans-3,5-dimethyl- 1,2,4-trithiolane	1599	65.23	152(100), 92(59), 59(44), 88(43), 153(7), 154(12)
cis-3,5-dimethyl- 1,2,4-trithiolane	1614	131.90	152(100), 135(84), 120(58), 92(58) 153(8), 154(4)
2-acetyl thiazole	1621	111.16	127(100), 99(97), 43(70), 112(66)
benzothiazole	1944	127.16	135(100), 108(36), 69(16), 82(11), 91(7), 137(55)
3,5,6-trimethyl- 1,2,4-dithiazine	1739	230.55	163(100), 44(93), 71(56), 70(50) 103(38), 164(9.1), 165(9.1)
2,6-dimethyl-4-butyl- 1,3,5-dihydrodithiazine	1934	74.65	112(74), 86(61), 70(51), 205(41), 145(22), 59(20)

\*retention index on CP WAX 52CB column

longer than 1 h resulted in some degradation of 2-acetyl thiazole as observed in corbicule (22). Benzothiazole at low ppb concentration is a positive flavor of roast beef. During prolonged cold storage of ground beef, benzothiazole increased to ppb levels and resulted in meat flavor deterioration (23). It was also found in dried squid (24,25), fermented anchovy and hairtail, but not in shrimp paste (1), and was possibly formed from cysteine and glucose via Strecker degradation (26).

The quantity of 3,5,6-trimethyl-1,2,4-dithiazine was the highest among all the S-compounds detected in shrimp. It was formed in shrimp probably in a similar way as in dried squid, i.e. from  $\text{NH}_3$ ,  $\text{H}_2\text{S}$ , and aldehydes at alkaline pH (25). The compound 2,6-dimethyl-4-butyl-1,3,5-dihydro dithiazine was probably formed from thermal degradation of cysteine, which produced 2,6-dimethyl-4-ethylperhydro-1,3,5-dithiazine in a model system (21).

Trithiolane and thialdine derivatives were found after cooking of the raw and fermented shrimp products, and from the raw krill. These compounds were products of reactions between free amino acids or ammonia,  $\text{H}_2\text{S}$ , lipids, and carbonyl compounds (9).

Raw shrimps contained 102 mmole/100 g d.w. of free amino acids (Table III). After Likens-Nickerson distillation of the shrimp homogenate for 2 h, the content of



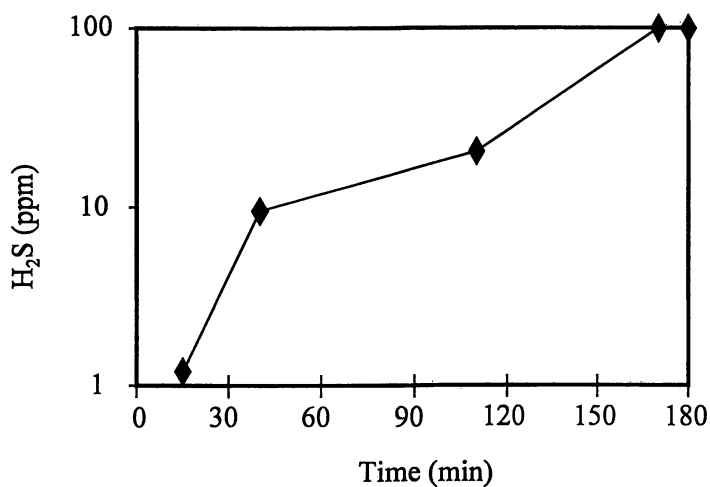


Figure 1. Hydrogen sulfide (H<sub>2</sub>S) formation during Likens-Nickerson extraction of shrimp (shrimp:distilled water, 1:1.5 vw/v).

free amino acids in the residue increased to 128 mmole/100g d.w. in which free cysteine was not detected. It could also be that cysteine reacted quickly to form H<sub>2</sub>S (Fig. 1) and cyclic S-containing compounds so that no free cysteine remained in the residue during steam distillation. Methionine increased from 1.88 to 3.07 mmole/100g d.w., while taurine remained between 5.29 to 5.69 mmole/100g d.w.. Level of H<sub>2</sub>S increased logarithmically with heating time (Fig. 1). The contribution of these three S-containing amino acids to seafood flavor was studied in model systems.

**Table III. Free sulfur-containing amino acid composition of raw and cooked tiger prawn (*Penaeus japonicus* Bate)**

Amino Acid	Frozen Raw (mmol/100 g)*	Cooked* (mmol/100 g)
cysteine	--- <sup>^</sup>	---
methionine	1.88 ± 0.25	3.07 ± 0.34
taurine	5.29 ± 0.33	5.69 ± 0.41
<b>total</b>	101.87	127.61

\*dry weight basis

\*residue of shrimp homogenate after Likens-Nickerson steam distillation for 2 h

<sup>^</sup>not detected

### Aroma Developed from S-Containing Amino Acids

Model systems containing the free amino acids abundant in shrimp, e.g. glycine, proline, and alanine (27), which contribute to sweet taste, showed less effect on odor development than the sulfur-containing amino acids.

Cysteine reacted with glucose in phosphate buffer producing an aroma similar to canned tuna (Table IV). In spite of the fact that free cysteine was not detected, it is still a constituent in the shrimp muscle protein, which is susceptible to autolysis.

**Table IV. Odor development in thermal reactions of sulfur-containing amino acids (0.2 M) and glucose (0.2 M) in 0.4 M phosphate buffer (pH 7.0) at 100°C for 2 h.**

Amino Acid	Odor Characteristic	Odor Strength*	Browning Index (A <sub>420nm</sub> )
cysteine	canned tuna in oil	6.0 <sup>a</sup>	4.11
methionine	biscuit, dried turnip, soysauce	5.7 <sup>a</sup>	36.30
taurine	caramel	4.3 <sup>a</sup>	46.00

\*Scale of 1 to 9 in increasing odor intensity; Numbers with same superscript in the column are not significantly different (p > 0.05) by Duncan's test.

In addition, cysteine is very important in flavor formation, since methionine produced an odor similar to biscuit, dried shrimp, and soy sauce. Taurine produced caramel-like odor. The three S-containing amino acids upon heating with glucose yielded odors of different characteristics but similar odor strengths. However, the resulting browning intensity varied greatly among the three model systems. Degree of browning and odor strength increased with thermal reaction time. Changes in odor during thermal reaction did not, however, correlate with browning intensity.

Prolonged reaction of S-containing amino acids and glucose altered odor characteristics. Cysteine and glucose reacted at 160°C for 5 minutes produced odor of canned tuna in oil and garlic odor, and later became roasted and meaty after reacting for 30 minutes. Methionine heated at 100°C for 15-120 minutes changed odor characteristics from sweet, dried turnip to salty and saucy. Taurine heated at 100°C for 15-120 minutes changed from a sweet to caramel odor.

Thermal reaction carried out at weakly acidic and basic pH values did not affect odor characteristics nor odor intensity, but affected browning intensity (Table V). At the pI of the amino acids browning intensity was the lowest. Reaction under nitrogen, air and oxygen did not influence odor development, but inhibited browning.

**Table V. Effect of pH on browning and odor development in thermal reaction of cysteine and glucose\***

pH	Browning Index ( $A_{420nm}$ )	Odor Characteristic	Odor Intensity*
5.07	0.27	boiled egg	4.8 <sup>a</sup>
7.00	4.11	canned tuna in oil	6.0 <sup>b</sup>
8.18	4.26	canned tuna in oil	5.5 <sup>b</sup>
10.40	3.04	canned tuna in oil, boiled egg	5.6 <sup>b</sup>

\*0.2 M of cysteine and 0.2 M of glucose in 0.4 M phosphate buffer reacted at 100°C for 2 h.

\*Scale of 1 to 9 in increasing odor intensity; Numbers with same superscript in the column are not significantly different ( $p > 0.05$ ) by Duncan's test.

The canned tuna in oil aroma produced from the thermal reaction of cysteine and glucose in phosphate contained 67% of 1-methylthio-2-propanone (Table VI). An unstable S-compound of low taste and odor threshold, 2-methyl-3-furanthiol gave a mustard, onion-like, and meaty flavor note. It was also found in roasted shrimp (11) and fermented fish sauce (28), and in canned tuna aroma (29). The formation of this S-containing compound involved thermal reaction between cysteine and ribose with or without phospholipid (30).

The compounds 2- and 3-thiophenethiol and 2-methylthiazolidine were found in the volatile profile resulting from reaction of cysteine and glucose at pH values from 2 to 7 with microwave heating (29). Formation of thiazole and its 2,5-dimethyl derivative was accelerated at pH 9.0 (31).

A proposed mechanism for seafood aroma developed from S-containing amino acids reacting with reducing sugar or carbonyl compounds derived from lipid oxidation is shown in Fig. 2.

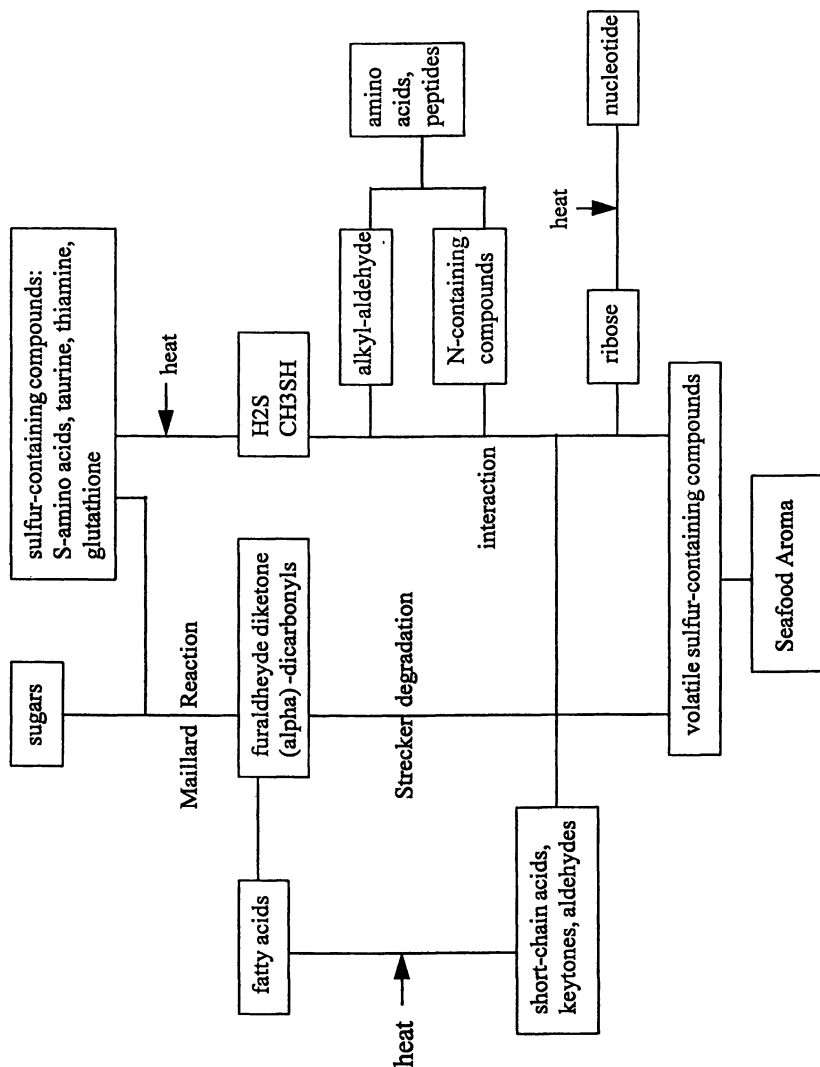


Figure 2. Proposed pathway for the formation of volatile sulfur-containing compounds of seafood during heat processing.

**Table VI. Major volatile compounds identified by GC/MS from the reaction of cysteine and glucose\***

Compound	RI <sup>†</sup>	Conc <sup>‡</sup> ( $\mu\text{g}/100\text{ mL}$ )	Mass Spectral Data
1-methylthio-propanone	1293	766.81	61(100), 43(82), 107(74), 60(31), 105(4.4), 106(3.6)
2-acetylpyrrole	1942	89.91	94(100), 109(76), 66(48), 39(11), 110(5.5)

\*0.2 M of cysteine and 0.2 M of glucose in 0.4 M phosphate buffer reacted at 100°C for 2 h

†retention index on CP-WAX 52CP column

‡hexadecene was used as internal standard

### Relations of Lipoxygenase to Seafood Aroma

Two interesting occurrences were observed during the studies of shrimp flavor. First, total number of volatiles obtained by the steam distillation of whole shrimp was greater than the sum of those volatiles extracted from shrimp heads and that from the shrimp tails (18). Second, the flavor note of shrimp oil did not resemble shrimp, while the residue after the solvent extraction of shrimp oil smelled shrimp-like with less intensity than the aqueous extract or the aqueous homogenate of the whole shrimp (Table VII). These two observations pointed to the possibility of interactions between the aqueous fraction and oil in shrimp resulting in the characteristic shrimp odor. In addition, lipoxygenase (LOX) was previously found to intervene in fresh fish aroma formation (32). LOX activity in the hemolymph of shrimp was identified and found to contribute to shrimp flavor formation (33-35).

**Table VII. Odor characteristics and odor strengths developed after heating of tiger prawn extracts at 100°C for 2 h.**

Extract	Odor characteristic	Odor Strength <sup>†</sup>
aqueous homogenate <sup>‡</sup>	shrimp	7.0 <sup>a</sup>
0.6N KCl extract <sup>‡</sup>	shrimp	6.3 <sup>a</sup>
aqueous extract <sup>‡</sup>	shrimp	6.3 <sup>a</sup>
solvent extract residue <sup>‡</sup>	shrimp	4.5 <sup>b</sup>
shrimp oil <sup>‡</sup>	fishy	3.5 <sup>c</sup>

†Scale of 1 to 9 in increasing odor intensity; Numbers with same superscript in the column are not significantly different ( $p > 0.05$ ) by Duncan's test.

‡Five volumes of distilled water was mixed with prawn and homogenized to obtain aqueous homogenate, then filtered to obtain aqueous extract; KCl (0.6 N) replaced distilled water to prepare the 0.6 N KCl extract.

‡Chloroform:methanol 2:1 (v/v) was used to extract oil from shrimp.

Model systems using partially purified LOX from fish waste, i.e. cultured grey mullet gill (36), were tested. Odor developed from polyunsaturated fatty acids treated with mullet gill LOX was green and grassy for 18:2 and 18:3, grassy and fishy for 20:4, fresh fish and slightly fishy for 20:5, fresh-fish for 22:6 and fish oil (Table VIII). The compound 1-octen-3-ol, having a mushroom and grassy odor, was formed from 20:4 treated with mullet gill crude extract. When mullet gill extract was incubated with total lipid or polar lipid extracted from shrimp then microwaved, it produced cooked-shrimp aroma. When it was incubated with neutral lipid, it produced a mild fishy odor. Fish oil was incubated with mullet gill lipoxygenase and amino acids then cooked by microwave heating, the flavor notes that resulted were similar to those of cooked shrimp or cooked fish (data not shown).

**Table VIII. Odor development from mullet gill lipoxygenase catalyzed dioxygenation of unsaturated fatty acids**

Fatty Acid	Odor
blank	odorless
C18:2	grassy
C18:3	grassy
C20:4	grassy, fishy
C20:5	fresh fish, slightly fishy
C22:6	fresh fish
fish oil*	fresh fish, slightly fishy

\*Super EPA 1000 (Advance Nutrition Technology Inc., Elizabeth, NJ)

By inhibition of LOX the formation of 1-octen-3-ol and tetradecatrienone in shrimp distillate was reduced by 77.3% and 23.9%, respectively (33). The latter was a keynote compound of cooked shrimp odor (37).

## Conclusion

Thermal reaction of S-containing amino acids and reducing sugars contributes to flavor formation. The odor characteristics and odor strength do not correlate with browning intensity. Inhibition of browning does not necessarily reduce odor formation.

1-Methylthio-2-propanone was identified in our model system containing cysteine and glucose and produced an aroma of canned tuna in oil. The formation mechanism is probably via reaction between 1-hydroxy-2-propanone and methanethiol. Prolonged heating of shrimp and fish produces excessive H<sub>2</sub>S and changes the composition of cyclic S-containing compounds leading to seafood flavor deterioration.

Fresh seafood aromas are associated with secondary products derived from reactions of highly unsaturated fatty acids initially catalyzed by endogenous LOX. Flavor modification of fish oil and seafood flavor simulation can be achieved by

addition of LOX from external sources even by using recovered LOX from seafood wastes.

### Literature Cited

1. Cha, Y.J.; Cadwallader, K.R. *J. Food Sci.* **1995**, *60*, 19-24.
2. Choi, S.H.; Kato, H. *Agric. Biol. Chem.* **1984**, *48*, 545-547.
3. Choi, S.H.; Kato, H. *Agric. Biol. Chem.* **1984**, *48*, 1479-1486.
4. Choi, S.H.; Kobayashi, A. *Nippon Shokuhin Kogyo Gakkaishi* **1983**, *30*, 404-408.
5. Choi, S. H.; Kobayashi, A.; Yamanishi, T. *Agric. Biol. Chem.* **1983**, *47*, 337-342.
6. Kubota, K.; Kobayashi, A.; Yamanishi, T. *Agric. Biol. Chem.* **1980**, *44*, 2677-2682.
7. Kubota, K.; Kobayashi, A.; Yamanishi, T. *Nippon Shokuhin Kogyo Gakkaishi* **1981**, *28*, 457-460.
8. Kubota, K.; Kobayashi, A.; Yamanishi, T. *Nippon Noeikagaku Kaishi* **1982**, *56*, 1049-1052.
9. Kubota, K.; Kobayashi, A.; Yamanishi, T. *Agric. Biol. Chem.* **1982**, *46*, 2835-2839.
10. Kubota, K.; Matsufusa, K.; Yamanishi, T.; Yanase, M. *Nippon Noeikagaku Kasishi* **1980**, *54*, 1-5.
11. Kubota, K.; Kobayashi, A.; Yamanishi, T. *Agric. Food Chem.* **1986**, *50*, 2867-2872.
12. Kubota, K.; Kobayashi, A. *J. Agric. Food Chem.* **1988**, *36*, 121-123.
13. Tokunaga, T.; Iida, H., Nakamura, K. *Bull. Jap. Soc. Sci. Fish* **1977**, *43*, 1209-1217.
14. Whitfield, F.B.; Freeman, D.J.; Bannister, P.A. *Chemistry & Industry* **1981**, Oct 3, 692-693.
15. Tanchotikul, U.; Hsieh T.C.-Y. *J. Food Sci.* **1989**, *54*, 1515-1520.
16. Shye, S.C.; Pan, B.S.; Wu, C.M. *J. Chinese Agric. Chem. Soc.* **1988**, *26*, 545-551.
17. Pan, B.S.; Shye, S.C.; Kuo J.M.; Wu, C.M. National Taiwan Ocean University, unpublished results.
18. Shye, S.C.; Pan, B.S.; Wu, C.M. *J. Chinese Agric. Chem. Soc.* **1987**, *25*, 169-176.
19. Vejaphan, W.; Hsieh, T.C.-Y.; Williams, S.S. *J. Food Sci.* **1988**, *53*, 1666-1670.
20. Schomburg, G.; Dielman, G. *J. Chromatogr. Sci.* **1973**, *11*, 151-154.
21. Zhang, Y.; Chien, M.; Ho, C.T. *J. Agric. Food Chem.* **1988**, *36*, 992-996.
22. Kubota, K.; Nakamoto A.; Moriguchi, M.; Kobayashi, A.; Ishii, H. *J. Agric. Food Chem.* **1991**, *39*, 1127-1130.
23. Vercellotti, J.R.; Kuan, J.W.; Spanier, A.M.; St. Angelo, A.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; 452-459.
24. Kawai, T.; Ishida, Y. *J. Agric. Food Chem.* **1989**, *37*, 1026-1031.
25. Kawai, T.; Ishida, Y.; Kakiuchi, H.; Ikeda, N.; Higashida, T.; Nakamura S. *J. Agric. Food Chem.* **1991**, *39*, 770-777.

26. Zhang, Y.; Ho, C.T. *J. Agric. Food Chem.* **1991**, *39*, 760-763.
27. Tsai, M.-J.; Pan, B.S. *J. Fish. Soc. Taiwan* **1988**, *15(1)*, 49-58.
28. McIver, R.C.; Brooks, R.; Reineccius, G.A. *J. Agric. Food Chem.* **1982**, *30*, 1017-1022.
29. Withycombe, D.A.; Mussian, C.J. *J. Food Sci.* **1988**, *53*, 658.
30. Farmer, C.J.; Mottram, D.S.; Whitfield, F.B. *J. Sci. Food Chem.* **1989**, *49*, 347 (from Lindsay, R.C. *Food Rev. Inter.* **1990**, *6*, 450).
31. Yeo, H.C.H.; Shibamoto, T. *J. Agric. Food Chem* **1991**, *39*, 370-373.
32. Kuo J.M.; Pan, B.S. *Agric. Biol. Chem.* **1991**, *55*, 847-848.
33. Kuo, J.M.; Pan, B.S. *J. Chinese Biochem. Soc.* **1992**, *21*, 9-16.
34. Kuo, J.M.; Pan, B.S.; Zhang, H.; German, B. *J. Agric. Food Chem.* **1994**, *42*, 1620-1623.
35. Hsu, H.H.; Pan, B.S. *J. Agric. Food Chem* **1996**, *44*, 741-745.
36. Kobayashi, A.; Kubota, K.; Iwarnoto, M.; Tamura, H. *J. Agric. Food Chem.* **1989**, *37*, 151-154.
37. Josephson, D.B.; Lindsay, R.C.; Stuiber, D.A. *J. Agric. Food Chem.* **1984**, *32*, 1344-1347.



## Chapter 8

# Thermally Generated Flavors from Seal Protein Hydrolysate

Chin-Fa Hwang<sup>1</sup>, Fereidoon Shahidi<sup>2</sup>, A. C. Onodenaloré<sup>2</sup>, and Chi-Tang Ho<sup>1,3</sup>

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

<sup>2</sup>Department of Biochemistry, Memorial University of Newfoundland, St. John's, Newfoundland A1B 3X9, Canada

Seal meat is a rich source of nutritionally-valuable proteins with a well-balanced amino acid composition. Production of seal protein hydrolysate (SPH) provides a means for better utilization of seal meat. Seal protein hydrolysate alone or in the presence of glucose was heated in a Parr reactor at 150°C for 2 hours. Considerable amounts of Maillard reaction compounds, such as Strecker aldehydes, pyrazines, thiazole, and furans were found in these thermally treated reaction mixtures. The addition of glucose to the hydrolysate significantly increased the concentration of most flavor compounds. In addition, small amounts of sulfur-containing compounds, such as 3,5-dimethyl-1,2,4-trithiolane and dimethyldisulfide were also found. These results showed that seal protein hydrolysate may be used as a flavor precursor to improve the flavor quality of processed foods.

Enzymatic protein hydrolysates have become popular ingredients in the food and flavor industry since they contain a large portion of peptides. Compared to thermal treatment of amino acids, thermal treatment of peptides with reducing sugars can generate more specific aroma compounds, which give some characteristic odors (3, 6-7). Considerable research on enzymatic protein hydrolysis has been carried out in the past few decades. Almost all these studies have focused on the modification of functional properties of proteins, so that the protein hydrolysates could be incorporated into foods for their flavor, functionality, and nutritional value (2). The proteins used most commonly are casein, whey protein, and soy protein.

Harp seals (*Phoca groenlandica*) is the most common species of Northern seal. Although a quota system for seal hunting was introduced in Canada, and permitted 186,000 seals annually until 1996, only 50,000-70,000 animals were hunted annually (9). In 1996, the quota was increased to 250,000 and this was further increased to 275,000 in 1997. This provides approximately 5.5-8.3 million kilograms of seal meat in Newfoundland each year (13). Limited consumption of

<sup>3</sup>Corresponding author

seal meat may be due to its dark color and intense flavor. The dark color was originated from the high amounts of myoglobin and hemoglobin in the muscle tissues. The consequence of oxidation of unsaturated lipids catalyzed by hemoproteins gives rise to flavor deterioration. The present use of seal meat is limited to preparation of silage, meat, or animal feed (9). Production of seal salami and pepperoni on a pilot scale has been achieved recently.

Seal meat is a nutritionally valuable source of protein because of its well-balanced amino acid composition (10, 11). In order to achieve a better utilization of seal meat, proteolytic hydrolysis of seal meat was developed, and protein hydrolysates with a bland taste and off-white color were prepared using a commercially available microbial enzyme Alcalase (12). This seal protein hydrolysate contained 77.3% crude proteins, 0.74% lipid, and 20.67% minerals (mainly sodium chloride), and was very soluble over a wide pH range. The content of free amino acids in the resulting hydrolysates (2.1–2.9%) was much higher than in the original meat (0.1–0.2%) (9). Thus, these products may participate in flavor generation through Maillard reaction more effectively than unhydrolyzed proteins. The purpose of this study is to determine the volatile compounds generated from the thermal treatment of seal protein hydrolysate alone or in the presence of glucose, and to investigate the possibility of using this hydrolysate as a flavor precursor to improve the quality of processed foods.

## Experimental

**Seal protein hydrolysate.** Protein hydrolysate from Harp seals (*Phoca groenlandica*) meat was prepared according to the method described by Shahidi et al. (12). Microbial proteases Alcalase 2.4L (Novo Industrie AS, Bagsvaerd, Denmark) was used to partially hydrolyze seal proteins at 60 °C, pH 8.5 for 50 min. Heme groups were removed, and the hydrolyzed proteins were bleached with charcoal, neutralized to pH 7.0. and dehydrated. The degree of hydrolysis was determined according to the ninhydrin method (14).

**Thermal reaction and volatile isolation.** Seal protein hydrolysate (3 g) alone or with the addition of 0.9 g glucose were dissolved in 200 ml distilled water in the reaction vessel. The pH was adjusted to 7.0 by 1N NaOH. The thermal reaction was carried out in a Parr reactor at 150 °C for 2 hr and stirred at the speed of 140–150 rpm. After the reaction, the pH of the reaction mixture was adjusted to 9 and an internal standard, tridecane, was added. The reaction mixture was then extracted three times with 100 ml methylene chloride. Any trace of water in the solvent phase was removed by adding anhydrous sodium sulfate. The solvent extract was concentrated to about 5 ml using Kuderna-Danish concentrators, and the concentrate was then blown with a stream of nitrogen to remove the remaining solvent until the sample was at a concentration appropriate for GC & GC/MS analysis.

**Gas chromatographic (GC) analysis.** A Varian 3400 GC equipped with DB-WAX column (I.D. 0.25mm, 30m, 0.25 µm film thickness, J & W Scientific) and a flame ionization detector (FID) was used to separate and quantify the volatile compounds.

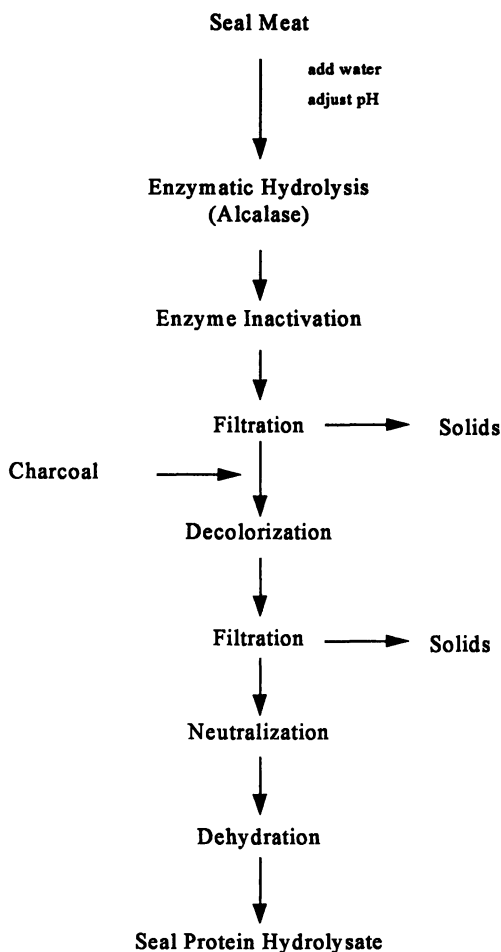


Figure 1. Flowsheet for production of seal protein hydrolysate.

The injector temperature was set at 200 °C, and the detector temperature was set at 230 °C. The oven temperature was programmed linearly from 40 °C to 210 °C at 2 °C/min, and then held at 210 °C for 30 min. A split ratio of 25:1 was used. The flow rate of carrier gas, helium, was 1ml/min. Linear retention indices of the volatile components were calculated with n-paraffins (C6-C25) as references (4). The paraffin standards were analyzed using the same GC conditions as those mentioned above.

**Gas chromatography-mass spectrometry (GC-MS) analysis.** A GC-MS analysis was performed on a Varian 3400 gas chromatograph coupled with a Finnigan Mat Ion Trap Detector using a DB-WAX column (I.D. 0.25 mm, 30 m, 0.25 µm film thickness, J & W scientific). The operation conditions were the same as those used in the GC analysis.

**Identification of Volatile Compounds.** Identification of volatile compounds in the condensate was based primarily on gas chromatography-mass spectrometry (GC-MS) analysis, and information from the GC retention indices of the compounds against the C6-C25 n-paraffins were used as references. The structure assignment of volatile compounds was accomplished by comparing the mass spectral data of individual compounds with those of authentic compounds available from the National Institute of Standards and Technology, Central Institute for Nutrition and Food Research TNO, the on-line computer library (NB) in ITD M/S software program, or those published previously in the literature.

## Results and Discussion

Figure 1 summarizes the procedures for preparation of seal protein hydrolysate from mechanically separated seal meat. The main steps in the preparation of seal protein hydrolysate were enzymatic hydrolysis at a constant pH, adjusted by addition of base, inactivation of enzyme under acidic conditions, separation of heme and other residues, and decolorization by charcoal followed by neutralization and dehydration (12). The yield of the hydrolysate and the degree of hydrolysis, using Alcalase, under optimum conditions, were 92.75% and 19.5%, respectively (12). The characteristics of seal protein hydrolysate were determined by the degree of hydrolysis (DH). A bitter taste hydrolysate may be formed at high degrees of hydrolysis (5). Therefore, preparation of seal protein hydrolysate was set at DH = 19.5% to obtain products with a near-bland taste. The dehydrated seal protein hydrolysate contained 77.3% crude proteins, 0.74% lipids, and 20.67% minerals, mainly sodium chloride. The amino acid composition of seal protein hydrolysate was similar to that of the original seal meat. The products so obtained were bland in taste and off-white in appearance.

Table I describes the odor description of products from the thermal reaction of seal protein hydrolysate alone or together with glucose. The hydrolysate sample alone had a weak seafood note. On the other hand, in the presence of glucose, the flavor notes of reaction product are seafood-like, nutty, cereal-like, chocolate, indole-like, and sweet. The overall flavor of product with glucose addition is more

pleasant and intense, and more acceptable than that of the hydrolysate alone, especially in sweetness and nutty notes.

**Table I. Odor description of products from the thermal reaction of seal protein hydrolysate alone or in the presence of glucose**

<i>Samples</i>	<i>Flavor Notes</i>
Seal protein hydrolysate	weak seafood
Seal protein hydrolysate + glucose	seafood, nutty, cereal-like, chocolate, animal note (indole-like), and sweet

After identification of compounds by GC/MS, many flavor compounds were generated as shown in Table II. Also shown in Table II is the semi-quantitative data of the compounds identified. These include pyridines, pyrazines, thiazoles, aldehydes, furan derivatives, sulfur-containing compounds, and others. The number of flavor compounds found in protein hydrolysate with glucose is greater than that of protein hydrolysate alone. The difference in the number of compounds generated from these two samples is mostly attributed to the pyrazines. This is because pyrazine compounds are thermally generated via Maillard reaction of amino acids with reducing sugars. In the presence of glucose, the formation of pyrazine is promoted in the reaction mixtures containing seal protein hydrolysates. On the other hand, many Maillard reaction products, such as pyrazines, aldehydes, and thiazole are also found in the thermal reaction of seal protein hydrolysate alone. This suggests that free amino acids are possibly present together with reducing sugars and thiamine possibly in the hydrolysate. The content of free amino acids in the hydrolysate was about 2.1-2.9%, which is significantly higher than that in the original meat. Small amounts of thiamine (14.5 mg%) were also found in seal meat (10). Thiamine is considered as a flavor precursor in meat products. Its degradation compounds, like furans, can further interact with amino acids or other components to generate meaty aroma in heat-processed products (8). On the other hand, no information about sugar content in seal was reported until now, although it may exist only in trace amounts.

Higher total amounts of compounds were generated from the samples with glucose than without glucose addition. In the thermal reaction of protein hydrolysate alone, aldehydes were dominantly formed, while pyrazines concentration was less than that of aldehydes. However, in the thermal reaction of protein hydrolysate with glucose, the concentration of pyrazine compounds was highest, while aldehyde concentration was less. Aldehydes are formed either from Strecker degradation of amino acids with carbonyl compounds, or from thermal degradation of lipids. Yasuhara and Shibamoto (15) reported that many volatile aldehydes, including formaldehyde, acetaldehyde, propanal, isobutanal, isopentanal, pentanal, hexanal, heptanal, octanal, and nonanal, were generated from various kinds of fish flesh during

**Table II. Volatile compounds identified and quantified from the thermal reaction of seal protein hydrolysate (SPH) or seal protein hydrolysate with glucose**

Compounds	Concentration (ppm)		
	IK (DB-WAX)	SPH	SPH + glucose
pyrazine	1202	0.14	29.91
2,5-dimethylpyrazine	1313	7.081	173.79
2,6-dimethylpyrazine	1319	4.07	14.1
2,3-dimethylpyrazine	1336	0.82	3.26
2-ethyl-6-methylpyrazine	1377	1.05	2.42
2-ethyl-5-methylpyrazine	1382	4.33	11.22
trimethylpyrazine	1395	12.74	24.03
2-vinylpyrazine	1425	nd	1.16
3-ethyl-2,5-dimethylpyrazine	1438	8.34	9.56
2-vinyl-6-methylpyrazine	1478	nd	0.97
2-vinyl-5-methylpyrazine	1484	nd	3.5
2-methyl-6(1-propenyl)pyrazine	1528	0.95	2.92
<b>SUBTOTAL</b>		<b>104.27</b>	<b>280.94</b>
pyridine	1183	2.2	0.52
4-methylpyridine	1255	21.83	73.3
2-acetylpyridine	1582	nd	trace
thiazole	1239	2.2	1.48
<b>SUBTOTAL</b>		<b>26.23</b>	<b>75.3</b>
2,3-dihydro-4-methylfuran	1188	2.72	3.37
2-furfural	1452	13.62	27.06
5-methyl-2-furfural	1473	nd	0.343
2-acetylfuran	1492	nd	2.2
2-furfuryl alcohol	1656	8.44	19.91
<b>SUBTOTAL</b>		<b>26.98</b>	<b>51.93</b>
2-butenal	1029	9.06	2.92
hexanal	1074	18.05	6.38
heptanal	1177	2.27	0.56
paraldehyde	1273	9.75	5.19
dodecanal	1386	23.54	2
benzaldehyde	1506	111.25	30.6
phenylacetaldehyde	1625	104.64	62.75
2,4-decadienal	1798	3.48	0.37
<b>SUBTOTAL</b>		<b>285.92</b>	<b>142.25</b>
dimethyldisulfide	1063	2.95	2.8
dimethyltrisulfide	1363	12.67	1.71
<i>syn</i> -3,5-dimethyl-1,2,4-trithiolane	1578	nd	trace
<i>anti</i> -3,5-dimethyl-1,2,4-trithiolane	1605	nd	0.75
2-furfuryl mercaptan	1791	0.85	0.73
thieno[3,2-B]thiophene	1850	trace	trace
<b>SUBTOTAL</b>		<b>16.47</b>	<b>5.99</b>
3-hexanone	1051	2.53	10.46
methional	1442	nd	4.05
2-cyclohex-1-one	1568	nd	3.24

heat treatment. As Table II indicates, these aldehydes were mainly formed from lipid degradation. In the presence of glucose, because more carbonyl compounds might be produced, more pyrazine compounds would be generated via Maillard reaction of amino acids with carbonyl compounds. In addition, it is interesting to note that the concentration of sulfur-containing compounds also decreased in the presence of glucose. It is possible that some sulfur-containing free amino acids or amino acid residues in the hydrolyzed proteins, such as methionine and cysteine, participated in the reaction, or formed polymers or colored pigments. The increase in the concentration of pyridine compounds may be due to the increase in the content of acetaldehyde which is produced from the degradation of glucose. Formation of pyridine compounds is known to be directly related to the amounts of acetaldehyde, ammonia, and amino acids.

Among the nitrogen-containing compounds, pyrazines were predominant. In the presence of glucose, the concentration of pyrazine compounds, especially 2,5-dimethylpyrazine and pyrazine, was significantly increased. Because most pyrazine compounds have roasted, toasted, or nutty flavor, the reaction product from the reaction of protein hydrolysate with glucose has nutty flavor notes due to its high content of pyrazines. In addition to 2,5-dimethylpyrazine, 2-ethyl-5-methylpyrazine, 2-ethyl-6-methylpyrazine, 2-vinyl-5-methylpyrazine and 2-vinyl-6-methylpyrazine, which have strong flavor notes and a low threshold values, are also very important flavor compounds.

Other nitrogen-containing compounds, such as pyridines and thiazole, were found in smaller amounts than pyrazine compounds. The significant increase in the concentration of 4-methylpyridine is possibly due to the presence of glucose, which enhances the formation of this compound from the Maillard reaction. In addition, trace amounts of 2-acetylpyridine were found from the reaction of hydrolysate with glucose. This compound has a popcorn-like flavor.

Furan-derived compounds generally have sweet or caramel-like flavor notes. Table II shows some furan-derived compounds found from the reaction products. Both 2-furfural and 2-furfuryl alcohol are well-known sugar degradation products. These two compounds were increased significantly when glucose was added as a reaction precursor. 2-Furfural is also the precursor of 2-furfuryl mercaptan which has strong coffee-like aroma.

The aldehydes, as shown in Table II, were formed either from lipid degradation or from Strecker degradation of amino acids. In the presence of glucose, the concentrations of aldehyde compounds decreased, as mentioned earlier. Due to the high amount of aldehydes generated from these two reactions, the flavor notes of these compounds are noteworthy. Benzaldehyde has a cherry-like flavor. Hexanal has a green, grassy, or apple-like flavor. 2,4-Decadienal, which might be derived from the oxidative breakdown of linoleic acid, has a fatty flavor note.

Another interesting group of compounds is sulfur-containing substances. Although only small amounts of these compounds were generated from both reactions, these are critically important for possessing meat-like flavors. For example, the *syn* and *anti* isomers of 3,5-dimethyl-1,2,4-trithiolane is also found in beef broth (1) or boiled meat (16). 2-Furfuryl mercaptan is an important coffee flavor compound.

In conclusion, the flavor notes of most volatiles generated from thermal reaction of seal protein hydrolysate alone or in the presence of glucose are desirable. Selected compounds in Table III such as hexanal with green, grassy, and apple flavor note; benzaldehyde with cherry or vanilla-like flavor; 2-furfural with strong sweet and roasted flavor; 2-furfuryl alcohol with sweet and fruity flavor; 3,5-dimethyl-1,2,4-trithiolane with meaty flavor note; and 2-furfuryl mercaptan, 2,5-dimethylpyrazine, 3-ethyl-2,5-dimethylpyrazine and 2-vinyl-5-methylpyrazine with strong coffee-like or roasted flavor notes are important contributors to the overall flavor of these products. Based on the flavor notes of generated compounds, seal protein hydrolysate may be used as a flavor precursor to improve flavor quality of selected processed foods.

**Table III. Flavor notes of selected volatile compounds**

<i>Compounds</i>	<i>Primary flavor notes</i>
hexanal	green, grassy, apple
benzaldehyde	cherry, vanilla
2-furfural	sweet, roasted (coffee)
2-furfuryl alcohol	sweet, fruity
3,5-dimethyl-1,2,4-trithiolane	meaty, mushroom
2-furfuryl mercaptan	coffee-like, roasted meat
2,5-dimethylpyrazine	roasted, nutty
3-ethyl-2,5-dimethylpyrazine	peanut-like
2-vinyl-5-methylpyrazine	roasted

### Literature Cited

1. Brinkman, H. W.; Copier, H.; de Leun, J. M.; Tjan S. B. *J. Agric. Food Chem.* **1972**, *20*, 177-181.
2. Constantinides, A.; Adu-Amankwa, B. *Biotechnol. Bioeng.* **1980**, *22*, 1543-1549.
3. Izzo, H. V.; Ho, C. -T. *Trends Food Sci. Technol.* **1992**, *3*, 253-257.
4. Majlat, P.; Erdor, Z.; Takacs, J. *J. Chromatogr.* **1974**, *91*, 89-103.
5. Olsen, H. S. *Z. Lebensm. - Technol. Verfahrenstechnik.* **1983**, *34*, 51-60.
6. Oh, Y. C.; Shu, C. -K.; Ho, C. -T. *J. Agric. Food Chem.* **1992**, *40*, 111-117.
7. Oh, Y. C.; Hartman, T. G.; Ho, C. -T. *J. Agric. Food Chem.* **1992**, *40*, 1878-1880.
8. Shahidi, F. In *Flavor Chemistry: Trends and Developments*; Teranishi, R.; Buttery, R. G. and Shahidi, F., Eds.; ACS Symposium Series 388, **1989**, pp.188-201.
9. Shahidi, F.; Synowiecki, J. *Food Rev. Int.* **1996**, *12*, 283-302.
10. Shahidi, F.; Synowiecki, J. *Food Chem.* **1993**, *47*, 41-46.



11. Shahidi, F.; Synowiecki, J.; Naczek, M. *Can. Inst. Food Sci. Technol. J.* **1990**, *23*, 137-139.
12. Shahidi, F.; Synowiecki, J.; Balejko, J. *J. Agric. Food Chem.* **1994**, *42*, 2634-2638.
13. Synowiecki, J.; Shahidi, F. *J. Food Sci.* **1991**, *56*, 1295-1297.
14. Chen, Q. Ph.D. Dissertation, Rutgers University, New Brunswick, NJ, **1995**.
15. Yasuhara, A.; Shibamoto, T. *J. Agric Food Chem.* **1995**, *43*, 94-97.
16. Wilson, R. A.; Mussinan, C. J., Katz, I.; Sanderson, A. *J. Agric Food Chem.* **1973**, *21*, 873-876.

## Chapter 9

# Character-Impact Aroma Compounds of Crustaceans

H. H. Baek<sup>1</sup> and Keith R. Cadwallader<sup>2,3</sup>

<sup>1</sup>Department of Food Engineering, Dankook University, Chunan 330-714, Korea

<sup>2</sup>Department of Food Science and Technology, Mississippi Agricultural and Forestry Experiment Station, Mississippi State University, Box 9805, Mississippi State, MS 39762-5953

Character-impact aroma compounds of cooked crustaceans, such as crab, crayfish, lobster, freshwater prawn, and shrimp, are reviewed as well as previous findings on fresh aroma and off-flavor of crustaceans. Character-impact aroma compounds of cooked crustaceans include 2,3-butanedione, 2-methyl-3-furanthiol, 2-acetyl-1-pyrroline, 3-(methylthio)propanal, and 2-acetyl-2-thiazoline. These compounds are the most potent aroma compounds responsible for desirable meaty, nutty/popcorn, and salty aroma notes. 1-Octen-3-one, (Z)-4-heptenal, and 2-acetylthiazole also contribute to the aroma of crustaceans. These compounds were present in all crustaceans evaluated.

Crustaceans, such as crab, crayfish, lobster, prawn, and shrimp, are important and popular seafoods and have unique aromas and tastes. Cooked crustaceans have different aroma properties from other seafoods, such as fish and mollusks. It is well-known that cooked crustaceans have meaty and nutty/popcorn notes. Volatile and non-volatile flavor compounds responsible for crustacean aroma have been intensively studied (1-16).

Volatile flavor compounds are usually generated by enzymatic reaction, lipid autoxidation, microbial action, environmental contamination, and thermal reaction. Of these, the lipoygenase-mediated enzymatic reaction seems to play the most significant role in flavors of fresh seafoods (17). Fishy flavors of seafoods are associated with autoxidation of fish lipids and trimethylamine formation (18). Recently, it was found that bromophenols were responsible for sea-, brine-, and iodine-like aromas of saltwater fishes (19). The occurrence of off-flavor associated with bromophenols in prawns and lobsters has been reported (20).

While fresh crustaceans possess mild fishy aromas, desirable and most familiar aromas of crustaceans are generated by thermal reaction during cooking. Thermally generated aroma compounds from crustaceans have been investigated (7-

<sup>3</sup>Corresponding author

12, 15, 16); however, character-impact aroma compounds of cooked crustaceans are not well-understood as compared with the chemistry of fresh seafood flavors.

Gas chromatography/olfactometry (GC/O), in particular aroma extract dilution analysis (AEDA), has been successfully applied to many foods (21, 22). The results from AEDA can be used to indicate potent aroma-active compounds. Also, AEDA allows for detection of important aroma-active compounds present at trace levels. Flavor dilution (FD) factors are used to compare potency of aroma-active compounds. An FD factor is the last dilution at which an aroma-active compound is detected by GC/O. In this paper, previous studies on volatile aroma compounds from crustaceans are reviewed and recent findings on character-impact aroma compounds of cooked crustaceans will be discussed.

### **Volatile Aroma Compounds of Crustaceans**

**Lipoxygenase-Mediated Reaction of Polyunsaturated Fatty Acids.** The mild aromas of freshly harvested seafoods are generated by lipoxygenase-mediated reaction. Volatile 6-, 8-, and 9-carbon carbonyls and alcohols formed by the reaction of 12- or 15-lipoxygenases and hydroperoxide lyase contribute to fresh aroma of seafoods (17, 23). Lipoxygenase has been found in fish (24, 25). The mechanism involves lipoxygenase action on eicosapentaenoic acid (17). 1,5-Octadien-3-ol was formed by the action of 12-lipoxygenase and hydroperoxide lyase. 1-Octen-3-ol and (5Z)-1,5-octadien-3-ol have been reported as mushroom-metallic and metallic off-flavors in uncooked prawn and sand lobsters (3). Their ketones, 1-octen-3-one and (5Z)-1,5-octadien-3-one have lower threshold values, contributing strongly to fresh crustacean aroma.

**Lipid Autoxidation.** In addition to attack by lipoxygenase, polyunsaturated fatty acids of crustaceans may undergo autoxidation. After lipid autoxidation takes place, fresh aroma will be replaced by stale and oxidized aromas. Hexanal, 2,4-heptadienal, 3,5-octadien-2-one, and 2,4-decadienal modify fresh aroma and contribute stale aromas. (E,Z)-2,4-Heptadienal and (E,Z)-3,5-octadien-2-one were formed via autoxidation of eicosapentaenoic acid (23). (Z)-4-Heptenal is responsible for the off-flavor of cold stored cod (26). This compound is formed from the retro-aldol condensation of (E,Z)-2,6-nonadienal (23). Highly oxidized fish aroma is related to 2,4,7-decatrienals (18).

**Thermally Generated Aroma Compounds.** Pyrazines and sulfur-containing compounds have been shown to play important roles in both roasted and boiled shrimp (8). Dithiazine compounds were found to be important to the aroma of cooked krill and small shrimp (9, 10, 12). Both (5Z,8Z,11Z)- and (5E,8Z,11Z)-5,8,11-tetradecatrien-2-one have been described as having a characteristic aroma of cooked shrimp (7, 11). These compounds were suggested to be formed through thermal decomposition of  $\beta$ -keto acids produced by  $\beta$ -oxidation of long chain fatty acids (18).

**Aroma Compounds from Environment.** It has been shown that iodoform-like flavor defects exist in the flavor of fresh prawns and saltwater fishes (2, 19). The compounds responsible for this off-flavor were identified as 2- and 4-bromophenol, 2,4- and 2,6-dibromophenol, and 2,4,6-tribromophenol (2). Algae and bryozoa, from diet of prawns, were considered to be the possible origin of bromophenols (2). It is known that these compounds are found primarily in saltwater seafoods (19).

**Microbial Action.** Garlic-like off-flavors have been detected in deep-water prawn and sand lobster (2). The causative agents were identified as bis-(methylthio) methane and trimethylarsine. These compounds are believed to be produced by microbial action in the gut of these crustaceans. Trimethylamine, which is responsible for the fish-house like odor of seafoods, is a product of microbial reduction of trimethylamine oxide (18).

### Cooked Crustacean Aroma: Character-Impact Aroma Compounds

Compared with fresh seafood aroma and off-flavors associated with the environment via diet and microbial action, cooked aroma of crustaceans is of recent research interest. Recently, various cooked crustaceans, such as crab, lobster, and freshwater prawn have been evaluated using AEDA to identify the potent character-impact aroma compounds (4-6, 27). Also presented in this paper will be some recent findings on AEDA of crayfish and shrimp, in which we employed previously described procedures (4).

Aroma-active compounds in cooked meats from crustaceans are listed in Table I. Lipid-derived compounds contributed to the aroma of both cooked and fresh crustaceans. Among these, (Z)-4-heptenal (Fig. 1, I), 1-octen-3-one (Fig. 1, II), and (E,Z)-2,6-nonadienal were detected with relatively high FD factors (4). These compounds are considered undesirable to the cooked aroma of crustaceans. Pyrrolidine (alkaline/raw egg aroma note) and (E)-4-decenal (potato aroma note) were detected in only crab (5). Trimethylamine was detected in lobster, crab, and freshwater prawn (4, 5, 27). Thermally generated aromas, such as pyrazines and thiazoles, were important for the nutty/popcorn aroma note of lobster (4). A large number of unknowns having this type of aroma note, as well as meaty aroma note, were detected in other crustaceans evaluated (5, 27). Some compounds were commonly detected in cooked crustaceans, strongly suggesting a common precursor system. The commonly detected aroma-active compounds with high FD factors were 2,3-butanedione (Fig. 1, III), (Z)-4-heptenal, 1-octen-3-one, 2-methyl-3-furanthiol (2-MF, Fig. 1, IV), 2-acetyl-1-pyrroline (2-AP, Fig. 1, V), 3-(methylthio)propanal (3-MP, Fig. 1, VI), 2-acetylthiazole (Fig. 1, VII), and 2-acetyl-2-thiazoline (2-AT, Fig. 1, VIII). Most of these character-impact compounds were thermally generated. Due to their high FD factors and aroma properties, 2,3-butanedione, 2-MF, 2-AP, 3-MP, and 2-AT are considered the most important character-impact aroma compounds in cooked crustaceans.

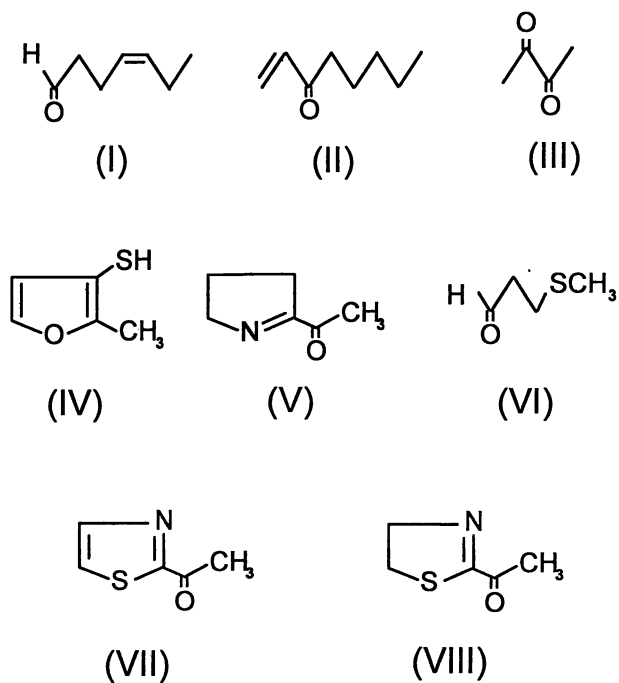


Figure 1. Commonly detected aroma-active compounds in cooked crustaceans

Table I. Aroma-active compounds detected in cooked crustaceans

compound name	RI <sup>a</sup>	crab <sup>b</sup>	crayfish	lobster <sup>c</sup>	prawn <sup>d</sup>	shrimp	aroma description
trimethylamine	<700	+ <sup>e</sup>	? <sup>f</sup>	+	+	?	fishy/ammonia
2,3-butanedione	974	+	+	+	+	+	buttery
pyrrolidine	1006	+	?	?	?	?	alkaline/raw egg
hexanal	1075	?	?	+	?	?	green/cut grass
2-heptanone	1161	?	?	+	?	?	moldy/bitter
2-heptanal	1176	?	?	+	?	?	nutty/chocolate
3-methyl-1-butanol	1196	?	?	+	+	?	dark chocolate
(Z)-4-heptenal	1242	+	+	+	+	+	rancid/fishy
1-octen-3-one	1302	+	+	+	+	+	mushroom
2-methyl-3-furanthiol	1314	+	+	+	+	+	cooked rice/meaty
2-acetyl-1-pyrroline	1340	+	+	+	+	+	popcorn
2,4,5-trimethylthiazole	1366	?	?	+	?	?	metallic/sulfury
2,3,5-trimethylpyrazine	1398	?	?	+	?	?	nutty
2-ethyl-3,6-dimethylpyrazine	1434	?	?	+	?	?	nutty
3-(methylthio)propanal	1453	+	+	+	+	+	soy sauce/baked potato
(E)-4-decenal	1535	+	?	?	?	?	potato
(E,Z)-2,6-nonadienal	1597	?	?	+	?	?	cucumber
2-acetyl-3-methylpyrazine	1615	?	?	+	?	?	burnt/popcorn
2-acetylpyrazine	1619	?	?	+	?	?	nutty/popcorn
2-acetylthiazole	1659	+	+	+	+	+	nutty/popcorn
2-acetyl-2-thiazoline	1764	+	+	+	+	+	popcorn
benzo(b)thiophene	1799	?	?	+	?	?	mothball
1-methylnaphthalene	1939	?	?	+	?	?	mothball

<sup>a</sup> retention indices calculated from DB wax column.

<sup>b</sup> from reference 5. <sup>c</sup> from reference 4. <sup>d</sup> from reference 27.

<sup>e</sup> positively identified based on aroma property, RI, and mass spectrum.

<sup>f</sup> presence was uncertain or contribution to the overall aroma was negligible due to low FD factor.

Table II. Aroma threshold values for character-impact aroma compounds commonly detected in cooked crustaceans

Compound	Aroma threshold (ppb)	Reference
(Z)-4-heptenal (I)	0.04	26
1-octen-3-one (II)	0.1	3
2,3-butanedione (III)	2.6	30
2-methyl-3-furanthiol (IV)	0.005-0.01	21
2-acetyl-1-pyrroline (V)	0.1	31
3-(methylthio)propanal (VI)	0.2	32
2-acetylthiazole (VII)	10	33
2-acetyl-2-thiazoline (VIII)	1	34

Table III. Mass spectral data for character-impact aroma compounds commonly detected in cooked crustaceans<sup>a</sup>

Compound	Mass Spectral data
(Z)-4-heptenal (I)	41(100), 55(79), 68(74), 84(61), 39(48)
1-octen-3-one (II)	55(100), 70(98), 43(22), 41(15), 83(12)
2,3-butanedione (III)	43(100), 86(15)
2-methyl-3-furanthiol (IV)	114(100), 85(29), 113(25), 71(22), 43(21)
2-acetyl-1-pyrroline (V)	43(100), 41(51), 83(37), 68(17), 111(17)
3-(methylthio)propanal (VI)	48(100), 104(57), 76(34), 61(33), 45(34)
2-acetylthiazole (VII)	43(100), 127(47), 99(43), 58(35), 112(31)
2-acetyl-2-thiazoline (VIII)	43(100), 129(67), 60(58), 101(13), 87(10)

<sup>a</sup> data were obtained using method described in reference 4

**2,3-Butanedione.** This compound had the second or third highest FD factors in lobster (4) and crab (5), and was predominant in other crustaceans evaluated (27). It is generally known that this compound is formed via Maillard reaction (28). This thermally generated compound has been found in other meats (29). Despite its relatively high aroma threshold value (Table II), this compound is believed to be important in cooked crustaceans due to its potency and desirable buttery aroma.

**2-Methyl-3-furanthiol (2-MF).** Recently, it was found that 2-MF contributed to the aroma of cooked freshwater prawn (27). Although this compound has not been reported in previous work on lobster (4) and crab (5), its presence in these products has been recently confirmed by us after re-evaluation (data not shown). This compound also was found in shrimp and crayfish with relatively high FD factors (data not shown). Its aroma was described as cooked rice-like, vitamin-like, and meaty. This compound was believed to be responsible for much of the meaty aroma of cooked crustaceans.

2-MF was reported to be a character-impact aroma compound in cooked beef (35) and has been identified in canned tuna fish (36) and yeast extract (37). Meaty and cooked rice aroma was detected in the RI region of 1310 for the DB-WAX column and in the RI region of 890 for the DB-5MS column in all crustaceans evaluated. It was not possible to obtain a mass spectrum of 2-MF due to its low abundance; however, characteristic  $m/e$  114 (Table III) of 2-MF was obtained using mass chromatography in the same RI region. 2-MF can be formed by thermal degradation of thiamin (38), by Maillard reaction involving cysteine and ribose (39-41), and by reaction of inosine 5'-monophosphate (IMP) with cysteine (42). 2-MF has been reported as an important compound imparting meaty aroma to boiled beef, pork, and chicken (21, 43, 44). 2-MF has the lowest threshold value among aroma-active compounds identified in crustaceans evaluated thus far (Table II).

**2-Acetyl-1-pyrroline (2-AP).** 2-AP was the most potent aroma compound in cooked crab (5) and lobster (4). 2-AP also was predominant in freshwater prawn (27), shrimp, and crayfish (data not shown). 2-AP has nutty/popcorn aroma note. This attribute is consistent with the nutty/popcorn aroma note of boiled crustaceans. 2-AP is a character-impact aroma compound in rice (31), and plays an important role in bread crust (22), beef (21), and boiled trout (29). This compound can be formed via Maillard reaction of glucose-proline reaction (45). It also was proposed that 2-AP was formed from the reaction of 2-oxopropanal (a sugar degradation product) and 1-pyrroline (degradation product of proline) (46). Threshold value and mass spectral data are listed in Table II and III, respectively.

**3-(Methylthio)propanal (3-MP).** 3-MP showed the highest or second highest FD factor in all crustaceans evaluated. The aroma property was described as salty/soy sauce/baked potato-like. This compound is formed via Strecker degradation of methionine (47). It was believed that 3-MP was a necessary and important component of the desirable aroma of cooked lobster and crab (4, 5). Threshold value and mass spectral data are listed in Table II and III, respectively.



**2-Acetyl-2-thiazoline (2-AT).** 2-AT has been described as having a nutty/popcorn-like note (48). This compound was reported for the first time in beef broth (49). A nutty/popcorn aroma note has been detected in crab (6) and lobster (4) in the RI region of 1760-1766. Although this compound was unknown at that time previous research was done, we have recently identified this compound in prawn and shrimp. The mass spectrum of 2-AT was not obtained in this RI region but characteristic m/e 129 (Table III) was found in all crustaceans evaluated. 2-AT was known to contribute to the aroma of boiled trout (29). 2-AT was a key aroma-active compound significantly contributing to the aroma of roast beef (50) and was formed from the reaction of cysteamine and 2-oxopropanal (48). Its threshold value was relatively high compared with other aroma-active compounds in crustaceans (Table II).

**Marine Aroma in Lobster.** Interestingly, bromophenols were not detected in saltwater crustaceans like shrimp, crab, and lobster (4-6, 7-12). However, marine and leather-like aromas were detected in the region of RI 2100-2300 in lobster (4). While the identities of these compounds are unknown, they impart seafood-like aroma notes to spiny lobster (4). These aroma notes also have been noticed in American lobster (data not shown). We are currently investigating the identity of these compounds and their importance to the aroma of spiny lobster.

## Conclusions

Character-impact aroma compounds present in all crustaceans include 2,3-butanedione, (Z)-4-heptenal, 1-octen-3-one, 2-MF, 2-AP, 3-MP, 2-acetylthiazole, and 2-AT. Of these, 2,3-butanedione, 2-MF, 2-AP, 3-MP, and 2-AT are believed to contribute to the desirable meaty, nutty/popcorn, and salty aroma notes of cooked crustaceans. All these compounds could be formed by Maillard reaction of amino acids and reducing sugars, Strecker degradation of amino acids, or thermal degradation of thiamin. These important character-impact compounds have been found in other meats such as beef and tuna. These compounds play a central role in cooked crustaceans and impart meat-like note to other meats. However, a great number of important aroma-active compounds still remain unknown. Further investigation is needed to better understand the aroma of cooked crustaceans.

## Acknowledgments

Approved for publication by the Mississippi Agricultural and Forestry Experiment Station as manuscript No. PS-9008. Partial support for this study was provided by a USDA Special Grant No. 91-34231-5940.

## Literature Cited

1. Konosu, S; Yamaguchi, K. 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. 367-404.

2. Whitfield, F.B. *Food Reviews Int.* **1990**, *6(4)*, 505-519.
3. Whitfield, F.B.; Freeman, D.J.; Last, J.H.; Bannister, P.A.; Kennett, B.H. *Aust. J. Chem.* **1982**, *35*, 373-383.
4. Cadwallader, K.R.; Tan, Q.; Chen, F.; Meyers, S.P. *J. Agric. Food Chem.* **1995**, *43*, 2432-2437.
5. Chung, H.Y.; Cadwallader, K.R. *J. Agric. Food Chem.* **1994**, *42*, 2867-2870.
6. Chung, H.Y.; Chen, F.; Cadwallader, K.R. *J. Food Sci.* **1995**, *60*, 289-291, 299.
7. Kubota, K.; Kobayashi, A. *J. Agric. Food Chem.* **1988**, *36*, 121-123.
8. Kubota, K.; Shijimaya, H.; Kobayashi, A. *Agric. Biol. Chem.* **1986**, *50*, 2867-2873.
9. Kubota, K.; Kobayashi, A.; Yamanishi, T. *Agric. Biol. Chem.* **1980**, *44*, 2677-2682.
10. Kubota, K.; Kobayashi, A.; Yamanishi, T. *Agric. Biol. Chem.* **1982**, *46*, 2835-2839.
11. Kobayashi, A.; Kubota, K.; Iwamoto, M.; Tamura, H. *J. Agric. Food Chem.* **1989**, *37*, 151-154.
12. Kubota, K.; Watanabe, K.; Kobayashi, A. *Agric. Biol. Chem.* **1988**, *52*, 1537-1540.
13. Choi, S.H.; Kato, H. *Agric. Biol. Chem.* **1984**, *48*, 1479-1486.
14. Choi, S.H.; Kobayashi, A.; Yamanishi, T. *Agric. Biol. Chem.* **1983**, *47*, 337-342.
15. Vejaphan, W.; Hsieh, T.C.-Y.; Williams, S.S. *J. Food Sci.* **1988**, *53*, 1666-1670.
16. Matiella, J.E.; Hsieh, T.C.-Y. *J. Food Sci.* **1990**, *55*, 962-966.
17. Josephson, D.B.; Lindsay, R.C. In *Biogenesis of Aroma*; Parliment, T.H.; Croteau, R., Eds.; ACS Symposium Series No. 317; American Chemical Society: Washington DC, 1986; pp. 201-219.
18. Lindsay, R.C. *Food Reviews Int.* **1990**, *6(4)*, 437-455.
19. Boyle, J.L.; Lindsay, R.C.; Stuibler, D.A. *J. Food Sci.* **1992**, *57*, 918-922.
20. Whitfield, F.B.; Last, J.H.; Shaw, K.J.; Tindale, C.R. *J. Sci. Food Agric.* **1988**, *46*, 29-42.
21. Gasser, U.; Grosch, W. *Z. Lebensm.-Unters. Forsch.* **1988**, *186*, 489-494.
22. Schieberle, P.; Grosch, W. *Z. Lebensm.-Unters. Forsch.* **1987**, *185*, 111-113.
23. Josephson, D.B. In *Volatile Compounds in Foods and Beverages*; Maarse, H. Ed.; Marcel Dekker, Inc.: New York, NY, 1991; pp. 179-202.
24. Hsieh, R.J.; German, J.B.; Kinsella, J.E. *J. Agric. Food Chem.* **1988**, *36*, 680-685.
25. German, J.B.; Kinsella, J.E. *J. Agric. Food Chem.* **1985**, *33*, 680-683.
26. McGill, A.S.; Hardy, R.; Burt, J.R. *J. Sci. Food Agric.* **1974**, *25*, 1477-1489.
27. Cadwallader, K.R.; Baek, H.H.; G.H. Chang-Lee. IFT annual meeting, **1996**, abstract no. 80D-20.
28. Hurrell, R.F. In *Food Flavours, Part A. Introduction*; Morton, I.D., MacLeod, A.J., Eds.; Elsevier Publishing: New York, NY, 1982; pp. 399-437.
29. Milo, C.; Grosch, W. *J. Agric. Food Chem.* **1993**, *41*, 2076-2081.
30. Fors, S. In *Maillard Reaction in Food and Nutrition*; Waller, G.R.; Feather,

- M.S., Eds.; ACS Symposium Series 215; American Chemical Society: Washington DC, 1983; pp. 185-286.
31. Buttery, R.G.; Turnbaugh, J.G.; Ling, L.C. *J. Agric. Food Chem.* **1988**, *36*, 1006-1009.
  32. Guadagni, D.; Buttery, R.G.; Turnbaugh, J.G. *J. Sci. Food Agric.* **1972**, *23*, 1435-1444.
  33. Schutte, L. *CRC Crit. Rev. Food Technol.* **1974**, *4*, 457-505.
  34. Guth, H.; Grosch, W. *J. Agric. Food Chem.* **1994**, *42*, 2862-2866.
  35. MacLeod, G.; Ames, J.M. *Chem. Ind.* **1985**, *5*, 175-177.
  36. Withycombe, D.A.; Mussinan, C.J. *J. Food Sci.* **1988**, *53*, 658, 660.
  37. Ames, J.M.; MacLeod, G. *J. Food Sci.* **1985**, *50*, 125-131, 135.
  38. Hartman, G.J.; Carlin, J.T.; Scheide, J.D.; Ho, C.-T. *J. Agric. Food Chem.* **1984**, *32*, 1015-1018.
  39. Mottram, D.S.; Whitfield, F.B. In *Thermally Generated Flavors, Maillard, Microwave, and Extrusion Processes*; Parliment, T.H.; Morello, M.J.; McGorin, R.J., Eds.; ACS Symposium Series No. 543; American Chemical Society: Washington DC, 1994; pp. 180-191.
  40. Güntert, M.; Brüning, J.; Emberger, R.; Hopp, R.; Köpsel, M.; Surburg, H.; Werkhoff, P. In *Flavor Precursors, Thermal and Enzymatic Conversions*; Teranishi, R.; Takeoka, G.R.; Güntert, M., Eds.; ACS Symposium Series No. 490; American Chemical Society: Washington DC, 1994; pp. 140-163.
  41. Grosch, W.; Zeiler-Hilgart, G. In *Flavor Precursors, Thermal and Enzymatic Conversions*; Teranishi, R.; Takeoka, G.R.; Güntert, M., Eds.; ACS Symposium Series No. 490; American Chemical Society: Washington DC, 1994; pp. 183-192.
  42. Zhang, Y.; Ho, C.-T. *J. Agric. Food Chem.* **1991**, *39*, 1145-1148.
  43. Gasser, U.; Grosch, W. *Z. Lebensm.-Unters. Forsch.* **1990**, *190*, 3-8.
  44. Gasser, U.; Grosch, W. *Lebensmittelchemie*, **1991**, *45*, 15.
  45. Roberts, D.D.; Acree, T.E. In *Thermally Generated Flavors, Maillard, Microwave, and Extrusion Processes*; Parliment, T.H.; Morello, M.J.; McGorin, R.J., Eds.; ACS Symposium Series No. 543; American Chemical Society: Washington DC, 1994; pp. 71-79.
  46. Schieberle, P. *J. Agric. Food Chem.* **1995**, *43*, 2442-2448.
  47. Ballance, P.E. *J. Sci. Food Agric.* **1961**, *12*, 532-536.
  48. Hofmann, T.; Schieberle, P. *J. Agric. Food Chem.* **1995**, *43*, 2946-2950.
  49. Tonsbeek, C.H.Th.; Copier, H.; Plancken, A.J. *J. Agric. Food Chem.* **1971**, *19*, 1014-1016.
  50. Cerny, C.; Grosch, W. *Z. Lebensm.-Unters. Forsch.* **1992**, *194*, 322-325.

## Chapter 10

# Flavor Characteristics and Lipid Composition of Atlantic Salmon

Linda J. Farmer<sup>1,2</sup>, Janice M. McConnell<sup>1,3</sup>, and William D. Graham<sup>2</sup>

<sup>1</sup>Department of Food Science, The Queen's University of Belfast, and <sup>2</sup>Department of Agriculture for Northern Ireland, Newforge Lane, Belfast BT9 5PX, United Kingdom

Wild and farmed Atlantic salmon (*Salmo salar*) were harvested from eight sources around the shores of Northern Ireland. Sensory evaluation of the flavor characteristics of the salmon was conducted using a sensory profiling method. The total lipids and fatty acid compositions of the salmon muscle were also determined. The results showed that the main differences in flavor occurred between wild, river and sea-caught salmon (whether farmed or wild). There was little difference in flavor between wild and farmed salmon when both were from the sea. In contrast, the main differences in fatty acid composition occurred between farmed and wild salmon. While both contained a similar proportion of n-3 fatty acids, farmed salmon contained higher concentrations of n-6 fatty acids and, therefore, a lower n-3/n-6 ratio than wild salmon. No direct correlation was found between fatty acid precursors and flavor.

The Atlantic salmon (*Salmo salar*) has long been valued as a luxury food and over the last 25 years has become widely available as a farmed product. However, it is frequently asserted that wild salmon possess more flavor than their farmed equivalents. It is, therefore, of considerable interest to both consumers and the salmon farming industry to determine whether this perception is based on fact.

Farmed salmon will inevitably be compared with its wild equivalent and must duplicate or improve on the quality of the wild fish if it is to compete favourably on the market (1). The possible quality differences between wild and farmed salmon have been the subject of research for both the Atlantic salmon and the various species of Pacific salmon (*Oncorhynchus sp.*); these studies have yielded conflicting results, with some authors reporting that the flavor of farmed salmon was similar or preferred to that of wild (2,3) while another study found that the wild salmon had more 'delicate, fresh fish flavor' (4).

<sup>3</sup>Current address: Evron Foods Limited, Portadown, Co. Antrim, Northern Ireland, United Kingdom

Many of the volatile compounds contributing to the odour and flavor of salmon and other fish are derived from lipid oxidation (5-8). In addition, the oily fatty acids which, when incorporated into the human diet, have been shown to exert a protective effect against cardiovascular disease (9). For this reason considerable interest has focused on the fatty acid composition of edible species of fish (e.g. 10). The effect of fish farming on fatty acid composition has also received attention; in general, wild salmon appear to have a higher n-3 to n-6 fatty acid ratio than their farmed equivalents (e.g., 2,11). Thus, the fatty acids present in Atlantic salmon are of interest both as potential flavor precursors and as nutritional food components.

Most of the above studies compare only one or two sources of farmed and wild salmon of any species. In order to reduce the impact of the sensory differences which occur between fish from separate farms, this paper presents some of the results of an investigation involving eight sources of Atlantic salmon. The full sensory results for salmon from 1993 and 1994, including those for texture and appearance, will be reported elsewhere. This paper reports the results of sensory profiling of flavor-related characteristics and the analyses of lipid composition for salmon harvested during 1994 and examines evidence for any correlation between the concentrations of lipid and fatty acid precursors and sensory scores for flavor attributes.

## Materials and Methods

### Salmon

**Comparison of Salmon from Eight Sources.** Farmed and wild Atlantic salmon from eight sources in the UK and Republic of Ireland (ROI) were obtained during the wild salmon season (June to September) of 1994. These sources are listed in Table I. All salmon were held on ice until transported to the laboratories of the Food Science Department, within 4 days of harvest. Salmon were eviscerated after the resolution of *rigor mortis* and ten or more 25mm steaks were immediately cut from the main body between the pectoral fins and the cloaca (labelled A to ca. J, as shown in Figure 1). The steaks were washed under running water and individually vacuum-packed in

**Table I. Sources of salmon**

<i>Name</i>	<i>Source type</i>	<i>Location</i>
Farm 1	Sea-farmed (SF)	Pens in enclosed sea-water inlet.
Farm 2	Sea-farmed (SF)	Pens in off-shore location
Farm 3	Sea-farmed (SF)	Onshore tanks with pumped sea-water
Sea 1	Sea-wild (SW)	Saltwater location near mouth of River 1
Sea 2	Sea-wild (SW)	Saltwater location near mouth of Rivers 2&3
River 1	River-wild (RW)	3 km from river mouth
River 2	River-wild (RW)	24 km from river mouth
River 3	River-wild (RW)	4 km from river mouth

laminated vacuum pouches (nylon 20 $\mu$ m, polythene 60 $\mu$ m; Brow Packaging, Belfast) within 15 mins of cutting, frozen and stored at  $-24^{\circ}\text{C} \pm 3^{\circ}\text{C}$  until required.

