Chapter 11

Lipid Oxidation

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Chapter Outline

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I. INTRODUCTION

In all foods, the first mode of spoilage is microbial. However, after microbes have been controlled by processing, oxidation becomes the set of chemical reactions most limiting shelf-life and degrading the quality of foods. Consumers commonly recognize lipid oxidation by characteristic painty or oily 'rancid' off-flavors and odors, but the impact of the reaction is far greater than this. As the most sensitive chemical functional groups in biological molecules, unsaturated fatty acids essentially harvest oxidizing potential from the atmosphere and transform it into highly reactive chemical species. Once initiated, the free radical chains of lipid oxidation are responsible for a cascade of oxidations that affect structural proteins and enzymes, nucleic acids, polysaccharides, vitamins, and lipids. The overall effect is to alter physical properties and degrade molecular functionality as well as to destroy the palatability of foods.

Lipid oxidation is not limited merely to foods, as radicals, hydroperoxides, epoxides, and aldehydes are involved in normal physiology and pathological processes in living tissues, including aging (Pryor, 1985), cancer (McBrien and Slater, 1982), atherosclerosis (Uchida, 2000), Alzheimer's disease (Sayre *et al.*, 1997) and other dementias, inflammatory bowel disease (Kruidenier and Verspaget, 2002), and macular degeneration (Gu *et al.*, 2003; Ebrahem *et al.*, 2006).

Most of the time, lipid oxidation is considered to be a toxic process leading to decomposition of membranes, inactivation of enzymes, adduct formation and strand scission in DNA, and impairment of cell functions (Logani and Davies, 1980; Borg *et al.*, 1981; McBrien and Slater, 1982; Borg and Schaich, 1983, 1984; Fadeel *et al.*, 2007). However, there are some positive impacts, as low levels of oxidation products play important roles in signal transduction in tissues, such as regulating responses to environmental oxygen (Suzuki *et al.*, 1997; Gutierrez *et al.*, 2006). One example of this is that when *Phanerochaete chrysosporium* (white rot fungi) are grown in high oxygen, lipid oxidation in membranes upregulates production of lignan peroxidases that utilize the excess oxygen and of antioxidant enzymes (catalase and glutathione peroxidase) that reduce oxidation products (C. Frenkel and K. M. Schaich, unpublished data, 1992). Hence, lipid oxidation has moved beyond the traditional purview of food science and has become a hot research topic in biology and medicine as well.

Whatever the field of application, new information is showing lipid oxidation to be a complex series of reactions with some fascinating chemistry and effects that move far beyond the neighboring lipid molecule or release of rancid odors. This chapter provides an introduction to lipid oxidation as a dynamic chemical process. The main focus is on lipid oxidation in foods rather than medicine, although the principles discussed are broadly applicable to all living tissues, both plant and animal.

II. WHERE DOES LIPID OXIDATION OCCUR?

Lipid oxidation primarily involves the reaction of oxygen with unsaturated fatty acids (fatty acids with double bonds), although secondary reactions with saturated fatty acids (no double bonds) cannot be ruled out. Lipid oxidation in foods is most commonly associated with fats and oils in bulk or in oil phases of emulsions, and certainly that is where the reaction is most obvious to consumers. However, it is important to recognize that oxidation occurs wherever unsaturated fatty acids are found, and that means in:

- triacylglycerols: in adipose tissue and marbling fats (Watts, 1954; Ladikos and Lougovois, 1990), oil bodies in grains, essential oils in fruits, chocolate (Rossi-Olson, 2011), bulk oils (Schaich, 2005a), and oil phases of emulsions (Sun *et al.*, 2011)
- phospholipids: in membranes (particularly muscle foods, organ meats, vegetables), bran layers, egg yolks, and natural and synthetic emulsifiers (Corliss and Dugan, 1971; Igene *et al.*, 1980; Mead, 1980; Yamamoto *et al.*, 1984; Porter and Wagner, 1986)

- free fatty acids: hydrolysis products of triacylglycerols and phospholipids resulting from acids, bases, heat with and without water, and lipase action (Heaton and Uri, 1961; deGroot *et al.*, 1973; Campbell *et al.*, 1974; Miyashita and Takagi, 1986); found especially in foods that are heated, have microbial contamination, or contain dairy or tropical lipids
- fatty acids esterified to other molecules such as sterols and alcohols (as in waxes).

This means that lipid oxidation can be rather pervasive in foods, even in low-lipid formulations.

