



Antioxidants in Food and Biology
Facts and Fiction



Also in the Oily Press Lipid Library:

Volume 19. Lipids: Structure, Physical Properties and Functionality
Written by Kare Larsson, Peter Quinn, Kiyotaka Sato and Fredrik Tiberg

Volume 18. Lipid Oxidation
Written by Edwin N. Frankel

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

**Antioxidants in
Food and Biology**
Facts and Fiction

EDWIN N. FRANKEL
University of California, California, USA



THE OILY PRESS
An imprint of PJ Barnes & Associates
Bridgwater, England

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 978-0-9552512-0-7

Copyright © 2007 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 20** 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

The field of antioxidants has expanded over the past six decades into a wide variety of multidisciplinary areas that affect foods and health. This book conveys the complexity of antioxidant chemistry by providing an appreciation of the various phenomena that affect oxidation and its inhibition in foods and biological systems. By emphasizing mechanistic aspects of antioxidants and lipid oxidation, this book also attempts to sort out facts from fiction, by identifying the many problem areas requiring further research to improve our understanding of complex antioxidant effects and to stimulate better designed methodology and dietary studies for the future.

The introductory Chapter 1 provides an overview of past, present and future aspects to initiate readers into the broad interdisciplinary fields of antioxidants in foods and biology. There is a vast basic literature on how antioxidant structures affect activity in solutions, but our knowledge on how these structural effects apply to multiphase foods and biological systems is limited. Knowledge on the sites of antioxidant action in foods and biological systems is necessary for a better understanding of their effects on their stability and susceptibility to oxidation. In foods, the activities of antioxidants are often difficult to predict and control, because their interactions with metal–protein complexes may either inhibit or promote oxidation. In biology, the activity of antioxidants is even more difficult to predict on the basis of *in vitro* studies, because interfacial interactions occur between different cellular sites and the complex effects of enzyme cofactors and inhibitors, and immune systems.

Chapter 2 deals with the classical chemistry necessary to understand more fully how antioxidants operate and the main aspects of the mechanisms of lipid oxidation and antioxidants. In addition to inhibiting the initiation and the propagation of oxidation, other multiple effects of antioxidants are discussed, including inhibiting the decomposition of hydroperoxides, inactivating prooxidant metals, reducing hydroperoxides and scavenging oxygen. Due to the multiplicity of factors influencing antioxidants' activities in complex foods and biological systems, the common use of artificial and non-relevant azo initiators to evaluate antioxidants is discouraged, because it may be misleading.

Chapter 3 presents details on how the activity of antioxidants is affected at the interface of complex multiphase lipid systems. This chapter introduces the concept of *interfacial antioxidation* that depends on the partition of antioxidants

between the aqueous phase, lipid phase and surfactant-enriched interface in foods and biological systems, and the colloidal chemistry of different types of emulsions affecting activity. Knowledge on the sites of antioxidant and prooxidant actions in multicomponent systems is essential to predict more successfully their activity in complex foods and biological systems.

Chapter 4 discusses the problems of evaluating the activities of antioxidants in foods and biological systems. Because antioxidant activity is strongly affected by the physical composition of the target systems, valid methods to evaluate antioxidants require the control of a multitude of parameters. A judicious choice of several methods is also necessary to determine the effects of different products of lipid oxidation. The many important questions are discussed for the careful choice of antioxidant protocols in foods and biological systems. This chapter ends with recommended protocols based on several substrate properties for valid antioxidant evaluations.

Chapter 5 on antioxidants in different foods deals with their interactions between food lipids, proteins and sugars, synergistic effects of phospholipids, and plant and beverage sources of phenolic compounds. This chapter includes information on the natural antioxidants in vegetable oils, milk, meat, fish and cereal products, special foods, herbs and spices. Plant polyphenols constitute the most important dietary antioxidants evaluated by a multitude of *in vitro* tests. The many analytical problems are discussed regarding the widespread use of one-dimensional methods to evaluate multifunctional food and biological antioxidants, and the caution required in making nutritional recommendations based on the so-called *antioxidant capacity* values of foods.