**Effect of freezing.** To compare fresh and frozen salmon, farmed salmon from Farms 2 and 3 were processed and packaged as above. Salmon to be analysed fresh were refrigerated at  $4^{\circ}\text{C}$  for one to two days prior to analysis. The remainder were frozen at  $-24^{\circ}\text{C} \pm 3^{\circ}\text{C}$  for 3-14 days prior to sensory analysis. To examine the effect of length of frozen storage, salmon from Farm 2 were harvested, processed and frozen 33, 15, 8 and 4 weeks before the commencement of sensory analysis. Panels were conducted on two consecutive weeks.

**Sensory Evaluation.** Salmon steaks were thawed (at  $4^{\circ}\text{C}$ , overnight), washed and cooked using a *bain marie* method, as follows: The steaks were wrapped in grease-proof paper, fold uppermost and placed individually on inverted, perforated aluminium trays (180mm x 120mm) which were placed in stainless steel baking trays. Tap water was added to a depth of 10mm and the whole tray covered in aluminium foil. The steaks for the comparison of fish from different sources were cooked for 20 min at  $200^{\circ}\text{C}$ . A cooking temperature of  $180^{\circ}\text{C}$  was used for the studies on the effect of frozen storage due to the smaller number of steaks in the oven. The ultimate internal temperature ranged from  $76^{\circ}\text{C}$  to  $85^{\circ}\text{C}$  depending on the size of steaks. Each panellist received one half steak served on a heated porcelain plate. A small portion from the ventral region was served separately in a heated, lidded porcelain dish (80 mm diameter) for odour assessment. Tap-water, filtered through a domestic water filter (Boots the Chemist, UK) to remove any extraneous flavors, and water biscuits were supplied as palate cleansers.

The eating quality of salmon from the eight sources was compared using quantitative descriptive analysis (sensory profiling). The same method was used to compare fresh and frozen salmon and fish stored at  $-24^{\circ}\text{C}$  for four to 33 weeks. A list of 37 attributes describing the appearance, aroma, flavor, texture and aftertaste of cooked salmon was agreed by a trained panel of 8 people. Of these, 19 described aroma, flavor or aftertaste. Table II lists the agreed definitions for these attributes.

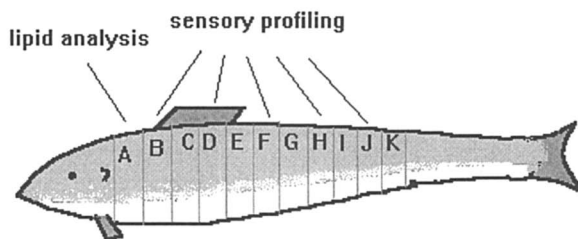


Figure 1. Allocation of salmon steaks

**Table II. Descriptors used for sensory profiling of salmon aroma and flavor**

<i>GROUPINGS</i>	<i>ATTRIBUTES</i>	<i>DEFINITIONS</i>
AROMA	Salmon-like	Intensity of distinctive salmon-like odour.
	Oily	Intensity of fish oils odour.
	Salty	Intensity of sea-like/salty odours.
	Earthy	Intensity of any earthy or peaty odours.
	Stagnant	Intensity of stagnant water odour.
	Farmyard	Intensity of manure/cow-dung odour.
FLAVOR	Salmon-like flavor	Intensity of distinctive salmon-like flavor.
	Salty flavor	Intensity of salt-like flavor.
	Oily Flavor	Intensity of fish oil flavor.
	Fishy flavor	Intensity of any other fish-like flavors.
	Fishy flavor (skin)	Intensity of non-salmon fishy flavor near the skin of the salmon steak.
	Earthy flavor	Intensity of earthy or peaty flavor.
AFTERTASTE	Farmyard flavor	Intensity of manure/cow-dung flavor.
	Time	Time when aftertaste starts.
	Overall aftertaste	Intensity of aftertaste
	Chicken-like a'taste	Intensity of chicken-like aftertaste.
	Oily aftertaste	Intensity of fish oil aftertaste
	Earthy aftertaste	Intensity of earthy aftertaste.
	Metallic aftertaste	Intensity of metallic aftertaste.

Panellists examined a total of ten salmon from each source and scored each attribute using a 100 mm linescale. All panels were conducted in ventilated booths under N-sky lighting and the data collected using PSA 1.64 data collection software (Oliemans, Punter & Partners, Utrecht, The Netherlands).

**Analysis of Total Lipids and Fatty Acids.** Five salmon from each source were analysed for total lipids and for fatty acid composition. Steak A (Figure 1) was used in all cases except two; in these cases, steak C was used and was shown to have a similar lipid content to steak A. The two halves of the steak were homogenised separately to give duplicate samples. Analyses were conducted to determine the nature of any changes in lipid composition over the length of a salmon.

The total lipid was extracted from the salmon homogenate (5g) using chloroform:methanol 2:1 (25ml x 2) by the method of Folch *et al.* (12). After filtration (Whatman No. 1) and washing with NaCl solution (0.37%) the extract was allowed to separate overnight, the organic layer removed and made up to 100 ml. Aliquots (10 ml) were taken for gravimetric analysis of the total lipids, while a volume (containing 150-200 mg lipid) was taken for fatty acid analysis.

Saponification of the lipids, by refluxing with methanolic sodium hydroxide (0.5M) and methylation with boron trifluoride methanol (14%), was conducted by the method of Metcalf *et al.* (13) Heptane (5ml) containing butylated hydroxytoluene ( $0.1 \text{ mg ml}^{-1}$ ) and internal standard (fatty acid methyl ester 22:0,  $1 \text{ mg ml}^{-1}$ ) was added and the samples refluxed for a further 1 minute. The heptane layer was dried with  $\text{Na}_2\text{SO}_4$  (anhydrous) and analysed by gas chromatography.

Gas chromatography was conducted using a Hewlett Packard 5890A gas chromatograph equipped with a HP7673A automatic injector, a HP3396A integrator and a fused silica capillary column coated with CP SIL 88 (50m x 0.25mm i.d. x 0.2 $\mu\text{m}$  film thickness; Chrompak UK Ltd., London). Quantification of the fatty acids was achieved by comparison with the known concentration of internal standard and the results analysed by analysis of variance. The fatty acids were identified by comparison with authentic fatty acid methyl ester standards from marine and animal sources (Supelco Ltd). Identities were confirmed by analysis of selected samples by chemical ionisation GC-MS using a Hewlett Packard 5890A gas chromatograph connected to a HP5971 mass selective detector operating in full scanning mode over mass range 100 to 500 atomic mass units. The reagent gas was methane.

## Results and Discussion

**Effect of Freezing and Storage on Flavor Quality.** The sensory investigations reported in this paper were conducted on frozen stored salmon. This was necessary due to the relatively short season over which the salmon were collected and because the supply of wild fish was dependent on river flows and was, therefore, sporadic. It was, therefore, essential to evaluate the effect of freezing and of frozen storage on eating quality characteristics. Profiling studies comparing fresh and frozen salmon indicated that, of the odour and flavor attributes, only 'oily flavor' was significantly altered; the mean score for 'oily flavor' was reduced by freezing ( $P < 0.001$ ). The period of frozen storage, from eight to 33 weeks, had no significant effect on the odour or flavor of the cooked salmon. However, appearance and/or texture were affected by freezing or frozen storage (data not shown).

**Effect of Origin of Salmon on Flavor Quality.** Table III shows the results for the flavor-related attributes of sensory profiling for salmon from the eight sources. The river-caught fish tended to receive lower scores for 'salmon-like odour' and 'salmon-like flavor' and higher scores for 'earthy odour', 'earthy flavor' and 'earthy aftertaste' than the sea-caught fish, whether farmed or wild. These results may be due to the masking of the 'salmon-like' attributes by earthy notes derived from freshwater microflora, by depuration of flavor compounds on entering freshwater (16) or due to the onset of maturation. Sexual maturation has been reported to cause a reduction in odour and flavor of the boiled salmon (17). While no visual changes associated with maturation were observed in the river fish, it is possible that the metabolic changes associated with maturation had commenced.

In contrast, a comparison of the sensory scores for sea-caught salmon from the three farmed and two wild sources show little difference between them in odour or flavor attributes. These results broadly agree with those reported for Pacific coho



Table III. Mean sensory scores for aroma and flavor attributes of salmon from eight sources

Attribute <sup>a</sup>	Farm 1	Farm 2	Farm 3	Sea 1	Sea 2	River 1	River 2	River 3
Salmon-like aroma *	24.93 <sup>b</sup>	25.08 <sup>b</sup>	24.74 <sup>ab</sup>	22.87 <sup>ab</sup>	25.18 <sup>b</sup>	21.04 <sup>a</sup>	22.62 <sup>ab</sup>	20.90 <sup>a</sup>
Oily aroma ***	27.10 <sup>c</sup>	26.62 <sup>c</sup>	27.06 <sup>c</sup>	25.66 <sup>bc</sup>	26.39 <sup>c</sup>	23.11 <sup>ab</sup>	25.10 <sup>bc</sup>	21.58 <sup>a</sup>
Salty aroma <sup>ns</sup>	19.48	21.74	21.93	19.34	21.09	19.71	20.68	19.65
Earthy aroma ***	27.77 <sup>ab</sup>	25.80 <sup>a</sup>	26.77 <sup>ab</sup>	27.15 <sup>ab</sup>	27.65 <sup>ab</sup>	31.95 <sup>c</sup>	29.95 <sup>bc</sup>	33.81 <sup>c</sup>
Stagnant aroma ***	25.41 <sup>a</sup>	23.47 <sup>a</sup>	25.79 <sup>a</sup>	23.55 <sup>a</sup>	24.39 <sup>a</sup>	30.15 <sup>b</sup>	25.99 <sup>a</sup>	30.27 <sup>b</sup>
Farmyard aroma ***	20.84 <sup>a</sup>	20.23 <sup>a</sup>	19.20 <sup>a</sup>	20.53 <sup>a</sup>	23.40 <sup>a</sup>	23.45 <sup>a</sup>	22.69 <sup>a</sup>	29.78 <sup>b</sup>
Salmon-like flavor ***	28.66 <sup>def</sup>	30.55 <sup>ef</sup>	31.05 <sup>f</sup>	26.56 <sup>cde</sup>	26.02 <sup>bed</sup>	18.59 <sup>a</sup>	24.42 <sup>bc</sup>	22.33 <sup>ab</sup>
Oily flavor *	27.89 <sup>b</sup>	27.99 <sup>b</sup>	27.74 <sup>b</sup>	25.76 <sup>ab</sup>	26.29 <sup>ab</sup>	24.36 <sup>ab</sup>	23.37 <sup>a</sup>	23.50 <sup>a</sup>
Salty flavor *	20.45 <sup>bc</sup>	19.52 <sup>abc</sup>	19.15 <sup>abc</sup>	17.59 <sup>a</sup>	20.49 <sup>bc</sup>	19.99 <sup>abc</sup>	20.66 <sup>c</sup>	18.10 <sup>ab</sup>
Fishy flavor ***	24.96 <sup>d</sup>	23.48 <sup>cd</sup>	24.33 <sup>cd</sup>	21.44 <sup>abc</sup>	22.64 <sup>bcd</sup>	18.64 <sup>a</sup>	20.20 <sup>ab</sup>	19.52 <sup>a</sup>
Fishy flavor (skin) ***	30.86 <sup>bed</sup>	30.31 <sup>bc</sup>	33.19 <sup>d</sup>	30.06 <sup>bc</sup>	31.60 <sup>cd</sup>	24.95 <sup>a</sup>	28.79 <sup>b</sup>	29.66 <sup>bc</sup>
Earthy flavor ***	33.12 <sup>bed</sup>	29.39 <sup>ab</sup>	32.00 <sup>abc</sup>	27.48 <sup>a</sup>	28.81 <sup>ab</sup>	38.21 <sup>d</sup>	36.73 <sup>cd</sup>	38.03 <sup>d</sup>
Farmyard flavor ***	25.66 <sup>bc</sup>	21.84 <sup>ab</sup>	23.67 <sup>ab</sup>	19.98 <sup>a</sup>	22.61 <sup>ab</sup>	32.25 <sup>d</sup>	29.85 <sup>cd</sup>	30.65 <sup>d</sup>
Time of aftertaste ***	14.44 <sup>bc</sup>	15.71 <sup>bc</sup>	15.15 <sup>bc</sup>	22.64 <sup>d</sup>	18.43 <sup>c</sup>	10.10 <sup>a</sup>	12.64 <sup>ab</sup>	12.11 <sup>ab</sup>
Overall a'taste ***	40.37 <sup>ab</sup>	34.44 <sup>a</sup>	37.01 <sup>a</sup>	33.56 <sup>a</sup>	40.53 <sup>ab</sup>	52.75 <sup>c</sup>	47.53 <sup>bc</sup>	50.87 <sup>c</sup>
Chicken-like a'taste ***	12.70 <sup>b</sup>	14.05 <sup>bc</sup>	12.48 <sup>b</sup>	15.01 <sup>c</sup>	13.90 <sup>bc</sup>	8.93 <sup>a</sup>	9.95 <sup>a</sup>	10.11 <sup>a</sup>
Oily a'taste ***	27.61 <sup>bed</sup>	29.00 <sup>d</sup>	28.34 <sup>cd</sup>	24.60 <sup>abc</sup>	24.52 <sup>abc</sup>	20.64 <sup>a</sup>	23.47 <sup>ab</sup>	22.84 <sup>a</sup>
Earthy a'taste ***	32.25 <sup>bc</sup>	26.16 <sup>ab</sup>	28.20 <sup>ab</sup>	25.04 <sup>a</sup>	28.53 <sup>ab</sup>	42.36 <sup>d</sup>	37.10 <sup>cd</sup>	40.78 <sup>d</sup>
Metallic a'taste ***	27.56 <sup>cde</sup>	22.29 <sup>ab</sup>	24.45 <sup>abc</sup>	21.44 <sup>a</sup>	26.15 <sup>bed</sup>	32.54 <sup>f</sup>	30.42 <sup>ef</sup>	29.79 <sup>def</sup>

<sup>ns</sup> etc. Scores are on a scale of 0 to 100 (increasing intensity); scores within a row without common superscripts differ significantly according to Duncan's test ( $P < 0.01$ )(26).

Significance according to analysis of variance: \*, \*\*, \*\*\* =  $P < 0.05$ , 0.01, 0.001, respectively, ns = not significant

salmon (one wild and two farmed sources) which were given similar scores for flavor when assessed using trained panellists, though the precise method used is unclear (2). In addition, when comparing three sources of Atlantic salmon, consumer panels found that the flavor of tank-reared salmon was significantly preferred over pen-reared and wild Atlantic salmon (3). However, wild chinook salmon was found to possess more 'delicate/fresh fish flavor' than two sources of farmed salmon by an untrained consumer panel using hedonic methods (4). 'Flavor intensity', 'sweet flavor', 'buttery flavor' 'gamey flavor' and 'fish oil flavor' did not differ.

'Oily aroma', 'oily flavor', 'oily aftertaste' and 'fishy flavor' were generally highest in the farmed fish and lowest in the river fish, with sea-caught wild fish in between. However, there were few significant differences between the sources of wild and farmed sea-caught salmon for these attributes.

It has been suggested that the characteristic sea-like, brine-like flavor of seafood is caused by bromophenols and that the depuration of these compounds from salmon on entering freshwater may be responsible for the loss of high quality sea-like flavor (16). In the sensory study reported herein, the panellists evaluated the salmon for 'salty', (or 'sea-like') aroma and 'salty flavor'. 'Salty aroma' showed no significant differences between sources while the scores for 'salty flavor' was perceived to be highest in salmon from Farm 1, Sea 2 and, surprisingly, River 2. Thus, this attribute did not appear to be related to the saltwater or freshwater origin of the salmon.

These data demonstrate that, in terms of flavor, there is little difference between wild and farmed sea-caught salmon. However, there were significant differences between river and sea-caught salmon.

**Analyses of Lipids and Fatty Acids.** The scope of this study did not permit the analysis of flavor compounds, with the exception of those responsible for off-flavors (18). However, studies elsewhere have proposed which of the many volatile compounds are important contributors to the desirable odours and flavors detected in salmon. The pleasant planty, green, melon-like aromas and flavors of freshly harvested salmon and other seafood are believed to be formed from long-chain polyunsaturated fatty acids by the action of endogenous lipoygenases (19,20). Milo and Grosch (8) recently found that, of the compounds contributing to cooked salmon flavor, many are derived from n-3 or n-6 polyunsaturated fatty acids. These authors have proposed that the additional 'fish-oil' character present in cooked salmon (8) compared with trout (*Salmo fario*)(7) may be due to higher concentrations of flavour compounds formed from n-3 fatty acids. They suggest that the increased quantities of these odour compounds detected in salmon may be due to the higher amounts of these fatty acids in salmon compared with trout (8). It was, therefore of interest to examine the fatty acid composition of salmon from the eight sources.

Table IV shows the lipid and fatty acid analyses for the steak (A) immediately behind the gills of salmon from the eight sources as well as the mean weights and lengths of the salmon analysed. A short study was conducted to investigate how the lipid content of steaks changed along the length of a fish. Steaks from two fish were analysed for total lipid and fatty acids. The total lipid content

Table IV. Fatty acid composition (as percentage of total fatty acids) of muscle from salmon from eight sources

Fatty acids <sup>t</sup>	Farm 1	Farm 2	Farm 3	Sea 1	Sea 2	River 1	River 2	River 3
14:0 ***	4.00 <sup>c</sup>	3.64 <sup>bc</sup>	3.61 <sup>bc</sup>	3.18 <sup>ab</sup>	3.32 <sup>ab</sup>	2.94 <sup>a</sup>	2.89 <sup>a</sup>	2.88 <sup>a</sup>
16:0 ***	11.93 <sup>ab</sup>	13.34 <sup>bc</sup>	11.53 <sup>a</sup>	14.84 <sup>cd</sup>	15.13 <sup>d</sup>	12.23 <sup>ab</sup>	15.17 <sup>d</sup>	14.57 <sup>cd</sup>
16:1 ***	3.83 <sup>a</sup>	3.69 <sup>a</sup>	4.00 <sup>ab</sup>	5.32 <sup>c</sup>	5.43 <sup>c</sup>	5.00 <sup>c</sup>	4.70 <sup>bc</sup>	4.89 <sup>c</sup>
18:0 ***	2.76 <sup>a</sup>	3.19 <sup>ab</sup>	2.67 <sup>a</sup>	4.00 <sup>c</sup>	3.93 <sup>c</sup>	3.64 <sup>bc</sup>	4.07 <sup>c</sup>	3.94 <sup>c</sup>
18:1 Δ9 + Δ11 **	14.72 <sup>a</sup>	16.68 <sup>abc</sup>	15.35 <sup>ab</sup>	20.23 <sup>cd</sup>	17.72 <sup>abcd</sup>	19.01 <sup>bcd</sup>	20.91 <sup>d</sup>	20.61 <sup>cd</sup>
18:2 (n-6) ***	2.59 <sup>c</sup>	6.15 <sup>c</sup>	2.82 <sup>d</sup>	1.31 <sup>ab</sup>	1.22 <sup>ab</sup>	1.42 <sup>b</sup>	1.12 <sup>a</sup>	1.18 <sup>a</sup>
18:3 (n-3) ***	1.46 <sup>bc</sup>	1.32 <sup>b</sup>	1.49 <sup>c</sup>	0.83 <sup>a</sup>	0.74 <sup>a</sup>	0.86 <sup>a</sup>	0.79 <sup>a</sup>	0.81 <sup>a</sup>
18:4 ***	2.23 <sup>c</sup>	1.30 <sup>b</sup>	2.02 <sup>c</sup>	0.79 <sup>a</sup>	0.76 <sup>a</sup>	0.70 <sup>a</sup>	0.62 <sup>a</sup>	0.61 <sup>a</sup>
20:1 *	9.24 <sup>bc</sup>	7.30 <sup>a</sup>	9.09 <sup>abc</sup>	7.63 <sup>ab</sup>	7.48 <sup>ab</sup>	9.83 <sup>c</sup>	7.47 <sup>ab</sup>	7.65 <sup>ab</sup>
20:4 ***	0.52 <sup>a</sup>	0.52 <sup>a</sup>	0.57 <sup>ab</sup>	0.57 <sup>ab</sup>	1.09 <sup>c</sup>	0.71 <sup>b</sup>	1.00 <sup>c</sup>	1.06 <sup>c</sup>
20:5 (n-3) **	5.61 <sup>ab</sup>	4.47 <sup>a</sup>	5.53 <sup>ab</sup>	6.38 <sup>bc</sup>	7.75 <sup>c</sup>	6.90 <sup>bc</sup>	6.49 <sup>bc</sup>	6.44 <sup>bc</sup>
22:1 <sup>t</sup>	11.26 <sup>b</sup>	10.04 <sup>ab</sup>	10.09 <sup>ab</sup>	8.86 <sup>ab</sup>	8.80 <sup>a</sup>	9.72 <sup>ab</sup>	7.95 <sup>a</sup>	8.22 <sup>a</sup>
22:5 (n-3) ***	2.52 <sup>ab</sup>	2.31 <sup>a</sup>	2.95 <sup>c</sup>	2.87 <sup>bc</sup>	3.19 <sup>c</sup>	3.63 <sup>d</sup>	3.16 <sup>c</sup>	3.09 <sup>c</sup>
22:6 (n-3) <sup>ns</sup>	13.36	11.71	13.92	11.98	13.20	12.21	13.73	13.60
Σ saturated ***	18.69 <sup>ab</sup>	20.17 <sup>bc</sup>	17.81 <sup>a</sup>	22.01 <sup>cd</sup>	22.38 <sup>d</sup>	18.81 <sup>ab</sup>	22.13 <sup>d</sup>	21.38 <sup>cd</sup>
Σ monounsat. *	39.05 <sup>ab</sup>	37.71 <sup>a</sup>	38.53 <sup>ab</sup>	42.04 <sup>bc</sup>	39.43 <sup>ab</sup>	43.53 <sup>c</sup>	41.03 <sup>abc</sup>	41.37 <sup>abc</sup>
Σ 3 or more DBs **	25.70 <sup>bc</sup>	21.63 <sup>a</sup>	26.47 <sup>c</sup>	23.43 <sup>ab</sup>	26.72 <sup>c</sup>	25.01 <sup>bc</sup>	25.79 <sup>bc</sup>	27.37 <sup>c</sup>
Σ n-6 fatty acids ***	2.59 <sup>c</sup>	6.15 <sup>c</sup>	2.82 <sup>d</sup>	1.31 <sup>ab</sup>	1.22 <sup>ab</sup>	1.42 <sup>b</sup>	1.12 <sup>a</sup>	1.18 <sup>a</sup>
Σ n-3 fatty acids **	22.95 <sup>bc</sup>	19.81 <sup>a</sup>	23.89 <sup>bc</sup>	22.07 <sup>ab</sup>	24.88 <sup>bc</sup>	23.61 <sup>bc</sup>	24.17 <sup>bc</sup>	25.7 <sup>c</sup>
Ratio n-3/n-6 ***	8.87 <sup>b</sup>	3.22 <sup>a</sup>	8.49 <sup>b</sup>	17.01 <sup>cd</sup>	20.79 <sup>de</sup>	16.59 <sup>c</sup>	22.24 <sup>e</sup>	20.65 <sup>de</sup>
Total lipid (mg/g) ***	103.6 <sup>c</sup>	83.8 <sup>bc</sup>	105.4 <sup>c</sup>	135.8 <sup>d</sup>	68.4 <sup>ab</sup>	66.6 <sup>ab</sup>	54.2 <sup>a</sup>	54.0 <sup>a</sup>

<sup>ab</sup> etc. Values within a row without common superscripts differ significantly according to Duncan's test ( $P < 0.01$ ) (26).

<sup>t</sup> Significance according to analysis of variance: \*, \*\* =  $P < 0.05$ , 0.01, 0.001, respectively, ns = not significant

varied considerably, with Steaks B to D containing the most lipid (Figure 2). The variation in total lipid within each fish is indicated by the coefficient of variance (CV): 25% and 27% for the two fish. Further studies with more fish are needed to confirm this effect. These results agree with comments by Porter *et al.* (21) who reported that the lipid content of the flesh depends on the anatomical position sampled. The total lipids determined using steak A may, therefore, be an underestimate for the overall average lipid content. However, the values for % fatty acids varied much less; the CV was between 0.7% and 3.5% for all fatty acids except 20:6, for which the CV was between 6% and 7% for both fish. Thus, the values cited in Table IV are good estimates of those for the whole salmon.



Figure 2. Total lipid content of steaks (n=7 or 6) from two salmon: Fish X (Sea2) and Fish Y (River2)

As might be expected, the farmed salmon had a consistently higher level of muscle lipids than most of the wild sources. Salmon from Farm 2 had a rather lower total lipid content as they were fed a lower fat diet (16% oil compared with 26% and 21% for salmon from Farms 1 and 3). The Sea 1 salmon were very high in lipid while the Sea 2 and the river fish were much lower. As expected, there was a trend towards a decrease in lipid content as the season progressed.

All fatty acids, except 22:6, show significant differences between sources (Table IV). For most of the fatty acids, the most extreme differences are between the sea-farmed and the river-wild fish, with the values for sea-wild salmon lying in between. The farmed salmon have more of the fatty acids 14:0, 18:2, 18:3 and 18:4 and a lower proportion of 16:1, 18:0, 18:1 and 20:4 with the river salmon showing the opposite effect. The trends for the long chain n-3 fatty acids are less clear, although 20:5 and 22:5 tend to be less abundant in the farmed salmon. The 18:2 and 18:3 fatty acids are likely to originate from vegetable components of the diets fed to farmed salmon, while 14:0 and 18:4 are probably derived from dietary fish oils (10). Salmon from one of the farms (Farm 2) differed from those from the other farms; in terms of 16:0, 20:1 and 22:1. The fatty acid composition was unusually close to that of wild salmon, but the proportion of 18:2 was more than twice and four times that for the other farmed and wild sources, respectively.

The wild fish generally contain the highest percentages of total n-3 fatty acids, although the differences are relatively small. However, the amount of these fatty acids provided to a consumer depends also on the total lipid content of the muscle. Thus, those salmon containing the highest total lipid content also supply the greatest quantity of n-3 fatty acids (Figure 3), but this is at the expense of additional calorie intake. A high intake of n-3 fatty acids is believed to have a beneficial effect on blood pressure, clotting mechanisms and serum triglyceride levels (22). However, it has also been suggested that the ratio of n-3 to n-6 fatty acids in the Western diet should be increased (23) and that, where the diet already contains sufficient n-6 fatty acids, fish with a high n-3/n-6 ratio may be beneficial (11). This study demonstrates that there is a wide variation in n-3/n-6 fatty acid ratios between salmon from farmed and wild sources; ratios range from 3.2 to 8.9 in farmed salmon and from 16.6 to 22.2 in the wild fish (Table IV and Figure 3). These n-3/n-6 ratios are broadly in agreement with those reported in the literature for wild and farmed Atlantic and Pacific salmon (Table V). However, two of the farmed sources investigated in this study have achieved unusually high concentrations of n-3 fatty acids and n-3/n-6 fatty acid ratios compared with data reported for other farmed salmon (Table V), although this is partly due to the elevated amounts of 18:3 in these fish.

It is uncertain whether the relatively small amounts of n-6 fatty acids in salmon from any of the sources will have a significant effect on the n-3/n-6 fatty acid ratio in the overall human diet. However, if and when the ratio of n-3 to n-6 fatty acids is of crucial importance, this study suggests that wild salmon show much higher ratios than the farmed fish.

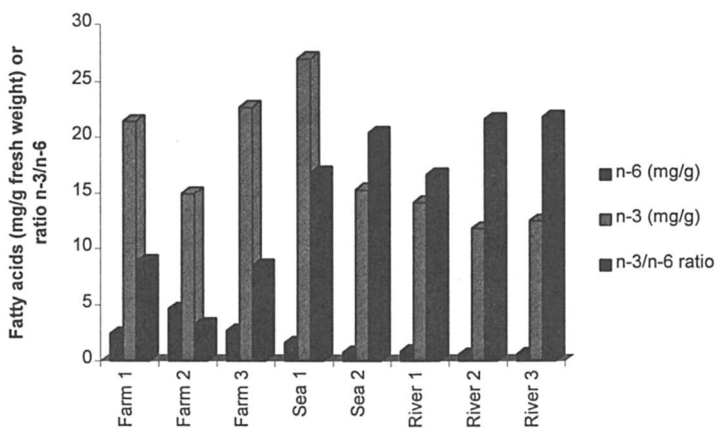


Figure 3. n-3 and n-6 Fatty acids and n-3/n-6 ratio for eight salmon sources.

**Linear Regression Analyses.** As a result of the studies described above, fatty acid analyses were available for 40 salmon and mean profiling scores from 8 panellists were available for 36 of these. It was, therefore, possible to examine the data for correlations between sensory attributes and measures of lipid composition. Although this approach is simplistic, it would indicate if there was any direct relationship

between content of n-3 fatty acids and flavor formation. Regression analyses were conducted between the percentage or content ( $\text{mg g}^{-1}$ ) of any of the fatty acids or classes of fatty acids listed in Table IV, the weight and length of the of the fish before evisceration and the sensory scores for flavor attributes. Many correlations existed between fatty acids when measured as  $\text{mg g}^{-1}$  fresh weight, related to the total lipid content; these are not discussed further. Only those correlations between individual fatty acids, as % total fatty acids, and between flavor scores and lipid composition are presented.

**Table V. n-3 Fatty acids (% and mg/g wet weight) and n-3/n-6 fatty acid ratios reported in the literature for wild and farmed salmon<sup>a</sup>**

Species, source of salmon	$\Sigma$ n-3 fatty acids (% total FA)		$\Sigma$ n-3 fatty acids (mg/g wet wt)		n-3/n-6 fatty acid ratio		Ref.
	wild	farmed	wild	farmed	wild	farmed	
Atlantic, UK+ROI	22.1 - 25.7	19.8 - 23.9	11.8 - 27.0	14.9 - 22.7	16.6 - 22.2	3.2 - 8.9	<i>ibid</i> <sup>b</sup>
Atlantic, ROI	18.4	16.1	12.4	11.7	17.5	2.7	(11)
Atlantic, Norway		19.7		17.7		3.7	(24) <sup>c</sup>
Atlantic, Europe	20	17	18	24	11	6	(25)
Coho, Canada	28	16	17	14	16	4	(2) <sup>d</sup>
Sockeye, Alaska			8.7		13.1		(21) <sup>c</sup>

a All studies included at least the major fatty acids in these two classes, 20:5 (n-3), 22:6 (n-3) and 18:2 (n-6), in their calculations.

b Ranges of the mean values for eight sources.

c Values for white muscle only.

d Estimated from graphical presentation of data.

**Correlations between fatty acids.** High correlation coefficients (R) were observed between many of the fatty acids. Some correlation, positive or inverse, is to be expected as the sum of all fatty acids is constrained to equal 100%. Some of the correlation was also found to be due to the substantial differences between wild and farmed salmon in terms of fatty acid content and the relationships between fatty acids. For example, Figure 4 shows the relationship between 18:3 and three other n-3 fatty acids. The data are very clearly grouped into those for farmed and wild salmon. The fatty acids 20:5, 22:5 and 22:6 all show positive correlation with 18:3 (R = 0.72, 0.65, 0.73) in the farmed salmon only, largely due to differences between the three

farms studied. This relationship does not apply for the wild fish and an inverse correlation is observed for 22:6 ( $R=-0.60$ ,  $P<0.01$ ).

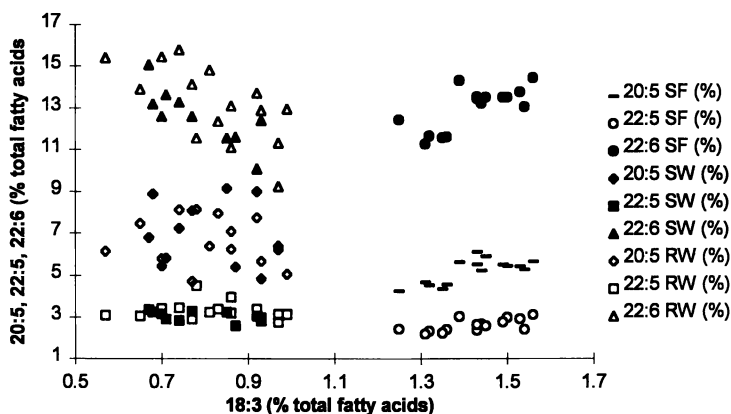


Figure 4. Relationships between n-3 fatty acids

As the fatty acid compositions of the farmed salmon differed considerably from those of the wild fish, as well as varying from farm to farm, the data for the wild salmon were considered separately. The main positive correlations were between 18:4 or 20:5 and 14:0 ( $R = 0.80, 0.79$ ), 18:0 and 16:0 ( $R = 0.74$ ), 20:5 and 18:4 ( $R = 0.77$ ) and between 22:1 and 20:1 ( $R = 0.78$ ). The main negative correlations were between 20:1 or 22:1 and 16:0 ( $R = -0.90, -0.84$ ), between the same two fatty acids and 18:0 ( $R = -0.72, -0.77$ ), and between 18:4 or 20:5 and 18:1 ( $R = -0.74, -0.82$ ). All these correlations were significant ( $P<0.001$ ) when compared with critical values for the Pearson product-moment correlation coefficient (26).

**Is there any relationship between fatty acid precursors and flavor?** Of particular interest was the possibility of a relationship between the amounts of n-3 fatty acids and any of the flavor attributes. Only weak correlations were observed, and these occurred solely between the sensory scores for salmon flavor and some fatty acids. Salmon flavor appeared to be positively correlated with 18:3 ( $R = 0.58$ ) and negatively correlated with 20:5 ( $R = -0.56$ ) and 22:5 ( $R = -0.63$ ). Although these correlations are significant ( $P<0.01$  or  $P<0.001$ ), if compared with critical values for the Pearson product-moment correlation coefficient, they do not obey the assumptions required for the use of this significance test (26); examination of the results indicates that the data are grouped according to whether the source of the salmon was river or sea. Figure 5 shows that any apparent correlation between salmon flavor and 18:3 was due entirely to the different scores for the three types of salmon; within each source type there is no correlation. As mentioned earlier, the low scores for 'salmon flavor' in river fish was probably due to other factors than the lack of availability of precursors. Given the likely importance of the polyunsaturated n-3 fatty acids for the formation of some of the important flavor compounds it is

surprising that salmon flavor was inversely correlated with 20:5 and 22:5. However, again, these relationships appear largely due to the influence of the river fish; if the river fish are omitted the correlation is very low.

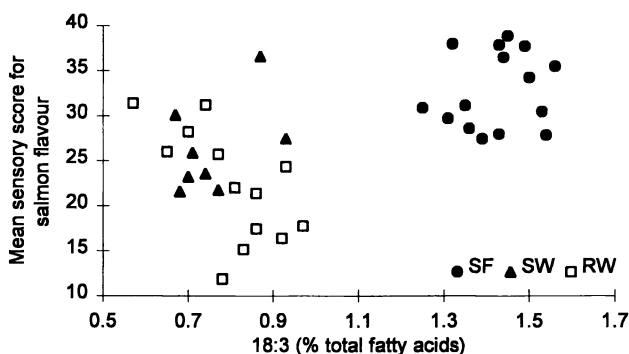


Figure 5. Plot of salmon flavour against fatty acid 18:3 (%total fatty acids)

Thus, such direct correlations between lipid or fatty acid concentrations and flavor attributes cannot explain the differences in flavor between individual salmon; the amounts of these precursors have little effect on flavor. Other factors must be critical for flavor development. These may include the fatty acid composition of a particular lipid component, such as the membrane phospholipids. Studies on meat flavor have shown that phospholipids play a more important role than the triglycerides in the generation of aroma compounds (27,28). The amount and nature of antioxidants present or differences in enzyme activity may also play a critical role. Further studies are required to elucidate the prerequisites for desirable salmon flavor.

## Conclusions

Assertions that wild Atlantic salmon have more flavor than farmed are not supported by the results of this study. The main difference in flavor was observed between the sea-caught and river-caught salmon; the latter had less salmon flavor and odour and higher scores for earthy attributes. There was no significant difference in flavor between the wild and farmed sea-caught salmon. In contrast, the fatty acid composition differed most between wild and farmed salmon. Fatty acid analyses showed that although wild salmon had a higher percentage of n-3 fatty acids, the generally higher lipid contents of the farmed salmon made them good sources of n-3 fatty acids. However, the wild fish had much lower amounts of n-6 fatty acids and higher n-3/n-6 ratio than farmed fish.

An examination of the correlations between measures of lipid composition and sensory scores indicated that there is no direct relationship between overall concentrations of fatty acid precursors and the perceived flavor.



### Acknowledgments

Funding from the EU Interreg Initiative and the Department of the Environment for Northern Ireland - Environment Service is gratefully acknowledged. The authors also wish to thank Miss E. Flanagan and Miss J. Sinton for conducting the lipid analyses, Mr T. Hagan and Mrs D. Rea for assistance with the preparation and sensory analysis of the salmon, Mr J. Hamilton and Mr C. McRoberts for the mass spectrometric analyses, Dr D. Kilpatrick and Mr A. Gordon for the statistical analyses and the numerous people whose co-operation facilitated the procurement of the salmon.

### Literature Cited

1. Moe, H.N. *Proc. Nutr. Soc. NZ* **1990**, *15*, 16-22.
2. Higgs, D.A.; Skura, B.J.; Dosanjh, B.S.; Yan, D.; Powrie, W.D.; Donaldson, E.M. *Can. Aquacult.* **1989**, *5*, 51-53.
3. Bartos, G.E. MSc Thesis, The Pennsylvania State University, College Station, PA. **1989**.
4. Sylvia, G.; Morrissey, M.T.; Graham, T.; Garcia, S. *J. Aquat. Food Prod. Technol.* **1995**, *4*, 51-64.
5. Josephson, D.B.; Lindsay, R.C.; Stuibler, D.A. *J. Agric. Food Chem.* **1984**, *32*, 1344-1347.
6. Lindsay, R.C. In *A Partnership of Marine Interests. IROC Oceans '88*. 1988, pp 61-65.
7. Milo, C.; Grosch, W. *J. Agric. Food Chem.* **1993**, *41*, 2076-2081.
8. Milo, C.; Grosch, W. *J. Agric. Food Chem.* **1996**, *44*, 2366-2371.
9. Herold, P.M.; Kinsella, J.E. *Am. J. Clin. Nutr.* **1986**, *43*, 566-598.
10. Hearn, T.L.; Sgoutas, S.A.; Hearn, J.A.; Sgoutas, D.S. *J. Food Sci.* **1987**, *52*, 1209-1211.
11. Cronin, D.A.; Powell, R.; Gormley, R. *J. Food Sci. Technol.* **1991**, *15*, 53-62.
12. Folch, J.; Lees, M.; Sloane Stanley, G.H. *J. Biol. Chem.* **1957**, *226*, 497-509.
13. Metcalf, L.D.; Schmitz, A.A. *Anal. Chem.* **1961**, *33*, 363.
14. Yu, T.C.; Sinnhuber, R.O.; Crawford, D.L. *J. Food Sci.* **1973**, *38*, 1197-1199.
15. Andersen, H.J.; Bertelsen, G.; Christophersen, A.G.; Ohlen, A.; Skibsted, L.H. *Z. Lebensm. unters. Forsch.* **1990**, *191*, 119-122.
16. Boyle, J.L.; Lindsay, R.C.; Stuibler, D.A. *J. Food Sci.* **1992**, *57*, 918-922.
17. Aksnes, A.; Gjerde, B.; Røald, S.O. *Aquaculture* **1986**, *53*, 7-20.
18. Farmer, L.J.; McConnell, J.M.; Hagan, T.D.J.; Harper, D.B. *Water Sci. Technol.* **1995**, *31*, 259-264.
19. Josephson, D.B.; Lindsay, R.C. In *Biogenesis of Aromas*; Parliment, T.H.; Croteau, R., Eds.; American Chemical Society: Washington, DC, 1986; pp 201-219.
20. Lindsay, R.C. *Food Rev. Int.* **1990**, *6*, 437-455.
21. Porter, P.J.; Kramer, D.E.; Kennish, J.M. *Int. J. Food Sci. Technol.* **1992**, *27*, 365-369.
22. Hornstra, G. In *The Role of Fats in Human Nutrition*; Vergroesen, A.J.; Crawford, M., Eds.; Academic Press, **1989**; pp 151-235.

23. Simopoulos, A. *J. Nutr.* **1989**, *119*, 521-528.
24. Aursand, M.; Bleivik, B.; Rainuzzo, J.R.; Jorgensen, L.; Mohr, V. *J. Sci. Food Agric.* **1994**, *64*, 239-248.
25. van Vliet, T.; Kahan, M.B. *Am. J. Clin. Nutr.* **1990**, *51*, 1-2.
26. O'Mahoney, M. *Sensory Evaluation of Foods: Statistical Methods and Procedures*. Marcel Dekker, New York, **1986**.
27. Mottram, D.S.; Edwards, R.A. *J. Sci. Food Agric.* **1983**, *34*, 517-522.
28. Farmer, L.J.; Mottram, D.S. *J. Sci. Food Agric.* **1990**, *53*, 505-525.

## Chapter 11

# Potent Odorants in Boiled Cod as Affected by the Storage of Raw Material

C. Milo and W. Grosch

Deutsche Forschungsanstalt für Lebensmittelchemie, Lichtenbergstrasse 4,  
D-85748 Garching, Germany

The potent odorants of boiled cod, fresh and after prolonged storage at  $-13^{\circ}\text{C}$ , were screened by two GC-sniffing methods in order to cover the whole range of volatility; these were aroma extract dilution analysis (AEDA) and gas chromatography/olfactometry of static headspace samples (GCO-H). Quantification of 19 odorants by means of an isotope dilution assay and calculation of odor activity values (ratio of the concentration to the threshold) revealed that methanliol and dimethylsulfide followed by (E,Z)-2,6-nonadienal and 3-methylbutanal as being the most potent odorants for the nasal perception of fresh boiled cod, whereas (Z)-1,5-octadien-3-one and methional showed the highest retronasal odor activity values (OAV). After storage for 14 weeks at  $-13^{\circ}\text{C}$  a drastic increase in the concentration, and therefore the OAVs, of 3-methylbutanal and 2-methylbutanal as well as a decrease in the content of dimethylsulfide was noticed. Based on these results 3-methylbutanal was found to be primarily responsible for the malty odor defect in the frozen stored cod.

The aroma of fish has been the subject of much research over the past decades. Several studies focused on raw fish and differences among species (1-3). So far more than 300 volatiles have been identified in fresh and processed fish (4). However, the contribution of certain odorants to the aroma remains largely unknown, due to the absence of sufficient quantitative data. Furthermore, either extracts or headspace samples have been analyzed, thereby neglecting the impact of the highly volatile or low volatile compounds, respectively. The aroma of cooked fish has been studied (5-8), sometimes under drastic cooking conditions (5,6). Since the boiling affects the aroma, the cooking procedure should reflect the common preparation method in order to allow production of the odorants in a "ready to eat" product. A thorough study of the volatiles in cooked

cod was performed by McGill et al. (8) who also followed quantitative changes in odorants depending on the time of frozen storage of raw cod.

Fish and fish products are usually stored and handled frozen, a necessity that arises from the short shelf life of these products. Although oxidative susceptibility of fish lipid and enzymatic hydrolysis of fish proteins in frozen products is well documented as being responsible for potential aroma defects (9), our understanding of the chemical basis of the off odors so produced is rather limited. In search for odorants that are mainly responsible for aroma changes in frozen stored cod, it was reported that freezing and thawing cycle *per se* does not affect the aroma (8). Gas chromatographic (GC) sniffing of extracts prepared, using a Likens-Nickerson apparatus, from frozen stored cod indicated three regions in the chromatogram that were associated with the odor defect described as the "cold storage flavor" (CSF). Odorants that were identified as the cause of this defect were (E)-2-heptenal, (E,E)-2,4-heptadienal and particularly (Z)-4-heptenal. The authors reported a good correlation between the sensory scores as reflected in CSF intensity and (Z)-4-heptenal concentration and concluded that this aldehyde is primarily responsible for the odor defect. (Z)-4-Heptenal was also associated with the fishy aroma quality of oxidized soybean oil (10). (Z)-4-Heptenal, together with (E,Z,Z)-2,4,7-decatrienal at high concentration (> 1ppm) imparted a burnt-fishy aroma in deodorized oils (11). We have previously determined that certain lipid peroxidation products namely, (Z)-3-hexenal, (Z,Z)-3,6-nonadienal and also (Z)-4-heptenal as being mainly responsible for the unpleasant fishy, oily aroma of frozen stored trout (12).

This work examines the potent odorants of boiled cod as affected by frozen storage of the raw material. Prior to storage, skinned cod fillets were homogenized to ensure a uniform material. Furthermore, flavor changes of cod, a lean saltwater fish, as compared to frozen stored trout (12) was tested. The medium and higher boiling odorants were screened by aroma extract dilution analysis (AEDA) and the lower boiling volatiles were examined using gas chromatography/olfactometry of static headspace samples (GCO-H). After quantification of the odorants, according to the results of the two GC sniffing methods, odor activity values (OAV = ratio of concentration to odor threshold) were calculated. Based on these results branched aldehydes derived from amino acid, were found responsible for the odor defect of stored cod and not the lipid peroxidation products.

## Experimental

**Material.** Skinned cod fillets (4 kg) were ground in a meat grinder. The raw mince was stored in polyethylene bags at -13°C for up to 26 weeks. The reference material was kept at -60°C until analyzed and is referred to as fresh sample. The storage temperature of -13°C was chosen in order to suppress microbiological spoilage and to keep the storage time reasonably short. Propanal, methylpropanal, 2,3-pentanedione, dimethyltrisulfide and (Z,Z)-3,6-nonadienal were synthesized as deuterium labeled compounds. The other labeled odorants used in the isotope dilution assays were prepared as described elsewhere (13).

**Isolation of volatiles.** The poached fish (250 g in aluminum foil, 15 min at 80°C) was frozen in liquid nitrogen, ground to a fine powder, mixed with an equal amount (w/w) of anhydrous sodium sulfate, soaked in diethyl ether (1000 ml) and extracted, after 24 h, in a Soxhlet apparatus over a 7 h period. A fat free organic solvent extract was obtained by high vacuum distillation. After the separation into a neutral/basic and an acidic fraction AEDA was performed as described earlier (14). The highly volatile odorants were analyzed by GC sniffing using a series of gradually decreasing volumes of static headspace samples (15). Identification and quantification of odorants was performed using gas chromatography - mass spectrometry (GC-MS) after an initial clean up by column chromatography on silica gel (12).

## Results and Discussion

The aroma profile of fresh boiled cod was described by a sensory panel as mild-fishy, vegetable-like with resemblance to cooked rice. This pleasant aroma changed to an unpleasant fishy, malty odor after 20 weeks of storage at -13°C.

The final extract of the neutral volatiles in fresh, boiled cod analyzed by AEDA had a distinct fish-like odor, but lacked the unique vegetable, cooked rice-like top note. As summarized in Table I, 15 odorants were detected by GC sniffing, 9 of which possessed a cod-like odor quality. To the best of our knowledge, 8 odorants, marked with astrices in Table I, were identified for the first time in cod. High FD factors were determined for methional, (Z)-1,5-octadien-3-one, (E,Z)-2,6-nonadienal and 1-octen-3-one in both fresh and stored samples. Greater differences in FD factors between fresh and stored cod samples were found for 2,3-pentandione, 2,4-nonadienal, dimethyltrisulfide, (Z)-1,5-octadien-3-one and (E,Z)-2,6-nonadienal. The aroma notes responsible for the unique fresh cod aroma as well as the aroma changes, observed after improper frozen storage, could not be entirely explained by the AEDA results. Since highly volatile compounds may be lost during the preparation of extracts for AEDA and/or may be hidden under the solvent peak, and therefore not being perceived by GC sniffing, gas chromatography/olfactometry of static headspace samples was performed in order to complement the results of AEDA.

As summarized in Table II, in a volume of 10 ml headspace from a cod sample, stored for 14 weeks at -13°C, 14 odorants were detected by GC-sniffing compared to 9 in the fresh sample. The additional odorants in the stored cod included methylpropanal, 3-methylbutanal and 2-methylbutanal, all of which exhibited a malty-like odor at the sniffing port. The importance of these branched aldehydes to the aroma-defect of cod, boiled after prolonged storage, is reflected by a drastic decrease of headspace volumes sufficient for their perception in GCO-H. The compound 3-methylbutanal was the only odorant perceivable in a headspace volume of 0.5 ml. Both screening methods (i.e. AEDA and GCO-H) did not indicate any contribution from (Z)-4-heptenal to the odor defect in the stored cod used in these experiments.

Accurate quantification of odorants was also performed in order to gain further insight into the actual contribution of individual compounds to the overall aroma. The method of choice for the determination of trace components and/or unstable compounds, as it is often the case with potent odorants, is the isotope dilution assay. Based on the

**Table I. Potent odorants (FD > 8) in boiled cod<sup>a</sup> and influence of storage of raw material on the FD factors**

No.	Compound <sup>a</sup>	Odor description <sup>b</sup>	FD-Factor <sup>d</sup>		
			RI <sup>c</sup> on SE 54	A (fresh)	B (stored)
6	diacetyl	buttery-like	595	<8	16
16	2,3-pentandione*	buttery-like	700	32	<8
17/18	hexanal/(Z)-3-hexenal <sup>c</sup>	green	800	16	8
19	(Z)-4-heptenal	creamy-fatty	900	8	8
10	methional*	boiled potato-like	908	128	256
20	2-acetyl-1-pyrrolin*	roasty	925	16	32
11	dimethyl trisulfide*	cabbage-like	957	8	32
12	1-octen-3-one	mushroom-like	981	256	64
13	(Z)-1,5-octadien-3-one*	geranium-like	985	64	256
21	(E,Z)-2,6-nonadienal	cucumber-like	1154	64	16
22	2,4-nonadienal	fatty	1193	32	<8
15	dimethyl tetrasulfide*	cabbage-like, putrid	1212	16	32
23	(E,E)-2,4-nonadienal*	fatty	1216	32	32
24	(E,E)-2,4-decadienal	fatty	1328	32	16
25	4,5-epoxy-(E)-2-decenal*	metallic	1384	16	8

<sup>a</sup>The material was stored for 20 weeks at -60°C (A) or at -13°C (B) before boiling.

<sup>b</sup>Odor description at the GC sniffing port.

<sup>c</sup>Retention indices according to Van den Dole and Kratz (1969).

<sup>d</sup>Flavor Dilution determined on SE 54 capillary column.

\*The following compounds 6,10-13, 16-19, 21-24 and 26-28 were identified by comparison with the reference standards on the basis of the following criteria: Odor quality at the GC sniffing port, RI on DB 5 and DB 1701 and mass spectra obtained by MS-EI and MS-CI. For numbers 15, 20 and 25 the mass spectra were too weak for an unequivocal interpretation and hence were identified based on the remaining criteria.

\*Compounds identified for the first time in boiled cod.

results of GCO-H and AEDA, IDAs for 16 odorants was developed. As shown in Figure 1 for quantification of (Z)-1,5-octadien-3-one, the labeled standard was distinguished from the analyte by specific mass traces after GC/MS-CI. The two fold deuterated standard showing the parent ion at 127 upon chemical ionization eluted 2 scans earlier than the mass 125 originating from the analyte. Thus, the high selectivity of this method is evident which is advantageous for quantification of compounds in complex mixtures.

The quantification procedure confirmed the conclusions reached from the results of sniffing experiments. As shown in Table III the concentration of 2- and 3-methylbutanal increased by a factor of 12. The concentration of other compounds such as 2,3-pentandione, hexanal and dimethylsulfide decreased by a factor of at least 3. By far the lowest concentration of all odorants was determined for (Z)-1,5-octadien-3-one (0.1 mg/kg in fresh cod).

**Table II. The minimum headspace volumes required to detect odorants of boiled cod by GCO-H<sup>a</sup> and the influence of storage of raw material**

No.	Compound <sup>b</sup>	Odor <sup>c</sup>	Volume <sup>d</sup> (ml)		
			RI on RTX 5	A (fresh)	B (fresh)
1	acetaldehyde	sweet	<500	5	2.5
2	methanethiol	sulfurous	<500	10	20
3	trimethylamine	ammoniacal, fishy	<5005	<20	5
4	dimethylsulfide	cabbage-like	<500	5	10
5	2-methylpropanal	malty	550	<20	5
6	diacetyl	buttery-like	595	10	2.5
7	3-methylbutanal	malty	653	20	0.5
8	2-methylbutanal	malty	663	>20	5
9	unknown	vegetable-like	785	>20	10
10	methional	boiled potato-like	908	10	5
11	dimethyltrisulfide	cabbage-like	977	1.0	1.0
12	1-octen-3-one	mushroom-like	980	10	5
13	(Z)-1,5-octadien-3-one	geranium-like	983	5	5
14	one	earthy, mushroom-like	1077	>20	10
15	unknown	like	1232	10	10
	dimethyltetrasulfide	cabbage-like, putrid			

<sup>a</sup>The material was stored for 26 weeks at -60°C (A) or at -13°C (B) before boiling.

<sup>b</sup>Compounds 1-8 and 9 were identified based on the following criteria: matching odor quality, retention indices and MS (EI, CI) spectra with the corresponding reference compounds. No MS spectra were obtained for odorants 9, 10 which were identified based on the remaining criteria.

<sup>c</sup>Odor description assigned during GCO-H.

<sup>d</sup>The smallest headspace volume sufficient to perceive an odorant at the GC sniffing port.

Odor activity values for the 16 odorants were calculated on the basis of their nasal and retronasal odor thresholds in water. The results listed in Table IV indicate that depending on the thresholds, nasally versus retronasally, used for the calculation of OAV, different ranking of the potent odorants may be obtained. In the fresh boiled cod the nasal OAV suggests that the cabbage sulfury smelling methanethiol and dimethylsulfide belong to the most potent odorants, followed by (E,Z)-2,6-nonadienal and 3-methylbutanal. The odor characteristics of these odorants are likely to cause the vegetable-like top note in the aroma profile of fresh boiled cod. Because of the differences in the nasal and retronasal thresholds the sulfury odorants do not contribute as much to the retronasal perception contrary to those of (Z)-1,5-octadien-3-one and

methional. The importance of the latter two compounds for the flavor of fish is underscored by the observation that an aqueous solution of them at the concentration levels found in our sample had a fishy odor. The increase in concentration of 3-methylbutanal by a factor of 12, resulted in the highest OAV of all odorants nasally as well as retronasally. Its malty character is further enhanced by 2-methylbutanal. On the other hand, the cabbage-like odor of dimethylsulfide decreased by a factor of 3. These results confirm the observation made by McGill et al. (8) who noted a decrease in the cabbage-like odor note of stored cod as evaluated by GC-sniffing. So far 3-methylbutanal has been just once reported as being a possible cause of odor defect in fish (16). Our results clearly demonstrate that 2-methylbutanal and particularly 3-methylbutanal are primarily responsible for the off-odor that develops upon frozen storage of raw cod.

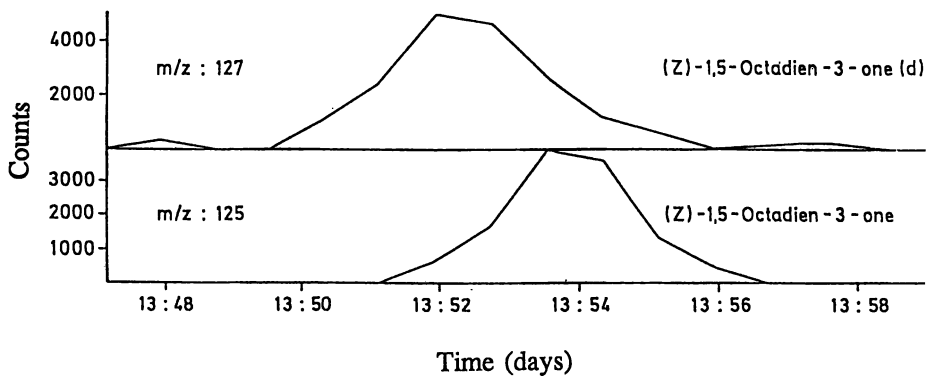
**Table III. Concentration of potent odorants ( $\mu\text{g}/\text{kg}$ ) in boiled cod<sup>a</sup> and influence of storage of raw material**

Compound	A (fresh)	B (stored)
acetaldehyde	1300	2400
methanthiole	100	130
dimethylsulfide	77	25
2-methylpropanal	27	n.b. <sup>c</sup>
2-methylbutanal	20	270
3-methylbutanal	51	620
diacetyl	200	596
2,3-pentandione	86	26
hexanal	115	28
methional	11	10
dimethyltrisulfide	0.15	0.4
1-octen-3-one	0.7	0.2
(Z)-1,5-octadien-3-one	0.1	0.16
(E,Z)-2,6-nonadienal	3.5	2.8
(E,E)-2,4-nonadienal	3.2	2.0
(E,E)-2,4-decadienal	3.5	2.2

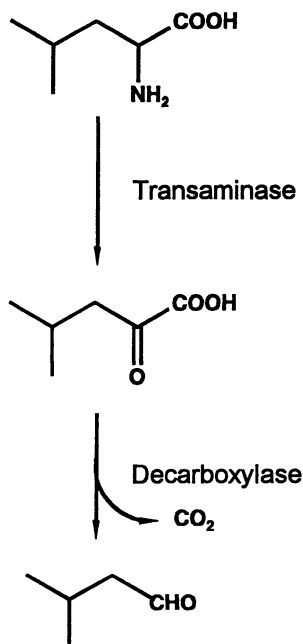
<sup>a</sup> Before boiling the cod, the raw material was stored for 14 weeks at  $-60^{\circ}\text{C}$  (A) or at  $-13^{\circ}\text{C}$  (B). Data are means of duplicate run.

Both 2- and 3-methylbutanal are well known as amino acid degradation products and may be generated either thermally (Strecker degradation) or enzymatically from isoleucine and leucine. Changes in free amino acids and their enzymatic conversion to odorants has been reported in shrimps (17). Since branched aldehydes were present





**Figure 1.** Quantification of (Z)-1,5-octadien-3-one using a deuterated (d) standard.



**Figure 2.** Enzymatic formation of 3-methylbutanal from leucine.

in equally high concentrations in the stored cod prior to cooking (unpublished data), their thermal generation could be ruled out. Figure 2 shows the formation of 3-methylbutanal from leucin in the presence of a transaminase and a decarboxylase. The content of free amino acids in cod has been previously reported (18).

**Table IV. Odour activity values for potent odorants in boiled cod and influence of storage of raw material**

Compound	n-OAV <sup>a</sup>		m-OAV <sup>a</sup>	
	A	B	A	B
acetaldehyde	130	240	130	240
methanliol	500	650	50	65
dimethylsulfide	260	83	39	13
2-methylpropanal	39	n.d.	39	n.d.
2-methylbutanal	15	208	22	386
3-methylbutanal	145	177	204	2480
diacetyl	13	0	40	119
2,3-pentandione	3	40	17	5
hexanal	26	1	11	3
methional	55	6	275	250
dimethyltrisulfide	25	50	19	50
1-octen-3-one	14	67	70	21
(Z)-1,5-octadien-3-one	83	4	250	400
(E,Z)-2,6-nonadienal	175	133	175	140
(E,E)-2,4-nonadienal	32	140	53	33
(E,E)-2,4-decadienal	16	20	70	44
		11		

<sup>a</sup>The nasal odor activity values (n-OAV) were calculated by dividing the concentration by the nasal threshold in water; the retronasal values (m-OAV) are based on retronasal thresholds in water.

In order to elucidate the role of (Z)-3-hexenal, (Z,Z)-3,6-nonadienal and (Z)-4-heptenal which were reported to cause an odor defect in boiled trout after prolonged storage (12), these compounds were quantified in cod although AEDA and GCO-H results did not indicate their contribution to the off odors in the products. There was only a slight increase (2-3 fold) for the content of these aldehydes in stored cod (Table V). Under similar storage conditions, the stored and then boiled trout showed a concentration increase of 5-fold for (Z)-4-heptenal, and 18-fold for each of (Z)-3-hexenal and (Z,Z)-3,6-nonadienal. Based on their low OAV, these unsaturated aldehydes are not regarded as significant contributors to the overall odor of cod. This might be explained by the very low fat content (0.6 %) in samples examined in this work.

It should be mentioned that highly volatile amines do not play a significant role in frozen stored cod. The content of dimethylamine (DMA) increases rapidly due to enzymatic breakdown of trimethylamine oxide in frozen fish muscle (19), but this was not detectable from the GCO-H studies. Considering the pH of fish tissue (6.6) and the rather high odor threshold of DMA (17.6 mg/l H<sub>2</sub>O) at pH 10.7 (20), the low impact of this compound on the aroma is evident. Although trimethylamine was perceived by GCO-H, it did not belong to the most important odorants in the stored fish.

**Table V. Concentration ( $\mu\text{g}/\text{kg}$ ) and OAV of aldehydes in cod, known to cause odor defects in trout**

Compound	Cod		Trout		m-OAV for cod	
	Fresh	Stored	Fresh	Stored	Fresh	Stored
(Z)-3-hexenal	1.3	4.3	1.4	24	43	143
(Z,Z)-3,6-nonadienal	1.3	2.8	1.1	22	26	84
(Z)-4-heptenal	1.6	2.8	1.1	6.0	27	47

The trout and cod samples were stored during 14 weeks at  $-13^{\circ}\text{C}$  and then boiled.

## Conclusions

A malty odor defect was observed in frozen stored cod caused by a rapid increase in 3-methylbutanal. Peroxidation products of omega unsaturated fatty acids are primarily responsible for flavor changes in frozen stored trouts, but these were unimportant to the off odor in cod which is a lean fish. A slight increase in the content of (Z)-4-heptenal was noticed upon storage, but this compound had no effect in the overall odor of stored cod.

## Literature Cited

1. Josephson, D.B.; Lindsay, R. C.; Stuiber, D.A. *J. Agric. Food Chem.* **1983**, *31*, 326-330.
2. Josephson, D.B.; Lindsay, R.C.; Stuiber, D.A. *J. Agric. Food Chem.* **1984**, *32*, 344-347.
3. Hirano, T.; Zhang, C.H.; Morishita, A.; Suzuki, T.; Shirai, T. *Nippon suisa Gakkaishi* **1992**, *58*, 547-557.
4. Maarse, H.; Visscher, C.A. Eds., *Volatile Compounds in Food. Qualitative and Quantitative Data*; TNO Biotechnology and Chemistry Institute: Zeist, The Netherlands, 1996; 7<sup>th</sup> edition.
5. Hughes, R.B. *J. Sci. Food Agric.* **1961**, *12*, 822-826.
6. Hughes, R.B. *J. Sci. Food Agric.* **1963**, *14*, 893-903.
7. Shimomura, M.; Yoshimatsu, F.; Matsumoto, F. *Kaseigaku Zasshi* **1971**, *22*, 106-112.
8. McGill, A.S.; Hardy, R.; Gunstone, F.D. *J. Sci. Food Agric.* **1977**, *28*, 200-205.

9. Jones, N. R. In *Symposium on Foods: The Chemistry and Physiology of Flavors*. Schultz HW, Day EA, Libbey L, Eds., Avi Publishing Co, Inc.: Westport, CT, 1967, 267-290.
10. Seals, R. G.; Hammond, E. G. *J. Am. Oil Chem. Soc.* **1970**, *47*, 278-280.
11. Karahadian, C.; Lindsay, R. C. *J. Am. Oil Chem. Soc.* **1989**, *66*, 953-960.
12. Milo, C.; Grosch, W. *J. Agric. Food Chem.* **1993**, *41*, 2076-2081.
13. Schieberle, P. In *Characterization of Food: Emerging Methods*, Gaonkar A. G.; Ed; Elsevier Science, Amsterdam, **1995**, 403-431.
14. Ullrich, F.; Grosch, W. *Z. Lebensm. Unters. Forsch.* **1987**, *184*, 277-282.
15. Milo, C.; Grosch, W. *J. Agric. Food Chem.* **1995**, *43*, 459-462.
16. Kasahara, K.; Nishibori, K. *Bul. Jap. Soc. Sci. Fish* **1975**, *41*, 1009-1013.
17. Pedraja, R. R. *Food Technol.* **1970**, *24*, 1355-1360.
18. Partmann, W.; Schlaszus, H. *Z. Lebensm. Unters. Forsch.* **1973**, *152*, 8-17.
19. Castell, C.H.; Neal, W.; Smith, B. *J Fish Res Bd Canada* **1970**, *27*, 1685-1690.
20. Amooe, J.E.; Forrester, L.J.; Buttery, R.G. *J. Chem. Ecol.* **1975**, *1*, 299-310.

## Chapter 12

# Manipulating the Flavor of Freshwater Crustacea Using Postharvest Seawater Acclimation

H.-J. Weng<sup>1</sup>, Keith R. Cadwallader<sup>1,3</sup>, H. H. Baek<sup>1</sup>, L. R. D'Abramo<sup>2</sup>,  
and J. A. Sullivan<sup>2</sup>

Departments of <sup>1</sup>Food Science and Technology and <sup>2</sup>Wildlife and Fisheries,  
Mississippi Agricultural and Forestry Experiment Station, Mississippi State  
University, Box 9805, Mississippi State, MS 39762-5953

The effects of seawater acclimation on the chemical and sensory properties of adult freshwater prawn (*Macrobrachium rosenbergii*) were studied. Salt and free amino nitrogen content increased steadily throughout the test period in seawater acclimated prawns. Moisture content of seawater acclimated prawns immediately decreased (<18 h) and then gradually increased during the remainder of the acclimation period. Lipid and protein content remained constant throughout the test period. No compositional changes were observed for control (freshwater) prawns during the same period. Significant ( $p < 0.05$ ) flavor differences were detected between baseline (0-time) and seawater acclimated prawns; however, sensory panelists could not differentiate among prawns acclimated for 18, 36, and 54 h. Affective testing (hedonic scale) revealed that panelists preferred seawater acclimated prawns over baseline prawns. Seawater acclimation, even for a short period of time (18 h), significantly improves the flavor of freshwater prawns.

Since about the turn of the century (ca. 1908) it has been known that amino acids impart distinct tastes to foods (1). Amino acids are especially important in the flavor of seafoods, such as boiled crab (2) and shrimp (3). The characteristic taste of boiled snow crab has been successfully reproduced using 12 components that include five amino acids (4). Even subtle differences in levels of amino acids may noticeably affect the flavor of seafoods. For example, the content of specific amino acids differs between the body and claw meat of blue crab (5) and may account for the different flavor properties of the two crabmeats (6,7).

The difference between the tastes of freshwater and saltwater crustaceans may be partially due to differences in the levels of free amino acids and nucleotides (8,9). These compounds may also influence the aroma because they serve as precursors to volatile Maillard and Strecker degradation products that are important "character-impact" aroma components in cooked saltwater crustaceans (7,10,11).

<sup>3</sup>Corresponding author

Levels of free amino acids and other water soluble components vary in aquatic invertebrates during osmoregulation (12-14). For example, levels of free amino acids increase in freshwater crawfish (*Procambarus clarkii*) muscle following acclimation to seawater and were close to those found in marine species of prawns and lobsters (15). Osmotic balance was similarly achieved by freshwater prawn, *Macrobrachium rosenbergii* (16), shrimp (13,17,18) and oyster (19). The major free amino acids involved in osmoregulation in *M. rosenbergii* were glycine, alanine, proline, and glutamate (16). As mentioned previously, these amino acids are important due to the flavor imparted to seafoods. Some researchers also have studied the role of D-amino acids in the osmoregulation of crustaceans (14,20,21). The contribution of the D-amino acids to the overall flavor of seafoods may be significant because these compounds typically impart sweet tastes to foods (22).

The temperate zones of the U.S. have the potential to produce freshwater prawns (*M. rosenbergii*). Research at Mississippi State University has led to the optimized cultural practices for freshwater prawns (23). Production of prawns has economic potential if the aquaculture facilities are already available (24). However, freshwater prawns have a relatively bland flavor and the option of modifying the flavor exists.

The importance of osmoregulation and the resulting increase in amino acids on the flavor of seafoods has been suggested by some researchers (13,17,18); however, no reports have been published regarding degree of flavor change or flavor improvement after seawater acclimation. It was our hypothesis that saltwater acclimation of freshwater prawns would lead to products with improved and/or potentiated flavor as a result of intermuscular increases in free amino acids and other osmoregulators, which serve as aroma precursors and taste-active compounds. The present study was designed to test this hypothesis 1) by measuring changes in osmoregulators, namely free amino acid nitrogen and sodium chloride, in tail meat of prawns at various time intervals post-acclimation to seawater versus freshwater, and 2) by comparing the sensory properties of seawater acclimated prawns to those of untreated controls.

## Materials & Methods

**Materials.** Adult prawns (*M. rosenbergii*) were bred and reared at the Aquaculture Research Unit of the Mississippi Agriculture and Forestry Experiment Station, Mississippi State University. Prawns were harvested from experimental earthen production ponds ranging in surface area of 0.04 to 0.05 ha during late September of 1995.

**Seawater acclimation.** The experimental design employed was based upon the goal of minimizing the holding period while still effecting a significant change in taste. This approach would minimize the logistics, labor and cost of operation of the post harvest holding facilities. Therefore, the acclimation period was limited to just over 48 h because thereafter the practice would become prohibitively expensive as the animals would probably have to be fed and water would need to be either replaced or biologically treated to maintain their viability.

After harvest prawns were stocked at a density of 4 g/L into two separate holding tanks containing fresh well water (approximately 1000 individuals averaging 30 g each). Prawns of various sizes in a presumed  $\approx 1:1$  male-female ratio were removed from both tanks to provide a baseline for sensory evaluation and chemical analyses. One tank served as freshwater control and held about 10% of the total number of prawns. The second tank held the remaining prawns and was used for seawater acclimation. The salinity of the acclimation tank was gradually increased by addition of a commercial salt mixture (Hawaiian Marine Salts). The initial salinity was made up to 10‰. At each 2 h interval the salinity was increased by 5‰ until a final concentration of 30‰ was achieved (8 h). Prawns were removed from each tank at 18, 36 and 54 h intervals after initial stocking into tanks. After sampling, prawns were immediately frozen under liquid nitrogen and stored at  $-20^{\circ}\text{C}$  until analysis.

**Chemical analysis.** For each treatment group chemical analysis were conducted on composite samples consisting of abdominal tissue (5 g subsample of entire muscle of each prawn) of five prawns. Moisture, fat, salt, and protein content were determined according to the following methods: moisture by AOAC 950.46 (25); lipid by AOAC 948.15 (25); salt by AOAC 976.25 (25); and protein by semimicro Kjeldahl method (26) which is an official method approved by the AOAC. Free amino nitrogen (FAN) was determined by the copper procedure described by Cobb et al. (27). All analyses were performed in duplicate.

**Sensory evaluation.** Prawns were prepared for sensory evaluation as follows: Frozen prawns were thawed under tap water and then deheaded. The remaining tail portions were cooked in boiling water for 8 minutes and then immediately cooled in an ice-water bath. Prawns from different treatments were cooked separately to minimize cross contamination. After peeling, each cooked tail was halved and presented to panelists in a plastic cup.

Sensory panel consisted of staff and students of the MSU Department of Food Science and Technology and had prior sensory evaluation experience. Sensory evaluation was conducted using established procedures (28). In order to determine possible flavor differences between cooked prawns post-acclimation versus freshwater, difference-from-control (DFC) and ranking tests were employed. For the DFC test, each panelist was provided with a tray containing the baseline control (labeled as control) and four coded samples representing the baseline control and 18, 36, and 54 h seawater acclimated prawns. Panelists were instructed to indicate the flavor difference between control sample and each of the coded samples using a scale that ranged from 0 (no difference) to 10 (extreme difference). For the ranking test, panelists were provided with four coded samples representing baseline and acclimated prawns and were asked to rank the samples according to their flavor intensity, where 4 = most intense and 1 = least intense.

Since significant flavor differences were detected between control and seawater acclimated prawns, sample acceptability (preference) was measured using a nine-point hedonic scale (where 9 = like extremely and 1 = dislike extremely) on four coded samples representing baseline control and acclimated prawns.

**Statistics.** Results of ranking tests were analyzed using the tables of Basker (29). Other data were analyzed by analysis of variance (ANOVA). Whenever F-values were significant, Fisher's LSD procedure or Student's t-test was used to separate means at  $p \leq 0.05$  (30).

## Results & Discussion

**Chemical changes during seawater acclimation.** The effect of seawater acclimation on the moisture content of freshwater prawn is shown in Figure 1. Initial moisture content was close to that previously reported for freshly harvested *M. rosenbergii* (31). After 18 h the seawater acclimated prawn had a significantly lower ( $p \leq 0.05$ ) moisture content than that of control. Throughout the test period no change in moisture content of control was observed. In the seawater acclimated prawns a rapid decrease in moisture initially occurred followed by a gradual increase after 18 h. Similar results were observed by Papadopoulos and Finne (18), who explained that moisture content seemed to play a role in osmoregulation of *M. rosenbergii* only during the early stages of seawater acclimation. These researchers demonstrated that during prolonged exposure to seawater moisture content would eventually revert back to normal levels regardless of the salinity level tested.