III. HOW DOES LIPID OXIDATION OCCUR?

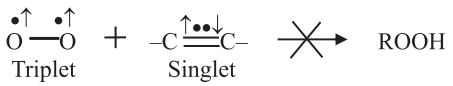
A. Lipid Oxidation Mechanisms: Traditional Free Radical Chain

Lipid oxidation has long been recognized as a free radical chain reaction which occurs in three stages: initiation, propagation, and termination (Figure 11.1) (Farmer *et al.*, 1943; Farmer and Sutton, 1943; Bolland, 1945, 1949; Swern, 1961). The radical chain reaction is responsible for several unique kinetic characteristics that present distinct challenges in measuring and controlling lipid oxidation, and are part of the reason why lipid oxidation is a major problem in storage stability of foods:

- Lipid oxidation is autocatalytic: once started, the reaction is self-propagating and self-accelerating.
- Many more than one lipid molecule is oxidized and more than one LOOH is formed per initiation. Chain lengths of up to several hundred lipid molecules have been measured (Hyde and Verdin, 1968; Cosgrove *et al.*, 1987).
- Very small amounts of pro-oxidants or antioxidants cause large rate changes.
- The reaction produces multiple intermediates and products that change with reaction conditions and time.

1. Initiation ($LH \rightarrow L^{\bullet}$)

Because lipid oxidation occurs so readily and is found so ubiquitously, it is often referred to as a spontaneous process (Anonymous, 1981). However, lipid oxidation is not thermodynamically spontaneous, i.e. it cannot happen on its own. Normal oxygen is in a triplet spin state (odd electrons parallel) while double bonds are in singlet spin states (electrons with opposite spin), so atmospheric oxygen cannot react directly with lipid double bonds:



(11.1)

Thus, lipid oxidation always *requires* an initiator or catalyst to remove an electron from either the lipid or oxygen, creating radicals, or to change the electron spin of the oxygen so that it can add to the double bond directly to form hydroperoxides that break down to radicals. Whatever the initiator, the final result is formation of initial lipid alkyl radicals that react with oxygen to start the oxidation process. Reactions of the most common initiators are described in Section IV, C.

2. Propagation and Branching

a. Basic reactions

Propagation is the heart of the oxidation process (Kochi, 1973a). In it, oxygen adds at diffusion controlled rates (almost instantaneously) to relatively unreactive lipid alkyl radicals, $L^{\bullet}$, converting them to reactive peroxyl radicals, $LOO^{\bullet}$ (Reaction 2 in Figure 11.1), that establish the free radical chain and keep it going (Ingold, 1969a). Peroxyl radicals abstract hydrogens from adjacent lipid molecules to form hydroperoxides, LOOH, and generate new $L^{\bullet}$ radicals in the process (Reaction 3, Figure 11.1). Each new $L^{\bullet}$ radical in turn adds oxygen, forms a peroxyl radical, abstracts a hydrogen from another lipid, forms another hydroperoxide, and generates a new $L^{\bullet}$ radical to provide the driving force in the chain reaction (Reaction 4, Figure 11.1). The process continues indefinitely until no hydrogen source is available or the chain is intercepted.

Peroxyl radicals are the main chain carriers in early oxidation. Their abstractions are rather slow and specific ($k = 36-62$ l/mol/s) (Gaddis *et al.*, 1961; Howard and Ingold, 1967; Gebicki and Bielski, 1981), which contributes

to an initial slow period in which lipid oxidation may or may not be detected. The chain continues one abstraction at a time from the initiation point. Without forces that decompose hydroperoxides, this process can continue indefinitely at a slow rate. However, reactions accelerate when hydroperoxides accumulate and then are decomposed to alkoxyl radicals, peroxyl radicals, and hydroxyl radicals by metals, heat, and ultraviolet (UV) light (Reactions 5, 6, and 7 in Figure 11.1). An important distinction in hydroperoxide decompositions is that metal reactions are heterolytic, yielding one radical and an ion, while heat and UV light induce homolytic scission that generates two radicals, alkoxyl ($LO^{\bullet}$) and hydroxyl ($HO^{\bullet}$), both of which react much more rapidly and more generally than $LOO^{\bullet}$. Once formed, these radicals greatly increase the rate of recycling in the chain reaction, and they attack more sites on lipids.

