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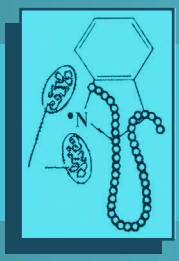

Food Oxidants and Antioxidants

Chemical, Biological, and Functional Properties

EDITED BY

Grzegorz Bartosz

CRC Press
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Chemical and Functional Properties of Food Components Series

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Preface

Food antioxidants are of primary importance for the preservation of food quality during processing and storage. They are also of considerable interest for consumers, who seek antioxidant-rich food items hoping for the prevention of diseases and postponement of aging. However, the status of food depends on the balance of prooxidants and antioxidants, the former being usually neglected when discussing food antioxidants. This book comprises, in one volume, a selection of topics covering both prooxidants and antioxidants occurring in food, which can be interesting, first of all, for food technologists and chemists and students in these fields.

After a general introduction to the problem (Chapter 1), Chapter 2 characterizes the main oxidants present in food, including both nonenzymatic oxidants (hemoproteins, redox active metals, and photosensitizers) and enzymes (lipoxygenases, myeloperoxidases, lactoperoxidases, and polyphenol oxidases). While the idea of antioxidant activity/capacity/potential has become popular, that of oxidation potential, equally useful, is much less known; it is presented in Chapter 3. Chapters 4 and 5 discuss the mechanisms of oxidation of main food components, lipids and proteins, respectively, including factors affecting this process, such as the presence of prooxidants and antioxidants, light, temperature, oxygen, and food composition. In many cases, exogenous oxidants are added to food during processing; this practice, as well as the effects of such physical agents as irradiation, freeze–thawing, and high hydrostatic pressure during food processing, is presented in Chapter 6. Chapter 7 discusses the effects of oxidation on the sensory characteristics of food components; these effects are usually unwanted, but there are also cases where they can be beneficial. Chapter 8 analyzes how the oxidation of main food components (lipids, proteins, and carbohydrates) and antioxidants affects the nutritive and health-promoting features of food components. Chapter 9 discusses natural antioxidants present in food, especially those that are less known, such as antioxidant amino acids, peptides, proteins, and polysaccharides and oligosaccharides. Chapter 10 presents antioxidants generated in food as a result of processing. The mechanisms of antioxidant activity and the main antioxidant enzymes are discussed in Chapter 11. The next two chapters deal with the measurement of the antioxidant activity of food components (Chapter 12) and their application to a specific material (apple products, Chapter 13). Many food components are classified as antioxidants but under certain conditions may have prooxidant activity; this question is described in Chapter 14. The bioavailability and antioxidant activity of two important groups of antioxidants, curcuminoids and carotenoids, are discussed in Chapter 15. Chapter 16 deals with case studies on selected natural food antioxidants, presenting novel extraction methods for optimal preservation of antioxidant activities, such as supercritical fluid extraction, pressurized liquid extraction, subcritical water extraction, and microwave- and ultrasound-assisted extraction, as well as their application to specific raw materials. Functional antioxidant foods and beverages are presented in Chapter 17.

The last chapter contains some general ideas concerning mainly the effects of food on the redox homeostasis of the organism.

The authors of the book are renowned scientists from Australia, Denmark, Germany, Italy, Lithuania, Poland, Slovakia, Spain, and the United States. I am deeply indebted for their contributions and cooperation. I hope that the book can provide basic information for students and newcomers to the field but also be of use to more experienced readers interested in the problems of food prooxidants and antioxidants.

Editor

Grzegorz Bartosz received his MS and PhD degrees from the University of Łódź and his DSc degree from the Jagiellonian University of Cracow (Poland). He spent his postdoctoral fellowship at Texas A&M University; was a research fellow at the University of Düsseldorf and Macquarie University in Sydney; and for short terms, visited various European universities and institutions. Presently, he is a professor at the Department of Molecular Biophysics of the Faculty of Biology and Protection of the Environment at the University of Łódź and at the Department of Biochemistry and Cell Biology of the Faculty of Biology and Agriculture of the University of Rzeszów (Poland). His research interest concentrates on reactive oxygen species and antioxidants. He is a corresponding member of the Polish Academy of Sciences and of the Polish Academy of Arts and Sciences and chairman of the Committee of Biochemistry and Biophysics of the Polish Academy of Sciences. He is on the editorial boards of *Acta Biochimica Polonica*, *Acta Physiologiae Plantarum*, *Free Radical Biology and Medicine*, and *Free Radical Research*. In 2011–2012 he was president of the Society for Free Radical Research–Europe. He is an author of more than 300 journal publications, 2 books, and 8 book chapters.

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Abbreviations

AAA:	α-aminoadipic acid
AAS:	α-aminoadipic semialdehyde
ABTS:	2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
ACA:	aldehyde/carboxylic acid
ACE:	angiotensin converting enzyme
ADP:	adenosine diphosphate
AGEs:	advanced glycation end products
ALA:	alpha-lipoic acid
ALEs:	advanced lipid oxidation end products
AMP:	adenosine monophosphate
AOX:	antioxidant
APCI:	atmospheric pressure chemical ionization
ARA:	arachidonic acid
AsA:	ascorbic acid
ATP:	adenosine triphosphate
AUC:	area under curve
AVA:	avenanthramide
BHA:	butylated hydroxyanisole
BHT:	butylated hydroxytoluene
CAP:	captopril
Car:	carotenoid
cGMP:	cyclic guanosine monophosphate
CLA:	conjugated linoleic acid
CMLys:	carboxymethyllysine
CMP:	caseinomacropeptide
COPs:	cholesterol oxidation products
CPP:	casein phosphopeptides
CumOOH:	cumene hydroperoxide
CYP:	cytochrome P450
DAG:	diacylglycerol(s)
DHA:	docosahexaenoic acid
DHLA:	dihydrolipoic acid
DNPH:	dinitrophenylhydrazine
DOPA:	3,4-dihydroxyphenylalanine
DP:	degree of polymerization
DPPH:	1,1-diphenyl-2-picrylhydrazyl
DSF:	defatted soy flour
DTNB:	5,5′-dithiobis-(2-nitrobenzoic acid), Ellman's reagent
ECD:	electron-capture detector
EDTA:	ethylenediaminetetraacetic acid
EFB:	empty fruit bunches
EGC:	epigallocatechin
EGCG:	epigallocatechin gallate
EPA:	eicosapentaenoic acid
ESR:	electron spin resonance
FA:	fatty acid(s)

FI: flow ice
FID: flame ionization detection
FFA: free fatty acid(s)
FOS: fructo-oligosaccharide
FOX: ferrous oxidation-xylenol orange
FRAP: ferric reducing/antioxidant power
FPH: fish protein hydrolysates
FTC: ferric thiocyanate
GC: gas chromatography
GGC: γ-glutamylcysteine
GGS: γ-glutamic semialdehyde
GPx: glutathione peroxidase
GRAS: generally recognized as safe
GSH: glutathione
GST: glutathione *S*-transferases
Hb: hemoglobin
HCA: hydroxycinnamic acid(s)
HDLc: high-density lipoprotein-associated cholesterol
HHE: 4-hydroxy-2-hexenal
HHP: high hydrostatic pressure
HMF: hydroxymethylfurfural
HMW: high molecular weight
HNE: 4-hydroxy-2-nonenal
H(p)ETE: hydro(pero)xyeicosatetraenoic acid
HPL: hydroperoxide lyase(s)
HPLC: high-performance liquid chromatography
HPSEC: high-performance size-exclusion chromatography
La: lactalbumin
LA: linoleic acid
LDH: lactate dehydrogenase
LMW: low molecular weight
LOX: lipoxygenase(s)
LPO: lactoperoxidase
MAE: microwave-assisted extraction
MAG: monoacylglycerol(s)
MALDI-TOF: matrix-assisted laser desorption ionization-time of flight
MAP: modified atmosphere packaging
Mb: myoglobin
MCL: methyl conjugated linoleate
MDA: malondialdehyde
metHb: methemoglobin
metMb: metmyoglobin
ML: methyl linoleate
MMP: matrix metalloproteinase
MPO: myeloperoxidase
MRP: Maillard reaction product(s)
MS: mass spectroscopy
MUFA: monounsaturated fatty acids
MW: molecular weight
NAC: N-acetylcysteine
NQO1: NAD(P)H-quinone oxidoreductase

OFI: flow ice system including ozone
OMWW: olive-mill wastewater
ONE: 4-oxo-2-nonenal
OP: oxidation potential
ORAC: oxygen radical absorbance capacity
OS: oligosaccharide(s)
PAD: pulsed amperometric detection
PAGE: polyacrylamide gel electrophoresis
PDA: photodiode array detection
PEF: pulsed electric field
PHWE: pressurized hot water extraction
PIR: protein interaction report
PLE: pressurized liquid extraction
POBN: (4-pyridyl-1-oxide)-*N*-*tert*-butylnitrone
POD: polyphenol peroxidase
PPF: palm pressed fibers
PPO: polyphenol oxidase(s)
PS: polysaccharide(s)
PUFA: polyunsaturated fatty acid(s)
PV: peroxide value
RNS: reactive nitrogen species
ROS: reactive oxygen species
RTE: ready to eat
SDG: secoisolariciresinol diglucoside
SDS: sodium dodecyl sulfate
SECO: secoisolariciresinol
Sen: sensitizer
SFE: supercritical fluid extraction
SO: sunflower oil
SPI: soy protein isolate
SWE: subcritical water extraction
TAC: total antioxidant capacity
TAG: triacylglycerol(s)
TAnC: total anthocyanin content
TBA: thiobarbituric acid
TBARS: thiobarbituric acid reactive substances
TBHQ: tertiary butyl hydroquinone
TE: Trolox equivalent(s)
TEAC: Trolox equivalent antioxidant capacity
TO: Tonalin oil
TPC: total phenolic content
TRAP: total radical-trapping antioxidant parameter
TRX: thioredoxin
UAE: ultrasound-assisted extraction
UGT: uridine-5′-diphospho glucuronosyltransferase
UV: ultraviolet
WEPO: water extraction and particle formation online
WOF: warmed-over flavor
WPC: whey protein concentrate
WSP: water-soluble proteins

1 Oxidation of Food Components
An Introduction

Anna Kołakowska and Grzegorz Bartosz

CONTENTS

> Remember that thou goest in the midst of snares,
> and that thou walkest upon the battlements of the city
>
> **Sirach 9.13**

1.1 INTRODUCTION

Oxidation in food has its source in the physiological mechanisms of the oxidation processes in plants and animals, which are raw materials for food products. In the chain of food production, under the influence of biological, environmental, and technological factors and the additives used, the status of the oxidation and antioxidant

changes. Oxidation in food systems is a detrimental process. It deteriorates the sensory quality and nutritive value of a product and poses health hazards by the presence of toxic oxidation products. Oxidation affects all food components, but its impact on food quality is not uniform.

Oxidation processes that occur naturally in the human body contribute to the development of most major diseases due to an insufficient defense system. The presence of toxic oxidation products in food and its reduced nutritional value and decreased antioxidant content, which are supplied in the diet, can significantly affect the health of the consumer. It can be expected that a diet rich in oxidized food components leads to a lowering of the antioxidant or oxidant status in an organism, increasing the risk of disease.