Chloride ion content was expressed on a dry weight basis to compensate for changes in moisture which occurred in the seawater acclimated prawns. The concentration of chloride ion (expressed as percent NaCl on a dry weight basis) increased steadily throughout the test period in seawater acclimated prawns, while no significant change was observed in control prawns (Figure 2). These results are in good agreement with previous reports (14,18). The possible role of inorganic ions in the regulation of haemolymph osmotic balance of *M. rosenbergii* has been described elsewhere (14).

In addition to increases in chloride ion concentration, several free amino acids are known to act as intracellular osmoregulators in *M. rosenbergii* during seawater acclimation (16,18). The major amino acids involved include glycine, alanine, proline, and glutamic acid (16). In the present study, free amino nitrogen (FAN) content increased steadily as a function of acclimation time and more than doubled by 54 h (Figure 3). Meanwhile, no change in FAN was observed for the control prawns. These results support those of other researchers (18). It has been suggested that prawns adjust to hyperosmotic conditions primarily through changes in the metabolism of haemolymph proteins and free amino acids. Subsequent osmotic equilibrium of individual cells is achieved by hydrolysis of serum proteins to amino acids which are actively transported across the cell membrane (16). However, in certain crustaceans osmotic balance may be achieved through *de novo* synthesis or regulation of free amino acids (32).

There was no difference in lipid content between control and seawater acclimated prawns (Figure 4) and lipid content remained unchanged throughout the acclimation period. Protein content of seawater acclimated prawns seemed to increase slightly during the test period (Figure 5) but this increase was not statistically significant.



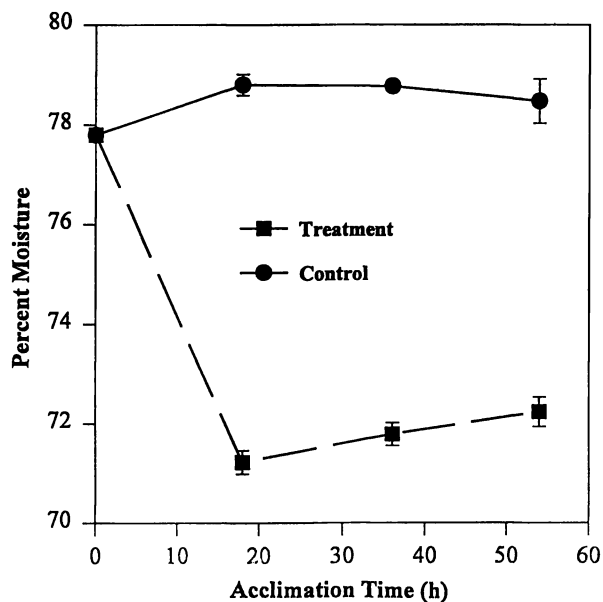


Figure 1. Moisture content of composite samples of abdominal muscle of *M. rosenbergii* as a function of duration of seawater acclimation. Values are means  $\pm$  SE ( $n = 3$ ).

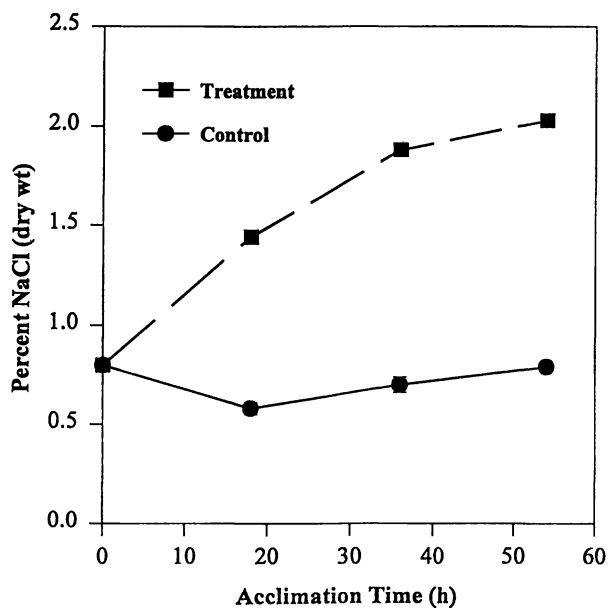


Figure 2. Sodium chloride content of composite samples of abdominal muscle of *M. rosenbergii* as a function of duration of seawater acclimation. Values are means  $\pm$  SE ( $n = 2$ ).

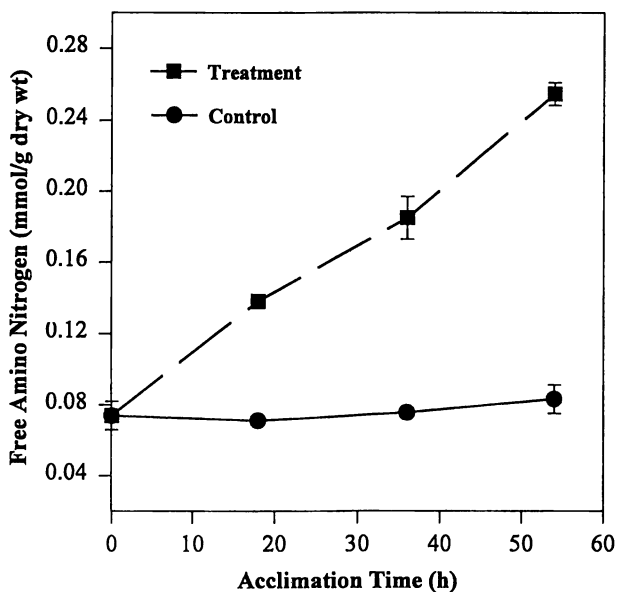


Figure 3. Change in free amino nitrogen (FAN) content of composite samples of abdominal muscle of *M. rosenbergii* as a function of duration of seawater acclimation. Values are means  $\pm$  SE ( $n = 2$ ).

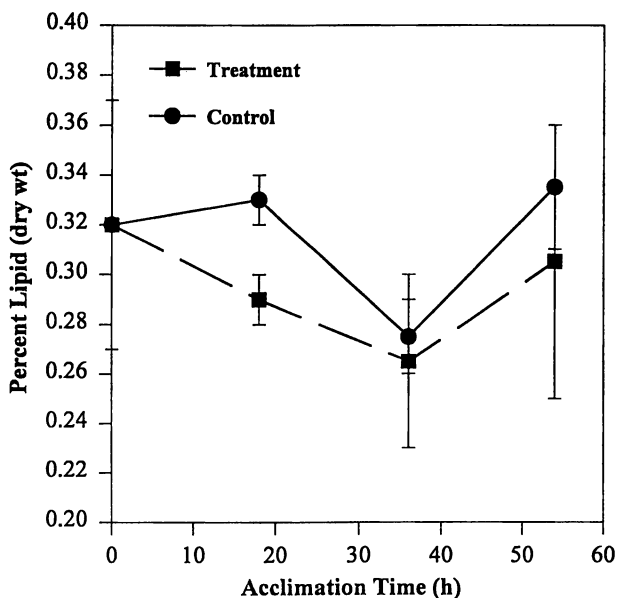


Figure 4. Lipid content of composite samples of abdominal muscle of *M. rosenbergii* as a function of duration of seawater acclimation. Values are means  $\pm$  SE ( $n = 2$ ).

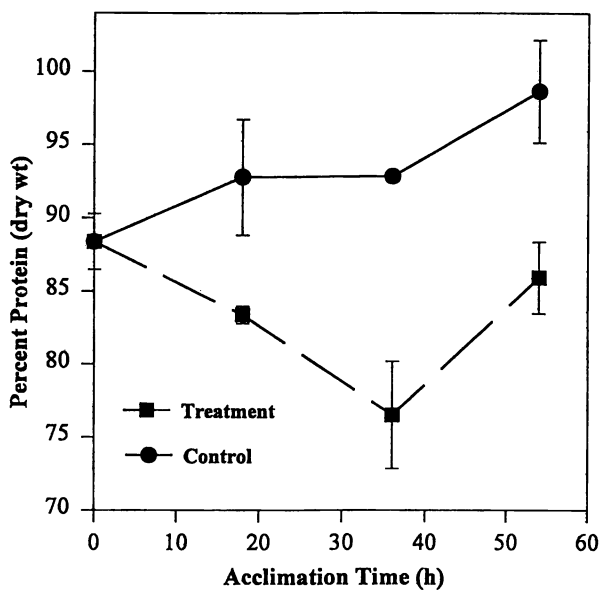


Figure 5. Protein content (as total Kjeldahl nitrogen) of composite samples of abdominal muscle of *M. rosenbergii* as a function of duration of seawater acclimation time. Values are means  $\pm$  SE ( $n = 2$ ).

**Sensory changes during seawater acclimation.** Results of sensory evaluation are provided in Table I. With the DFC test, panelists could readily detect a difference among the baseline and seawater acclimated prawns; however, panelists could not distinguish between prawns acclimated for 18, 36, and 54 h. Similar results were observed for the ranking test, with a notable exception that panelists were able to distinguish prawns acclimated for 54 h from those removed after 18 and 36 h acclimation. Evidently a significant flavor difference existed between baseline and seawater acclimated prawns; however, sample preference could not be determined using the above statistical procedures. Therefore, a preference (hedonic) test was conducted and indicated that panelists preferred seawater acclimated prawns over baseline prawns. Panelists did not indicate a preference among prawns acclimated for 18, 36, and 54 h.

The flavor change in seawater acclimated prawn is most likely the result of increased levels of NaCl and free amino acids (as FAN). The sensory techniques employed in this study were not designed to determine sample flavor attributes. Therefore, the relative impact that NaCl and free amino acids had on the flavor of seawater acclimated prawns cannot be addressed. It is known that the individual amino acids have different taste properties and intensities (22). Amino acids possessing fairly intense sweet tastes include L-alanine and L- and D-glycine. These amino acids are among those found essential for snow crab flavor (4). Glutamic acid, having a "UMAMI" taste (34), also was essential for snow crab flavor (4). Glycine content was shown to be an important component of shrimp flavor (33) and was the most abundant amino acid in boiled crab (2). Arginine, has been described as having a "flat" taste (22); however, this amino acid was reported to be important in snow crab flavor (4) and was the second most abundant amino acid in crab (2). The influence of amino acids on seafood flavor is well recognized and glycine, alanine, and arginine are common constituents of synthetic flavors used for crab analogues or surimi based products (9).

**Table I. Sensory evaluation of cooked tail meat of prawns acclimated to seawater for different periods of time**

	Seawater acclimation time (h)				MSE <sup>1</sup>
	0	18	36	54	
Difference-from-control <sup>2</sup>	1.057a <sup>3</sup>	5.486b	5.571b	5.829b	2.370
Rank sums <sup>4</sup>	42a	92b	92b	131c	(27.7) <sup>5</sup>
Hedonic <sup>2</sup>	4.514a	6.314b	6.285b	7.000b	1.610

<sup>1</sup>MSE=root mean square error; <sup>2</sup>mean scores (n = 35); <sup>3</sup>Values with different bold letters in each row are statistically different (p<0.05); <sup>4</sup>sum of rank scores (n = 35); <sup>5</sup>critical value of difference for rank sums (29).

Amino acids are not only important taste-active components in seafoods, but may serve as precursors to the many desirable "character-impact" aroma compounds generated during cooking. Hayashi and coworkers (9) studied the mechanism(s) of aroma formation in crab leg meat and found that levels of specific water extractible components, including amino acids, decreased during cooking. In addition, by enriching the extract with these components more volatile flavor compounds were produced after heating. It is well known that amino acids and reducing sugars react via Maillard reactions (35) to yield volatile heterocyclic compounds with desirable aroma properties (36). Amino acids also may react with other reductones such as alkanals (37) or lipid-derived compounds (38) to yield volatile flavor compounds. The nature of the nitrogen source (amino acid, ammonia, etc.) affects the types and levels of aroma compounds formed during heating (39). Sulfur amino acids may react to form potent sulfur-containing aroma compounds. For example, 3-(methylthio)propanal (methional), derived from methionine during cooking, is an important "character-impact" flavor component of both cooked crab (7,11) and spiny lobster (10). Other thermally generated heterocyclic nitrogen- and sulfur-containing compounds that are derived from amino acids make significant contributions to the flavor of cooked freshwater prawns (40).

## Conclusion

The results of this study demonstrate that *M. rosenbergii* responds to changes in salinity by a combination of mechanisms that include increases in osmoregulators such as chloride ions and free amino acids as well as a decrease in moisture content. Sensory evaluation further indicated that these physiological changes resulted in a significant preferred change in flavor.

## Acknowledgements

Approved for publication by the Mississippi Agricultural and Forestry Experiment Station as manuscript No. PS-9007. Partial support for this study was provided by the Mississippi Agricultural and Forestry Experiment Station under project MIS-0855.

## Literature Cited

1. Kato, H.; Rhue, M.R.; Nishimura, T. In *Flavor Chemistry: Trends and Developments*. Teranishi, R., Buttery, R., and Shahidi, F., Eds.; ACS Symposium Series 388; American Chemical Society: Washington, DC; 1989, pp 158-174.
2. Konosu, S.; Yamaguchi, K.; Hayashi, T. *Bull. Japan. Soc. Sci. Fish.* **1978**, *44*, 505-510.
3. Chung, S.-Y.; Lee, E.-H. *Bull. Korean Fish. Soc.* **1976**, *9*, 79-110.
4. Hayashi, T.; Yamaguchi, K.; Konosu, S. *J. Food Sci.* **1981**, *46*, 479-483,493.
5. Thompson, M.H.; Farragut, R.N. *Comp. Biochem. Physiol.* **1966**, *17*, 1065-1078.
6. George, C.; Gopakumar, K. *Fish. Technol.* **1987**, *24*, 57-61.

7. Chung, H.Y.; Chen, F.; Cadwallader, K.R. *J. Food Sci.* **1995**, *60*, 289-291,299.
8. Jones, N.R. *J. Agric. Food Chem.* **1969**, *17*, 712-716.
9. Hayashi, T.; Ishii, H.; Shinohara, A. *Food Rev. Internat.* **1990**, *6*, 521-536.
10. Cadwallader, K.R.; Tan, Q.; Chen, F.; Meyers, S.P. *J. Agric. Food Chem.* **1995**, *43*, 2432-2437.
11. Chung, H.Y.; Cadwallader, K.R. *J. Agric. Food Chem.* **1994**, *42*, 2867-2870.
12. Cobb, B.F. III; Conte, F.S.; Edwards, M.A. *J. Agric. Food Chem.* **1975**, *23*, 1172-1174.
13. McCoid, V.; Miget, R.; Finne, G. *J. Food Sci.* **1984**, *49*, 327-330,355.
14. Funge-Smith, S.J.; Taylor, A.C.; Whitley, J.; Brown, J.H. *Comp. Biochem. Physiol.* **1995**, *110A*, 357-365.
15. Okuma, E.; Abe, H. *Comp. Biochem. Physiol.* **1994**, *109A*, 191-197.
16. Tan, C.H.; Choong, K.Y. *Comp. Biochem. Physiol.* **1981**, *70A*, 485-489.
17. Dalla Via, G.J. *Aquaculture* **1986**, *55*, 307-316.
18. Papadopoulos, L.; Finne, G. *J. Agric. Food Chem.* **1985**, *33*, 1174-1177.
19. Powell, E.N.; Kasschau, M.; Chen, E.; Koenig, M.; Pecon, J. *Comp. Biochem. Physiol.* **1982**, *71A*, 591-598.
20. D'Aniello, A.; Giuditta, A. *Comp. Biochem. Physiol.* **1980**, *66B*, 319-322.
21. Preston, R.L. *Comp. Biochem. Physiol.* **1987**, *87B*, 55-62.
22. Solms, J. *J. Agric. Food Chem.* **1969**, *17*, 686-688.
23. D'Abramo, L.R.; Daniels, W.H.; Fondren, M.W.; Brunsen, M.W. Management Practices for Culture of Freshwater Prawn (*Macrobrachium rosenbergii*) in Temperate Climates. Mississippi Agricultural and Forestry Experiment Station Bull. 1030. 1995.
24. Bauer, L.L.; Sandifer, P.A.; Smith, T.I.; Jenkins, W.E. *Aquac. Engineering* **1983**, *2*, 181.
25. AOAC. *Official Methods of Analysis*, 15th ed; Association of Official Analytical Chemists: Washington, DC; 1990.
26. Hambleton, L.G.; Noel, R.J. *J. Assoc. Anal. Chem.* **1975**, 143-145.
27. Cobb, B.F. III; Alaniz, I.; Thompson Jr., C.A. *J. Food Sci.* **1973**, *38*, 431-436.
28. Meilgaard, M.; Civille, G.V.; Carr, B.T. *Sensory Evaluation Techniques*, 2nd Ed. CRC Press, Inc.: Boca Raton, FL; 1991.
29. Basker, D. *Food Technol.*, **1988**, *42(2)*, 79-84.
30. *SAS User's Guide: Statistics*. Version 6 Edn. SAS Institute, Inc.: Cary, NC, 1987.
31. Angel, S.; Basker, D.; Kanner, J.; Juven, B.J. *J. Food Tech.* **1981**, *16*, 357.
32. Schoffeniels, E.; Gilles, R. In *Chemical Zoology*, Vol 1. Florkin, M. and Scheer, B.T. Eds. Academic Press: New York; 1970, pp. 255-286.
33. Simudu, W.; Huijita, M. *Bull. Japan. Soc. Fish.* **1954**, *20*, 720.
34. Kotani, K.; Toyota, T.; Yasumatsu, K. In *Off-Flavors in Foods and Beverages*. Charalambous, G. Ed. Elsevier: New York; 1992, pp 523-546.
35. Mills, F.D.; Baker, B.G.; Hodge, J.E. *J. Agric. Food Chem.* **1969**, *4*, 723-727.
36. Maga, J.A. *CRC Crit. Rev. Food Sci. Nutr.* **1982**, *16*, 1-115.
37. Pokorny, J.; Luân, N.-T.; Kondratenko, S.S.; Janicek, G. *Die Nahrung* **1976**, *20*, 267-272.

38. Ho, C.-T.; Bruechert, L.J.; Zhang, Y.; Chiu, E.-M. In *Thermal Generation of Aromas*. Parliment, T.H. and McGorin, R.J. (Eds.). ACS Symposium Series 409, American Chemical Society: Washington, D.C.; 1989, pp 105-113.
39. Wong, J.M.; Bernhard, R.A. *J. Agric. Food Chem.* **1988**, *36*, 123-129.
40. Cadwallader, K.R.; Back, H.H.; Chang-Lee, G.H. IFT Annual Meeting, **1996**, abstract no. 80D-20.

## Chapter 13

# Aroma-Active Compounds in Salt-Fermented Anchovy

Y. J. Cha<sup>1</sup>, G. H. Lee<sup>2</sup>, and Keith R. Cadwallader<sup>2,3</sup>

<sup>1</sup>Department of Food Science and Nutrition, Changwon National University, Changwon, 641-773, Korea

<sup>2</sup>Department of Food Science and Technology, Mississippi Agricultural and Forestry Experiment Station, Mississippi State University, Box 9805, Mississippi State, MS 39762-5953

Volatile flavor compounds in salt-fermented anchovy with (koji) and without (control) added koji were analyzed by simultaneous steam distillation-solvent extraction/gas chromatography/mass spectrometry and gas chromatography/olfactometry. Ninety-eight volatile compounds were detected in control and 96 in koji. These included 59 odor-active compounds, such as 16 aldehydes, 8 esters, 4 ketones, 1 sulfur, 1 alcohol, 1 acid and 28 unknowns. Aldehydes and esters were found in the highest abundance in both samples. Alkylpyrazines (5) were found in koji only. Furthermore, the alcohols 3-methyl-1-butanol, 1-octen-3-ol and 2-phenylethanol were generally at higher levels in koji than in control. Based on odor intensity and odor values of volatile compounds in both samples, the most potent odorants were 1-octen-3-one (mushroom, earthy), (Z)-4-heptenal (rancid, boiled potato), (E,Z)-2,6-nonadienal (cucumber, melon), 3-methylbutanal (dark chocolate), 3-(methylthio)propanal (nutty, baked potato), ethyl 2-methylbutanoate (fruity, ripe apple), and ethyl 3-methylbutanoate (fruity, green apple). Other odorants, such as 1-penten-3-one (plastic bottle) and two unknowns (RI 1092 and RT 5.1 min), were more intense in control than koji treated samples.

Salt-fermented fish, having a unique and desirable aroma and taste, has been favored by Koreans for centuries. These products are important protein and nitrogen sources because they contain about 10% (w/w) nitrogen, of which 80% is in the form of amino acids (1). The characteristic aroma and taste of these products are primarily formed during fermentation through protein and lipid degradation by autolytic and bacterial enzymes (2). Fermented fish products have been consumed as a condiment or as a seasoning added during the manufacture of Kimchi, a typical Korean pickled vegetable. Most fermented fish contain 25-30% salt and are matured for over 6 months by traditional methods. Economically, it would be more advantageous if fermentation time could be shortened

<sup>3</sup>Corresponding author



without causing undesirable spoilage or off-flavors. Furthermore, the common ripening agent in fermented fish products, sodium chloride, is known to be one factor that increases the potential of hypertension and renal disease (3). High salt content has effectively lessened the intake of fermented fish products (4,5). Therefore, attempts to reduce salt content and to reduce fermentation time of fermented fish products have been made (5-8). In certain areas of Korea addition of malt powder or cooked cereals to fermented fish is thought to enhance its flavor quality (1). Kunimoto et al. (9) reported that *Aspergillus oryzae*, which is useful in making koji, reduced fishy odor. Application of koji to make sardine meal was attempted by Kim et al. (10).

As a consequence of consumer demands for natural seasonings rather than synthetic flavoring agents in processed foods, the Korean seafood industry has recently focused on developing natural seasoning agents from traditional salt-fermented fish products. This trend has in turn initiated additional studies of fermented fish products to improve their flavor quality. However, except for a few reports (11-13), the volatile flavor of fermented fish has not been fully investigated. An investigation of volatile flavor is essential if modified fermented fish products are to be successfully developed.

The objective of this study was to identify and to compare odor-active components in low salt-fermented anchovy with (koji) and without (control) added koji during fermentation.

## Materials & Methods

**Sample Preparation.** Fresh anchovy (*Engraulis japonica*) was purchased from a fish market in Chungmu, Korea and transported on ice in polyethylene bags to the laboratory within 1 h. A medium for koji production was prepared with 1:1:0.3 ratio (w/w) of boiled soybean, roasted barley and powdered dried anchovy, respectively. Fifty g of sterilized mixture (121°C, 15 min) was inoculated with a culture of *Aspergillus oryzae* var. *oryzae* Murakami [made from about 0.1g freeze dried *A. oryzae* (American Type Culture Collection, ATCC No. 22788) dissolved into 0.1 mL of sterilized saline water] and then incubated at 25°C for 3 days until mold growth was observed throughout the material. This procedure was repeated several times to attain the required koji feedstock. Salt-fermented anchovy was prepared as follows: control was produced by adding various additives to fresh anchovy, namely, 15% (w/w) salt, 6% (w/w) sorbitol, 0.5% (v/w) lactic acid and 5% (v/w) ethanol as preservatives and flavor enhancers; and koji was prepared as control except 5% (w/w) glucose and 10% (w/w) koji were added. These samples were ripened at 30°C for up to 4 weeks. Samples were homogenized using a Waring blender (Waring Products Co., Winsted, CT) before flavor analysis.

**Simultaneous steam distillation-solvent extraction (SDE).** Homogenized fish paste (500g), distilled water (1.5 L) and 90.8µg of internal standard 2,4,6-trimethylpyridine (TMP; Aldrich Chemical Co., Milwaukee, WI) were placed in a Lickens-Nickerson (14) type SDE apparatus (Cat. No. K-523010-0000, Kontes, Vineland, NJ) to extract volatile flavor compounds into redistilled diethyl ether (100 mL). The procedure has been described elsewhere (15). Extracts were concentrated to 1.5 mL under a gentle stream of nitrogen. Each sample was extracted in duplicate.

**Gas chromatography/mass spectrometry (GC/MS).** A 4 $\mu$ L aliquot from each SDE extract was injected into an HP 5790GC/5970B mass selective detector (MSD) (Hewlett Packard Co., Palo Alto, CA) by splitless mode (155°C injector temperature; 30s valve delay). Separation of volatile components was achieved on a fused silica open tubular (FSOT) column (Supelcowax 10; 60 m long x 0.25 mm i.d. x 0.25 $\mu$ m d; Supelco Inc., Bellefonte, PA). The linear velocity of the helium carrier gas was 25.7 cm/s. Oven temperature was programmed from 40°C to 175°C at a rate of 2°C/min with initial and final hold times at 5 and 30 min, respectively, then further increased to 195°C at a rate of 5°C and maintained for 25 min. Electron ionization energy was 70 eV, mass range was 33-300 a.m.u., electron multiplier voltage was 2000 V, and scan rate was 1.6/s. Other details of GC/MSD procedure have been described elsewhere (16). Duplicate analyses were performed on each SDE extract.

**Gas chromatography/olfactometry (GC/O).** GC/O system consisted of a Varian series 3300 GC (Varian, Walnut Creek, CA) equipped with a flame ionization detector (FID) and a sniffing port. One  $\mu$ L of each extract was injected (splitless mode) into a 30 m x 0.32 mm i.d. x 0.25 $\mu$ m d<sub>r</sub> Supelcowax 10 column. Effluent from the end of the GC column was split 1:1 between FID and sniffing port. Further details of procedure have been reported elsewhere (17). Oven temperature was programmed from 40°C to 200°C at a rate of 6°C/min with initial and final hold times at 5 and 30 min, respectively. FID temperature was 250°C. Injector, sniffer port, and transfer line temperatures were maintained at 200°C. GC/O was performed on each extract by two trained panelists. Panelists were asked to assign odor properties and rate odor intensity of each compound using an 8-point scale (where 0 = no odor detected, 7 = very strong).

**Compound identification.** Volatile compounds were identified by matching retention indices (RI) (18) and mass spectra of samples with those of authentic standards (Aldrich Chemical Co.). Tentative identifications were based on standard MS library data (Hewlett-Packard Co, 1988). The relative abundance of each compound was expressed by the ratio of its total ion peak area to that of the internal standard.

## Results & Discussion

Volatile flavor components and odor intensity in salt-fermented anchovies made with (koji) and without (control) added koji were examined during fermentation. One hundred and fifteen volatile compounds were detected (Table I), including 34 aldehydes, 11 ketones, 19 alcohols, 24 esters, 6 nitrogen-containing compounds, 5 aromatic hydrocarbons, and 16 miscellaneous compounds (88 compounds were positively identified). As shown in Table I, 98 compounds were detected in control and 96 compounds in koji. Fifty-nine odor-active compounds, including 16 aldehydes, 8 esters, 4 ketones, 1 sulfur, 1 alcohol, 1 acid and 28 unknown compounds, were detected in both samples. Identification and odor description of these compounds are presented in Table II.

Thirty-four aldehydes were identified in the control and koji (Table I). Levels of aldehydes increased with fermentation time in both koji and control. The aldehydes, 3-methylbutanal, hexanal, heptanal, (Z)-4-heptenal, (E,E)-2,4-heptadienal,

Table I. Changes in volatile flavor components in salt-fermented anchovy with (Koji) and without (control) koji during storage at 30 °C

No.	Compound name by class	RI <sup>a</sup> (RT)	Control						Koji						
			0 day		13 day		30 day		0 day		13 day		30 day		
			MAR <sup>b</sup>	S.D. <sup>c</sup>	MAR	S.D.	MAR	S.D.	MAR	S.D.	MAR	S.D.	MAR	S.D.	
<b>Aldehydes</b>															
1	2-Methylpropanal	5.3min	3.42	1.93	2.63	1.62	4.50	1.52	1.50	1.52	1.52	4.42	2.20	4.75	3.30
4	Butanal	858	9.98	7.22	6.26	3.46		6.59	3.08			2.50	1.29	5.00	1.15
7	2-Methylbutanal	897	3.13	2.25	12.70	8.06									
8	3-Methylbutanal	900	11.63	8.73	71.03	33.94	33.06	19.14	14.46	19.08	34.54	38.51	96.00	45.14	
13	Pentanal	971			12.82	4.44	4.00	2.04						3.75	0.50
19	(E)-2-Butenal	1043			3.65	2.96	1.06	0.31							
23	Hexanal	1078	18.00	8.55	82.86	28.10	12.44	6.85	10.90	13.10	9.25	5.85	15.00	4.76	
24	2-Methyl-(E)-2-butenal	1095			4.76	1.83								3.25	0.50
26	(E)-2-Pentenal	1125	2.75	1.04	16.13	5.07	4.00	1.83	1.70	1.36	3.67	3.62	4.50	0.58	
31	2-Methyl-(E)-2-pentenal	1155					0.88	0.25							
35	Heptanal	1181	4.25	2.02	20.66	4.26	2.31	1.28	2.58	1.65	1.96	0.75	2.75	0.96	
37	(E)-2-Hexenal	1214	6.25	3.18	21.65	7.10	6.50	1.73	3.51	2.13	3.83	1.45	4.50	1.00	
41	(Z)-4-Heptenal	1240	4.13	1.93	6.96	2.63	2.06	1.36	1.73	0.79	1.33	0.47	2.25	0.50	
43	Octanal	1287	0.74	0.88	7.65	3.46	1.44	1.05	0.46	0.39	1.21	0.63	1.25	0.50	
48	(E)-2-Heptenal	1323	0.88	0.25	4.15	2.75	1.94	2.05	0.84	0.32	1.04	0.67	1.00	0.00	
56	Nonanal	1392			9.90	4.37									
58	(E,E)-2,4-Hexadienal	1410	0.38	0.13	1.25	0.50	1.75	0.96	1.17	1.10	4.25	2.99	2.50	1.74	
59	(E)-2-Octenal	1429	2.38	1.49	4.84	2.30	2.13	1.44	1.87	0.34	2.92	2.17	3.25	0.96	
63	3-(Methylthio)propanal	1453	0.85	0.52	2.00	1.15	3.00	2.37	2.52	1.54	5.67	3.74	10.00	3.74	
66	2-Furancarboxaldehyde	1466	1.88	0.63	4.68	2.55	2.50	1.78	2.24	0.52	12.58	4.40	18.50	3.00	
69	(E,E)-2,4-Heptadienal	1493	16.50	10.78	17.78	12.12	14.56	5.32	4.63	1.28	7.54	4.77	13.00	4.83	
71	Benzaldehyde	1523			21.11	7.10	20.25	6.45	3.38	1.28	15.04	9.64	12.75	9.03	
74	(E)-2-Nonenal	1540	2.00	1.15	18.18	6.31	3.06	0.97	4.21	2.03	2.63	1.11	3.50	1.00	

Continued on next page

Table I. Continued

No.	Compound name by class	RI <sup>a</sup> (RT)	Control						Koji						
			0 day		13 day		30 day		0 day		13 day		30 day		
			MAR <sup>b</sup>	S.D. <sup>c</sup>	MAR	S.D.	MAR	S.D.	MAR	S.D.	MAR	S.D.	MAR	S.D.	
77	(E,Z)-2,6-Nonadienal	1582	6.00	2.71	17.93	7.31	2.44	7.69	2.44	2.05	1.41	3.96	2.06	6.00	0.00
78	(E,E)-2,4-Octadienal <sup>e</sup>	1586	3.00	1.78	7.34	2.53	4.31	1.21	1.39	0.71	2.42	1.26	3.75	1.26	
84	(E)-2-Decenal	1639	2.50	1.00	17.65	5.39	1.88	0.63							
85	Phenylacetaldehyde	1642	0.99	0.62	7.34	2.01	6.25	3.23	2.09	0.81	7.00	1.83	8.50	0.58	
93	2-Undecenal <sup>e</sup>	1746	1.63	1.11	12.13	4.25	0.94	0.13	1.14	0.50	2.00	0.82	1.50	0.58	
95	(E,E)-2,4-Decadienal	1806	4.25	2.60	11.91	4.06	4.69	1.38	2.55	1.77	3.96	2.06	6.50	1.29	
96	2-Chlorobenzaldehyde <sup>e</sup>	1813							0.64	0.49	1.58	0.50	1.50	0.58	
103	Tetradecanal <sup>e</sup>	1918	31.75	31.84	11.53	8.53	4.94	2.07	6.03	3.00	12.29	2.58	11.75	3.30	
104	$\alpha$ -Ethylidenephenylacetaldehyde <sup>e</sup>	1928							1.08	0.94					
108	Pentadecanal <sup>e</sup>	2021	7.00	3.83	3.38	1.61	1.00	0.00					1.50	0.58	
110	Hexadecanal <sup>e</sup>	2128	145.75	40.15	86.87	73.16	24.94	14.14	14.19	5.22	38.96	15.37	45.25	26.00	
	<b>Ketones</b>														
12	2,3-Butanedione	961	4.13	1.89					2.05	1.02	4.50	4.04	3.75	0.50	
15	1-Penten-3-one	1015	0.39	0.41	3.50	3.10									
21	2,3-Pentanedione	1057	0.45	0.39											
33	2-Heptanone	1177							0.87	0.46					
44	1-Octen-3-one	1296	10.81	6.63	11.75	4.31	3.52	1.74	10.42	8.74	14.92	13.06	32.50	13.38	
55	2-Nonanone	1389	2.38	0.95	6.53	2.99	1.50	0.58	1.52	0.42	1.33	0.47	1.75	0.50	
72	1-(2-Furanyl)-ethanone <sup>e</sup>	1534					6.13	10.59							
76	(E,E)-3,5-Octadien-2-one <sup>e</sup>	1567	3.88	1.93	6.98	2.52	3.31	0.85	1.74	0.98	1.33	0.47	3.00	0.82	
79	2-Undecanone	1593	2.25	0.29	5.62	0.87	1.44	1.05	1.34	0.55	2.25	1.26	2.00	0.82	
98	Geranylacetone	1850							0.71	0.20	2.13	1.31	2.50	1.29	
107	Pentadecanone <sup>e</sup>	2012	3.13	3.97					0.51	0.16	2.13	1.31	1.00	0.00	

Continued on next page

Table I. Continued

No.	Compound name by class	RI <sup>a</sup> (RT)	Control						Koji								
			0 day		13 day		30 day		0 day		13 day		30 day				
			MAR <sup>b</sup>	S.D. <sup>c</sup>	MAR	S.D.	MAR	S.D.	MAR	S.D.	MAR	S.D.	MAR	S.D.			
<b>Alcohols</b>																	
30	Butanol	1139															
32	1-Penten-3-ol	1156	27.00	12.94	76.74	16.49	31.25	10.50	0.49	0.16	15.00	6.22	21.50	1.73			
36	3-Methyl-1-butanol	1204			3.63	2.30	0.88	0.25	14.54	10.97	14.54	4.86	23.00	6.27			
42	Pentanol	1246	1.38	0.48	4.83	3.54	1.19	0.55	6.43	4.46	1.25	0.50	2.25	1.89			
45	(Z)-2-Penten-1-ol	1310	2	0.82	1.28	0.53	1.69	1.03	0.81	0.36							
46	(E)-2-Penten-1-ol	1318	5.75	3.69	6.21	1.81	2.44	1.48	2.54	1.18							
51	Hexanol	1351							1.30	0.57	1.21	0.25	1.50	0.58			
61	1-Octen-3-ol	1447	5.75	2.72	11.44	3.44	5.88	2.32	5.15	0.73	9.50	3.70	14.25	3.86			
64	Heptanol	1454			2.79	0.85	3.50	2.89	1.75	0.50	2.08	1.07	4.25	3.86			
68	2-Ethyl-1-hexanol	1484	8.25	5.68	7.93	2.45	4.63	2.50	2.83	0.67	5.67	3.68	6.75	2.06			
75	Octanol	1552					0.88	0.25									
81	(E)-2-Octen-1-ol	1608											1.00	0.00			
86	2-Furanmethanol	1656	11.75	5.27	1.80	0.40	6.81	2.41	2.24	1.92	29.46	13.48	49.50	6.19			
92	Decanol	1744	4.25	3.84	1.28	0.53	1.13	0.25	0.52	0.23	1.58	0.96	1.75	0.50			
99	Benzylalcohol	1874	1.00	0.41	1.70	1.01	1.69	1.55	0.48	0.43							
102	2-Phenylethanol	1907							0.39	0.09	1.33	0.47	1.25	0.50			
106	Dodecanol	1971	2.5	1.08													
113	Pentadecanol	2299					2.50	3.00									
115	Hexadecanol	2376					5.00	2.97									
<b>Esters</b>																	
5	Ethyl acetate	867	358.75	287.26	59.93	47.85	219.56	140.22	125.51	108.38	302.88	172.30	313.00	121.58			
10	Ethyl propanoate	951	3.16	4.09	9.50	7.32	8.25	2.50	8.23	2.50	4.50	3.57	15.50	9.85			
11	Ethyl 2-methyl propanoate	956							3.48	2.18	30.79	20.99	39.50	5.69			

Continued on next page

Table I. Continued

No.	Compound name by class	RI <sup>a</sup> (RT)	Control						Koji					
			0 day		13 day		30 day		0 day		13 day		30 day	
			MAR <sup>b</sup>	S.D. <sup>c</sup>	MAR	S.D.	MAR	S.D.	MAR	S.D.	MAR	S.D.	MAR	S.D.
17	Ethyl butanoate	1031	2.21	2.00	4.78	2.34	2.19	2.54	2.82	1.50	15.38	3.82	40.50	11.56
20	Ethyl 2-methyl butanoate <sup>e</sup>	1047	2.53	1.19	3.00	1.37	3.75	2.22	3.80	2.58	7.45	2.63	19.50	4.19
22	Ethyl 3-methyl butanoate <sup>e</sup>	1062	8.07	4.63	10.38	6.97	4.25	3.86	9.75	7.51	4.33	2.18	10.10	1.93
27	Ethyl pentanoate	1130	2.13	2.07	2.50	1.50	1.44	0.72	1.65	1.20	2.88	1.55	2.25	0.50
40	Ethyl hexanoate	1230	1.63	0.75	10.02	3.32	4.50	3.54	1.59	0.47	4.46	3.52	11.25	7.27
49	Ethyl heptanoate	1333	1.00	0.41	1.84	1.09	1.31	0.47	1.03	0.53	1.46	0.42	2.25	0.50
50	Ethyl 2-hydroxypropanoate <sup>e</sup>	1344	102.00	50.73	22.73	7.35	48.94	36.96	29.73	19.22	33.38	23.61	28.75	4.57
60	Ethyl octanoate	1435	1.63	0.75	4.68	2.55	2.31	1.60	3.66	0.46	7.00	2.71	11.25	0.96
73	Ethyl nonanoate	1535	2.00	1.58			4.56	6.97	1.18	0.56	1.58	0.50	2.75	0.96
83	Ethyl decanoate	1633	3.75	1.19	3.14	1.76	3.69	0.99	3.03	1.92	8.42	5.10	12.25	2.06
88	Ethyl 3-(2-furyl)propanoate <sup>e</sup>	1674					11.19	2.64						
91	Ethyl undecanoate <sup>e</sup>	1736	37.88	65.12	3.21	1.31	1.31	0.47	1.28	0.71	5.88	3.12	4.50	0.58
94	Ethyl phenylacetate <sup>e</sup>	1784	9.00	4.53	24.87	8.88	6.06	1.88	6.70	4.09	38.46	18.39	70.75	11.70
97	Ethyl dodecanoate <sup>e</sup>	1840	75.63	54.26	101.78	32.21	69.50	33.76	23.54	6.76	121.58	49.48	146.00	31.84
100	Methyl 2,8-dimethylundecanoate <sup>e</sup>	1892							0.88	0.65	4.96	2.08	4.25	3.20
105	Ethyl tridecanoate <sup>e</sup>	1939	10.00	7.93	11.73	5.72	7.75	2.87	3.54	1.46	15.83	6.69	12.75	9.46
109	Ethyl tetradecanoate <sup>e</sup>	2049	252.75	282.57	1360.67	411.40	653.63	360.60	221.38	75.72	643.71	274.33	1194.25	363.60
111	Ethyl pentadecanoate <sup>e</sup>	2143	48.75	16.46	115.58	79.22	32.13	9.75	14.13	5.80	58.04	12.49	85.50	53.31
112	Ethyl hexadecanoate <sup>e</sup>	2255	104.63	72.35	1432.64	700.63	326.88	271.93	219.30	77.94	469.42	172.43	215.75	60.29
114	Ethyl heptadecanoate <sup>e</sup>	2357	19.13	11.01	16.70	10.68	11.69	9.13	2.24	0.62	16.38	9.91	21.50	3.70
116	Ethyl octadecanoate <sup>e</sup>	2456	29.00	13.34	35.83	35.06	22.56	6.36	4.69	2.46	25.54	12.85	62.00	41.86

Continued on next page

Table I. Continued

No.	Compound name by class	RI <sup>a</sup> (RT)	Control						Koji									
			0 day		13 day		30 day		0 day		13 day		30 day					
			MAR <sup>b</sup>	S.D. <sup>c</sup>	MAR	S.D.	MAR	S.D.	MAR	S.D.	MAR	S.D.	MAR	S.D.				
<b>N-Containing compounds</b>																		
38	2,4-Dimethylpyridine <sup>e</sup>	1216			5.70	3.16												
47	2,6-Dimethylpyrazine	1320																
52	2,4,6-Trimethylpyridine (i.s.) <sup>d</sup>	1363	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00
54	2-Ethyl-6-methylpyrazine	1384																
57	Trimethylpyrazine	1400																
65	2-Ethyl-3,5-dimethylpyrazine	1461																
67	Tetramethylpyrazine	1471																
<b>Aromatic hydrocarbons</b>																		
18	Toluene	1037			13.31	4.80	3.81	2.19	3.81	2.19	3.81	2.19	3.81	2.19	3.81	2.19	3.81	2.19
25	Ethylbenzene	1119			2.20	1.05	1.19	0.55	1.19	0.55	1.19	0.55	1.19	0.55	1.19	0.55	1.19	0.55
28	<i>p</i> -Xylene	1132			2.61	1.34	1.50	0.71	1.50	0.71	1.50	0.71	1.50	0.71	1.50	0.71	1.50	0.71
29	<i>m</i> -Xylene	1134			4.58	2.73												
34	<i>o</i> -Xylene	1178			2.77	1.70	1.69	1.55	1.69	1.55	1.69	1.55	1.69	1.55	1.69	1.55	1.69	1.55
<b>Miscellaneous compounds</b>																		
2	Octane	812			48.79	23.68	4.00	2.42	4.00	2.42	4.00	2.42	4.00	2.42	4.00	2.42	4.00	2.42
3	1,2-Dimethylcyclohexane <sup>e</sup>	827			4.49	2.48	9.44	10.56	9.44	10.56	9.44	10.56	9.44	10.56	9.44	10.56	9.44	10.56
6	Nonane	891			15.83	14.53	9.06	6.04	9.06	6.04	9.06	6.04	9.06	6.04	9.06	6.04	9.06	6.04
9	2-Ethylfuran	940			130.75	51.17												
14	Decane	1002			5.43	2.03	3.19	1.97	3.19	1.97	3.19	1.97	3.19	1.97	3.19	1.97	3.19	1.97
16	2-Propylfuran	1024			9.69	3.30	1.06	0.13	1.06	0.13	1.06	0.13	1.06	0.13	1.06	0.13	1.06	0.13
39	2-Pentylfuran	1227	2.25	1.19	26.83	9.29	5.69	2.17	5.69	2.17	5.69	2.17	5.69	2.17	5.69	2.17	5.69	2.17
53	2,4,5-Trimethylthiazole	1374	0.46	0.45	0.88	0.25	1.00	0.00	0.38	0.18	0.83	0.33	1.00	0.00	0.38	0.18	0.83	0.33

Continued on next page

Table I. Continued

No.	Compound name by class	R <sub>t</sub> <sup>a</sup> (RT)	Control						Koji					
			0 day		13 day		30 day		0 day		13 day		30 day	
			MAR <sup>b</sup>	S.D. <sup>c</sup>	MAR	S.D.	MAR	S.D.	MAR	S.D.	MAR	S.D.	MAR	S.D.
62	Acetic acid	1449	0.85	0.65			3.75	2.06			3.66	3.15	4.00	1.41
70	Pentadecane	1501	40.38	27.90	3.32	1.49	16.50	9.98			7.09	2.26	12.42	5.76
80	Hexadecane	1598	2.50	1.47	7.54	2.57	2.44	1.78			0.99	0.47	3.96	5.37
82	3,5-Dimethyl-1,2,4-trithiolane <sup>e</sup>	1618									0.50	0.36		
87	2,6,10,14-Tetramethylpentadecane	1667	9.38	5.56	2.84	1.58	4.31	4.03			2.67	1.36	10.71	6.30
89	Heptadecane	1698	84.38	73.00	538.23	166.50	294.75	164.65			59.13	18.86	282.08	146.54
90	Heptadecene	1717					13.00	7.97						
101	Nonadecane	1899									0.46	0.25	1.83	1.45
														1.00
														0.00

<sup>a</sup> R<sub>t</sub>(RT) = retention index (retention time)<sup>b</sup> MAR = mean area ratio; compound peak area/I.S. peak area from the average of 2 SDE extractions, and 2 injections of each extract.<sup>c</sup> S.D. = Standard deviation of mean area ratio.<sup>d</sup> I.S. = internal standard.<sup>e</sup> Compound tentatively identified by MS data only.

SOURCE: Reprinted with permission from ref. 33. Copyright 1994



(E,Z)-2,6-nonadienal, and (E,E)-2,4-decadienal were found in high abundance in both samples during fermentation. All of these aldehydes, except for heptanal and (E,E)-2,4-heptadienal, had high odor intensities in both control and koji. In particular, (Z)-4-heptenal and (E,Z)-2,6-nonadienal had the highest odor intensities. (E,Z)-2,6-Nonadienal, having a desirable sweet, cucumber, and melon-like aroma, has been shown to be derived from omega-3 fatty acids (19) and can be readily converted to (Z)-4-heptenal through the Retro-Aldol degradation reaction (20). Triqui and Reineccius (13) reported that two aldehydes, (E,Z)-2,6-nonadienal and (Z)-4-heptenal, contributed cucumber-like, fatty-fishy odors to the flavor of fermented anchovy. These straight chain alkenals might have arisen from the oxidation of polyunsaturated fatty acids (21,22). Cha et al. (23) reported that fermented anchovy contains a high proportion of omega-3 fatty acids (18:3, 20:5, 22:5, 22:6) and omega-6 fatty acids (18:2, 20:4), which are highly susceptible to lipid oxidation. Moreover, sea salt used in fermented anchovy could act as a source of metal catalysts which could both decrease the induction period as well as increase the rate of lipid oxidation (24). Two branched aldehydes, 2-methylpropanal and 3-methylbutanal, both having chocolate-like aromas, have been shown to originate from Strecker or microbiological degradation of amino acids (25). The C8 and C9 series of short-chain carbonyls and alcohols have been reported to be derived from omega-3 and omega-6 series fatty acids by action of lipoxygenase in fish (20,21,26). Although benzaldehyde and phenylacetaldehyde were detected at high levels in both samples, only phenylacetaldehyde was detected by GC/O. Benzaldehyde, having a sweet and almond-like aroma, has been identified in some fermented foods (12,27). Phenylacetaldehyde was reported to have a strong and sweet floral aroma in many cooked foods (27). The sulfur containing aldehyde, 3-(methylthio)propanal, with its nutty, baked potato and soysauce aroma, was in higher abundance in koji than in control, and increased with fermentation time. Based on their odor intensities and levels of identified flavor compounds, the aldehydes derived from polyunsaturated fatty acids may play a major role in fermented anchovy flavor.

A total of 11 ketones were detected in both koji and control. These ketones may be produced by thermal and oxidative degradation of polyunsaturated fatty acids (21). 1-Octen-3-one, having a mushroom and earthy odor, had the highest odor intensity among the ketones, followed by 2,3-butanedione. The odor intensity of 1-octen-3-one increased in koji during fermentation.

1-Penten-3-one, having a plastic bottle-like odor, which may negatively affect flavor quality, was detected in control only. In general, Ketones have low aroma threshold values (21). However, the ketones, with the exception of 1-octen-3-one, may not play a significant role in the characteristic flavor of salt-fermented anchovy because of the low levels present in both samples.

Certain alcohols, such as 1-penten-3-ol, 3-methyl-1-butanol, 1-octen-3-ol and 2-furanmethanol, were detected at high levels in both samples. However, only 3-methyl-1-butanol, with its dark chocolate aroma, was detected by GC/O in koji and in control after 13 days. This may be because most alcohols have high threshold values (28). Among the alcohols, 3-methyl-1-butanol and 2-phenylethanol, which are known to have suppressive effects on fishy odor (27,29), were detected in koji and in control only after 13 days. Kunimoto et al. (9) reported that koji made from *A. oryzae* reduced the content of trimethylamine that significantly contributes to fishy odor. In wheat meal medium, *A.*

*oryzae* produced 1-octen-3-ol, having a strong mushroom-like aroma (30).

Twenty-four esters were detected in koji and control during fermentation. A series of fatty acid ethyl esters, from dodecanoate to octadecanoate, which were not detected by GC/O, were found in high concentration in both samples. On the other hand, low molecular weight esters, from ethyl propanoate to ethyl octanoate, were detected by GC/O at high intensities with sweet, fruity, bubble gum, candy and ripe apple odors. In particular, ethyl 2- and ethyl 3-methylbutanoate had higher odor intensities in koji than in control. Esters may have arisen from the esterification of various alcohols and carboxylic acids formed from microbial decomposition of lipid and protein in fermented fish products. Cha and Cadwallader (12) reported that over 20 esters were detected in Korean salt fermented fish products. High levels of esters are not necessarily desirable. Presence of high levels of esters has been reported to cause a fruity defect in cheese flavor (27). More research is needed to determine the sensory role of esters in fermented fish products.

Among the 5 alkylpyrazines detected, all were detected in koji only. Tetramethylpyrazine was in highest abundance, followed by 2-ethyl-3,5-dimethylpyrazine and trimethylpyrazine. However, these compounds were not detected by GC/O because of their low abundance compared with other compounds. MacLeod and Ames (31) identified many alkylpyrazines, having nutty, roasted and toasted aromas, in soybean, which was used as an ingredient for making koji in this study. Pyrazines can be formed by Maillard and pyrolysis reactions through Strecker degradations in heat processed foods (32). Further research is needed to determine the origin of pyrazines, i.e., whether they arise from enzymic action of *A. oryzae* or from the koji medium.

Five aromatic hydrocarbons were detected in control and 4 in koji. These compounds slightly decreased and were found only in small abundance in both samples during fermentation. Among 16 miscellaneous compounds detected, only 2,4,5-trimethylthiazole, having a metallic and sulfurous odor, had a high odor intensity by GC/O.

A total of 28 unidentified odor-active compounds were detected in both samples during fermentation (Table II). Unknown compounds (RI 1092 and 5.1 min), having plastic bottle odors that may impart off-flavor to fermented anchovy, were detected at higher levels in control than in koji. On the other hand, unknown compounds (RI = 1260, 1327, 1446, 1537, 1686, 1745, and 1928), having sour, nutty, popcorn, stale, rancid, fatty and chicken broth-like odors, had higher odor intensities in koji than in control.

The relative concentrations and odor values for predominant odor-active compounds detected in both samples are presented in Table III. Most compounds were present at parts per million levels except for 2,4,5-trimethylthiazole and octanal. Two esters, ethyl 2- and ethyl 3-methylbutanoate, had the highest odor values followed by 3-methylbutanal, (E,Z)-2,6-nonadienal, (Z)-4-heptenal, 1-octen-3-one and 3-(methylthio)propanal. For the most part, odor values of these compounds coincided with their odor intensities except for ethyl 2- and ethyl 3-methylbutanoate.

## Conclusions

Based on flavor profiles and odor intensity data, adding koji during the processing of salt-fermented anchovy was an effective alternative to existing traditional procedures.

Table II. Odor-active compounds in salt-fermented anchovy

No. <sup>a</sup>	Compound <sup>b</sup>	RI/RT <sup>c</sup>	Mean odor intensity <sup>d</sup>						Odor description <sup>e</sup>
			Control			Koji			
			0day	13day	30day	0day	13day	30day	
1	2-Methylpropanal	2.4min	1.0	0.5	1.0	1.5	0.5	1.0	chocolate
4	Butanal	3.0min	0.5	0.25	ND <sup>f</sup>	0.5	0.25	0.75	chocolate
8	3-Methylbutanal	3.4min	3.0	2.75	2.75	2.5	3.0	2.5	dark chocolate, roasted bean
10	Ethyl propanoate	4.1min	0.75	0.5	0.5	0.5	0.5	0.5	sweet, ethyl acetate-like
11	Ethyl 2-methylpropanoate	4.4min	ND	ND	ND	2.25	0.5	0.5	sweet, cherry candy
12	2,3-Butanedione	4.6min	1.0	ND	ND	1.0	1.5	1.25	sour, butter, diacetyl
	unknown	5.1min	1.25	1.5	0.75	0.5	1.0	1.25	plastic bottle odor, plastic pool
	unknown	1006	3.0	1.25	1.25	3.0	2.5	2.5	sour, rotten onion, garlic
15	1-Penten-3-one	1011	1.25	1.25	ND	ND	ND	ND	plastic water bottle (empty)
17	Ethyl butanoate	1030	1.0	1.25	1.25	1.75	2.0	1.75	fruity, bubble gum, candy
20	Ethyl 2-methylbutanoate	1046	1.5	1.0	1.5	2.25	3.25	1.75	fruity, ripe apple, bubble gum
21	2,3-Pentanedione	1057	0.75	ND	ND	ND	ND	ND	sour, buttery
22	Ethyl 3-methylbutanoate	1064	1.5	1.75	2.0	2.0	3.25	2.75	fruity, green apple
23	Hexanal	1074	2.5	2.0	1.75	2.75	2.25	2.25	sour, cut grass (hexanal)
	unknown	1092	2.5	3.25	3.25	2.5	2.5	1.5	plastic bottle odor
	Ethyl pentanoate	1132	2.0	1.5	1.5	0.5	0.75	0.5	fruity, pear
27	unknown	1139	0.75	0.75	0.5	1.25	ND	0.25	sour, rubber tire, grassy
	unknown	1162	0.75	0.5	0.25	ND	ND	ND	nutty, soysauce, dried fish
35	Heptanal	1182	0.75	0.5	1.0	0.5	1.75	1.0	fruity, sweet, spicy, wine
	unknown	1189	0.75	1.5	1.25	1.5	1.0	1.5	fruity, spicy
36	3-Methyl-1-butanol	1206	ND	1.0	0.25	1.25	1.25	0.75	dark chocolate
40	Ethyl hexanoate	1231	1.0	1.5	1.25	1.25	1.25	1.75	fruity
41	(Z)-4-Heptenal	1238	4.25	4.25	3.75	3.75	3.5	3.0	rancid, boiled potato
	unknown	1260	0.25	ND	ND	2.25	1.0	1.0	sour, onion

Continued on next page

Table II. Continued

No. <sup>a</sup>	Compound <sup>b</sup>	Mean odor intensity <sup>d</sup>						Odor description <sup>e</sup>
		Control			Koji			
		0day	13day	30day	0day	13day	30day	
43	Octanal	3.25	2.75	2.0	2.25	2.0	1.75	sweet, wine-like, candy
44	1-Octen-3-one	4.25	4.25	4.25	4.5	3.5	5.25	mushroom, earthy
	unknown	2.25	1.0	1.5	ND	ND	ND	nutty, malt, roasted bean
	unknown	1.5	1.25	ND	ND	ND	ND	sweet, plastic
	unknown	ND	ND	ND	0.25	0.75	1.75	nutty, popcorn
	unknown	0.75	0.25	0.5	ND	ND	ND	floral, mushroom
	unknown	2.5	3.25	1.75	2.25	2.0	1.25	fresh air, melon-like
53	2,4,5-Trimethylthiazole	4.0	3.25	3.25	3.25	2.0	4.0	metallic, sulfurous
	unknown	0.25	0.75	ND	0.5	0.75	1.0	PVC, plastic
58	(E,E)-2,4-Hexadienal	0.25	1.5	0.75	1.5	1.75	1.5	sweet, grainy, fruity
59	(E)-2-Octenal	1.25	0.5	0.5	ND	ND	1.0	nutty, stale, grainy, fishy
60	Ethyl octanoate	3.25	2.25	2.0	2.0	2.0	2.25	sweet, fruity, wine
62	Acetic acid	0.5	ND	0.75	0.75	0.75	ND	vinegar (acetic acid)
63	3-(Methylthio)propanal	2.0	2.25	1.0	2.75	2.75	3.25	nutty, baked potato, soysauce
	unknown	ND	ND	ND	1.75	2.75	2.5	stale, vitamin, earthy
	unknown	3.25	2.0	1.5	1.25	1.25	2.0	mushroom
69	(E,E)-2,4-Heptadienal	0.5	0.5	2.0	ND	ND	ND	stale, grainy
	unknown	2.25	2.5	2.5	2.75	2.0	2.0	stale, mildew, grainy, woody
	unknown	ND	ND	ND	1.25	1.25	2.0	sweet, stale, fresh air
74	(E)-2-Nonenal	2.75	3.0	2.75	2.75	2.5	1.75	grainy, stale, hay-like, cucumber
	unknown	2.25	0.5	ND	0.5	1.75	1.25	floral, spicy, peppery
77	(E,Z)-2,6-Nonadienal	3.75	3.5	5.25	3.5	3.5	3.75	cucumber, melon, sweet
	unknown	1.5	0.5	0.5	1.0	2.25	1.5	rancid, crabby
78	(E,E)-2,4-Octadienal	1.25	0.5	ND	3.25	2.25	1.75	rancid, fishy, amine

Continued on next page

Table II. Continued

No. <sup>a</sup>	Compound <sup>b</sup>	RI/RT <sup>c</sup>	Mean odor intensity <sup>d</sup>						Odor description <sup>e</sup>
			Control			Koji			
			0day	13day	30day	0day	13day	30day	
85	Phenylacetaldehyde	1648	4.0	3.0	3.75	2.75	3.25	4.0	floral, honeysuckle
	unknown	1671	4.0	3.0	3.75	3.25	3.25	4.25	sour, mildew, dried fruit
	unknown	1686	ND	ND	ND	2.0	2.25	1.0	rancid, crabby
	unknown	1695	1.75	2.25	2.0	ND	ND	ND	sweet, floral, grainy
	unknown	1720	2.0	2.25	1.75	1.25	3.0	1.75	fatty, stale, fishy, hay-like
	unknown	1745	1.25	ND	ND	3.5	0.75	1.75	sulfurous, pungent
	unknown	1771	1.75	ND	ND	1.25	1.75	1.0	stale, grainy, fatty
95	(E,E)-2,4-Decadienal	1822	1.0	0.5	2.25	2.0	2.5	2.5	sweet, fatty, grainy
	unknown	1874	2.75	1.0	3.25	3.25	2.75	3.25	sweet, fatty, pipe tobacco
	unknown	1928	1.25	0.75	ND	2.5	1.75	3.5	fatty, chicken broth
	unknown	1984	1.25	3.0	1.75	1.5	0.5	3.25	sweet, fatty, boiled chicken

<sup>a</sup> Numbers correspond to those in Table I.<sup>b</sup> Compounds identified by comparison of their MS, RI, and odor properties with authentic standards.<sup>c</sup> Retention index on Supelcowax 10 OTGC column.<sup>d</sup> Mean odor intensity from four replications.<sup>e</sup> Odor as perceived during olfactometry.<sup>f</sup> No odor detected.

Levels of 3-methyl-1-butanol and 2-phenylethanol, which have masking effects on fishy odor, increased in koji treated samples. Other odor-active compounds described as rancid, stale, mushroom, nutty and chicken broth-like, also increased in koji treated samples. However, optimization and standardization of the procedure need to be done prior to commercial application. For this purpose, it would be helpful to identify additional key flavors that affect the quality of these products.

Table III. Relative concentrations and odor values for selected odor-active compounds in salt-fermented anchovy

No. <sup>a</sup>	Compound	Concn Range <sup>b</sup> (ng/g)	Odor Threshold (ng/g)	Odor Value <sup>c</sup>
8	3-Methylbutanal	2112-17434	0.35 <sup>d</sup>	6034-49811
11	Ethyl 2-methylbutanoate	459-3541	0.006 <sup>e</sup>	76500-590166
22	Ethyl 3-methylbutanoate	772-1834	0.01 <sup>e</sup>	7200-183400
23	Hexanal	1680-3269	5 <sup>f</sup>	336-653
41	(Z)-4-Heptenal	242-1264	0.04 <sup>f</sup>	6050-31600
43	Octanal	84-1389	0.7 <sup>g</sup>	120-1984
44	1-Octen-3-one	639-5902	0.09 <sup>h</sup>	7100-15433
53	2,4,5-Trimethylthiazole	69-181	50 <sup>f</sup>	1-4
60	Ethyl octanoate	296-2043	N/A <sup>i</sup>	
63	3-(Methylthio)propanal	154-1816	0.2 <sup>f</sup>	770-9080
74	(E)-2-Nonenal	363-3301	1 <sup>h</sup>	363-3301
77	(E,Z)-2,6-Nonadienal	372-3256	0.1 <sup>h</sup>	3720-32560
85	Phenylacetaldehyde	180-1544	4 <sup>g</sup>	45-386

<sup>a</sup> Numbers correspond to those in Tables I and II.

<sup>b</sup> Relative concentration range of each compound during storage based on SDE data.

<sup>c</sup> Odor value = compound concentration divided by odor threshold.

<sup>d</sup> Threshold in water (34)

<sup>e</sup> Threshold in water (35)

<sup>f</sup> Threshold in water (36)

<sup>g</sup> Threshold in water (37)

<sup>h</sup> Threshold in water (38)

<sup>i</sup> Not available

### Acknowledgments

Approved for publication as Journal Article No. PS-9015 of the Mississippi Agricultural and Forestry Experiment Station. This work was supported in part by the Mississippi Agricultural and Forestry Experiment Station under project MIS-0855.

## Literature Cited

1. Lee, C. H.; Lee, E. H.; Lim, M. H.; Kim, S. H.; Chae, S. K.; Lee, K. W.; Koh, K. H. *Korean J. Dietary Culture*. **1986**, *1*, 267.
2. Saisithi, P.; Kasemsarn, B.-O.; Liston, J.; Dollar, A. M. *J. Food Sci.* **1966**, *31*, 105.
3. Dahl, L. K. *Am. J. Clin. Nutr.* **1972**, *25*, 231.
4. Sanceda, N. G.; Kurata, T.; Arakawa, N. *J. Food Sci.* **1990**, *55*, 983.
5. Chayovan, S.; Rao, R. M.; Liuzzo, J. A.; Khan, M. A. *J. Agric. Food Chem.* **1983**, *31*, 859.
6. Cha, Y. J.; Lee, E. H. *Bull. Korean Fish. Soc.* **1985**, *18*, 206.
7. Cha, Y. J.; Lee, E. H.; Kim, J. S.; Joo, D. S. *J. Korean Agric. Chem. Soc.* **1990**, *33*, 330.
8. Sanceda, N. G.; Kurata, T.; Arakawa, N. *J. Food Sci.* **1996**, *61*, 220.
9. Kunitomo, M.; Terao, K.; Kaneniwa, M. *Nippon Suisan Gakkaishi*. **1992**, *58*, 1471.
10. Kim, D. S.; Kim, Y. M.; Koo, J. G.; Lee, Y. C.; Woo, S. G. *Bull. Korea Fish. Soc.* **1990**, *23*, 69.
11. Choi, S. H.; Kato, H. *Agric. Biol. Chem.* **1984**, *48*, 1479.
12. Cha, Y. J.; Cadwallader, K. R. *J. Food Sci.* **1995**, *60*, 19.
13. Triqui, R.; Reineccius, G.A. *J. Agric. Food Chem.* **1995**, *43*, 453.
14. Likens, S. T.; Nickerson, G. B. *Amer. Soc. Brew. Chem. Proc.* 1964, pp 5-13.
15. Tanchotikul, U.; Hsieh, T. C.-Y. *J. Food Sci.* **1991**, *56*, 327.
16. Cha, Y. J.; Cadwallader, K. R.; Baek, H. H. *J. Food Sci.* **1993**, *58*, 525.
17. Chung, H. Y.; Cadwallader, K. R. *J. Agric. Food Chem.* **1994**, *42*, 2867.
18. van den Dool, H.; Kratz, P. D. *J. Chromatogr.* **1963**, *2*, 463.
19. Josephson, D. B.; Lindsay, R. C.; Stuber, D. A. *J. Agric. Food Chem.* **1984**, *32*, 1344.
20. Josephson, D. B.; Lindsay, R. C. *J. Am. Oil Chem. Soc.* **1987**, *64*, 132.
21. Josephson, D. B.; Lindsay, R. C. In *Biogenesis of Aromas*; Parliament, T. H.; Croteau, R., Eds.; ACS Symposium Series 317, American Chemical Society, Washington, D.C., 1986, pp 201-219.
22. Bailey, M. E.; Eining, R. G. In *Thermal Generation of Aroma*; Parliament, T. H.; McGorin, R. J.; Ho, C. T., Eds.; ACS Symposium Series 409, American Chemical Society, Washington, D.C., 1989, pp 421-432.
23. Cha, Y. J.; Lee, E. H.; Kim, H. Y. *Bull. Korean Fish. Soc.* **1985**, *18*, 511.
24. Grosch, W. In *Food Flavours. Part A. Introduction. Developments in Food Science 3A*; Morton, I. D.; Macleod, A. J., Eds.; Elsevier Scientific Publishing, Amsterdam, 1982, pp 325-397.
25. Collin, S.; Osman, K.; Delcambre, S.; El-Zayat, A. I.; Dufour, J. P. *J. Agric. Food Chem.* **1993**, *41*, 1659.
26. Hang, C. H.; Hirano, T.; Suzuki, T.; Shirai, T. *Nippon Suisan Gakkaishi*. **1992**, *58*, 559.
27. Lee, K. C. M.; Shi, H.; Huang, A. S.; Carlin, J. T.; Ho, C. T.; Chang, S. C. In *Biogenesis of Aromas*; Parliament, T. H.; Croteau, R., Eds.; ACS Symposium Series 317, American Chemical Society, Washington, D.C., 1986, pp 370-378.
28. Kawai, T. *Crit. Rev. Food Sci. Nutr.* **1996**, *36*, 257.

29. Kasahara, K.; Nishibori, K. *Nippon Suisan Gakkaishi*. **1991**, *57*, 737.
30. Charpf, L. G.; Seitz, E. W.; Morris, J. A.; Farbood, M. I. In *Biogenesis of Aromas*; Parliament, T. H.; Croteau, R., Eds; ACS Symposium Series 317, American Chemical Society, Washington, D.C., 1986, pp 323-346.
31. MacLeod, G.; Ames, J. *CRC Crit. Rev. Food Sci. Nutr.* **1988**, *27*, 219.
32. Whitfield, F. B. *CRC Crit. Rev. Food Sci. Nutr.* **1992**, *31*, 1.
33. Cha, Y. J. *J. Korean Soc. Food Nutrion.* **1994**, *23*, 481.
34. Semmelroch, P.; Grosch, W. *J. Agric. Food Chem.* **1996**, *44*, 537.
35. Takeoka, G. R.; Buttery, R. G.; Turnbaugh, J. G.; Benson, M. *Lebensm.-Wiss. u.-Technol.* **1995**, *28*, 153.
36. Cadwallader, K. R.; Tan, Q.; Chen, F.; Meyers, S. P. *J. Agric. Food Chem.* **1995**, *43*, 2432.
37. Buttery, R. G.; Turnbaugh, J. G.; Ling, L. G. *J. Agric. Food Chem.* **1988**, *36*, 1006.
38. Cadwallader, K. R.; Baek, H. H.; Chung, H. Y.; Moody, M. W. In *Lipids in Food Flavors*; Ho, C. T.; Hartman, T. G., Eds; ACS Symposium Series 558, American Chemical Society, Washington, D. C., 1994, pp 186-195.



## Chapter 14

# Impact of Dietary Peroxides and Tocopherols on Fillet Flavor of Farmed Atlantic Salmon

R. G. Ackman<sup>1</sup>, M. P. M. Parazo<sup>1</sup>, and S. P. Lall<sup>2</sup>

<sup>1</sup>Canadian Institute of Fisheries Technology, DalTech, Dalhousie University,  
P.O. Box 1000, Halifax, Nova Scotia B3J 2X4, Canada

<sup>2</sup>Institute for Marine Biosciences, National Research Council Canada,  
1411 Oxford Street, Halifax, Nova Scotia B3H 3Z1, Canada

The Atlantic salmon (*Salmo salar*) is a suitable model for animal research on the impact of oxidation of feedstuff oils and fish meal lipids on product quality. This species not only has an absolute requirement for dietary long-chain n-3 polyunsaturated fatty acids (20:5n-3, 22:6n-3) for the subcellular membrane lipids, but also deposits these same fatty acids in the muscle depot fats. Peroxides may retard growth and potentially affect fillet flavor. Traditionally only alpha-tocopherol has been boosted in salmon diets, but we have now shown that gamma-tocopherol may also be deposited in salmon muscle total lipids. It appears that if both tocopherols are fed,  $\alpha$ -tocopherol will be found in phospholipid-rich organ tissues and  $\gamma$ -tocopherol in the adipocytes which known to be the principal sites for triacylglycerol storage in the muscle. Our initial studies suggest that in any long-term post-slaughter storage of salmon  $\gamma$ -tocopherol could be an asset in preventing rancidity.

The lipids of the marine world are highly varied in different phyla and species. Fish and shellfish (poikilotherms) are almost always at ambient water temperature. Their lipids are notorious for containing 5-45% of oxidation-susceptible eicosapentaenoic (EPA or 20:5n-3) and docosahexaenoic (DHA or 22:6n-3) fatty acids. Ironically, it has lately been determined that such fatty acids may be nutritionally important in man (1). The fatty acid biochemistry in fish is now well understood (2), but it may not be appreciated that most of the fatty acids originate in phytoplankton (3,4). Figure 1 compares a typical algal fatty acid composition with that of a fish depot fat, in this case that of menhaden. These two are selected for comparison because the menhaden feeds directly on mixed phytoplankton and obviously selects the fatty acids it needs for what should be considered a "basic" fish oil fatty acid composition (5). At the same time, the menhaden is supplied with protein for growth and can

presumably utilize carbohydrates for energy or for biosynthesis of the saturated and monoethylenic fatty acids it may need. Other filter-feeding organisms such as the sea scallop *Placopecten magellanicus* may accumulate algal lipids in the digestive gland or gonad triacylglycerols, with EPA > DHA, but in phospholipids and organ lipids the two may be equal or DHA may exceed EPA (6). Table I shows that other commercial fish oils differ from menhaden oil in having variable EPA and DHA proportions. They differ primarily in having, in addition to the basic fatty acids, 20:1n-9 and 22:1n-11. These are introduced from the oils of calanoid copepods, as the corresponding long-chain fatty alcohols, and on digestion are oxidized by the fish to fatty acids (12). The copepods feed mostly on the phytoplankton but may biosynthesize the fatty alcohols to offset the weight of their exoskeleton since long-chain fatty alcohols have a lower specific gravity than the corresponding fatty acids. Moreover long-chain monoethylenic (C<sub>22</sub>) fatty acids also have a lower specific gravity than C<sub>16</sub> and C<sub>18</sub> fatty acids (13,14). These oils are usually considered to have EPA and/or DHA primarily in the 2-position of the triacylglycerols (15). Table I summarizes the fact that the principal fatty acids of most fish oils are essentially similar and that this includes the depot fats of seals which are really "biofiltered" fish fats. Because they are mammals the seals do rearrange the fatty acids on the glycerol molecule, although any biochemical advantage of having EPA

**Table I. Percent (w/w) of Principal Fatty Acids of Some Commercial Marine Oils**

Fatty acid	Sand Lance <sup>a</sup> <i>Ammodytes</i> <i>americanus</i>	Cod liver <sup>b</sup> <i>Gadus</i> <i>morhua</i>	Menhaden <sup>c</sup> <i>Brevoortia</i> <i>tyrannus</i>	Pacific Salmon <sup>d</sup>	Grey Seal <sup>e</sup> <i>Halichoerus</i> <i>grypus</i>
14:0	7.67	3.3	10.8	5.2	3.52
16:0	12.90	13.4	23.2	15.1	9.16
16:1	13.54	9.6	11.4	5.5	19.84
18:1	7.47	23.4	10.6	20.0	32.91
20:1	12.71	7.8	1.3	9.5	6.65
22:1	14.01	5.3	0.2	7.5	0.73
18:2n-6	0.79	1.4	1.8	1.4	0.97
18:3n-3	0.41	0.6	1.7	0.9	0.27
18:4n-3	2.87	1.0	2.1	1.7	0.69
20:4n-6	0.14	1.4	2.3	0.5	0.66
20:5n-3	10.11	11.5	11.9	8.5	5.23
22:5n-3	1.30	1.6	0.8	1.9	4.94
22:6n-3	6.81	12.5	8.0	11.3	7.12

<sup>a</sup> Ref. 7

<sup>b</sup> Ref. 8

<sup>c</sup> Ref. 9

<sup>d</sup> unpublished data for mixed oil from canneries

<sup>e</sup> Ref. 10

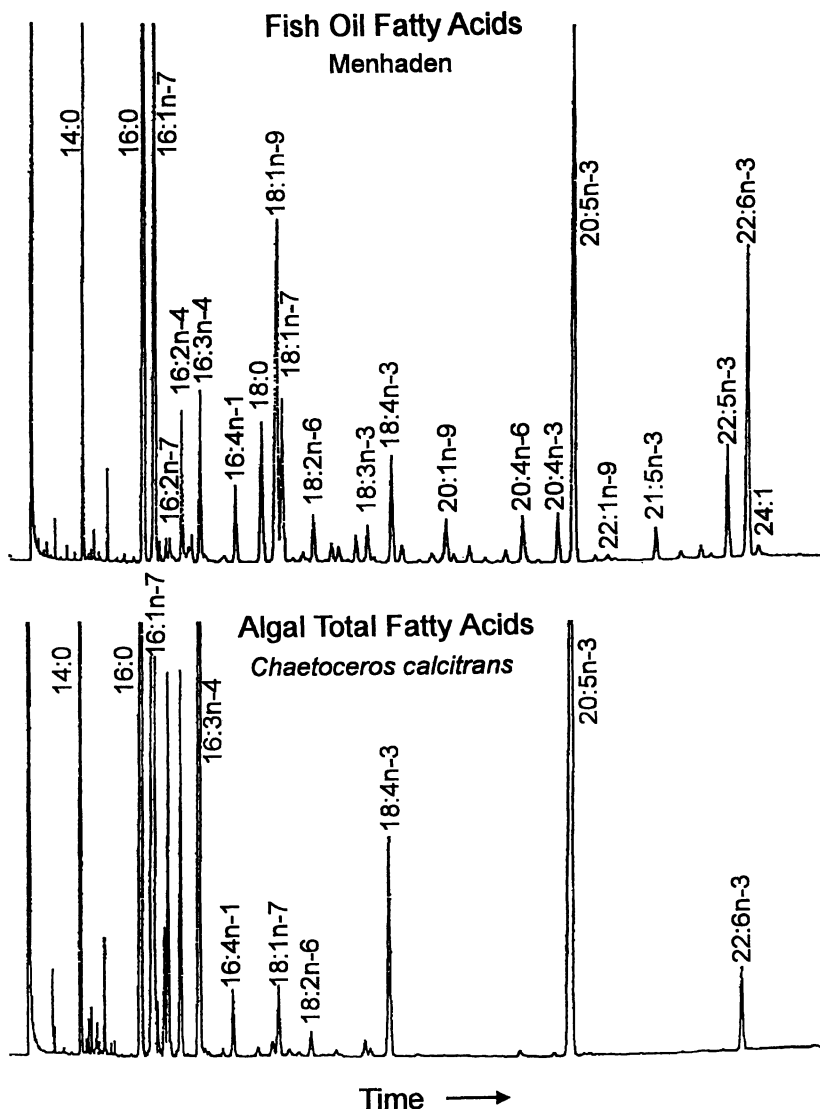


Figure 1. Principal fatty acids of body oil of a large herbivorous fish, menhaden (*Brevoortia tyrannus*) and total fatty acids of a typical marine phytoplankter. Analyses of methyl esters is on an Omegawax open-tubular column. Adapted from Ref. 4.

in the 1- and 3-position on triacylglycerol is not clear and the difference probably reflects a different triacylglycerol assembly pathway.