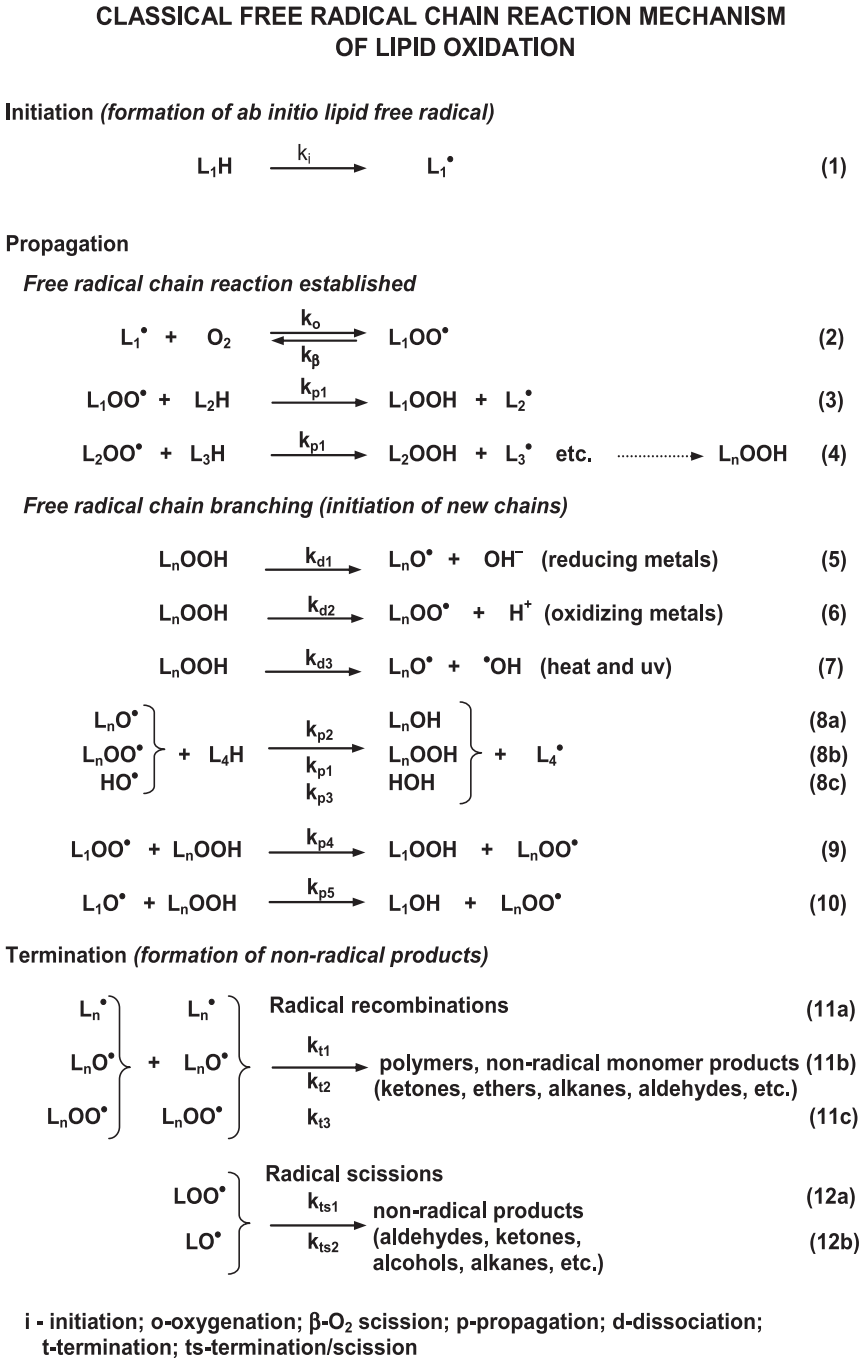


FIGURE 11.1 Classical free radical chain reaction of lipid oxidation as traditionally understood. *(From Schaich, 2005b; used with permission.)*

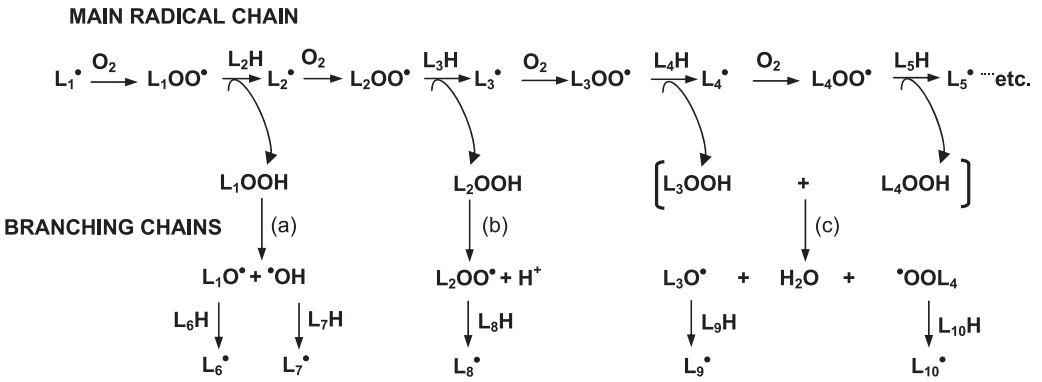


FIGURE 11.2 Expansion of the lipid oxidation chain reaction by chain branching. Propagation extends the original chain from the first radical by hydrogen abstraction (top chain). Chain branching occurs when hydroperoxide products of the original chain decompose by (a) reduction, (b) oxidation, or (c) bimolecular dismutation to multiple radicals that all initiate new chains. Propagation rates are faster on chains with LO• as the chain carrier. *(Adapted from Schaich, 2005b; used with permission.)*