1.2 FREE RADICALS AND REACTIVE OXYGEN SPECIES

The sources of oxidative processes in living organisms are free radicals and other reactive oxygen species (ROS), which are formed in every living cell. A free radical is any atom or molecule that has at least one unpaired electron in its outermost shell. Any free radical containing oxygen is then referred to as a ROS, but a ROS can also include species that are not free radicals (such as hydrogen peroxide H_2O_2, singlet oxygen $^1O_2$, ozone O_3, hypochlorite ^-OCl, and peroxynitrite $ONOO^-$). The most commonly formed ROS are the superoxide anion radical $\left(O_2^{\bullet -}\right)$ and hydrogen peroxide, and the hydroxyl radical is the most reactive ROS. Nitric oxide ($NO^{\bullet}$) is also a free radical. A reaction $O_2^{\bullet -}$ anion with $NO^{\bullet}$ produces peroxynitrite $ONOO^-$, a strong oxidant (Figure 1.1). The main source superoxide is the one-electron leakage of the mitochondrial respiratory chain and, in plant cells, of the chloroplasts redox

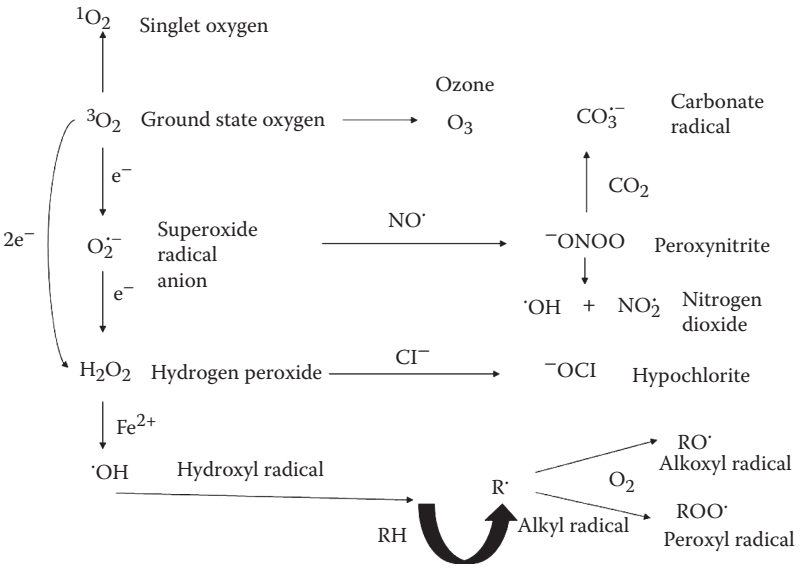

FIGURE 1.1 Main ROS occurring in biological systems

system. Free radicals can also be produced by many cells as a defensive mechanism (phagocytes) or for signaling purposes. Neutrophils produce free radicals to attack and destroy pathogens. $NO^{\bullet}$ plays mainly a signaling role in the body.

While ROS are predominantly implicated in causing cell damage, they also play a major physiological role in several aspects of intracellular signaling and regulation. ROS interfere with the expression of a number of genes and signal transduction pathways and, depending on the concentration, cause either a positive response (cell proliferation) or a negative cell response (growth arrest or cell death). ROS can thus play a very important physiological role as secondary messengers (Valko et al. 2006; Bartosz and Kołakowska 2011).

1.3 PEROXIDATION OF LIPIDS

1.3.1 Initiation

All types of biological molecules are damaged by ROS, but lipid oxidation plays a special role in this process because the energy barrier to initiate lipid peroxidation is lower than those to initiate oxidation of proteins, carbohydrates, or nucleic acids. Nevertheless, there is interplay between oxidative reactions of various biomolecules. Free radicals of other molecules may initiate lipid oxidation while products of lipid oxidation modify proteins and nucleic acids and deplete antioxidants. Different mechanisms are able to induce lipid peroxidation: free-radical reactions, photooxidation, enzyme action, and oxidation catalyzed by trace metals.

1.3.2 Autoxidation

Autoxidation is a radical-chain process involving three steps: initiation, propagation, and termination. In the initiation step, a hydrogen atom is removed from a molecule LH and a free-radical form of lipid alkyl radical $L^{\bullet}$ is formed. Heat, ultraviolet (UV), and visible light and metal catalysts can accelerate this step. It is usually initiated by hydroxyl, peroxyl, or hydroperoxyl radicals (but not superoxide or hydrogen peroxide) or hemoproteins activated to the ferryl state.

In the propagation step, an alkyl radical reacts with oxygen to form a peroxyl radical ($LOO^{\bullet}$):

$$L^{\bullet} + O_2 \rightarrow LOO^{\bullet}$$

which, in turn, can abstract a hydrogen atom from another lipid molecule:

$$LOO^{\bullet} + LH \rightarrow LOOH + L^{\bullet}$$

thus causing an autocatalytic chain reaction. The peroxyl radical combines with H to give a lipid hydroperoxide.

Alternatively, peroxyl radicals [especially those formed from polyunsaturated fatty acids (PUFAs), such as arachidonic acid (ARA) or eicosapentaenoic acid (EPA)] can be transformed in the organism into cyclic peroxides or even cyclic endoperoxides.

Reaction between two radicals terminates the reaction

$$LOO^{\bullet} + L^{\bullet} \rightarrow LOOL$$

$$L^{\bullet} + L^{\bullet} \rightarrow LL$$

1.3.3 Photooxidation

Oxygen can be excited to the singlet state ($^1O_2$) by light energy (photooxidation) promoted by pigments that act as sensitizers. As singlet oxygen ($^1O_2$) is highly electrophilic, it can react rapidly with unsaturated lipids but by a different mechanism than free-radical autoxidation. In the presence of sensitizers (chlorophyll, porphyrins, myoglobin, riboflavin, bilirubin, erythrosine, rose bengal, methylene blue, etc.), the double bond of a fatty acid (FA) residue interacts directly with singlet oxygen produced from O_2 by light or UV radiation. The photooxidation is a faster reaction than autoxidation; it was demonstrated that photooxidation can be 30,000 times faster than autoxidation in the case of oleic acid and can be 1000–1500 times faster in the case of polyenes.

Lipid hydroperoxides, the main intermediates of the peroxidation reactions, accumulate in the bilayer and induce changes in the structure and biophysical organization of membrane lipid components, especially oxidation of phospholipid FA residues, including the loss or rearrangement of double bonds. The main biophysical consequences of the lipid membrane include changes in membrane fluidity and permeability, alteration of membrane thermotropic phase properties, and membrane protein activities (Mosca et al. 2011).

1.3.4 Enzymatic Peroxidation

Lipoxygenases (LOXs) (EC 1.13.11.12), a family of non-heme iron-containing FA dioxygenases, are widely distributed in plants, animals, and microorganisms. LOX-catalyzed lipid oxidation differs from the free-radical reaction by the formation of hydroperoxides in a defined position of FA chains. LOXs use molecular oxygen to catalyze the stereospecific and regiospecific oxygenation of PUFAs with 1-*cis*,4-*cis*-pentadiene moieties. The newly formed FA peroxy free radical abstracts hydrogen from another unsaturated FA molecule to form a conjugated hydroperoxy diene. Hydroperoxy dienes are responsible for the off-flavor in frozen vegetables; for lipid oxidation in cereal products, rapeseed, pea, and avocado; and for "beany" and bitter flavors. If the oxidation reactions proceed to a low degree only, they may be considered as desirable. For example, the typical flavors of cucumbers and virgin olive oil are results of lipid oxidation products (Pokorny and Schmidt 2011). LOXs in fish are also responsible for the formation of a desirable fresh fish flavor, the seaweed flavor, from n-3 PUFA. Soybean LOX has been used to bleach flour to produce white bread crumbs and to improve the dough-forming properties and baking performance of wheat flour by oxidizing free lipids.

In both plants and animals, cyclooxygenase enzymes catalyze the addition of molecular oxygen to various polyunsaturated FAs, which are thus converted into biologically active endoperoxides, intermediates in the transformation of FAs to

prostaglandins. Among the cytochrome-P450 catalyzed reactions, the FA epoxygenase activity produces epoxide derivatives. These monoepoxides can be metabolized into diepoxides, epoxy-alcohols, or oxygenated prostaglandins.

1.3.5 Peroxidation Catalyzed by Trace Metals and Heme Compounds

Transition metal ions, first, participate in the Fenton reaction believed to be the main source of the hydroxyl radical (•OH) in biological systems. Another contribution of transition metal ions to lipid peroxidation is a result of their role in the decomposition of lipid hydroperoxides. These primary products of peroxidation may slowly decompose spontaneously, especially at elevated temperatures, but transition metal ions accelerate their decomposition, resulting in formation of alkoxyl (RO•) and peroxyl (ROO•) radicals (Bartosz 2003):

$$ROOH + Fe^{2+} \rightarrow RO^{\bullet} + HO^{-} + Fe^{3+} \quad \text{(fast reaction)}$$

$$ROOH + Fe^{3+} \rightarrow ROO^{\bullet} + H^{+} + Fe^{2+} \quad \text{(slow reaction)}$$

Metal ions found naturally in food components and gained from the environment or metal equipment can initiate lipid peroxidation in foods. Higher concentrations of hemoglobin and myoglobin iron in meat and in fish are associated with higher rates of lipid oxidation. In meat, lipid oxidation and myoglobin oxidation can occur concurrently. The oxidation of oxymyoglobin (OxyMb) to metmyoglobin (MetMb) generates reactive intermediates capable of enhancing further oxidation of OxyMb and/or unsaturated FAs. Specifically, a superoxide anion is formed and dismutates enzymatically or nonenzymatically, producing hydrogen peroxide. The latter can react with the MetMb concurrently generated in this oxidation sequence to form an activated MetMb complex capable of enhancing lipid oxidation. Heme or iron can be released from the myoglobin and hemoglobin during postmortem handling and storage, thereby also promoting lipid oxidation (Min et al. 2010; Faustman et al. 2010).

Compared to LOX, iron and hemoglobin cause slower lipid oxidation in the initial phase but result in a severe oxidized oil odor, and LOX is associated with a strong fishy odor (Fu et al. 2009).

1.3.6 Secondary Oxidation Products

Lipid hydroperoxides, the primary products of oxidation, are highly unstable and, under conditions of elevated temperature and illumination and in the presence of prooxidants, tend to decompose via β-scission reaction, giving rise to secondary products, such as aldehydes, ketones, lactones, alcohols, keto acids, hydroxy acids, epidioxides, and other volatile compounds. Some of these secondary products can be toxic to humans and are responsible for the undesirable rancid odor typical of oxidized oils (Decker 2010).

The most important of these products are short-unsaturated aldehydes because of the reactivity of the aldehyde group. They exhibit biological activity *in vivo* and interact easily with cell and food components, causing a loss of the nutritional value

of food. For years, malonodialdehyde (MDA), the most abundant aldehyde, which results from lipid peroxidation in food, has been found to be the main culprit. Its concentration in meat and fish products could even reach 300 μM (Kanner 2007). Since the 1990s, trans-4-hydroxy-2-nonenal (HNE) has been viewed as the quintessential toxic lipid peroxidation product. In addition to the formation of HNE, the hydroperoxides of ARA and linoleic acid are precursors of the highly electrophilic γ-keto aldehyde trans-4-oxo-2-nonenal (4-ONE). Lipid oxidation of n-3 PUFA generates a closely related compound, 4-hydroxy-2-hexanal (HHE), whose concentration in several foods, such as fish products, could reach 120 μM (Long and Picklo 2010). Analysis of aldehyde products resulting from docosahexaenoic acid (DHA) oxidation identified at least 15 aldehydes, including HHE, glyoxal, malondialdehyde, 4-hydroxy-2,6-nonadienal, 2-pentenal, and others (Kanner 2007).

These aldehydes are capable of producing damage to the biological macromolecules even at a distance from the formation site (Traverso et al. 2010). They are durable and easily absorbed from food (Gracanin et al. 2010; Goicoechea et al. 2011).

Because of its ability to interact with DNA and proteins, such as MDA, HNE has often been considered as a potentially genotoxic agent able to cause mutations. Moreover, MDA toxicity also is directed toward cardiovascular system stability through the intermolecular crosslinking of collagen, which contributes to the stiffening of cardiovascular tissue.

There seems to be a dual influence of 4-HNE on the physiology of cells: Lower intracellular concentrations (around 0.1–5.0 μM) seem to be beneficial to cells, promoting proliferation, and higher concentrations (around 10–20 μM) have been shown to be toxic and involved in the pathology of several diseases.

1.3.7 Oxysterols

Oxidized derivatives of cholesterol and phytosterols can be generated in the human organism through different oxidation processes, some requiring enzymes. Furthermore, oxysterols are also present in food as a result of lipid oxidation reactions caused by heat treatments, contact with oxygen, exposure to sunlight, etc. Cholesterol oxides are present in our diet, particularly in foods high in cholesterol. Storage, cooking, and processing tend to increase the cholesterol oxidation products contain. Their concentration is particularly high (10–100 μM, i.e., 10–150 μg/g dry weight) in dried egg, milk powders, heated butter (ghee), precooked meat and poultry products, and heated tallow. Oxysterols can be absorbed from the diet at different rates, depending on their side chain length. In the organism, oxysterols can follow different routes: They may be secreted into the intestinal lumen, esterified, and distributed by lipoproteins to different tissues or degraded, mainly in the liver (Kanner 2007). Cholesterol oxidation products show cytotoxicity (especially the 7-oxygenated species) and apoptotic and proinflammatory effects, and they have also been linked with chronic diseases, including atherosclerotic and neurodegenerative processes. In the case of phytosterol oxidation products, more research on their toxic effects is needed. Nevertheless, current knowledge suggests they may also exert cytotoxic and proapoptotic effects although at higher concentrations than oxycholesterols (Otaegui-Arrazola et al. 2010; Wąsowicz and Rudzińska 2011).