Chapter 6 covers extensive worldwide research on biological antioxidants, generally based on the hypothesis that the health of an individual is influenced by the efficiency of various protection systems against oxidant damage. The nutritional approach to antioxidant therapy is, however, poorly understood due to the multiple interacting factors that relate degenerative diseases to diet and to oxidation. Because of a lack of reliable biomarkers of oxidative stress, animal and human feeding studies have produced controversial and mixed results. Although there is extensive evidence that diets high in fruits and vegetables rich in phenolic antioxidants are associated with a lower incidence of cardiovascular disease, very few studies have shown that flavonoids are directly responsible for health effects in the diet. Because of complexities in the behavior of natural phenolic antioxidants in different systems, the true impact of oxidation processes in biological tissues is controversial. Results of most *in vitro* and *in vivo* studies to assess the effects of phenolic antioxidants in biological systems are extremely difficult to interpret, because questionable methodology has been used to measure oxidation and the oxidative susceptibility of polyunsaturated lipids and other biological targets. The chapter ends with the many questions that remain to be researched to understand and predict the effectiveness of phenolic antioxidants better in various biological systems.

Chapter 7 covers the renewed attention in biochemistry on the Maillard browning reaction, developed early in food chemistry, and on the interactions of reducing sugars, proteins and lipid secondary oxidation products. The complex biological modification of proteins by glucose in blood known as *glycation* and *glycosylation* involves oxidative and non-oxidative processes, producing low-molecular weight aldehydes that may initiate cardiovascular diseases and are implicated in age-related chronic diseases, including obesity, diabetes and renal disorders. The cross-linking between proteins and carbohydrates generates lifelong products of *advanced glycation end products* (AGEs) at later stages of the Maillard reactions, contributing to tissue degeneration. Advanced glyco-sylation has been shown to affect a number of proteins and has been implicated in the pathogenesis of several diabetes and age-related diseases. Glycation products that are formed in heated foods and a number of recently developed inhibitors are discussed in detail to control their formation in biological systems.

The final chapter, Chapter 8, addresses the important question that concerns food scientists and nutritionists today, as to whether additional or more effective natural phenolic antioxidants are needed in our diet to reduce oxidative stress from dietary and environmental factors, and the risk of cardiovascular disease. There is much *in vitro* evidence supporting a possible beneficial role for polyphenols in preventing cardiovascular disease and cancer. Because oxidative damage is involved in atherosclerosis and other degenerative diseases, antioxidants have been generally thought to contribute to cardiovascular protective effects. However, intervention trials with vitamin E and different phytochemicals produced confusing results. The beneficial nutritional effects of fruits and vegetables have been tied up to increased levels of antioxidants in the body. However, very few studies provide direct evidence that the benefits of eating fruits and vegetables are actually due to *in vivo* antioxidant activity. Many phenolic compounds recognized for their antioxidant activity *in vitro* might have different and additional *in vivo* properties. Discussions include the evidence for several non-antioxidant activities of vitamin E, flavonoids and other phenolic compounds. Looking at future research, the nutritional and health properties of plant foods are examined, with a list of the many unsettled questions that deserve additional research using more relevant and reliable bioassays for clarification of the interactions between dietary polyphenols and health effects. Another section discusses recent claims for the health benefits of organic compared to conventional plant foods. These claims have been difficult to prove due to the complex environmental and agricultural factors known to influence the contents of phenolic compounds in plants, and because very little reliable data have been published on their corresponding antioxidant activity. A final section deals with *nanotechnology*, a new development in food technology that deals with extremely small structures that

have unique, novel and potentially useful functional properties caused by modified interfacial phenomena with potential applications for antioxidants.