The change in propagation rate and abstraction specificity marks progression into a second stage of propagation called branching, in which the radical chain reaction expands, establishing new chains at faster rates. The effect of branching is shown diagrammatically in Figure 11.2. Lipid oxidation gathers steam, increasing in rate and extent as LO• becomes the dominant, faster chain carrier ($k = 10^6 - 10^7$ l/mol/s) (Pryor, 1986; Erben-Russ *et al.*, 1987) and secondary chains dramatically amplify and broadcast lipid oxidation beyond the initial radical chain. In this manner, a single initiating event can lead to sequential oxidation of literally hundreds of molecules in the primary chain and in secondary branching chains (Hyde and Verdin, 1968; Cosgrove *et al.*, 1987).

One final point about propagation must be made before moving on. In the early stages of lipid oxidation when hydroperoxides are in low concentrations, hydroperoxide decompositions occur monomolecularly (one at a time) as shown in Reactions 5, 6, and 7 of Figure 11.1. However, as oxidation progresses and hydroperoxides accumulate, decomposition shifts to bimolecular mechanisms in which two hydroperoxides interact to induce decomposition. The traditional explanation proposes that the hydroperoxides hydrogen bond and then undergo concerted hydrolysis to yield two radicals (Reaction 11.2); these go on to initiate branching reactions and accelerate the overall oxidation rate (Sliwiok *et al.*, 1974; Hiatt and McCarrick, 1975):

$$2\ \text{LOOH} \longrightarrow \text{LOOH}\cdots\text{HOOL} \longrightarrow \text{LO}^{\bullet} + \text{H}_2\text{O} + {}^{\bullet}\text{OOL} \qquad (11.2)$$

An alternative explanation suggests that the dramatic increase in oxidation is kinetically more likely when one slowly reacting radical reacts with a non-propagating hydroperoxide to generate a powerful cascade of *three* very reactive radicals: LO•, epoxy-LO•, and •OH (Elson *et al.*, 1975):

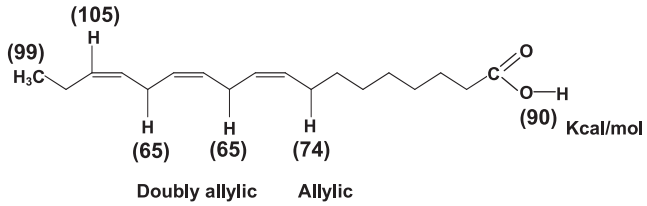
$$\text{LOO}^{\bullet} + \text{R}_1\text{-CH}_2\text{-CH=CH-CH(OOH)-CH}_2\text{-} \longrightarrow \text{R}_1\text{-CH}_2\text{-CH(LOO)-CH}^{\bullet}\text{-CH(OOH)-CH}_2\text{-} \qquad (11.3)$$

$$\text{R}_1\text{-CH}_2\text{-CH(O)-CH(}{}^{\bullet}\text{O)-CH}_2\text{-} + {}^{\bullet}\text{OH} \longleftarrow \text{R}_1\text{-CH}_2\text{-CH(O)-CH(OOH)-CH}_2\text{-} + \text{LO}^{\bullet} \qquad (11.3a)$$

Whichever mechanism is operative, the initial effect of bimolecular decomposition is to dramatically accelerate lipid oxidation. However, as oxidation progresses, eventually the hydroperoxide breakdowns and termination reactions, such as radical recombination or alkoxyl radical scission, become faster than the initiation of new chains. Oxidation then slows as stable secondary products form and off-flavors and odors become detectable.

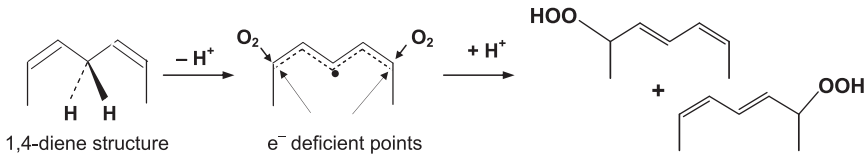
b. Sites of Hydrogen Abstraction and L•/LOOH Formation in Unsaturated Fatty Acids

During propagation, lipid oxyl free radicals abstract hydrogens from carbon positions with the weakest bonding. The lowest C—H bond energies in unsaturated fatty acids are at the allylic hydrogens (next to double bonds) (Kerr, 1966) so these become the preferred sites for H removal and formation of a free radical. The $-CH_2-$ groups between two double bonds in lipids (called doubly allylic) are doubly activated so C—H bond energies drop tremendously, as shown in the structure below (Scott, 1965; Kerr, 1966).