The similarity of the fatty acid compositions of the fish depot fats (Table I) suggests a high degree of functionality. In other words, each species is offered a variety of fatty acids and will catabolize some for energy, or break them down temporarily to acetate units to be reformed into any necessary saturated and monounsaturated fatty acids and reformulate these and others into triacylglycerols or phospholipids as required. Many, but not all, fish can elongate 18:3n-3 to 20:5n-3 and 22:6n-3 (2), an important factor in salmonid aquaculture (16), but this process is most likely to be related to size and age in the wild.

The interesting feature of the biosyntheses of triacylglycerols in fish is that position 3 of the glycerol probably accepts whatever fatty acids are available from the blood, thus offering the fatty acids of food prey a direct chance to modify the depot fat. Atlantic and Pacific herring oils differ slightly in fatty acid composition (17), and in each ocean the more northerly catches provide oils with additional amounts of highly unsaturated fatty acids such as EPA and DHA. Paradoxically, the free ranging bonito tuna (*Euthynnus pelamis*) caught in relatively warm (20-30 °C) Pacific waters have lipids with very high 22:6n-3 (Table II), supposedly reflecting the fatty acids of squid and other organisms in their diets (18,19). This may not however be totally a definite case of modulation of depot fat by species-specific diet fats as these fish have elevated body temperatures and no doubt a higher metabolic activity in the muscle. Both body and orbital triacylglycerols of tuna are extremely high in DHA (Table II) compared to most other fish (Table I).

### Fish Cellular Lipids

The membranes of the muscle cells of marine fish are almost always rich in DHA (25-45%), with lesser amounts of EPA (5-10%), the balance being mostly palmitic (16:0) and oleic (18:1n-9) fatty acids (Table III). Obviously these lipids are extremely functional (2,6,20-22). This is the ostensible reason that omega-3 fatty

**Table II. Some Fatty Acids of Commercial Tuna Oils (w/w%) from an Industrial Oil Source**

Fatty acid	Body <sup>a</sup>		Orbital <sup>b</sup>		
20:4n-6	1.8 <sup>c</sup>	1.1 <sup>c</sup>	2.2 <sup>c</sup>	3.4 <sup>d</sup>	2.3 <sup>c</sup>
20:5n-3	5.3	7.3	6.8	6.0	6.4
22:5n-6	1.6	0.6	1.2	-	1.2
22:5n-3	1.1	1.1	1.4	-	1.3
22:6n-3	20.6	20.1	26.2	28.2	25.5

<sup>a</sup> Crude oils

<sup>b</sup> Refined oils

<sup>c</sup> Unpublished data

<sup>d</sup> Ref. 11

**Table III. Fatty Acid Composition (w/w%) of Total Phospholipid in Muscle of Atlantic Salmon Fed Four Experimental Diets Containing Different Oil Sources<sup>a</sup>**

Fatty acid	Diet lipid source			
	Herring oil	Canola oil	EPA/DHA concentrate	Egg lipid
14:0	3.3	1.6	2.1	1.6
16:0	16.0	12.1	13.9	12.8
16:1	5.4	1.8	3.3	3.4
18:1	12.6	27.9	10.3	27.8
20:1	5.4	2.1	2.4	2.3
22:1	2.8	0.7	1.0	0.9
18:2n-6	4.5	11.2	4.9	7.7
18:3n-3	0.9	3.5	0.7	0.7
18:4n-3	1.3	1.3	2.5	0.5
20:4n-6	0.8	1.3	0.7	3.7
20:5n-3	6.8	3.5	9.4	2.6
22:5n-3	1.9	1.9	2.8	1.3
22:6n-3	33.6	27.2	42.6	30.2

<sup>a</sup> Adapted from ref. 23.

acids, especially EPA and DHA are considered "essential" diet components of salmonids. As Table III shows, the total phospholipids can be sensitive to dietary fatty acids, allowing some replacement of EPA and DHA by polyunsaturated C<sub>18</sub> fatty acids. The arachidonic acid (AA or 20:4n-6) in the phospholipids of the fish fed egg lipid is also of dietary origin, although in those fish fed canola oil some conversion of 18:2n-6 to 20:4n-6 is apparent.

Not all stages of fish life have identical fatty acid requirements but have instead adapted to those available from the diets. For example the juvenile Atlantic salmon living in freshwater may ingest insect fats with a high proportion of AA in addition to linoleic acid (24). The menhaden is a carnivore when young and living in marshes and estuaries before becoming the deep-water phytoplankton-feeding adult already mentioned.

### Tocopherols in Marine Fish

This should be phrased in the singular. It is known from long experience that only  $\alpha$ -tocopherol (alpha) appears to be present in fish lipids out of the four possible tocopherol structures (25). This finding was supported by contemporary Japanese and Norwegian scientists with general agreement that all marine fish oils seemed to contain approximately 300  $\mu\text{g/g}$ . Subsequent analyses in the era of modern HPLC have confirmed that only  $\alpha$ -tocopherol is present in our North Atlantic fish lipids

(26-28). In a one-year study of lipids of blue mussels (*Mytilus edulis*) we have verified that only  $\alpha$ -tocopherol is concentrated from phytoplankters by this species of marine invertebrate (29). The actual  $\alpha$ -tocopherol levels vary widely among different phytoplankters (30), but we presume that this concentration effect applies broadly to filter-feeding invertebrates that often have little or no reserve of depot fat and accordingly have lipids that are dominated by membrane phospholipids. These are usually not very dissimilar in fatty acid composition from those of fish (Table III) and it is possible that our dependence on  $\alpha$ -tocopherol as an essential vitamin rests on evolution from the presumed physical "fit" of this particular tocopherol among the phospholipids of membrane bilayers, a subject of much speculation (31). The transport and distribution of "vitamin E" in fish tissues has often been studied (32), but has not always been linked to the content of highly unsaturated fatty acids such as DHA. In tuna heads, the source of the orbital oil of Table II, it has been noted that a high vitamin E content conferred exceptional stability on the lipids (33).

The importance of "vitamin E" in aquaculture has been stressed in a review by Watanabe (34). The last line of the abstract can be quoted: "Vitamin E is also important in maintaining the flesh quality of frozen fish". The work reported here is in fact directed towards this objective and specifically on Atlantic salmon *Salmo salar*. In a recent review of tocopherols (35) an acknowledgement credited the incentive to write the review to a question posed in a Ph.D. defense "Why is  $\alpha$ -tocopherol a more potent antioxidant than  $\gamma$ -tocopherol (gamma) *in vivo* and vice versa *in vitro*?" Our work has in fact been devoted to determining if  $\gamma$ -tocopherol can be deposited in salmon muscle, and if so, does it have special frozen storage antioxidant powers equivalent to or better than  $\alpha$ -tocopherol.

### Potential of $\gamma$ -Tocopherol

The terrestrial plant kingdom developed seeds, often containing oils, and three other tocopherols supplement the  $\alpha$ -tocopherol in these oils. Table IV lists edible seed oils and their concentrations of different tocopherols. The oils such as canola or soybean containing the most  $\alpha$ -linolenic acid (18:3n-3) seem to be richer in  $\gamma$ -tocopherol than in  $\alpha$ -tocopherol but  $\delta$ -tocopherol (delta) usually plays a lesser role. This suggests that  $\gamma$ -tocopherol is superior to  $\alpha$ -tocopherol as a natural antioxidant in the milieu of vegetable seed triacylglycerols (37, reviewed by 38). In an examination of the effect of tocopherols on the induction period for autoxidation of a polyunsaturated fatty acid  $\gamma$ -tocopherol was 1.4 times as effective as  $\alpha$ -tocopherol (39). Fortuitously, the deodorization of vegetable oils produces, as a byproduct, ample quantities of natural tocopherol mixtures (40). Two companies sell these for food use under trade names such as Covi-Ox (Henkel) or Tenox GT-1 or GT-2 (Kodak). In these mixtures, probably mostly derived from soybean oils, the percentages of tocopherols could be about  $\alpha:\beta:\gamma:\delta::12:2:56:30$  and we selected for our research on salmon muscle keeping quality the Covi-Ox T-70 mixture (70% total tocopherols).

### Tocopherols and Atlantic Salmon

The farmed salmon industry adds  $\alpha$ -tocopherol acetate generously to salmon diets. The National Research Council (41) recommendation is 30 mg/kg diet, but this is

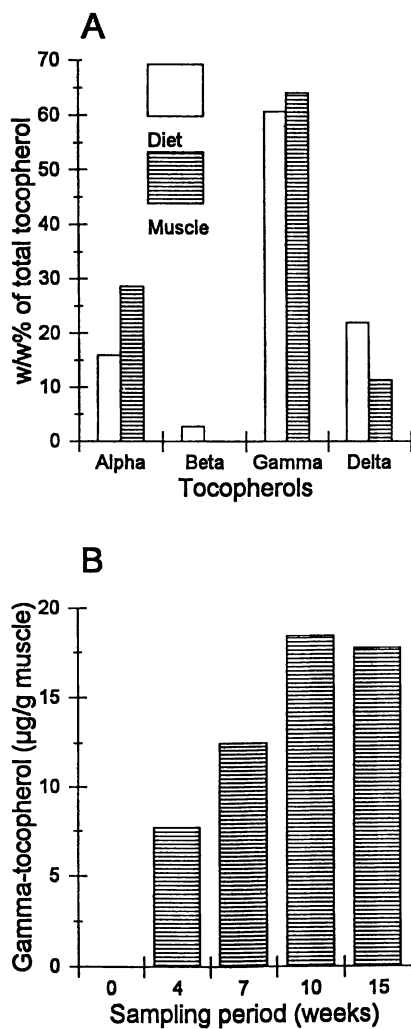


Figure 2. A. Tocopherol content in feed and muscle of fish fed a diet containing a natural tocopherol mixture for 15 weeks. B.  $\gamma$ -Tocopherol content of salmon muscle in fish fed a diet containing a natural tocopherol mixture. Adapted from Ref. 42.

Table IV. Tocopherol Contents of Some Selected Vegetable Oils<sup>a</sup>

Oil	Tocopherols (mg/kg)			
	$\alpha$	$\beta$	$\gamma$	$\delta$
HEAR <sup>b</sup>	268	-	426	-
HEAR	160	-	431	-
LEAR <sup>c</sup> (Canbra)	192	-	431	40
LEAR (Primor)	260	-	613	-
Soybean (refined)	116	34.0	737	275
Soybean	55	-	435	149
Soybean	90	-	680	230
Safflower	223	7.0	33	3.9
Sunflower (refined)	608	17.0	11	-
Peanut	169	5.4	144	13
Peanut	210	-	15	-
Corn	134	18.0	412	39
Cottonseed	402	1.5	572	7.5
Olive	93	-	7.3	-

<sup>a</sup> Adapted from ref. 36

<sup>b</sup> High erucic acid rapeseed oil

<sup>c</sup> Low erucic acid rapeseed oil

for fish health and any antioxidant effect is secondary. Locally  $\alpha$ -tocopherol acetate is usually added to salmon feeds at 300 mg/kg.

In our preliminary research with  $\gamma$ -tocopherol we used Atlantic salmon smolts as described in two published papers (42, 43). By analysis, the addition of Covi-Ox T-70 provided, in mg tocopherol/kg of feed:  $\alpha$ , 236 mg;  $\beta$ , 25 mg;  $\gamma$ , 921 mg;  $\delta$ , 333 mg. The control diet contained 10 mg/kg of  $\alpha$ -tocopherol from the natural components of the feed. The results showed that in 10 weeks the balances approached an equilibrium condition (Figure 2A and B). By the triangle test different tocopherols had minor effects on the flavour of salmon muscle according to sensory panelists (43). There was no clear effect on fatty acid compositions. Table V shows the tocopherols present and the results of a single accelerated oxidation test following Frigg et al. (44) for TBA values. However the large excess of total tocopherols in two diets merely confirmed the findings of others that any tocopherols offer protection against oxidation in frozen stored fish muscle samples (44-49) or in fish oil (50). Some experimental fish diets rich in vegetable components have included both  $\alpha$ - and  $\gamma$ -tocopherols (51).

It is important to note that one view of lipid oxidation in frozen fish muscle is that slow lipid hydrolysis, primarily of phospholipids (52), sets free polyunsaturated fatty acids and that these are more readily oxidized than are the same fatty acids in intact lipids. Table VI shows how this lipid class can develop in Atlantic salmon fillets. Total fat levels in farmed Atlantic salmon can reach 20% (53), but our experiments have been conducted on smaller, leaner fish



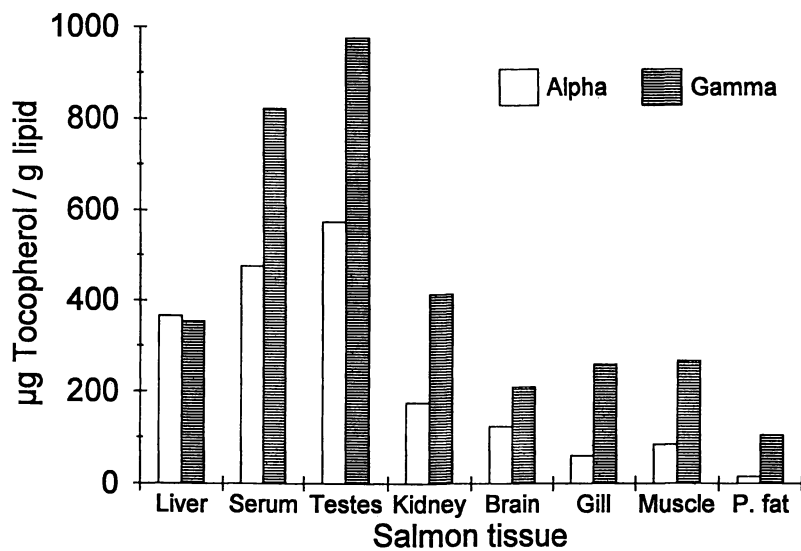


Figure 3. Concentration of  $\alpha$ - and  $\gamma$ -tocopherol in selected tissues of Atlantic salmon fed a  $\gamma$ -tocopherol diet for 36 weeks. Adapted from Ref. 55.

**Table V. Thiobarbituric Acid Reactive Compounds (Expressed as  $\mu\text{mol}$ s Malondialdehyde/kg Muscle) and Tocopherol ( $\mu\text{g/g}$  muscle) in Muscle of Atlantic Salmon after 15 Weeks of Feeding on Experimental Diets <sup>a</sup>**

Diet <sup>b</sup>	TBA	$\alpha$ -tocopherol	$\gamma$ -tocopherol	$\delta$ -tocopherol
1	6.2	8.1	17.8	3.0
2	9.5	4.1	0	0
3	5.5	7.6	13.0	1.8
4	8.9	2.9	0	0

<sup>a</sup> Adapted from ref. 43.

<sup>b</sup> Diets 1 and 2 contained astaxanthin; Diets 1 and 3 contained a natural mix of tocopherols (Covi-Ox T-70) added tocopherols; Diet 4 was not supplemented with either astaxanthin or tocopherols.

(23,42,43,54,55). Ingemansson et al. (56,57) have shown that in frozen stored rainbow trout (*Oncorhynchus mykiss*) the free fatty acids increased after 34 weeks to up to 5-15% of total muscle lipids, but more so in light muscle than in dark muscle. Free EPA and DHA were especially noteworthy as hydrolysis products, as reported by an independent method (TLC-FID) for cod (*Gadus morhua*) by Ohshima et al (58). An historically interesting effect in frozen stored cod was the development of a "cardboardy" flavor identified as due to *cis*-hept-4-enal from autoxidation of DHA or EPA (59). However as yet we have no concept of whether the recent increase in our understanding of the distribution of fat in salmon muscle (60,61) will alter the effect of tocopherols. The triacylglycerol is found primarily in adipocytes and not in muscle cell walls. The adipocytes could act as local depots for tocopherols.

Accordingly, a more comprehensive study of the intestinal adsorption and distribution of  $\gamma$ - and  $\delta$ -tocopherols in Atlantic salmon was developed (55). Intestinal absorption of specific tocopherols fed as part of a semisynthetic diet was uniformly good-to-excellent for all tocopherols, including  $\gamma$ - and  $\delta$ -tocopherols and  $\alpha$ -tocopherol from the acetate. The deposition results for  $\alpha$ - and  $\gamma$ -tocopherols after 36 weeks of feeding salmon are given in Table VII for selected tissues. From tocopherol analyses and comparisons of the lipid classes in several tissues it appears that  $\alpha$ -tocopherol is stored in phospholipid-rich tissues and is low in the perivisceral fat, mostly triacylglycerols, whereas the  $\gamma$ -tocopherol is relatively high in that fat (P. fat in Figure 3).

The opportunity to add the natural vitamin E mixture to salmon diets has both plus and minus features. As shown in the footnotes to Table VII that diet provided 15 mg/kg of  $\alpha$ -tocopherol for a feeding period of 36 weeks. These values were from actual analyses. The diet was semisynthetic and provided other essential vitamins and minerals, and included casein, gelatin and krill and herring meals as protein sources, as well as 14% natural herring oil. Over the 36 weeks of feeding no obvious symptoms of tocopherol deficiency appeared in the fish of any diet

**Table VI. Lipid Classes (g/100g Tissue) of Muscle of Atlantic Salmon Fed Four Experimental Diets Containing Different Fat Sources and Analyzed Before and After Frozen Storage <sup>a</sup>**

Diet fat	Treatment	Lipid Class			
		Phospholipid	Cholesterol	Triacylglycerol	FFA
Herring oil	F	0.67	0.07	5.14	tr
	S	0.58	0.02	5.09	0.19
Canola oil	F	0.66	0.02	5.95	tr
	S	0.65	0.02	5.73	0.23
EPA/DHA concentrate	F	0.54	0.05	3.76	0.04
	S	0.43	0.04	3.75	0.16
Egg lipid	F	0.56	0.06	3.81	0.01
	S	0.44	0.05	3.72	0.23

F = frozen briefly at  $-30^{\circ}\text{C}$ ; S = first frozen at  $-30^{\circ}\text{C}$ , then stored at  $-12^{\circ}\text{C}$  for 3 months; FFA = free fatty acids

<sup>a</sup> Adapted from ref. 54.

group, and specific growths were comparable. For the fish fed no supplemental tocopherol it must be considered that the  $6\ \mu\text{g}/\text{kg}$  of  $\alpha$ -tocopherol was as adequate for health as the  $36\ \mu\text{g}/\text{kg}$  of the diet specific for  $\alpha$ -tocopherol, and the  $15\ \text{mg}/\text{kg}$  included in the diet providing mostly  $\gamma$ -tocopherol (Table VII). No claim can be made that  $\gamma$ -tocopherol spares  $\alpha$ -tocopherol in a mixture, although the inclusion of  $\gamma$ -tocopherol equal to  $\alpha$ -tocopherol in the liver of the group fed that tocopherol is potentially of biochemical interest as the total approaches the level for liver observed in the diet group fed only  $\alpha$ -tocopherol. Our objective was to study the muscle deposition of the tocopherols, and we confirmed (Figure 3) that the muscle of these relatively lean fish (total lipid 1.6% w/w) responds well to diet. Our new work thus confirms that salmon muscle tocopherols can be modified by depositing  $\gamma$ -tocopherol from the diet (Figure 3).

The crude deodorizer concentrate, the source of food grade products such as Covi-Ox T-70, and the Kodak equivalent, is a mixture of vegetable sterols, free acids, triglycerides, etc. (40), and need not be as highly purified for aquaculture use as are the food grade materials. The short period to equilibrium suggests that a finisher diet of ten weeks might be able to deposit useful and adequate  $\gamma$ -tocopherol (plus any accompanying  $\delta$ -tocopherol). A drawback is that the free tocopherol form, unlike tocopherol acetate, is subject to oxidation, but the vacuum infusion process for feed manufacture (see below), or microencapsulation, could overcome this problem if the conversion of natural tocopherols to their acetate were impractical.

In terms of TBARS values at the end of a frozen storage ( $-40^{\circ}\text{C}$ ) period of six months the malondialdehyde equivalents of muscle were similar (respectively  $\sim 0.04$  and  $\sim 0.03\ \text{mg}/\text{kg}$ ) for the fish fed  $\alpha$ - and  $\gamma$ -tocopherols, and 0.07 for the tocopherol deficient fish. At this point it would therefore be imprudent to suggest

**Table VII. Tocopherol Contents ( $\mu\text{g/g}$  Lipid) of Various Tissues of Atlantic Salmon Fed Diets Containing  $\alpha$ -Tocopherol,  $\gamma$ -Tocopherol or No Added Tocopherol for 36 Weeks <sup>a</sup>**

Tissue	Tocopherol	Diet type		
		No added Tocopherol <sup>b</sup>	$\alpha$ -Tocopherol <sup>c</sup>	$\gamma$ -Tocopherol <sup>d</sup>
Liver	$\alpha$	110.7	1379	365.5
	$\gamma$	-	-	354.3
Muscle	$\alpha$	23.7	217.1	85.2
	$\gamma$	-	-	267.3
Perivisceral fat	$\alpha$	6.2	28.5	15.3
	$\gamma$	-	-	105.1
Serum	$\alpha$	202.7	1139	475.2
	$\gamma$	-	-	822.8
Testes	$\alpha$	340.3	1340	574.2
	$\gamma$	-	-	976.9

<sup>a</sup> Adapted from ref. 55.

<sup>b</sup> Diet contained 6 mg  $\alpha$ -tocopherol/kg; none was added

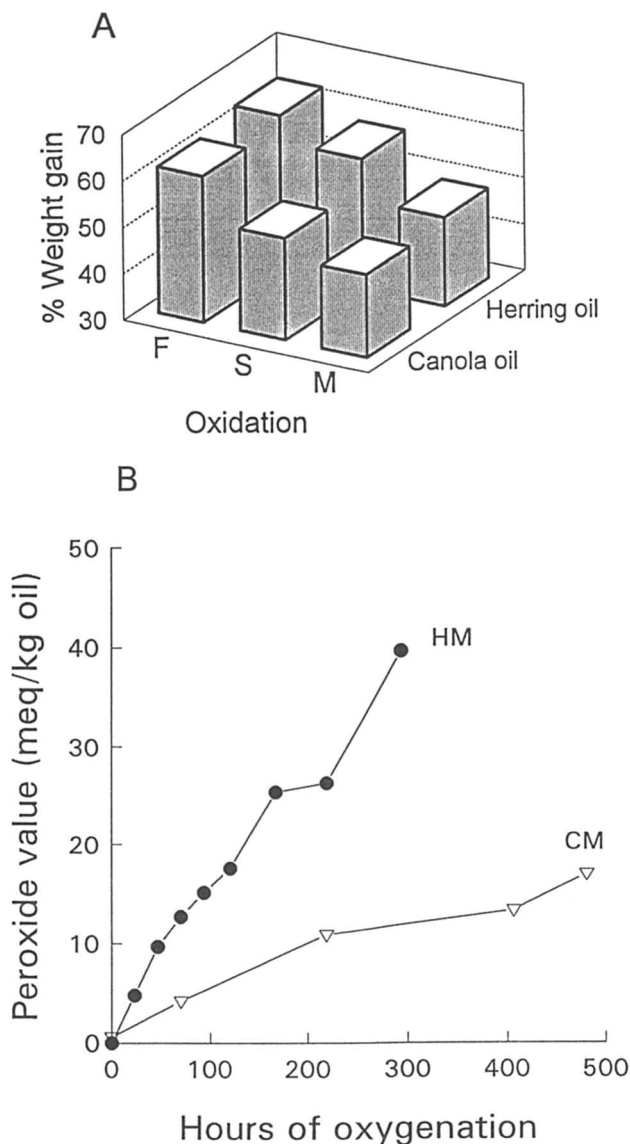
<sup>c</sup> Diet contained 36 mg  $\alpha$ -tocopherol/kg

<sup>d</sup> Diet contained 15 mg  $\alpha$ -tocopherol/kg and 78 mg  $\gamma$ -tocopherol/kg

that  $\gamma$ -tocopherol is equivalent to  $\alpha$ -tocopherol as an antioxidant during frozen storage of salmon muscle. It is however proposed that the natural tocopherol mixtures available from vegetable oil deodorization are able to provide adequate total tocopherols for fish health, do not interfere with fatty acid metabolism and flavor in fresh fish, and may or may not enhance lipid stability in frozen storage.

### Oxidized Dietary Fats in Aquaculture

It is difficult to imagine a more awkward nutrition problem than that of incorporating the oxidation-sensitive "essential" fatty acids into fish feed. The quality indexes for fish oil are not all easy to determine and are as follows: iodine value, free fatty acids, unsaponifiables, peroxide value, anisidine value, color and polymer. Of these indices the peroxide value is ephemeral, starting with no change in fatty acid oxidation status for an induction period, then a sharp rise to something of the order of 30 meq/kg, and then a decline as the peroxides decay into aldehydes, acids or polymers. The anisidine value reflects the total aldehydes from prior peroxidized fatty acids and will in consequence rise to some number such as 25. Many of the aldehydes contain ethylenic bonds (62,63) and will be quite reactive with each other or with the protein and carbohydrates when they are blended into fish feed materials. To overcome the surface exposure problem for fats in "high-energy" feeds a process called vacuum infusion has recently been introduced in our area by Corey Feed Mills



**Figure 4.** A. Percent weight gain of atlantic salmon fed diets containing fresh oil (F), slightly oxidized oil (S) and mildly oxidized oil (M). B. Development of oxidation in herring and canola oils. HM, mildly oxidized herring; CM, mildly oxidized canola. Adapted from Ref. 65.

of Fredericton, New Brunswick. The free fat is partially pushed into pores in the feed pellets by atmospheric pressure, a process reminiscent of the safe storage of oxidation sensitive methyl esters in glass capillary tubing (64).

In regard to Atlantic salmon smolts, Koshio et al. (65) fed both herring oil and retail canola (low erucic acid rapeseed oil) with maximum peroxide values of 40 and 17 respectively (Figure 4A), as well as fresh oil and slightly oxidized oils as intermediates. These were added to feeds at 13.8% of total feed. Figure 4B shows that, although there was no mortality and no obvious disability, weight gain was definitely reduced for both "rancid" oils over 50 days. An interesting aspect of the gas-liquid chromatography analyses of the fatty acids from the feed made up from these oils was that both  $C_{18}$  and the EPA and DHA polyunsaturated fatty acids did not differ quantitatively before and after oxidation. The effect of the oxidation must have been more subtle, possibly in reducing the palatability of the oils. However digestibility was not examined and it is possible that oxidized oils interfere with the proper functioning of the surface cells of the intestinal villae, reducing absorption of nutrients. Toxicity of the secondary oxidation products from fish oil in fish seems to be an unexplored area. Canola oil, as noted above, is a good source of  $\gamma$ -tocopherol as well as containing  $\alpha$ -tocopherol at approximately 250  $\mu\text{g/g}$  of oil before mixing into the diets. Unfortunately the  $\gamma$ -tocopherol was not determined in the fish muscle or oxidized canola oil.

The effect of feeding these oxidized oils on the taste of Atlantic salmon was evaluated using a triangle test with 10 panelists (66). Cooked fillets of Atlantic salmon randomly selected from each diet group were supplied to the panelists. Since only 3 or 4 marked the fillet meat from fish fed oxidized oils as "different" the results were not statistically significant.

Subsequently Koshio et al (67) fed similar oxidized oils to *Penaeus japonicus* and found that the adverse effects on larvae were greater than on juvenile stages. The results of Murai et al. (68) and of Sakai et al. (69) on fingerling yellowtail (*Seriola quinqueradiata*) and of Messager et al. (70) for sea bass *Dicentrarchus labrax*, include physiological changes not included in our salmon studies but some of these are known for rainbow trout (71,72). In an interesting study with turbot *Scophthalmus maximus*, Stéphan et al. (73) showed that even 320 mg of  $\alpha$ -tocopherol/kg of feed was insufficient to prevent TBARS developing in muscle during 6 months of frozen storage at  $-20^{\circ}\text{C}$ . These were small fish (310 g after 34 weeks on diet) with low muscle lipid ( $\sim 1\%$ ). The relevance of much more of the literature for species such as catfish *Ictalurus punctatus* (74,75) to Atlantic salmon studies is limited but it appears that  $\alpha$ -tocopherol has merits in stabilizing muscle lipids of these fish post-mortem. Synthetic  $\alpha$ -tocopherol is, however, a costly choice that could perhaps be partially replaced by the deodorizer materials with less  $\alpha$ -tocopherol but high levels of  $\gamma$ -tocopherol, providing for both health and frozen storage benefits.

The general adverse impression created by fish odors and flavors is misleading. An oil with a high peroxide value ( $\geq 30$  meq/kg) may still be a valuable source of the highly unsaturated fatty acids such as EPA and DHA for Atlantic salmon (65). These are necessary for cellular membrane functions which may be relatively independent of the status of the triacylglycerols locked up in the adipocytes. Horrobin (76) has expressed the view that the "toxic" metabolites or

secondary oxidation products released by autoxidation may not seriously damage cells. His view of the problem is that of the loss of the "highly unsaturated essential fatty acids from membranes. If this view is correct, then antioxidant and anti-free radical therapy will be inadequate to prevent and reverse such damage. Treatment must include measures to replace the missing essential fatty acids". Of course, this does not reduce the need for additional  $\alpha$ -tocopherol, vitamin C, selenium etc. for animal health, but it is important to recall that many of our own most acceptable food flavors are in fact produced by degradation of cooking fats and the interaction of secondary oxidation products materials with proteins and carbohydrates.

### Conclusion

In several studies we have established that  $\gamma$ -tocopherol is readily taken up by Atlantic salmon and deposited in the muscle. Atlantic salmon muscle differs from the homogeneous cellular tissues of cod and other familiar fish and at present we consider it possible that  $\gamma$ -tocopherol may distribute more into the fat-rich adipocytes than does the  $\alpha$ -tocopherol which is traditionally found in the muscle cell membrane bilayers. At a whole fillet level the  $\gamma$ -tocopherol appears to have a modest potential for reduction of autoxidation during frozen storage. Natural mixtures of tocopherols may be a low-cost alternative to synthetic  $\alpha$ -tocopherols in salmonid nutrition, and probably would provide adequate  $\alpha$ -tocopherol for fish health.

Highly oxidized herring oil or canola oil are not toxic to aquaculture salmonids as long as dietary  $\alpha$ -tocopherol is adequate. However such oils may reduce diet intake, or food consumption, and thus slow down smolt growth. In accord with the experience of other workers, flavors in fillets of farmed Atlantic salmon are not obviously altered by different tocopherol types, dietary fats and oils, or oxidation status of those fats and oils.

### Literature Cited

1. Ackman, R.G. *J. Aquatic Food Product Tech.* **1996**, *5*(4), 7-26.
2. Sargent, J.R. In *Fish Oil: Technology, Nutrition and Marketing*; Hamilton, R.J., Rice, R.D, Eds; PJ Barnes & Associates: High Wycombe, England, 1995; pp 67-94.
3. Ackman, R.G.; Tocher, C.S.; McLachlan, J. *J. Fish. Res. Board Canada* **1968**, *25*, 1603-1620.
4. Ackman, R.G.; Kean-Howie, J. In *Nutrition and Utilization Technology in Aquaculture*; Lim, C.E., Sessa, D.J., Eds; AOCS Press: Champaign IL, 1995; pp 82-104.
5. Ackman, R.G.; Ratnayake, W.M.N.; Olsson, B. *J. Am. Oil Chem. Soc.* **1988**, *65*, 136-138.
6. Napolitano, G.E.; Ackman, R.G. *J. Shellfish Res.* **1993**, *12*, 267-277.
7. Ackman, R.G.; Eaton, C.A. *J. Fish. Res. Board Canada* **1971**, *28*, 601-606.
8. Jangaard, P.M.; Ackman, R.G.; Sipos, J.C. *J. Fish. Res. Board Canada* **1967**, *24*, 613-627.
9. Sebedio, J-L.; Ackman, R.G. *J. Am. Oil Chem. Soc.* **1983**, *60*, 1986-1991.
10. Ackman, R.G.; Hooper, S.N. *J. Fish. Res. Board Canada* **1974**, *31*, 333-341.

11. Nakajima, S.; Sakuta, T.; Tsuchiya, T. *Nippon Suisan Gakkaishi* **1995**, *61*, 769-770.
12. Pascal, J.-C.; Ackman, R.G. *Chem. Phys. Lipids* **1976**, *16*, 219-223.
13. Ackman, R.G.; Eaton, C.A. *J. Fish. Res. Board Canada* **1970**, *27*, 1669-1683
14. Ackman, R.G.; Eaton, C.A. *J. Am. Oil Chem. Soc.* **1977**, *54*, 435.
15. Litchfield C. *Analysis of triglycerides*. Academic Press: New York, 1972; p 355.
16. Olsen, R.E.; Ringø, E. *Fish. Physiol. Biochem.* **1992**, *9*, 393-399.
17. Ackman, R.G.; Eaton, C.A. *J. Fish. Res. Board Canada* **1976**, *33*, 1634-1638.
18. Saito, H.; Watanabe, T.; Murase, T. *Biosci. Biotech. Biochem.* **1995**, *59*, 2186-2188.
19. Watanabe, T.; Murase, T.; Saito, H. *Comp. Biochem. Physiol.* **1995**, *111B*, 691-695.
20. Greene, D.H.S.; Selivonchick, D.P. *Aquaculture* **1990**, *89*, 165-182.
21. Lie, Ø; Hemre, G-I.; Lambertsen, G. *Lipids* **1992**, *27*, 770-775.
22. Medina, I.; Aubourg, S.P.; Martín, R.P. *Lipids* **1995**, *30*, 1127-1135.
23. Polvi, S.M.; Ackman, R.G. *J. Agric. Food Chem.* **1992**, *40*, 1001-1007.
24. Ackman, R.G.; Takeuchi, T. *Lipids* **1986**, *21*, 117-20.
25. Ackman, R.G.; Cormier, M.G. *J. Fish Res. Board Canada* **1967**, *24*, 357-373.
26. O'Keefe, S.F.; Ackman R.G. *Proc. N.S. Inst. Sci.* **1987**, *37*, 1-7.
27. Bhuiyan, A.K.M.A.; Ratnayake, W.M.N.; Ackman, R.G. *J. Food Composit. Anal.* **1993**, *6*, 172-184.
28. Lie, Ø; Lied, E.; Maage, A.; Njaa, L.R.; Sandnes, K. *Fisk. Dir. Skr. Ser. Ernæring* **1994**, *6(2)*, 83-105.
29. Sigurgisladdottir, S.; Ackman, R.G.; O'Keefe, S.F. *J. Food Lipids* **1993**, *1*, 97-109.
30. De Roeck-Holtzhauer, Y.; Quere, I.; Claire, C. *J. Appl. Phycol.* **1991**, *3*, 259-264.
31. Parker, R.S. In *Advances in Food and Nutrition Research*; Kinsella, J.E., Ed.; Academic Press, Inc.: San Diego, 1989; Vol. 33, pp 157-232.
32. Lie, O.; Sandvin, A.; Waagbø, R. *Fish Physiol. Biochem.* **1994**, *13*, 241-247.
33. Watanabe, T.; Sugii, K.; Yamada, J.; Kinumaki, T. *Bull. Tokai Reg. Fish. Res. Lab.* **1989**, *No. 127*, 69-80.
34. Watanabe, T. *Yukagaku* **1990**, *39*, 299-306.
35. Kamal-Eldin, A.; Appelqvist, L-A. *Lipids* **1996**, *31*, 671-701.
36. Ackman, R.G. In *High and Low Erucic Acid Rapeseed Oils*; Kramer, J.K.G., Sauer, F.D., Pigden, W.J., Eds; Academic Press: Toronto, 1983; pp 85-129.
37. Gottstein, T.; Grosch, W. *Fat Sci. Technol.* **1990**, *92*, 139-144.
38. Huang, S-W; Frankel, E.N.; German, J.B. *J. Agric. Food Chem.* **1995**, *43*, 2345-2350.
39. Wu, G-S., Stein, R.A., and Mead, J.F. *Lipids* **1979**, *14*, 644-650.
40. Ramamurthi, S.; McCurdy, A.R. *J. Am. Oil Chem. Soc.* **1993**, *70*, 287-295.
41. National Research Council. *Nutrient requirements of fish*; National Academy Press: Washington, DC, 1993; p 114.
42. Sigurgisladdottir, S.; Parrish, C.C.; Ackman, R.G.; Lall, S.P. *J. Food Sci.*, **1994**, *59*, 256-259.



43. Sigurgisladdottir, S.; Parrish, C.C.; Lall, S.P.; Ackman, R.G. *Food Res. Internat.* **1994**, *27*, 23-32.
44. Frigg, M.; Prabucki, A.L.; Ruhdel, E.U. *Aquaculture* **1990**, *84*, 145-158.
45. Murata, H.; Yamauchi, K. *Nippon Suisan Gakkaishi* **1989**, *55*, 1435-1439.
46. Nishina, A. *Nippon Suisan Gakkaishi* **1991**, *57*, 2073-2076.
47. Gatlin, D.M.; Bai, S.C.; Erickson, M.C. *Aquaculture* **1992**, *106*, 323-332.
48. Waagbø, R.; Sandnes, K.; Sandvin A.; Lie, Ø. *Fisk. Dir. Skr. Ser. Ernæring* **1991**, *IV(1)* 51-63.
49. Waagbø, R.; Sandnes, K.; Torrissen, O.J.; Sandvin A.; Lie, Ø. **1993**. *Food Chem.* **1993**, *46*, 361-366.
50. Aoyama, M.; Kanematsu, H.; Tsukamoto, M.; Tokairin, S.; Niiya, I. *Yukagaku* **1993**, *42*, 680-684.
51. Fair, P.A.; Williams, W.P. *J. Food Lipids* **1995**, *2*, 1-23.
52. Braddock, R.J.; Dugan, L.R. Jr. *J. Food Sci.* **1972**, *37*, 426-429.
53. Wold, J.P.; Jakobsen, T.; Krane, L. *J. Food Sci.* **1996**, *61*, 74-77.
54. Polvi, S.M.; Ackman, R.G.; Lall, S.P.; Saunders, R.L. *J. Food Process. Preserv.* **1991**, *15*, 167-181.
55. Parazo, M.P.M. Biochemical effects of dietary tocopherols in Atlantic salmon (*Salmo salar*). Ph.D. Thesis, Technical University of Nova Scotia, Halifax, Canada, 1995, p 266.
56. Ingemansson, T.; Pettersson, A.; Kaufmann, P. *J. Food Sci.* **1993**, *58*, 513-518.
57. Ingemansson, T.; Kaufmann, P.; Ekstrand, B. *J. Agric. Food Chem.* **1995**, *43*, 2046-2052.
58. Ohshima, T.; Ratnayake, W.M.N.; Ackman, R.G. *J. Am. Oil Chem. Soc.* **1987**, *64*, 219-223.
59. Hardy, R.; McGill, A.S.; Gunstone, F.D. *J. Sci. Food Agric.* **1979**, *30*, 999-1006.
60. Zhou, S.; Ackman, R.G.; Morrison, C. *Can. J. Fish. Aquatic Sci.* **1996**, *53*, 326-332.
61. Zhou, S.; Ackman, R.G.; Morrison, C. *Fish Physiol. Biochem.* **1995**, *14*, 171-179.
62. Ke, P.J.; Ackman, R.G.; Linke, B.A. *J. Am. Oil Chem. Soc.* **1975**, *52*, 349-353.
63. Karahadian C.; Lindsay, R.C. *J. Am. Oil Chem. Soc.* **1989**, *66*, 953-960.
64. Sipos, J.C.; Ackman, R.G. *J. Gas Chromatogr.* **1967**, *5*, 215-216.
65. Koshio, S.; Ackman, R.G.; Lall, S.P. *J. Agric. Food Chem.* **1994b**, *42*, 1164-1169.
66. Larmond, E. *Laboratory Methods for Sensory Evaluation of Food*. Canada Department of Agriculture, Research Branch: Ottawa, 1982, Publication 1637, pp 19-24.
67. Koshio, S.; Teshima, S.; Kanazawa, A. *Fish. Sci.* **1994b**, *60*, 283-288.
68. Murai, T.; Akiyama, T.; Ogata, H.; Suzuki, T. *Nippon Suisan Gakkaishi* **1988**, *54*, 145-149.
69. Sakai, T.; Murata, H.; Yamauchi, K. *Nippon Suisan Gakkaishi* **1992**, *58*, 1483-1486.

70. Messenger, J.-L.; Stéphan, G.; Quentel, C.; Laurencin, F.B. *Aquat. Living Resour.* **1992**, *5*, 205-214.
71. Hung, S.S.O.; Cho, C.Y.; Slinger, S.J. *J. Nutr.* **1981**, *111*, 648-657.
72. Cowey, C.B.; Adron, J.W.; Youngson, A. *Aquaculture* **1983**, *30*, 85-93.
73. Stéphan, G.; Guillaume, J.; Lamour F. *Aquaculture* **1995**, *130*, 251-268.
74. Erickson, M.C. *J. Sci. Food Agric.* **1992**, *59*, 529-536.
75. Erickson, M.C.; Thed, S.T. *Internat. J. Food Sci. Tech.* **1994**, *29*, 585-591.
76. Horrobin, D.F. *Medical Hypotheses* **1991**, *35*, 23-26.

## Chapter 15

# Effect of Sodium Potassium Phosphate (Carnal 2110) on Acceptability and Color of Hot Smoked White Sturgeon (*Acipenser transmontanus*)

Frank A. Chapman, Susan L. Lucas, and Sean F. O'Keefe<sup>1</sup>

Departments of Food Science and Human Nutrition and Fisheries and Aquatic Sciences, University of Florida, Gainesville, FL 32611-0370

The purpose of this investigation was to determine the effect of phosphate (sodium/potassium phosphate, Carnal 2110) on the chemical, physical and sensory properties of hot smoked White sturgeon (*Acipenser transmontanus*). White sturgeon were obtained from a commercial aquafarm and were shipped overnight on ice immediately after slaughter. Fish were filleted and were brined at 20°C with either 20% (w/v) NaCl or 20% (w/v) NaCl + 10% (w/v) Carnal 2110. Fish were hot smoked in a commercial Koch smokehouse. Target final water phase salt content was 2.5% w/w and heating was maintained at the fillet center at  $\geq 63^\circ\text{C}$  monitored by thermocouple for  $\geq 30$  min. Weight recovery (yield), color (L,a,b), and sensory acceptability panel testing were measured on freshly smoked fish. Phosphate greatly improved color development and improved slightly the acceptability and yield of the fish.

Sturgeon stocks worldwide were depleted in the late 19th and early 20th centuries due to over-fishing and habitat destruction. The large caviar fishery in the Caspian Sea has been largely stabilized by an extensive hatchery program in Russia (1). In the past 15 years, there has been increased interest in sturgeon due to developments in white sturgeon (*Acipenser transmontanus*) aquaculture (2). The white sturgeon is now commercially grown worldwide and is a source of caviar and sturgeon meat. The wholesale price of sturgeon meat (skinless, boneless) is currently about US \$11-\$15/kg and for caviar is  $> \$500/\text{kg}$ . Sturgeon clearly represent an unusually attractive species for food fish aquaculture.

Traditionally sturgeon meat is smoked and smoking increases the value of the meat considerably. Thus, smoking can be considered a *value added* process. Smoking can be divided into two main types depending on the temperatures the fish are exposed

<sup>1</sup>Corresponding author

to during smoking: hot and cold smoking. Hot smoking requires a temperature of at least 63°C for 30 min to ensure destruction of pathogens such as *Listeria monocytogenes*. However, 30 min is too short to result in good color and smoke flavor development. Smoking is therefore conducted until an acceptable color and flavor are formed. Any process that increases the rate of color and flavor development while maintaining quality will have a strong impact on the economics of smoking.

### Phosphates in Seafood Processing

Phosphates have been used extensively for fish and seafood processing for many years (3,4). Phosphates allow processors to maintain quality in frozen and fresh seafood by preventing or decreasing drip loss (water loss) on freeze-thaw, prevention of oxidation and struvite formation (5). Phosphates are used extensively in frozen shrimp where they facilitate mechanical peeling, promote maintenance of weight during frozen storage, and improve acceptability and shelf life. Thus, phosphates have been a very important aid to maintain seafood quality. However, there is little information available on the role of phosphates in smoked fish.

Phosphates can be divided into categories depending on the polymerization of the phosphates in the molecules (4,5). The most commonly used phosphates in seafood processing are the condensed phosphates, sodium tripoly- and hexameta- phosphates (5). Sodium tripolyphosphate (STPP) has been used to control fish shrinkage in hot smoked fish. Addition of 2% (w/v) STPP to concentrated brine resulted in improved yields in halibut, silver salmon and black cod (6). The weight loss in halibut was reduced from 29.5 to 24.8%, in silver salmon from 28.7 to 25.2% and black cod from 25.6 to 24.0% after smoking at 21°C for 14 h then at 113°C for 1 h (6). Other than this report, there is little information available in the literature on the role of phosphates in hot smoked fish.

The solubility of Na/Kphosphates in concentrated brines is much better than for STPP (data not shown). For this reason, Na/Kphosphate blends such as Carnal 2110 would be easier to use in commercial practice than STPP, by allowing more flexibility in brine concentration and temperature. The purpose of this study was to investigate the effect of Carnal 2110 on color development, acceptability, and recovery (yield) in hot smoked white sturgeon.

### Experimental Procedures

**Chemicals.** All chemicals were obtained from Fisher Scientific and were reagent grade. Sodium tripolyphosphate was obtained from Rhone Poulenc (Atlanta, GA) and Budenheim's sodium/potassium phosphate (Carnal 2110) from Gallard-Schlesinger Industries Inc. (Carle Place, NY).

**Fish.** White sturgeon (*Acipenser transmontanus*) were obtained from Stolt Sea Farm Inc. (Seattle, WA) on three separate occasions. Each shipment consisted of 2 bullets (deheaded, detailed, definned, gutted) which were evenly matched in weight (bullets weighted between 3–4 kg). The fish were obtained within 3 days of slaughter and were maintained on ice. The bullets were skinned and filleted, stored in plastic zip-lock bags and held on ice until smoking. Fillets were cut and trimmed to remove all cartilaginous bone. The lateral fat layer was not removed (not deep skinned).

Fish were smoked within 4-6 days of slaughter. The right and left fillets from each fish were subjected to the two brining treatments using the right fillet of one fish and the left fillet of the other fish were controls, and the corresponding fillets were treatments.

**Smoking.** A Koch commercial smokehouse was used with dimensions 1 m x 1 m x 1.5 m (height). The smoke generator was used with hickory sawdust (Koch, Kansas City, MO) and smoke was transferred to the smoking chamber using a blower. Temperature was controlled at 75°C using a thermocouple with feedback to the heater.

Brines were made of the following concentrations. Control brine was 20% (w/v) NaCl (no iodine, Publix, FL) and treatment was 20% (w/v) NaCl with 10% (w/v) Carnal 2110. The treatment brine was made by dissolving the phosphate first and the NaCl afterwards, since this increased the speed of dissolution.

Fillets were cut into thirds by length (front, middle, back). Fillets were brined in control or treatment brines for 45 min at 20°C with mixing at 5 min intervals. The ratio of fish to brine was about 0.3 kg/L. After brining, the fish were removed and were placed on screened racks (1.5 cm screen size) for drying at ambient temperature (25-30 °C) for 1 h. After drying, the fish were placed in the smokehouse which had been preheated to operating temperature. Fish were smoked for 3 h at a smokehouse temperature of 75 °C. Fish were removed, weighed and placed in plastic bags overnight on ice in a cold store (5 °C) before analysis.

## Analyses

**Sodium content.** Sodium was measured using a Ross Sodium electrode model 86-11 (Orion Research, Boston MA). Fish was chopped and a composite sample (10 g) was blended (Waring commercial blender model 33BL79) with 100 ml distilled deionized (2D) water for 2 min. The blended samples were transferred to a 200 ml volumetric flask and the blender rinsed with ca. 80 ml 2D water and the rinsing added to the volumetric flask. The flask was brought to volume. The instrument was calibrated and sodium concentration determined (ppm) as described in the electrode manual. Data were converted to water phase sodium chloride (% w/w) using the moisture content and atomic/molecular weights of Na and NaCl.

**Color evaluation.** Color of smoked fillets was measured using the Lab scale with a CR-200 Chroma Meter (Minolta, Japan). Averages of 10 spot readings per section were obtained and the lightness (L), red-green (a) and yellow-blue (b) scores were averaged.

Moisture (24.003) and ash (31.012) were measured using AOAC Methods (7). Analytical measurements were replicated. For each treatment, fillets were identified as front (F), middle (M), or back (B) portions. Only front and middle pieces were analyzed since the back pieces were thinner and narrower than the others and this affected rate of moisture loss and salt penetration in preliminary experiments. Each portion was evenly quartered, and each quarter was split into three layers, top (T), middle (M) and bottom (B). The bottom was the skin side of the fillet. Two of the quarters (diagonals) were analyzed separately in duplicate. Data for different sections were analysed separately and were pooled when there were no significant differences.

**Sensory analysis.** Sensory evaluation was conducted using a likability test (Table I) as described by Larmond (8). Sensory evaluations were performed in the Food Science Sensory Laboratory at the University of Florida. Panelists were faculty, staff and student volunteers. The panel consisted of about equal numbers of men and women and ranged in age from 19-57 (median age 22). The sensory facility consisted of partitioned booths with running water, sinks and adjustable lighting.

Front or middle sections were used for sensory analysis and each panelist received portions of fish cut from the same position on the fillets to avoid differences in location or thickness on acceptability. Fillets were cut into 1.5 cm cubes and panelists received 3 cubes per sample on white plastic plates along with an unsalted cracker and distilled water. Panelists were asked to taste the fish in the order presented (presentation was randomized and three digit coding used). Samples were kept on ice and served immediately when panelists arrived. Three sensory panels were conducted and the data were pooled then statistically analysed.

**Statistics.** Analysis of variance was conducted using Statistica for Windows version 4.5 (Statsoft, Tulsa, OK) running on an IBM compatible personal computer (Zenon Inc., City of Industry, CA). Means were separated where main effects were significant using Tukey's Honest Significant Difference test at  $p < 0.05$ .

## Results and Discussion

**Smoking and fish yield.** The solubility of sodium/potassium phosphate (Carnal 2110) in a 20% NaCl brine at 20°C was better than STPP (data not shown). The solubility of Carnal 2110 was best when solutions were made with stock solutions rather than by direct dissolution. Addition of NaCl before phosphate greatly increased time for dissolving so phosphate was dissolved before salt was added. The solubility of phosphates at lower temperatures was not investigated. Because of solubility, experiments were conducted using Carnal 2110 rather than STPP.

The bullets yielded about  $64.7 \pm 1.2$  % (mean + SEM) meat after skinning and filleting. The fillets obtained were skinless and were trimmed to remove all cartilage. These yields are similar to those obtained for cultured Gulf of Mexico (*Acipenser oxyrinchus desotoi*) sturgeon, which represented recoveries of about 40% of the live weight (9-11). Additional meat could be obtained by deboning the racks. As well, the skin of sturgeon is attractive and would appear to have potential as a decorative "leather".

The average yields of fillets after smoking were  $88.4 \pm 4.6$ % for control fish and  $90.8 \pm 3.7$ % for phosphate treated fish ( $p=0.04$ ). The yields were highest for the thick middle section and lowest for the thinner back (tail) sections (Figure 1). The average moisture contents of the smoked fish are illustrated in Figure 2. There was no significant effect of piece, treatment or any of the interactions ( $p > 0.05$ ). The effect of section was significant ( $p=0.007$ ). Moisture was lowest on the skin side (bottom) and highest in the middle section. The moisture content was consistently slightly higher in the phosphate treated fish, but the variability resulted in a non-significant treatment effect even though recovery (which should closely parallel moisture) was affected by

**Table I. Ballot for 9-point hedonic test****INSTRUCTIONS**

Please evaluate one sample at a time working from the top to bottom.  
 Proceed when you are ready. Please indicate age and sex category.

---

Male	Female		Under 18	18-29	30-44	45-65	>65
------	--------	--	----------	-------	-------	-------	-----

---

**FOR EACH SAMPLE**

Take a bite of cracker and sip water to rinse your mouth. Taste the first sample and circle the number that best describes your opinion of the sample. Repeat for the second sample.

Sample Code \_\_\_\_\_

Dislike  
extremely

Neither like  
nor dislike

Like  
extremely

1      2      3      4      5      6      7      8      9

Sample Code \_\_\_\_\_

Dislike  
extremely

Neither like  
nor dislike

Like  
extremely

1      2      3      4      5      6      7      8      9

---

Describe the reasons for your choice:

Sample Code \_\_\_\_\_

Sample Code \_\_\_\_\_

---

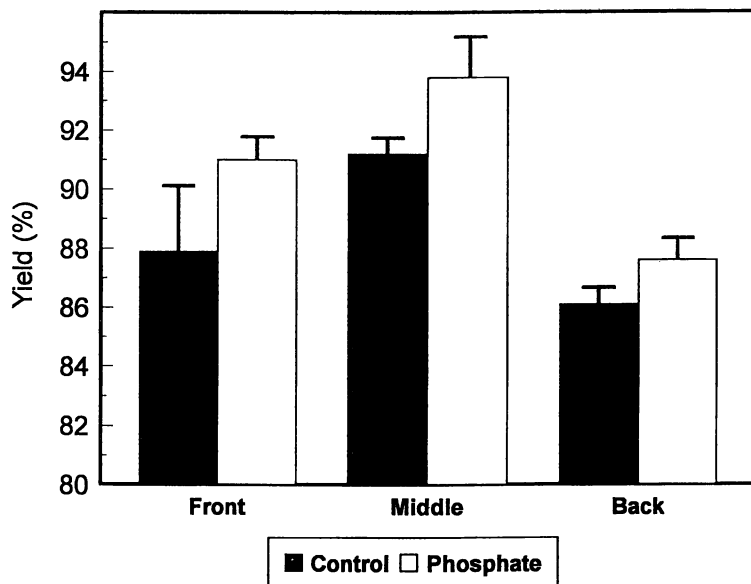


Figure 1. Weight yield of smoked white sturgeon fillets .

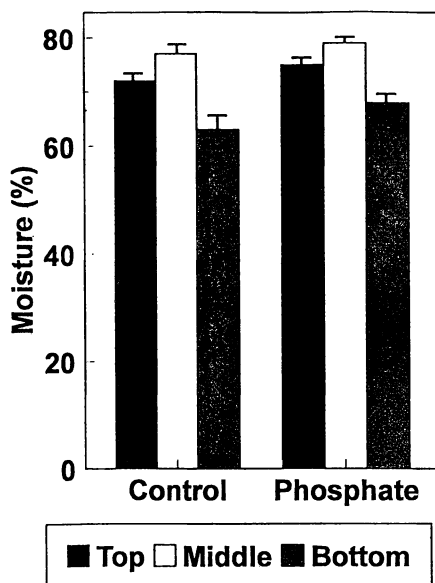


Figure 2. Moisture (% w/w) in smoked white sturgeon fillets .



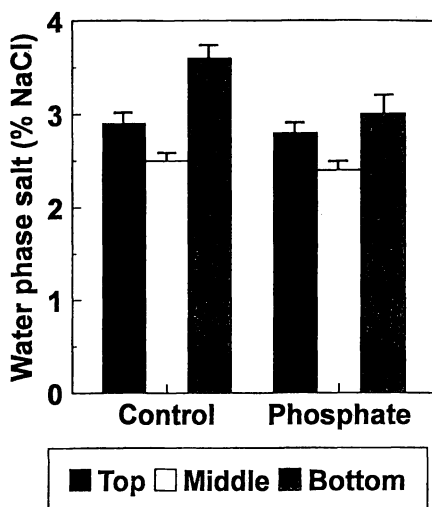


Figure 3. Water phase salt (% w/v) in smoked white sturgeon fillets .

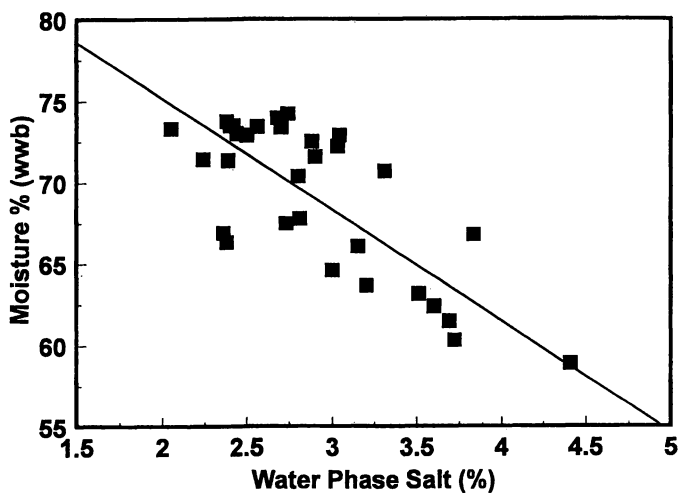


Figure 4. Relationship between moisture and water phase salt in smoked white sturgeon.

treatment. Weight loss was lower than reported for other fish species (6), probably a result of the differences in time-temperature of smoking. Barrett *et al.* (6) smoked for 14 h at 21°C then 1 h at 113°C vs 4 h at 75°C in our experiments.

**Water phase salt.** Water phase salt (Figure 3) was significantly affected by piece ( $p=0.036$ ), treatment ( $p=0.024$ ) and section ( $p=0.000006$ ). The interactive effects of treatment by section ( $p=0.045$ ) and treatment by fish ( $p=0.0057$ ) were significant. These interactions probably result from differences in fish thickness and surface smoothness. The potassium in the Carnal 2110 may have been involved in the decreased penetration of sodium into phosphate treated fish. Although the sodium levels were lower in phosphate treated fish (Figure 3), the differences were relatively minor, except for the bottom section.

The relationship between water phase salt and moisture is illustrated in Figure 4. The negative correlation ( $-0.77$ ) was significant ( $p<0.05$ ). The data points shown represent different portions, sections and treatments. The relationship illustrates the inverse relationship between water phase salt (WPS) and moisture. Regulations requiring a relatively high WPS will result in lower moisture in hot smoked sturgeon. This may result in differences in perceived tenderness, moistness or other desirable sensory attributes, although these quality attributes were not assessed in this study.

**Color.** The color of the sturgeon smoked with phosphate was improved by phosphate treatment. The fish were darker and more evenly colored after phosphate treatment. The L values were  $56.4 \pm 0.27$  for control and  $49.1 \pm 1.0$  for treatment fish ( $p<0.05$ ). As well, there were some significant differences in hue with significantly higher a ( $17.6 \pm 0.46$  vs  $17.0 \pm 0.8$ ) and lower b ( $13.6 \pm 0.86$  vs  $17.9 \pm 0.84$ ) values in phosphate treated fish compared to controls ( $p<0.05$ ). The Lab values would represent a darker, more red-yellow color in phosphate treated fillets compared to controls, which agreed with visual observations. The increased development of color in phosphate treated smoked fish could be related to surface moisture contents since surface wetness will increase smoke adsorption (12). The moisture content on the top section of the phosphate treated fish was not significantly higher than control fish but the moisture at the surface itself was not measured. Other factors such as protein solubilization or electrostatic attraction of smoke particles may also be important.

**Sensory evaluations.** Sensory analysis was conducted on fish from all replications of the experiment. The difference in acceptability between control and phosphate fish was not significant ( $p>0.05$ ) but approached significance at the 5% level with the average acceptability for phosphate treated fish being higher than control. Acceptability testing was used for these preliminary studies to determine whether phosphate treatment had an effect on consumer acceptability of smoked sturgeon. From our studies, there are no statistically significant differences in acceptability of hot smoked sturgeon processed with or without Carnal 2110. However, more panelists preferred the phosphate treated samples over controls in all sensory panels, perhaps because of the higher moisture in the phosphate treated fish.

## Conclusions

Use of Carnal 2110 significantly improves color development in smoked white sturgeon. Yields are improved with phosphate treatment and there is no significant effect on consumer acceptability. Further studies should investigate the microbiological and chemical stability of packaged and stored fillets as well as more detailed sensory evaluations.

## Acknowledgments

The financial support of Budenheim (Chemische Fabrik Budenheim Rudolf A. Oetker, Budenheim, Germany) is appreciated. W. Steven Otwell provided expert advice for various portions of the work.

## Literature Cited

1. Sternin, V.; Dore, I. . Caviar. *The Resource Book*; Cultura: Moscow, Russia, 1993, pp. 256.
2. Doroshov, S.I.; Clark, W.H.; Lutes, P.B.; Swallow, R.L.; Beer, K.E.; McGuire, A.B.; Cochran, M.D. *Aquaculture* **1983**, *32*, 93-104.
3. Hung, S.S.O.; Lutes, P.B. *Aquaculture* **1987**, *65*, 307-317.
4. Molins, R.A. Phosphates in Food. CRC Press: Boca Raton, FL, 1991.
5. Lampila, L.E. *J. Aquat. Food Prod. Technol.* **1992**, *1*, 29-41.
6. Barnett, H.J.; Nelson, R.W.; Dassow, J.A. *Fishery Industrial Research* **1969**, *5*, 103-106.
7. Williams, S. Official Methods of Analysis of the Association of Official Analytical Chemists, 14th Ed., 1984.
8. Larmond, E. *Laboratory Methods for Sensory Evaluation of Food*; Publication No. 1637; Canada Department of Agriculture: Agriculture Canada, ON, 1977.
9. Chapman, F.A.; O'Keefe, S.F., University of Florida, unpublished observations.
10. Chen, I-C.; Chapman, F.A.; Wei, C.I.; O'Keefe, S.F. *J. Food Sci.* **1996**, *61*, 533-535,539.
11. Chen, I-C.; Chapman, F.A.; Wei, C.I.; Portier, K.M.; O'Keefe, S.F. *J. Food Sci.* **1995**, *60*, 631-635.
12. Storey, R.M. Smoking. In *Fish Handling and Processing*; Aitken, A.; MacKie, I.M.; Merritt, J.H.; Windsor, M.L. Eds.; Her Majesty's Stationar: Edinburgh, Scotland, 1982.

## Chapter 16

# Influence of Microenvironment on Oxidative Susceptibility of Seafood Lipids

Marilyn C. Erickson and Ramani V. Sista

Center for Food Safety and Quality Enhancement, University of Georgia,  
Griffin, GA 30223

Lipid microenvironment plays a critical role in dictating the susceptibility of lipids to oxidize with triacylglycerols and membrane lipids of channel catfish serving as the major site of oxidation in early and late stages of storage, respectively. Both mincing and abused storage temperatures ( $-6^{\circ}\text{C}$ ) accelerate the contribution of triacylglycerols to the oxidative process through acceleration of phospholipase activity and subsequent inhibition of phospholipid oxidation. Furthermore, it has been shown that intercellularly distributed ascorbic acid will accelerate oxidation of triacylglycerols while protecting membrane phospholipids. In contrast, intracellularly distributed ascorbic acid did not accelerate oxidation of triacylglycerols but again protected membrane phospholipids. This relationship of antioxidant/lipid proximity to the efficacy of an applied antioxidant needs to be explored further in model systems where oxidant stress is varied. It is advocated that mathematical models of oxidative stability will be strengthened by incorporation of information pertaining to the lipid's microenvironment.

Lipid oxidative reactions in seafood are responsible for the generation of both fresh flavors and off-flavors. Despite this knowledge, little progress has been made in correlating a product's lipid composition to the generation of these flavors. While pro- and antioxidant levels should no doubt also be considered in the product's potential for flavor generation, it is contended that a lipid's microenvironment is the primary force directing lipid oxidative pathways and rates. Typically, however, these microenvironments and their activities are overshadowed by examination of the entire tissue's composition. The objective of this chapter will therefore be to review those studies which demonstrate the

role of microenvironment in the susceptibility of lipids to oxidize and hence generate flavor volatiles. Following these illustrations, a case is made to systematically explore the impact of microenvironment following improvements in design of model systems.

Multiple environments potentially exist in tissue since the compounds involved in lipid oxidation are often distributed nonhomogeneously throughout the tissue. Accessibility of oxygen to all lipid fractions, for example will vary based on location. Hence, in channel catfish, it was demonstrated that oxidation was less in internal environments than it was in surface environments (1).

Location of free fatty acids (FFA) has also been shown to create microenvironmental differences not only in its own susceptibility to oxidize but in the surrounding lipids to oxidize. When FFA are present in depot or bulk lipids, it is generally accepted that they will oxidize more readily than if esterified in that same location (2). On the other hand, if FFA are generated in a membrane environment, structural rearrangements occur that leads to decreased rates of free radical chain (3-4).

The organization state of the lipid itself influences the rate of lipid oxidation. In the case of autoxidation of unsaturated lipids in micelles, oxidizability rates were twice those found in homogeneous organic solutions (5). Increased rates in this situation were attributed to the high local concentration of oxidizable substrate. Similarly, in liposomes, Strahm et al. (6) hypothesized that the membrane arrangement of lipids facilitated propagation reactions. Enhancement of oxidation, however, could be achieved by partial breakup of the liposomal structure with detergent. Apparently, partial breakup of the liposome led to enhanced exposure of the lipids to the dissolved catalyst while leaving the membrane structure intact for enhanced propagation. Further addition of detergent while increasing the exposure of the lipids to catalyst and oxygen, however, decreased the distance between adjacent fatty acids and hence decreased propagation and overall rates of oxidation.

In a few studies, lipid susceptibility has been found to be dependent on the source of oxidant stress. Both cobalt and copper ions have been effective catalysts when emulsions of fatty acids were examined (7), but ineffective catalysts when phospholipid liposomes served as the oxidative substrate (8).

In another study examining the dependence of lipid susceptibility on oxidant stress, three types of initiation were applied to liposomes including the enzymic initiation system of NADPH, ADP and iron, the water-soluble azo compound AAPH and the lipid soluble azo compound AMVN (8). By observing both the tocopherol and TBARS concentrations as the prooxidant varied, different temporal relationships existed between the loss of tocopherol and the observance of lipid. A substantial depletion of about 70% of endogenous tocopherol preceded the propagation phase when induced by either of the azo compounds while only 20% of the antioxidant had disappeared before peroxidation began when induced by the enzymic system.

The ability to identify variations in lipid oxidizabilities within microenvironments of tissues is more difficult than it is in model systems. As a

first step, relative susceptibilities of the lipid classes were identified in frozen minced muscle tissue from two strains of channel catfish (9). In both the samples, a slow accumulation of oxidative products occurred during the first 6 months followed by a dramatic increase in the next 6 months (Figure 1). Examining the tissue tocopherol levels of these samples simultaneously (Figure 2), no tocopherol degradation was statistically seen during the first 3 months of storage, whereas, after 9 months, 75 to 90% of the original tocopherol present had been degraded. In the later stages of storage, it was presumed that the oxidation was accelerated because tocopherol pools had become depleted to a point where they were no longer sufficient to protect membrane lipids. Hence oxidation of phospholipids was ascribed as the major contributor to the exponential accumulation of oxidative products. In contrast, since losses of membrane tocopherol were not seen before 3 months, losses of membrane phospholipids would also not be expected during this period. By deduction then, it was believed that the bulk or depot lipids were the major source of oxidative products seen in early stages of storage.

As confirmation that volatiles were generated from depot lipids early in storage, fatty acid composition of the phospholipid and FFA fractions from the two strains of channel catfish were also examined over the storage periods (Figure 3). Five times more FFA were generated than were lost from phospholipids, implying that the major source of the FFA were the triglycerides. Knowing that FFA hydrolyzed from triglycerides have a greater susceptibility to oxidize than when esterified to the triglyceride (2), it was deduced that it is the FFA pool in the bulk lipids that is the best candidate for the source of oxidative volatiles in 3 month samples. If that is the case, then the accessibility of hydrolytic enzymes to their lipid substrate would be a major factor in the susceptibility of lipids to oxidize with both increases in lipase and phospholipase activity increasing the contribution of bulk lipids and decreasing the contribution of phospholipids as the source of oxidative products.

A study which would support lipid hydrolysis as minimizing the contribution of membrane phospholipids to volatile generation was reported by Brannan and Erickson (10). In this study, minced tissue lipids had increased exposure to oxygen than intact tissue lipids. Under those conditions, oxidative products would have been expected to be generated to a greater degree in the minced material than in intact material. This trend, however, was not observed over the time periods examined. There were no differences between mince and intact fillets up to 5 months and a statistically greater amount of oxidative volatiles were observed at 6 months in the intact fillets than in the mince (Figure 4). Measurement of tocopherol after 6 months, however, saw no differences between these samples. Consequently, oxidation of components at the membrane level, both tocopherol and phospholipids, are believed to have been similar in the intact and minced samples. Increased oxidizability of the bulk lipids, on the other hand, must therefore account for the differences in volatile concentrations at 6 months.

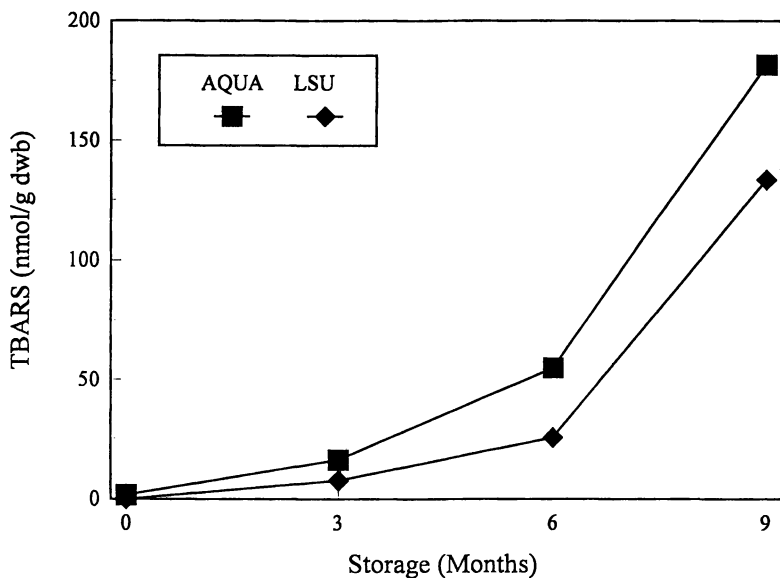


Figure 1. Levels of TBARS in minced muscle tissue of two channel catfish strains during frozen storage. Adapted from Ref. 9.

