

# **Lipid Oxidation**

## **Also in the Oily Press Lipid Library:**

**Volume 17.** Bioactive Lipids

Edited by Anna Nicolaou and George Kokotos

**Volume 16.** Advances in Lipid Methodology – Five

Edited by Richard O. Adlof

**Volume 15.** Lipid Analysis (third edition)

Written by William W. Christie

**Volume 14.** Confectionery Fats Handbook

Written by Ralph E. Timms

**Volume 13.** Lipids for Functional Foods and Nutraceuticals

Edited by Frank D. Gunstone

**Volume 12.** Lipid Glossary 2

Written by Frank D. Gunstone and Bengt G. Herslöf

**Volume 11.** Lipids in Nutrition and Health: A Reappraisal

Written by Michael I. Gurr

**Volume 10.** Lipid Oxidation

Written by Edwin N. Frankel

**Volume 9.** *Trans* Fatty Acids in Human Nutrition

Edited by Jean Louis Sébédio and William W. Christie

**Volume 8.** Advances in Lipid Methodology – Four

Edited by William W. Christie

**Volume 7.** Advances in Lipid Methodology – Three

Edited by William W. Christie

**Volume 6.** Waxes: Chemistry, Molecular Biology And Functions

Edited by Richard J. Hamilton (out of print)

**Volume 5.** Lipids: Molecular Organization, Physical Functions and Technical Applications

Written by Kåre Larsson

**Volumes 1– 4.** Out of print

# Lipid Oxidation

Second Edition

EDWIN N. FRANKEL

*University of California, Davis, California, USA*



**THE OILY PRESS**

An imprint of PJ Barnes & Associates  
Bridgwater, England

**Lipid**  
Technology

Published in association with  
*Lipid Technology*

Published by **The Oily Press**  
an imprint of PJ Barnes & Associates  
PO Box 200, Bridgwater TA7 0YZ, England  
Tel: +44-1823-698973, Fax: +44-1823-698971  
E-mail: editor@pjbarnes.co.uk  
Web site: <http://www.pjbarnes.co.uk>

ISBN 0-9531949-8-1

Copyright © 2005 PJ Barnes & Associates

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted by any form or by any means, electronic, mechanical, photocopying, recording or otherwise, without prior permission in writing from the publisher.

All reasonable care is taken in the compilation of information for this book. However, the author and publisher do not accept any responsibility for any claim for damages, consequential loss or loss of profits arising from the use of the information.

This book is **Volume 18** in **The Oily Press Lipid Library**

**British Library Cataloguing-in-Publication Data**

A catalogue record for this book is available from the British Library

Typeset in 10½/12pt Times New Roman by  
Ann Buchan (Typesetters), Shepperton, Middlesex, UK

# Preface

## Preface to Second Edition

After more than six decades, the field of lipid oxidation continues to be very active and has expanded into broad areas of food science and technology, free radical chemistry, nutrition, biochemical and biomedical interests. Although there have been significant advances in this challenging field, many important problems remain unsolved. This second edition follows the example of the first edition in offering a summary of the many unsolved problems that need further research. New developments in lipid oxidation research and technology and new dietary guidelines have resulted in difficult and challenging problems of control with nutritional and biological implications. Various processed foods have been reformulated with oils rich in long-chain polyunsaturated fatty acids because of their recognized nutritional benefits, but lipid oxidation has seriously limited their utilization. Because partial hydrogenation produces *trans* fatty acids now considered nutritionally harmful, much effort has been made in developing alternate methods for the preparation of oxidatively stable oils. To avoid hydrogenation, special plant breeding has been used to develop several high-oleic and low-linolenic acid oils, and has improved their oxidative stability and nutritional value. In the last decade, enormous worldwide attention has been given to natural phenolic antioxidants, including flavonoids and various phytochemicals found in many fruits and vegetables and beverages. Although some new knowledge has been acquired about the absorption of these antioxidants in humans, their *in vivo* activity is still not well understood. The development of reliable biomarkers that can be better related to degenerative disease presents one of the most difficult challenges in this field.