Thus, the order of preference for hydrogen abstractions in fatty acids is H between two double bonds (which partially explains why oxidizability of fatty acids increases with the number of double bonds) > singly allylic H next to double bonds > > H adjacent to the —COOH group > H on methylene groups further down the acyl chains (Patterson and Hasegawa, 1978).

When a hydrogen is abstracted between two double bonds, the free electron remaining (i.e. the radical) becomes distributed across a resonance stabilized double bond system (Reaction 11.4). The highest electron density concentrates in the center (weakest C—H bond), so the outside positions become relatively electron deficient, providing enhanced targets for oxygen addition and hydroperoxide formation.



(11.4)

Linoleic acid thus forms hydroperoxides almost exclusively at external positions carbons 9 and 13 during autoxidation. In polyunsaturated fatty acids with more than two double bonds and multiple 1,4-diene structures, the dominant hydroperoxides of autoxidizing fatty acids also are found at the external positions (Figure 11.3), regardless of the number of double bonds.

Two notable exceptions to this pattern are: (1) hydrogens are abstracted (and hydroperoxides are formed) equally at both double bond carbons and both neighboring carbons in isolated double bonds, such as in oleic acid; and (2) internal hydroperoxides, e.g. carbons 10 and 12 in linoleic acid, are formed during photosensitized oxidation by singlet oxygen, as will be discussed further in Section IV, D, 1.

Another important point to recognize about lipid oxidation is that hydrogen abstraction does not break the double bonds: when radicals are formed, the double bonds migrate to the next carbon and invert from *cis* to *trans*, even in isolated double bonds such as oleic acid (Reaction 11.5) (Farmer *et al.*, 1943; Porter, 1990; Porter *et al.*, 1995). In the

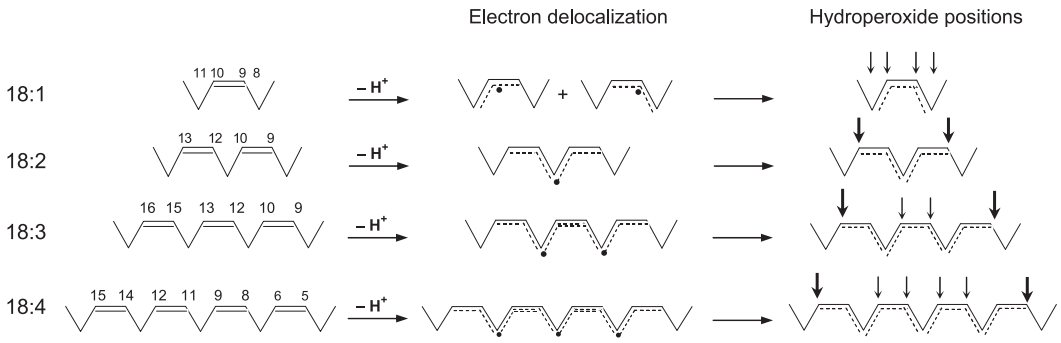


FIGURE 11.3 Electron resonance distributions and corresponding locations of hydroperoxides formed when hydrogens are abstracted from unsaturated fatty acids. Heavy arrows denote dominant positions for hydroperoxide formation. *(From Schaich, 2005b; used with permission.)*

1,4-diene systems of the polyunsaturated fatty acids, migration of the double bond generates a conjugated diene (Reaction 11.6).

$$RCH_2CH_2\text{-}CH{=}CH\text{-}CH_2(CH_2)_6\,COOH \longrightarrow RCH_2CH{=}CH\text{-}CH\text{-}CH_2(CH_2)_6\,COOH + RCH_2CH_2\dot{C}H\text{-}CH{=}CH(CH_2)_6\,COOH \quad (11.5)$$

$$\text{-}CH_2CH{=}CH\text{-}CH_2\text{-}CH{=}CHCH_2\text{-} \longrightarrow \text{-}CH_2CH{=}CH\text{-}CH{=}CH\text{-}\dot{C}HCH_2\text{-} + \text{-}CH_2\dot{C}H\text{-}CH{=}CH\text{-}CH{=}CHCH_2\text{-} \quad (11.6)$$

Conjugated dienes have two important consequences in lipid oxidation. They are the first detectable chemical change in lipid oxidation, so are important intermediates for following early stages of lipid oxidation by their UV absorption at 234 nm (Parr and Swoboda, 1976). In addition, the conjugated system primes the chain for further reactions (see Section III, B), and converts the acyl chain from bent and flexible to straight and rigid. In membranes, therefore, even early oxidation alters membrane fluidity and disrupts function.