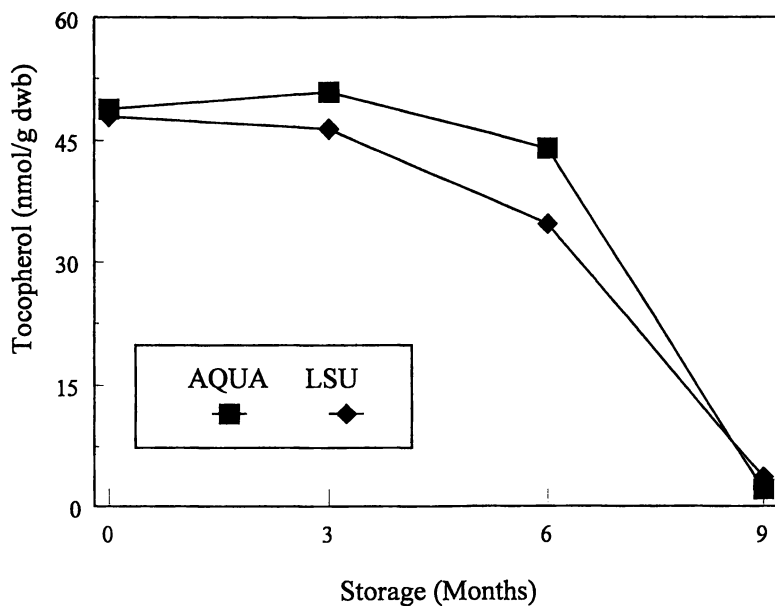


Figure 2. Levels of tocopherol in minced muscle tissues of two channel catfish strains during frozen storage. Adapted from Ref. 9.

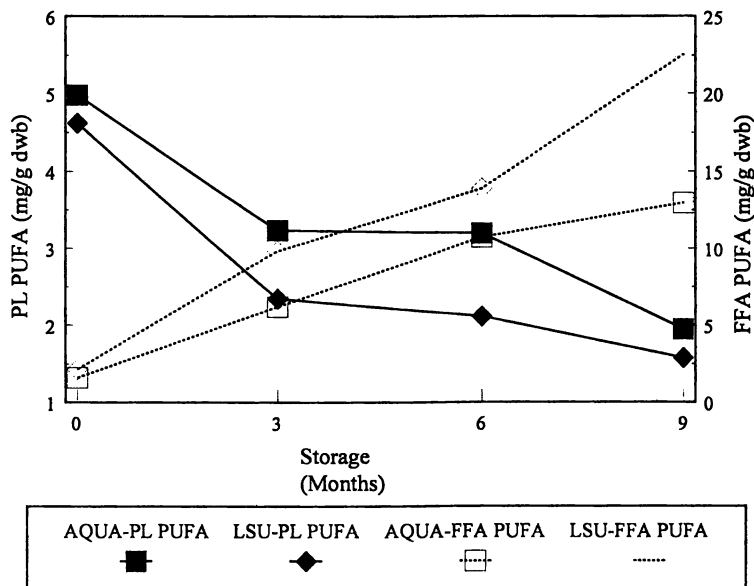


Figure 3. PUFA levels in phospholipid and FFA fractions of minced catfish muscle tissue during frozen storage. Adapted from Ref. 9.

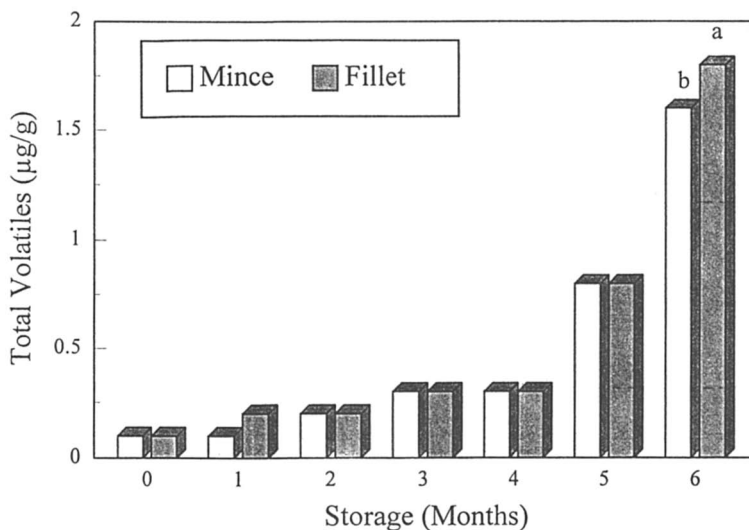


Figure 4. Levels of headspace volatiles in frozen channel catfish muscle tissue. Letters above bars for a given month indicate significant differences ( $P < 0.05$ ). Adapted from Ref. 10.



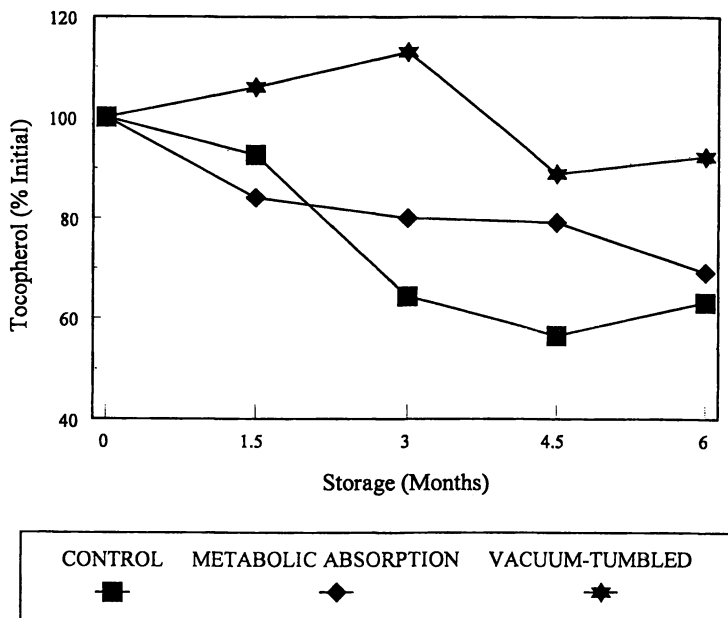


Figure 5. Tocopherol degradation in channel catfish muscle tissue in response to endogenous and exogenous application of ascorbic acid. Adapted from Ref. 13.

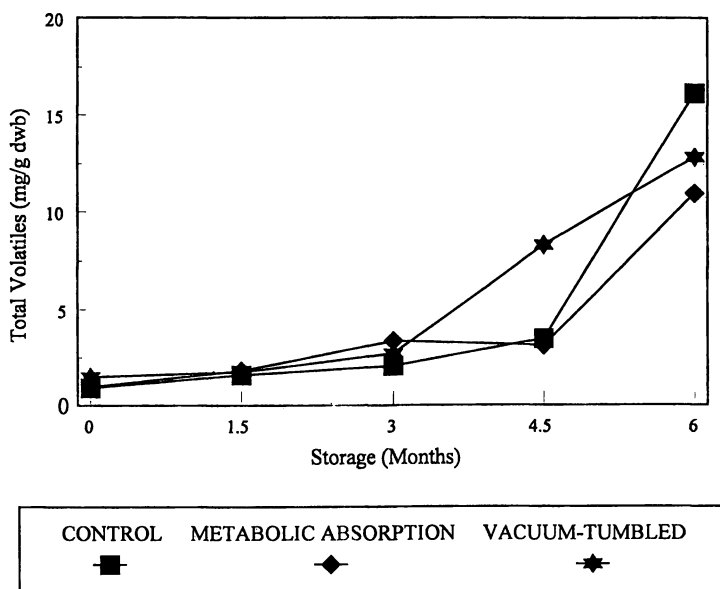


Figure 6. Generation of headspace volatiles in channel catfish muscle tissue in response to endogenous and exogenous application of ascorbic acid. Adapted from Ref. 13.

Another category of compounds which has a major impact on the susceptibility of lipids to oxidize are antioxidants or free radical scavengers. In examining the influence of individual antioxidants, however, two different research groups have observed a phenomenon described as a "polar paradox" (11-12). Briefly, this "polar paradox" effect accounts for the observance that polar antioxidants are more effective toward nonpolar lipids and nonpolar antioxidants are more active in membranes. Differences observed in the efficiency of the antioxidants in the two types of systems have been explained by their affinities toward the air-oil interface in bulk oil and the oil-water interface in emulsion or membranes. To date, the "polar paradox" effect has only been demonstrated in oil and model system studies. Studies in tissue systems, such as fish muscle, is complicated by the structural and cellular compartmentation of the system which limits accessibility of applied antioxidants to all lipids sites.

Application of antioxidants to structurally compartmentalized tissues, in fact, would not be expected to deliver a homogeneous distribution of that antioxidant on a microenvironmental level. Exogenous application, whether it be through injection, dipping, or vacuum tumbling, would lead to intercellular distribution of the antioxidant, that is between the muscle cells. Endogenous application of an antioxidant, on the other hand, could occur through dietary supplementation or water supplementation, in which case metabolic uptake would lead to intracellular distribution or within the muscle cell. Consequently, different responses to these two types of application could be expected were microenvironment a critical factor in oxidizabilities of lipids.

To compare the response of exogenous and endogenous application of antioxidants, Erickson (13) conducted a study using ascorbic acid as the antioxidant. For the exogenous application, vacuum tumbling was the medium chosen and for the endogenous application, metabolic absorption. In metabolic absorption, ascorbic acid was added to the water in which live fish were immersed. Uptake of this antioxidant and distribution to the muscle tissue has been demonstrated (14) and levels 10 times those of initial can be achieved in as little as 24 hours (data not shown). In this particular study, fish were sacrificed when muscle concentrations were twice what they were prior to treatment. A similar final concentration of ascorbic acid was obtained in vacuum tumbled fillets. Fillets from both treatment groups as well as control fillets were then subjected to frozen storage at -6°C for periods from 0 to 6 months.

By observing tocopherol levels of the tissues (Figure 5), it was seen that 30% of the tocopherol was lost by 6 months of storage in control and metabolically absorbed fillets. In contrast, tocopherol levels in vacuum tumbled fillets did not change significantly over storage.

For total oxidative headspace volatiles generated during storage, a lag phase was seen for all treatments (Figure 6). In the case of metabolically absorbed and control fillets, this lag phase extended to 4.5 months, but for vacuum tumbled fillets, it extended only to 3 months. Despite the earlier generation of headspace volatiles in vacuum tumbled fillets, control fillets had the

4.5 MONTHS

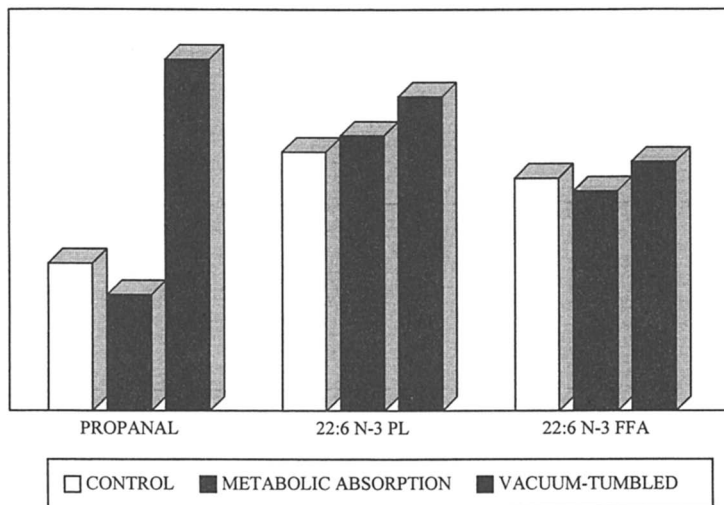


Figure 7. Levels of propanal after 4.5 months frozen storage in relationship to phospholipid and FFA levels of 22:6 n-3 in channel catfish fillets previously treated with endogenous and exogenous applications of ascorbic acid. Adapted from Ref. 13.

6 MONTHS

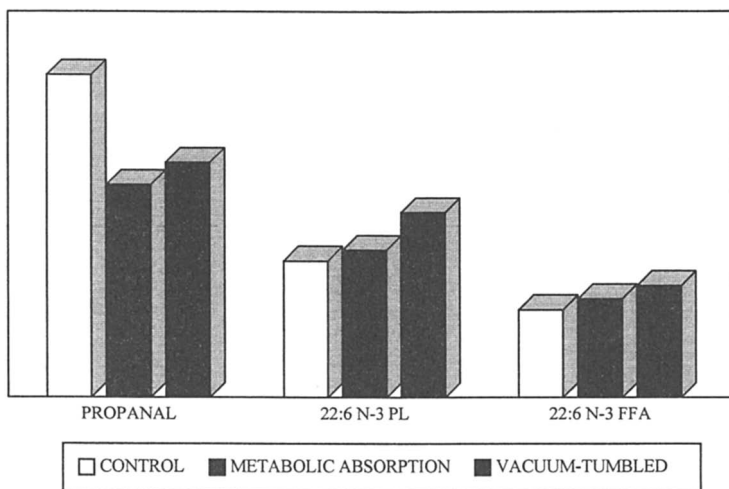


Figure 8. Levels of propanal after 6 months frozen storage in relationship to phospholipid and FFA levels of 22:6 n-3 in channel catfish fillets previously treated with endogenous and exogenous applications of ascorbic acid. Adapted from Ref. 13.

highest concentrations by 6 months of storage due to a faster rate in generation of volatiles over the period of 4.5 and 6 months of storage.

To understand the response to these treatments, fatty acid compositions of each lipid class were also examined in the study of Erickson (13). Large variability in the initial triglyceride contents of the fish samples prevented detecting any differences in fatty acid composition of the triglyceride fraction. For the FFA and phospholipid fractions, however, differences in fatty acid composition between samples were noted. Figure 7 summarizes the 22:6 n-3 fatty acid data for 4.5 month samples while also displaying the data for the specific oxidative volatile, propanal. Like the total volatile data, propanal concentrations were highest in vacuum-tumbled fillets. At the same time, the major n-3 fatty acid from which propanal is generated, 22:6 n-3, was higher in concentration in the phospholipid and FFA fractions of vacuum-tumbled fillets than they were in those fractions of control and metabolically absorbed fillets. Neither of these fractions could be expected to have served as the major site of generation of the propanal in the vacuum-tumbled fillets since the concentrations of 22:6 would have been expected to have been lower in these samples. Membrane tocopherol would also have been expected to have been oxidized in vacuum tumbled fillets were membrane phospholipids being oxidized. By ruling out the phospholipid and FFA fractions, therefore, the site of generation of propanal in 4.5 months samples was presumed to be the triglyceride fraction.

In contrast, examining the 6 month propanal and 22:6 n-3 fatty acid data together (Figure 8), the highest concentrations of propanal were seen in control fillets. At the same time, however, the quantities of 22:6 n-3 in the phospholipid and FFA fractions of control fillets were lower than vacuum-tumbled and metabolically absorbed fillets, implying that one or both of these fractions could have been the major source for propanal generated. The contribution of phospholipids or FFA in control fillets at 6 months and the contribution of triglycerides in vacuum-tumbled fillets at 4.5 months suggests that exogenously added ascorbic acid created a different microenvironment than endogenously added ascorbic acid. Consequently, in changing the microenvironment, the oxidizability of the triglyceride fraction was changed.

To systematically continue in the evaluation of the impact of microenvironment on oxidizability of lipids, model systems are going to be needed to be developed that have the basic features of the real system but in addition have the capability to have well-defined compositions and structural properties. To date, the types of model systems that have been studied as a simulation of tissue systems, include oils, emulsion, liposomes, microsomes, muscle fibers and tissue slices. It may be noted that as the complexity of the system increases, its similarity to the original tissue system increases, but this complexity is at the expense of the ability to manipulate the composition of the system. With tissue slices, for instance, application of catalysts or antioxidants to a medium surrounding a tissue system may occur, but there is no assurance that the compounds are getting to the site they would encounter *in vivo*. In our laboratory, therefore, we are attempting to develop a system that would fall

somewhere between muscle fibers and tissue slices in complexity. Basically this system would use delipidated muscle fibers as its base, after which catalysts, lipids and antioxidants would be added in defined proportions. Where this system would differ from those used previously is in the addition of water to concentrations found in tissue systems, addition of phospholipids as membrane lipids, and gelation of the final system to impart immobility and compartmentation. With this system, we aim to identify first which lipids are the most susceptible to oxidation as the oxidant stress is varied, and secondly to evaluate the ability of antioxidants to protect the susceptible lipids under the imposed microenvironmental conditions. The information generated will subsequently be incorporated into mathematical models of oxidative stability, from which we can derive the conditions for optimal stability.

In summary, it will be necessary to have a thorough understanding of the mechanisms of lipid oxidation and how these are affected by the lipid's microenvironment to develop methods of preventing, or at least retarding lipid oxidation in seafood. In the examples presented in this chapter, it was shown that FFA in the bulk lipid fraction is primarily responsible for oxidative products in minced samples generated during the lag phase. In the exponential phase of oxidation, phospholipids appeared to be the primary contributor to oxidative volatiles generated in minced tissue. As microenvironment of the lipid changed, however, through mincing or application of vacuum-tumbled ascorbic acid, so also did the susceptibility of the lipids to oxidize. Microenvironmental effects, such as the type of oxidant stress, still needs to be clarified and this information will best be obtained from model systems. Ultimately, incorporation of information pertaining to the lipid's microenvironment will strengthen mathematical models of oxidative stability, models which the food manufacturer can use to improve their product's stability or to predict shelf-life.

### Literature Cited

1. Freeman, D.W. and Hearnberger, J.O. *J. Food Sci.*, **1994**, *59*, 60-63, 84.
2. Labuza, T.P., Tsuyuki, H. and Karel, M. *J. Am. Oil Chem. Soc.*, **1969**, *46*, 409-416.
3. Mazeaud, F. and Bilinski, E.. *J. Fish. Res. Board Can.*, **1976**, *33*, 1297-1302.
4. Shewfelt, R.L. and Hultin, H.O. *Biochim. Biophys. Acta*, **1983**, *751*, 432-438.
5. Barclay, L.R.C.; MacNeil, J.M.; Van Kessel, J.A.; Forrest, B.J.; Porter, N.A.; Lehman, L.S.; Smith, K.H.; Ellington, J.E. *J. Am. Chem. Soc.*, **1984**, *106*, 6740-6742.
6. Strahm, A.; Sorensen, J.; Karel, M. *Lebensm.-Wiss.u.-Technol* , **1981**, *14*, 192-197.
7. Wills, E.D. *Biochim. Biophys. Acta* **1965**, *98*, 238.
8. Palozza, P.; Moualla, S.; Krinsky, N.I. *Free Radical Biol. Med.* **1992**, *13*, 127-136.

9. Erickson, M.C. *J. Agric. Food Chem.* **1993**, *41*, 1213-1218.
10. Brannan, R.G.; Erickson, M.C. *J. Agric. Food Chem.*, **1996**, *44*, 1361-1366.
11. Porter, W.L.; Black, E.D.; Drolet, A.M. *J. Agric. Food Chem.* **1989**, *37*, 615-624.
12. Frankel, E.N.; Huang, S.-W.; Kanner, J.; German, J.B. *J. Agric. Food Chem.*, **1994**, *42*, 1054-1059.
13. Erickson, M.C. *J. Food Lipids*, In Press.
14. Thed, S.T.; Erickson, M.R.; Shewfelt, R.L. *J. Food Sci.*, **1993**, *58*, 75-78.

## Chapter 17

# Marine Lipids and Their Stabilization with Green Tea and Catechins

Fereidoon Shahidi<sup>1</sup>, Udaya N. Wanasundara<sup>1</sup>, Y. He<sup>1</sup>, and V. K. S. Shukla<sup>2</sup>

<sup>1</sup>Department of Biochemistry, Memorial University of Newfoundland, St. John's, Newfoundland A1B 3X9, Canada

<sup>2</sup>International Food Science Center, 7 Sonderskovvej, DK 8520 Lystrup, Denmark

Marine Lipids are highly susceptible to autoxidation due to their high content of long-chain omega-3 fatty acids which contain 5 and 6 double bonds. In the process of oxidation during storage, off-flavors and off-odors are produced in fish, shellfish and marine oils. Thus, it is prudent to employ strategies to control oxidative deterioration of such products. Green tea leaves, crude catechin extracts of green tea and individual tea catechins were used to arrest oxidation of ground light muscles of mackerel (GLMM), menhaden oil (MHO) and seal blubber oil (SBO). While ground green tea leaves (GGTL), as such, or their extracts were highly effective in controlling oxidation of GLMM, they exhibited a pro-oxidant effect in marine oils, perhaps due to the catalytic effect of their chlorophyll constituents. The antioxidant activity of individual tea catechin, namely (-) epicatechin, EC; (-) epicatechin gallate, ECG; (-) epigallocatechin, EGC; and (-) epigallocatechin gallate, EGCG, in the systems examined was in the order of  $ECG \approx EGCG > EGC > EC$ .

Seafoods are rich in polyunsaturated fatty acids (PUFA) and play an important role in human health and nutrition. Presence of relatively large amounts of long-chain  $\omega$ 3 fatty acids, especially eicosapentaenoic (EPA, 20:5  $\omega$ 3), docosapentaenoic (DPA, 22:5  $\omega$ 3) and docosahexaenoic (DHA, 22: 6 $\omega$ 3) acids in seafoods and marine oils is responsible for many of their beneficial effects (1). This is because humans cannot easily synthesize these fatty acids and must acquire them through the diet. The beneficial effects of  $\omega$ 3 PUFA have been ascribed to their ability to lower serum triacylglycerol and cholesterol (1). Furthermore, DHA is essential for proper development and functioning of the retina and may play a structural role in nerve and brain tissues. Meanwhile, EPA has been recognized as a precursor to eicosanoids (2) and possesses therapeutic benefits in treatment of coronary heart disease (CHD; 1, 3). Thus, marine lipids play an essential

role in prevention and possible treatment of CHD, diabetes, high blood pressure, and auto-immune diseases (4). Unfortunately, the highly unsaturated fatty acids (HUFA) in seafoods and marine lipids are prone to rapid oxidation (5,6) and thus their protection against such deteriorative processes is mandatory.

The oxidation of HUFA occurs via a free-radical chain mechanism leading to the formation of hydroperoxides, that are considered to be detrimental to health, and proceeds to their degradation to secondary products that produce off-flavors and off-odors. The reactions involved require low activation energies and their rates are not changed significantly by lowering the storage temperatures (7,8). Therefore, antioxidants are often used to control the oxidation of food lipids (9,10).

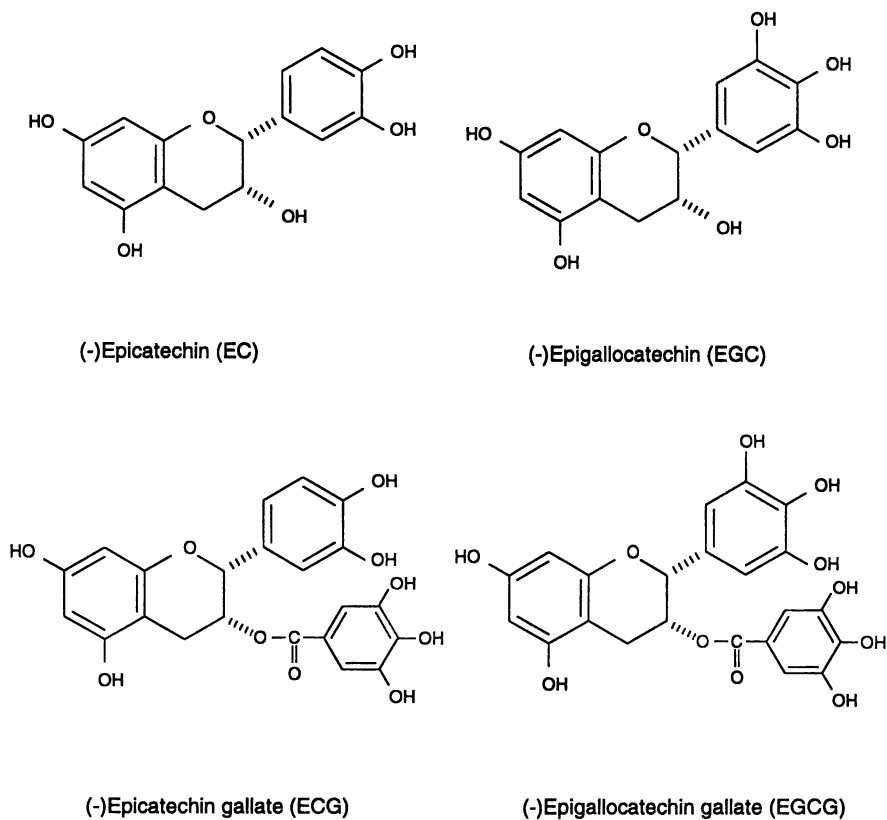
Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and *tert*-butylhydroquinone (TBHQ) have been used for several decades by the food industry. However, there is concern about the use of some synthetic antioxidants due to their potential harmful effects (10). As a result, Food and Drug Administration (FDA) has removed BHA from the list of GRAS (generally recognized as safe) compounds (11). In addition, TBHQ has not been approved for use in Europe, in Japan and in Canada. Thus, natural antioxidants have gained popularity in recent years. Natural antioxidants to stabilize seafoods and marine oils may include tocopherols, other phenolic compounds including flavonoids, as well as phospholipids and polyfunctional organic acids (12,13). Use of rosemary extract is also commonplace. Green tea leaves contain some 25% catechins, namely (-) epicatechin (EC), (-) epicatechin gallate (ECG), (-) epigallocatechin (EGC), and (-) epigallocatechin gallate (EGCG) (Figure 1, 14-16). Tea catechins are considered as being useful food ingredients due to their antimutagenic, antitumorogenic and anticarcinogenic activities (17,18). These compounds, similar to other flavonoids, are efficient antioxidants with the ability to scavenge oxygen radicals and to chelate pro-oxidative metal ions (19-25).

The superior antioxidative properties of crude extracts of green tea in a lard model system has been reported (26). The antioxidant activity of individual catechins, evaluated in the same manner, was in the order of EGCG > ECG > EGC > EG. Recently, Amarowicz and Shahidi (16) and Wanasundara and Shahidi (27) found that green tea catechins were able to delay the onset of oxidation in a  $\beta$ -carotene-linoleate model system as well in marine oils. Thus, dechlorophyllized crude tea extracts and individual catechins may provide a viable alternative to synthetic antioxidants for stabilizing HUFA.

### **Antioxidant Activity of Ground Tea Leaves and their Extracts in Muscle Foods**

In preliminary experiments, green tea leaves were ground and applied to a meat model system, as such, or as extracts in water, absolute methanol, 85% methanol, 95% ethanol and ethyl acetate. The sample used contained  $7.1 \pm 0.1\%$  fat. The meat with or without added green tea leaves or extracts thereof was cooked at  $85 \pm 1^\circ\text{C}$  and then stored at  $4^\circ\text{C}$  for up to 4 weeks. Results given in Table I indicate that green tea leaves were most effective, at 500 ppm, in inhibiting oxidation of the meat system. All extracts had a lower activity than the original sample, thus indicating that either all





**Figure 1.** Chemical structures of green tea catechins.

active components were not extracted or that the extract also contained compounds which did not possess antioxidant activity. The effectiveness of solvents used for extraction of the antioxidative catechins was in the order of MeOH (85%) > MeOH > EtOH (95%) > H<sub>2</sub>O > EtOAc. However, in view of potential presence of residual organic solvents, in subsequent experiments water extracted samples from ground green tea leaves were used.

**Table I.** The 2-Thiobarbituric acid reactive substances (TBARS mg malonadehyde equivalents/kg sample) of cooked meat treated with ground green tea leaves (GGTL) or its extracts and stored at 4°C<sup>1</sup>

GGTL or Extracts (ppm) <sup>1</sup>	Storage period, Days				
	0	1	4	7	28
Control (no additive)	2.07	6.36	8.84	9.61	13.77
GGTL (200)	0.22	0.33	0.43	0.63	3.66
GGTL (500)	0.14	0.14	0.14	0.16	0.57
H <sub>2</sub> O (500)	0.23	0.70	0.88	1.25	4.88
MeOH (500)	0.16	0.33	0.49	0.60	2.78
MeOH (85%, 500)	0.09	0.28	0.36	0.59	2.38
EtOH (95%, 500)	0.36	0.36	0.50	0.86	3.00
EtOAc (500)	0.29	0.91	1.12	1.26	6.48

<sup>1</sup>Addition levels of solvent extracted samples are equivalent values of ground green tea leaves used prior to extraction. Symbols are H<sub>2</sub>O, water; MeOH, methanol; EtOH, ethanol; and EtOAc, ethyl acetate.

In another set of studies, light muscles of mackerel (*Scomber scumbrus*) were separated manually and ground. The samples were cooked with or without additives and stored at 4°C for 14 days. GGTL were used at 200 and 1265 ppm (equivalent to 200 ppm catechin with a 25% catechins in GGTL). The ground light muscles of mackerel (GLMM) contained 10.1 ± 0.04% lipid, of which approximately 2.5% was phospholipid. While the fatty acid composition of the extracted lipids of GLMM, immediately after cooking or 14 days storage at 4°C, remained unchanged, the content of PUFA in the phospholipid fraction of its lipids changed with time (Table II). Furthermore, the 2-thiobarbituric acid reactive substances (TBARS) values of the GLMM sample, without any additive, changed from 8.81 to 12.9 mg/kg over the entire storage period (Table III). Corresponding values for propanal content over a 7 day storage period was increased by a factor of 1.5 (Table IV).

While TBARS values are non-specific, propanal, a breakdown product of omega-3 fatty acids, and hexanal, a breakdown product of omega-6 fatty acids, provide specific information about the oxidative stability of dominant HUFAs of oils. Thus, while propanal serves as an important marker for monitoring the stability of seafoods and marine oils which are rich in omega-3 fatty acids, hexanal may be used as an index of oxidative stability for products with dominance of omega-6 fatty acids, mainly linoleic acid.

**Table II. Changes in the content of selected fatty acids of phospholipid (PL) fraction of ground light muscles of mackerel after cooking**

Fatty acid	Freshly cooked	Cooked and stored
18:3	0.9 ± 0.03	0.4 ± 0.02
20:4	0.7 ± 0.03	0.6 ± 0.03
20:5	10.6 ± 0.49	9.1 ± 0.35
22:5	2.0 ± 0.10	1.6 ± 0.07
22:6	27.6 ± 1.31	23.3 ± 1.10
Total PUFA in PL	43.6	37.0

**Table III. The content of 2-thiobarbituric acid reactive substances (mg malonaldehyde equivalents/kg sample) of cooked ground light muscles of mackerel, treated with ground green tea leaves (GGTL) and its extracts<sup>1</sup>**

Antioxidant (ppm)	Storage time at 4°C, days				
	0	1	5	7	14
Control	8.81	9.41	11.14	12.03	12.90
α-Tocopherol (296)	7.34	7.86	8.89	9.76	9.85
GGTL (1265)	4.09	4.24	4.19	4.25	4.03
P-G (200)	5.05	4.58	5.87	5.58	6.32
P-G (1265)	4.03	3.91	3.75	4.05	4.11
P-30 (200)	5.05	4.92	5.37	5.58	6.22
P-30 (1053)	3.55	3.81	3.69	3.84	3.90
P-60 (200)	4.55	3.82	3.97	4.64	5.28
P-60 (527)	3.73	3.85	3.89	4.05	3.91
NPP-60 (200)	4.14	4.47	4.35	4.19	4.25
NPP-60 (527)	3.51	3.94	3.87	4.09	4.11
EC (200)	5.81	6.13	6.57	6.53	6.30
EGC (211)	3.91	4.11	4.16	4.46	4.23
ECG (304)	3.38	3.39	3.69	3.90	3.59
EGCG (316)	3.29	3.29	3.60	3.84	3.65

<sup>1</sup>Addition levels of solvent extracted samples are equivalent values of ground green tea leaves used prior to extraction. Symbols are: GGTL, ground tea leaves; P-G, 25% crude catechins; P-30, 30% crude catechins; P-60, 60% crude catechins; NPP-60, 60% crude catechins; EC, (-)epicatechins; EGC, (-)epigallocatechin; ECG, (-)epicatechin gallate; and EGCG, (-)epigallocatechin gallate.

**Table IV. Propanal (P) and total volatiles (TV) contents (mg/kg sample) of cooked ground light muscles of mackerel treated with ground green tea leaves (GGTL) and its extracts (values over a 7-day storage at 4°C)<sup>1</sup>**

System	0		1		3		7	
	P	TV	P	TV	P	TV	P	TV
Control	17.1	67.0	20.1	74.1	23.1	79.8	25.4	87.8
GGTL	1.0	4.1	1.1	5.2	1.6	5.8	2.5	8.8
$\alpha$ -Toc	8.4	26.8	9.7	32.3	13.1	48.8	14.1	80.0
P-25	1.0	3.9	1.1	4.6	1.5	6.6	2.1	9.6
P-30	0.6	3.3	0.9	4.5	1.4	6.7	2.6	10.9
P-60	0.6	2.8	1.1	4.4	1.5	7.0	2.3	10.3
NPP-60	0.7	3.3	0.8	4.4	0.9	6.5	2.4	11.0
EC	7.7	27.6	8.2	31.5	9.5	40.0	10.3	38.4
EGC	2.3	13.3	2.8	13.3	3.0	13.3	3.2	14.3
ECG	3.1	18.6	3.3	19.6	3.8	19.2	4.6	24.3
EGCG	2.0	11.7	2.2	13.7	2.7	13.9	3.4	14.4

<sup>1</sup>Addition levels and symbols are as footnoted to Table III; 2-heptanone was used as the internal standard.

The stabilization of GLMM with ground green tea leaves as well as commercially-available crude green tea extracts namely Polyphenon G (25% catechins), Polyphenon 30 (30% catechin) and Polyphenon 60 (60% catechins) (Mitsui Norin Co., Japan) and Nikken Polyphenon 60 (Nikken Foods, Japan) was also studied. Results assembled in Table III indicate that ground green tea leaves (GGTL) as well as Polyphenon extracts, at similar mole content of their catechins, were effective in controlling oxidation of mackerel lipids.

The activity of individual tea catechins was also tested in the mackerel system. The mean activity value of catechins was in the order of EGCG  $\approx$  ECG > EGC > EC. Thus, structural characteristics of catechins play an important role in their activity.

### Antioxidant Activity of Tea Extracts and Catechins in Marine Oils

In preliminary experiments, crude commercial extracts of green tea leaves, namely Polyphenon G (P-G) and Polyphenon 60 (P-60) and Nikken Polyphenon 60 (NP-60) were used to stabilize an unknown refined, bleached and deodorized (RBD) fish oil against oxidative deterioration. All samples tested in this medium exhibited marked prooxidative effect as reflected in peroxide values. It is possible that this prooxidative effect was caused by the presence of chlorophyll in the crude extracts (see Table V).

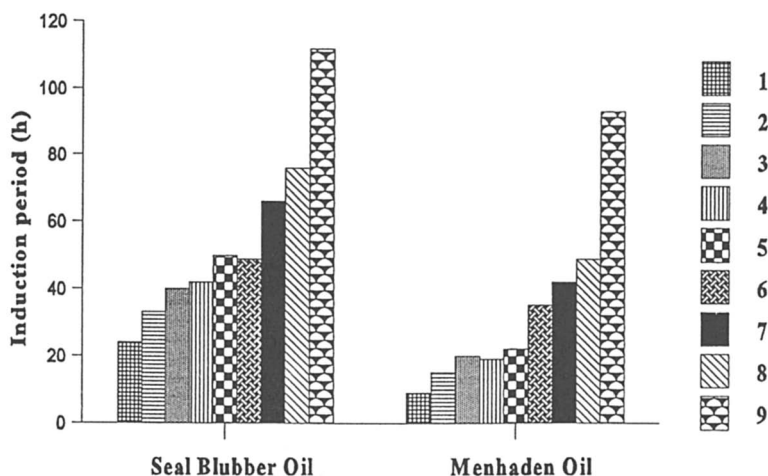
In the follow up experiments, the crude green tea extracts, prepared in the laboratory, were used to stabilize RBD seal blubber oil (SBO) and menhaden oil (MHO). These oils had iodine values of 154.4 and 171.8 g per 100 g oil, respectively. The corresponding content of long-chain omega-3 fatty acids was 20.7% for SBO and 25.6% for MHO. The crude green tea extracts were again pro-oxidative in both systems. Hence complete dechlorophyllization of green tea extracts were necessary so that their catechin components could become effective.

**Table V. Peroxide values (meq/kg) of a treated fish oil with tea extracts and stored at 60°C**

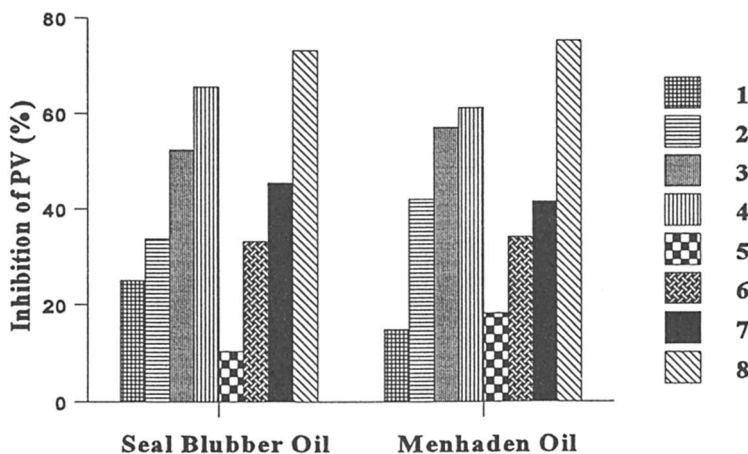
Additive (ppm) <sup>1</sup>	Storage time at 60°C, days			
	0	1	3	7
Control	2.6	11.0	11.2	31.6
Polyphenon G (P-25; 500)	2.6	10.5	17.9	61.8
Polyphenon 60 (P-60; 500)	2.6	8.1	16.9	43.0
Nikken Polyphenon 60 (NPP-60; 500)	2.6	8.2	17.0	40.9

<sup>1</sup> Polyphenons are from Mitsui Norin Co. and Nikken Polyphenon 60 from Nikken Foods.

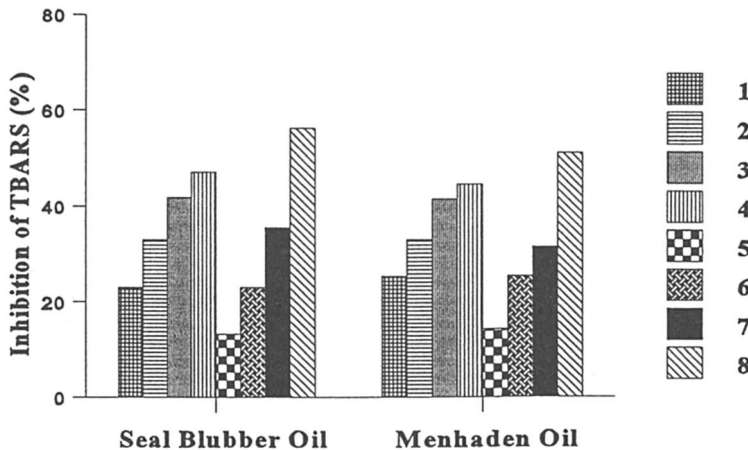
Because of pro-oxidant activity of crude green tea extracts in marine oils, a column chromatographic technique was employed to remove the chlorophylls. The resultant dechlorophyllized green tea extracts (DGTE) were applied to seal blubber and menhaden oils at 100, 200, 500 and 1000 ppm levels. A control sample devoid of any additive and oils containing  $\alpha$ -tocopherol (500 ppm) and *t*-butylhydroquinone (TBHQ, 200 ppm) were used for comparative purposes in this experiment. The time to attain a 0.5% weight gain was considered as the induction period for oxidation to begin. Samples for this study were kept under Schaal oven conditions at 65°C for 144-h. Under these conditions, each day of storage is equivalent to 1 month storage at ambient temperatures (28). Results so obtained are summarized in Figure 2. While the efficacy of DGTE at 200 ppm exceeded that of  $\alpha$ -tocopherol, BHA and BHT, TBHQ at 200 ppm



**Figure 2.** Induction period (0.5% weight gain) of seal blubber oil and Menhaden oil treated with 1, no additive; 2,  $\alpha$ -tocopherol (500 ppm); 3, dechlorophyllized green tea extract (DGTE, 100 ppm); 4, butylated hydroxyanisole (BHA, 200 ppm); 5, butylated hydroxytoluene (BHT, 200 ppm); 6, DGTE (200 ppm); 7, DGTE (500 ppm); 8, DGTE (1000 ppm); and 9, *t*-butylhydroquinone (TBHQ, 200 ppm).



**Figure 3.** Percentage inhibition of formation of hydroperoxide (PV) by dechlorophyllized green tea extract (DGTE) and conventional antioxidants during storage of seal blubber and menhaden oils. 1, DGTE, 100 ppm; 2, DGTE, 200 ppm; 3, DGTE, 500 ppm; 4, DGTE, 1000 ppm; 5,  $\alpha$ -tocopherol, 500 ppm; 6, BHA, 200 ppm; 7, BHT, 200 ppm; 8, TBHQ, 200 ppm.



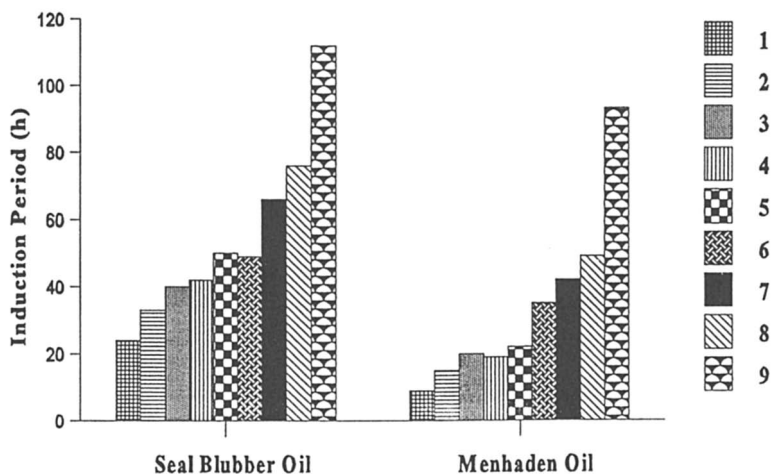
**Figure 4.** Percentage inhibition of formation of 2-thiobarbituric acid-reactive substances (TBARS) by dechlorophyllized green tea extract (DGTE) and conventional antioxidants during storage of seal blubber and menhaden oils. 1, DGTE, 100 ppm; 2, DGTE, 200 ppm; 3, DGTE, 500 ppm; 4, DGTE, 1000 ppm; 5,  $\alpha$ -tocopherol, 500 ppm; 6, BHA, 200 ppm; 7, BHT, 200 ppm; 8, TBHQ, 200 ppm.

was found to be more effective than DGTE, even at a 1000 ppm level. However, on a mole basis, the effect might have been different if catechin content of the extract and its dominant contributor namely (-)epigallocatechin gallate as well as existing differences in the molecular weight of catechins were considered.

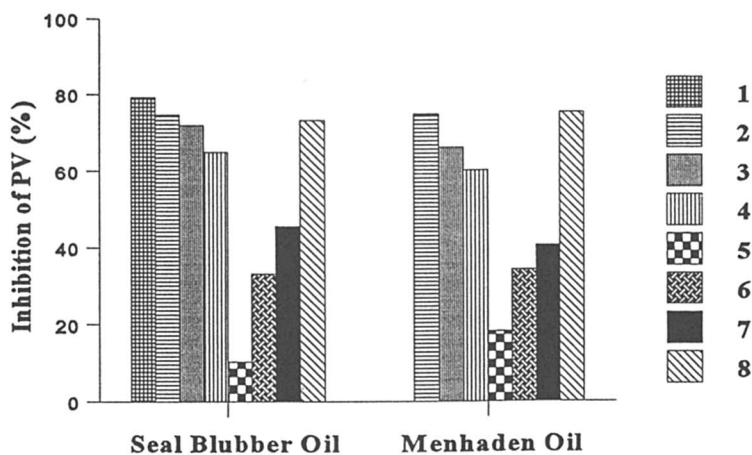
Results summarized in Figures 3 and 4 indicate that inhibition of formation of peroxides (PV) and 2-thiobarbituric acid reactive substances (TBARS), respectively, followed a similar trend to that of the weight gain data. Thus, DGTE may be used as a natural antioxidant for controlling the oxidation of marine oils. Addition of DGTE to marine oils not only protects the oil against oxidation, it would also serve as a beneficial health factor for inhibiting cancer development.

Similar studies were also carried out using individual tea catechins prepared in our laboratory (27), or obtained from Mitsui Norin Co. (Japan). Figure 5 indicates that the induction period, as 0.5% weight gain, for the oxidation of seal blubber and menhaden oils treated with individual catechins and stored at 65°C under Schaal oven conditions was extended considerably as compared with those of the control samples. Furthermore, the activity of TBHQ which is the most effective and commonly used antioxidant was overtaken by that of ECG and EGCG.

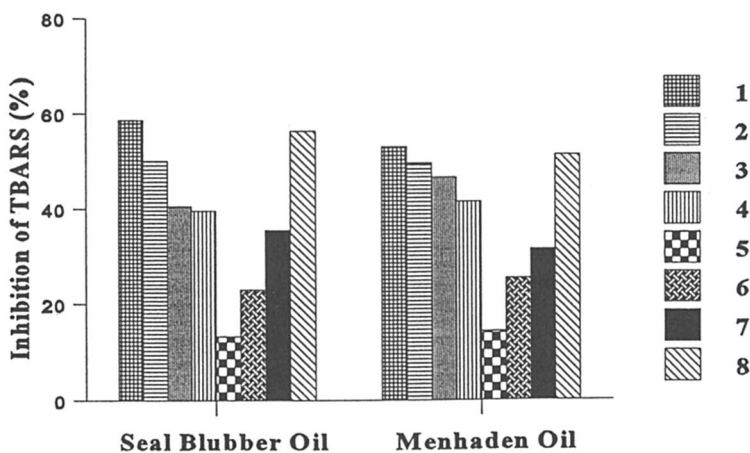
The results for PV and TBARS values of the oils examined, expressed as percent inhibition of their formation are given in Figures 6 and 7. The PV of seal blubber and menhaden oils treated with tea catechins decreases by at least by 60% during their storage. However, samples treated with  $\alpha$ -tocopherol, BHA and BHT showed higher PV as compared to tea catechins under similar experimental conditions. Among tea catechins tested, ECG and EGCG exhibited >50% inhibition of TBARS formation in treated seal blubber oil, whereas for menhaden oil, only ECG-treated samples showed a similar inhibition of oxidation as reflected in the TBARS values. The most commonly used synthetic antioxidants, namely BHA and BHT, were not as effective as tea



**Figure 5.** Induction period (0.5% weight gain) of seal blubber oil and Menhaden oil treated with 1, no additive; 2,  $\alpha$ -tocopherol; 3, butylated hydroxyanisole; 4, butylated hydroxyanisole; 5, (-) epicatechin; 6, (-) epigallocatechin; 7, *t*-butylhydroquinone; 8, (-) epigallocatechin gallate ; and 9, (-)epicatechin gallate at 200 ppm under Schaal oven conditions at 65°C.



**Figure 6.** Percentage inhibition of formation of hydroperoxide (PV) by individual tea catechins and conventional antioxidants during storage of seal blubber and menhaden oils. 1, (-)epicatechin gallate, 200 ppm; 2, (-)epigallocatechin gallate, 200 ppm; 3, (-)epigallocatechin, 200 ppm; 4, (-)epicatechin, 200 ppm; 5,  $\alpha$ -tocopherol, 500 ppm; 6, BHA, 200 ppm; 7, BHT, 200 ppm; 8, TBHQ, 200 ppm.



**Figure 7.** Percentage inhibition of formation of 2-thiobarbituric acid-reactive substances (TBARS) by individual tea catechins and conventional antioxidants during storage of seal blubber and menhaden oils. 1, (-)epicatechin gallate, 200 ppm; 2, (-)epigallocatechin gallate, 200 ppm; 3, (-)epigallocatechin, 200 ppm; 4, (-)epicatechin, 200 ppm; 5,  $\alpha$ -tocopherol, 500 ppm; 6, BHA, 200 ppm; 7, BHT, 200 ppm; 8, TBHQ, 200 ppm.



catechins in inhibiting the formation of hydroperoxides as well as flavor-active secondary oxidation products. Thus, prevention of oxidative deterioration of marine oils may be achieved using individual tea catechins or dechlorophyllized green tea extracts.

In summary, crude tea extracts containing chlorophyll and green tea leaves as such may act as pro-oxidants in marine oils. Nonetheless, the pro-oxidative effects of such additives or their extracts was system dependent. Therefore, green tea leaves and their crude extracts were effective in controlling oxidation of muscle foods.

### Literature Cited

1. Simopolus, A.P. *Am. J. Clin. Nutr.* **1991**, *54*, 438-463.
2. Branden, L.M.; Carroll, K.K. *Lipids* **1986**, *21*, 285-288.
3. Mehta, J.; Lopez, L.M.; Lowton, D.; Wargovich, T. *Am. J. Med.* **1988**, *84*, 45-52.
4. Dyerberg, J. *J. Nutr. Rev.* **1986**, *44*, 125-134.
5. Cho, S.-Y.; Miyashita, M.; Miyazawa, T.; Fujimoto, K.; Keneda, T. *J. Am. Oil. Chem. Soc.* **1987**, *64*, 876-880.
6. Shahidi, F.; Wanasundara, U.N.; Brunet, N. *Food Res. Int.* **1994**, *27*, 555-562.
7. Labuza, T.P. *CRC Crit. Rev. Food Technol.* **1971**, *2*, 355-405.
8. Hamilton, R.J. In *Rancidity in Foods*; Allen, J.C.; Hamilton, R.J., Eds.; Applied Science Pub. New York, **1983**, pp. 1-20.
9. Sherwin, E.A. In *Food Additives*, Branen, R., Ed.; Dekker, New York, **1990**, pp. 139-193.
10. Dziezak, J.D. *Food Technol.* **1986**, *40(9)*, 94-102.
11. Nieto, S.; Garrido, A.; Sanhueza, J.; Loyola, L.A.; Morales, G.; Leighton, F.; Velenzuela, A. *J. Am. Oil Chem. Soc.* **1993**, *70*, 773-778.
12. Dugan, L.R. In *Autoxidation in Food and Biological Systems*, Simic, M.G.; Karel, M., Eds.; Plenum Press, New York, **1980**, pp. 261-282.
13. Wanasundara, U.N.; Amarowicz, R.; Shahidi, F. *J. Agric. Food Chem.* **1994**, *42*, 1285-1290.
14. Shahidi, F.; Ke, P.J.; Zhao, X.; Yang, Z.; Wanasundara, P.K.J.P.D. In *Proceedings of the 38th International Congress of Meat Science and Technology*, August 23-28. Clermont-Ferrand, France, **1992**, pp. 599-602.
15. Ho, C.-T.; Ferraro, T.; Chen, Q.; Rosen, R.T.; Huang, M.-T. In *Food Phytochemicals for Cancer prevention II - Tea, Spices and Herbs*, Ho, C.-T.; Osawa, T.; Huang, M.-T.; Rosen, R.T., Eds.; ACS Symposium Series 547, American Chemical Society, Washington, DC, **1994**, pp. 2-19.
16. Amarowicz, R.; Shahidi, F. *J. Food Lipids* **1995**, *2*, 47-56.
17. Wang, Z.Y.; Cheng, S.J.; Zhou, Z.C.; Athar, M.; Khan, W.A.; Bickers, D.R.; Mukhtar, M. *Mutat. Res.* **1989**, *223*, 273-285.
18. Kim, M.; Hagiwara, N.; Smith, S.J.; Yamamoto, Y.; Yamane, Y.; Takahashi, T. In *Food Phytochemicals for Cancer Prevention II - Tea, Spices and Herbs*, Ho, C.-T.; Osawa, T.; Huang, M.T.; Rosen, R.T., Eds.; ACS Symposium Series 547, American Chemical Society, Washington, DC, **1994**, pp. 51-55.
19. Sorata, Y.; Takahama, U.; Kimura, M. *Biochim. Biophys. Acta* **1984**, *799*, 313-317.
20. Husain, S.R.; Cillard, J.; Cillard, P. *Phytochemistry* **1987**, *26*, 2489-2491.
21. Chen, Y.; Zheng, R.; Zhongjian, J.; Yong, J. *Free Radical Biol. Med.* **1990**, *9*, 19-21.

22. Shahidi, F.; Wanasundara, P.K.J.P.D.; Hong, C. In *Phenolic Compounds in Food and Their Effects on Health I*, C.-T. Ho; Lee, C.-Y.; Huang, M.-T., Eds.; ACS Symposium series 506, Washington, DC, 1991, pp. 214-222.
23. Shahidi, F.; Wanasundara, P.K.J.P.D. *CRC Crit. Rev. Food Sci. Nutr.* **1992**, *32*, 67-103.
24. Shahidi, F.; Yang, Z.; Saleemi, Z.O. *J. Food Lipids* **1993**, *1*, 69-78.
25. Wanasundara, U.N.; Shahidi, F. *Food Chem.* **1994**, *50*, 393-396.
26. Hara, Y., In *Food Phytochemicals for Cancer prevention II - Tea, Spices and Herbs*, Ho, C.-T.; Osawa, T.; Huang, M.-T.; Rosen, R.T., Eds.; ACS Symposium Series 547, American Chemical Society, Washington, DC, 1994, pp. 34-50.
27. Wanasundara, U.N.; Shahidi, F. *J. Am. Oil Chem. Soc.* **1996**, *73*, 1183-1190.
28. Evans, C.D.; List, G.R.; Moser, H.A.; Cowan, J.C. *J. Am. Oil Chem. Soc.* **1973**, *50*, 218-222.

## Chapter 18

# Analysis of Polyunsaturated Fatty Acid Isomeric Hydroperoxides by High-Performance Liquid Chromatography with Post-Column Fluorescence Detection

Toshiaki Ohshima, Hideki Ushio, and Chiaki Koizumi

Department of Food Science and Technology, Tokyo University of Fisheries,  
Konan 4, Minato-ku, Tokyo 108, Japan

Determination of lipid hydroperoxides is important to the understanding of the mechanism of lipid oxidation in seafood. Isomeric hydroperoxides generated from photosensitized oxidation of polyunsaturated fatty acids found in seafood, *i.e.* arachidonic acid, eicosapentaenoic acid and docosahexaenoic acid, were determined by high-performance liquid chromatography coupled with post-column fluorescence detection based on reaction between lipid hydroperoxide and diphenyl-1-pyrenylphosphine. Using this system, it was possible to determine not only non-conjugated diene isomeric hydroperoxides but also isomeric monoene hydroperoxides, both of which are undetectable by ultraviolet absorption.

Lipid oxidation occurring during storage and cooking of food leads to changes in food quality and nutritional value. Unsaturated lipid is easily oxidized to form reactive peroxides such as hydroperoxide as a primary oxidation product. The primary oxidation products often further decompose to derivatives such as malondialdehyde (1). It is still unknown whether malondialdehyde in food is a human health risk (2,3). However, it has been established that certain oxysterols, *e.g.* 25-hydroxycholesterol and cholestane triol, are powerful carcinogens (4,5). Hydroxy fatty acids can contribute bitter tastes (6). Furthermore, oxidized products are volatile and can influence the aroma of foods (7-11). The volatile compounds, especially carbonyls, can react with amino compounds via the Maillard reaction to form colored compounds (12). The peroxides can react with protein to form cross-linked proteins and which leads to changes in texture and functionality of foods (13,14). The peroxides can also react with vitamins and decrease the nutritive value. This may also result in the discoloration of food.

The patterns of volatile compounds generated from polyunsaturated fatty acids are much more complex compared to those obtained from linoleic and linolenic acid oxidation (15). Eicosapentaenoic acid is a characteristic polyunsaturated fatty

acid found in seafood. Its autoxidation is initiated by hydrogen abstraction at four doubly allelic positions to produce eight hydroperoxide isomers (16,17). For each hydroperoxide, there are two positions for homolytic cleavage to generate volatile compounds (18). This is one reason why the pattern of volatile compounds generated from seafood lipid oxidation is so complex. Hydroperoxides are the primary oxidation products and, therefore, are key to the understanding the mechanisms of quality and nutritional changes in foods. Thus, it is necessary to develop a simple method for determination of unstable and reactive isomeric hydroperoxides.

Diphenyl-1-pyrenylphosphine (DPPP) is a fluorescent reagent recently developed by Akasaka *et al.* (19). The DPPP reagent reacts with a hydroperoxy group to form a hydroxy group and a DPPP oxide. The DPPP oxide is excited by UV rays at 352 nm and emits fluorescent light at 380 nm. The oxidation of DPPP is quantitative, and the reaction proceeds in numerous organic solvents, including non-polar solvents such as *n*-hexane (20). Therefore, DPPP is useful for post-column derivatization in HPLC.

In this study, HPLC coupled with DPPP fluorescence detection was applied for the determination of fatty acid hydroperoxides, especially those from polyunsaturated fatty acids common in seafood. The results were compared to previous studies based on GC/MS and HPLC coupled with UV detection to evaluate the efficiency of the present methodology.

### Experimental Procedures

**Lipids.** Methyl oleate, methyl linoleate, and methyl linolenate (all of >99% purity), methyl arachidonate (>90% purity), and docosahexaenoic acid (>99% purity) were purchased from Nu-Chek Prep (Elysian, MN). Cumene hydroperoxide (85% purity) and linoleic acid (>99% purity) were obtained from Sigma (St. Louis, MO). Epadel (a pharmaceutical capsule of ethyl eicosapentaenoate, >99% purity) was purchased from Mochida Pharmaceutical (Tokyo, Japan). Fatty acids and their esters were purified by passage through a Sep-Pak silica cartridge (Waters Associate, Milford, MA) just prior to use (21).

**Chemicals.** Diphenyl-1-pyrenylphosphine was purchased from Tokyo Kasei Kogyo (Tokyo, Japan). Methylene blue trihydrate and NaBH<sub>4</sub> (Wako Pure Chemicals, Osaka, Japan) were of reagent grade. *n*-Hexane of HPLC grade was used after distillation in glass. Diethyl ether of reagent grade was purified by first washing with a ferrous sulfate-sulfuric acid solution to remove peroxide impurities followed by distillation in glass. Methanol, 1-butanol, water and acetone (all HPLC grade) were used as purchased.

**Autoxidation of Lipids.** One hundred mg of linoleic acid or methyl linoleate was dissolved in *n*-hexane and placed in a test tube (10 mm x 130 mm). *n*-Hexane was flash evaporated under a nitrogen stream. Autoxidation was carried out at 35°C in the dark. The autoxidized lipid was dissolved in methanol for reversed-phase HPLC or in *n*-hexane for normal phase HPLC.

**Photosensitized Oxidation of Lipids.** The oxidation of a fatty acid methyl or ethyl ester was done by first sensitizing the compound with methylene blue (1 mg/g ester) in methanol solution with continuous gentle stirring in a jacketed flask cooled to 3°C. The solution was then exposed to a 1000 watt tungsten light source which was first passed through a 10 cm path of water to filter out infrared radiation (22). Methanol was evaporated under a nitrogen stream in the dark and the residual photosensitized-oxidized lipid was dissolved in 25% diethyl ether in *n*-hexane. The solution was filtered through a Sep-Pak silica cartridge to remove the photosensitizer. The eluent was made up to 10 mL with *n*-hexane and 20  $\mu$ L was injected into the HPLC column (23).

**HPLC Coupled with DPPP Detection System.** A schematic diagram of the HPLC system used for detection of fatty acid isomeric hydroperoxides is shown in Figure 1. The HPLC pump was a Shimadzu model LC-9A (Kyoto, Japan). The eluent from the column was passed through a diode array detector (Hewlett Packard model 1050, Boise, ID) to monitor conjugated dienes and then to a stainless steel reaction coil (0.5 mm i.d. and 10 m length for normal phase HPLC or 20 m length for reversed-phase HPLC). The DPPP reagent solution was added at the inlet of the reaction coil using an auxiliary HPLC pump (model LC-9A) and then mixed with the column eluent in a T-connector. The reaction coil was immersed in a water bath heated at 80°C. The eluent from the coil was cooled to room temperature by passing through a stainless steel coil (0.5 mm i.d. and 0.5 m length) immersed in a water bath and then led to a fluorescence spectrophotometer (Shimadzu model RF535). The fluorescence intensity of the DPPP oxide was monitored at 380 nm with excitation at 352 nm (20).

**Determination of Total Fatty Acid Hydroperoxides.** To elute all fatty acid hydroperoxide isomers as a single peak, a C<sub>18</sub> reversed-phase column (Econosphere C18, 4.6 mm i.d. x 250 mm, 5  $\mu$ m, Alltech, Deerfield, IL) was used. A mixture of 400 mL of methanol and 40 mL of water was used as a mobile phase at a flow rate of 1 mL/min. The DPPP solution, 3 mg DPPP in a mixture of 300 mL methanol and 100 mL acetone, was pumped at a flow rate of 0.6 mL/min.

**Detection of Isomeric Hydroperoxides.** The column used for separation of fatty acid isomeric hydroperoxides was a Supelcosil LC-Si (2.1 mm i.d. x 250 mm, 5  $\mu$ m, Supelco, Bellefonte, PA). Mobile phase was a mixture of 34 mL diethyl ether and 500 mL *n*-hexane. The flow rate of the mobile phase was 0.6 mL/min. The DPPP solution was prepared by dissolving 3 mg of DPPP in a mixture of 200 mL of 1-butanol and 200 mL of methanol. The flow rate of the DPPP reagent was 0.6 mL/min (23).

**Identification of Fatty Acid Hydroperoxide by GC/MS.** Isomeric hydroperoxides separated by the normal phase HPLC column and monitored with the diode array detector were fractionated before mixing with the DPPP solution by disconnecting the flow tubing at the inlet of the T-connector below the diode array detector. To suppress formation of secondary oxidation products, 200 ppm butylated hydroxytoluene (BHT) was added to the eluent. The hydroperoxides

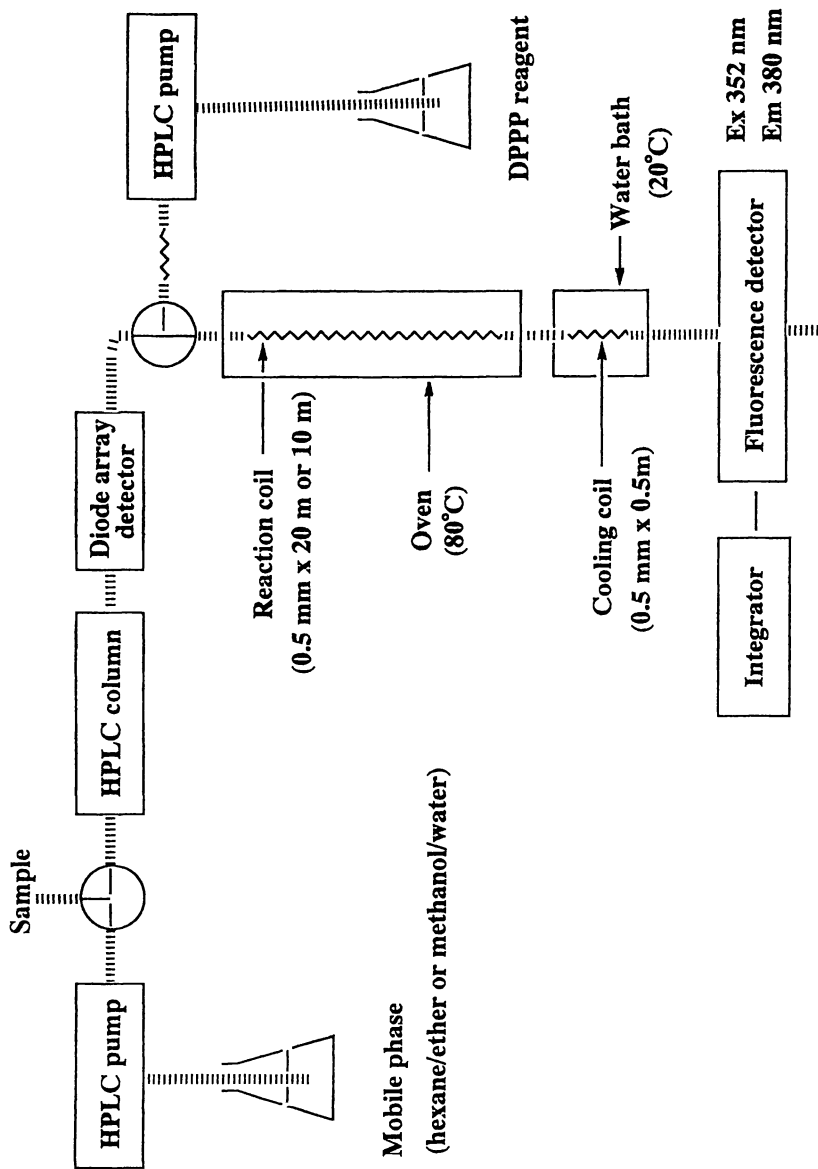


Figure 1. Diagram of the high-performance liquid chromatography coupled with diphenyl-1-pyrenylphosphine detection system.

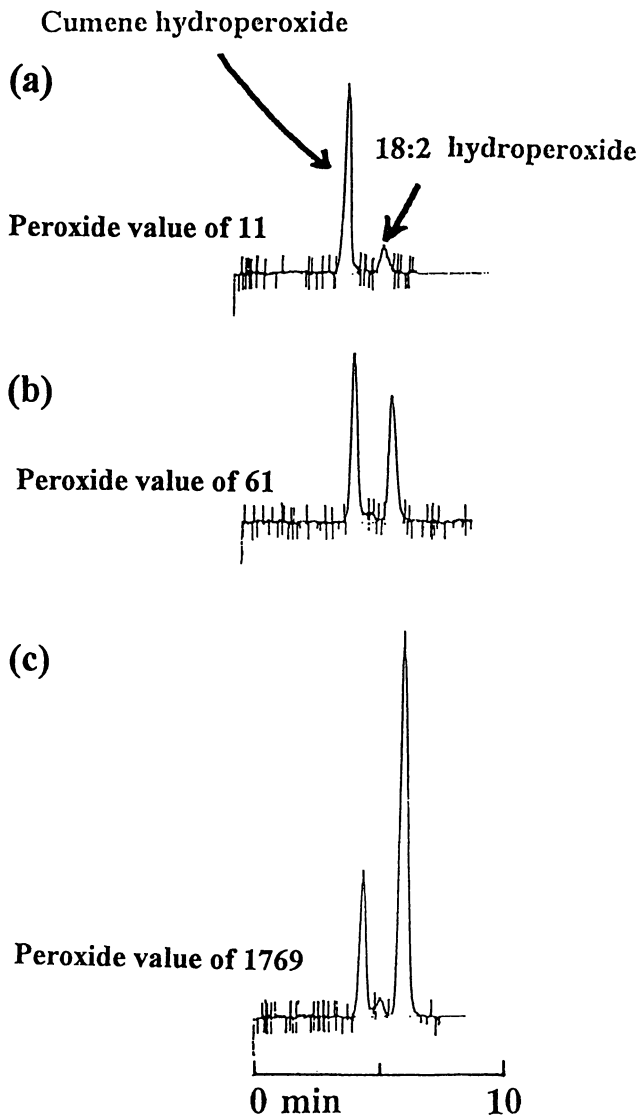


Figure 2. Changes in peak area of linoleic acid hydroperoxides generated from autoxidation by reversed-phase HPLC/DPPP method ((a)-(c)). All isomeric hydroperoxides appear as a single peak. Cumene hydroperoxide was the internal standard.

were reduced to the corresponding hydroxy esters with  $\text{NaBH}_4$  (24), followed by hydrogenation to avoid rearrangement of hydroperoxides, and finally converted to trimethylsilyl (TMS) ether derivatives by mixing with hexamethyldisilazane and trimethylchlorosilane (22).

The TMS derivatives were separated with a gas chromatograph (Shimadzu model GC 17A, Kyoto, Japan) equipped with a Supelcowax-10 fused silica open-tubular column (0.25 mm id x 25 m, 0.25  $\mu\text{m}$  film thickness, Supelco Japan, Tokyo). The outlet of the column was connected directly to an electron impact ion source of a quadrupole mass spectrometer (Shimadzu model QP 5000). The column oven temperature was programmed from 150 to 220°C at a rate of 1°C/min. Injection port temperature was 250°C. Helium was used as carrier gas. Mass spectra were acquired with 3 kV accelerating energy, 70 eV electron beam energy and a source temperature of 260 using a Shimadzu Class-5000 computer system (23).

**Determination of Peroxide Value.** The peroxide value of oxidized lipids was determined by the ferric thiocyanate method (25).

**Statistical Analysis.** Statgraphics software (STCC Inc., Rockville, ML) was used for the statistical analysis of the HPLC data.

## Results and Discussion

### Quantitative Determination of Total Amount of Linoleic Acid Hydroperoxides.

Typical HPLC chromatograms of linoleic acid hydroperoxides generated by autoxidation and using cumene hydroperoxide as internal standard are shown in Figure 2. Following elution of cumene hydroperoxide, four isomeric hydroperoxides generated by autoxidation of linoleic acid eluted as a single peak at 6 min. The peak area of linoleic acid hydroperoxides increased with increasing peroxide values (Figure 2(a)-(c)). There was a significant relationship ( $r^2=0.999$ ,  $n=21$ ) between the ratio of peak heights of linoleic acid hydroperoxides generated by autoxidation to cumene hydroperoxide and the molar ratio of the two components. Detection limit of the hydroperoxides was in the picomole range under the experimental conditions (data not shown).

One application of this methodology is shown in Figure 3. Three systems of linoleic acid with and without different amounts of BHT were subjected to autoxidation at 35°C and changes in the levels of hydroperoxides were determined by the HPLC/DPPP method. These results suggest that this methodology would be useful for determining peroxide values of lipids instead of the traditional iodometric determination.

### Isomeric Hydroperoxides of Methyl Oleate, Methyl Linoleate and Methyl Linolenate.

Four peaks were detected by the HPLC/DPPP method using a normal phase column for photosensitized-oxidized methyl linoleate (23). In this case, four isomeric hydroperoxides, namely 13-*c,t*-, 12-*c,t*-, 10-*c,t*-, and 9-*c,t*-hydroperoxides, should be theoretically produced (26). To identify each isomer, the fractionated lipid sample was reduced by reaction with  $\text{NaBH}_4$ , followed by



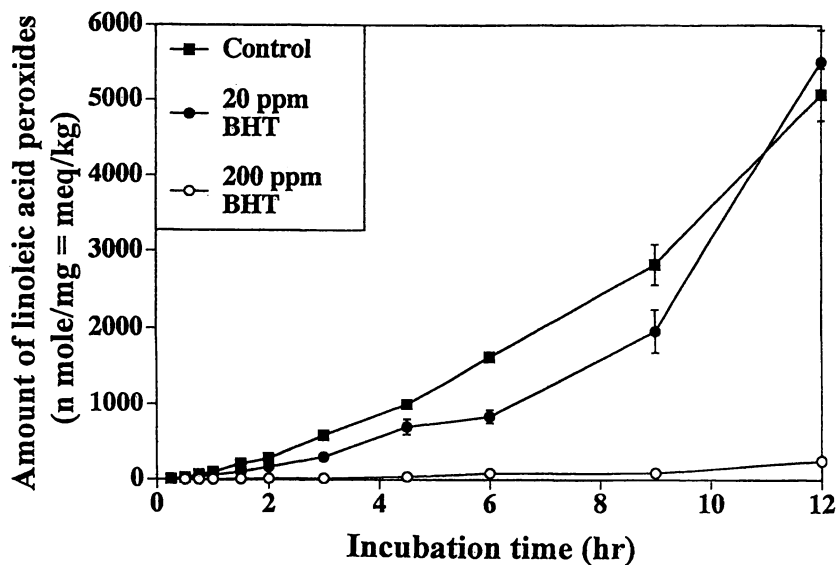


Figure 3. Changes in the levels of linoleic acid hydroperoxides generated from autoxidation of linoleic acid at 35°C in the dark with and without butylated hydroxytoluene as antioxidant. Levels of linoleic acid hydroperoxides were determined by the reversed-phase HPLC/DPPP method.

hydrogenation to avoid secondary formation of artifacts, and then converted to trimethylsilyl ether derivatives of the fatty acid hydroperoxides. These derivatives were analyzed by mass spectrometry. The compound derived from the first peak yielded fragment ions of  $m/z$  173 and of  $m/z$  315. These characteristic fragment ions suggested the corresponding compound was methyl linoleate 13-*c,t*-hydroperoxide (27). The possibility for the determination of non-conjugated dienes is a strong advantage of the HPLC/DPPP method. As shown in Figure 4(a), it not possible to detect non-conjugated diene structures by UV at 234 nm. However, these non-conjugated dienes, 12-*c,t*- and 10-*c,t*- hydroperoxides in this case, were detected by the HPLC/DPPP (Figure 4(b)).

The HPLC/DPPP method is also applicable for the determination of monohydroperoxides generated by autoxidation of methyl linoleate. As shown in Figure 5(a), four positional isomers of monohydroperoxides, all with conjugated diene structures, were detected by UV. Hydroperoxides with *t,t*- configurations, e.g., 13-*t,t*- and 9-*t,t*-isomers, also were detected by the HPLC/DPPP method (Figure 5(b)).

A typical HPLC chromatogram of monohydroperoxides generated by photosensitized oxidation of methyl linolenate is shown in Figure 6. With UV detection, non-conjugated diene hydroperoxides, 15-*c,t*- and 10-*c,t*- isomers, did not appear on the chromatogram (23). However, these non-conjugated diene hydroperoxides could be detected by the HPLC/DPPP method.