The second edition includes important developments in the characterization by capillary gas chromatography-olfactometry of aroma and flavor impact of volatile decomposition products from polyunsaturated fatty acids and esters. Discussions are included on various mechanisms for the formation from linoleate of 4-hydroxy-2-nonenal, which has received much attention in the biochemical literature because of its cytotoxic properties, and its occurrence in oxidized LDL and in vegetable oils heated at frying temperatures. Some of the volatiles produced from fish oils are responsible for major problems in their utilization, because they produce very powerful 'fishy' odors and flavors perceptible at extremely low levels of oxidation.

This second edition offers new material on methods of sensory detection (nasal: through the nose) or (retronasal: through the mouth and back of the oral cavity), different flavor release phenomena in the headspace versus the mouth, and matrix in flavor release from oils compared to emulsion systems. Advanced gas chromatographic methods are included, such as solid phase microextraction for the volatile analyses in foods and vegetable oils, gas chromatography-olfactometry, and aroma extraction dilution analyses.

A number of advanced instrumental methods have emerged for analysing mixtures of complex products of lipid oxidation. The second edition has a new chapter that examines these research methods, and how they provide new information on the stereochemistry of hydroperoxides from oleate and linoleate, polyunsaturated phospholipids and triacylglycerols, oils extracted from fried foods and their decomposition products (Chapter 6). The formation of the nonconjugated bis-allylic 11-hydroperoxide intermediate from linoleate was established unambiguously for the first time using  $\alpha$ -tocopherol acting as hydrogen donor. Conjugated linoleic acid (CLA) has been claimed to be bioactive and to have beneficial nutritional effects. The mechanism of CLA autoxidation is discussed, together with the complex isomeric mixtures of endoperoxides and thromboxanes (prostaglandin-like compounds) produced from arachidonic acid, phospholipids and cholesterol. Many of these new research methods are useful for the direct mass spectral identification of intact labile polyunsaturated hydroperoxides and other oxidation products without the necessity of derivatization, but their quantitation has not been achieved without the availability of authentic standards. This new chapter includes important advances in the development of very sensitive methods for the analysis of volatile flavor compounds, linking their relative release to sensory perception in foods.

A new section in the chapter on Stability Methods (Chapter 7) covers electron spin resonance spectrometry and spin-trapping for measuring the tendency of foods to form radicals during early stages of oxidation, and a discussion on its merits and pitfalls. The chapter on Control Methods (Chapter 8) has new material on physical refining, and soft column deodorization, more structural details of oxidative deterioration compounds formed during refining of different vegetable oils obtained by high performance size exclusion chromatography, and *trans* isomers of polyunsaturated fatty acids, oxidized sterols and cyclic monomers. Novel processing technology includes special membranes for the separation of impurities in crude oil extracts and for refining oils, cold pressing and hot expeller-pressing oils that have attracted interest for environmental reasons as they avoid the use of chemical solvents in the extraction of seeds. There is also information on high-oleic and mid-oleic canola and sunflower oils now commercially available as substitutes for partially hydrogenated oils, and an expanded section on packaging.

Chapter 9 on Antioxidants presents more detailed information on metal

inactivators including ethylenediamine tetraacetic acid (EDTA), new material on lactoferrin, an expanded discussion of synergism, problems with methodology to evaluate antioxidants in multi-phase systems, and more on the mechanism of rosemary antioxidants at elevated temperatures. A new section on the evaluation of natural antioxidants in plants was motivated by the explosion of published research on the antioxidant properties of phytochemicals, including new tables comparing free radical trapping methods and lipid oxidation methods for the evaluation of natural antioxidants.

Chapter 10 on Multiphase Systems includes the effects of food proteins that can inhibit oxidation of oil-in-water emulsions by several mechanisms and how pH affects their activity. The metal complexing activity of bovine lactoferrin in inhibiting oxidation in emulsions and liposome systems is compared with that of EDTA. Antioxidants are greatly influenced by the colloidal properties of lipid systems. Interactions of emulsifiers with antioxidants, hydrogen bondings, interphase transport and surface access are important parameters determining their activity in lipid-containing systems differing in their colloidal characteristics. Proteins have strong binding properties with flavonoid antioxidants in foods and biological systems, and generally increase the oxidative stability of liposomes. The partitioning behavior of different phenolic antioxidants in emulsions is greatly affected by interactions between oils and emulsifiers that determine their solubilization and their activity in inhibiting lipid oxidation.