3. Termination Processes

In lipid oxidation, 'termination' is almost a misnomer since lipid oxidation never fully stops. A specific radical may be terminated and form some product, but usually there is another radical left behind, so the chain reaction continues. Net oxidation slows down when radical quenching processes exceed the rate of new chain production, and the momentum of oxidation shifts from radical propagation and chain expansion to generation of stable products. Thus, 'termination' in the discussion below refers to converting an individual lipid radical to a product, not stopping the overall reaction. The cumulative effect on a food system is determined by the number of radical chains being terminated.

Lipid free radicals terminate to form non-radical products by four major mechanisms (Schaich, 2005b):

- radical recombinations
- α and β scission reactions of alkoxyl radicals when proton sources (e.g. water) are present to stabilize the products
- co-oxidation of non-lipid molecules such as proteins
- group eliminations or dismutation.

The mechanisms dominating in a given food are influenced by the nature and concentration of the radicals, the temperature and oxygen pressure, and the solvent. Basic aspects of the reaction chemistry will be covered here; effects of co-oxidation reactions will be covered in more detail in Section V.

a. Radical Recombinations

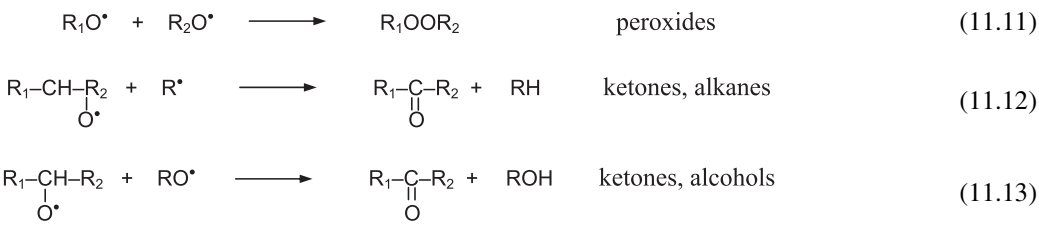
Radicals formed from oxidizing lipids can recombine in limitless combinations to generate a broad range of oxidation products. Nevertheless, recombinations are not random, and distinct patterns of favored recombinations have been identified. Some of the most important recombinations responsible for major lipid oxidation products are:

alkyl radicals: $R_1^{\bullet} + R_2^{\bullet} \longrightarrow R_1\text{-}R_2$ alkane polymers (11.7)

peroxyl radicals: $2\ R_1CHR_2(OO^{\bullet}) \longrightarrow R_1CH(R_2)\text{-}OOOO\text{-}CH(R_1)R_2 \longrightarrow R_1CHR_2(OH) + R_1CR_2(=O) + O_2$ alcohols and ketones (11.8)

$ROO^{\bullet} \xrightarrow{O_2} R^{\bullet} \xrightarrow{ROO^{\bullet}} ROOR$ alkyl peroxides (11.9)

alkoxyl radicals: $R_1O^{\bullet} + R_2^{\bullet} \longrightarrow R_1OR_2$ ethers (11.10)

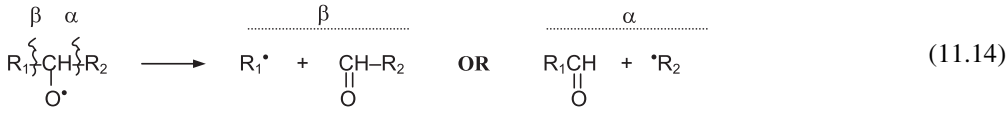


Temperature and oxygen pressure are key determinants of radical recombination pathways. $L^{\bullet}$ recombinations dominate under low oxygen pressures ($pO_2 = 1$ to about 80−100 mmHg) and high temperatures (reduced oxygen solubility) (Figure 11.4) (Lundberg and Chipault, 1947; Labuza, 1971). High oxygen favors $LOO^{\bullet}$ reactions, but then addition to double bonds becomes competitive with combination. $LO^{\bullet}$ contributions to the product mix dominate in secondary stages of oxidation and at moderate temperatures and oxygen pressures when LOOH or $LOO^{\bullet}$ decompositions are faster than their formation (Bolland, 1949). Radical recombinations increase in importance relative to other products as oxidation increases and intermediates accumulate. For example, alkoxyl radicals from hydroperoxyepidioxides heated at 40°C generate > 90% dimers (Neff *et al.*, 1988).