Another advantage of the DPPP detection system is that both polyene monohydroperoxides and monoene monohydroperoxides are detectable. Typical HPLC chromatograms of methyl oleate monohydroperoxides generated from photosensitized oxidation are shown in Figure 7((a)-(d)). Two isomeric hydroperoxides of methyl oleate, 9-*c,t*- and 10-*c,t*-hydroperoxides, were not detected by UV (23), but were successfully detected by the HPLC/DPPP method.

Table I summarizes the peak area ratios of methyl oleate hydroperoxides compared with different peroxide values. The peak area ratios of two hydroperoxide isomers generated from photosensitized oxidation were almost 1:1 even though the peroxide values were different from sample to sample. These ratios between isomers coincided well with previously reported results obtained using other analytical methods (21,22,27).

As shown in Table II, the ratios of four isomeric monohydroperoxides of methyl linoleate, namely 13-*c,t*-, 12-*c,t*-, 9-*c,t*- and 10-*c,t*-hydroperoxides which were generated by photosensitized oxidation, were almost 2:1:2:1. These ratios were comparable with those of previous researchers who employed other analytical methods (21,22,28).

Reproducibility of the HPLC/DPPP method was comparable to that of GC/MS analysis in the case of methyl oleate and methyl linoleate hydroperoxides (23) as shown in Table III.

**Isomeric Hydroperoxides of Methyl Arachidonate, Ethyl Eicosapentaenoate and Methyl Docosahexaenoate.** The structures of all eluted peak components of the isomeric methyl arachidonate hydroperoxides generated from photosensitized oxidation were identified (Figure 8). Only hydroperoxides with conjugated structures were detected by UV (Figure 8(a)). In addition to these conjugated

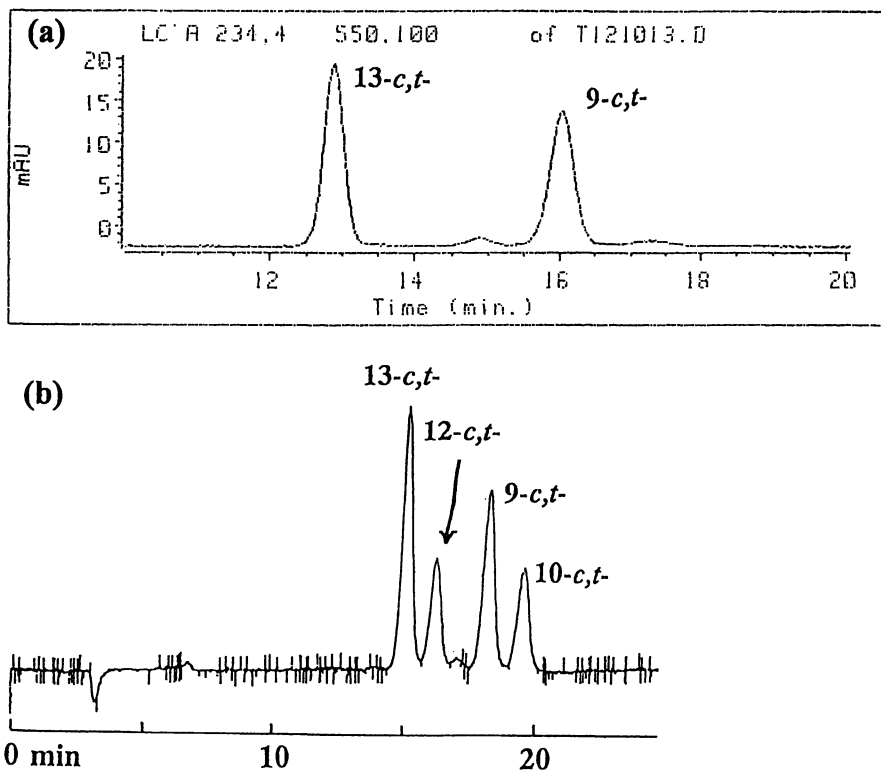


Figure 4. Typical HPLC chromatograms of methyl linoleate isomeric hydroperoxides generated from photosensitized oxidation. Separation was carried out using normal phase HPLC and UV detection at 234 nm (a) or the DPPP detection system (b). (Reproduced with permission from ref. 23. Copyright 1996 The American Oil Chemists' Society).

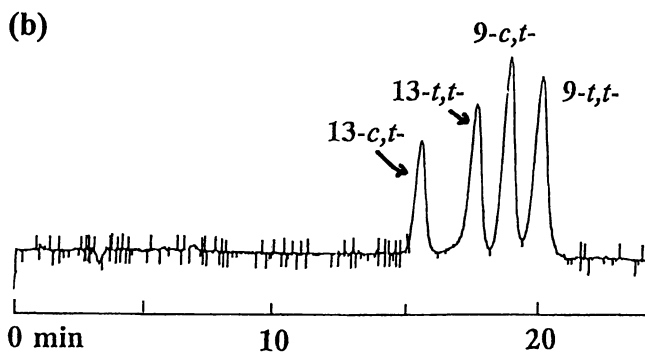
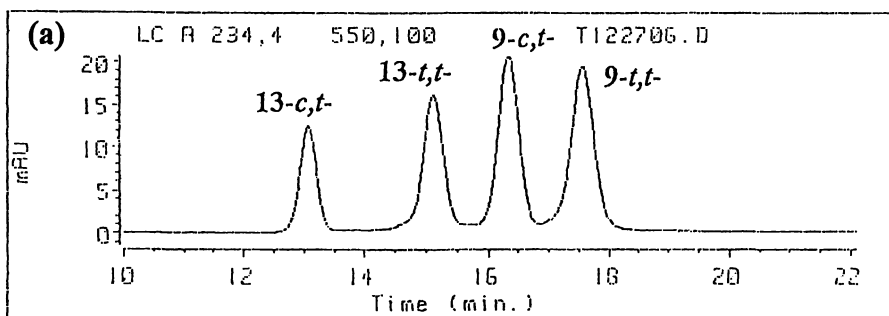


Figure 5. Typical HPLC chromatograms of methyl linoleate isomeric hydroperoxides generated from autoxidation. Separation was carried out using normal phase HPLC and UV detection at 234 nm (a) or the DPPP detection system (b). (Reproduced with permission from ref. 23. Copyright 1996 The American Oil Chemists' Society).

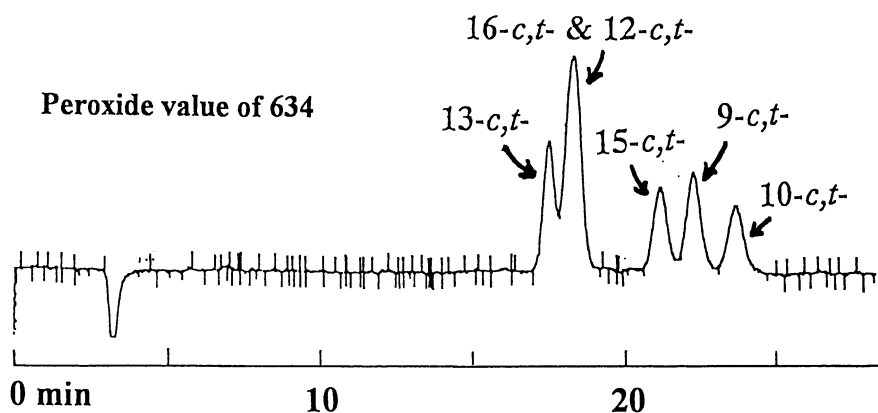


Figure 6. Typical HPLC chromatograms of methyl linolenate isomeric hydroperoxides generated from photosensitized oxidation. Separation was carried out using normal phase HPLC and the DPPH detection system. The photosensitized-oxidized methyl linolenate with a peroxide value of 634 meq/kg was diluted six fold prior to analysis.

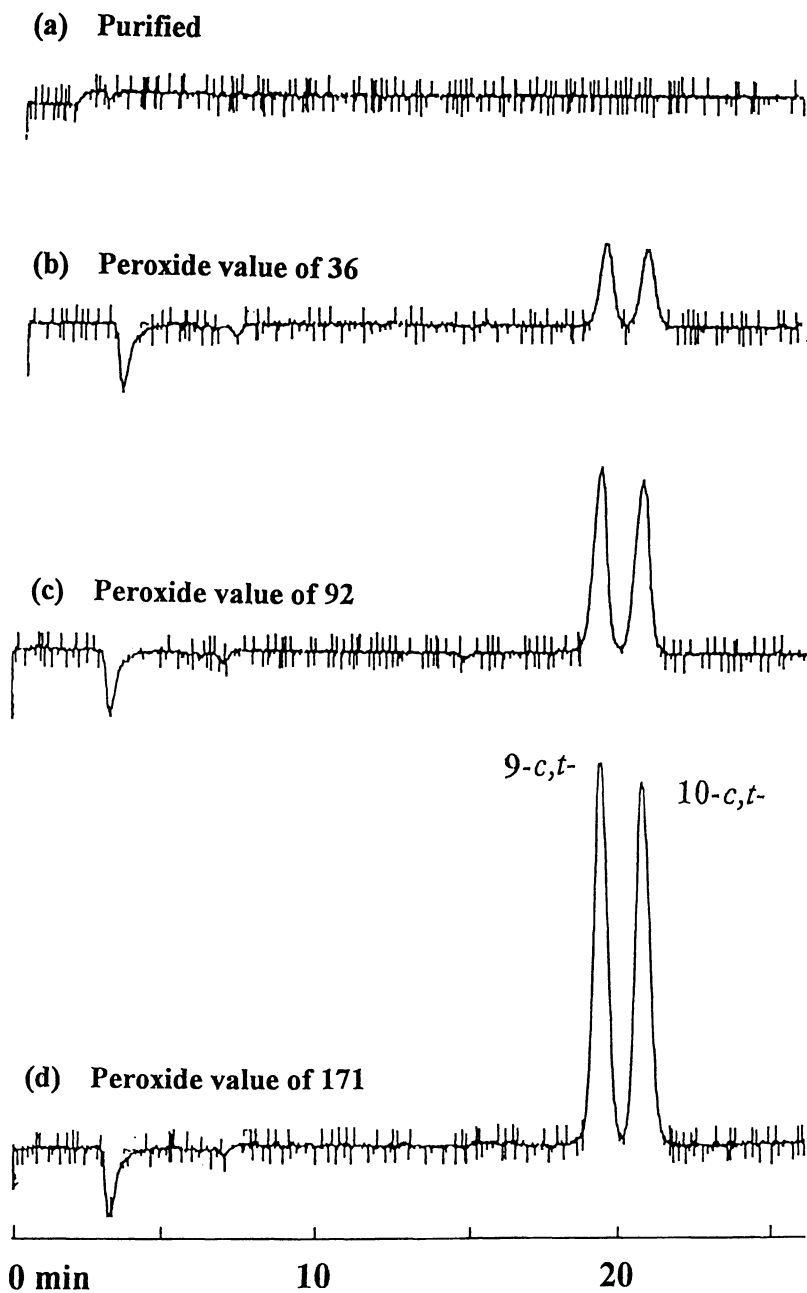


Figure 7. Typical HPLC chromatograms of methyl oleate (a) and its isomeric hydroperoxides generated from autoxidation ((b)-(d)). Separation was carried out using normal phase HPLC and the DPPP detection system.

**Table I. Composition of Isomeric Hydroperoxides Generated from Photosensitized-Oxidized Methyl Oleate**

Method	PV <sup>a</sup>	9- <i>c,t</i>	10- <i>c,t</i>	Reference
HPLC/DPPP	36	50.0	50.0	
	38	47.5	52.5	
	92	51.3	48.7	
	98	50.3	49.7	
	171	50.5	49.5	
	179	52.0	48.0	
HPLC <sup>b</sup>	1727	47.7	52.3	(21)
GC/MS <sup>c</sup>	1727	51	49	(22)
GC/MS <sup>c</sup>	--	50	50	(27)

SOURCE: Adapted from ref. 23.

<sup>a</sup>Peroxide value (meq/kg).

<sup>b</sup>Separated corresponding hydroxystearate and detected by UV at 212 nm.

<sup>c</sup>Analyzed corresponding trimethylsilylated hydroxystearate.

**Table II. Composition of Isomeric Hydroperoxides Generated from Photosensitized-Oxidized Methyl Linoleate**

Method	PV <sup>a</sup>	13- <i>c,t</i> -	12- <i>c,t</i> -	9- <i>c,t</i> -	10- <i>c,t</i> -	Reference
HPLC/DPPP	106	36.2	15.1	33.3	15.5	
	107	34.2	12.8	36.0	17.0	
	163	33.5	17.5	31.5	17.6	
	164	30.3	16.6	33.5	19.5	
	283	30.5	18.5	30.7	20.2	
	274	29.2	17.8	32.4	20.8	
	357	30.8	19.3	29.5	20.4	
	340	29.1	18.3	31.2	21.4	
	HPLC <sup>b</sup>	1124	35	17	32	17
HPLC <sup>c</sup>	--	31.1	20.7	33.3	14.7	(28)
GC/MS <sup>d</sup>	1124	34.5	17.0	31.9	16.7	

SOURCE: Adapted from ref. 23.

<sup>a</sup>Peroxide value (meq/kg).

<sup>b</sup>Separated corresponding hydroxystearate and detected by UV at 212 nm.

<sup>c</sup>Separated corresponding hydroperoxide and detected by chemiluminescence.

<sup>d</sup>Analyzed corresponding trimethylsilylated hydroxystearate.

**Table III. Reproducibility of the Isomeric Hydroperoxide Analyses by HPLC Coupled with DPPP Detection System**

Isomeric Hydroperoxide	Relative Ratio (%) <sup>a</sup>	S.D. <sup>b</sup>
Methyl oleate hydroperoxide, PV <sup>c</sup> =92		
9- <i>c,t</i> -	50.5±0.89	2.1
10- <i>c,t</i> -	49.5±0.89	
Methyl linoleate hydroperoxide, PV=123		
13- <i>c,t</i> -	30.8±0.70	0.5
12- <i>c,t</i> -	19.4±0.18	
9- <i>c,t</i> -	29.3±0.59	
10- <i>c,t</i> -	20.6±0.26	

SOURCE: Adapted from ref. 23.

<sup>a</sup>Mean ± S.D. (n=5).

<sup>b</sup>Standard deviation on HPLC, adapted from ref. 21.

<sup>c</sup>Peroxide value (meq/kg).

hydroperoxides, two isomeric hydroperoxides with non-conjugated diene structures, 14-*c,t*- and 6-*c,t*-hydroperoxides, were detected by the HPLC/DPPP method. Thus, all eight theoretically-produced isomers (29) were detected by the HPLC/DPPP method (Figure 8(b)). In the case of polyunsaturated fatty acids, detection of monohydroperoxides is made difficult because the decomposition rate of the hydroperoxides is higher than the formation rate (Figure 9(a)). However, in the presence of antioxidants such as BHT, monohydroperoxides generated by photosensitized-oxidized methyl arachidonate accumulate gradually (Figure 9(b),(c)). This is probably due to suppression of hydroperoxide decomposition by the antioxidant.

Comparison of UV and DPPP for detection of photosensitized oxidation products of ethyl eicosapentaenoate is shown in Figure 10. Ten isomeric hydroperoxides of ethyl eicosapentaenoate were identified, although some isomers did not separate completely. Here again, non-conjugated isomers, 17-*c,t*- and 6-*c,t*-hydroperoxy ethyl eicosapentaenoate, were not detected by UV at 234 nm (Figure 10(a)). On the other hand, all ten isomeric monohydroperoxides, which theoretically should be produced (16), were detected by the HPLC/DPPP method (Figure 10(b)).

All twelve of the theoretically generated isomeric hydroperoxides of photosensitized-oxidized methyl docosahexaenoate having different peroxide values were detected by the HPLC/DPPP method (Figure 11(a)-(d)). This HPLC detection technology also may be useful for the study of the decomposition mechanism of polyunsaturated hydroperoxides.

There have been many methods proposed for the determination of lipid hydroperoxides. A normal-phase HPLC technique has been applied to separate isomeric hydroperoxides (30). Usually, UV absorption at 234 nm has been used to detect the separated isomeric hydroperoxides (31). However, there are a few



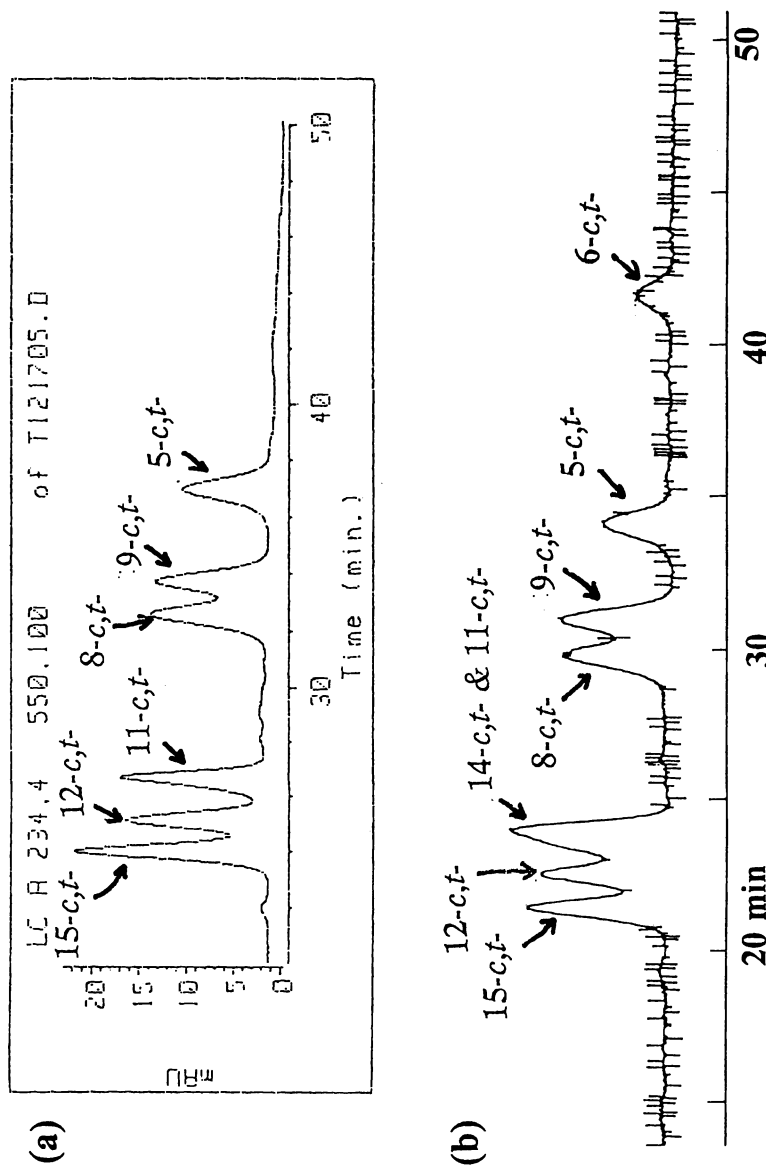


Figure 8. Typical HPLC chromatograms of methyl arachidonate isomeric hydroperoxides generated from photosensitized oxidation. Separation was carried out using a normal phase HPLC and UV detection at 234 nm (a) or the DPPPP detection system (b).

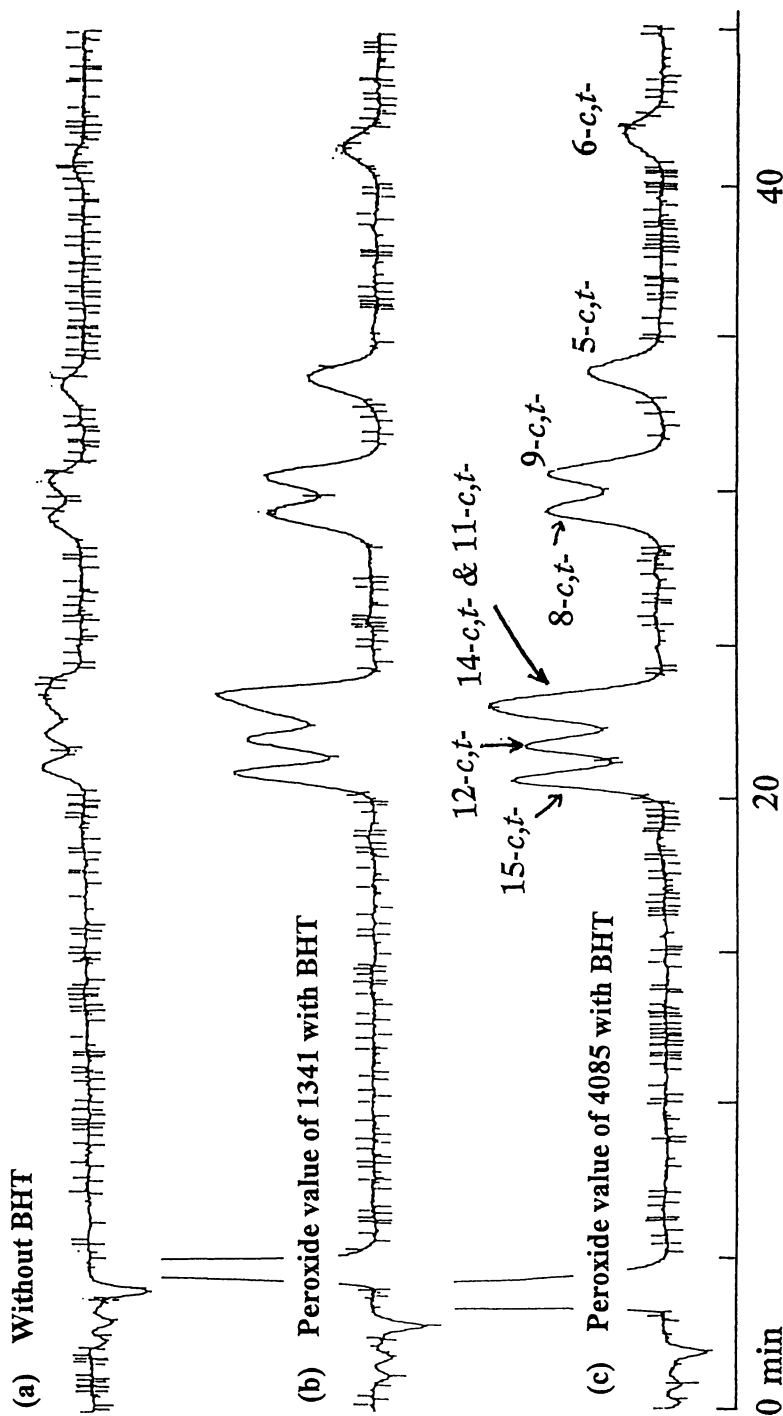


Figure 9. Accumulation of methyl arachidonate isomeric hydroperoxides generated from photosensitized-oxidation in the presence of butylated hydroxytoluene.

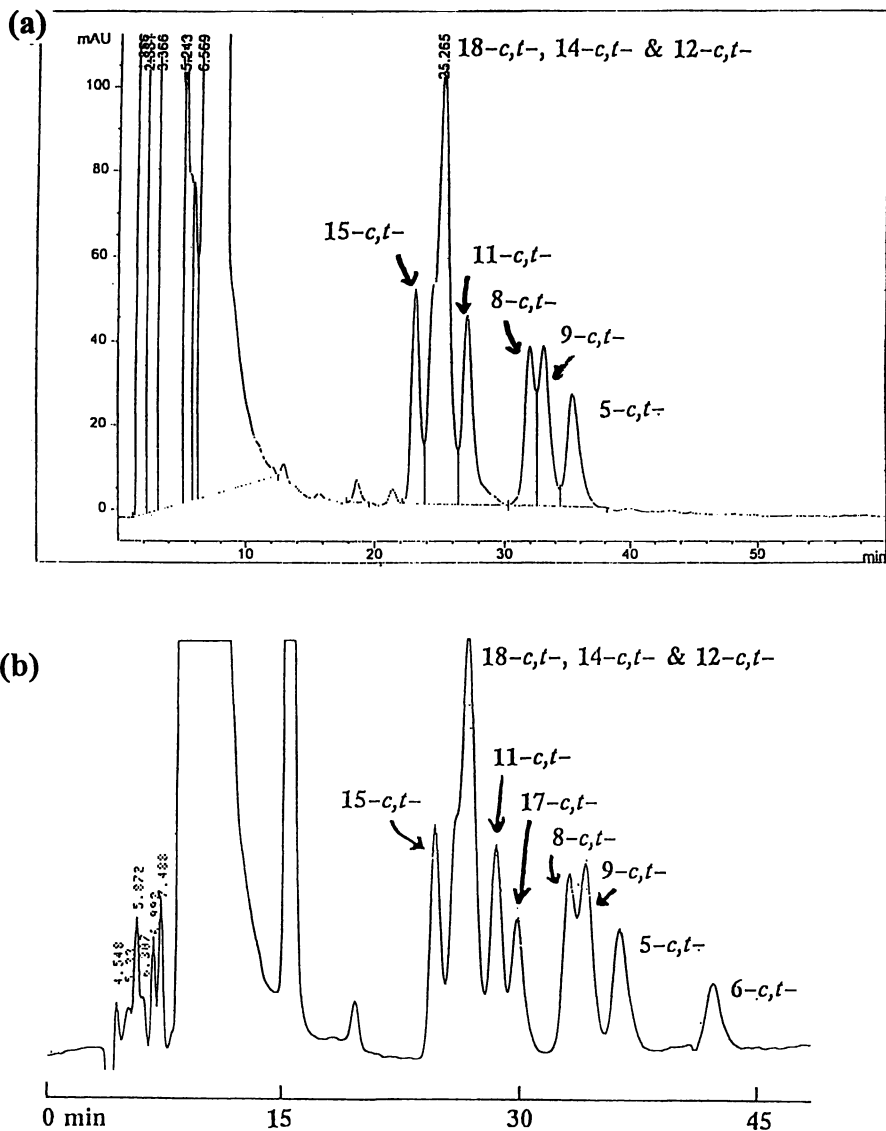


Figure 10. Typical HPLC chromatograms of ethyl eicosapentaenoate isomeric hydroperoxides generated from photosensitized oxidation in the presence of butylated hydroxytoluene. Separation was carried out using a normal phase HPLC and UV detection at 234 nm (a) or the DPPP detection system (b).

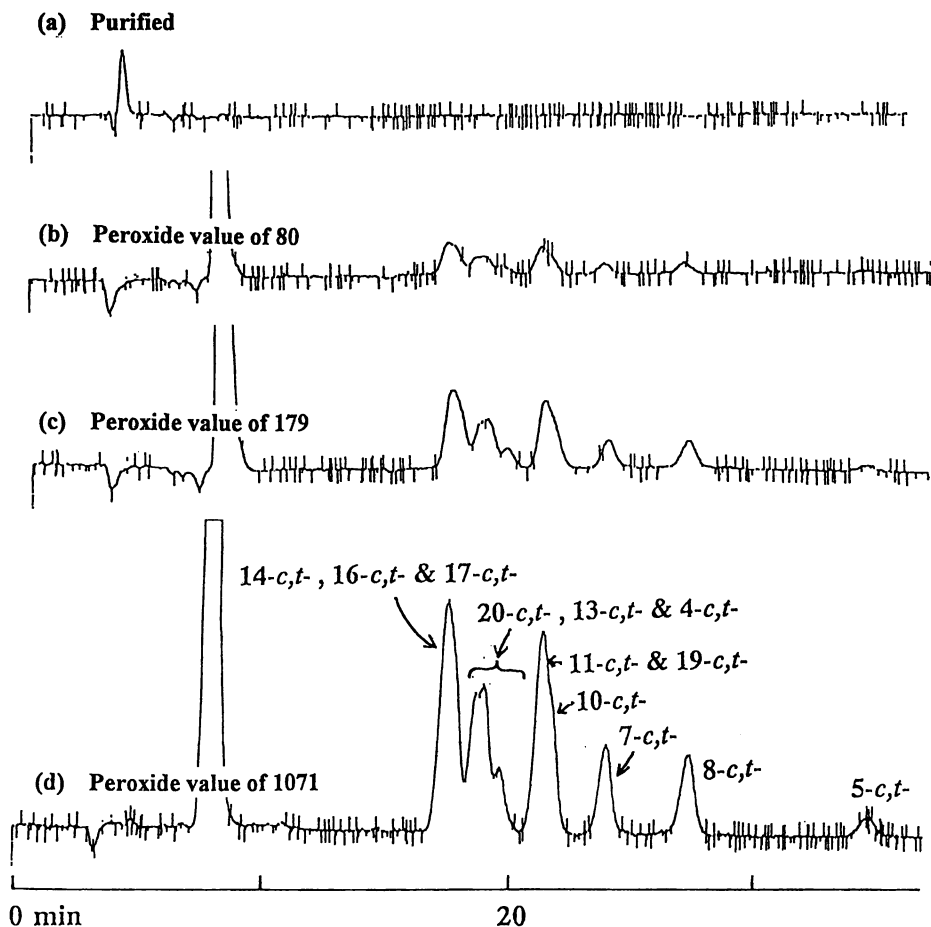


Figure 11. Accumulation of methyl docosahexaenoate isomeric hydroperoxides generated from photosensitized oxidation in the presence of butylated hydroxytoluene. Separation was carried out using a normal phase HPLC and the DPPP detection system.

problems with the UV detection of hydroperoxides. Among eight isomeric hydroperoxides which can be theoretically generated by photosensitized oxidation of arachidonic acid, non-conjugated diene hydroperoxides, such as 14-*c,t*-hydroperoxide and 6-*c,t*-hydroperoxide, are not detectable by UV at 234 nm (32). Furthermore, UV at 234 nm is not specific for hydroperoxides. Hydroxy fatty acids with conjugated diene structures also absorb UV energy at 234 nm (30). Because of this, mass spectrometry has been widely used to identify the structures of hydroperoxide isomers previously fractionated by HPLC methodology (16,24,33,34). This methodology, however, includes many time-consuming pre-treatment steps, such as reduction of hydroperoxides to the corresponding alcohols, hydrogenation, and silylation. Post-column chemiluminescence detection has been recently applied to help solve these problems (35,36). With this system, luminol or isoluminol is oxidized by several kinds of oxygen radicals and excited oxygen. Therefore, this reaction system is not specific for lipid hydroperoxides alone. The formation of radicals from lipid hydroperoxides and cytochrom c is inhibited by several kinds of radical scavengers and this system is not useful for studies on the effects of antioxidants on lipid peroxidation (35).

In conclusion, in the present study we have shown that HPLC coupled with DPPP fluorescence detection is highly specific and sensitive to lipid hydroperoxides. This methodology is also useful for determination of hydroperoxides with non-conjugated structures. Once an HPLC column or configuration is changed, then other hydroperoxide classes such as phospholipids, cholesterol esters, and triglyceride hydroperoxides could be detectable. This means the present HPLC/DPPP method also may be useful for the determination of lipid hydroperoxides in biological systems.

### Acknowledgments

Partial financial support by a Grant-in-Aid from the Ministry of Education, Science, Sports and Culture of Japan is acknowledged. Dr. Akasaka of the Tohoku University provided the DPPP reagent. This study was carried out partly in conjunction with Dr. E.N. Frankel and Dr. B.J. German of the University of California at Davis, and Dr. Anu Hopia of the University of Helsinki.

### Literature Cited

1. Stocks, J.; Offerman, E.L.; Modell, C.B.; Dormandy, TL. *Br. J. Haematol.* **1972**, *23*, 713-724.
2. Reiss, U.; Tappel, A.L. *Lipids* **1973**, *8*, 199-202.
3. Levin, D.E.; Lovely, T.T.; Klekowski, E. *Mutat. Res.* **1982**, *103*, 283-288.
4. Wrensch, M.R.; Petrakis, N.L.; Hauck, W.W.; Craig, J.C.; Goodson, W.H. *Cancer Res.* **1989**, *49*, 2168-2174.
5. Kendall, C.W.; Koo, M.; Sokoloff, E.; Rao, A.V. *Cancer Letter* **1992**, *66*, 248.
6. Baur, C.; Grosch, W. *Z. Lebensm.-Unters. Forsch.* **1977**, *165*, 82-84.
7. Ke, P.J.; Linke, B.A.; Ackman, R.G. *J. Am. Oil Chem. Soc.* **1975**, *52*, 349-353.

8. McGill, A.S.; Hardy, R.; and Gunstone, F.D. *J. Sci. Food Agric.* **1977**, *28*, 200-214.
9. Swoboda, P.A.T.; Peers, K.E. *J. Sci. Food Agric.* **1977**, *28*, 1010-1018.
10. Tressl, R.; Bahri, D.; Engel, K.H. In *Quality of Selected Fruits and Vegetables of North America*; Teranishi, R., Ed.; ACS Symp. Ser. 170.; American Chemical Society: Washington, DC., 1981; pp 213-232.
11. Josephson, D.B.; Lindsay, R.C.; and Stuibler, D.A. *J. Agric. Food Chem.* **1984**, *32*, 1344-1347.
12. Williams, J.C. In *Intermediate Moisture Foods*; Davies, R; Birch, G.G.; Parker, K.J., Ed.; Applied Sci. Publ.: Barking, Essex, 1976; pp 100-119.
13. Schaich, K.M.; Karel, M. *Lipids* **1976**, *11*, 392-400.
14. Logani, M.K.; Davies, R.E. *Lipids* **1980**, *15*, 485-495.
15. Hsieh, R.J.; Kinsella, J.E. In *Oxidation of polyunsaturated fatty acids: mechanism, products, and inhibition with emphasis on fish*; Kinsella, J.E., Ed.; Advances in Food and Nutrition Research; Academic Press: San Diego, CA, 1989, Vol. 33; pp 233-341.
16. Yamaguchi, R.; Yamada, T; Kato, K.; Ueno, Y. *Agric. Biol. Chem.* **1983**, *47*, 2897-2902.
17. Yamaguchi, R.; Yamada, T; Kato, K.; Ueno, Y. *Agric. Biol. Chem.* **1985**, *49*, 3311-3313.
18. Frankel, E.N. *Prog. Lipid Res.* **1983**, *22*, 1-33.
19. Akasaka, K; Suzuki, T.; Ohruai, H.; Meguro, H. *Anal. Lett.* **1987**, *20*, 731-745.
20. Akasaka, K; Suzuki, T.; Ohruai, H.; Meguro, H. *Anal. Lett.* **1987**, *20*, 797-807.
21. Neff, W.E.; Frankel, E.N. *Lipids* **1980**, *15*, 587-590.
22. Frankel, E.N.; Neff, W.E.; Bessler, T.R. *Lipids* **1979**, *14*, 961-967.
23. Ohshima, T.; Hopia, A.; German, B.J.; Frankel, E.N. *Lipids* **1996**, *31*, 1091-1096.
24. Chan, H.W.S.; Levett, G. *Chem. Ind. (London)* **1977**, 692-693.
25. Chapman, R.A.; Mackay, K. *J. Am. Oil Chem. Soc.* **1949**, *26*, 360-363.
26. Frankel, E.N. *J. Am. Oil Chem. Soc.* **1984**, *61*, 1908-1917.
27. Terao, J; Matsushita, S. *Agric. Biol. Chem.* **1977**, *41*, 2401-2405.
28. Kondo, Y.; Kawai, Y.; Miyazawa, T.; Mizutani, J. *Biosci. Biotech. Biochem.* **1993**, *57*, 1575-1576.
29. Terao, J; Matsushita, S. *Agric. Biol. Chem.* **1981**, *45*, 595-599.
30. Neff, W.E.; Frankel, E.N.; Weisleder, D. *Lipids* **1981**, *16*, 439-448.
31. Chan, H.W.S.; Levett, G. *Lipids* **1977**, *12*, 99-104.
32. Coxon, D.T.; Price, K.R.; Chan, H.W.S. *Chem. Phys. Lipids* **1981**, *28*, 365-378.
33. Toyada, I.; Terao, J.; Matsushita, S. *Lipids* **1982**, *17*, 84-90.
34. Van Rollins, M.; Murphy, R.C. *J. Lipid Res.* **1984**, *25*, 507-517.
35. Yamamoto, Y.; Brodsky, M.H.; Baker, J.C.; Ames, B.N. *Anal. Chem.* **1987**, *160*, 7-13.
36. Miyazawa, T.; Yasuda, K.; Fujimoto, K. *Anal. Lett.* **1987**, *20*, 915-925.

## Chapter 19

# Evaluation Method for Lipid Oxidation by Nuclear Magnetic Resonance

H. Saito

Laboratory of Lipid Chemistry, National Research Institute of Fisheries Science,  
Ministry of Agriculture, Forestry, and Fisheries, 2-12-4, Fuku-ura,  
Kanazawa-ku, Yokohama-shi 236, Japan

Oxidative deterioration of oil from marine products was examined using nuclear magnetic resonance (NMR) spectroscopy. Peroxide value (PV) and acid value (AV) are generally used to determine the extent of oxidative deterioration of edible oils from plant and animal sources. However, these methods are not always useful for evaluation of oil deterioration because PV rises at the initial stage of oxidation, reaches a peak and then declines, and subsequently changes little during further storage.

The ratios of olefinic protons (Ro) and diallylmethylene protons (Rm) to aliphatic protons in fish oil, as determined by NMR, decreased steadily with increasing storage time. A comparison of Ro and Rm with PV and AV demonstrated their usefulness as an index of oxidative deterioration of fish oils, particularly in cases where PV is decreasing.

From recent epidemiological, clinical and nutritional studies on animals and humans, it is accepted that fish oils containing high amount of n-3 polyunsaturated fatty acids (PUFA) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) may be responsible for preventing atherosclerosis, cardiovascular diseases, aging, and certain forms of cancer (1-5). With growing recognition of the beneficial uses of dietary fish oils by consumers and industry, the seafood market is expanding considerably. Marine products are an important source of dietary n-3 fatty acids while long-chain n-3 PUFA are generally absent in terrestrial organisms (6).

The marine oils are highly susceptible to atmospheric oxidation, because EPA and DHA have 5 and 6 double bonds, respectively, and are unstable in the presence of oxygen (7, 8). Deterioration of food often causes serious problems, and some food poisonings are attributed to the toxicity of autoxidized fats and their hydroperoxides. Therefore, measurements of the peroxide value (PV) and acid value (AV) are used

as accepted indices for deterioration of edible oils. These indices are suitable for quality evaluation of vegetable and animal fats and may be used for estimating the deterioration of fish oils. In fact, PV of marine oils often exceeds hundreds of meq/kg at the initial stage of oxidation, reaches a peak, and then decreases during the storage (9-14). A low PV indicates either a very early or a late stage of development of rancidity. Hence, quality judgment using a simple PV measurement is sometimes misleading. AV does not change much in processed marine products such as dried fish and fish meal and thus is not useful for assessing oil deterioration. The numerical change in the measurement of AV during the course of storage is not generally significant because it reflects only the amount of fatty acids which are enzymatically generated. The enzymes responsible for lipid hydrolysis are generally lost due to extraction with organic solvents or by drying and heating (15-19). A suitable method for evaluation of oxidative deterioration of fish oils and those in the marine products is urgently required for the fisheries industry.

Investigation on fatty acids and their triacylglycerols using NMR was first reported by Hopkins and Bernstein (20, 21). Recently, various applications of NMR technique for the analyses of fish oils have been expanded (22-30). In this contribution, the use of NMR methodology for estimation of the oxidative deterioration of fish oils during storage and its practical application to oil from marine products such as fish meal and dried fish is also examined.

## Experimental

### Lipid Extraction and Storage Condition.

**Model and Purified Fish Oils.** Purified ethyl eicosapentaenoate (EPA) (95.7% purity) was donated by Nippon Suisan Kaisha Ltd., Tokyo, Japan. Methyl linoleate was purchased from Wako Pure Chemical Industries Ltd., Osaka, Japan.

Fresh sardine (*Sardinops melanostictus*) and pollack (*Theragra chalcogramma*) were purchased from the central whole fish market at Tsukiji in Tokyo, Japan. Crude fish oils were extracted with a mixture of chloroform/methanol (2:1, v/v) according to the procedure of Folch *et al.* (31). The purified neutral oils were separated from the crude oils with silicic acid (Merck and Co. Ltd., Kieselgel 60, 70-230 mesh) column chromatography by elution with a mixture of dichloromethane and diethyl ether (10:1, v/v). Both the model purified neutral fish oils were allowed to stand in the dark at 40°C.

**Fish Meal.** Sardine press cake was prepared in the ordinary way by pressing thoroughly boiled fish (*Sardinops melanostictus*) with a hydraulic press. The moisture and lipid contents of the press cake were 29.4 and 10.8%, respectively. The press cake was crushed with a meat chopper and dried in two different ways without addition of any antioxidant. Meal I was prepared by drying under forced ventilation at 80°C for 2 h; Meal II was prepared by heating in static air at 90°C for 5 h. The meals were allowed to stand at 20°C in contact with air during the experiment and their oils were extracted with a mixture of chloroform/methanol (2:1, v/v) as described by Folch *et al.* (31).



**Boiled and Dried Fish.** Two different samples of Niboshi were prepared by boiling juvenile sardines (*Sardinops melanostictus*) and anchovies (*Engraulis japonica*) for 30 min and drying under forced ventilation at 50°C for 18 h. The samples were pulverized with a meat chopper into fine uniform granules and left at 20°C in contact with air throughout the experiment for about 30 days, and the oil was periodically extracted from each pulverized sample as described earlier for fish meal.

**Salted Dried Fish.** *Scomber japonicus*, *Trachurus japonicus*, and *Cololabis saira*, commonly called mackerel, Japanese horse mackerel, and Pacific saury, were purchased from the wholesale fresh market at Tsukiji in Tokyo. Fish were cleaned and filleted prior to salting; fillets were immersed in 20% brine for up to 15 min at 25°C. After water was drained off, the salted fillets were dried under forced ventilation at 35°C for 3h. The samples prepared in the laboratory were tentatively called experimental models. The ordinary salted dried fish (mackerel, horse mackerel, and Pacific saury) were purchased from retail outlets at Tsukiji, and called ordinary market products and used for the storage experiments. The experimental models and the ordinary market products were allowed to stand at -10°C and -5°C, respectively, and the oil in every sample was extracted similar to that used for fish meal.

**Analytical Methods.** The ratios of olefinic ( $\delta$  5.1-5.6 ppm) and diallylmethylene protons ( $\delta$  2.6-3.0 ppm) to that of aliphatic protons ( $\delta$  0.6-2.5 ppm) were designated Ro and Rm, respectively. The NMR data as well as PV and AV were periodically measured during storage of the samples, and the relationships between the Ro and Rm with PV were examined during the active oxygen absorption period.

**PV, AV, and carbonyl value (CV) Measurements.** PV and AV were determined according to the Official Methods and Recommended Practices of the American Oil Chemists' Society (32, 33). CV was determined according to the procedure of Kumazawa and Oyama (34).

**Measurement of NMR.** Spectra were recorded on a JEOL GSX-270 NMR spectrometer in the pulsed Fourier transform mode at 270 MHz in deuterated chloroform using tetramethylsilane (TMS) as an internal standard (the frequency length, 3001.2 Hz; the digital resolution, 0.18 Hz; pulse, 90°). A solution containing the oil (about 20.0 mg) from marine products in CDCl<sub>3</sub> was introduced into a 5-mm NMR sample tube, and the spectra were recorded for a total of 16-64 readings of 10 sec pulse delays, to attain accurate equalization and integration. Since concentration of fish oil (20.0 mg in 0.4 ml of deuteriochloroform) is high enough, the signal of noise is negligible. The error in the integral calculation was below 0.2% in every Ro and Rm even though the integration was repeated more than ten times.

**The Preparation of Methyl Esters and Gas-liquid Chromatography (GLC) of the Esters.** The purified neutral fish oils (triacylglycerols) were converted to fatty acid methyl esters by direct transesterification with boiling methanol containing 1% concentrated hydrochloric acid with reflux for 1.5 h under argon atmosphere,

respectively. The methyl esters so obtained were purified by column chromatography using silica gel (Merck and Co. Ltd., Kieselgel 60, 70-230 mesh) and elution with a mixture of dichloromethane and diethyl ether (10:1, v/v). Analysis of fatty acid methyl esters was performed on a Shimadzu GC-8A gas chromatograph (Shimadzu Seisakusho Co. Ltd., Kyoto, Japan) equipped with a DEGS (15% diethylene glycol succinate in chromosorb W, mesh 80-100) packed columns (2m (2.6 mm i. d., GL Science Co. Ltd., Tokyo, Japan). The temperatures of the injector and the column were held at 250 and 190°C, respectively. Nitrogen was used as the carrier gas at a constant inlet rate of 40 mL/min. Quantitative analyses were performed on capillary columns by means of a Shimadzu Model C-R3A (Shimadzu Seisakusho Co. Ltd, Kyoto, Japan) electronic integrator.

### Results and Discussion

**Autoxidation of PUFA.** The autoxidation of PUFA is a free radical chain reaction, in which hydroperoxides are produced as the primary products. Hydroperoxides of PUFA are easily decomposed to a complex mixture of secondary products with the decrease of olefinic protons. Finally, cyclic and/or decomposition products of hydroperoxides such as alcohols and ketones are produced (7, 8). For example, in linolenic acid with three double bonds, six olefinic protons are ultimately reduced to two protons or less at the final stage of these reactions, while aliphatic protons, relatively stable against the attack by the radicals, are hardly reduced (7).

**The Characteristic of the Peaks in NMR Measurement.** NMR absorption peaks of the olefinic protons of fatty acids in fish oils appeared at 5.1-5.6 ppm as compared to their relative position to TMS (0.00 ppm) used as an internal standard. The number of protons could be easily calculated by integrating the area under each peak. For example, ethyl eicosapentaenoate (EPA) has ten olefinic protons at  $\delta$  5.38 ppm (multiplet), eight diallylmethylene protons at  $\delta$  2.84 ppm (multiplet), and sixteen aliphatic protons from  $\delta$  0.6 to 2.5 ppm.

According to the mechanism of autoxidation, the ratios of olefinic ( $\delta$  5.1-5.6 ppm) and diallylmethylene protons ( $\delta$  2.6-3.0 ppm) to that of aliphatic protons ( $\delta$  0.6-2.5 ppm) in NMR decreases during oil oxidation. The ratios were designated "Ro" and "Rm," respectively.

$$R_o (\%) = (\text{Olefinic protons}/\text{Aliphatic protons}) \times 100$$

$$R_m (\%) = (\text{Diallylmethylene protons}/\text{Aliphatic protons}) \times 100$$

where olefinic, diallylmethylene, and aliphatic protons are the relative numbers obtained from the integration calculation of their peaks in the NMR, respectively.

**Estimation of the Oxidation of Model and Natural Fish Oils by NMR.** At first, the model fish oils, the various mixtures of ethyl eicosapentaenoate and methyl linoleate, were examined. The model oils were allowed to stand at 40°C, and both PV and NMR

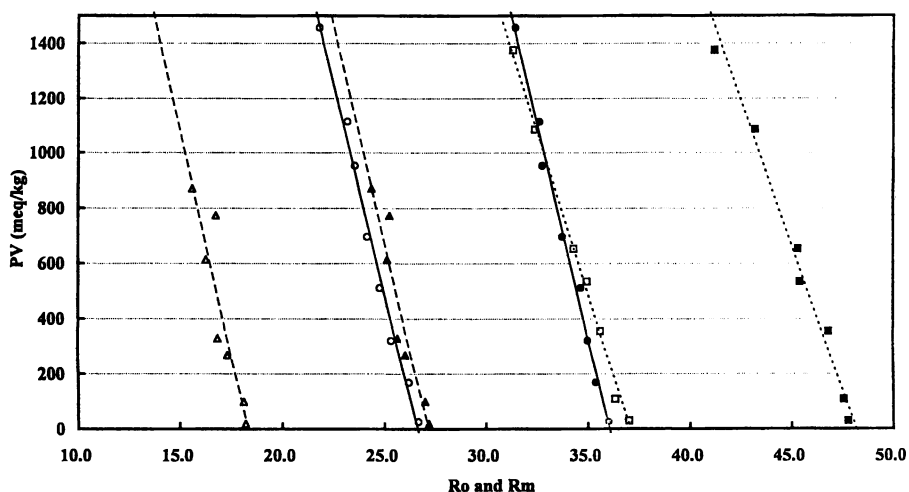


Figure 1. Relationships between Ro and Rm with PV for various kinds of model fish oils prepared from ethyl EPA and methyl linoleate.

▲ and Δ; Ro and Rm for 40% of EPA in methyl linoleate, respectively.

● and ○; Ro and Rm for 60% of EPA in methyl linoleate, respectively.

■ and □; Ro and Rm for 80% of EPA in methyl linoleate, respectively.

spectra were periodically recorded. Ro and Rm decreased with progression of oxidative deterioration, while PV generally increased steadily at the active oxygen absorption period. Values of Ro and Rm were plotted against corresponding PV. The relationship between each Ro, Rm and PV for the model oils was examined during the active oxygen absorption period when PV monotonously increased. In Figure 1, linear correlations existed between Ro and Rm with PV in various concentrations of EPA (40, 60, and 80%) in methyl linoleate, respectively. For example, the correlation coefficients were -0.953 for the Ro of 40% of EPA, -0.995 for that of 60% of EPA, and -0.996 for that of 80% of EPA in methyl linoleate, respectively.

Subsequently, the purified neutral lipids of the pollack liver and sardine oils were also allowed to stand at 40°C, and both PV and NMR spectra were recorded, and the relationship between each Ro and Rm with PV for fish oil were calculated. Linear correlations were also observed between Ro and Rm with PV of the natural fish oils (Figure 2). The correlation coefficients were -0.864 for Ro of sardine oil, -0.932 for Ro of pollack oil, -0.883 for Rm of sardine oil and -0.954 for Rm of pollack oil.

**Generalization of the Relationships of Ro and Rm with PV for Various Model and Natural Fish Oils.** Although the regression curves of Ro and Rm for the different model oils were almost parallel to each other, the points obtained by extrapolating the PV plots to zero differed according to the oils which contained different ratios of EPA in methyl linoleate. For example, numerical values of Ro were 27.1 for the model oil containing 40% of EPA, 36.1 for that containing 60% of EPA, and 48.2 for that containing 80% of EPA in methyl linoleate. The differences among these values depended on the ratio of the olefinic protons to that of the total protons in PUFA in these model oils. A straight line existed between the PV and value  $Ro/p[EPA]$  obtained by dividing Ro by each original percentage of EPA (abbreviated to  $p[EPA]$ ) in the oils (Figure 3a). This attempt was made for a generalization of the relationship applicable to fish oils of different species containing various ratios of PUFA. The relationships between PV and  $Ro/p[EPA]$ , and PV and  $Rm/p[EPA]$  were nearly linear with little dispersions in comparison with the case of their Ro and Rm, and the correlation coefficients were -0.812 and -0.934, respectively.

Similarly, the regression curves of Ro and Rm for different samples of the natural oils were also parallel to each other (Figure 3), however, the points obtained by extrapolating the PV to zero differed according to fish oils used from different species. The difference between the two values may depend upon the ratio of the olefinic protons to the total protons in PUFA in the two samples of fish oil. As shown in Figure 4, linear relationships were also observed between values of  $Ro/p[PUFA]$  and  $Rm/p[PUFA]$ , with the PV in fish oils. For convenience,  $p[PUFA]$  was substituted for the percentage of EPA plus DHA which are the main source of olefinic protons in PUFA of fish oils. Although the relationships between Ro and Rm with PV were linear for natural oils and high correlations were obtained as shown in Figure 2, the relationships between  $Ro/p[PUFA]$ ,  $Rm/p[PUFA]$  and PV were linear for whole sample oils in different fish species (pollack, sardine, sablefish *Anoplopoma fimbria*, and rockfish *Sebastolobus macrochir*) examined, and the correlation coefficients for  $Ro/p[PUFA]$ ,  $Rm/p[PUFA]$

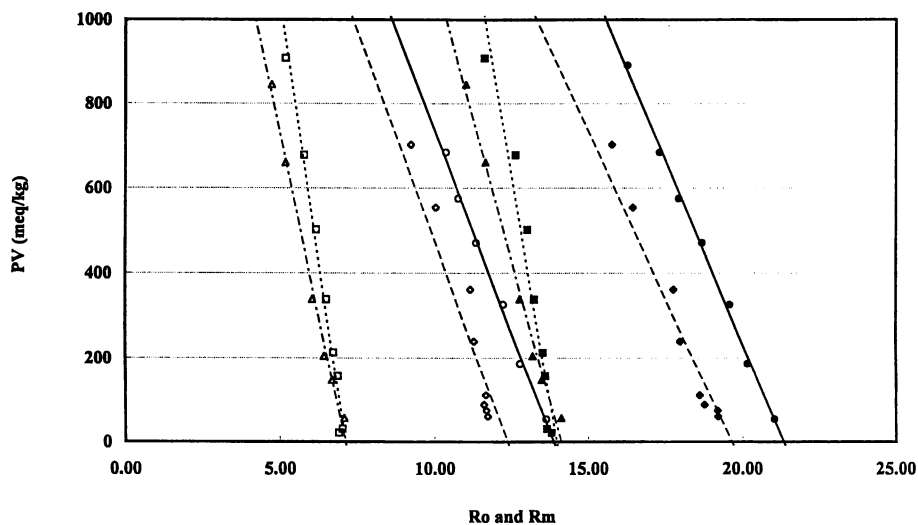


Figure 2. Relationships between Ro and Rm with PV for pollack liver oils (p1 and p2) and sardine oils (s1 and S2).

■ and □; Ro and Rm for the pollack liver oil 1, respectively.

▲ and △; Ro and Rm for the pollack liver oil 2, respectively.

● and ○; Ro and Rm for the sardine oil 1, respectively.

◆ and ◇; Ro and Rm for the sardine oil 2, respectively.

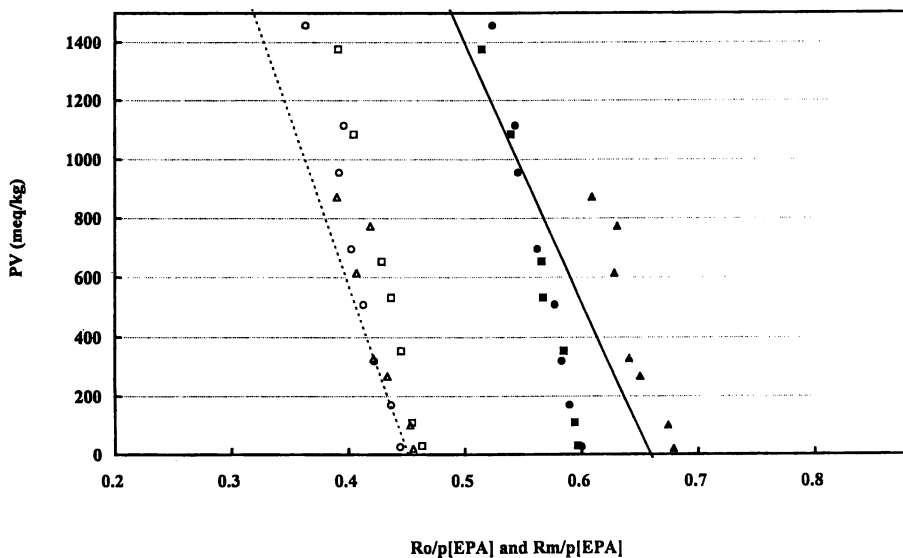


Figure 3. Relationships between Ro/p[EPA] and Rm/p[EPA] with PV for various kinds of model fish oils prepared from ethyl EPA and methyl linoleate.

▲ and △; Ro/p[EPA] and Rm/p[EPA] for 40% of EPA in methyl linoleate, respectively.

● and ○; Ro/p[EPA] and Rm/p[EPA] for 60% of EPA in methyl linoleate, respectively.

■ and □; Ro/p[EPA] and Rm/p[EPA] for 80% of EPA in methyl linoleate, respectively.

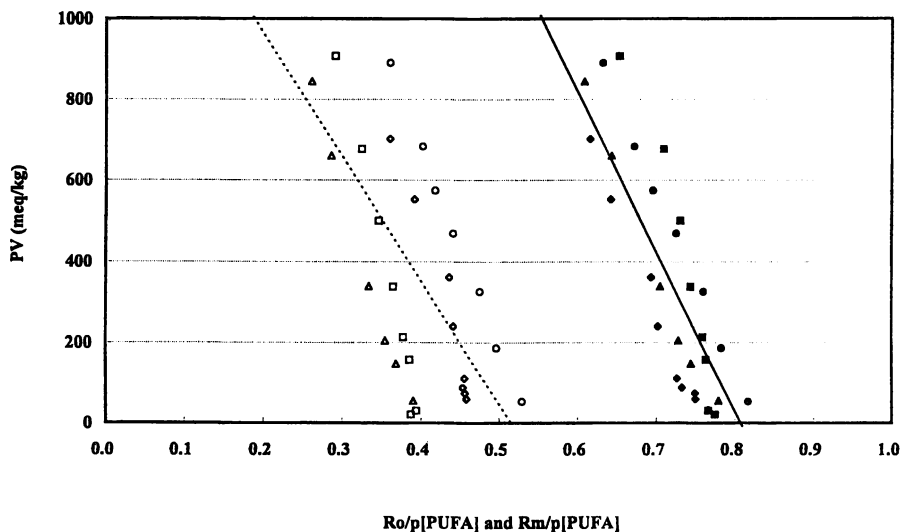


Figure 4. Relationships between Ro/p[PUFA] and Rm/p[PUFA] with PV for pollack liver oils (P1 and P2) and sardine oils (S1 and S2).

■ and □; Ro/p[PUFA] and Rm/p[PUFA] for the pollack liver oil 1, respectively.

▲ and △; Ro/p[PUFA] and Rm/p[PUFA] for the pollack liver oil 2, respectively.

● and ○; Ro/p[PUFA] and Rm/p[PUFA] for the sardine oil 1, respectively.

◆ and ◇; Ro/p[PUFA] and Rm/p[PUFA] for the sardine oil 2, respectively.

and PV were -0.875 and -0.675, which are somewhat lower when compared with those of Ro and Rm. From these results PV might be roughly derived from  $Ro/p[PUFA]$ , where Ro is easily obtained from the NMR measurement and  $p[PUFA]$  was determined by gas chromatographic analysis (Figure 5) (35, 36).

The NMR method was at least as useful as PV, however, accurate measurement of NMR is required because decreases in Ro and Rm are quite small as compared with large increases in PV. Nonetheless, NMR methodology is useful for measuring oxidative deterioration of fish oil even after PV begin to decline. Use of NMR methodology for evaluation of oxidative stability of vegetable and marine oils has also been reported (37-41). Excellent correlations existed between totox values and NMR (38, 41).

**Application of NMR to Evaluate the Oxidative Deterioration of Fish Oils during Storage.** It is well known that peroxide values monotonously increase during the active oxygen absorption period at the initial stage of oxidative deterioration, however, they start to decline as peroxides begin to decrease during the following steps of oxidation, where secondary products of oxidation are formed rapidly (9-14). Ro and Rm were assumed to decrease continuously with the time in contrast to PV, because they reflect directly the total amount of oxidative products. To investigate the possibility of NMR methodology for estimating the deterioration of fish oils, Ro, Rm and PV were periodically measured during long-term storage. As shown in Figure 6, Ro and Rm values decrease continuously with time. The fish oil deterioration proceeds rapidly, because it contains a large amount of unstable PUFA. In Figure 6, PV rises at the initial stage of oxidative deterioration, and reaches a maximum, fluctuates, and then declines during further storage. In contrast, Ro and Rm values decreased continuously and monotonously. It is suggested that the NMR method should be useful for measuring the oxidative deterioration of fish oils, even after PV begins to decline (42).

Marine products are often stored for a long period, and PV in the oil fraction from dried products often decreases during processing and storage (9-14). Practical applications of the NMR method to evaluating the oxidative deterioration of several processed marine products were, as described below.

**Application of NMR for Evaluation of Oxidative Deterioration of Fish Meal.** Fish meal is an important product and a chief source of dietary protein for chicken (broilers and layers), turkey, pig, and fish (43, 44). Brown fish meal produced from red-meat fish such as sardine (*Sardinops melanostictus*) and mackerel (*Scomber japonicus*) is extremely susceptible to lipid oxidation.

PV and AV have been conventionally used as indices of oxidative deterioration of oil in fish meal, which is commonly stored for extended periods of time, however, these indices are not always appropriate (10, 45). Thus practical applications of NMR methodology for the evaluation of oxidative deterioration of brown meal in comparison with other methods was examined.

PV and AV of the oil from meal I were periodically measured during 50 days of storage. As shown in Figure 7, PV increased very rapidly at the beginning and began to decrease within 10 days. The AV hardly changed throughout the storage period.



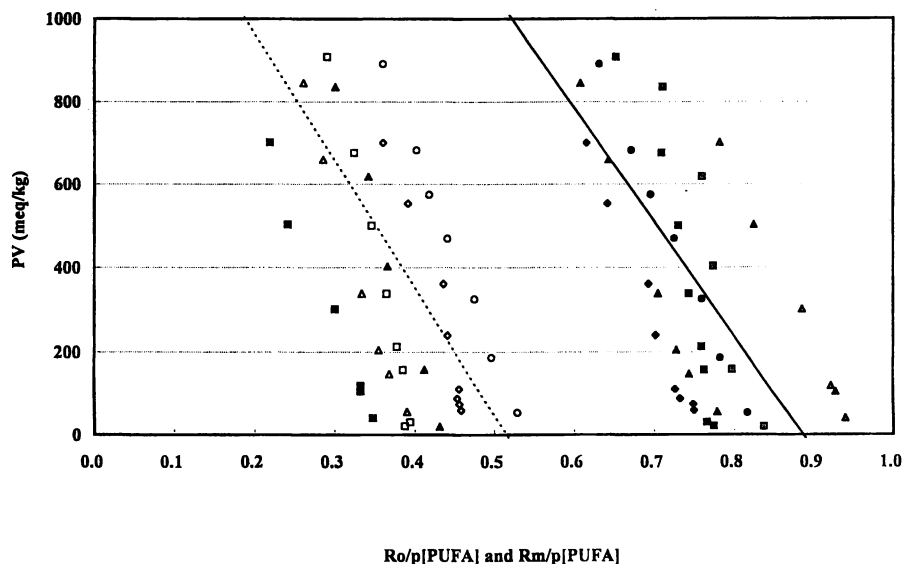


Figure 5. Relationships between Ro/p[PUFA] and Rm/p[PUFA] with PV for several kinds of fish oils.

■ and ▲; Ro/p[PUFA] for the pollack liver oils 1 and 2, respectively.

● and ◆; Ro/p[PUFA] for the sardine oils 1 and 2, respectively.

■ and ▲; Ro/p[PUFA] for the rockfish and the sablefish oils, respectively.

□ and Δ; Rm/p[PUFA] for the pollack liver oils 1 and 2, respectively.

○ and ◇; Rm/p[PUFA] for the sardine oil 1 and 2, respectively.

▲ and ■; Rm/p[PUFA] for the rockfish and the sablefish oils, respectively.

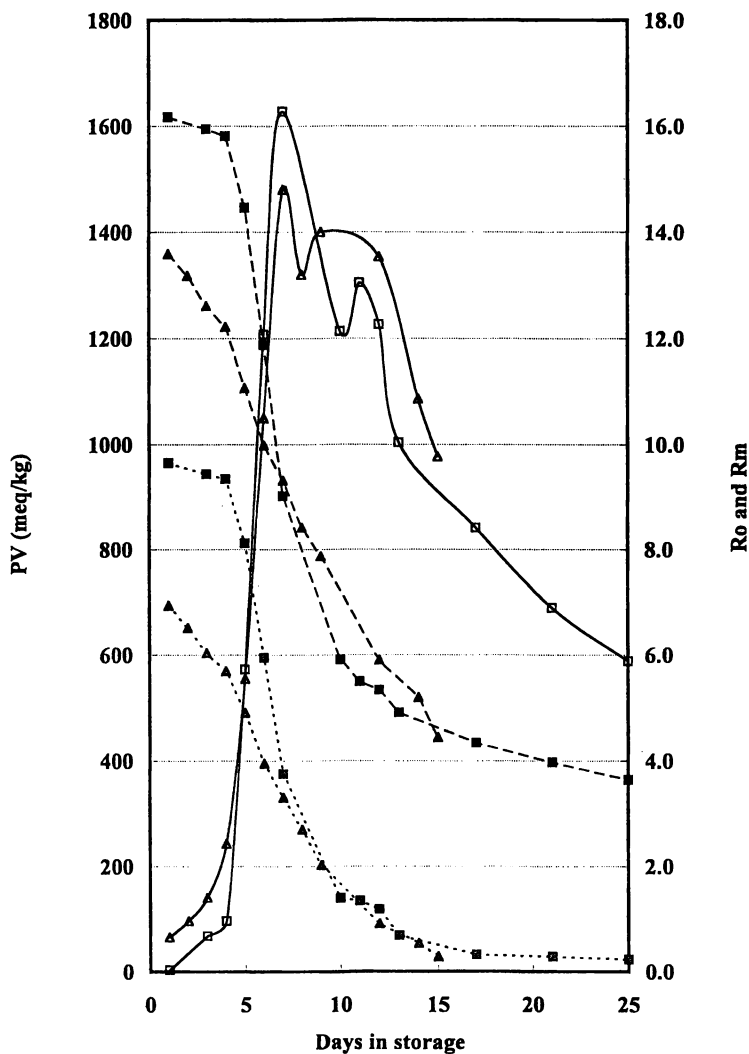


Figure 6. Changes in Ro, Rm and PV for pollack liver and sardine oils during storage at 40°C.

△ and □; PV for the pollack liver and the sardine oils, respectively.

▲ and ■; Ro for the pollack liver and the sardine oils, respectively.

▲ and ■; Rm for the pollack liver and the sardine oils, respectively.

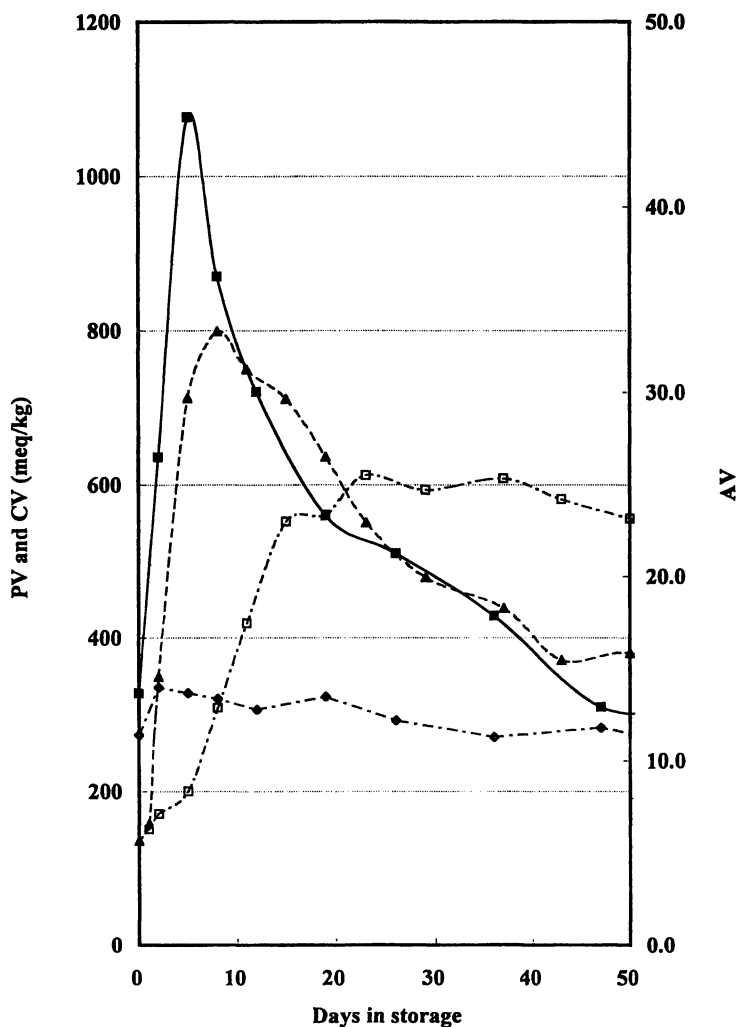


Figure 7. Changes in PV, CV and AV of oil in brown fish meals I and II during storage at 20°C.  
 ■ and ▲; PVs for the meal I and II, respectively.  
 □ and ◆; CV for the meal II and AV for the meal I, respectively.

The PV and AV were measured for meal II in which the PV changed in a similar manner to that of meal I. These findings indicate that PV and AV are hardly suitable parameters for the evaluation of oil deterioration in fish meal during long-term storage. The CV increased steadily over 20 days and thus may be useful as a quality parameter within the time scale used. In contrast to these methods, the Ro and Rm values decreased continuously and steadily as shown in Figure 8. For example, the Ro value and PV were 15.1 and 328.5 meq/kg at the initial stage of oxidation, respectively. The Ro decreased to 8.0 after 5 days of storage, whereas the PV increased and reached a maximum value of 1076.4 meq/kg (Figure 7). Figures 7 and 8 show that Ro decreases gradually to reach a value of 4.5 during 40 days of storage whereas the PV decrease to about the same value of the starting material (310.7 meq/kg). The steady decrease in Ro suggests that the results of the NMR methodology may directly reflect the total oxidation of products. Oxidative deterioration of oil in fish meal is complicated, and production of hydroperoxides is accompanied by generation of many secondary products. This finding suggests that the Ro value may better reflect the quality of fish meal than PV (46).

The Rm measured in two different fish meal specimens decreased in much the same way as Ro. This is a consequence of the fact that behavior of olefinic proton groups is closely related to that of diallylmethylene proton groups, possibly because of the interchangeability of allyl radicals.

The final values of Ro and Rm over a 40-day storage were approximately 4.5 and 1.0, respectively. That is to say, fish meal having an Ro of about 4.5 and/or below, or an Rm of 1.0 and/or below may be judged as being deteriorated, while it is difficult to offer such a definitive evaluation using the PV, AV, or CV methods.

**Use of NMR to Evaluate Oxidative Deterioration of Niboshi, Boiled and Dried Fish.** Drying has been used for many years to preserve fish products that are boiled and dried. For example, boiled and dried products of small fish such as juvenile sardines (*Sardinops melanostictus*) and Japanese anchovies (*Englauris japonica*), known as Niboshi, are typical marine dried products that are widely used for preparing soup stock in Japan (13).

Since these fish as raw material contain a large amount of fat and Niboshi boiled and dried fish is generally stored for a long period, it may be exposed to deterioration due to lipid oxidation during processing and preservation. An appropriate method for estimation of the degree of oxidative deterioration of Niboshi has long been required by processors and traders. For measuring the degree of oxidative degradation of lipids in Niboshi, PV and AV are conventionally used, however, they are not appropriate as discussed earlier (10-12).

Thus, Ro, Rm, and PV of the extracted oil of Niboshi were measured. As shown in Figure 9, PVs of oils extracted from sardine and anchovy Niboshi increased very rapidly at the beginning of storage and reached a maximum value, and then decreased. For example, the PV in the oil of sardine Niboshi increased to 600 meq/kg upon 3 days of storage, and decreased gradually to below 250 meq/kg after 20 days, which is similar to the PV of the starting material. On the contrary, Ro and Rm values in the NMR decreased continuously and monotonously; the Ro decreased rapidly from 17.0% to

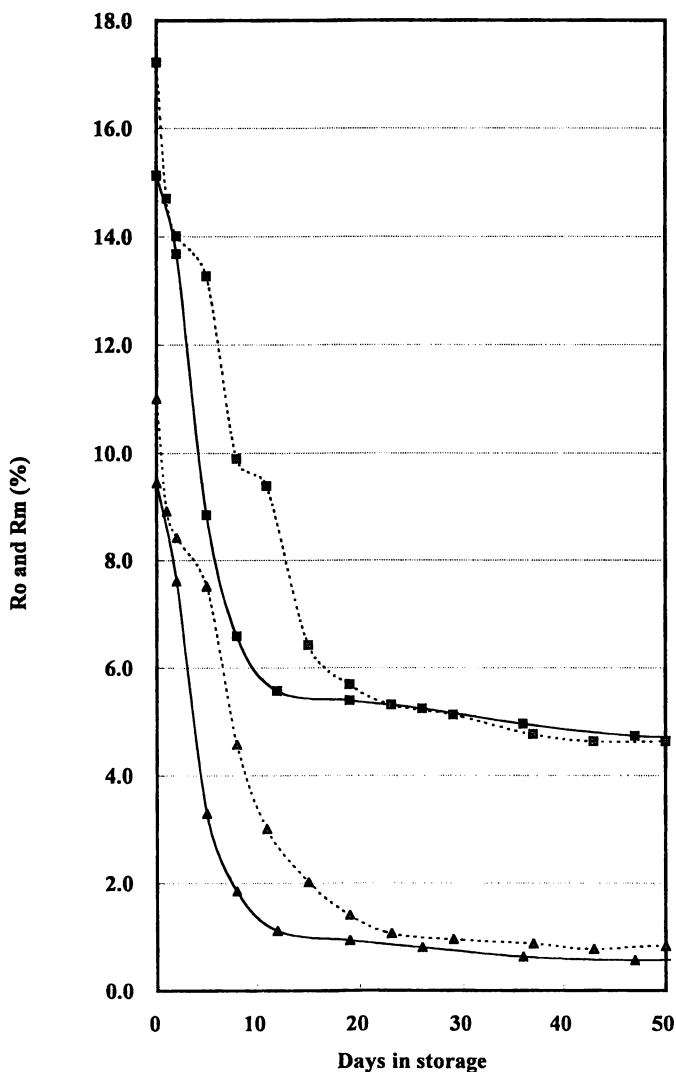


Figure 8. Changes in Ro and Rm of oil in brown fish meals I and II during storage at 20°C.

