Lipid Oxidation

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Lipid Oxidation

Second Edition

EDWIN N. FRANKEL
University of California, Davis, California, USA

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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 α-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.

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