Radical recombinations are responsible for many of the characteristics of oxidized oils or lipids. Recombinations are diffusion and concentration controlled (Erben-Russ *et al.*, 1987; Tsentalovich *et al.*, 1998), so they occur most readily when viscosity and radical concentrations are both high, i.e. in neat oils after extensive oxidation; recombinations decrease with lipid dilution and are probably unimportant in polar solvents. Primary alkyl radical recombinations generate the dimers and polymers that account for the increased viscosity in oxidized oils. Perhaps most importantly, recombinations of alkoxyl radicals and their fragmentation products (see discussion below) generate low levels of volatile compounds and flavor components that augment those produced in scission reactions and provide the undertones and secondary notes that round out flavors (Grosch, 1987). Ketones and dialkyl peroxides, in particular, result uniquely from recombination reactions.

b. Scission Reactions of $LO^{\bullet}$

Scission of alkoxyl radicals probably has the greatest practical consequences in lipid oxidation because the products generated are responsible for the distinctive volatile off-odors and off-flavors that are the strong markers of rancidity (Grosch, 1987). Lipid alkoxyl radicals undergo scission of the C−C bond on either side of the alkoxyl group to yield a mixture of carbonyl final products (typically aldehydes and oxo-esters from the initial alkoxyl radicals) and alkyl free radicals that can continue the chain reaction:



Unsaturated radical fragments oxidize further and then undergo secondary scissions to produce carbonyls and alkanes of shorter chain length. Consequently, product mixtures that accumulate in oxidized lipids can become quite complex.

Scission of alkoxyl radicals requires a strong proton donor (Russell, 1959). Hydrogen atoms hydrogen bond to both intermediate transition states and final polar cleavage products, reducing the activation energy for bond rupture

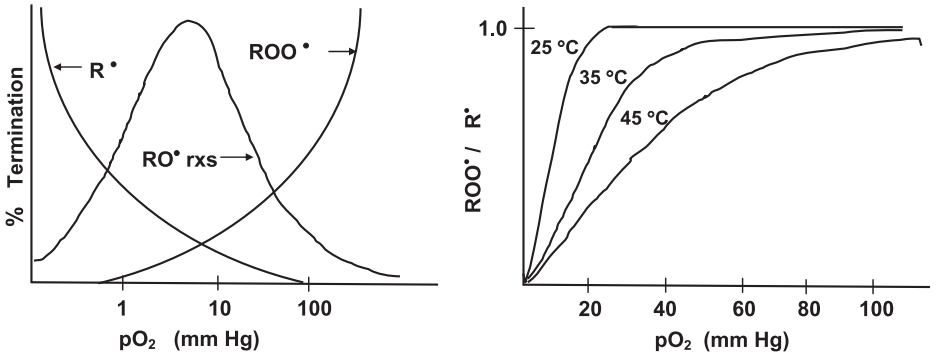
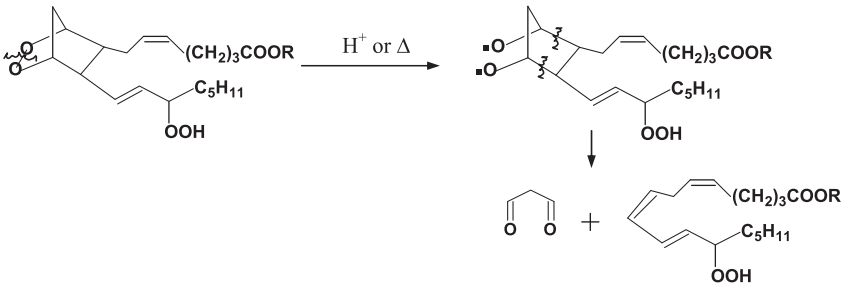


FIGURE 11.4 Effects of oxygen and temperature on termination processes in lipid oxidation. More oxygenated products are favored by high oxygen and low temperatures; alkyl reactions and dimerizations are favored by low oxygen and elevated temperatures (Schaich, 2005b). *(Redrawn from Labuza, 1971; used with permission.)*

(Walling and Padwa, 1963; Walling and Wagner, 1963; Kochi, 1973b; Avila *et al.*, 1993; Tsentalovich *et al.*, 1998); H^+ from the solvent then adds immediately to the scission radicals to provide the driving force for the reaction (Schauenstein, 1967). Thus, alkoxyl radical scissions are quite rapid in the presence of water (e.g. in emulsions) and indeed account for about half the alkoxyl radical reactions in polar media, especially in dilute solutions of lipids where there is reduced competition from hydrogen abstraction (Bors *et al.*, 1984). However, the greatest contribution of this termination (and propagation) reaction is at elevated temperatures (Kochi, 1973b) because $LO^{\bullet}$ scission has a large E_a and log A (Arrhenius factor) (Horner *et al.*, 2000). Heat accelerates alkoxyl radical scissions in all solvents, although the pattern of cleavage may change as temperature increases. Scission is a minor process in neat lipids at room temperature.

Figures 11.5−11.7 show some of the multiple scissions that occur leading to complex mixtures of products in oxidizing oleic acid, linoleic acid, and linolenic acids, respectively. Further details of the scission reactions responsible for the hundreds of volatile products in lipid oxidation may be found in reviews by Frankel and by Grosch, pioneers in this field (Frankel, 1982, 1984, 1985, 1987; Grosch, 1987).