1.4 PROTEIN OXIDATION

Reactive oxidants generated continuously in biological systems are expected to react mainly with proteins as a result of the high abundance of proteins (approximately 70% of the dry mass of cells) and rapid rates of their reactions with many oxidants. The occurrence of protein oxidation in biological systems has been known and studied for approximately 50 years as a result of the connection between the oxidative damage to proteins and the development of age-related diseases. The oxidation of food proteins is one of the most innovative research topics within the food science field having only been studied for approximately 20 years (Estévez 2011).

Protein oxidation occurs as a result of either direct attack by ROS or photooxidation or indirectly through peroxidation of lipids that further degrade and attack proteins.

Numerous ROS, such as the superoxide $\left(O_2^{\bullet -}\right)$, the hydroperoxyl $\left(HO_2^{\bullet}\right)$, and hydroxyl ($HO^{\bullet}$) radicals and other nonradical species, such as hydrogen peroxide (H_2O_2) and hydroperoxides (ROOH), are potential initiators of protein oxidation. As a direct consequence of the abstraction of a hydrogen atom from a susceptible target (PH), a carbon-centered protein radical ($P^{\bullet}$) is formed. The initial $P^{\bullet}$ is consecutively converted into a peroxyl radical ($POO^{\bullet}$) in the presence of oxygen and to an alkyl peroxide (POOH) by abstraction of a hydrogen atom from another susceptible molecule. Further reactions with ROS, such as the $HO_2^{\bullet}$ radical or with reduced forms of transition metals (Mn^{+}), such as Fe^{2+} or Cu^{+}, lead to the formation of an alkoxyl radical ($PO^{\bullet}$) and its hydroxyl derivative (POH) (Estévez 2011):

$$PH + HO^{\bullet} \rightarrow P^{\bullet} + H_2O$$

$$P^{\bullet} + O_2 \rightarrow POO^{\bullet}$$

$$POO^{\bullet} + PH \rightarrow POOH + P^{\bullet}$$

$$POOH + HO_2^{\bullet} \rightarrow PO^{\bullet} + O_2 + H_2O$$

$$POOH + M^{n+} \rightarrow PO^{\bullet} + HO^{-} + M^{(n+1)+}$$

$$PO + HO_2^{\bullet} \rightarrow POH + O_2$$

$$PO^{\bullet} + H^{+} + M^{n+} \rightarrow POH + M^{(n+1)+}$$

The common protein targets for ROS are the peptide backbone and the functional groups located in the side chains of amino acid residues. It has been shown that a single hydroxyl radical is capable of causing damage of up to 15 amino acids of a peptide chain. Certain amino acids, such as cysteine and methionine, would be first oxidized because of the high susceptibility of their sulfur centers. Tryptophan residues are also promptly oxidized. Susceptible to oxidation are also amino acids with a free amino, amide, and hydroxyl group (lysine, arginine, and tyrosine).

Lipid radicals abstract hydrogen mainly from the side chains of the protein molecule, in particular, from lysine, arginine, histidine, tryptophan, cysteine, and cystine residues, to form protein radicals ($P^{\bullet}$) that initiate formation of further radicals interacting with the protein, causing formation of protein radicals or protein–protein and protein–lipid adducts, or they react also with other food components (Sikorski 2007):

$$P + L^{\bullet} \rightarrow P^{\bullet} + L$$

$$P + LO^{\bullet} \rightarrow P^{\bullet} + LOH,\ LO^{\bullet}P$$

$$P + LOO^{\bullet} \rightarrow P^{\bullet} + LOOH,\ LOO^{\bullet}P$$

$$P + LOH \rightarrow LO^{\bullet} + P^{\bullet} + {}^{\bullet}OH + H^{\bullet}$$

These processes generate various byproducts, among them oxidized amino acids, carbonyls, and fragmentation products. The formation of carbonyl compounds is principally a result of the oxidation of threonine, proline, arginine, and lysine residues. The total protein carbonyl content is estimated to be ≈1–2 nmol/mg protein in a variety of human and animal tissues, which represent modification of about 10% of the total cellular protein. The result of protein oxidation is the loss of the native structure and functionality of protein molecules (Estevez 2011; Gracanin et al. 2010).

1.5 CONSEQUENCES OF LIPID AND PROTEIN OXIDATION *IN VIVO*

Once free radicals are generated, they are often capable of giving rise to chain reactions, that is, reactions that create new radicals that, in turn, trigger new reactions. Despite the numerous lines of defense, protection against free radicals is never complete, and more or less severe random damage continually takes place within living organisms.

Considering that the sites responsible for the greatest production of oxygen radicals are localized on biological membranes, the components of the membrane themselves (phospholipids and proteins) are among the principal targets. The fundamental roles of free radicals have been suggested in aging and numerous pathological situations regarding several organs; among these, for instance, are inflammation; ischemia–reperfusion syndromes; atherosclerosis; degenerative cerebral syndromes, particularly Alzheimer's disease; cataracts; retinopathy; diabetic complications; and cancer (Traverso et al. 2010; Bartosz and Kołakowska 2011).

Proteins are structurally altered by oxidation under oxidative stress conditions; their oxidation leads to the generation of disulfide bridges, unfolding and increasing exposure of the polypeptide chain to the hydrophilic environment (Chaudhuri et al. 2002).

As a result of oxidation, carbonyls are introduced into proteins either by direct oxidation of amino acids or, indirectly, by covalent attachment of a carbonyl-containing moiety, such as HNE or MDA. The proteins, when oxidized at the level

of sulfur-containing amino acids, can be repaired. Cells have limited mechanisms for protein repair and mechanisms for getting rid of damaged proteins (mainly proteasomes). Protein damage may include, for example, disulfide bridges; cysteine sulfenic acid; methionine sulfoxide; hydroxylation and carbonylation of Arg, Lys, Pro, Thr, Leu, etc.; nitrosylation of Cys; nitration of Tyr, Try, and His; lipid peroxidation adducts to His, Cys, and Lys; glycation/glycoxidation adducts to Lys and Arg; and protein aggregation (Friguet 2006). Accumulation of oxidized forms of protein is observed in aging and age-related diseases (Valko et al. 2006).

A consequence of the harmful protein oxidation processes is the formation of lipofuscin, which accumulates intracellularly with age (e.g., in the liver, kidney, heart muscle, adrenals, nerve cells, eyes, and brain). It is an aggregate containing highly oxidized and covalently cross-linked proteins (30%–58%), oxidized lipids (19%–51%), and low amounts of saccharides. These yellowish-brown pigment granules are products that result from the interaction of oxidatively modified proteins and lipids, in which a major cross-linking agent are carbonyls (aldehydes as MDA, HNE, HHE) of lipids and proteins. Carbohydrates form only a minor structural component (Höhn et al. 2011; Traverso et al. 2010). Oxidative stress is a major promoter for lipofuscin formation *in vitro* and *in vivo* (Breyer and Pischetsrieder 2009).

Similarly colored melanoidin-like polymers from oxidized lipids and proteins are formed in food. In these reactions, the main active amino acid is the lysine as it possesses a free amine group, and aldehydes are the most active groups in oxidized lipids. Another sensitive amino acid is tryptophan because of the indole group (Pokorny et al. 2011).

1.6 FACTORS AFFECTING LIPID AND PROTEIN OXIDATION IN FOOD

Susceptibility of lipids and/or protein oxidation in food depends on the composition of the foods: content and composition of lipids and proteins, the presence of prooxidants and antioxidants, oxygen levels, light, temperature, and a number of biological and technological factors.

FA composition. The hydrogen atom bound to the carbon atom separating the C=C bond is the easiest to detach; therefore, PUFAs containing such residues are most prone to peroxidation. If we assume the rate of this reaction for stearic acid as 1, it would be 100 for oleic acid, 250 for linoleic acid, and 2500 for linolenic acid. FAs may be autoxidized either in free form or, mostly, as components of glycerolipids or glycolipids. PUFAs were shown to be more stable to oxidation when located at the *sn*-2 position of triacylglycerol compared to *sn*-1.

Fortification of food with LC n-3 PUFAs can make it more prone to protein oxidation. For example, dairy products enriched with n-3 PUFAs can lead to severe changes, including oxidation of the side chain groups, backbone fragmentation, aggregation, and loss of nutritional value and functional properties of the proteins (Cucu et al. 2011).

Temperature. The increase in temperature during storage and food processing accelerates the oxidation process and changes its course. Primary lipid oxidation products decompose more easily and interact more quickly with other components

of food. Heating above 100°C is critical for the oxidation of cholesterol. The frying temperature above 170°C causes oxidation of not only the frying oil but also of lipids in the fried food, especially in foods rich in PUFAs.

Water activity. Rate of oxidation decreases as the water activity (a_w) is lowered. The rate of many lipid oxidation reactions increases under very low a_w (<0.2). Rancidity becomes a major problem in dehydrated foods and in frozen foods rich in PUFAs, such as fish.

Light. Light is a source of energy that can lead to the formation of radical initiators. UV irradiation is particularly harmful. The influence of light on lipid oxidation depends on the wavelength, the depth of penetration into the product, sensitizer content, and the content of carotenoids, which are a barrier to the photooxidation. Reduced-fat dairy products often seem more sensitive to oxidation compared to dairy foodstuffs with a higher lipid content, demonstrating the important role of proteins and lipid–protein interaction in this chemical decay (Mestdagh et al. 2011). In the processing of fish intended for long frozen storage, where the process-initiated lipid oxidation cannot be effectively inhibited, certain technological operations are performed in rooms with artificial lighting. The appropriate choice of food packaging, forming a barrier against oxygen and light, is important.

Technological process. Susceptibility to oxidation of raw materials (plant and animals) is affected by many biological factors (species, variety, race, sex, age, etc.) and many others. For example, environmental pollutants may induce oxidative stress (Braconi et al. 2011; Grosicka-Maciąg 2011). In technological processes, increased susceptibility to oxidation through mechanical processing (milling, mixing) and salt addition may occur (Sakai et al. 2004). This problem may be especially important in technologies such as irradiation, drying, and microwaves that produce ROS (Zanardi et al. 2009). Microwaves can break disulfide bonds, thus inducing subunit disaggregation, which can cause protein unfolding and formation of smaller aggregates in the solution (Guan et al. 2011). Lipid oxidation in microwave-cooked food is difficult to detect because of the participation of lipid oxidation products in interactions with proteins (Kołakowska 2011). As a result of microwave heating of fish's lipids–albumin system, almost half of the lipids were not available for extraction (covalently bound to a protein). In a system containing partly oxidized lipids, as much as 76% of the lipids after microwave heating have been covalently bound to a protein; DHA was bound.

1.7 WHAT IS OXIDIZED FIRST: LIPIDS OR PROTEINS?

Until recently, protein oxidation in food was primarily interpreted as a secondary result of lipid oxidation. In the presence of linoleate, bovine serum albumin was not oxidized by the direct action of HO• radicals but was undergoing a secondary oxidation by nondienic lipid hydroperoxides and/or lipid radical intermediates, arising from the HO•-induced linoleate oxidation. However, linoleate was secondarily oxidized by oxidized species of albumin (Collin et al. 2010). However, the •OH radical would react faster and preferentially with certain proteins, such as albumin ($k = 8 \times 10^{10}$ dm^3 mol^{-1} s^{-1}) or collagen ($k = 4 \times 10^{11}$ dm^3 mol^{-1} s^{-1}), than with unsaturated lipids, such as linoleic acid ($k = 9 \times 10^9$ dm^3 mol^{-1} s^{-1} (Davies 2005). According

to Soyer and Hultin (2000), reactions of ROS with lipids and proteins in fish tissue proceed simultaneously. Lipid and protein oxidation appeared to occur simultaneously in chicken meat during frozen storage (Soyer et al. 2010) and in minces from horse mackerel during processing and storage, but it was not possible to determine at which level these two reactions were coupled (Eymard et al. 2009). During storage of frozen fatty fish, lipid oxidation symptoms occur significantly ahead of changes in proteins; therefore, the last were assumed to be a result of lipid oxidation. But in lean fish, where the ratio of protein to lipid is about 20:1, protein denaturation changes were observed already in the initial period of frozen storage while rancidity was noticeable after a period of several times longer. With the increase of lipid oxidation in herring, the antioxidant activity of tissue decreases. Therefore, even in fatty fish rich in PUFAs, the primary target of ROS appears to be protein (Kołakowska 2011; Aranowska 2011).