There is an expanded discussion in Chapter 11 on Foods on the interactions between metmyoglobin, deoxyhemoglobin and deoxymyoglobin and their mechanism of initiating lipid oxidation in meat and fish. The antioxidant properties produced by the Browning products are discussed, together with the methodology problems involved with model systems generally used in the literature. New information is added on the structure of volatile compounds and their impact on the flavor of cooked meat. The effect of thermal processing of foods and beverages (tomato juice, wine, coffee and tea) are subject to complex interactions between lipid oxidation and reducing browning reaction products, proteins, phospholipids, and phenolic antioxidants that are influenced by multifactorial changes during processing and storage. The methodological problems of using simplistic model systems and unidimensional methods to evaluate antioxidants in foods are discussed here and throughout the second edition. New material addresses the problems of iron supplementation in infant formulas and the control of oxidation by lactoferrin and EDTA, and the relative oxidative stability of long chain polyunsaturated fish and algae oils and emulsions, including the antioxidant activity of roasted foods.

Chapter 12 on Frying contains new material on complex interaction compounds produced by non-enzymatic browning reactions between thermally oxidized lipids and amines, amino acids and proteins. This chapter

also discusses the new varieties of vegetable oils containing higher levels of oleate and lower levels of linolenate being used for frying, because partial hydrogenation produces *trans* fatty acids, which are now considered nutritionally undesirable. New tables include aroma extraction dilution analysis of volatiles from French fries; analyses of total polar compounds and tocopherol in frying oils versus fried potatoes; fatty acid composition of new varieties of soybean, sunflower, and canola oils suitable for frying without partial hydrogenation. The controversial subject of whether or not natural antioxidants can account for the strong epidemiological evidence that fruits and vegetable consumption is associated with reduced coronary heart disease, cancer and other diseases is covered in Chapter 13 on Biological Systems. The absorption of antioxidants in human blood as metabolites, their molecular mechanisms of protection *in vivo*, and of disease intervention are under intensive investigation around the world. Important developments are included in discussions of new pathways of LDL oxidation, the metabolic interactions between LDL and HDL on cholesterol transport mechanisms, new evidence on the role of antioxidant enzymes in HDL, which can break down oxidized lipids and neutralize their proinflammatory effects, and the close link between the oxidation and inflammation hypotheses of cardiovascular disease. The new evidence of core aldehyde formation in oxidized LDL is examined, together with the bioactivity of oxidized phospholipids on the surface of LDL particles that are recognized by macrophages. More information is discussed on the cyclic oxidation products of arachidonate described as isoprostanes in biological samples as an index of oxidative status. Important nutritional topics have been added to the second edition, including the significant role of docosahexaenoic acid (DHA) in neural membrane phospholipid metabolism, immune responses and the aging process. The difference in antioxidant testing results between animal and human studies and between *in vitro* and *in vivo* effects is discussed in terms of different end-points of oxidation. This chapter concludes with seven important unsettled nutritional questions that need future research. With so many unanswered questions, the conclusion is drawn that it would be imprudent to make dietary recommendations to the public before the mechanisms of polyunsaturated lipid nutrition, *in vivo* activity of antioxidants, and *in vivo* lipid peroxidation are better understood.

The author gratefully acknowledges the invaluable editorial work of Bill Christie and Frances Daniel.

Edwin Frankel  
Department of Food Science and Technology  
University of California  
Davis, California 95616 USA  
enfrankel@ucdavis.edu  
January 2005

## Preface to First Edition

The oxidation of unsaturated fatty acids is one of the most fundamental reactions in lipid chemistry. When unsaturated lipids are exposed to air, the complex, volatile oxidation compounds formed cause rancidity, decreasing the quality of foods containing lipids as well as foods in which oils are used as ingredients. Another important process in the oxidation of unsaturated fats proceeds through activated species of oxygen. Singlet oxygen produced by photooxidation, in the presence of a sensitizer such as chlorophyll, is an important reactant that has attracted much interest to organic and biological chemists. Products of lipid oxidation have been implicated in many vital biological reactions. The revival of the field of lipid oxidation in the last two decades may be attributed in large part to the accumulating evidence that free radicals and reactive oxygen species participate in tissue injuries and in degenerative diseases.