■ and □; Ro for the meal I and II, respectively.

▲ and △; Rm for the meal I and II, respectively.

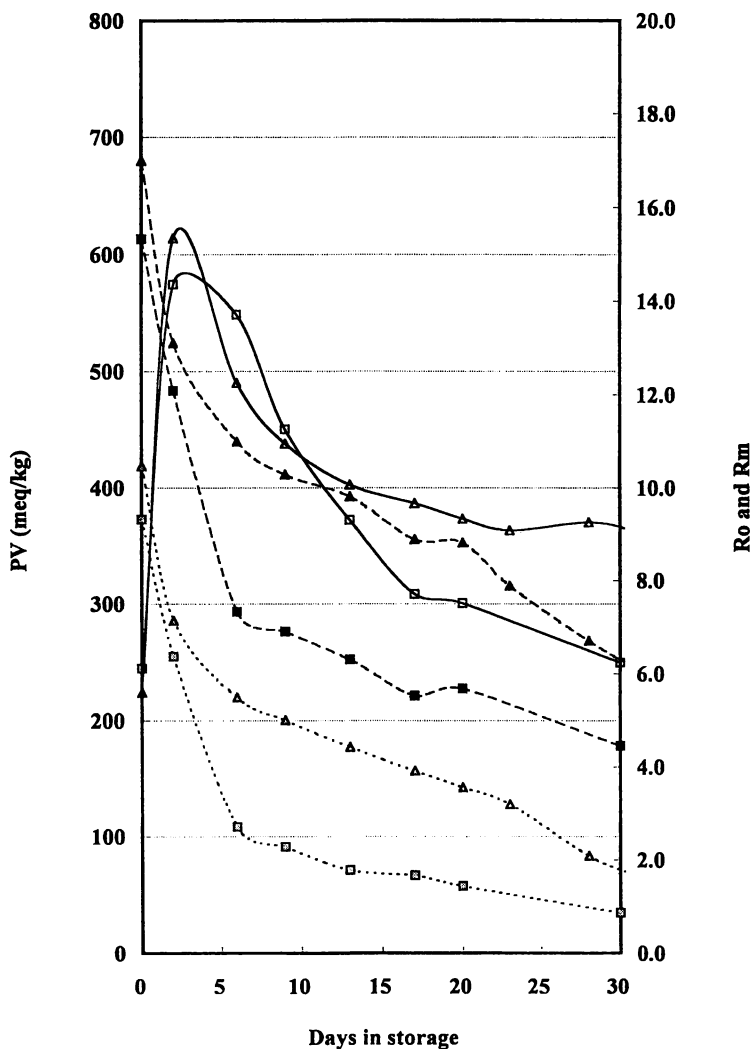


Figure 9. Changes in Ro, Rm and PV for the oils of sardine and anchovy Niboshi (boiled and dried fish).

□ and △; PV for the anchovy and the sardine oils, respectively.  
 ■ and ▲; Ro for the anchovy and the sardine oils, respectively.  
 ▣ and ▴; Rm for the anchovy and the sardine oils, respectively.

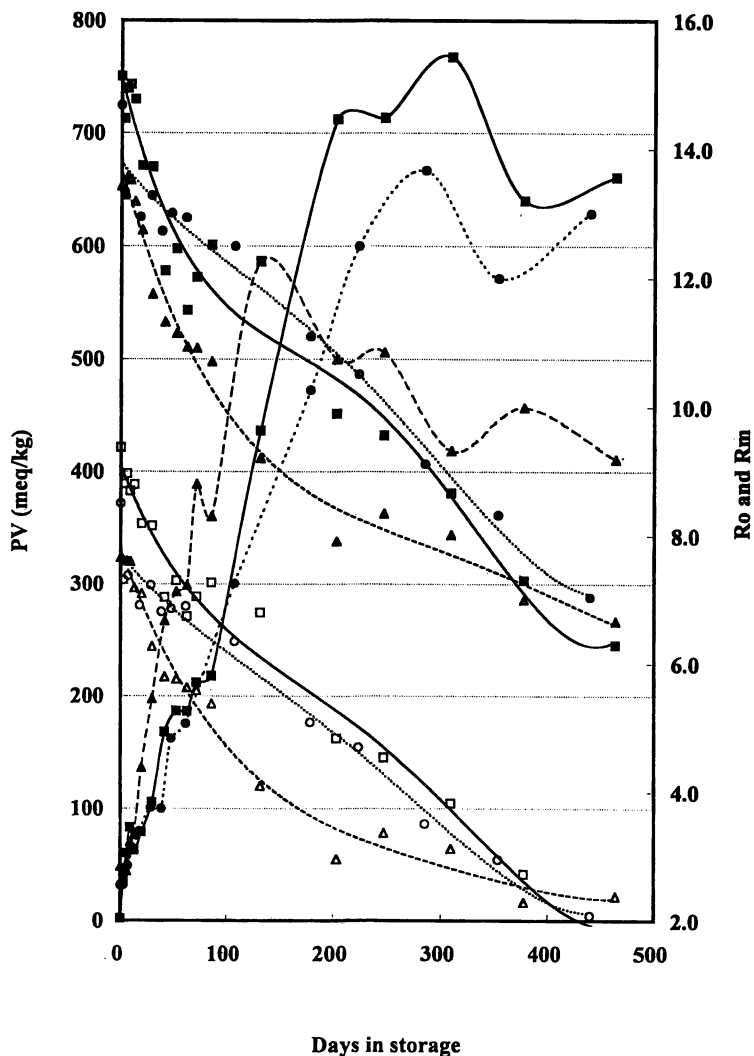


Figure 10. Changes in Ro, Rm and PV for the experimental models (salted dried fish) during storage at  $-10^{\circ}\text{C}$ .

■, ▲, and ●; PV for the dried fishes of horse mackerel, mackerel, and sardine, respectively.

▣, ▴, and ⊙; Ro for the dried fishes of horse mackerel, mackerel, and sardine, respectively.

□, △, and ○; Rm for the dried fishes of horse mackerel, mackerel, and sardine, respectively.

11.0% after the first 3 days of storage, while PV showed a rapid increase, then decreased gradually to 6.1% during the next 27 days, while PV decreased moderately (Figure 7).

The values of Ro and Rm closely paralleled the oxidative deterioration which generally proceeds steadily with time, while the PV does not always reflect the exact stage of oil deterioration in Niboshi (47).

#### **Application of NMR to Evaluate Oxidative Deterioration of Salted Dried Fish.**

Fatty fish, such as mackerel, are extremely important sources of food and dietary fish oil. These are generally preserved by salting and drying. Salted dried fish have been consumed for generations, and constitute an important part of the staple diet in Japan and developing countries in of South-east Asia (48-50). The oil in dried fish products is very susceptible to autoxidation, as the salt may act as a pro-oxidant (16). Some lipid oxidation products are toxic and cured products bought in the market are occasionally unsatisfactory. The NMR methodology is expected to offer a reliable index of oxidation, but PV and AV are temporarily used as quality indicators (9-13, 18, 19).

The PV of oil from salted dried fish (experimental models) was periodically measured over a one year storage period at -10°C. As shown in Figure 10, PV of the extracted oil from salted dried fish (mackerel, horse mackerel, and saury) increased gradually during the first 200 days of storage, reached a maximum (about 600-700 meq/kg), and then gradually decreased. The oil from salted dried mackerel attained PV of 389.0 after 72 days, a maximum value of 586.5 after 132 days, and a value of 456.7 meq/kg after 377 days of storage (initial value 48.8 meq/kg), but Ro and Rm values decreased steadily. The Ro values were 13.43 on day 0, 10.92 on day 72, 9.21 on day 132, and 7.00 on day 377, showing a steady decrease with time. Thus Ro reflects oxidative deterioration of fish oil in dried fish. The change in AV of the oil of the ordinary market products (Figure 11) was too small to permit assessment of the oil deterioration as enzymatic hydrolysis proceeds slowly at low temperatures and low water activity in dried products. Figure 12 shows variation in PV of the three ordinary market products; these were essentially similar to the experimental models in Figure 10. Ro and Rm of oil from the market products decreased linearly with storage time similar to that in the experimental models (51).

**Convenience of NMR Measurement.** The NMR measurement is a convenient method for estimating the overall extent of oxidative deterioration of fish oils during storage as the percentage of olefinic protons in fish oils containing a large amount of PUFA is relatively high. In addition, the NMR method is simple and requires only 20 mg of specimen.

#### **Conclusion**

Linear correlations existed between Ro and Rm with PV at the active oxygen absorption period. Although the changes of Ro and Rm are very small, the PV could be roughly determined as the Ro/p[PUFA] and Rm/p[PUFA] fractions during this period.



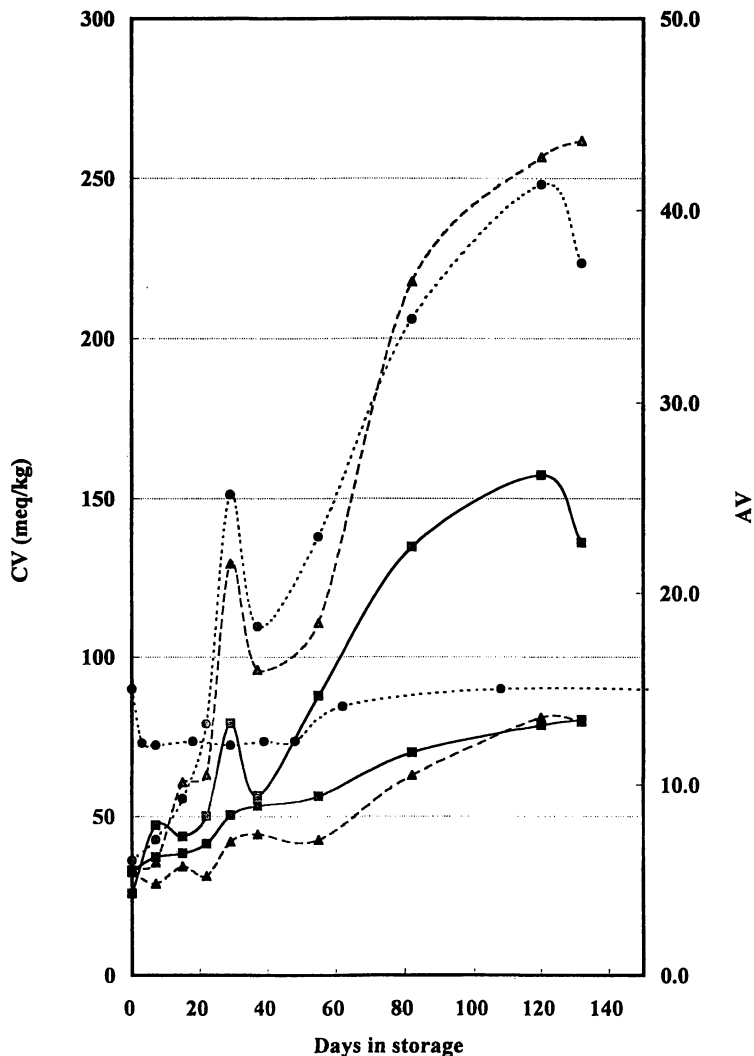


Figure 11. Changes in CV and AV for the ordinary market products (salted dried fish) during storage at  $-5^{\circ}\text{C}$ .

□, △, and ○; CV for the ordinary market products of horse mackerel, mackerel, and sardine, respectively.

■, ▲, and ●; AV for the ordinary market products of horse mackerel, mackerel, and sardine, respectively.

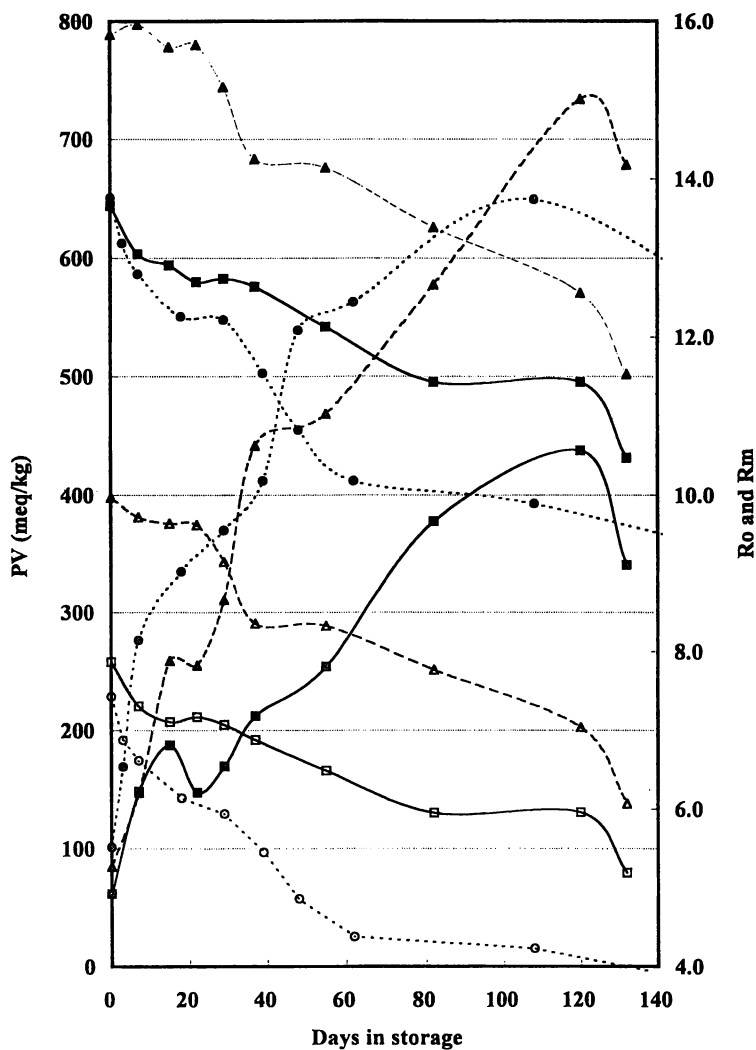


Figure 12. Changes in Ro and Rm for the ordinary market products (salted dried fishes) during storage at  $-5^{\circ}\text{C}$ .

■, ▲, and ●; PV for the ordinary market products of horse mackerel, mackerel, and sardine, respectively.

■, ▲, and ●; Ro for the ordinary market products of horse mackerel, mackerel, and sardine, respectively.

□, Δ, and ○; Rm for the ordinary market products of horse mackerel, mackerel, and sardine, respectively.

The NMR method estimates the progression of oxidation of oil and offers a more suitable index for comparing the storage condition of marine products and for estimating the effects of antioxidants in them. More accurate estimation of oxidative deterioration may be realized using both PV and NMR methodologies, especially in case of fish oils and fish products.

### Acknowledgment

The author thanks Mr. Kunisuke Nakamura and Mrs. Miho Udagawa, for their valuable technical assistance.

### Literature Cited

1. Karmali, R.A. *Am. J. Clin. Nutr.* **1987**, 225-229.
2. Carroll, K.K. *Lipids* **1992**, 27, 793-797.
3. Neuringer, M.; Anderson, G.J.; Connor, W.E. *Ann. Rev. Nutr.* **1988**, 8, 517-541.
4. Kinsella, J.E. *Food Technol.* **1988**, 42, 124-146.
5. Harris, W.S. *J. Lipid Res.* **1989**, 30, 785-807.
6. Ackman, R.G. *Food Technol.* **1988**, 151-155.
7. Pryor, W.A.; Stanley, J.P. *J. Org. Chem.* **1975**, 40, 3615-3617.
8. Porter, N. A. *Acc. Chem. Res.* **1986**, 19, 262-268.
9. Smith, G.; Hole, M. Browning of salted sun-dried fish. *J. Sci. Food. Agric.* **1991**, 57, 291-301.
10. Flick, Jr., G.J.; Hong, G.-P.; Knobl, G.M. In *Lipid Oxidation in Food*; St. Angelo, A.J., Ed.; ACS Symposium Series 500; American Chemical Society: Washington, DC, 1992, pp 183-207.
11. Ackman, R.G.; Gunnlaugsdottir, H. In *Lipid Oxidation in Food*; St. Angelo, A.J., Ed.; ACS Symposium Series 500; American Chemical Society: Washington, DC, 1992, pp 208-230.
12. Fritsche, K.L.; Johnston, P.V. *J. Nutr.* **1988**, 118, 425-426.
13. Takiguchi, A. *Fisheries Sci.* **1996**, 62, 240-245.
14. Perez-Villarreal, B.; Howgate, P. *J. Sci. Food Agric.* **1991**, 57, 455-469.
15. Deng, J.C. *J. Food Sci.* **1978**, 43, 337-340.
16. May J.; Kinsella, J.E. *J. Food Sci.* **1979**, 44, 1619-1624.
17. Awad, A. *J. Food Sci.* **1968**, 33, 227-235.
18. Maruf, F.W., Ledward, D.A., Neale, R.J., and Poulter, R.G. *Int. J. Food Sci. Technol.* **1990**, 25, 66-77.
19. Akande, G.R.; Knowles, M.J.; Taylor, K.D.A. *Int. J. Food Sci. Technol.* **1988**, 23, 495-500.
20. Hopkins, C.Y.; Bernstein, H.J. *Can. J. Chem.* **1959**, 37, 775.
21. Johnson, L.F.; Shoolery, J.N. *Anal. Chem.* **1962**, 34, 1136-1139.
22. Gunstone, F.D. *Chem. Phys. Lipids* **1991**, 59, 83-89.
23. Aursand, M.; Grasdalen, H. *Chem. Phys. Lipids* **1992**, 62, 239-251.
24. Aursand, M.; Rainuzzo, J.R.; Grasdalen, H. *J. Am. Oil Chem. Soc.* **1993**, 70, 971-981.

25. Sacchi, R.; Medina, I.; Aubourg, S.P.; Giudicianni, I.; Palillo, L.; Addeo, F. *J. Agric. Food Chem.* **1993**, *41*, 1247-1253.
26. Sacchi, R.; Medina, I.; Aubourg, S.P.; Addeo, F.; Palillo, L. *J. Am. Oil Chem. Soc.* **1993**, *70*, 225-2228.
27. Medina, I.; Sacchi, R. *Chem. Phys. Lipids* **1994**, *70*, 53-61.
28. Aursand, M.; Jørgensen, L.; Grasdalen, H. *J. Am. Oil Chem. Soc.* **1993**, *72*, 293-297.
29. Cunnane, S.C.; Likhodii, S.S.; Moine, G. *Lipids* **1996**, *31*, 127-130.
30. Bergana, M.M.; Lee, T.W. *J. Am. Oil Chem. Soc.* **1996**, *73*, 551-556.
31. Folch, J.; Lees, M.; Stanley, G.H.S. *J. Biol. Chem.* **1957**, *226*, 497-509.
32. AOCS. *Official Methods and Recommended Practices*. American Oil Chemists' Society; 4<sup>th</sup> ed.; Am. Oil Chem. Soc.: Champaign, IL, 1989, Cd8-53.
33. AOCS. *Official Methods and Recommended Practices*. American Oil Chemists' Society; 4<sup>th</sup> ed.; Am. Oil Chem. Soc.: Champaign, IL, 1989, Cd3a-63.
34. Kumazawa, H.; Oyama, T. *Yukagaku (Japan)* **1965**, *14*, 167-171.
35. Saito, H. *Agric. Biol. Chem.* **1987**, *51*, 3433-3435.
36. Saito, H.; Nakamura, K. *Bull. Japan. Soc. Sci. Fish. (Japan)* **1989**, *55*, 1663.
37. Shahidi, F. *Inform* **1992**, *3*, 543.
38. Wanasundara, U.N.; Shahidi, F. *J. Food Lipids* **1993**, *1*, 15-24.
39. Shahidi, F.; Wanasundara, U.N.; Brunet, N. *Food Res. Inter.* **1994**, *27*, 555-562.
40. Wanasundara, U.N.; Shahidi, F.; Jablonski, C.R. *Food Chem.* **1995**, *52*, 249-253.
41. Shahidi, F.; Wanasundara, U.N. In *Natural Antioxidants: Chemistry, Health Effects and Applications*; Shahidi, F., Ed.; American Oil Chemists' Society Press: Champaign, IL, 1997, pp 397-404.
42. Saito, H.; Nakamura, K. *Agric. Biol. Chem.*, **1990** *54*, 533-534.
43. Walisundera, M.N.; Ratnayake, M.N.; Ackman, R.G. *J. Sci. Food Agric.* **1989**, *49*, 59-74.
44. Bimbo, A.P.; Crowther, J.B. *J. Am. Oil Chem. Soc.* **1992**, *69*, 221-227.
45. Gunstone, F.D.; Wijesundera, R.C. *J. Sci. Food Agric.* **1978**, *29*, 28-32.
46. Saito, H.; Udagawa, M. *J. Sci. Food Agric.* **1992**, *58*, 135-137.
47. Saito, H.; Udagawa, M. *Biosci. Biotechnol. Biochem.* **1992**, *56*, 831-832.
48. Berhimpon, S.; Souness, R.A.; Buckle, K.A.; Edwards, R.A. *Int. J. Food Sci. Technol.* **1990**, *25*, 409-419.
49. Lubis, Z.; Buckle, K.A. *Int. J. Food Sci. Technol.* **1990**, *25*, 295-303.
50. Wootton, M.; Ismail, N. *J. Sci. Food Agric.* **1986**, *37*, 399-408.
51. Saito, H.; Udagawa, M. *J. Am. Oil Chem. Soc.* **1992**, *69*, 1157-1159.

## Chapter 20

# Structural Characteristics of Marine Lipids and Preparation of $\omega$ 3 Concentrates

Udaya N. Wanasundara and Fereidoon Shahidi<sup>1</sup>

Department of Biochemistry, Memorial University of Newfoundland, St. John's, Newfoundland A1B 3X9, Canada

Marine oils, rich in  $\omega$ 3 fatty acids, have been recognized as desirable dietary components. Intestinal absorption of these long chain fatty acids is somewhat dependent on their arrangement in the triacylglycerol (TAG) molecules. In this study, positional distribution of  $\omega$ 3 fatty acids namely eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) in the TAG of seal blubber oil (SBO) and menhaden oil (MHO) was determined via reaction with Grignard reagent. EPA, DPA and DHA occurred mainly in the *sn*-1 and *sn*-3 positions of the TAG in SBO. The relative amounts in the *sn*-1 and *sn*-3 positions were: EPA, 8.36 and 11.2; DPA, 3.99 and 8.21; and DHA, 10.5 and 17.9%, respectively. In MHO, DPA and DHA occurred mainly in the *sn*-2 position of the TAG at 3.11 and 17.2%, respectively. However, EPA was equally distributed in the *sn*-2- and *sn*-3 positions and was present only in minute amounts in the *sn*-1 position. Enzyme assisted hydrolysis of SBO and MHO was carried out to increase the content of  $\omega$ 3 fatty acids in the acyglycerol form. Among lipases tested, *Candida cylindracea*-lipase was most active and resulted in highest increase in the content of total  $\omega$ 3 fatty acids, EPA and DHA, in the non-hydrolysed fraction of both SBO and MHO.

Epidemiological studies in the early 1970's postulated that the low incidence of coronary heart disease of Greenland Eskimos might be related to their distinctive dietary habit and use of lipids rich in polyunsaturated fatty acids (PUFA; 1). Eskimos were also found to have a reduced prevalence of other chronic and inflammatory diseases such as arthritis, psoriasis, asthma and diabetes. Several sources of information suggest that humans originally consumed a diet with a ratio of  $\omega$ 6 to  $\omega$ 3 fatty acids of about 1:1 whereas this ratio is now ranging from 10:1 to 20-25:1 in the affluent western societies. Therefore, the western diets are deficient in  $\omega$ 3 fatty acids

<sup>1</sup>Corresponding author

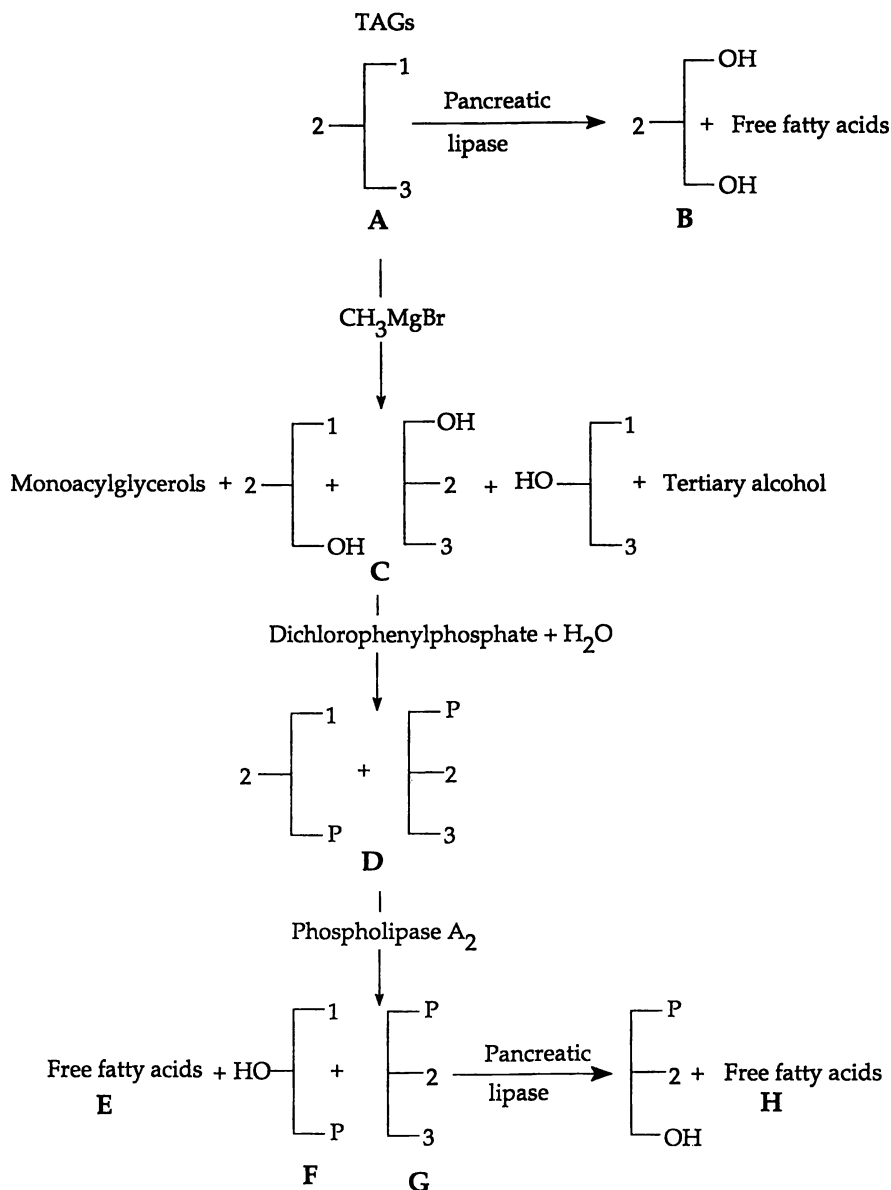
compared with the diet on which humans were evolved and their genetic patterns established (2).

The beneficial effects of PUFA have been ascribed to their ability to lower serum triacylglycerol (TAG) and cholesterol levels and enhance their excretion, to increase membrane fluidity and by conversion to eicosanoids to reduce thrombosis (3). The  $\omega 3$  fatty acids are considered essential for normal growth and development throughout the life cycle and may play an important role in the prevention and treatment of coronary artery disease (3,4).

A significant amount of docosahexaenoic acid (DHA) is found in human milk (5). High levels of DHA are also found in human brain and retina. Studies on nonhuman primates and human newborns indicate that DHA is essential for the normal functional development of the retina and brain, particularly in premature infants. Therefore, DHA in breast-milk or infant formula is supposed to meet the requirements of developing human brain and visual parts (6). Because  $\omega 3$  fatty acids are essential for growth and development throughout the life cycle, they should be included in the diets of humans of all age groups. The  $\omega 3$  and  $\omega 6$  fatty acids are not interconvertible in the human body and are important components of practically all cell membranes. Whereas cellular proteins are genetically determined, the PUFA composition of cell membranes is, to a great extent, dependent on the dietary intake. Therefore, appropriate amounts of  $\omega 3$  and  $\omega 6$  fatty acids need to be considered in making dietary recommendations and these two classes of PUFA should be distinguished because they are metabolically and functionally distinct and have opposing physiological functions. The balance is important for homeostasis and normal development (2).

In the USA, it is recommended that the total fat intake be approximately 30% of the total calories with equal distribution among saturated, monounsaturated and polyunsaturated fatty acid groups, the latter being equally divided between  $\omega 6$  and  $\omega 3$  fatty acids. The 1990 Canadian Nutrition Recommendations have included separate values for the two of long-chain  $\omega 3$  PUFA; eicosapentaenoic acid (EPA) and DHA (7). The amount of  $\omega 3$  and  $\omega 6$  fatty acids are given in grams, based on the energy expressed as daily requirements, for the various age groups from birth to 75+ years. During pregnancy additional amounts of  $\omega 3$  and  $\omega 6$  fatty acids are recommended in amounts that increase from the first to the second trimester. There is no increase between the second and the third trimester. Additional amounts of dietary  $\omega 3$  and  $\omega 6$  fatty acids are recommended during lactation.

The long-chain  $\omega 3$  fatty acids (EPA or DHA) may be obtained mainly from seafoods or derived from dietary  $\alpha$ -linolenic acid by chain elongation and desaturation. EPA and DHA are synthesized mainly by both uni- and multicellular marine plants such as phytoplankton and macro algae. They are eventually transferred through the food web and are incorporated into lipids of aquatic species such as fish and marine mammals, particularly those living in low temperature waters, probably due to these acids' ability to maintain fluidity in such environments. Therefore, increased consumption of marine lipids has been suggested in order to increase the intake of  $\omega 3$  fatty acids from dietary sources.



**Figure 1.** Procedure for the stereospecific analysis of triacylglycerols of seal blubber and menhaden oils

## Absorption of Fatty Acids

Intestinal absorption of fatty acids has been reported to be dependent on their arrangement in the TAG molecules. Investigation of the absorption of fatty acids in a canine model suggested that the positional distribution of fatty acids within the TAG (*sn*-1, *sn*-2 and *sn*-3) might affect the metabolic fate of fatty acids (8). Filer *et al.* (9) and Tomarelli *et al.* (10) made a similar observation in human infants. On the other hand, different microbial lipases have been used to prepare  $\omega$ 3 concentrates from marine lipids via a hydrolysis process. Some microbial lipases have both positional and acyl-chain specificities (11). Therefore, knowledge of the fatty acid distribution in TAG molecules may provide useful information when selecting appropriate microbial lipases for hydrolysis of specific fatty acids from TAG in order to obtain  $\omega$ 3-enrichment. Stereospecific analysis allows determination of the distribution of fatty acids over the three positions of TAG molecules (12). The analysis is based on the modification of TAG by Grignard degradation followed by synthesis of phosphatides and subsequent hydrolysis of fatty acids by stereospecific phospholipase A<sub>2</sub>.

## Stereospecific Analysis of Marine Oils

Stereospecific analyses of seal blubber oil (SBO) and menhaden oil (MHO) and the intermediates involved are shown in Figure 1. In the first step, TAG (A) of both SBO and MHO were hydrolysed by porcine pancreatic lipase in order to split fatty acids at the *sn*-1 and *sn*-3 positions, yielding 2-monoacylglycerols (B), which accurately provides the fatty acid composition of the *sn*-2 position of TAG. In the second step, TAG of both SBO and MHO were modified by Grignard degradation using methyl magnesium bromide (CH<sub>3</sub>MgBr). Laakso and Christie (13) and Itobashi *et al.* (14) used Grignard reaction to obtain partially deacylated triacylglycerols, i.e., diacylglycerols. The products of the reaction are a ketone and a magnesium salt of acylglycerol. In the presence of an aqueous acid, the magnesium salt of acylglycerols yields acylglycerols. The resulting ketone reacts further with the Grignard reagent to yield a tertiary alcohol. The products of the Grignard degradation of TAG may be separated on TLC plates; these consisted of monoacylglycerols, 1,2- and 2,3-diacylglycerols (C), 1,3-diacylglycerol and a tertiary alcohol. Among these bands, 1,2- and 2,3-diacylglycerols were isolated and used to prepare synthetic racemic phosphatides by reacting them with dichlorophenylphosphate (Figure 1). This reaction produced 1,2-diacylglycero-3-phosphatide (L-isomer) and 2,3-diacylglycero-1-phosphatide (D-isomer). These phosphatides (D) were hydrolysed by stereospecific phospholipase A<sub>2</sub> enzyme extracted from snake venom. This enzyme reacts only with 1,2-diacylglycero-3-phosphatide (L-isomer; naturally present) and releases free fatty acids from the *sn*-2 position (15). The products of phospholipase A<sub>2</sub> hydrolysis (free fatty acids from *sn*-2 position and L-lysophosphatide) and the unchanged 2,3-diacylglycero-1-phosphatide (D-isomer; un-natural) were separated on a TLC plate. The separated bands were identified as being free fatty acids from the *sn*-2 position



of 1,2-diacyl-3-phosphatide (E), unchanged 2,3-diacylglycero-1-phosphatide (G) and L-lysophosphatide (F). From these bands L-lysophosphatide was isolated, extracted into chloroform/methanol (1:1, v/v) and then used for fatty acid analysis. These allowed identification of the fatty acid composition at the *sn*-1 position of TAG of SBO and MHO.

In order to determine the fatty acid composition at the *sn*-3 position of TAG, unchanged 2,3-diacylglycero-1-phosphatide was isolated and extracted into chloroform/methanol (1:1, v/v). The latter compound was subjected to porcine pancreatic lipase hydrolysis, the products of which were 2-monoacylglycero-1-phosphatide and free fatty acids (H) released from the *sn*-3 position of TAG of SBO and MHO.

Fatty acid distribution at three different positions of the TAG of SBO and MHO are given in Table I. In SBO, saturated fatty acids such as 14:0, 15:0, 16:0 and 17:0 were preferentially located at the *sn*-2 position, followed by the *sn*-1 and *sn*-3 positions. However, the saturated fatty acids in MHO were randomly distributed over the three positions. SBO contained very high amounts (over 59%) of monounsaturated fatty acids, in which 18:1 was preferentially located at the *sn*-1 position, whereas 16:1 and 20:1 were abundant at the *sn*-2 and *sn*-3 positions, respectively. In MHO, monounsaturated fatty acids were randomly distributed. SBO and MHO were also different in the dominancy and distribution of long-chain  $\omega$ 3 fatty acids in their TAG molecules. In SBO, EPA (20:5 $\omega$ 3), docosapentaenoic acid (DPA, 22:5 $\omega$ 3) and DHA (22:6 $\omega$ 3) occurred mainly at the *sn*-1 and *sn*-3 positions of TAG and their quantities were: EPA, 8.36 and 11.2%; DPA, 3.99 and 8.21%; and DHA, 10.5 and 17.9%, respectively. In MHO, DPA and DHA occurred mainly at the *sn*-2 position of TAG at 3.11 and 17.2%, respectively. However, EPA was equally distributed at the *sn*-2 (17.5%) and *sn*-3 (16.3%) positions and was present only in small amounts (3.12%) at the *sn*-1 position. Therefore, EPA, DPA and DHA from SBO might be assimilated in the body more effectively than those from MHO. During digestion, fatty acids in the *sn*-1 and *sn*-3 positions of the TAG are liberated by a position specific enzyme such as pancreatic lipase, but the fatty acids attached to the *sn*-2 position of the TAG are then absorbed and distributed in the body in the chylomicron form.

Brockerhoff *et al.* (16) have pointed out the general tendency of long-chain PUFA to be preferentially esterified at the *sn*-2 position of fish oil TAG, but these are mainly located at the *sn*-1 and *sn*-3 positions of marine mammal lipids. Recently, Aursand *et al.* (17) determined the positional distribution of  $\omega$ 3 fatty acids in cod liver and Atlantic salmon oils (fish) and seal blubber oil (marine mammal) by high-resolution  $^{13}\text{C}$  nuclear magnetic resonance spectroscopy and found that in cod liver and salmon oils DHA was dominant at the *sn*-2 position of TAG, whereas EPA was nearly randomly distributed in all three positions. In seal blubber oil, DHA was primarily present at the *sn*-1 and *sn*-3 positions. Similar observations were made by Brockerhoff *et al.* (16,18) for fish (herring and mackerel) and marine mammal depot lipids (harbor seal blubber oil). However, the major drawback of the method employed is that results for the *sn*-3 position are subject to cumulative error because fatty acid distribution in this position was not determined directly. Ando *et al.* (19)

Table I. Fatty acid distribution in different positions of triacylglycerols of seal blubber and menhaden oils

Fatty acids (w/w %)	Seal blubber oil (SBO)			Menhaden oil (MHO)		
	<i>sn</i> -1	<i>sn</i> -2	<i>sn</i> -3	<i>sn</i> -1	<i>sn</i> -2	<i>sn</i> -3
	14:0	2.23±0.12	8.08±0.01	0.12±0.00	12.1±0.10	10.5±0.09
14:1 $\omega$ 9	0.23±0.00	2.35±0.03	0.22±0.00	0.30±0.01	0.60±0.00	0.16±0.00
15:0	0.10±0.00	1.25±0.01	0.41±0.01	1.02±0.01	0.31±0.00	0.80±0.01
16:0	3.12±0.13	12.4±0.15	2.99±0.04	24.1±0.55	19.9±0.13	6.91±0.01
16:1 $\omega$ 7	8.22±0.16	35.3±0.80	12.3±0.12	18.1±0.32	10.6±0.18	9.20±0.10
17:0	0.79±0.01	1.80±0.01	0.80±0.01	4.39±0.12	3.65±0.03	1.01±0.00
17:1	0.20±0.00	1.30±0.00	0.12±0.00	2.11±0.09	2.23±0.13	1.23±0.03
18:0	1.01±0.01	1.64±0.06	0.71±0.02	1.21±0.02	2.06±0.00	4.12±0.01
18:1	38.3±1.00	22.6±1.00	18.0±0.17	13.1±0.21	7.47±0.01	13.6±0.09
18:2 $\omega$ 6	0.32±0.00	1.24±0.09	2.01±0.08	0.81±0.00	0.49±0.00	3.11±0.03
18:3 $\omega$ 3	0.96±0.01	0.45±0.01	1.21±0.01	1.12±0.01	0.41±0.01	0.99±0.03
18:4 $\omega$ 3	1.82±0.16	0.45±0.02	0.33±0.09	4.51±0.02	0.34±0.03	4.94±0.01
20:0	0.10±0.00	0.43±0.00	0.09±0.00	0.21±0.00	0.11±0.00	1.21±0.01
20:1 $\omega$ 9	13.0±0.10	4.33±0.13	17.2±0.11	0.71±0.00	0.50±0.01	2.20±0.03
20:2 $\omega$ 6	0.21±0.00	0.15±0.00	0.20±0.00	0.40±0.00	0.14±0.00	0.10±0.00
20:3	0.19±0.00	0.13±0.00	0.22±0.01	0.59±0.00	0.42±0.00	0.61±0.00
20:4 $\omega$ 6	0.21±0.02	0.14±0.00	0.91±0.01	1.11±0.01	1.13±0.02	0.27±0.01
20:5 $\omega$ 3	8.36±0.12	1.60±0.09	11.2±0.04	3.12±0.03	17.5±0.19	16.3±0.18
22:0	-	-	-	0.21±0.02	0.08±0.00	0.21±0.02
22:1 $\omega$ 11	3.00±0.04	0.13±0.01	3.25±0.03	0.13±0.00	0.12±0.00	0.09±0.00
22:2	-	-	-	0.20±0.00	0.15±0.00	0.18±0.00
22:4 $\omega$ 6	0.01±0.00	0.05±0.00	0.22±0.00	0.20±0.01	0.31±0.02	0.04±0.00
22:5 $\omega$ 3	3.99±0.05	0.79±0.06	8.21±0.08	1.21±0.01	3.11±0.02	2.31±0.05
22:6 $\omega$ 3	10.5±0.10	2.27±0.12	17.9±0.11	4.11±0.03	17.2±0.19	6.12±0.02

have determined the positional distribution of fatty acids in TAG of fish oils (capelin, herring, menhaden, sardine and saury) by high-performance liquid chromatography using a chiral stationary phase and found that DHA in these oils was present mainly at the *sn*-2 position of the TAG. However, Ota *et al.* (20) found that in flounder liver and flesh lipids there was no preference for the *sn*-2 position in contrast to the general tendency for distribution of long-chain PUFA of fish oils in this position.

### Polyunsaturated Fatty Acid Concentrates

It has been suggested that PUFA concentrates devoid of the saturated fatty acids are much better than marine oils themselves since they would keep the daily total lipid intake as low as possible. With the growing public awareness of the nutritional benefits of consuming PUFA concentrates the market for these products is expected to grow in the future. In response to this demand, pharmaceutical industries have used different methods for preparing PUFA concentrates from marine oils. Chromatographic separation, fractional distillation, low temperature crystallization, supercritical fluid extraction and urea complexation are currently practised for preparation of PUFA concentrates from marine oils. However, these methods involve extreme conditions such as high or low pH, high temperature and organic solvents which may partially destroy  $\omega$ 3 PUFA by oxidation, *cis-trans* isomerization or double-bond migration and polymerization. Selective enzymatic hydrolysis of marine oils in order to remove saturated fatty acids and concentrate the  $\omega$ 3 PUFA in the acylglycerol form has gained popularity in recent years.

The acylglycerol form of PUFA is considered to be nutritionally more favorable than methyl or ethyl esters of fatty acids due to the impaired intestinal absorption of alkyl esters of  $\omega$ 3 fatty acids as observed in laboratory animals (21-23). It has also been shown that methyl and ethyl esters of unsaturated fatty acids hydrolyse at a slower rate than their corresponding TAG (24). From a marketing point of view, mono-, di- and triacylglycerols are often promoted as being more "natural" than other forms such as free fatty acids and their methyl or ethyl esters (25). On the other hand, enzymatic method involves mild conditions and thus provides a promising alternative to the drastic conditions applied for the traditional concentration methods. In addition, use of enzymes in the preparation of  $\omega$ 3-PUFA concentrates may save energy and increase productivity. Therefore, in this study, preparation of  $\omega$ 3 PUFA enriched acylglycerols from SBO and MHO was attempted via enzymatic hydrolysis.

### Enzymatic Hydrolysis of Marine Oils

Hydrolysis of SBO and MHO by microbial lipases (*Aspergillus niger*, AN; *Candida cylindracea*, CC; *Chromobacterium viscosum*, CV; *Geotricum candidum*, GC; *Mucor miehei*, MM; *Rhizopus niveus*, RN; *Rhizopus oryzae*, RO; and *Pseudomonas* spp., PS) and separation of the  $\omega$ 3-enriched fraction was carried out according to the scheme

given in Figure 2. Oil and phosphate buffers containing lipase were placed in a glass container. The container was flushed with nitrogen and sealed with a rubber cap and parafilm and then placed in a gyrotory water bath shaker at  $35\pm 1^\circ\text{C}$  operating at 200 rpm. Hydrolysed samples were removed periodically (separate sample container for each time) to determine the percentage of hydrolysis.

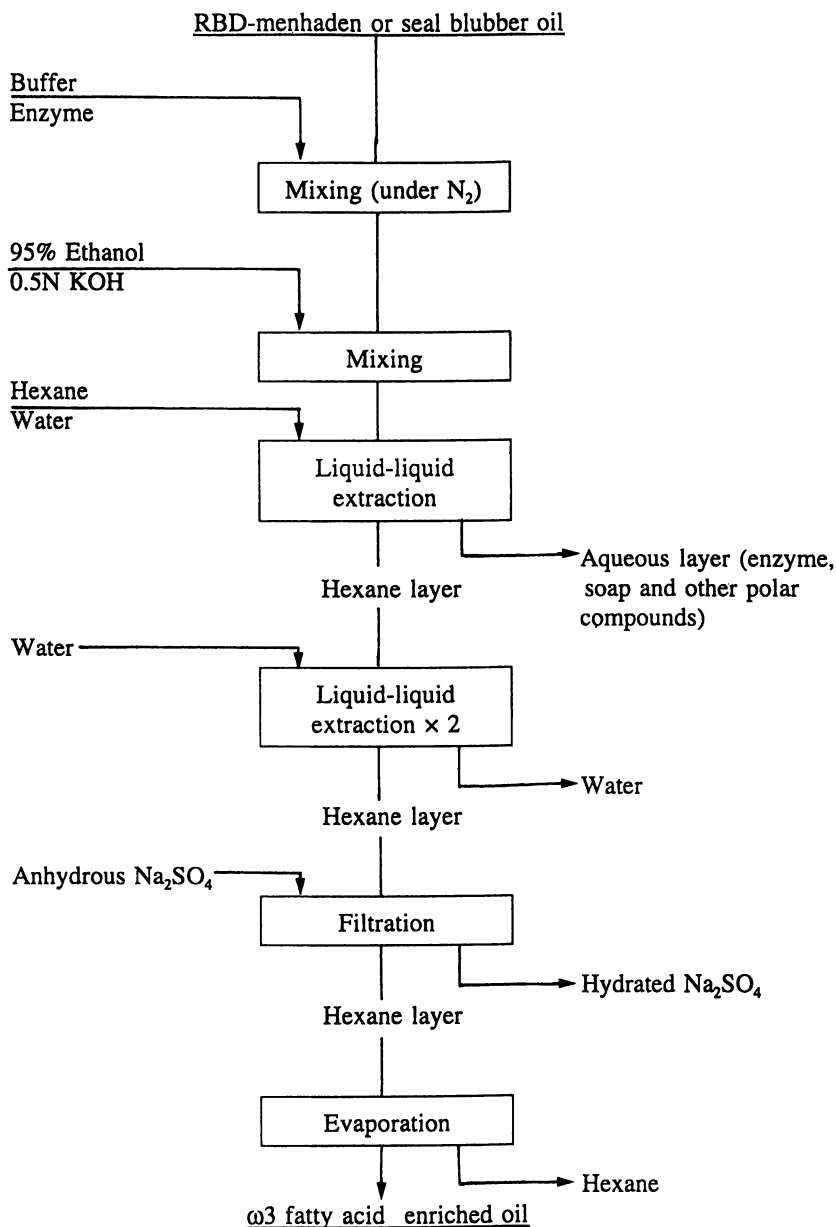
Unhydrolysed oil, acylglycerols and free fatty acids from the hydrolysed acylglycerols (SBO and MHO) were extracted into hexane and used for determination of the acid value. The hydrolysis percentage of the oils, after enzyme treatment, was calculated as:

$$\text{Hydrolysis (\%)} = 100(\text{Acid value}_{(\text{Hydrolysed oil})} / \text{Saponification value})$$

Separation of acylglycerols and free fatty acids upon enzymatic hydrolysis was carried out after adding the required amount of KOH to neutralize fatty acids released during hydrolysis, the mixture was then transferred into a separatory funnel and thoroughly mixed with hexane and distilled water (Figure 2). The lower aqueous layer was separated and discarded. The upper layer (hexane) containing monoacylglycerols, diacylglycerols and triacylglycerols was washed with distilled water and then passed through a bed of anhydrous sodium sulfate. The acylglycerols were subsequently recovered following hexane removal at  $45^\circ\text{C}$  using a rotary evaporator and their fatty acid composition then determined.

Percentage hydrolysis of SBO and MHO, as a function of time was then determined. All microbial lipases tested were able to hydrolyse fatty acids in both oils, but at different rates. Among the lipases tested *CC*-lipase gave the highest degree of hydrolysis of SBO followed by *RO*-lipase; however, in MHO, it was the *RO*-lipase that afforded the highest degree of hydrolysis. The degree of hydrolysis of both oils by other lipases studied was less than when *CC* and *RO* were used. At a given time, all lipases gave a higher degree of hydrolysis of SBO than of MHO. This difference may be due to the presence of higher amounts of PUFA, especially EPA and DHA in MHO than in SBO which exhibit resistance to enzymatic hydrolysis. The degree of hydrolysis of SBO by *CC*-lipase after 9 h was 70% and the same degree of hydrolysis was achieved after 60 h when *RO*-lipase was used. However, none of the lipases used were able to afford 70% hydrolysis in MHO over a 75 h period.

Figures 3 and 4 show the changes in the content of total  $\omega 3$  fatty acids, EPA and DHA in the non-hydrolyzed fraction (acylglycerols) of both oils upon enzymatic (lipase) hydrolysis. Among the lipases tested *CC*-lipase significantly ( $P < 0.05$ ) increased the total content of  $\omega 3$  fatty acids and DHA of SBO as the hydrolysis reaction progressed. After 75 h of hydrolysis, *CC*-lipase doubled the total content of  $\omega 3$  fatty acids in SBO. In MHO this lipase was able to increase the content of total  $\omega 3$  fatty acids only by 16% (from 30% in original oil to 46% after hydrolysis) during the same hydrolysis period. In MHO, the highest content of total  $\omega 3$  fatty acids and DHA were obtained by *RO*-lipase-assisted hydrolysis. But using this lipase, the EPA content of both oils decreased during the course of the reaction. This may be due to the fact that *RO*-lipase selectively hydrolyses EPA in the oils by exhibiting acyl-chain



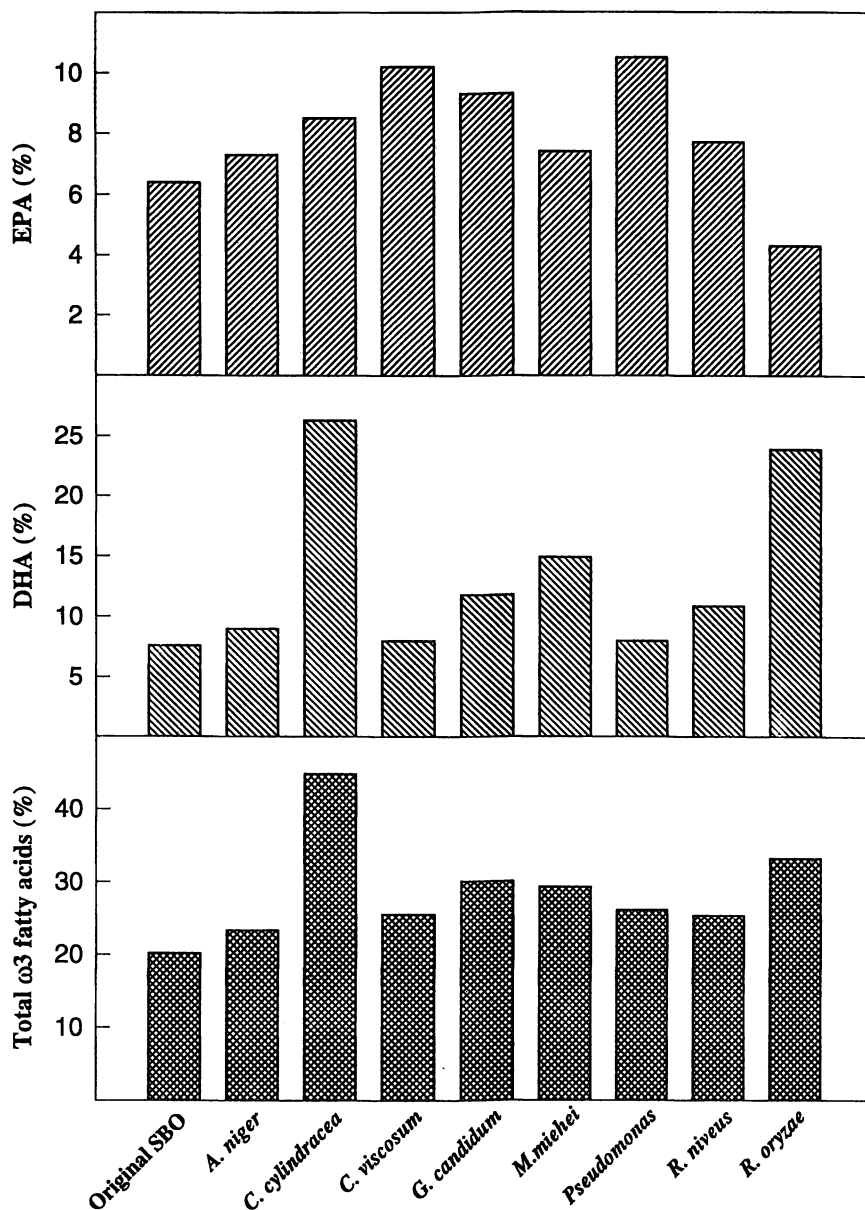
**Figure 2.** Flowsheet for preparation of  $\omega$ 3 fatty acid concentrates by enzymatic hydrolysis

specificity. Tanaka *et al.* (26) have also reported that EPA content of tuna oil was decreased upon hydrolysis by lipase from *Rhizopus* spp; *R. delemer* (RD) and *R. javanicus* (RJ). Kotting and Eibl (27) have reported that lipases from *Rhizopus* spp are *sn*-1 and *sn*-3 position specific. In SBO the EPA content was decreased from 6.4 to 4.3% during a 75 h RO-lipase-assisted hydrolysis. In MHO, the content of EPA decreased from 13.2 to 12.5% during the same period of hydrolysis. Therefore, the rate of hydrolysis of EPA from SBO was much higher than that from MHO. This may be due to the cumulative effect of both 1,3-positional and acyl-chain specificity of RO-lipase. In the stereospecific analysis, it was demonstrated that EPA was located mainly in *sn*-1 and *sn*-3 positions of the TAG in SBO, however, EPA in MHO was equally distributed over the *sn*-2 and *sn*-3 positions and was present only in small amounts in the *sn*-1 position (Table I). Therefore, the higher hydrolysis rate of EPA in SBO may reflect the abundance of this fatty acid in the *sn*-1 and *sn*-3 positions which is highly vulnerable to 1,3-specific RO-lipase hydrolysis.

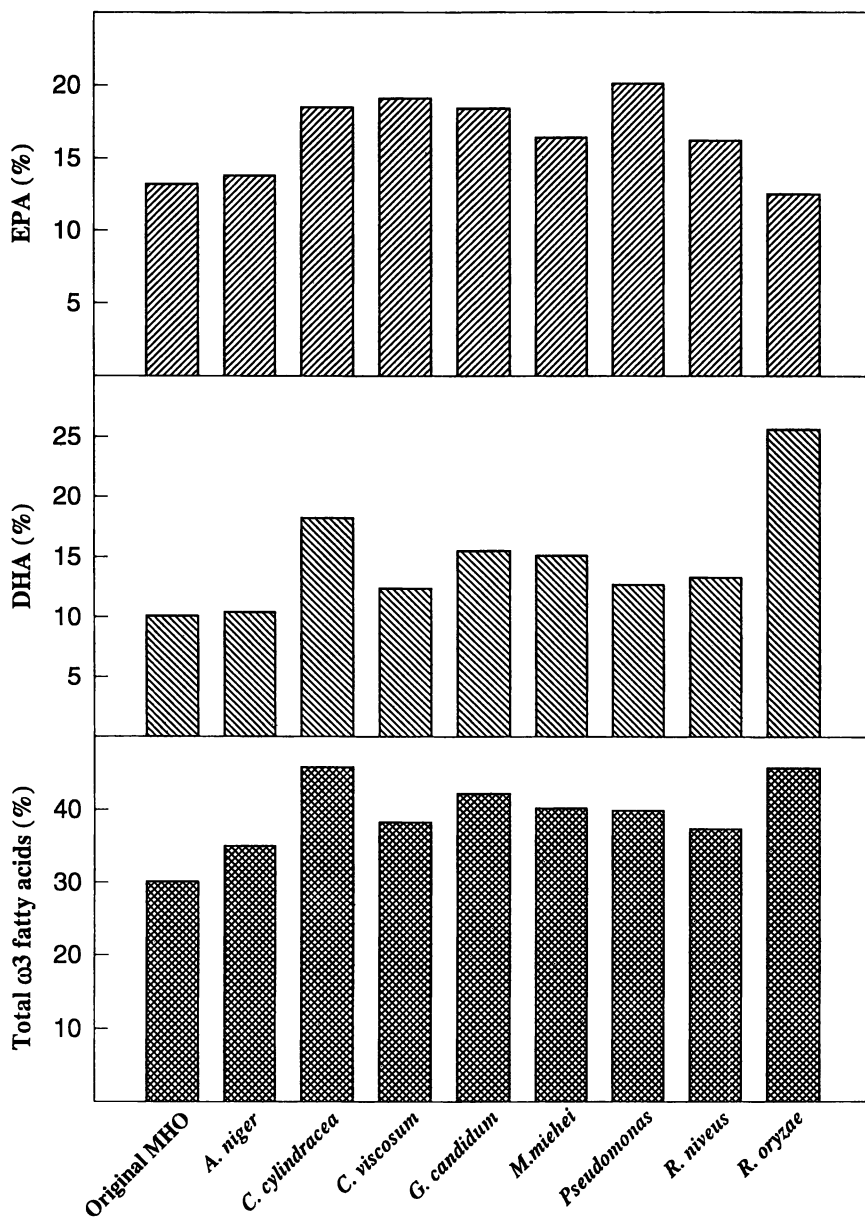
Among the lipases tested in this experiment, AN-lipase gave the lowest total  $\omega$ 3 fatty acids in the non-hydrolysed fraction of both oils. Even though this lipase is 1,3-specific, it was not able to hydrolyse much of the saturated fatty acids present in the *sn*-1 position of MHO (about 43.3% of saturated fatty acids were present in the *sn*-1 position of MHO, Table I). Similarly, 1,3-specific RN-lipase was unable to hydrolyse much of the saturated fatty acids from MHO. Therefore, no correlation was found between positional specificity and ease of hydrolysis of fatty acids in the oils studied. This implies that the concept of positional specificity of lipases alone cannot be used to explain the observed hydrolysis differences of marine oils. Hoshino *et al.* (28) have pointed out that the course of hydrolysis of marine oils by lipases is decided by cumulative effects of various factors such as differences in substrate specificities, including fatty acid and positional specificity of lipases, differences in the rate of the reverse reaction which occurs during hydrolysis, differences in fatty acid composition of the oil and reactivity of each lipase towards partial acylglycerols (monoacyl- and diacylglycerols).

Bottino *et al.* (29) have illustrated the mechanism of resistancy of lipases towards the long-chain  $\omega$ 3-PUFA in marine oils. The presence of *cis* carbon-carbon double-bonds in the fatty acids results in bending of the chains (Figure 5). Therefore, the terminal methyl group of the fatty acid lies close to the ester bond which may cause a steric hinderance effect on lipases. The high bending effect of EPA and DHA due to the presence of 5 and 6 double-bonds, respectively, enhanced the steric hinderance effect, therefore, lipases cannot reach the ester-linkage of these fatty acids and glycerol. However, saturated or monounsaturated fatty acids do not present any barriers to lipase and they could be easily hydrolysed. Therefore, fatty acid selectivity of a lipase for EPA and DHA allows separation and concentration of these fatty acids from others in the remaining portion of marine oils. In addition, lipases have been frequently used to discriminate between EPA and DHA in concentrates containing both of these fatty acids, thus providing the possibility of producing  $\omega$ 3 fatty acid concentrates with dominance of either EPA or DHA.

Use of microbial enzymes to produce  $\omega$ 3 fatty acid concentrates has received much attention in recent years; both hydrolysis and transesterification reactions may

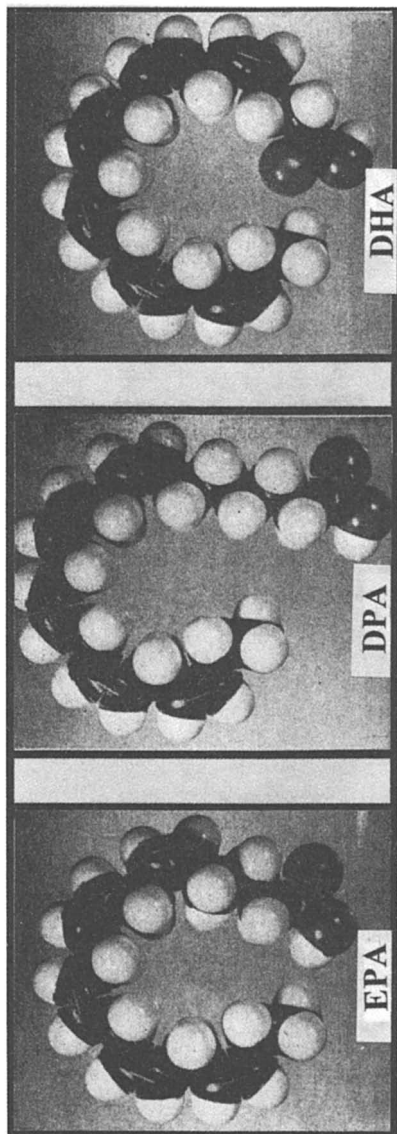


**Figure 3.** Enrichment of total  $\omega$ 3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) after 75h hydrolysis of seal blubber oil by different microbial lipases



**Figure 4.** Enrichment of total  $\omega$ 3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) after 75h hydrolysis of menhaden oil by different microbial lipases





**Figure 5.** Molecular models of eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA).

be considered. Tanaka *et al.* (26) used six types of microbial lipases (*AN*, *CC*, *CV*, *RD*, *RJ* and *PS*) to hydrolyse tuna oil and found that *CC*-lipase was the most effective one in increasing the DHA content in the preparation. It increased the DHA content in the non-hydrolysed fraction to three times of that present in the original tuna oil; however, other lipases did not increase the DHA content of the oil. Hoshino *et al.* (28) have also used several lipases for selective hydrolysis of cod liver and sardine oils. The highest degree of hydrolysis was reached when non-regiospecific *CC*- and *sn*-1 and *sn*-3 specific *AN*-lipase were used, but none of the lipases were able to increase the EPA content of the acylglycerols to any great extent. However, concentrates with over 50% of total  $\omega$ 3 fatty acids were produced when these two lipases were employed. Shimada *et al.* (30) have reported that hydrolysis of tuna oil by *GC*-lipase increases the content of total EPA and DHA from 32.1 to 57.5%. Osada *et al.* (31) have employed *CV*- and *CC*-lipases for direct esterification of glycerol with individual free fatty acids, including EPA and DHA. The *CV*-lipase was superior to *CC*-lipase and 89-95% incorporation levels were obtained. With the latter lipase, 71-75% incorporation was obtained for all fatty acids, except DHA which reached 63% incorporation. More recently, Akoh *et al.* (32) reported the incorporation of EPA into evening primrose oil (EPO) using *Candida antarctica*-lipase. These researchers were able to incorporate up to 43% EPA in EPO.

## Conclusions

The  $\omega$ 3 fatty acids in blubber oil from seal were primarily located at the *sn*-1 and *sn*-3 positions of the TAG molecules while in fish oils they occurred dominantly in the *sn*-2 position of the TAG molecules. Preparation of  $\omega$ 3 fatty acid concentrates may be achieved by hydrolysis of marine oils with different microbial lipases. Among the lipases tested in this study, *CC*-lipase seems to be the most active one for increasing the content of  $\omega$ 3 fatty acids in non-hydrolysed fraction of both SBO and MHO. This method of concentration of  $\omega$ 3 fatty acids involved mild conditions and thus provides a promising alternative to the traditionally employed concentration methods which involves drastic conditions.

## Literature Cited

1. Bang, H.O.; Dyerberg, J.; Hjorne, N. *Acta. Med. Scand.* **1976**, *200*, 59-73.
2. Simopoulos, A.P. *Am. J. Clin. Nutr.* **1991**, *54*, 438-463.
3. Kinsella, J.E. *Food Technol.* **1986**, *40*(2), 89-97.
4. Branden, L.M.; Carroll, K.K. *Lipids* **1986**, *21*, 285-288.
5. Yongmanitchai, W.; Ward, O.P. *Prog. Biochem.* **1989**, *24*, 117-125.
6. Neuringer, M.; Anderson, G.J.; Connor, W.E. *Ann. Rev. Nutr.* **1988**, *8*, 517-521.
7. Canadian Scientific Review Committee, Nutrition Recommendations: Minister of National Health and Welfare, Ottawa, ON, **1990**, (H49-42/1990E).

8. Jensen, R.G.; Dejong, F.A.; Clark, R.M. *Lipids* **1983**, *18*, 239-252.
9. Filer, L.J.; Mattson, F.H.; Formon, S.J. *J. Nutr.* **1969**, *99*, 293-298.
10. Tomarelli, R.M.; Meyer, B.J.; Weaber, J.R.; Bernhart, F.W. *J. Nutr.* **1968**, *95*, 583-590.
11. Brockerhoff, H. *Biochim. Biophys. Acta* **1970**, *212*, 92-101.
12. Brockerhoff, H. *Lipids* **1971**, *6*, 942-956.
13. Laakso, P.; Christie, W.W. *Lipids* **1990**, *25*, 349-353.
14. Itobashi, Y.; Kuksis, A.; Marai, L.; Tagaki, T. *J. Lipid Res.* **1990**, *31*, 1711-1717.
15. Verheij, H.M.; Dijkstra, B.W. In *Lipase*; Wooley, P.; Petersen, S.B., Eds.; Cambridge University Press: Cambridge, **1994**, pp. 119-138.
16. Brockerhoff, H.; Hoyle, R.J.; Huang, P.C. *Can. J. Biochem.* **1966**, *44*, 1519-1525.
17. Aursand, M.; Jorgensen, L.; Grasdalen, H. *J. Am. Oil Chem. Soc.* **1995**, *72*, 293-297.
18. Brockerhoff, H.; Ackaman, R.G.; Hoyle, R.J. *Arch. Biochem. Biophys.* **1963**, *100*, 9-12.
19. Ando, Y.; Nishimura, K.; Aoyanagi, N.; Takagi, T. *J. Am. Oil Chem. Soc.* **1992**, *69*, 417-424.
20. Ota, T.; Kawabata, Y.; Ando, Y. *J. Am. Oil Chem. Soc.* **1994**, *71*, 475-478.
21. Hamazaki, T.; Hirai, A.; Terano, T.; Sajiki, J.; Kondo, S.; Fujita, T.; Tamura, Y.; Kumagai, A. *Prostaglandins* **1982**, *23*, 557-567.
22. El-Boustani, S.; Colette, C.; Monnier, L.; Descomps, B.; Paulet, C.A.; Mendey, F. *Lipids* **1987**, *22*, 711-714.
23. Lawson, L.D.; Hughes, B.G. *Biochem. Biophys. Res. Comm.* **1988**, *152*, 328-335.
24. Yang, L.Y.; Kuksis, A.; Myher, J.J. *Biochem. Cell Biol.* **1989**, *67*, 192-204.
25. Haraldsson, G.G.; Hoskuldsson, P.A. *Tetrahed. Lett.* **1989**, *30*, 1671-1674.
26. Tanaka, Y.; Hirano, J.; Funada, T. *J. Am. Oil Chem. Soc.* **1992**, *69*, 1210-1214.
27. Kotting, J.; Eibl, H. In *Lipases*; Woolley, P.; Petersen, S.B., Eds.; Cambridge University Press: Cambridge, **1994**, pp. 289-313.
28. Hoshino, T.; Yamane, T.; Shimuzu, S. *Agric. Biol. Chem.* **1990**, *54*, 1459-1467.
29. Bottino, N. R.; Vandenburg, G.A.; Reiser, R. *Lipids* **1967**, *2*, 489-493.
30. Shimada, Y.; Murayama, K.; Okazaki, S.; Nakamura, M.; Sugihara, A.; Tominaga, Y. *J. Am. Oil Chem. Soc.* **1994**, *71*, 951-954.
31. Osada, K.; Nakamura, M.; Nonaka, M.; Hatano, M. *J. Jpn. Oil Chem. Soc.* **1992**, *41*, 39-43.
32. Akoh, C.C.; Jennings, B.H.; Lillard, D.A. *J. Am. Oil Chem. Soc.* **1996**, *73*, 1059-1062.

## Chapter 21

# Separation of $\omega$ 3 Polyunsaturated Fatty Acids from Fish Oil and Stabilization of the Oil Against Autoxidation

Daeseok Han, Hyun-Kyung Shin<sup>1</sup>, and Suk Hoo Yoon

Korea Food Research Institute, San 46-1, Baekhyundong, Bundanggu, Seongnamsi, Kyungido 463-420, Korea

Fatty acid fractions rich in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) could be obtained from sardine oil by solvent fractional crystallization and urea adduct formation methods. The former method was based on the solubility difference of saturated and unsaturated fatty acid salts in ethanol. Since the composition of EPA and DHA changed due to the kind of organic solvent used as the reaction medium for urea adduct formation, EPA and DHA could selectively be enriched. Ascorbic acid could be solubilized in fish oil *via* fish oil/lecithin/water reverse micelles. When 200ppm ascorbic acid was used together with 4,000ppm  $\delta$ -tocopherol, the induction period of the stabilized fish oil was extended 22 times as compared to that of a control sample. Combined use of tocopherol and ascorbic acid could inhibit the production of carbonyl and volatile compounds, and the oxidative polymerization of the polyunsaturated fatty acids.

In 1979, Dyerberg and Bang (1) reported that human populations consuming 200-400 g fish/day (e.g., Greenland Eskimos) were less prone to coronary heart diseases as compared with those who were consuming a lesser amount of fish. Later studies (2,3) showed that the important components yielding this epidemiological result were  $\omega$ 3 polyunsaturated fatty acids (PUFA) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

Considering the dietary habit of most people, however, it is rather impractical to consume such a large amount of fish on a daily basis. Therefore, concentrate forms of EPA and DHA, may reduce the intake volume of the oil, especially for those who dislike fish.

<sup>1</sup>Current address: Department of Nutrition and Food Science, Hallym University, Chunchonsi, Kangwondo, Korea

Lipids are sensitive to autoxidation which adversely affects the flavor and nutritive value of products. This is especially true for fish oils which contain a large amount of PUFA (4). To overcome the liability of fish oil to become rancid, the rate of free-radical chain reaction has to be slowed down by removing oxygen, sequestering metal ions and sensitizers, scavenging radicals and/or adding antioxidants. When applying antioxidant to fish oil, it is important to use several antioxidants in combination because such formulations usually result in a synergistic effect (5). Synthetic antioxidants have been commonly used to control oxidation. However, consumers are increasingly reluctant to accept synthetically derived additives in their foods (6). In addition, according to legislation in some countries such as Korea and Japan, incorporation of synthetic antioxidant to fish oil products is not permitted. Therefore, natural antioxidants are most desirable in this regard.

This contribution describes separation methods of EPA and DHA from fish oil by solubility differences of fatty acid salts in ethanol and by adduct formation of fatty acids with urea. It also describes a method to solubilize ascorbic acid in fish oil *via* reverse micelles and its synergistic antioxidative effect with tocopherol.

## Experimental

Sardine oil was purchased from a local market in Korea and was used after purification. Antioxidants were purchased from Sigma, and lecithin (Centrol 1FUB) was obtained from Central Soya Co. (Fort Wayne, IN).

Saponification of fish oil and extraction of fatty acid salts were done according to the procedure of a Japanese patent (7). Upon cooling the saponified solution, it was partially solidified. A liquid fraction enriched in particular fatty acids was obtained by filtration.

Fatty acids of fish oil was prepared according to the procedure of Haagsma *et al.* (8). To fatty acids, urea and a wetting agent were added, and the mixture was shaken overnight to allow adduct formation. Organic solvents used for adduct formation in order to achieve selective enrichment of EPA and DHA.

To solubilize ascorbic acid in fish oil, lecithin was dissolved in fish oil, and then ascorbic acid solution was injected to the mixture. Upon stirring, ascorbic acid solution was uniformly dispersed in the oil phase.  $\delta$ -Tocopherol was directly mixed with fish oil. Antioxidative effect was analyzed by measuring induction period monitored using Rancimat 679 (Metrohm CH-9100, Herisau, Switzerland). Peroxide value, carbonyl value and fatty acid composition were determined according to the AOCS methods (9). Headspace volatile compounds were analyzed using gas chromatography (10). All reagents and solvents used were of analytical grade, unless otherwise specified.

## Results and Discussion

**Separation of EPA and DHA from Fish Oil by Solubility Difference of Fatty Acid Salts in Ethanol.** It is well known that alkali salts of less unsaturated fatty acids crystallize more rapidly than those of polyunsaturated fatty acids containing four or more double bonds when the saponified solution is cooled (11). Effect of cooling temperature and the procedure used on the content of  $\omega$ 3 PUFA in the concentrate

and the yield were compared. As shown in Table I, EPA and DHA could be concentrated by more than 2.3-fold from fish oil with yields of at least 87% and 91%, respectively. Fatty acid compositions of PUFA concentrate which were prepared via different treatments indicated that cooling temperature and procedures employed did not influence significantly the yield and the contents of EPA, DHA, as well as other  $\omega$ 3 PUFA. Therefore, ambient temperature would be a practical choice for large-scale separation.

**Table I. Effect of Cooling Temperature and Procedure on the Enrichment of  $\omega$ 3 PUFA**

Cooling temperature(°C)	Cooling rate (C/min)	Fatty acid composition(%)			Concentrate obtained(g)**
		EPA	DHA	Other $\omega$ -3 PUFA	
Sardine oil		14.2	10.7	8.3	20.0
25	1	32.5	25.6	14.3	7.6
10	0.5	33.6	27.1	14.5	7.1
10	2	34.1	27.8	14.0	6.8
0	0.5	33.7	27.3	14.6	7.0
0	2	34.2	28.0	14.1	6.6
-15	2	34.6	28.3	14.6	6.6
-15*	2	35.7	28.8	14.9	6.2

\*The saponified solution was first cooled to 10C, and then the filtrate of it was cooled further to -15 C .