The free radical transfer from oxidizing lipids to protein and amino acids has been observed in dry (lyophilized) products. These interactions may initiate the reactions leading to protein degradation (Schaich 2008). On the other hand, free radicals may transfer from proteins to lipids and initiate lipid oxidation. Furthermore, free radicals may transfer from proteins to other biomolecules, such as other proteins and peptides. Hence, the free radical interactions in food systems may be important in determining the stability and shelf life of dry foods, in particular, in irradiated freeze-dried products. The interactions between the particles of different powdered ingredients in soup powder increased the rate of reactions, leading to a higher rate of radical reactions than in powdered ingredients stored separately (Raitio et al. 2011).

1.8 CARBOHYDRATE OXIDATION

Classical nonenzymatic browning (Maillard reactions) is traditionally attributed to reactions of reducing sugars with amine-containing compounds, and it is uncertain whether these free-radical reactions are accompanied by the oxidative processes of saccharides. Free radicals do not play a significant role in the browning reactions of amine groups of ethanolamine and PUFA and in a saccharide–lecithin system (Nguyen et al. 2002). The presence of radicals and the oxidation of saccharides have been shown in an oxidative model system copper–carbohydrate (Cerchiaro et al. 2005) and in the iron-containing xanthine oxidase and hypoxanthine (Fe-XO/HX)–saccharide system. Saccharide molecules, such as glucose, fructose, and sucrose, are essential for generating radicals ($R^{\bullet}$) as no $R^{\bullet}$ were detected in the absence of saccharides (Luo et al. 2001).

$$\text{Saccharide chain (R)} - \text{H} + \text{OH}^{\bullet} \rightarrow \text{saccharide derivatives} + \text{R}^{\bullet} + \text{H}_2\text{O}$$

Free radicals formed during irradiation of lactose (Lyutova and Yudin 2006) and during the industrial oxidation of starch (Łabanowska et al. 2011). Various stable and short-living radical species were formed upon thermal treatment (at 180°C–230°C) of pressurized starches (Błaszczak et al. 2008, 2010). A stable radical was detected in dark beers and in sweet wort produced with dark malt. The radical is formed during the roasting of malt (Jehle et al. 2011). When roasting coffee, free radical-mediated reactions could be important processes during both the heating and cooling phases

of a roasting cycle (Goodman et al. 2011). Interactions at the free-radical level were observed between dry ingredients in cauliflower soup powder, prepared by dry mixing of ingredients and rapeseed oil (Raitio et al. 2011). Spices are subjected to irradiation. In irradiated black pepper, cellulose, starch, phenoxyl, and peroxyl radicals were observed (Yamaoki et al. 2011). Therefore, the Maillard reaction and lipid oxidation follow parallel mechanisms. ROS are capable of activating glucose and other α-hydroxy aldehydes (or α-hydroxy ketones), rendering them more reactive and favoring the attack of biological macromolecules; ultimately, ROS are capable of accelerating the Maillard reaction. Equally, the reducing sugars, Amadori products, and other intermediaries of a Maillard reaction can, in the presence of metallic transition ions, lead to autoxidation generating oxygen free radicals (Adams et al. 2011; Traverso et al. 2010).

In comparison with the lipid and protein oxidation, oxidation of saccharides has no significant effect on ROS in mitochondria (Sanz et al. 2006). In the food, formation of saccharide radicals requires drastic conditions (irradiation, high temperature, the presence of strong metal catalysts).

Carbohydrates in foods act as antioxidants. Some of the Maillard reaction products (glycated proteins) and complexes of oxidized lipids with proteins, such as moderate properties, also have such properties (Pokorny et al. 2011). It has been reported that polysaccharides from different resources extracted from plants and seaweed have strong antioxidant properties (free-radical scavenging, transition-metal binding) and can be explored as novel potential antioxidants (Zhang et al. 2011; Waraho et al. 2011). Polysaccharides extracted from marine algae, chitosan, and its derivatives are effective antioxidants (Feng et al. 2008; Redouan et al. 2011). Scavenging ability on hydroxyl radicals was found to be in the order of chitosan > hyaluronan > starch (Yang et al. 2010).

1.9 CONSEQUENCES OF FOOD COMPONENT OXIDATION

While ROS play multiple, both beneficial and deleterious, roles in living organisms, their reactions in food are almost always harmful, leading to loss of sensory quality, nutritional value, and health risks. This also changes the usefulness of raw materials for processing. It is difficult to find a food component that would not be capable of affecting the oxidation process.

In food, $^1O_2$ reacts with vitamins and other compounds, causing a loss of nutritive value. Riboflavin is a photosensitizer but also reacts with $^1O_2$. Milk exposed to sunlight for 30 min may lose up to 30% of its riboflavin; an 80% loss has been reported in milk stored under light. Light of wavelength 450 nm (maximum absorption of riboflavin) is the most destructive to this compound. Ascorbic acid is also reactive with singlet oxygen ($k = 3.1 \times 10^8$ M^{-1} s^{-1}); as a result, vitamin C is also easily destroyed by light. Losses of 80%–100% of ascorbic acid have been reported upon 60 min of exposure of milk to sunlight (Min and Boff 2002). Vitamin D reacts effectively with $^1O_2$ (reaction rate $k = 2.2 \times 10^7$ M^{-1} s^{-1}), which leads to its photodestruction. Lipid-soluble vitamins are particularly susceptible to oxidation resulting from physical factors (temperature, sunlight, UV light, and oxygen or air), chemical factors (radicals, peroxides, metal ions, e.g., Cu^{2+}, Fe^{3+}), and enzymes, mainly

oxidases, for example, LOX, and the process is further accelerated by the presence of oxidized fats. These factors, acting jointly, could cause even greater vitamin losses in foodstuffs during technological processes and, afterward, during storage. Processes in which radicals are generated, such as irradiation and microwave heating, significantly affect vitamin losses, depending on the applied dose and the environmental conditions (Nogala-Kałucka 2011).

Oxidation of pigments (carotenoids, anthocyanins, myoglobin) in foods manifested by discoloration, or bleaching, lowers the attractiveness of the product. This takes place during the storage of raw materials and heat treatment, especially in the presence of oxygen, light, and copper or iron ions. Carotenoids in fruits, vegetables, some invertebrates, and fish (meat and skin), in the presence of PUFAs, undergo oxidation. Despite the nutritional and biological functions of carotenoids, several of the oxidation products of these pigments are deleterious (Benevides et al. 2011). It can also lead to a change in the composition of carotenoids. The dominant carotenoid in trout is astaxanthin with canthaxanthin representing approximately 20% of total carotenoids in freshly cooked trout. Trout cooked after 2 weeks of storage showed an increase in lipid oxidation, and canthaxanthin content increased up to 50% of total carotenoids (Kołakowska and Łomaszewska 2006).

It is generally accepted that, apart from microbial spoilage, lipid oxidation is the primary process by which muscle food spoilage occurs. The oxidative deterioration of lipids leads to the development of rancidity; off-odor, off-flavor compounds; polymerization; and other reactions causing the reduction of shelf life, nutritional quality, and safety. Lipid oxidation leads to loss of essential FAs. However, apart from bulk oils and fat, even in fish products, when rancidity is sensorily detectable, the LC n-3 PUFA losses are relatively small (Kołakowska 2011).

The western diet contains large quantities of oxidized lipids because a high proportion of the diet is consumed in a fried, heated, processed, or stored form. It is important to what extent lipid oxidation products contained in a diet may contribute to the *in vivo* destructive activity of ROS. The gastrointestinal tract is constantly exposed to dietary oxidized food compounds; after digestion, a part of them are absorbed into the lymph or directly into the bloodstream. Hydroperoxides are generally thought to be decomposed in the stomach from where they are not transported any further. On the other hand, the human gastric fluid may be an excellent medium for enhancing the oxidation of lipids and other dietary constituents. It is possible that, at low doses, FA hydroperoxides are converted to the corresponding hydroxy FA in the mucosal membrane before they are transported to the blood. Gastric mucosa, under stress conditions, exhibits intensification of lipid peroxidation (an increase of MDA and 4-HNE) (Kwiecień et al. 2010).

The secondary products of lipid autoxidation contain cytotoxic and genotoxic compounds; after digestion, a part of them is absorbed into the lymph or directly into the bloodstream and may cause an increase in oxidative stress and deleterious changes in lipoprotein and platelet metabolism. The aldehydes occur in free form or conjugates with amino acids being bioaccessible in the gastrointestinal tract and so are able to reach the systemic circulation. Besides, it was evidenced that during digestion of Maillard products, esterification and oxidation reactions take place (Goicoechea 2011). The absorbable aldehydes form adducts with protein from the

diet that are less toxic than free aldehydes. Oxidized cholesterol in the diet was found to be a source of oxidized lipoproteins in the human serum. Some of the dietary advanced lipid oxidation end products, which are absorbed from the gut to the circulatory system, seem to act as injurious chemicals that activate an inflammatory response, which affects not only the circulatory system but also the liver, kidney, lungs, and the gut itself. However, Ottestad et al. (2011) reported that 9 g/day daily intake of highly oxidized cod liver oil (capsules) for 7 weeks does not significantly change the level of circulating oxidation products or affect oxidative stress markers. This can probably be attributed to the specific composition of this oil and the role of LC n-3 PUFAs (Kołakowska et al. 2002).

Protein oxidation's effect on the loss of nutritional value of food by the loss of essential amino acids decreased proteolytic susceptibility and impaired digestibility (Soyer et al. 2010; Estévez 2011). Loss of digestibility was correlated with oxidative parameters of proteins resulting from hydrophobicity change, aggregation, and carbonylation. The analysis of the *in vitro* digestibility of semidry sausages showed no correlation between pepsin activity and protein oxidation; however, a highly significant correlation was observed with trypsin and α-chymotrypsin activity (Sun et al. 2011). The destruction of labile amino acids, such as cysteine and methionine, and their cross-linking of covalent bonds significantly reduces the biological value of protein (Sikorski 2007).

However, the most significant oxidation effect on the loss of nutritional value is a result of the interaction of oxidized components in food (Table 1.1). This applies in particular lipid–protein interactions (Hęś and Korczak 2007; Pokorny et al. 2011; Sikorski 2007). Most covalent bonds formed in the interaction are not hydrolyzed by proteases under the conditions of digestion in human subjects. The 6-amine group of bound lysine is particularly sensitive to interaction with carbonylic oxidation products, such as aldehydes or ketols, and the resulting imine bonds substantially reduce the lysine availability. Other amino acids, such as tyrosine, tryptophan, and methionine, are also partially converted into unavailable products (Pokorny et al. 2011). The interaction preferentially involves most unsaturated FAs. During microwave cooking of fish lipids (oxidized)-albumin, as much as 95% of DHA was covalently bonded. While in systems with fresh and added DHA, respectively, 81% and 75% DHA was bound covalently (Bienkiewicz 2001).

Oxidized lipids also interact with saccharides but generally form weak, reversible complexes. Polyunsaturated FAs, EPA, and DHA, in particular, proved to be most susceptible to binding amylose and amylopectin (Bienkiewicz and Kołakowska 2003, 2004). Up to 90% of the DHA from fish lipids are complexed with amylopectin as a result of homogenization. Compared to fresh fish lipids, those lipids, which were oxidized to a higher extent, were shown to be more amenable to complexing with amylopectin, but they were also more readily released from these complexes (Bienkiewicz and Kołakowska 2003). Thermal treatments, such as heating, microwave cooking, or freezing, exert a significant, differential effect on the fish lipid–starch interaction. This effect depends on the FA profile and degree of oxidation of lipids on the type of starch used.

Saccharide–protein interactions, although of great importance to food quality and shelf life, are not a direct result of oxidation.