Since the early 1960s, our understanding of the autoxidation of unsaturated lipids has advanced considerably as a result of the application of powerful new analytical tools. The characterization of cyclic peroxides from autoxidized linolenate, structurally related to the physiologically active prostaglandins, has also generated widespread interest in the field. Much of the work reported on the oxidative decomposition products of unsaturated fats emphasizes the role they play in causing rancidity in foods and cellular damage in the body. Decomposition of fat hydroperoxides creates a wide range of carbonyl compounds, hydrocarbons, furans, and other materials that contribute to flavor deterioration of foods. However, materials from secondary oxidation are also implicated in biological oxidation. Although fat hydroperoxides are the recognized precursors of volatile secondary products, the mechanism of their decomposition is not clearly understood.

This book aims at integrating a large body of interdisciplinary information on the oxidation of unsaturated lipids in order to develop the basic principles involved in the methodology and mechanisms of free radical oxidation. Understanding these principles is necessary for gaining insights into how unsaturated lipids in foods are subject to oxidative deterioration, and for developing appropriate control measures. The question of how biological systems undergo oxidative damage is very complex and the literature accumulates at a geometric rate.

This is an advanced textbook for graduate students, academic and industrial scientists concerned with the many phases of the complex series of lipid oxidation reactions. Starting from the basics of free radical and hydroperoxide chemistry, and methodology, the book progresses into topics of increasing complexity including control methods, antioxidants, and oxidation in multiphase systems, foods, frying fats and biological systems. Investigators working with polyunsaturated fatty acids and lipids must be seriously concerned with their

oxidation, since these products have caused numerous problems in analyses, particularly in stability tests. Unfortunately, in spite of the great strides made in the field of lipid oxidation, many contemporary studies employ nonspecific and unreliable methods to follow lipid oxidation. Lipid oxidation is often studied under drastic conditions and with simple model systems that do not simulate complex multi-phase foods and biological systems. Much of the classical concepts of lipid oxidation come as a result of elegant kinetic studies, in which a wholly theoretical picture is developed in support of mechanistic hypotheses. The conditions and systems often employed in these studies may be simplistic, however, and do not represent those of real complex food systems undergoing oxidation.

This book discusses these and other pitfalls inherent in basic studies that use artificial radical initiators with unrealistic models and homogeneous systems. The application of simple research models may lead to numerous problems in the ultimate interpretation of results, because lipid oxidation proceeds by a complex sequence of reactions influenced by many factors, all of which become extremely difficult to unravel in real food and biological systems. These systems are multi-phased and controlled by complex colloidal phenomena affecting different sites of oxidation and antioxidation. In interpreting the effects of prooxidant and antioxidant compounds their 'effective' concentrations in different phases must be considered. A dimension of lipid oxidation that is important to better understand control methods deals with the relative partition of oxidants and antioxidants in multiphase systems. This topic has not received sufficient attention.

One of the main objectives of this book is to develop the background necessary for a better understanding of what factors should be considered, and what methods and lipid systems should be employed, to achieve suitable evaluation and control of lipid oxidation in complex foods and biological systems. Now, and throughout its 50-year history, the field is still developing and more progress can be expected in improving our understanding of the complex phenomena of lipid oxidation.