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

Edwin Frankel
Department of Food Science and Technology
University of California
Davis, California 95616 USA
enfrankel@ucdavis.edu
April 2007

Contents

Preface	v
1 Introduction to antioxidants	1
A. Past aspects	1
1. Natural versus synthetic antioxidants	
2. Lipid peroxidation <i>in vivo</i>	
3. Tocopherols and vitamin E antioxidants	
4. Ascorbic acid or vitamin C	
B. Present aspects	6
1. Interfacial phenomena	
2. Oxidant–antioxidant balance	
3. Oxidation of low-density lipoproteins (LDL)	
4. Phytochemicals	
5. Antioxidant testing	
6. Nutritional effects of food ingredients	
7. Effect of antioxidants on aging	
C. Future aspects	15
Bibliography	17
2 Chemistry of antioxidation	21
A. Free radical mechanisms	21
1. Initiation	
2. Propagation	
3. Termination	
B. Classes of antioxidants	25
1. Initiation inhibitors	
2. Propagation inhibitors	
C. Structure-activity relationships	29
1. Inductive effects	
2. Steric effects	
D. Synergistic antioxidant systems	33
1. Homosynergism	
2. Heterosynergism	
3. Autosynergism	
E. Inhibition of photosensitized oxidation	35
F. Antioxidant enzymes in food systems	36
G. Inhibition of biological oxidation	37
1. Metal binders and chelators	

2. Antioxidant enzymes in biological systems	
3. Non-enzymatic reducing agents	
Bibliography	41
3 Antioxidant action in multiphase systems	43
A. Multiphase colloidal systems	43
1. Effect of antioxidants	
2. Effect of metal catalysts	
3. Effect of proteins	
B. Partition	65
C. Summary	71
Bibliography	72
4 Antioxidant protocols for foods and biological systems	77
A. Food and biological oxidation methods	79
B. Antiradical methods	83
1. DPPH assay	
2. TEAC or ABTS assay	
3. Linoleic acid TRAP assay	
4. β -Carotene bleaching method	
5. ORAC assay	
6. Superoxide anion scavenging assays	
7. FRAP assay	
C. Comparison of antiradical methods with <i>in vitro</i> LDL oxidation	93
D. Recommended protocols	96
Bibliography	98
5 Food antioxidants	105
A. Interactions of lipids with proteins and sugars	105
B. Synergism of phospholipids	110
C. Plant and beverage sources of phenolic compounds	111
D. Vegetable oils	114
1. Salad and fish oils	
2. Olive oils	
3. Frying oils	
E. Milk products	122
1. Tocopherols	
2. Phospholipids	
3. Ascorbic acid	
4. Other antioxidants	
5. Other components of milk	
6. Addition to other beverages containing antioxidants	

F. Meat products	124
G. Fish products	128
H. Cereal products	132
I. Special foods, fruits, plant extracts, herbs and spices	136
Bibliography	138
6 Antioxidants in biology	143
A. Biological antioxidant defense systems	145
B. Antioxidant enzymes	146
1. Superoxide dismutases (SOD)	
2. Catalase	
3. Glutathione peroxidases (GPx)	
4. Antioxidant network	
C. Inhibition of LDL oxidation and coronary heart disease by antioxidants	148
D. <i>In vitro</i> versus <i>in vivo</i> studies	150
E. Postprandial oxidative stress	155
F. Prooxidant chemistry of phenolic antioxidants	157
G. Bioavailability, absorption and pharmacokinetic studies	160
1. Tocopherols	
2. Ascorbic acid (vitamin C)	
3. Flavonoids	
4. Methodology	
H. 'Non-antioxidant' activities of phenolic compounds	182
I. Dietary recommendations	184
Bibliography	185
7 Browning and glycation reaction products in biology	193
A. Biological antioxidant activity of Maillard reaction products	194
B. Advanced lipoxidation and glycation end products	195
1. Biological effects of AGEs	
2. Glycation products in foods	
3. Analyses of glycation products	
C. Inhibition of AGE formation	203
1. Antioxidant therapy	
2. AGE inhibitors	
D. Future research	212
Bibliography	212
8 Future perspectives	217
A. Tocopherols	218
B. Flavonoids	220

1. Catechin	
2. Quercetin	
3. Tea catechins	
C. Nutrition studies	228
D. Activities in the gastrointestinal (GI) tract	229
E. What is an antioxidant?	231
F. Future research	232
1. Nutritional and health properties of plant foods	
2. Organic versus conventional plant foods	
3. Food nanotechnology	
Bibliography	239
Glossary	244
Abbreviations	248
Index	251