TABLE 1.1
Effect of Lipid, Protein, and Carbohydrate Oxidation and the Interaction between Them on the Quality of Food

	Lipid L	Protein P	Interactions (L–P)	Carbohydrate S	Interactions (P–S)
			Undesirable Effects		
Sensory Attributes					
Development of					
Off-odor	LLL	P	(LL–P)	S	(P–S)
Off-flavor	LLL	P	(LL–P)		(P–S)
Discoloration	LLL		(L–P)		(P–SS)
Undesirable texture	L	PPP	(L–PP)		(P–S)
Nutritional Value					
Losses in					
PUFA	L		(L–P)		
Vitamins	LL				
Carotenoids	LL				
Phytosterols	LL				
Other antioxidants	LL	PPP	(L–P)		
Digestibility	L	PPP	(L–PP)		(P–S)
Enzymatic activity		PPP	(L–PP)		(P–S)
Damage of					
Proteins		PPP	(L–PP)		(P–S)
Blocking of essential amino acids		PP	(LL–PP)		(P–S)
Oxidation of amino acids		PPP			
S–S bonding		PPP			
Toxicity					
Generation of					
Radicals	LLL	PP	(L–P)	S	
Peroxides	LLL				
Aldehydes	LLL	PP			
Epoxides	LL				
Oxycholesterols	LL				
Trans FAs	LL				
Maillard-type product			(L–P)	SS	(P–SS)
Technological Suitability					
Decrease in					
Stability of the emulsion	LL	PP	(L–P)		
Protein solubility		PPP	(L–PP)		(P–S)
Protein gel		PPP	(L–PP)		(P–S)

(*continued*)

TABLE 1.1 (Continued)
Effect of Lipid, Protein, and Carbohydrate Oxidation and the Interaction between Them on the Quality of Food

	Lipid L	Protein P	Interactions (L–P)	Carbohydrate S	Interactions (P–S)
			Desirable Effects		
Typical flavors of some vegetables, seafood	LL				
CLA	LL				
Antioxidants			(L–P)		(P–S)
Aroma compounds				SS	(P–S)
Blanching of flour	LL				
Improvement of breadmaking	L	P	(L–P)		(P–S) (L–P–S)
Bactericidal effects of ROS	L	P			

Note: Effects of L, P, S – weak; LL, PP, SS – medium; LLL, PPP, SSS – strong.

Oxidation affects all food components and their interactions. Determination of lipid oxidation and/or protein oxidation products does not sufficiently reflect the status of food oxidation. Similarly, instead of determining the antioxidant activity of individual antioxidants, antioxidant capacity assays are often used, and an overall estimate of the degree of oxidation is provided by the total content (in w.w.) of oxidation products in food (the oxidation index). There is always a very significant negative correlation between the total oxidation index and total antioxidant activity (Kołakowska and Bartosz 2011; Kołakowska 2011). *Antioxidative–oxidative status* (the ratio of total antioxidant activity to total oxidation index) allows us to control development of the oxidation process, taking into account changes in antioxidant activity (synergism, antagonism) during storage and processing of food (Aranowska 2011).

1.10 CONCLUDING REMARKS

Because of the natural presence of ROS in animal and plant raw material and the action of a catalytic factor, despite the presence of antioxidants, spontaneous oxidation reactions likely take place in each food. There are even reports on the harmless benefits of a small degree of food oxidation; however, in general, food oxidative processes are detrimental. The intensity and rate of these reactions are affected by the food composition and the processes to which food is subjected *from the farm to the plate*. Oxidation affects all food components, but their impact on food quality is not uniform. Among the main components, lipid and protein oxidation are the most important destructors of the quality of food. Carbohydrate oxidation does not play a significant role in determining the quality of food under conventional conditions, while the antioxidant properties of saccharides do. Oxidative processes apply

to all food components, and the interaction between them affects the course of oxidation. There has also been a radical transfer between the food ingredients. Therefore, *remember that thou goest in the midst of snares, and that thou walkest upon the battlements of the city* (Sirach 9.13).

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2 Oxidants Occurring in Food Systems

Manuel Pazos and Isabel Medina

CONTENTS

2.1 INTRODUCTION

Important food components such as unsaturated lipids, proteins, and vitamins are very prone to undergoing oxidative deterioration during processing and storage. Although several of these food constituents suffer important autoxidative reactions under certain conditions, in most foods, the rate and extent of oxidation are highly conditioned by the concentration and activity of oxidizing agents, which can be natural components of foods, food additives, and even substances generated during processing or storage. Food oxidation regularly causes a decrease in consumer acceptance, but in some cases, oxidative reactions of specific food components lead to an improvement in the product quality. An example is the enzymatic oxidation of polyunsaturated fatty acids (PUFAs) by lipoxygenases (LOXs) that is responsible for the fresh aromas in fish and vegetables or the oxidation developed during the ripening of several appreciated salted fish products that are related with the characteristic organoleptic properties of these foods (Andersen, Andersen and Baron 2007). On the contrary, the activity of oxidants, such as redox active metals and hemoproteins, on PUFA generates rancid off-flavors and diminishes nutritive value during processing and storage. This oxidative deterioration of PUFA dramatically restricts shelf life and consumer acceptance in a large variety of foodstuffs, such as meat, fish, milk products, and new functional products enriched in PUFA n-3. Therefore, the identification of the

TABLE 2.1
Principal Oxidants Occurring in Foods

Chemical Oxidants	Enzymatic Oxidants
Redox active metal (Fe, Cu)	LOX
Hemoproteins (hemoglobin, myoglobin)	MPO and LPOs
Singlet-oxygen sensitizers (riboflavin, porphyrins, and chlorophylls)	Catalase
Phenolics and reductants	PPOs

Note: LOX, lipooxygenases; MPO, myeloperoxidases; PPOs, polyphenol oxidase.

principal oxidants occurring in foods is essential to understanding the oxidative reactions suffered by food components and their implication on food quality.

Catalysts of oxidation in foods include both enzymatic and nonenzymatic compounds (Table 2.1). Most of these compounds exert vital functions for life in redox-balanced living organisms; however, foodstuffs exhaust their endogenous antioxidant defense systems during processing and storage, and in these conditions of impaired redox balance, the above substances are extremely reactive, promoting oxidation of lipids, proteins, and vitamins. The main aspects of their occurrence—pathways of oxidative action, influence of technological processing and storage on concentration and activity, and redox interactions with other food constituents—will be addressed in the following sections.

2.2 NONENZYMATIC OXIDANTS

2.2.1 Redox-Active Metals

The transition metals iron and copper are endowed with a labile d-electron system able to catalyze oxidative modifications in foods. The most common redox state for iron, the ferric ion, exhibits a reduced solubility at pH values near neutrality. For this reason, iron ions exist in food systems chelated to other compounds to form soluble low-molecular-weight (LMW) complexes. Inorganic phosphates, nucleotides [Adenosine triphosphate (ATP); Adenosine diphosphate (ADP); Adenosine monophosphate (AMP)], peptides (carnosine), amino acids (glycine, histidine), and carboxylic acids (citric acid, oxalic acid, pyruvic acid) are potential iron-chelating agents in foods (St. Angelo 1992). Metal chelation may alter the catalytic activity of oxidation by changing metal accessibility and/or its redox potential (Table 2.1). In general, chelators that coordinate Fe(II) via oxygen ligands promote the oxidation of Fe(II), whereas the oxidation of ligated Fe(II) is retarded by chelators with nitrogen ligands (Welch, Davis, and Aust 2002). The capacity of iron complexed to nucleotides, citrate, or pyrophosphate to stimulate oxidation has been reported (Rush, Maskos, and Koppenol 1990; Rush and Koppenol 1990; Soyer and Hultin 2000; Pazos et al. 2006). Carnosine, an endogenous β-alanyl-histidine dipeptide in meat, has been suggested as an important inhibitor for iron-mediated oxidation via metal chelation, although the antioxidant mechanism of carnosine seems also to be a result of free radical scavenging (Chan et al. 1994; Decker and Faraji 1990).

LMW-iron concentrations vary depending on the food system, processing conditions, and even on the species and type of muscle tissue. Levels of LMW iron are initially low, being only 2.4%–3.9% of total muscle iron in beef, lamb, pork, and chicken (Hazell 1982). The analysis of light and dark muscles from mackerel indicated that about 7%–10% of the iron and 7%–38% of the copper are associated with fractions with molecular weights lower than 10 kDa (Decker and Hultin 1990). In general, the total content of LMW Fe is notably higher in dark muscle than in light muscle. The dark muscle from mackerel has approximately fivefold more LMW Fe than the light muscle (Decker and Hultin 1990). The distribution of redox-active metals in foods is modified during storage and processing as a result of the release from metal-containing proteins. The delivery of iron from the heme pocket of hemoglobin (Hb) and myoglobin (Mb) is a principal source of LMW Fe in processed and/or stored muscle-based foods. There is evidence of the increment in the levels of LMW Fe during the chilled storage of chicken, turkey, and fish muscle (Decker and Hultin 1990; Kanner, Hazan, and Doll 1988). The reduction of the levels of heme iron and the parallel increase in the non-heme iron content are noticeable in the fish muscle from tilapia and sea bass stored under refrigeration (Thiansilakul, Benjakul, and Richards 2010). Previous freeze–thawing processes enhance the accumulation of free iron and copper in refrigerated fish muscle (Decker and Hultin 1990). Cooking and other thermal treatments are able to increase the concentration of non-heme iron in beef, lamb, and chicken (Purchas et al. 2003; Min et al. 2008). Heating decreased heme iron, and the severity of the losses can be controlled by using milder processing conditions that do not weaken the anchorage of the heme group to the protein (Lombardi-Boccia, Martinez-Dominguez, and Aguzzi 2002).

Food fortification to prevent iron-deficiency anemia in at-risk populations can be an additional source of LMW Fe (Theuer 2008). To avoid unacceptable taste, color, and stability derived from the fortification, iron is stabilized by applying principles of colloid chemistry (encapsulation), chelation, antioxidant compounds, and electrochemical chemistry (redox modulation) (Mehansho 2006).

The progress of lipid oxidation may also change the proportion of LMW Fe. The interaction of Hb with lipid hydroperoxides, primary lipid oxidation products, or trans-2-pentenal, an aldehyde product of the decomposition of lipid hydroperoxides, triggers the liberation of hemin (an oxidized form of the heme group) from the hemoprotein (Maestre, Pazos, and Medina 2009). The capacity of lipid hydroperoxides and trans-2-pentenal to promote the loss of the heme group from Hb may be ascribed to their ability to accelerate the oxidation of hemoproteins to met- forms (Maestre, Pazos, and Medina 2009). Methemoglobin (MetHb) is endowed with a higher propensity to release the heme group than the reduced oxyHb species (Hargrove, Wilkinson, and Olson 1996; Maestre, Pazos, and Medina 2009). The electrophilic character of unsaturated aldehydes favors the establishment of covalent bonds with the amine groups of amino acids through a nucleophile/electrophile mechanism. The analysis of Mb adducts with 4-hydroxy-2-nonenal (HNE), an unsaturated aldehyde generated by lipid peroxidation, indicates that HNE establishes covalent bonds with the proximal (HIS 93) and distal (HIS 64) histidine associated with the heme group (Alderton et al. 2003). This hemoglobin–aldehyde interaction destabilizes the linkage heme–protein favoring the loss of hemin. The free heme group out of the

protein globin core is notably made unstable by reaction with free radicals, resulting in the oxidative cleavage of the porphyrin ring and the liberation of ferric ion (Pazos, Andersen, and Skibsted 2008). Figure 2.1 illustrates the fast degradation of free hemin in the presence a cumene hydroperoxide, a source of free radicals by interaction by hemin.

In addition to the concentration, the redox state of metals is crucial for the oxidative stability of foods. The most common redox state for iron, the oxidized ferric [Fe(III)] ion, is significantly less prooxidant than the reduced ferrous [Fe(II)] state. However, several enzymatic and nonenzymatic food components or additives may convert the ferric iron to the more oxidizing ferrous form: enzymatic iron-reductase systems, ascorbic acid, glutathione, and phenolic compounds (Pierre and Fontecave 1999; Soyer and Hultin 2000; Petrat et al. 2003; Gülçin 2006). The reduction of the ferric to the ferrous state is the driving force for the catalysis of oxidation by iron, being thermodynamically possible for reductants whose one-electron reduction potential is lower than that of ferric iron. The redox potential of the Fe(III)/Fe(II) transition depends strongly on the chelating agent and pH. Iron coverts a wide physiological range of redox potentials, from approximately −0.5 to 0.6 V, depending on the ligand complexed to the metal (Table 2.2). Moreover, the reducing potential of the Fe(III)/Fe(II) pair is extremely pH-dependent. Acidic pH values favor the reduction of ferric because the transition to ferrous occurs at the standard redox potential of +0.77 V, whereas at pH 7.0, the transition redox potential is between +0.1 and +0.2 V. Accordingly, the reduction rate of ferric to ferrous iron by reducing agents increases noticeably as the pH decreases, and the prooxidative effect of phenolic

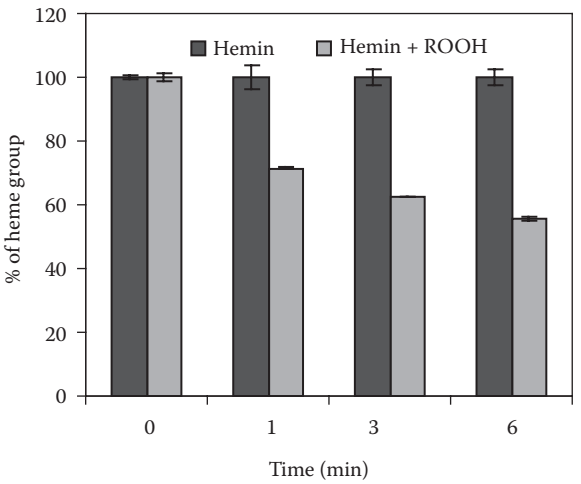

FIGURE 2.1 Effect of hydroperoxides, primary lipid oxidation products, on stability of free hemin: Hemin (40 μM) was incubated in presence or absence of 2000 μM cumene hydroperoxide (CumOOH), which was used as a model for lipid hydroperoxide. Hemin stability was monitored as absorbance at 390 nm, wavelength at which hemin is endowed of maximum absorption.