Edwin N. Frankel,  
California  
1998

# Contents

<b>Preface to Second Edition</b>	v
<b>Preface to First Edition</b>	ix
<b>Introduction</b>	<b>1</b>
A. Classification of lipids	1
1. Fatty acids	
2. Triacylglycerols (triglycerides)	
3. Phosphoglycerides (phospholipids)	
B. Minor components	6
C. Chemical reactions of unsaturated fatty acids	7
1. Heterolytic ionic reactions	
2. Homolytic radical reactions	
3. Radical stability	
D. Lipid oxidation and health	12
Bibliography	14
<b>1 Free radical oxidation</b>	<b>15</b>
A. Mechanism	15
1. Initiation	
2. Propagation	
3. Termination	
B. Kinetics	18
C. Relative rates of autoxidation of unsaturated fatty acids	21
D. Metal catalysis	22
Bibliography	23
<b>2 Hydroperoxide formation</b>	<b>25</b>
A. Oleate autoxidation	25
B. Linoleate autoxidation	29
C. Linolenate autoxidation	34
D. Arachidonate autoxidation	39
E. Autoxidation of other long-chain polyunsaturated fatty acids	43
F. Autoxidation of triacylglycerols	43
G. Autoxidation of phospholipids	45
H. Autoxidation of cholesterol	46
Bibliography	48

<b>3 Photooxidation of unsaturated fats</b>	<b>51</b>
A. Photosensitized oxidation	51
B. Hydroperoxide formation by singlet oxygen	52
C. Inhibition of photosensitized oxidation	59
Bibliography	64
<b>4 Hydroperoxide decomposition</b>	<b>67</b>
A. Monomeric products	67
1. Oleate	
2. Linoleate	
3. Linolenate	
B. Oligomeric products of methyl linoleate and methyl linolenate	72
C. Oligomeric products of triacylglycerols	73
D. Volatile products	75
1. Oleate	
2. Linoleate	
3. Linolenate	
4. Long-chain polyunsaturated fatty acids	
5. Heterolytic mechanisms	
E. Volatiles from secondary oxidation products	89
1. Aldehydes	
2. Dimers and oligomers	
3. Hydroperoxy epoxides, epidioxides and bicyclo-endoperoxides	
4. Triacylglycerols	
Bibliography	96
<b>5 Methods to determine extent of oxidation</b>	<b>99</b>
A. Sensory methods	99
B. Peroxide value	103
C. Conjugated dienes	106
D. Carbonyl compounds	106
E. 2-Thiobarbituric acid (TBA) value	108
F. Gas chromatographic methods	110
1. Static headspace method	
2. Dynamic headspace method	
3. Direct injection method	
4. Advanced GC methods	
G. Fluorescence methods	119
H. Octanoate method	122
I. Conjugatable oxidation products	122
Bibliography	124

<b>6</b>	<b>Research methods for lipid oxidation</b>	<b>129</b>
A.	Countercurrent distribution	129
B.	Conventional column liquid chromatography	131
C.	High performance liquid chromatography (HPLC)	134
D.	High performance size exclusion chromatography (HPSEC)	146
E.	Gas chromatography-mass spectrometry (GC-MS)	148
F.	High performance liquid chromatography and mass spectrometry (HPLC-MS)	151
G.	Coordination ion-spray-mass spectrometry (CIS-MS)	156
H.	Advanced instrumental techniques for volatile flavor analysis	157
	Bibliography	161
<b>7</b>	<b>Stability methods</b>	<b>165</b>
A.	Stability as parameter of quality control	165
B.	Development of rancidity in foods during storage	166
C.	Stability of different systems	168
D.	Accelerated parameters	168
E.	Effect of temperature	169
F.	Stability of oxidation products	170
G.	Standard accelerated stability tests	171
	1. Storage at ambient conditions	
	2. Storage at elevated temperatures	
H.	Alternative stability methods	173
	1. Gas analysis	
	2. Analyses of volatiles by gas chromatography	
	3. Hydroperoxides	
	4. Carbonyl compounds	
	5. Thermal analyses	
	6. Electron spin resonance (ESR) spectrometry and spin-trapping	
I.	Lipid oxidation methods	176
	1. Sensory methods	
	2. Analysis of volatiles by gas chromatography	
	3. Ultraviolet absorption measurements	
	4. Carbonyls and anisidine values	
	5. Peroxide values	
	6. Oxygen absorption methods	
	7. Thiobarbituric acid (TBA) method	
	8. Carotene bleaching	
	9. Volatile acids by Rancimat and OSI	
J.	Shelf life prediction	179
K.	Recommended stability testing protocol	182
	Bibliography	183