\*\*Concentrate obtained using 20 g of fatty acids from sardine oil.

SOURCE: Reproduced with permission from reference 11. Copyright 1987 Daeseok Han.

Separation of particular fatty acids is generally based on two factors, carbon chain length and degree of unsaturation. However, the principle of separation of this procedure can be more satisfactorily explained by using the ratio of the number of double bonds to carbon number. The concentration increase is then defined as the ratio of the percentage of a fatty acid in PUFA concentrate to that in sardine oil. Thus, the sodium salt of a fatty acid is more soluble in ethanol as the number of double bonds increases at a fixed carbon number, and as the chain length decreases when the degree of unsaturation remains the same.

**Selective Enrichment of EPA and DHA by Adduct Formation of Fatty Acids of Fish Oil and Urea.** This method is based on the principle that saturated fatty acids easily form adducts with urea, but unsaturated ones do not (12). Conventional method that used methanol as a wetting agent of urea caused unwanted methanolysis of  $\omega$ 3 fatty acids during the separation procedure. To overcome the problem, other solvents were examined in this study. When urea adduct formation was carried out in ethanol, methanol, water, formamide and acetonitrile as a wetting agent for urea, the content of EPA, DHA, and their precursors (short chain  $\omega$ 3 fatty acids are known

to be precursors of EPA and DHA) in non-adduct forming fractions was higher than those in the starting material. Thus, all solvents tested could be used as a wetting agent and water may provide the best choice because of its low cost and lack of toxicity.

**Table II. Effect of the Organic Solvent on the Enrichment of EPA, DHA and Their Precursors by Urea Adduct Formation Method**

Organic solvent	Fatty acid composition(area %)				Concentrate obtained(g)
	EPA	DHA	other $\omega$ -3 PUFA	Others	
Starting material	15.5	9.8	7.4	67.3	
Methylene chloride	35.8	24.2	16.3	23.7	10.0
Heptane	35.8	30.6	13.8	19.8	3.1
Benzene	35.3	26.4	16.0	22.3	2.9
Xylene	34.7	25.5	15.3	24.5	3.1
Ethyl ether	33.9	23.6	15.4	27.1	3.3
Cyclohexane	26.2	31.8	14.8	27.2	3.8
<i>n</i> -Hexane	22.5	42.8	15.0	19.7	2.5
Isooctane	19.3	48.0	14.5	18.2	2.0
Pentane	15.5	49.1	14.9	20.5	1.8
1,4-Dioxane	14.8	9.2	7.2	68.8	1.7
<i>n</i> -Hexane*	17.2	10.5	8.1	64.2	10.2
					8.0

\*In this case, urea adduct formation was carried out without a wetting agent.

SOURCE: Reproduced with permission from reference 12. Copyright 1990 Daeseok Han.

Another advantage of using water is that more diverse organic solvents can be used as the reaction medium for urea adduct formation because it is immiscible with most of organic solvents. Table II lists fatty acid compositions of non-adduct forming fraction when urea adduct was formed in 10 different solvents. It indicates that a wetting agent has to be present for adduct formation, and reaction medium has to be immiscible with the wetting agent.

The composition of EPA or DHA varied significantly according to the kind of organic solvent used, but the sum of their amounts remained nearly unchanged. It was envisaged that successive fractionation of fatty acids with pentane and then heptane may selectively enrich DHA and EPA, respectively. When urea adduct with fatty acids was formed in pentane, DHA content in non-adduct forming fraction was 49.1%, which corresponds to 5.1 times as much as that (9.8%) in the starting material. To the filter cake, 2 volumes of heptane were added. After refluxing the slurry for 1 h at the boiling point of the solvent to dissociate adduct, it was cooled to room temperature, and then kept overnight to reform adduct in heptane. EPA content in the second non-adduct forming fraction was 53.0%. From 10 g of fatty acids derived from fish oil, 1.7 g of DHA-enriched fraction and 1.7g of EPA-enriched fraction were obtained, respectively.

### Stabilization of Fish Oil

**Solubilization of Ascorbic Acid in Fish Oil via Reverse Micelles.** Amphiphilic molecules, when dissolved in non-polar media, form spherical or ellipsoidal aggregates. In these systems, often referred to as reverse micelles, hydrophobic carbon chains of the surfactants are arranged toward the non-polar medium, and hydrophilic groups are localized in the interior of the aggregates (13). Polar solvents, including water, can be solubilized in this polar core. With the help of polar solvents, hydrophilic compounds such as ascorbic acid can also be incorporated into that polar core (14).

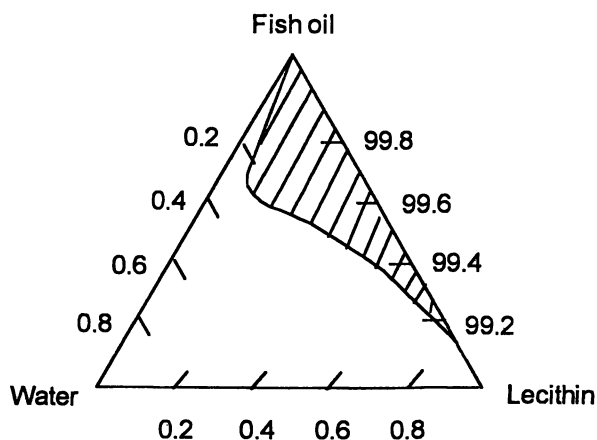
In this study (15), edible lecithin was used as a surfactant, and water was used as a carrier of ascorbic acid. The phase diagram of the fish oil/lecithin/water system is shown in Figure 1, in which the slashed region indicates that clear reverse micelles was maintained during the experimental period.

**Combined Effect of Ascorbic Acid and  $\delta$ -Tocopherol on Oxidative Stability of Fish Oil.** Induction period of sardine oil with 0.1% lecithin, determined by Rancimat at 80°C, was 4.4 h. Individual addition of ascorbic acid (0.04%) and  $\delta$ -tocopherol (0.2%) increased the length of the induction period of fish oil to 11.3 and 8.5 h, respectively. Figure 2 shows changes of induction periods and synergistic efficiencies when ascorbic acid was varied from 0 to 0.4% at a fixed content of  $\delta$ -tocopherol (0.2%). Considering that sum of the increments due to individual effects is 11.0 h{(8.5 h - 4.4 h) + (11.3 h - 4.4 h) = 11.0 h}, induction period of 40.0 h at 0.2%  $\delta$ -tocopherol and 0.04% ascorbic acid indicates that they have acted synergistically. In another experiment, upon varying the concentration of  $\delta$ -tocopherol (0 - 0.3%) at a fixed content of ascorbic acid (0.02%), the synergistic efficiency was more than 100% (16). If a similar experiment was done at 30°C, extension of induction period reached 24-fold. Synergistic efficiency at 80°C appeared to be lower, probably due to the thermal destruction of ascorbic acid at high temperatures.

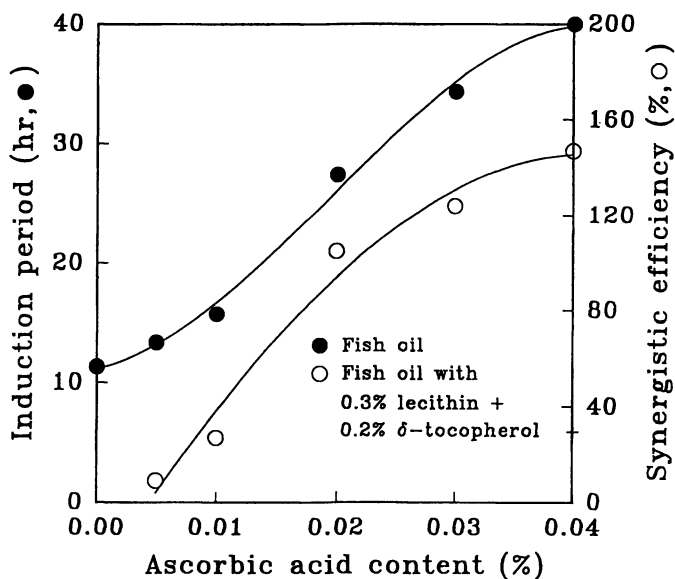
Figure 3 shows the changes of carbonyl values for a control and an oil sample with antioxidants. The carbonyl value of the control increased exponentially with storage time after a certain lag period, and then reached 45 after 36 days of storage in the dark at 30°C. For oils treated with ascorbic acid and  $\delta$ -tocopherol, the carbonyl value remained around 1.0 during the same period. When comparing the carbonyl values of oil samples with the same peroxide value, the level of these oxidation products in the oil with antioxidants was lower than that of the control, indicating that the decomposition of hydroperoxides to carbonyl compounds could be suppressed by the addition of the two antioxidants.

The effect of ascorbic acid and  $\delta$ -tocopherol on the formation of volatile compounds in the oils stored in serum vials were examined. Integrator readings of the headspace gases showed that these antioxidants, in combination, inhibited the occurrence of rancid flavor of fish oil (Figure 4). While the volatile compounds of the control increased rapidly to reach an integrator reading of  $1.4 \times 10^5$  after 36 days of storage, the oil stabilized with the two antioxidants produced one tenth of the level produced in the control. This effect may be ascribed to minimal formation of low molecular weight compounds, such as aldehydes and ketones (Figure 3). It is well

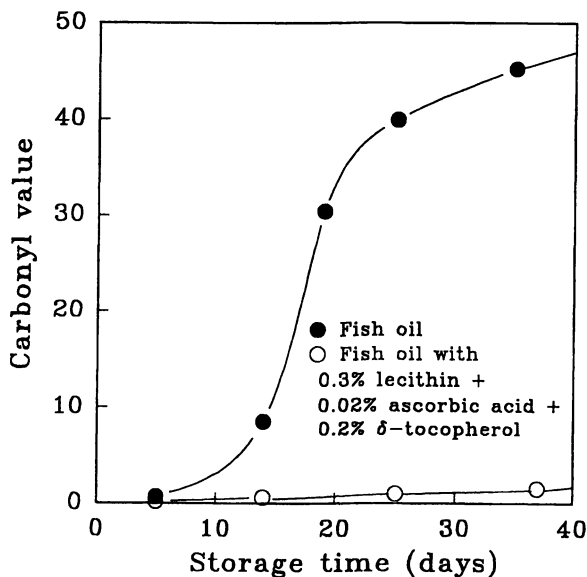




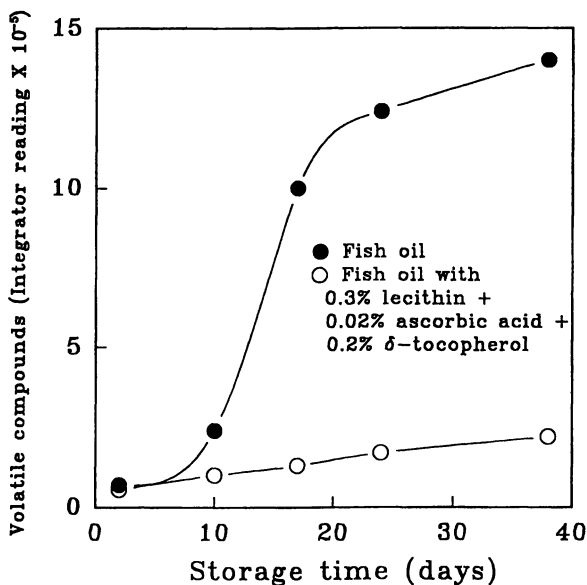
**Figure 1.** Phase Diagram of Fish Oil/Lecithin/Water System (Reproduced with permission from ref. 15. Copyright 1991 AOCS Press).



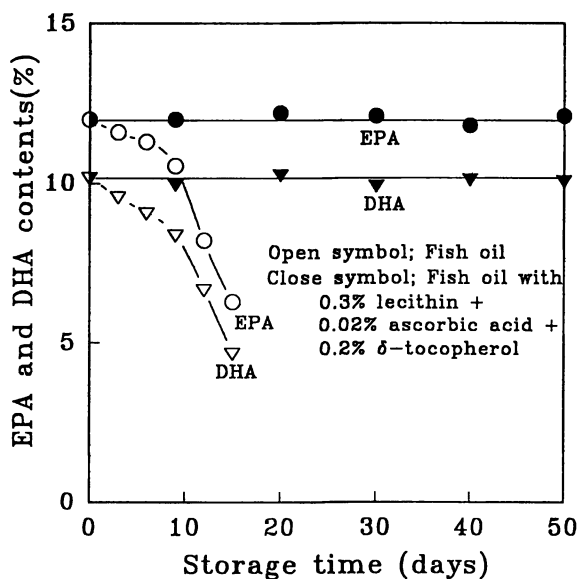
**Figure 2.** Dependence of Induction Period (Rancimat at 80) of Fish Oil and Synergistic Efficiency on Ascorbic Acid Content in the Presence of 0.2%  $\delta$ -Tocopherol (Reproduced with permission from ref. 16. Copyright 1991 AOCS Press).



**Figure 3.** Combined Effects of Ascorbic Acid and  $\delta$ -Tocopherol on the Carbonyl Value of Fish Oil Stored in Petri Dishes (Reproduced with permission from ref. 15. Copyright 1991 AOCS Press).



**Figure 4.** Combined Effects of Ascorbic Acid and  $\delta$ -Tocopherol on Volatile Compound Formation of Fish Oil Stored in Serum Vials (Reproduced with permission from ref. 15. Copyright 1991 AOCS Press).



**Figure 5.** Changes of EPA and DHA Compositions of Fish Oil Stored in Petri Dishes (Reproduced with permission from ref. 15. Copyright 1991 AOCS Press).

known that as the volatile compounds in soybean oil increases, the flavor quality of the product decreases (17,18). The characteristic objectionable rancid odor of fish oil in control was intense after nine days, whereas there was little discernible flavor in the oil treated with both antioxidants, even after 36 days.

Changes of fatty acid composition in the stored oils are shown in Figure 5. The initial contents of EPA (12.1%) and DHA (10.0%) in the control were reduced to about the amount after 15 days. However, stabilized oil (0.3% lecithin, 0.02% ascorbic acid and 0.2%  $\delta$ -tocopherol) remained unchanged over 50 days. Thus, the combined use of both antioxidants inhibits the destruction and/or polymerization of unsaturated fatty acids.

### Literature Cited

1. Dyerberg, J.; Bang, H. O. *Lancet*. 1979, *II*, 117-119.
2. Kromhout, D.; Boschietter, E. B.; Koulander, C. D. L. *New Engl. J. Med.* 1985, *312*, 1205-1209.
3. Sanders, T. A. B. *Proc. Nutr. Soc.* 1985, *44*, 391-397.
4. Cho, S.-Y.; Miyashita, K.; Miyazawa, T.; Fujimoto, T. *J. Am. Oil Chem. Soc.* 1987, *64*, 876-879.
5. Kanematsu, H.; Aoyama, M.; Maruyama, T.; Niya, I.; Tsukamoto, M.; Tokairin, S.; Matsumoto, T. *Yukagaku*, 1983, *32*, 695-699.
6. Dziezak, J. D. *Food Technol.* 1986, *40*(9), 94-102.
7. Kureha Chem. Co., Ind. 1984, Japanese patent 59-21641.
8. Haagsma, N.; van Gent, C. M.; Luten, J. B.; de Jong, R.W.; van Doorn, E. J. *Am. Oil Chem. Soc.* 1982, *59*, 117-118.
9. *Official and Tentative Methods of the American Oil Chemists' Society*, AOCS, 4<sup>th</sup> ed., Champaign, IL, 1989.
10. Yoon, S.H.; Jung, M.Y.; Min, D.B. *J. Am. Oil Chem. Soc.* 1988, *65*, 1652-1656.
11. Han, D.; Ahn, B.-H.; Shin, H.-K. *Kor. J. Food Sci. Technol.* 1987, *19*, 430-434.
12. Han, D.; Ahn, B.-H.; Shin, H.-K. *Kor. J. Food Sci. Technol.* 1990, *22*, 520-525.
13. Luisi, P. L. *Angew. Chemie*, 1985, *24*, 439-450.
14. Han, D.; Yi, O.-S.; Shin, H.-K. *J. Food Sci.* 1990, *55*, 247-249.
15. Han, D.; Yi, O.-S.; Shin, H.-K. *J. Am. Oil Chem. Soc.* 1991, *68*, 740-743.
16. Yi, O.-S.; Han, D.; Shin, H.-K. *J. Am. Oil Chem. Soc.* 1991, *68*, 881-883.
17. Min, D. B. *J. Am. Oil Chem. Soc.* 1983, *60*, 544-545.
18. Yoon, S.H.; Kim, J.G.; Min, D.B. *Kor. J. Food Sci. Technol.* 1985, *17*, 355-360.

## Chapter 22

# Microencapsulation and Oxidative Stability of Docosahexaenoic Acid

Pahn Shick Chang

Department of Food Science and Technology, Seoul National Polytechnic University, 172 Kongnung-Dong, Nowon-Ku, Seoul 139-743, Korea

Microcapsules of docosahexaenoic acid (DHA) in waxy corn starch were prepared. Microencapsulation was accomplished by extruding an oil/water emulsion, consisting of a non-aqueous solution dispersed in a mixture of liquefied waxy corn starch and emulsifier, under high pressure through an orifice submerged in a chilled dispersion fluid. Optimum conditions for the microencapsulation of DHA were attained when 0.5% (w/v) aqueous solution of waxy corn starch contained 3.0% (w/w) of a 1:1 to 1:2 ratio of emulsifiers Tween 85 and Tween 20. Maximum encapsulation yield was 97.5% under the experimental conditions employed. The microcapsules so obtained were stored at 35°C and their oxidative stability tested. The amount of DHA and peroxide value in the microencapsulated product remained unchanged after 2 weeks of storage.

The diet of Greenland Eskimos consists of high levels of fish and marine mammals and their lipids. Thus incidences of high blood pressure and cardiac infarction are rare in this population. Many nutritional biochemists have demonstrated that docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) in fish oil play an important role in reducing blood pressure and blood cholesterol and viscosity (1-6). However, these polyunsaturated fatty acids are very sensitive to environmental conditions, such as oxygen, light, and heat and, if not protected, acquire a fishy smell and a bitter taste (3). To prevent these highly unstable fatty acids from deterioration and development of a fishy flavor and taste, macroencapsulation of fish oil is employed (7-9). However, macrocapsules with diameters of larger than 5 mm have a disadvantage because of their large size. Moreover, digestibility of macrocapsules is very low since they have a thick shell, ranging between 0.5 and 1.0 mm. To solve these problems, microencapsulation, which is defined as the technology to produce microcapsules having diameters of less than several tens of micrometers has gained popularity. This study examines microencapsulation of DHA and storage stability of microencapsulated powders under environmental conditions of oxygen and heat.

The conditions for gas chromatographic analysis of DHA are shown in Table I.

**Table I. Experimental conditions for the assay of DHA with gas chromatography.**

Contents	Conditions
Instrument	HP 5890 <sup>+</sup> II
Detector	Flame Ionization Detector
Column	Omega wax 320 <sup>TM</sup> , 30mx0.25mm ID, 0.25 $\mu$ m film
Temperature	
Column	190 °C (0 min) $\rightarrow$ 230 °C (1 °C/min) $\rightarrow$ 230 °C for 5 min
Injector	250 °C
Detector	260 °C
Carrier gas	Helium (99.999%), 1.2 mL/min
Split ratio	80:1

**Microscopic Observation.** Samples of all microcapsules suspended in a dispersion fluid were observed under a light microscope to verify the presence of the capsules and to note characteristics such as carrier vacuole distribution and coalescence or stability of the capsules. The capsule size was estimated by a calibrated ocular grid.

**Storage Stability of Microcapsules.** The ability of microcapsules to retain their initial state at 35°C following encapsulation of DHA by freeze drying was tested. The powder of microcapsules was prepared and saponified with ethanolic KOH. The DHA in the microcapsules was extracted and then transmethylated using BF<sub>3</sub>-methanol and analyzed for changes in microencapsulation yield (see Figure 1).

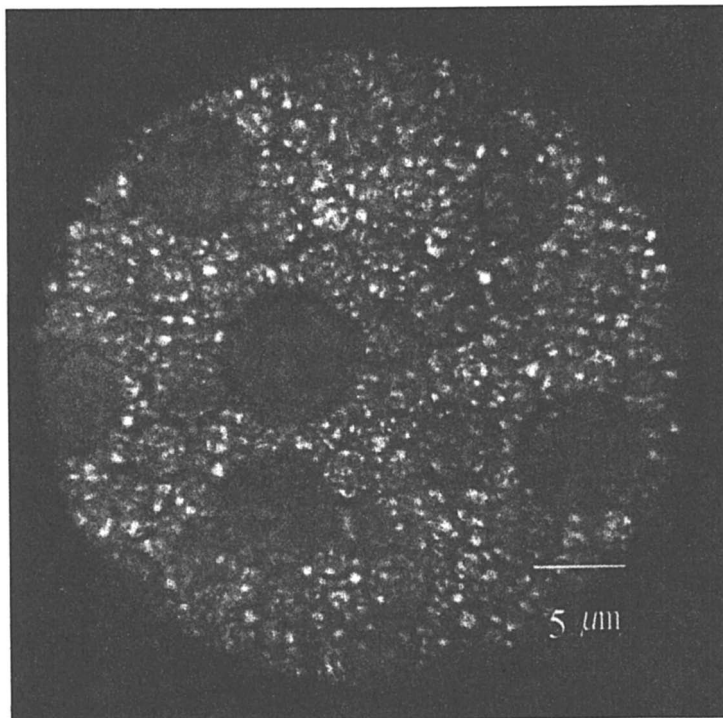
## Results and Discussion

**Photomicrographs of Microcapsules.** A representative photomicrograph of microcapsules in Figure 2 demonstrates that the capsule configuration was a multiple phase emulsion. An enlargement of a photomicrograph taken at 1,000 x magnification of the capsules is also shown (Figure 2). The continuous phase is water, and the apparent ring is a waxy corn starch shell surrounding microencapsulated non-aqueous carrier vacuoles.

**Microencapsulation Conditions.** The airless sprayer produced microcapsules with a maximum diameter between 10 and 15  $\mu$ m. Regardless of the apparatus or conditions to form the capsules, most microcapsules were 5  $\mu$ m or less in diameter with a small percentage being greater than 10  $\mu$ m in diameter. The percentage microencapsulation of DHA increased from as the orifice used for ejection increased in size (data are not shown.).

Preparation of powder of microcapsules using freeze dryer  
↓  
Sampling (5 g) after 2 weeks  
↓ Addition of C<sub>23:0</sub> fatty acid as an internal standard  
Saponification with 50 mL of 1N-KOH in ethanol for 30 min  
↓  
Extraction with 150 mL of distilled water and 100 mL of diethyl ether for 5 min  
↓  
Taking the diethyl ether phase  
↓  
Transesterification using BF<sub>3</sub>-methanol methodology  
↓  
Gas chromatographic analysis of DHA

**Figure 1.** Procedure for the analysis of oxidative state of microcapsules containing DHA



**Figure 2.** Photomicrograph of typical microcapsules composed of a waxy corn starch-emulsifier shell and encapsulated carrier vacuoles dispersed in water.

## Experimental

**Materials.** Waxy corn starch was obtained from Samyang Sugar Corp. (Daejeon, Korea) and DHA and EPA were procured from Dongwon Fishery Co. Ltd (Daejeon, Korea). All commercially available emulsifiers (purity > 95%) used in this study were gifts from Ilshin Emulsifier Co. Ltd (Seoul, Korea) and were used without any further purification. All chemicals used in this study were reagent grade. The dispersion fluid, into which the carrier/waxy corn starch emulsion was injected, was prepared by dissolving an amount of Span 60, equal to 0.01% (w/v) of the final dispersion fluid, in a small beaker of warm distilled water. The Span 60 solution was added to a tared vessel, and chilled water was added until the desired volume and temperature were reached. The total weight and volume of vessel was recorded before and after microcapsule formation.

**Preparation of Carrier in Waxy Corn Starch Emulsion.** Liquefied waxy corn starch was weighed into a beaker and placed in a water bath at 50°C and stirred at 10,000 rpm for 5 min with Ultra Turrax T25 (Janke & Kunkel Co., Ltd, Duesseldorf, Germany). A specified weight of emulsifier was added to the liquefied waxy corn starch and stirred for 5 min. Then, a designated weight of polyunsaturated fatty acid was added slowly to the waxy corn starch-emulsifier mixture with stirring at 10,000 rpm with Ultra Turrax T25 for 1 min. When the emulsion temperature reached 51 - 52°C, it was sprayed and dispersed into the dispersion fluid by an airless paint sprayer of Wagner PP-W400, which was obtained from Spray Tech. Corp. (Minneapolis, MN).

**Formation of Capsules with Airless Paint Sprayer.** The airless paint sprayer was used to form microcapsules. The sprayer generated a high pressure with a piston pump vibrating at 120 strokes per sec, which drew the carrier-waxy corn starch emulsion into a compression chamber and pumped it out through an orifice submerged in the dispersion fluid to form microcapsules. The sprayer was equipped with a nozzle extension, and a control screw was adjusted to produce a fine aerosol of water and maintained at this setting throughout each experiment. Before forming microcapsules, 20 g of carrier-waxy corn starch emulsion at 51°C was sprayed into an empty flask. Then, the orifice of the sprayer was submerged immediately into the dispersion fluid (approximately 1.4 L) containing 0.01% Span 60, and 80 g of emulsion was ejected to form microcapsules.

**Microencapsulation Yield.** Encapsulation yield of all microcapsules was measured as the difference between the carrier concentration in the dispersion fluid after removal of the intact capsules. The sample was stirred, and after centrifugation at 6,000 x g for 10 min, the supernatant of the dispersion fluid was analyzed for its content of DHA using the Folch extraction method (10) and gas chromatography.

Microencapsulation yield was expressed as the percentage of the carrier that was encapsulated according to the following equation.

$$\text{Microencapsulation yield (\%)} = \left(1 - \frac{\text{Unencapsulated DHA}}{\text{Initial DHA content}}\right) \times 100$$



**Effect of Dispersion Fluid Temperature and Various Emulsifier Blends on Percent Microencapsulation of DHA.** The yield for microencapsulation of DHA was highly dependent on the temperature of dispersion fluid and type of hydrophilic emulsifier in waxy corn starch for the microcapsules formed by the airless paint sprayer (Table II). A carrier-waxy corn starch emulsion ejected into the dispersion fluid at 15°C resulted in better microencapsulation than ejection into the fluid at 25 or 35°C. Waxy corn starch and the added emulsifiers with high melting temperature were solidified when ejected into dispersion fluid at 15°C to form a rigid and circular matrix that physically entrapped carrier vacuoles within the waxy corn starch shells. Ejection into the fluids below 15°C produced large cylindrical capsules.

The hydrophilic-lipophilic balance (HLB) of emulsifiers was used to select emulsifiers shown in Table II in order to maximize the microencapsulation yield. The microencapsulation yield with the emulsifier of HLB value of more than 15.0 was very high (more than 90.0%). However, the encapsulation yield with emulsifiers having HLB value of less than 5.0 was lower than 50.0%. This may be because emulsifiers with low HLB value of less than 5.0 are hydrophobic emulsifiers and tend to stabilize water in oil emulsions. But emulsifiers with high HLB value (greater than 15.0) are hydrophilic emulsifiers and may be related to the tendency of stabilizing oil in water emulsions.

The blending of Tween 20 with Tween 85 gave a microencapsulation yield of 97.5% as shown in Figure 3. The maximum yield for microencapsulation of DHA was attained between 1:1 and 1:2 ratios of Tween 85 and Tween 20. This efficient microencapsulation with Tween 85 and Tween 20 may be due to a synergistic effect (11-16).

**Table II. Effects of various kinds of emulsifiers and dispersion fluid temperature on the microencapsulation yield for DHA<sup>a</sup>**

Emulsifier <sup>b</sup>	Microencapsulation yield, %		
	Dispersion fluid temperature,		
	15	25	35
Glycerol monoricinoleate	41.2	35.1	27.3
Sorbitan trioleate	42.3	37.9	30.4
Glycerol distearate	39.4	32.5	25.0
Propyleneglycol monostearate	36.2	31.1	11.4
Glycerol monostearate	41.7	36.5	21.3
Sorbitan monostearate	54.9	49.6	40.2
Sorbitan monooleate	57.1	51.4	42.8
Sorbitan monopalmitate	80.6	74.5	67.2
Sorbitan monolaurate	74.8	70.2	51.8
Tween 85	87.4	82.1	65.9
Tween 20	92.1	87.8	72.9

<sup>a</sup>Experimental conditions are as follows: airless paint sprayer equipped with 0.8 mm orifice; carrier, purified fish oil; carrier/0.5% waxy corn starch solution, 0.25 (w/w); temperature of emulsion, 50°C; dispersion fluid, 0.01% Span 60.

<sup>b</sup>3.0 % (w/w) of each emulsifier in 0.5% waxy corn starch solution.

**Effect of Concentration of Waxy Corn Starch on Microencapsulation Yield.** The concentration of waxy corn starch is thought to be a very important factor in the yield and storage stability for the microencapsulation of DHA because the rigidity of the shell and retaining ability of the carrier are dependent on the content of waxy corn starch in the shell.

As shown in Figure 4, the maximum yield of microencapsulation of DHA was achieved when more than 0.4% (w/v) of waxy corn starch was used. Therefore, the optimum concentration of waxy corn starch for the microencapsulation was determined as being 0.5% (w/v).

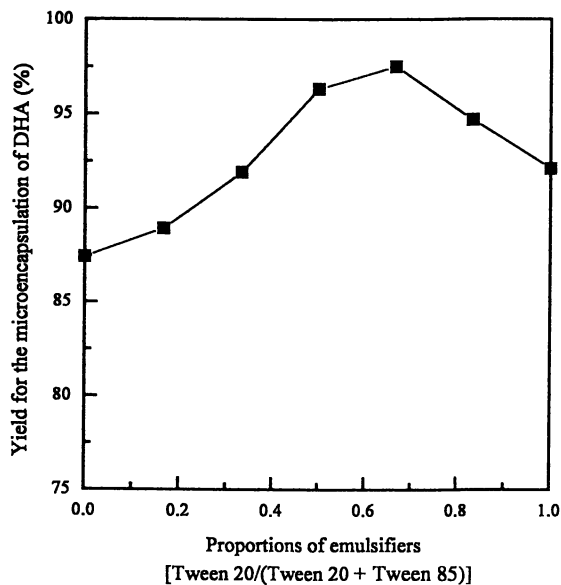
**Effect of Emulsifier Blending Ratios on the Microencapsulation Yield.** Requirement for the two emulsifiers to form microcapsules suggested the possibility that proportions of emulsifiers in a blend may influence the microencapsulation of carrier. It was evaluated by adding various proportions of Tween 20 and Tween 85 with maintaining the combined emulsifier weight at 3.0% (w/w) of the 0.5% waxy corn starch solution.

As shown in Figure 5, blends of emulsifiers were superior for microencapsulation of DHA as compared to either of the individual emulsifiers. Maximum encapsulation yield was attained when Tween 20 comprised 0.50 to 0.67 of total emulsifier; use of larger amounts of Tween 20 reduced the yield of microencapsulation.

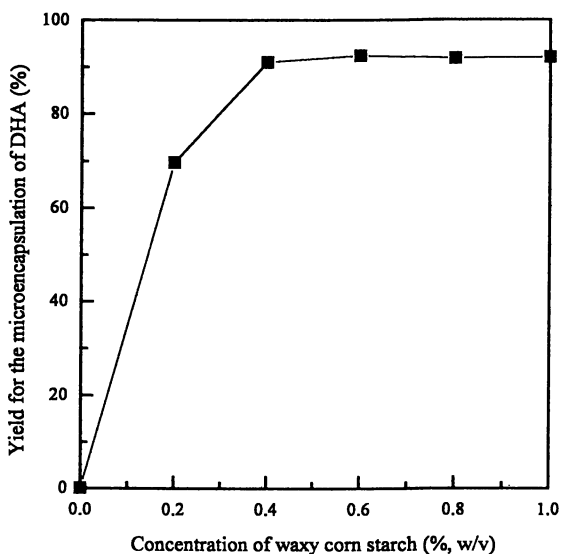
**Effect of Carrier/Waxy Corn Starch Solution Ratio on the Microencapsulation of DHA.** The carrier weight is important for emulsion formation and stability. The amount of carrier weight microencapsulated also will affect the density and rate of creaming of microcapsules, and the optimal concentration of waxy corn starch in the dispersion fluid. The capacity of microencapsulation was tested by adding increased weights of a fish oil containing DHA to waxy corn starch to establish a range of carrier to waxy corn starch ratios from 0.125 to 0.750 (w/w).

As shown in Figure 6, the yield of microencapsulation decreased considerably and there was a hyperbolic increase in the amount of microencapsulated DHA with increasing carrier weight. The ratio of carrier/waxy corn starch of greater than 0.250 (w/w) resulted in loss of retention of DHA and affected the rigidity of the shell. Therefore, the optimal ratio of carrier to waxy corn starch solution was established at 0.250 (w/w).

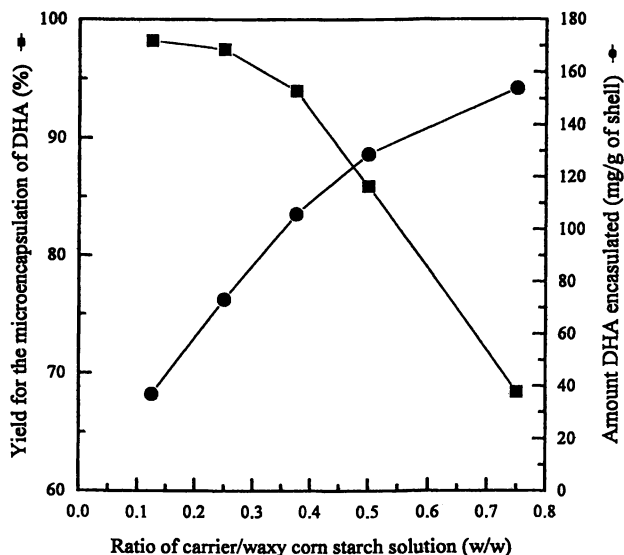
**Storage Stability of the Powder Form Microcapsules.** The powder of the microcapsules of DHA could be prepared using a freeze dryer and tested for storage stability at 35°C. As shown in Figure 7, 9.2% of the initial amount of DHA in the microcapsules was reduced after 2 weeks of incubation at 35°C due to the high stability of the shell structure. Also, the peroxide value of the DHA in microcapsules was measured using the official AOCS method (17) and the value did not increase much after 2 weeks of the storage at 35°C (data are not shown). Therefore, the shell served as a good protecting layer against oxygen and high temperatures. In addition, the shell material of waxy corn starch could be fully digested by the  $\alpha$ -amylase which was produced from porcine pancreas (data are not shown). Thus, if the microcapsules



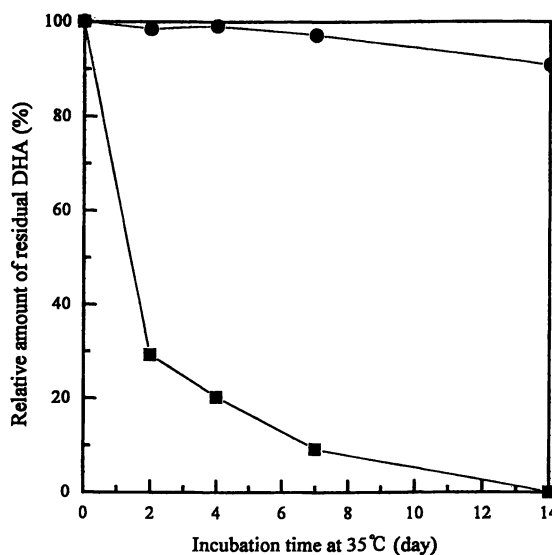
**Figure 3.** Effect of various proportions of Tween 20 and Tween 85 added to waxy corn starch on the microencapsulation yield. The other experimental conditions are the same as described in Table II.



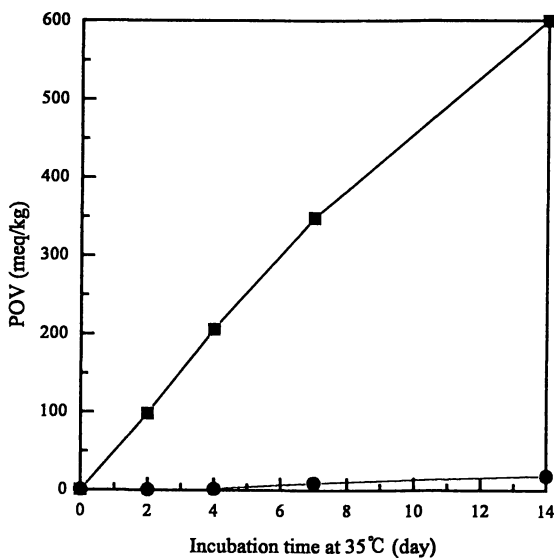
**Figure 4.** Effect of the concentration of waxy corn starch on the microencapsulation yield. Experimental conditions are the same as described in Table II.



**Figure 5.** Effect of various weights of carrier (DHA) added to 0.5% waxy corn starch solution on the microencapsulation yield and amount of DHA encapsulated. At all points, the absolute amount of emulsifiers (Tween 20:Tween 85 = 2:1, w/w) was fixed as the value of 3.0% (w/w) in the shell.



**Figure 6.** DHA changed as a function of incubation time at 35°C. (●) Fish oil in microcapsules; (■) fish oil not microencapsulated.



**Figure 7.** Time course of peroxide value increase at 35°C. (●) Fish oil in microcapsules; (■) fish oil not microencapsulated.

formed by this technology pass through the pancreas, the DHA in the microcapsules will be released by pancreatic amylase.

### Acknowledgments

Financial supports from the Korea Sciences and Engineering Foundations (KOSEF) during 1995-1996 post doctoral program and Seoul National Polytechnic University (1996-1997) are gratefully acknowledged. The author is indebted to his former graduate students, Hak J. Kim, Yong J. Lee, Kwang I. Lee, Hyung D. Kim, and Seung, Y. Lim, for their assistance and to Dr. Sun C. Kim for his advice about microencapsulation process.

### Literature Cited

1. Rice, R. *European Food Drink Rev.* **1991**, *30*, 27-28.
2. Kazumasa, P. *J. of Jpn. Oil Chem. Soc.* **1991**, *37*, 9-13.
3. Bimbo, A. P. In *Fish Oils in Nutrition*; Stansby, M. E. Ed.; Van Nostrand Reinhold Co.: New York, 1990, pp. 181-222.
4. Rice, R. *Lipid Tech.* **1992**, *4*, 14-18.
5. Taguchi, K.; Iwami, K.; Ibuki, F.; Kawabata, M. *Biosci. Biotech. Biochem.* **1992**, *56*, 672-673.
6. Taguchi, K.; Iwami, K.; Ibuki, F.; Kawabata, M. *Biosci. Biotech. Biochem.* **1992**, *56*, 560-563.
7. Monshipouri, M.; Neufeld, R. J. *Enz. Microb. Tech.* **1991**, *13*, 309-313.
8. Bell, F. L. *Food Processing* **1991**, *52*, 42-44.
9. Arshady, R. *J. Microencapsulation* **1992**, *9*, 187-190.
10. Folch, J.; Lees, M.; Stanley, G. H. S. *J. Biol. Chem.* **1957**, *226*, 497-509.
11. Berger, R.; Langhammer, G.; Nitzsche, C. *Germany Patent*, 142,720, **1980**.
12. Balke, W. J. In *The Use of Natural Hydrocolloids as Thickeners, Stabilizers, and Emulsifiers*, Agricultural Experiment Station: New York, U. S. A., 1984, pp. 31-34.
13. Jalil, R.; Nixon, J. R. *J. Microencapsulation* **1990**, *7*, 229-244.
14. Das, K. P.; Kinsella, J. E. *Adv. Food Nutr. Res.* **1990**, *34*, 81-201.
15. Kirby, C. J.; Whittle, C. J.; Rigby, N.; Coxon, D. J.; Law, B. A. *Int. J. Food Sci. Tech.* **1991**, *26*, 437-449.
16. Yan, N. X.; Zhang, M. Z. *J. Microencapsulation* **1992**, *9*, 143-151.
17. AOCS. *Official Methods and Recommended Practices of American Oil Chemists' Society*. 1990; 4th Ed. Am. Oil. Chem. Soc. Champaign, Illinois.

## Author Index

- Ackman, R. G., 148  
Alasalvar, C., 39  
Baek, H. H., 85, 120  
Boyd, Leon C., 9  
Cadwallader, Keith R., 1, 85, 120, 131  
Cha, Y. J., 131  
Chang, Pahn Shick, 264  
Chapman, Frank A., 166  
Chen, L. M., 64  
D'Abramo, L. R., 120  
Erickson, Marilyn C., 175  
Farmer, Linda J., 95  
Graham, William D., 95  
Grigor, J. M., 39  
Grosch, W., 110  
Guth, Helmut, 31  
Han, Daeseok, 255  
He, Y., 186  
Ho, Chi-Tang, 76  
Horiuchi, Masahiro, 20  
Hwang, Chin-Fa, 76  
Koizumi, Chiaki, 198  
Lall, S. P., 148  
Lee, G. H., 131  
Lee, Tung-Ching, 55  
Lucas, Susan L., 166  
McConnell, Janice M., 95  
Milo, C., 110  
Ohshima, Toshiaki, 198  
O'Keefe, Sean F., 166  
Onodenaloro, A. C., 76  
Pan, B. S., 64  
Parazo, M. P. M., 148  
Quantick, P. C., 39  
Saito, H., 218  
Shahidi, Fereidoon, 1, 76, 186, 240  
Shibamoto, Takayuki, 20  
Shin, Hyun-Kyung, 255  
Shukla, V. K. S., 186  
Sista, Ramani V., 175  
Sullivan, J. A., 120  
Triqui, Réda, 31  
Tsai, J. R., 64  
Ushio, Hideki, 198  
Wanasundara, Udaya N., 186, 240  
Weng, H.-J., 120  
Wu, C. M., 64  
Yoon, Suk Hoo, 255  
Zhang, Hui-Zhen, 55

## Affiliation Index

- Changwon National University, 131  
DalTech, Dalhousie University, 148  
Dankook University, 85  
Department of Agriculture for Northern  
Ireland, 95  
Der Yuh Junior College of Nursing  
and Management, 64  
Deutsche Forschungsanstalt für  
Lebensmittelchemie, 31, 110  
Food Industry Research and Development  
Institute (Republic of China), 64  
Institut Agronomique et Vétérinaire  
Hassan II, 31  
International Food Science Center  
(Denmark), 186  
Korea Food Research Institute, 255  
Memorial University of Newfoundland,  
1, 76, 186, 240  
Ministry of Agriculture, Forestry,  
and Fisheries (Japan), 218  
Mississippi State University, 1, 85, 120, 131  
National Research Council Canada, 148

National Taiwan Ocean University, 64  
 North Carolina State University, 9  
 Rutgers, The State University of New  
 Jersey, 55, 76  
 Seoul National Polytechnic University,  
 264

Tokyo University of Fisheries, 198  
 University of California—Davis, 20  
 University of Florida, 166  
 University of Georgia, 175  
 University of Lincolnshire and  
 Humberside, 39

## Subject Index

- 2-Acetyl-1-pyrroline, role in aroma  
 of crustaceans, 90*r*, 91
- 2-Acetyl-2-thiazoline, role in aroma  
 of crustaceans, 90*r*, 92
- Acid value, use for measurement  
 of deterioration of edible oils,  
 218–219
- Acipenser transmontanus*, *See* Hot  
 smoked white sturgeon
- L-Alanine, role in flavor of freshwater  
 crustaceans, 127
- Aldehyde, effect in cooked fish flavors  
 aldehyde analysis in headspace volatiles,  
 24*f*, 25  
 compounds from headspace of oil with  
 trimethylamine oxide, 27, 28*f*  
 cysteamine method for aldehyde  
 analysis, 24*f*, 25  
 experimental description, 21, 23*f*, 25  
 formation of flavor compounds after heat  
 treatment, 21, 22*f*  
 GC, 25, 26*f*, 27, 29*f*  
*n*-saturated aldehydes formed in  
 headspace, 25, 27*f*  
 quantitative analysis of aldehydes  
 formed in headspace, 27, 29–30  
 unsaturated aldehydes formed in  
 headspace, 27, 28*f*
- Alkylpyrazines, role in aroma of salt-  
 fermented anchovy, 131–145
- Amino acid effect  
 osmoregulation, 121  
 seafood flavor formation, 64–73,  
 120–121
- Anchovy  
 aroma-active compounds, 131–145  
 odorants, 31–37
- Anisidine value, measurement of role  
 of dietary peroxides and tocopherols in  
 fillet flavor of farmed Atlantic salmon,  
 148–162
- Antioxidant activity  
 ground tea leaves in muscle foods, 187,  
 189–191  
 tea extracts and catechins in marine oils,  
 191–196
- Antioxidant effect  
 oxidative susceptibility of seafood lipids,  
 180*f*, 181–183  
 seafood flavor, 12–13
- Aquaculture products, effect of processing  
 on flavor, 17–18
- Arachidonic acid, analysis using HPLC  
 with postcolumn fluorescence detection,  
 198–216
- Arginine, role in flavor of freshwater  
 crustaceans, 127
- Aroma  
 formation in seafood, 64–73  
 quality determinants of fresh fish, 39
- Aroma-active compounds  
 crustaceans, 85–92  
 fresh and stored mackerel  
 dynamic headspace analysis, 46, 48–52  
 experimental description, 41–42  
 sensory analysis, 42–43, 44*f*  
 static headspace analysis  
 cooked mackerel, 46, 47*t*



National Taiwan Ocean University, 64  
 North Carolina State University, 9  
 Rutgers, The State University of New  
 Jersey, 55, 76  
 Seoul National Polytechnic University,  
 264

Tokyo University of Fisheries, 198  
 University of California—Davis, 20  
 University of Florida, 166  
 University of Georgia, 175  
 University of Lincolnshire and  
 Humberside, 39

## Subject Index

- 2-Acetyl-1-pyrroline, role in aroma  
 of crustaceans, 90*r*, 91
- 2-Acetyl-2-thiazoline, role in aroma  
 of crustaceans, 90*r*, 92
- Acid value, use for measurement  
 of deterioration of edible oils,  
 218–219
- Acipenser transmontanus*, *See* Hot  
 smoked white sturgeon
- L-Alanine, role in flavor of freshwater  
 crustaceans, 127
- Aldehyde, effect in cooked fish flavors  
 aldehyde analysis in headspace volatiles,  
 24*f*, 25  
 compounds from headspace of oil with  
 trimethylamine oxide, 27, 28*f*  
 cysteamine method for aldehyde  
 analysis, 24*f*, 25  
 experimental description, 21, 23*f*, 25  
 formation of flavor compounds after heat  
 treatment, 21, 22*f*  
 GC, 25, 26*f*, 27, 29*f*  
*n*-saturated aldehydes formed in  
 headspace, 25, 27*f*  
 quantitative analysis of aldehydes  
 formed in headspace, 27, 29–30  
 unsaturated aldehydes formed in  
 headspace, 27, 28*f*
- Alkylpyrazines, role in aroma of salt-  
 fermented anchovy, 131–145
- Amino acid effect  
 osmoregulation, 121  
 seafood flavor formation, 64–73,  
 120–121
- Anchovy  
 aroma-active compounds, 131–145  
 odorants, 31–37
- Anisidine value, measurement of role  
 of dietary peroxides and tocopherols in  
 fillet flavor of farmed Atlantic salmon,  
 148–162
- Antioxidant activity  
 ground tea leaves in muscle foods, 187,  
 189–191  
 tea extracts and catechins in marine oils,  
 191–196
- Antioxidant effect  
 oxidative susceptibility of seafood lipids,  
 180*f*, 181–183  
 seafood flavor, 12–13
- Aquaculture products, effect of processing  
 on flavor, 17–18
- Arachidonic acid, analysis using HPLC  
 with postcolumn fluorescence detection,  
 198–216
- Arginine, role in flavor of freshwater  
 crustaceans, 127
- Aroma  
 formation in seafood, 64–73  
 quality determinants of fresh fish, 39
- Aroma-active compounds  
 crustaceans, 85–92  
 fresh and stored mackerel  
 dynamic headspace analysis, 46, 48–52  
 experimental description, 41–42  
 sensory analysis, 42–43, 44*f*  
 static headspace analysis  
 cooked mackerel, 46, 47*t*

- Aroma-active compounds—*Continued*  
fresh and stored mackerel—*Continued*  
static headspace analysis—*Continued*  
raw mackerel, 43, 45–46  
salt-fermented anchovy  
experimental description, 132–133  
odor-active compounds, 141,  
142–144*t*, 145*t*  
volatile compounds vs. koji content,  
133–141
- Aroma extract dilution analysis  
advantages, 86  
character-impact aroma compounds  
of crustaceans, 85–92  
limitations, 31  
odorants  
boiled cod, 112, 113*t*  
ripened anchovy, 31–37
- Ascorbic acid effect  
oxidative susceptibility of seafood  
lipids, 181  
stabilization of fish oil, 259–263
- Astaxanthin, role in seafood flavor, 18
- Atlantic salmon  
dietary peroxides and tocopherols,  
148–162  
experimental description, 96–99  
farming, 95  
fatty acids analysis, 101–104  
fatty acid precursor–flavor relationship,  
106–107  
fillet flavor  
fatty acids of tuna oils, 151  
fish cellular lipids, 151–152  
lipid classes, 155, 157, 158*t*  
oxidized dietary fats in aquaculture,  
159–162  
potential of  $\gamma$ -tocopherol, 153, 155*t*  
 $\alpha$ -tocopherol addition to diet, 153–155  
tocopherol content, 152–159  
flavor quality, 99–101  
linear regression analyses of fatty acids,  
104–106  
lipids analysis, 101–103  
sea-cught, 95–105
- Autoxidation  
role in flavor and nutritional quality, 256  
stabilization of  $\omega$ 3 polyunsaturated fatty  
acids, 255–26
- Boiled cod, odorants, 110–118
- Boiled fish, evaluation of lipid oxidation  
by NMR, 218–237
- Boiling, role in seafood flavor, 16
- Browning reaction, production of  
compounds affecting cooked fish  
flavors, 20–30
- 2,3-Butanedione, role in aroma of  
crustaceans, 90*t*, 91
- Butylated hydroxyanisole, 187
- Butylated hydroxytoluene, 187
- tert*-Butylhydroquinone, 187
- Candida cylindracea* lipase,  $\omega$ 3  
concentrate preparation, 240–253
- Carnal 2110, *See* Sodium–potassium  
phosphate, effect on acceptability and  
color of hot smoked white sturgeon
- Carrier/waxy corn starch solution ratio,  
role in microencapsulation, 269, 271*f*
- Catechins of tea  
advantages as antioxidants, 187, 188*f*  
antioxidant activity in marine oils,  
191–196  
stabilization of marine lipids, 186–196
- Catfish, role of microenvironment on  
oxidative susceptibility of lipids,  
175–184
- Character-impact aroma compounds  
of crustaceans  
2-acetyl-1-pyrroline, 90*t*, 91  
2-acetyl-2-thiazoline, 90*t*, 92  
2,3-butanedione, 90*t*, 91  
examples, 87–89  
experimental description, 86  
marine aroma in lobster, 92  
2-methyl-3-furanthiol, 90*t*, 91  
3-(methylthio)propanal, 90*t*, 91  
volatile aroma compounds, 86–87
- Chilling, role in seafood flavor, 10–11

- Chloride ions, role in flavor of freshwater crustaceans, 120–128
- Cod, odorants, 110–118
- Cold smoking, description, 166–167
- Cold storage flavor, odorants involved, 111
- Color measurement
- dietary peroxide and tocopherol role in fillet flavor of farmed Atlantic salmon, 148–162
  - sodium–potassium phosphate role in hot smoked sturgeon, 166–173
- Cooked crustaceans, volatile compounds, 85–92
- Cooked fish, factors affecting flavors, 20
- Coronary heart disease, role of marine lipids, 186–187
- Crab, volatile compounds, 85–92
- Crayfish, volatile compounds, 85–92
- Crustaceans
- aroma compounds, 85–92
  - flavor manipulation using postharvest seawater acclimation, 120–128
  - volatile components, 64
- Cryogenics, role in seafood flavor, 13
- Diallylmethylene proton/aliphatic proton ratio in fish oil, use in evaluation of lipid oxidation, 218–237
- Dietary supplementation, enhancement of flavor and storage stability, 18
- Dimethyl sulfide, role in seafood flavor, 17–18
- N,N*-Dimethylformamide, role in cooked fish flavors, 20–30
- Diphenyl-1-pyrenylphosphine, oxidation, 199
- Direct sample injection, seafood flavor analysis, 3
- Direct solvent extraction, seafood flavor analysis, 3
- Dispersion fluid temperature, role in microencapsulation, 268
- Docosahexaenoic acid
- analysis using HPLC with postcolumn fluorescence detection, 198–216
  - carrier/waxy corn starch solution ratio effect on microencapsulation, 269, 271f
  - dispersion fluid temperature effect on microencapsulation, 268
  - emulsifier blend effect on microencapsulation, 268–271f
  - experimental description, 264–267
  - generation, 241
  - microencapsulation conditions, 265
  - photomicrographs of microcapsules, 265, 266f
  - role in reducing blood pressure and blood cholesterol and viscosity, 264
  - separation from fish oil, 255–263
  - storage stability, 269, 272–273
  - structural characteristics, 240–253
  - waxy corn starch concentration effect on microencapsulation yield, 269, 270f
- Docosapentaenoic acid
- health effects, 1–2, 186
  - structural characteristics, 240–253
- Dried fish, evaluation of lipid oxidation by NMR, 218–237
- Dynamic headspace analysis, 3, 40–41
- Dynamic headspace analysis–gas chromatography–mass spectrometry, aroma compounds of fresh and stored mackerel, 39–52
- Eicosapentaenoic acid
- analysis using HPLC with postcolumn fluorescence detection, 198–216
  - autoxidation, 198–199
  - generation, 241
  - role in reducing blood pressure and blood cholesterol and viscosity, 264
  - separation from fish oil, 255–263
  - structural characteristics, 240–253
- Electronic aroma sensor, seafood flavor analysis, 4

- Emulsifier blend, role in  
microencapsulation, 268–271*f*  
*Engraulis encrasicolus* L., *See* Anchovy
- Enzymatic protein hydrolysates, 76
- Enzyme-assisted hydrolysis of fish oil,  $\omega$ 3  
concentrate preparation, 240–253
- (–)-Epicatechin, 186–196
- (–)-Epicatechin gallate, 186–196
- (–)-Epigallocatechin, 186–196
- Equilibrium headspace sampling, seafood  
flavor analysis, 3
- Eskimos, diet rich in polyunsaturated fatty  
acids, 240
- Ethyl methylbutanoate, role in aroma  
of salt-fermented anchovy, 131–145
- Evaluation method for lipid oxidation  
by NMR  
advantages, 235  
characteristic of peaks, 221  
experimental description, 219–221  
fish oil oxidation estimation, 221–223,  
224*f*  
oxidative deterioration evaluation  
fish meal, 227, 230–231, 232*f*  
fish oils, 227, 229*f*  
Niboshi, boiled, and dried fish, 231,  
233*f*, 235  
peroxide value relationship to ratios of  
olefinic proton and diallylmethylene  
proton to aliphatic proton, 223, 225–228  
polyunsaturated fatty acid autoxidation,  
221
- Fatty acid(s)  
composition in Atlantic salmon,  
95–107  
content of commercial marine oils,  
149, 151  
role in fish flavor, 96
- Fatty acid biochemistry in fish, menhaden  
vs. phytoplankton, 148–149, 150*f*
- Fatty acid composition, Atlantic salmon,  
95–107
- Fermentation, role in seafood flavor, 16
- Fermented fish products, 131–132
- Fish  
cooked, factors affecting flavor, 20  
determination of flavor quality and  
spoilage index, 55
- Fish meal, evaluation of lipid oxidation  
by NMR, 218–237
- Fish oils  
evaluation of lipid oxidation by NMR,  
218–237  
fatty acid composition, 20, 22*f*  
separation of  $\omega$ 3 polyunsaturated fatty  
acids, 255–263
- Fish quality assessment, GC–MS analysis  
of volatile flavor compounds in  
mackerel, 55–62, 36
- Flavor  
aldehyde effect in cooked fish, 20–30  
amino acid effect, 120–121  
aroma components, 2  
developments in analysis, 2–4  
food processing, 9–18  
taste components, 2  
thermal effects, 2
- Flavor dilution factors, description, 86
- Flavor-impact compounds, examples, 40
- Flavor manipulation of freshwater  
crustaceans using postharvest seawater  
acclimation  
chemical changes  
chloride ion content, 123, 124*f*  
free amino nitrogen content, 123, 125*f*  
lipid content, 123, 124*f*  
moisture content, 123, 124*f*  
protein content, 123, 126*f*  
experimental description, 120–123  
sensory changes, 127–128
- Flavor(s) thermally generated from seal  
protein hydrolysate, *See* Thermally  
generated flavors from seal protein  
hydrolysate
- Food additives, role in seafood flavor,  
12–13
- Food processing effect in seafood flavor  
chilling, 10–11  
cooking, 16

- Food processing effect in seafood flavor—*Continued*
- factors affecting intrinsic qualities of fish and shellfish, 10
  - fermentation, 16
  - food additives, 12–13
  - freezing and frozen storage, 10–11
  - high hydrostatic pressure, 15–16
  - irradiation, 15
  - marker compounds affecting quality, 9–10
  - modified atmospheric packaging, 13–15
  - techniques, 10
  - thermal processing, 16–17
- Free amino acids, role in flavor of freshwater crustaceans, 120–128
- Free fatty acids
- measurement of role of dietary peroxides and tocopherols in fillet flavor of farmed Atlantic salmon, 148–162
  - role in oxidative susceptibility of seafood lipids, 176
- Fresh mackerel, aroma compounds, 39–52
- Freshwater crustaceans, flavor
- manipulation using postharvest seawater acclimation, 120–128
- Freshwater prawn
- flavor manipulation using postharvest seawater acclimation, 120–128
  - optimized cultural practices, 121
  - volatile compounds, 85–92
- Gas chromatography, seafood flavor analysis, 3
- Gas chromatography–mass spectrometry seafood flavor analysis, 3
- volatile flavor compounds in mackerel for fish quality assessment
  - experimental description, 56–57
  - hypoxanthine content vs. storage times, 58, 61*t*
  - quantitative changes of volatile compounds vs. storage times, 58, 61*t*
  - volatile compounds vs. storage times, 58, 59–60*t*
- Gas chromatography–olfactometry
- boiled cod, 112, 113*t*
  - ripened anchovy, 31–37
  - seafood flavor analysis, 3
- Glucose oxidase–catalase, role in seafood flavor, 12
- Glutamic acid, role in flavor of freshwater crustaceans, 127
- Glycine, role in flavor of freshwater crustaceans, 127
- Green tea leaves, stabilization of marine lipids, 186–196
- Greenland Eskimos, diet, 264
- Ground tea leaves, antioxidant activity in muscle foods, 187, 189–191
- Harp seals, hunting quota system, 76
- (*Z*)-4-Heptenal
- aroma of salt-fermented anchovy, 131–145
  - odor of boiled cod, 117–118
- (*Z*)-3-Hexanol, role in odor of boiled cod, 117–118
- High hydrostatic pressure, role in seafood flavor, 15–16
- High-performance liquid chromatography with postcolumn fluorescence detection, polyunsaturated fatty acid isomeric hydroperoxide analysis, 198–216
- Highly unsaturated fatty acids, oxidation, 187
- Hot smoked white sturgeon, role of sodium–potassium phosphate on acceptability and color, 166–173
- Hot smoking, description, 166–167
- Hurdle-barrier concept, 14–15
- Hydrolysis, calculation, 247
- Hypoxanthine, role in GC–MS analysis of volatile flavor compounds in mackerel for fish quality assessment, 55–62
- Iodine value, measurement of role of dietary peroxides and tocopherols in fillet flavor of farmed Atlantic salmon, 148–162

- Isotope dilution assay for quantification of odorants in boiled cod, 112–113, 115*t*, 116*f*
- Koji, aroma-active compounds, 131–145
- Lipid(s)  
analysis and evaluation of oxidative status, 4–5  
quality deterioration, 4  
role in cooked fish flavors, 20–30  
role in microenvironment on oxidative susceptibility, 175–184  
variation in phyla and species, 148  
*See also* Marine lipid structures
- Lipid composition, Atlantic salmon, 95–107
- Lipid hydrolysis, role in volatile generation by membrane phospholipids, 177, 179*f*
- Lipid oxidation  
evaluation method by NMR, 218–237  
fresh flavor and off-flavor generation, 175  
role in food quality and nutritional value, 198  
volatile aroma compounds of crustaceans, 86
- Lipid stabilization with green tea and catechins, *See* Marine lipid stabilization with green tea and catechins
- Lipoxygenase, role in seafood flavor formation, 64–73
- Lipoxygenase-mediated reaction of polyunsaturated fatty acids, volatile aroma compounds of crustaceans, 86
- Lobster, volatile compounds, 85–92
- Long-chain  $\omega$ 3 fatty acids  
beneficial effects in seafoods, 186  
generation, 241
- Mackerel  
aroma compounds, 39–52  
GC–MS analysis of volatile flavor compounds for fish quality assessment, 55–62
- Mackerel—*Continued*  
lipid stabilization with green tea and catechins, 186–196
- Macrobrachium rosenbergii*, *See* Freshwater prawn
- Marine fish  
concentrations of trimethylamine oxide, 21  
role of tocopherols in flavor, 152–153
- Marine lipid(s), role in coronary heart disease, 186–187
- Marine lipid stabilization with green tea and catechins, antioxidant activity ground tea leaves, 187, 189–191  
tea extracts and catechins in marine oils, 191–196
- Marine lipid structures  
absorption of fatty acids, 243  
enzymatic hydrolysis of marine oils, 246–253  
polyunsaturated fatty acid concentrates, 246  
stereospecific analysis of marine oils, 242*f*, 243–246
- Marine oils  
antioxidant activity in marine oils, 191–196  
fatty acid content, 149, 151
- Menhaden oil  
aldehyde effect in flavors, 20–30  
lipid stabilization with green tea and catechins, 186–196  
stereospecific analysis, 242–246  
structural characteristics of lipids, 240–253
- 3-Methylbutanal  
aroma of salt-fermented anchovy, 131–145  
raw material storage on concentration, 110–118
- 3-Methyl-1-butanol, role in aroma of salt-fermented anchovy, 131–145
- 2-Methyl-3-furanthiol, role in aroma crustaceans, 90*t*, 91
- N*-Methylpyrrole, 20–30
- 3-(Methylthio)propanal effect on aroma crustaceans, 90*t*, 91  
salt-fermented anchovy, 131–145

- 1-(Methylthio)-2-propanone, role in seafood flavor formation, 64–73
- Microbial action, volatile aroma compounds of crustaceans, 87
- Microencapsulation  
definition, 264  
docosahexaenoic acid, 264–273  
yield, calculation, 267
- Microenvironment effect on oxidative susceptibility of seafood lipids  
antioxidants, 180*f*, 181–183  
experimental description, 175–176  
fatty acid composition vs. storage, 177, 179*f*  
free fatty acids, 176  
identification in tissues, 176–177, 178*f*  
lipid hydrolysis vs. volatile generation by membrane phospholipids, 177, 179*f*  
location, 176  
model system development, 183–184  
organization state of lipids, 176  
source of oxidant stress, 176
- Modified atmospheric packaging, 13–15
- Muscle foods, antioxidant activity of ground tea leaves, 187, 189–191
- Natural antioxidants, advantages as antioxidants, 187, 188*f*
- Niboshi fish, evaluation of lipid oxidation by NMR, 218–237
- Nitrogen-containing amino acids, role in flavor of freshwater crustaceans, 127–128
- Nitrogen-containing compounds, role in cooked fish flavors, 20–30
- NMR, evaluation of lipid oxidation, 218–237
- (*E,Z*)-2,6-Nonadienal, role in aroma of salt-fermented anchovy, 131–145
- (*Z,Z*)-3,6-Nonadienal, role in odor of boiled cod, 117–118
- n*-saturated aldehydes, role in cooked fish flavors, 20–30
- $\omega$ 3 polyunsaturated concentrates, preparation, 240–253
- $\omega$ 3 polyunsaturated fatty acid(s)  
content and composition in seafoods, 2  
health effects, 1–2, 255
- $\omega$ 3 polyunsaturated fatty acid separation from fish oil  
experimental description, 256  
selective adduct formation of fatty acids of fish oil and urea, 257–258  
separation by solubility of fatty acid salts in ethanol, 256–257  
stabilization of fish oil  
combined effect of ascorbic acid and  $\gamma$ -tocopherol, 259–263  
solubilization of ascorbic acid via reverse micelles, 259, 260*f*
- 1-Octen-3-ol, role in aroma of salt-fermented anchovy, 131–145
- 1-Octen-3-one, role in aroma of salt-fermented anchovy, 131–145
- Odorants  
boiled cod  
dimethylamine effect, 118  
enzymatic formation of 2- and 3-methylbutanal, 115–117  
experimental description, 111–112  
(*Z*)-4-heptenal role, 117–118  
(*Z*)-3-hexanol role, 117–118  
(*Z,Z*)-3,6-nonadienal role, 117–118  
odor activity values for odorants, 114–115  
volatiles, 112–113, 115*t*, 116*f*
- ripened anchovy  
experimental procedure, 32*t*, 33–34  
volatile compounds, 32*t*, 34–37
- Off-flavor compounds, examples, 40
- Olefinic proton/aliphatic proton ratio in fish oil, use in evaluation of lipid oxidation, 218–237
- Organization state of lipids, role in oxidative susceptibility of seafood lipids, 176
- Osmoregulation, influence of amino acids, 121
- Overwrap packaging, role in seafood flavor, 12

- Oxidation  
evaluation method by NMR, 218–237  
seafood lipids, 4–5
- Oxidative stability  
docosahexaenoic acid, 264–273:  
role of microenvironment, 175–184
- Oxysterols, carcinogenicity, 198
- Pasteurization, role in seafood flavor, 16
- 2-Pentanone, role in seafood flavor, 17–18
- 1-Penten-3-one, role in aroma of salt-fermented anchovy, 131–145
- Peroxide(s), role in fillet flavor of farmed Atlantic salmon, 148–162
- Peroxide value  
measurement of role of dietary peroxides and tocopherols in fillet flavor of farmed Atlantic salmon, 148–162  
use for measurement of deterioration of edible oils, 218–219
- pH, role in seafood flavor, 12–13
- 2-Phenylethanol, role in aroma of salt-fermented anchovy, 131–145
- Phoca groenlandica*, *See* Harp seals
- Phosphates, role in seafood flavor, 13
- Pickling, role in seafood flavor, 16
- Polar paradox, description, 181
- Polydextrose, role in seafood flavor, 13
- Polyunsaturated fatty acid(s)  
health effects, 218, 241  
dietary requirements, 241
- Polyunsaturated fatty acid isomeric hydroperoxide, analysis by HPLC with postcolumn fluorescence detection  
experimental procedure, 199–200, 201f, 203  
isomeric hydroperoxides of methyl arachidonate, ethyl eicosapentaenoate, and methyl docosahexaenoate, 205, 211–216  
isomeric hydroperoxides of methyl oleate, methyl linoleate, and methyl linolenate, 203, 205–211
- Polyunsaturated fatty acid isomeric hydroperoxide, analysis by HPLC with postcolumn fluorescence detection—*Continued*  
quantitative determination of total amount of linoleic acid hydroperoxides, 202f, 203, 204f
- Postcolumn fluorescence detection with high-performance liquid chromatography, polyunsaturated fatty acid isomeric hydroperoxide analysis, 198–216
- Prawn, freshwater, *See* Freshwater prawn
- Processing effect in seafood flavor, *See* Food processing effect in seafood flavor
- Propanal, role in oxidative susceptibility of seafood lipids, 182f, 183
- Propyl gallate, role in seafood flavor, 12
- Protein hydrolysate, thermally generated flavors, 76–83
- Quality assessment of fish, GC–MS analysis of volatile flavor compounds in mackerel, 55–62
- Raw materials, role of storage on odorants in boiled cod, 110–118
- Ripened anchovy, odorants, 31–37
- River salmon, flavor characteristics and lipid composition, 95–107
- Salmo salar*, *See* Atlantic salmon
- Salmon, *See* Atlantic salmon
- Salted dried fish, evaluation of lipid oxidation by NMR, 218–237
- Salt-fermented anchovy, aroma-active compounds, 131–145
- Sardine oil, separation of  $\omega$ 3 polyunsaturated fatty acids, 255–263
- Scomber scombrus*, *See* Mackerel
- Seafood  
content and composition in  $\omega$ 3 fatty acids, 2



- Seafood—Continued**  
determination of flavor quality and spoilage index, 55  
factors affecting consumer acceptance, 9  
flavor, 2–4  
lipids  
  quality deterioration, 4  
  role of microenvironment on oxidative susceptibility, 175–184
- Seafood flavor, food processing effects, 9–17**
- Seal blubber oil**  
stereospecific analysis, 242–246  
structural characteristics of lipids, 240–253
- Seal meat, source of protein, 77**
- Seal protein hydrolysate, thermally generated flavors, 76–83**
- Seawater acclimation, flavor manipulation of freshwater crustaceans, 120–128**
- Shrimp, volatile compounds, 85–92**
- Shrimp flavor formation**  
  aroma development from sulfur-containing amino acids, 69–72  
  experimental description, 65–66  
  lipoyxygenase relationship to aroma, 72–73  
  sulfur-containing components, 66–69
- Simultaneous steam distillation–solvent extraction**  
  analysis of volatile flavor compounds in mackerel for fish quality assessment, 55–62  
  seafood flavor analysis, 3
- Smoking, types, 166–167**
- Sodium acetate, role in seafood flavor, 12**
- Sodium glutamate, role in seafood flavor, 12–13**
- Sodium lactate, role in seafood flavor, 12**
- Sodium–potassium phosphate, effect on acceptability and color of hot smoked white sturgeon**  
  categories, 167  
  color, 173  
  experimental procedure, 167–169  
  sensory evaluations, 173  
  smoking vs. fish yield, 169–171, 173
- Sodium–potassium phosphate, effect on acceptability and color of hot smoked white sturgeon—Continued**  
  solubility, 167  
  water-phase salt  
    vs. moisture, 172, 173f  
    vs. treatment, 172f, 173
- Sodium tripolyphosphate, role in seafood flavor, 12**
- Sous vide processing, role in seafood flavor, 17**
- Stabilization**  
   $\omega$ 3 polyunsaturated fatty acids against autoxidation, 255–263  
  use of green tea and catechins, 186–196
- Static headspace analysis, 40–41**
- Static headspace analysis–gas chromatography–mass spectrometry, aroma compounds of fresh and stored mackerel, 39–52**
- Storage of raw materials, role in odorants in boiled cod, 110–118**
- Storage stability**  
  aquaculture products, 17–18  
  docosahexaenoic acid, 269, 272–273
- Storage temperature, role in oxidative susceptibility of seafood lipids, 175–184**
- Stored mackerel, aroma compounds, 39–52**
- Sturgeon**  
  role of sodium–potassium phosphate in acceptability and color, 166–173  
  worldwide stocks, 166
- Sublimation in vacuo, seafood flavor analysis, 3**
- Sucrose–sorbitol, role in seafood flavor, 13**
- Sulfur-containing amino acid effect**  
  seafood flavor formation, 64–73  
  freshwater crustacean flavor, 127–128
- Synthetic antioxidants, potential harmful effects, 187**
- Tea extracts, antioxidant activity in marine oils, 191–196**
- Thermal processing, role in seafood flavor, 16–17**

- Thermally generated aroma compounds, crustaceans, 86
- Thermally generated flavors from seal protein hydrolysate  
experimental description, 77, 79  
flavor notes of volatile compounds, 83  
odor description, 79–80  
seal protein hydrolysate  
degree of hydrolysis, 79  
preparation, 78*f*, 79  
volatile compounds generated, 80–83
- $\gamma$ -Tocopherol, role in stabilization of fish oil, 259–263
- Tocopherol  
fillet flavor of farmed Atlantic salmon, 148–162  
oxidative susceptibility of seafood lipids, 181–183  
seafood flavor, 18
- Triacylglycerol molecules, structural characteristics, 240–253
- Trimethylamine oxide  
concentrations in marine fish, 21  
formation of flavor compounds after heat treatment, 21, 22*f*  
role in cooked fish flavors, 20–30
- Unsaponifiables, measurement of role of dietary peroxides and tocopherols in fillet flavor of farmed Atlantic salmon, 148–162
- Vacuum infusion, description, 159, 161
- Vacuum-skin package, role in seafood flavor, 12
- Volatile aroma compounds  
crustaceans, 86–87  
determination of flavor quality and spoilage index, 55  
examples, 56  
generation, 85–86  
influencing factors, 56  
measurement methods, 55  
patterns, 198–199
- Waxy corn starch concentration, role in microencapsulation yield, 269, 270*f*
- Weight recovery, measurement of sodium–potassium phosphate effect on acceptability and color of hot smoked white sturgeon, 166–173
- White sturgeon, role of sodium–potassium phosphate on acceptability and color, 166–173