TABLE 2.2
Standard One-Electron Reduction Potential of Interest to Predict the Course of the Fe(III)/Fe(II) Transition by Interacting with Common Reducing Food Components

	Reduction Potential ($E^{\circ\prime}$; V)[a]	Reference
Fe(III)-citrate/Fe(II)-citrate	0.6	Pierre, Fontecave, and Crichton 2002
$Fe(III)_{(aq)}/Fe(II)_{(aq)}$	0.1–0.2	Buettner 1993
Fe(III)-EDTA/Fe(II)-EDTA	0.12	Buettner 1993
Fe(III)-ADP/ Fe(II)-ADP	0.1	Buettner 1993
Fe(III)-transferrine/Fe(II)-transferrine	–0.4	Pierre, Fontecave, and Crichton 2002
Semiubiquinone$^{\cdot}$/ubiquinol	0.2	Buettner 1993
Ascorbate$^{\cdot}$/Ascorbate	0.28	Buettner 1993
EGCG$^{\cdot}$/EGCG	0.43	Jovanovic et al. 1995
α-Tocopheryl$^{\cdot}$/α-tocopherol	0.48	Buettner 1993
Caffeyl$^{\cdot}$/caffeic acid	0.54	Laranjinha et al. 1995
Catechin$^{\cdot}$/Catechin	0.57	Jovanovic et al. 1995

[a] Reduction potentials at pH 7.0 (vs. NHE).

antioxidants in the presence of iron is especially relevant in mayonnaises and other food systems marked by acid pH (pH < 5) (Hsieh and Hsieh 1997). Caffeic acid has demonstrated prooxidant activity in oil-in-water emulsion at pH 3.0 and pH 6.0, whereas the phenolics with higher reduction potential than caffeic acid and, therefore, with lower reducing power, as naringenin, rutin, and coumaric acid, exhibit a prooxidative capacity only at pH 3.0 (Sorensen et al. 2008). Ascorbic acid is also known for its high efficiency for Fe(III) reduction at both neutral and acidic pH values. At acid pH (pH 2.6), 2 mol of Fe(III) are reduced by 1 mol of ascorbic acid (Hsieh and Hsieh 1997). The reduction rate of Fe(III) by ascorbic acid decreases as the pH value increases (Hsieh and Hsieh 1997), but still at pH near neutrality (pH 7.4), ascorbic acid has a strong capacity to reduce Fe(III)–ATP complexes to the Fe(II) state (Petrat et al. 2003). The supplementation of ethylenediaminetetraacetic acid (EDTA), a chelating agent generally used as a food additive, prevents Fe(III) from being reduced by ascorbic acid at pH 2.6–6.0 (Hsieh and Hsieh 1997). In turkey muscle, ascorbic acid is recognized to be the main reductant that affects the iron redox cycle because the destruction of ascorbic acid totally inhibits lipid oxidation (Kanner 1994). Table 2.3 collects some of the food systems or food model systems in which the ascorbic acid combined with traces of iron causes oxidation of important food components, for example, lipids, proteins, α-tocopherol, or ubiquinol.

Iron-reducing enzymatic systems are also a relevant font of the reactive ferrous ions in meat-based foods not subjected to thermal treatments. Cellular membranes of

TABLE 2.3
Oxidative Targets of LMW Fe and LMW Cu in Food Systems

Food System or Food Model System	Oxidizing System	Oxidative Target	Reference
Pork mitochondrial system	Fe(III)/ascorbic	Lipid, α-tocopherol, and ubiquinol	Tang et al. 2005
Pork myofibrillar proteins	Fe(III)-ascorbate	Protein	Xiong et al. 2010
Pork myofibrillar proteins	Fe(III) and Cu(II) combined with H_2O_2	Protein	Estévez and Heinonen 2010
Fish muscle membranes	Fe(III)/ADP/ascorbate Fe(III)-ADP/NADH	Lipid and protein	Soyer and Hultin 2000
Fish muscle membranes	Fe(III)-ADP/ascorbate Fe(III)-ADP/NADH	Lipid	Pazos et al. 2006
Fish myofibrillar and sarcoplasmic proteins	Fe(II)/ascorbate	Protein	Pazos et al. 2011
Whey-based and casein-based infant formulas	Fe(II)	Lipid	Satue-Gracia et al. 2000
Emulsion containing linoleic acid	Fe(II)	Lipid	Sugiarto et al. 2010
Water–oil mixtures	Cu(II)	Lipid	Alexa et al. 2011
Oil-in-water emulsions	Fe(II), Fe(II)-EDTA, Fe-lactoglobulin (beta), Fe(II)-caseinate	Lipid	Guzun-Cojocaru et al. 2011

muscle foods contain NAD(P)H-dependent enzymatic systems with the capacity to transfer one electron to convert ferric to ferrous iron, such as ferredoxin, thioredoxin, and cytochrome P450 (Petrat et al. 2003). It is also relevant that these enzymatic reducing systems of membranes generate the oxidizing ferrous iron in an environment fundamentally consisting of highly unsaturated lipids, an essential oxidative substrate in foods. In the presence of iron, the iron-reducing systems of fish sarcoplasmic reticulum and microsomes, small particles consisting of fragments of ribosomes and endoplasmic reticulum obtained by sedimentation at ultracentrifugation, exert an important activity promoting oxidation of both lipids and proteins (Table 2.3). Comparing the oxidative efficiency with nonenzymatic iron-reducing systems, the iron-reducing system from fish sarcoplasmic reticulum has shown a lower ability than the ascorbate–iron system to promote the oxidation of lipids and proteins (Soyer and Hultin 2000). A similar tendency has been observed by studying the iron-reducing enzymes contained in fish microsomes, causing the ascorbate–iron system a faster lipid oxidation system compared to those enzymatic complexes (Figure 2.2).

The oxidative behavior of ferrous ions is in part attributable to their peroxidase activity to decompose hydroperoxides generating reactive peroxyl ($ROO^{\bullet}$), alkoxyl ($RO^{\bullet}$), and alkyl ($R^{\bullet}$) free radicals. Ferrous iron is 14 times more active than ferric iron in producing free radicals by decomposition of lipid hydroperoxides, but free iron ions are significantly less effective than heme proteins (O'Brien 1969).

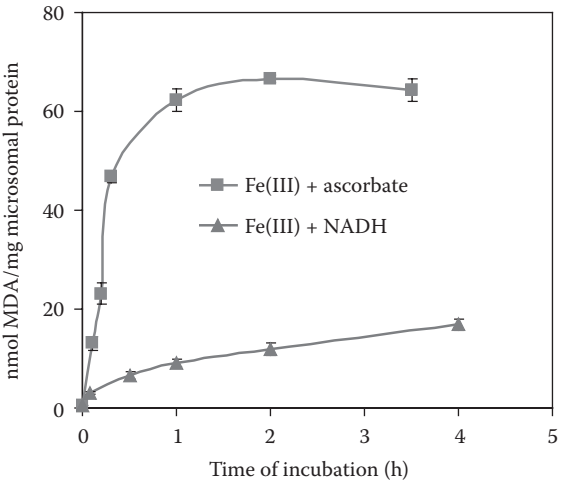

FIGURE 2.2 Activity of enzymatic iron [NADH-Fe(III)] and nonenzymatic iron [ascorbate-Fe(III)] in promoting lipid oxidation in fish microsomes. Fish microsomes were suspended in 0.12 M potassium chloride and 5 mM histidine (pH 6.8) to final concentration of 0.7 mg/mL. The membranes oxidized by enzymatic iron contained 20 μM Fe(III), 100 μM ADP, and 100 μM NADH, and NADH was substituted by 100 μM ascorbate in membranes oxidized by nonenzymatic iron. Lipid oxidation was monitored by the formation of aldehydes, secondary lipid oxidation products, by means of the thiobarbituric acid (TBA) reaction with aldehydes, using malondialdehyde (MDA) as standard.

Ferrous ions also have the capacity to generate the extremely powerful oxidizing hydroxyl radical ($HO^{\bullet}$) through the reaction with hydrogen peroxide via a Fenton-type mechanism:

$$Fe(II) + H_2O_2 \rightarrow Fe(III) + HO^{\bullet} + {}^{-}OH$$

The elevated oxidizing power of the hydroxyl radical is a consequence of its extremely high standard reduction potential ($E^{\circ\prime}HO^{\bullet}/H_2O = 2.31$ V) that makes it competent to steal one electron (or hydrogen atom) from compounds whose reduction potentials are lower, which are mostly lipids, proteins, and vitamins (Buettner 1993). The lower reduction potentials of allylic ($E^{\circ\prime}$ allylic$^{\bullet}$/allylic – H = 0.96 V) and *bis*-allylic ($E^{\circ\prime}$*bis*-allylic$^{\bullet}$/*bis*-allylic – H = 0.6 V) imply the capacity of a hydroxyl radical to initiate the oxidation of monounsaturated fatty acids (MUFAs) and PUFAs. Even though a hydroxyl radical is a very powerful oxidizing species with the ability to oxidize whichever of the 20 essential amino acids, it shows some selectivity in its reaction. The highest reaction rate of a hydroxyl radical is achieved by cysteine (3.4×10^{10} dm^3 mol^{-1} s^{-1}) and the aromatic amino acids tryptophan, histidine, and tyrosine (1.3×10^{10} dm^3 mol^{-1} s^{-1}), but it also provokes the fast oxidation of arginine, methionine, and phenylalanine [$(3.5–8.3) \times 10^9$ dm^3 mol^{-1} s^{-1}] (Davies and Dean 1997).

2.2.2 Hemoproteins

The hemoproteins, hemoglobin (Hb) and myoglobin (Mb), have a decisive contribution to the oxidative degradation of muscle foods as a result of their elevated content in muscle tissues and their behavior as oxidizing agents. Table 2.4 reports some of the food systems or food model systems in which hemoproteins promote oxidation of important food components. Hb is a tetramer composed of four globin chains containing one heme group inside each polypeptide chain. Mb consists of a globin chain along with a porphyrin heme group (Shikama 1998). *In vivo* and during the early postmortem stages, hemoproteins are typically present in the ferrous state either with molecular bound oxygen (oxygenated state) or without bound oxygen (deoxygenated state). Hemoproteins undergo oxidation to a ferric state, also called the metform, in muscle subjected to processing and long-term storage. The oxidized forms methemoglobin (metHb) and metmyoglobin (metMb) are unable to bind molecular oxygen, which gives a less compact structure of these proteins compared to that of their reduced ferrous forms.

The relative level of hemoproteins depends on the type of muscle and species. Pork and beef generally contain more Mb than Hb (Trout and Gutzke 1996), and Hb is the hemoprotein more abundant in chicken and some fish species (Kranen et al. 1999; Richards and Hultin 2002). Hb is the principal hemoprotein in mackerel light muscle (6 μmol Hb/kg muscle) and whole trout muscle (11 μmol Hb/kg). However, Mb is predominant in mackerel dark muscle, representing 65% of hemoproteins on a molar basis, although the tetrameric Hb supplies more heme units than monomeric Mb (*10*). Tuna also contains a higher proportion of Mb compared to Hb (Brown 1962; Sannier et al. 1996). Reddish-flesh fish as pelagic species have

TABLE 2.4
Oxidative Targets of Hb/Mb in Food Systems

Food System or Food Model System	Oxidizing System	Oxidative Target	Reference
Pork myofibrillar proteins	MetMb/H_2O_2	Protein	Xiong et al. 2010
Pork muscle membranes	Bovine oxyMb and metMb	Lipid	Bou et al. 2008; Bou et al. 2010
Pork myofibrillar proteins	Equine Mb/H_2O_2	Protein	Estévez and Heinonen 2010
Pork mitochondrial system	Equine oxyMb	Lipid, α-tocopherol, and ubiquinol	Tang et al. 2005
Isolated porcine myosin	Horse heart metMb/H_2O_2	Myosin	Frederiksen et al. 2008
Washed fish muscle	Fish Hb	Lipid	Richards and Hultin 2002
Washed fish muscle	Fish Hb	Lipid, protein and α-tocopherol	Larsson and Undeland 2010

more elevated Hb concentrations (3–12 μmol Hb/kg) than lean fish species, such as Atlantic cod (0.03–0.23 μmol Hb/kg) (Richards and Hultin 2002; Larsson, Almgren, and Undeland 2007; Maestre, Pazos, and Medina 2011). There is a direct dependence between Hb concentration and the ability to promote oxidation within the typical Hb concentrations found in muscle foods (Richards and Hultin 2002; Pazos, Medina, and Hultin 2005). Therefore, higher amounts of Hb cause a more rapid and extensive propagation of lipid oxidation. This observation can partially explain the poor oxidative stability of pelagic fish muscle during processing and storage and its underutilization to human food purposes compared to lean fish species. Experiments performed in a model system composed of washed muscle free of hemoproteins reveal the strong aptitude of the concentrations typically found in pelagic fish (3–12 μmol Hb/kg muscle) to activate lipid oxidation (Richards and Hultin 2002). The results also draw attention to the oxidizing activity of Hb concentrations as low as 0.06 μmol/kg of muscle.