<b>8 Control of oxidation</b>	<b>187</b>
A. Metal inactivation	187
B. Processing	189
C. Hydrogenation	196
D. Blending with monounsaturated oils	200
E. Breeding and genetic modification	202
F. Packaging	203
Bibliography	206
<b>9 Antioxidants</b>	<b>209</b>
A. Mechanism of antioxidants	209
1. Chain-breaking antioxidants (AH)	
2. Other classes of antioxidants	
B. Antioxidant evaluations	220
C. Synthetic antioxidants	222
D. Natural antioxidants	224
1. Tocopherols	
2. Ascorbic acid	
3. Rosemary and other spice extracts	
4. Flavonoids and green tea catechins	
E. Evaluation of natural antioxidants in plants	248
F. Antioxidant enzymes	253
Bibliography	253
<b>10 Oxidation in multiphase systems</b>	<b>259</b>
A. Nature of colloidal systems	260
B. Food lipid emulsions	263
1. Synthetic emulsifiers and surfactants	
2. Protein emulsifiers	
3. Phospholipid emulsifiers	
4. Mixtures of emulsifiers	
C. Lipid oxidation in emulsions	267
1. Metal catalysis	
2. Antioxidants	
Bibliography	294
<b>11 Foods</b>	<b>299</b>
A. Raw materials	299
1. Metals and metallo-proteins	
2. Enzymatic oxidation in plant foods	
3. Enzymatic and non-enzymatic volatile decomposition products	

4. Enzymatic and non-enzymatic oxidation in animal foods	
B. Processed foods	307
1. Water activity	
2. Interactions of lipids, proteins and sugars	
3. Antioxidant properties of browning reaction products	
4. Interaction products in foods	
C. Milk products	318
1. Metal catalysis	
2. Light oxidation	
3. Antioxidants	
4. Flavor compounds	
5. Factors affecting lipid oxidation	
D. Meat and poultry products	329
1. Iron catalysts	
2. Enzymatic lipid oxidation	
3. Volatile flavor compounds	
4. Antioxidants	
5. Other control measures	
E. Fish products	338
1. Metal catalysts	
2. Enzyme oxidation	
3. Volatile compounds in stored fish, fish oils and fish oil enriched foods	
4. Fish processing	
F. Cereal products	345
1. Hydrolytic rancidity	
2. Lipoxygenase action	
3. Non-enzymatic oxidation	
4. Factors affecting lipid oxidation	
Bibliography	350
<b>12 Frying fats</b>	<b>355</b>
A. Chemistry of frying	355
1. Volatile compounds	
2. Monomeric compounds	
3. Polymeric compounds	
4. Interaction compounds	
B. Fats used for frying	364
C. Methods to assess frying deterioration	366
1. Post-frying methods	
2. Rapid control methods	
3. Methods applied to fried foods	

D. Control measures	373
1. Temperature	
2. Turnover rate	
3. Filtration	
4. Antioxidants	
5. Polymerization inhibitors	
6. Fat uptake in fried foods	
E. Criteria for used frying fats	382
F. Health effects of frying fats	382
Bibliography	385
<b>13 Biological systems</b>	<b>391</b>
A. Biological peroxidation	392
B. Biological antioxidants	394
C. Oxidant–antioxidant balance	397
D. Plasma lipoproteins	399
E. Coronary heart disease	402
1. Development of the disease	
2. Oxidative modification of LDL	
3. The role of high-density lipoproteins (HDL)	
4. Methodology	
5. Autoxidation products of cholesterol	
6. Inhibition of LDL oxidation by antioxidants	
F. Nutritional consequences	422
1. Effect of saturated fatty acids	
2. Effect of polyunsaturated fatty acids	
3. Effect of monounsaturated fatty acids	
4. Effect of <i>trans</i> unsaturated fatty acids	
5. Effect of antioxidants	
6. Effect of lipid oxidation products	
7. Effect of iron supplementation	
G. Optimum dietary intakes	443
H. Conclusions and future perspectives	446
Bibliography	448
Glossary	455
<b>Abbreviations</b>	<b>459</b>
<b>Index</b>	<b>463</b>