On the other hand, the concentration is not the unique parameter relevant for understanding the oxidative action of hemoproteins. Hemoproteins have differential oxidizing power depending on the species considered but also on extrinsic factors, such as pH and thermal processing. The capacity of Hb from terrestrial animals, such as beef and chicken, in activating lipid oxidation is lower compared to that of fish species (Richards, Modra, and Li 2002; Richards and Dettman 2003; Aranda et al. 2009). Important differences are even found in the oxidizing ability of Hbs from different fish species. Pollock Hb is more effective than horse mackerel Hb in activating lipid oxidation, and both are less active than sea bass Hb (Maestre, Pazos, and Medina 2009). Other studies show the higher catalytic activity promoting lipid oxidation for pollock Hb followed, in decreasing order, by mackerel > menhaden > flounder (Undeland, Kristinsson, and Hultin 2004). Hb from mackerel and herring oxidizes fish muscle more rapidly as compared to trout Hb, whereas the latter is more oxidant than tilapia Hb, a warm-water fish species (Richards and Hultin 2003; Richards et al. 2007). The diversity of the prooxidative capacity of hemoproteins from different species has been related to two intrinsic factors of hemoproteins: (1) the redox instability to be converted to the ferric met- form and (2) the susceptibility to produce hemin. Hemin is the term used to describe the porphyrin ring coordinated to one ferric atom, and heme indicates the porphyrin ring with iron in the ferrous state. The ferric methemoglobin (metHb) and metmyoglobin (metMb) are formed spontaneously through the autoxidation of the corresponding reduced species, but their generation is accelerated in the presence of lipid oxidation products, such as hydroperoxides and aldehydes (Maestre, Pazos, and Medina 2009). Several lines of evidence indicate a straight correlation between the oxidizing activity of Hb and its vulnerability to be converted to metHb either spontaneously or when forced by the interaction with lipid oxidation products. Hbs with the most pronounced oxidizing activities are those that rapidly form metHb (Undeland, Kristinsson, and Hultin 2004; Pazos, Andersen, and Skibsted 2009). This observation is consistent with the stronger facility of metHb to promote oxidation in comparison to the reduced Hb form (Grunwald and Richards 2006). An extensive liberation of hemin has also been detected for those Hbs possessing greater prooxidative activity, in agreement with

the poor hemin affinity observed for metHb species (Maestre, Pazos, and Medina 2009).

The two inherent properties of hemoproteins related to their oxidizing power, metMb generation and hemin liberation, are involved in the efficiency of the two principal prooxidative pathways ascribed to hemoproteins: (1) the formation of oxidizing ferrylHb radicals and (2) the generation of free radicals via scission of lipid hydroperoxides, including both heterolytic and hemolytic cleavage of the peroxide bond and a peroxidase-type mechanism. The interaction of metHb with hydrogen peroxide or lipid hydroperoxide results principally in the formation of the hypervalent ferrylHb [HbFe(IV) = O] and perferrylHb [$^{\bullet}$HbFe(IV) = O], which can initiate lipid peroxidation via abstraction of a hydrogen atom from the *bis*-allylic position of PUFA (Reeder and Wilson 1998; Kanner 1994). Hypervalent ferryl species are also active in transferring oxidative damage to the relevant muscle protein myosin by inducing thiyl, tyrosyl, and other unidentified protein radical species (Lund et al. 2008). In regard to the peroxidase mechanism, there is no difference in the free radical-generating activity of metHb and reduced Hb species (Pazos, Andersen, and Skibsted 2008). Therefore, the propensity of hemoproteins to be converted to metHb enhances the oxidizing behavior of hemoproteins through the formation of the highly oxidizing ferrylHb. Free hemin displays a more rapid and extensive generation of free radicals than Hb under low hydroperoxide concentrations (hydroperoxide/heme molar ratios ≤ 1:4), conditions found at the initial stages of lipid oxidation. Figure 2.3 represents the rapid kinetics of free radicals caused by free hemin versus Hb in conditions of low hydroperoxide concentrations (hydroperoxide/heme molar ratios = 1:8).

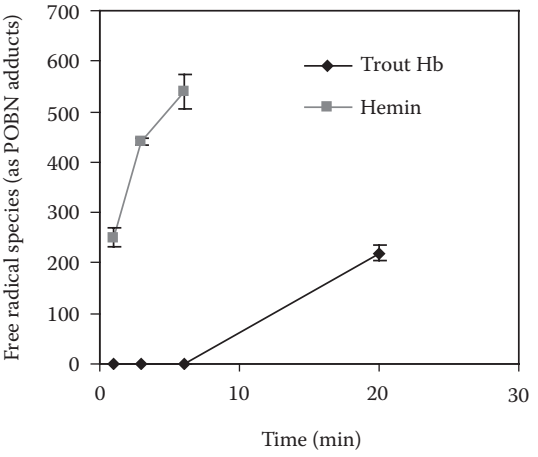

FIGURE 2.3 Capacity of free hemin (oxidized form of heme group) and Hb from rainbow trout (*Oncorhynchus mykiss*) to promote the formation of free radicals by interaction with low hydroperoxide levels (hydroperoxide/hemin molar ratio = 1:8). Free radicals were monitored as POBN-radical adducts, and cumene hydroperoxide (CumOOH) was used as a lipid hydroperoxide model. Heme-containing systems, CumOOH, and POBN were incubated at concentrations of 92 μM (heme basis), 11 μM, and 60 mM, respectively, in 50 mM phosphate buffer, pH 6.8.

By increasing the concentration of hydroperoxides or after successive exposures to hydroperoxides, hemin dramatically loses the efficiency to generate free radicals, and the radical generating activity of Hb is extraordinarily activated. The low free radical-generating efficiency of hemin in elevated free radical/hydroperoxide environments is ascribable to the hemin degradation and the subsequent liberation of the free ferric iron (Pazos, Andersen, and Skibsted 2008). Heme compounds are found to cleave hydroperoxides more rapidly than ferric or ferrous ions (O'Brien 1969). These data suggest a central role of free hemin during the initial stages of oxidation.

The oxidizing action of hemoproteins is often influenced by pH and thermal treatments. Several attempts have been made to identify the effect of heating on the oxidizing ability of hemoproteins. The capacity of several fish Hbs to generate hydroperoxide-derived free radicals has not been significantly affected by heating at 70ºC for 10 and 45 min (Pazos, Andersen, and Skibsted 2009). Studies performed with bovine oxyHb and metHb show similar oxidizing activity for native Hb and that heated to 45ºC, but the increment of heating temperature to 68ºC and 90ºC reduced the activity of Hb (Bou et al. 2010). The prooxidant activities of metHb heated at 68ºC and 90ºC were analogous, whereas the prooxidant activity of oxyHb heated at 68ºC was higher than that heated at 90ºC. The attenuation of the prooxidant activity of heat-denatured Hb is associated with a decrease in the solubility of heme iron and a reduced impact of free iron on the lipid oxidation. In the case of oxyMb, the native species is more efficient as a promoter of lipid oxidation than oxyMb heated at 45ºC–95ºC, being the heating temperatures of 90ºC–95ºC, which provoke lower oxidation (Bou et al. 2008). In contrast, bovine metMb incites a faster oxidation of lipids for Hb heated at 45ºC–60ºC than for the native form. Heating at temperatures immediately below the thermal denaturation of metMb induces structural changes in the heme protein, which increases its prooxidative activity (Kristensen and Andersen 1997). On the contrary, temperatures above the denaturation temperature of metMb decrease the prooxidant activity of the resulting species compared to native metMb.

It is important to bear in mind that Hb's oxygen binding affinity can decrease as conditions become acidic, a phenomenon known as the Bohr effect (Richards and Hultin 2000). Hbs from beef and chicken are fully oxygenated at pH 6.7 because of the low Bohr effect, and conversely, trout Hb is largely deoxygenated at the same pH value (Richards, Modra, and Li 2002). Hbs from fish species, such as rainbow trout and Atlantic cod, require pH values as high as 7.2–7.5 to be fully oxygenated (Richards, Modra, and Li 2002; Pazos, Medina, and Hultin 2005). In general, mammalian Hbs are endowed with a lower Bohr effect than those from fish species (Berenbrink 2006). The prevalence of the Bohr effect has been related to the proportion of anodic isoforms of Hb, whose oxygen affinity drops at acidic pH values, whereas cathodic Hb isoforms bind oxygen strongly independently of pH (Richards, Ostdal, and Andersen 2002). The Bohr effect enhances the oxidizing activity of Hb at the usual conditions of muscle-based food (pH value: 5–7) because the presence of deoxyHb accelerates metHb formation (Shikama 1998), and as indicated above, the ferric metHb is more oxidant than ferrous Hb species. Accordingly, the oxidizing activity of fish Hbs is pH-dependent, being more oxidant at acidic pH values (Pazos, Medina, and Hultin 2005; Richards and Hultin 2000). This pH dependence implies that the pH drop that naturally occurs during the rigor mortis of meat and fish may

activate the oxidizing action of the Hbs with a strong Bohr effect. Therefore, the slaughtering methods that include a previous stunning procedure, which diminish the rate and extent of the postmortem pH reduction, should contribute to improving the oxidative stability of meat-based food by lessening the oxidative activity of Hb.

2.2.3 Photosensitizers

Riboflavin, porphyrins, and chlorophyll derivatives are photosensitizers naturally occurring in foods. Meat, fruit juice, and vegetable oils, together with dairy products, such as cheese, butter, and milk, are susceptible to photooxidation resulting from their natural content of photosensitizers.

Photosensitizers absorb light, becoming excited to one or more energy-rich state(s) (Figure 2.4). The excited sensitizer (Sen*) undergoes internal reactions that ultimately result in the oxidative alteration of a second molecule. The direct oxidative action of light, or photolytic autoxidation, is principally restricted to the production of free radicals primarily from the exposure of the primary substrate to UV radiation (Airado-Rodriguez et al. 2011). However, photosensitizers promote the photooxidation of diverse substrates when foods are exposed to visible light. Photooxidation by a photosensitizer can proceed through two major pathways, type I or type II reactions (Figure 2.4). Type I photosensitization proceeds through a free-radical mechanism in which the excited photosensitizer (Sen*) subtracts an electron or hydrogen atom from another component of the system. This reaction generates two radicals, a photosensitizer radical ($Sen^{\bullet -}$) and a substrate radical. If oxygen is present, both of these radicals can further react to produce oxygenated products. This type of reaction can lead to a loss of the sensitizer. An additional reaction is the direct transfer of the extra electron of the sensitizer radical ($Sen^{\bullet -}$) to molecular oxygen to produce an oxidant superoxide radical $\left(O_2^{\bullet -}\right)$, which can be converted via a dismutation process into another reactive oxygen species, hydrogen peroxide (H_2O_2). This reaction regenerates the sensitizer to the original ground state (Sen). In a type II reaction, the energy from the excited sensitizer is transferred to triplet oxygen, and

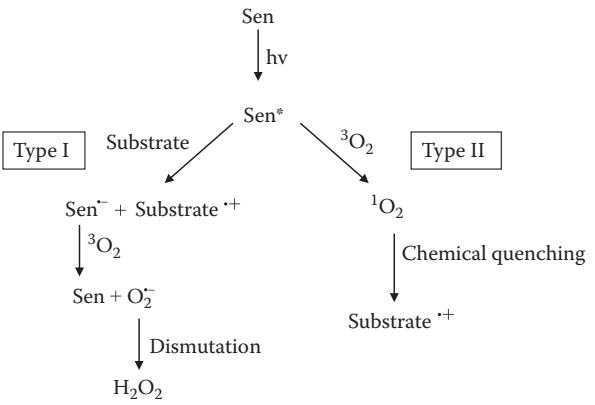

FIGURE 2.4 Type I and type II photosensitization process.

the very reactive singlet oxygen ($^1O_2$) is formed. Singlet oxygen reacts with electron-rich sites, such as unsaturated fatty acids, resulting in hydroperoxides and in the net reduction of oxygen (chemical quenching) (Huvaere et al. 2010). Singlet oxygen can also release its excess of energy (physical quenching). Compounds such as β-carotene and lycopene are able to remove the excitation energy of singlet oxygen without being damaged themselves. These reactions can occur at the same time in a competitive manner, the type II (singlet oxygen-mediated) mechanism being favored over the type I (radical) mechanism by lower substrate concentration and higher oxygen concentration (He, An, and Jiang 1998).

Lipids, proteins, DNA, or vitamins are chemical quenchers of singlet oxygen (Table 2.5). Ascorbate, α-tocopherol, and glutathione are among the vitamins or reductants oxidized by singlet oxygen. Guanosine is the DNA base most reactive with singlet oxygen. Unsaturated fatty acids and cholesterol are also targets of $^1O_2$-mediated oxidation because of the presence of electron-rich sites in their structure and the electrophilic nature of singlet oxygen. The triplet-excited riboflavin yields, directly or indirectly, radical species with the capacity to initiate oxidation of unsaturated fatty acid methyl esters, such as oleate, linoleate, and linolenate, but still the deactivation of the excited flavin by lipid derivatives is significantly slower than that observed by proteins (Huvaere et al. 2010; Huvaere and Skibsted 2009). The most susceptible places for proteins to quench singlet oxygen are cysteine, methionine, tryptophan, tyrosine, or histidine residues, which are holders of areas with high electron density, such as double bonds or sulfur moieties. Free amino acids have been reported to be superior to peptides in quenching singlet oxygen, and unfolded proteins have a higher quenching ability toward singlet oxygen than globular proteins (Dalsgaard et al. 2011).

Riboflavin has been traditionally considered as the active photosensitizer in dairy products. Riboflavin can initiate photooxidation of types I and II by exposure to UV radiation or visible light up to approximately 500 nm. Recent studies have shown the presence of five other photosensitizers in butter: protoporphyrin, hematoporphyrin, a chlorophyll a-like molecule, and two unidentified tetrapyrrols (Wold et al. 2006). Chlorophyll and porphyrin molecules absorb light in the UV and violet region with absorption peaks of approximately 410 nm (the Soret band). In addition, they absorb light pronouncedly in the red above 600 nm, and therefore, they may be responsible for the formation of off-flavors in dairy products exposed to light of wavelengths longer than 500 nm (Wold et al. 2006). Chlorophyllic compounds have also been recently suggested to contribute prominently to the major part of photooxidation in

TABLE 2.5
Principal Chemical Quenchers of Singlet Oxygen in Foods

Lipids	Proteins	Reductants	DNA
PUFA, cholesterol	Cysteine, histidine, methionine, tryptophan, tyrosine	ascorbate, glutathione, α-tocopherol	2′-deoxyguanosine

cow's milk (Airado-Rodriguez et al. 2011). In meats, heme porphyrins and riboflavin are the more relevant photosensitizers. Chlorophylls have importance in the photooxidation developed by vegetable oils.

2.2.4 Polyphenols and Other Reductants

As indicated above, the reducing ability of ascorbic acid to convert the less active ferric to the more oxidizing ferrous iron explains in part the oxidative effects observed by supplementing iron-rich foods with ascorbate. The dual antioxidant/prooxidant role of ascorbic acid is concentration-dependent. Ascorbic acid at higher doses acts as an antioxidant, but at lower concentrations, it enhances the catalytic effect of iron and copper (acting as a prooxidant) (Childs et al. 2001). Ascorbic acid can also induce oxidation because of its ability to induce lipid hydroperoxide decomposition to free radicals and aldehydes responsible for oxidative degradation of lipids, proteins, and vitamins (Lee, Oe, and Blair 2001).

Different phenolics are also able to generate oxidizing ferrous and cuprous ions, metal ions that yield the highly oxidizing hydroxyl radicals via a Fenton-type mechanism. Catechin, which is expected to have redox properties similar to epicatechin, gallic acid, and caffeic acid, has displayed prooxidant activity because of its ability to reduce ferric iron (Rodtjer, Skibsted, and Andersen 2006; Sorensen et al. 2008). Moreover, strongly reducing phenolic compounds, such as pyrogallol (three adjacent phenol groups) containing (–)-epigallocatechin gallate (EGCG) and (–)-epigallocatechin (EGC), are able to form the superoxide radical from molecular oxygen (Touriño et al. 2008). Recent studies have reported that EGCG and EGC cause protein carbonyl formation in proteins, and such prooxidant action has been related to their ability to be faster autoxidized to the quinone form (Ishii et al. 2010). The quinonic structure is reactive with the primary amine groups of proteins to render a protein–iminoquinone adduct, which undergoes hydrolysis to finally produce a protein carbonyl.

Therefore, the phenolic compounds that are commonly employed in antioxidant strategies to avoid deteriorative oxidations may also reduce the oxidative stability of foods. These antioxidant phenolic additives are endowed with one or more properties that result decisively to protect foods from oxidation, that is, radical-scavenging activity, metal-chelating action, regenerative ability on the endogenous antioxidant components, or deactivation of ferrylHb species (Iglesias et al. 2009). The promotion of oxidation by phenolics depends largely on the food system. Factors such as the type and concentration of prooxidants, antioxidants, oxidative substrates, pH, and T^a can modify the antioxidant effect of phenols and even render them prooxidants by favoring the pathways by which phenolics can activate oxidation: metal-reducing activity to ferrous or cuprous, hydroperoxide-derived free radicals through peroxidase activity, and generation of superoxide radicals via autoxidation.

2.3 ENZYMATIC OXIDANTS

Oxidative enzymes in food are of particular interest because they affect the flavor, color, and nutrient content of food. During food processing, their activity can be decreased but also enhanced, provoking off-flavor and off-odor in food because of

its reaction with unsaturated fatty acids. They are widely described in plants, fungi, invertebrates, and mammals. These enzymes catalyze oxidation by a range of different reactions. As a general distribution, the two most common types of oxidative enzymes are catalases and peroxidases, which use hydrogen peroxide as the oxidizing substrate, and peroxidases, which use molecular oxygen, such as LOXs or cyclooxygenases (Table 2.1).

2.3.1 Lipoxygenases

LOXs consist of a structurally related family of non-heme iron-containing dioxygenases. Depending on its origin, LOX catalyzes the insertion of oxygen into PUFAs with a (Z,Z)-1,4-pentadiene structural unit to generate position-specific hydroperoxides. The reaction is stereospecific and regiospecific. The first LOXs were found in soybean seeds in the early 1970s. Nowadays, it is known that they are widely distributed in plants, fungi, invertebrates, and mammals (Brash 1999; Shibata and Axelrod 1995). Recently, LOXs have also been described in bacteria (Porta and Rocha-Sosa 2001). Although there are several forms of LOXs in plant and animal tissues, all of them are composed of a single polypeptide chain with a molecular mass of 75–81 kDa (≈662–711 amino acids) in mammals and 94–103 kDa (≈838–923 amino acids) in plants (Prigge et al. 1996). LOXs are characterized by preserved domains and sequence patterns, both aspects that determine different structures and the binding of the catalytic iron. The tridimensional configuration of the structural domain that regulates its catalytic action is formed by an α-helix with a single atom of non-heme iron near its center. The non-heme iron atom is coordinated with five amino acids, three histidines, one asparagine, and the carboxyl group of the carboxy-terminal isoleucine. The carboxy-terminal domain harbors the catalytic site of the enzyme (Minor et al. 1996).

The LOX enzymes are usually in the inactive ferrous (Fe^{2+}) form when isolated and must be oxidized to Fe(III) by the reaction product, fatty acid hydroperoxides or hydrogen peroxide, before activating as an oxidation catalyst (Andreou and Feussner 2009), and this form drives the reaction. LOX catalyzes the stereospecific hydrogen subtraction from a doubly allylic methylene group followed by a radical rearrangement and accompanied by a Z,E diene conjugation, depending on LOX specificity. The last step is dependent on the stereospecificity and implies the introduction of molecular oxygen and the reduction of the hydroperoxyl radical intermediate to the corresponding anion. The non-heme iron in the catalytic center of the LOX catalyzes the one-electron transitions.

The enzyme occurs in a variety of isoforms, which often vary in optimum pH as well as product and substrate specificity. Animal LOXs are classified according to their specificity of arachidonic acid (C-20) oxygenation, and plant LOXs are classified according to their positional specificity of linoleic acid (C-18). 9- and 13-LOXs (in plants) and 5-, 8-, 12-, and 15-LOXs (in mammals) have been widely described (Andreou and Feussner 2009). The general nomenclature is aimed at identifying the position of the dioxygenation, so the 12-LOX is that which oxidizes arachidonic acid at the C12 position, and 13-LOX is that which oxidizes linoleic acid at the C-13 position. The generated hydroperoxides may be subsequently cleaved to shorter

chain length oxygenated products by LOXs themselves or by hydroperoxide lyases (HPLs). The cleavage products include volatile unsaturated aldehydes and alcohols and the corresponding unsaturated oxo fatty acids (Figure 2.5). Metabolites originating from these pathways are collectively named oxylipins. While higher plants use exclusively polyunsaturated C18 fatty acids for the production of oxylipins, animals and algae rely predominantly on the transformation of polyunsaturated C20 fatty acids, which are not ubiquitously found in the plant kingdom (Noordermeer, Veldink, and Vliegenthart 2001). Moreover, the formation of volatile short-chain aldehydes relies on the combined action of LOX and HPL species in higher plants, whereas animals and algae seem to be more flexible because they may use either the LOX/HPL system or specific LOX forms alone. The reaction is stereospecific with *S*-hydroperoxides being the predominant products of plant and mammalian LOXs, and *R*-epimers are formed predominantly by invertebrate LOXs. Most LOXs also exhibit high regiospecificity. Research on the sequence alignments has suggested that *S*-LOXs contain a conserved alanine residue at a critical position at the active site, but *R*-LOXs contain glycine (Gly) in this position. However, recent studies on the model vertebrate *Danio rerio* (zebra fish) by cloning have expressed a novel *R*-LOX isoform that carries Gly at this critical site, resulting in a predominant production of 12S-H(p)ETE [12S-hydro(pero)xyeicosatetraenoic acid] (Jansen et al. 2011). Research on the Ala-to-Gly exchange in human and animal LOXs resulted in different specificity for producing oxygenation products. In human LOXs, there was an increase of specific *R*-oxygenation products. However, in rabbits, orangutans, and mice, *S*-HETE (hydroxyeicosatetraenoic acid) isomers remained the major oxygenation products, whereas chiral *R*-HETEs contributed only 10%–30% to the total product mixture.

In plants, the most common LOX-resulting products from linoleic acid and linolenic acids lead to different bioactive mediators related to different functions, especially plant defense and development (Grechkin 1998). In mammals, the different

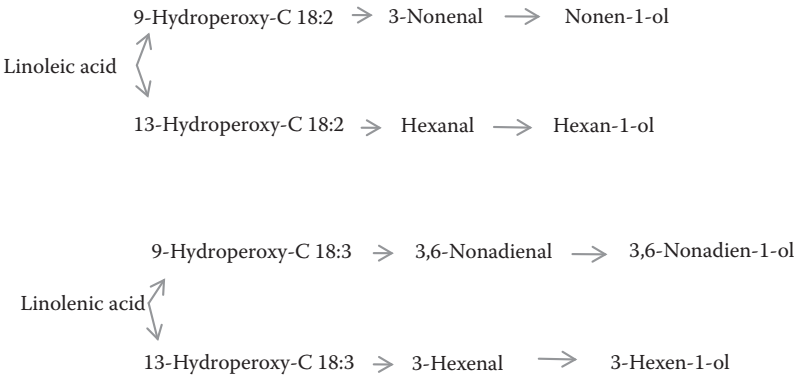

FIGURE 2.5 Representative oxidation products of linoleic (18:2 n-6) and linolenic (18:3 n-3) acids by action of 9-LOX and 13-LOX.