Discussion of scission reactions would be incomplete without mention of malondialdehyde (MDA), one of the most notorious products of lipid oxidation. MDA arises from multiple scissions of cyclic internal hydroperoxides formed in fatty acids with three or more double bonds (linolenic and higher) (Reaction 11.15) (Dahle *et al.*, 1962; Pryor *et al.*, 1976).



(11.15)

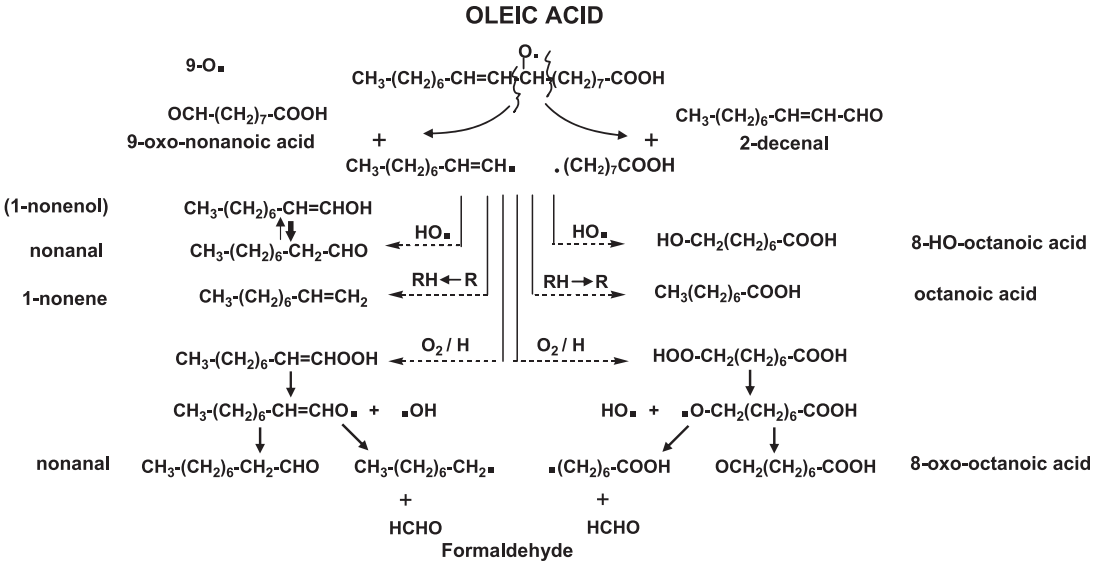
MDA is very commonly used − and misused − in assays of lipid oxidation because it can be detected without lipid extraction. However, it is not a universal product and not appropriate for assaying oxidation fatty acids with fewer than three double bonds. MDA formation is facilitated in pure lipid phases, by low lipid and oxygen, and by photosensitized oxidation (Porter *et al.*, 1984); it also requires mild heat and acid for endoperoxide cleavage (Frankel and Neff, 1983). In autoxidizing lipids, yields of authentic MDA are usually less than 0.1% (Shamberger *et al.*, 1977; Frankel and Neff, 1983), although in photosensitized fatty acids where internal hydroperoxides are formed in high concentrations MDA concentrations can reach 5% or higher (Frankel and Neff, 1983). Hence, this assay is not the best choice for lipid oxidation analysis in most food systems.

c. Co-oxidation of Non-lipid Molecules

While hydrogen transfer is needed to quench a particular lipid radical, the hydrogen atom can come from any molecule that has abstractable (loosely bonded) hydrogens and is located close to the lipid. In foods and in cells, lipids are closely associated with proteins, carotenoids and other pigments, starches, antioxidants, and vitamins, and radical transfer to any of these molecules via hydrogen abstraction or addition of $LOO^{\bullet}$ or $LO^{\bullet}$ to double bonds can lead to co-oxidation of these molecules.

Co-oxidations are a process in which the interception of lipid free radicals by non-lipid molecules stops propagation and forms lipid products on one side, while transferring radicals and oxidizing potential to proteins and other biomolecules (Schaich, 2008). This reaction is similar to that of antioxidants but differs critically in that the radicals formed are not stable. Most of these non-lipid radicals add oxygen to form peroxyl radicals that also abstract H from other molecules and lead to oxidative degradation of the molecular target. In this way, lipids serve to 'broadcast' oxidation damage to other molecules which then provide footprints of lipid oxidation in foods and biological systems (Pryor, 1978, 1989; Schaich, 1980a, 2008; Borg and Schaich, 1984).

Generic co-oxidation reactions of target molecules are shown in Reactions 11.16 and 11.17. TH is any target molecule and RH is any molecule with an abstractable hydrogen, either lipid or non-lipid. The conjugated double bonds in Reaction 11.17 can be in any molecule but are particularly numerous in carotenoids.



Following the same fragmentation pattern –

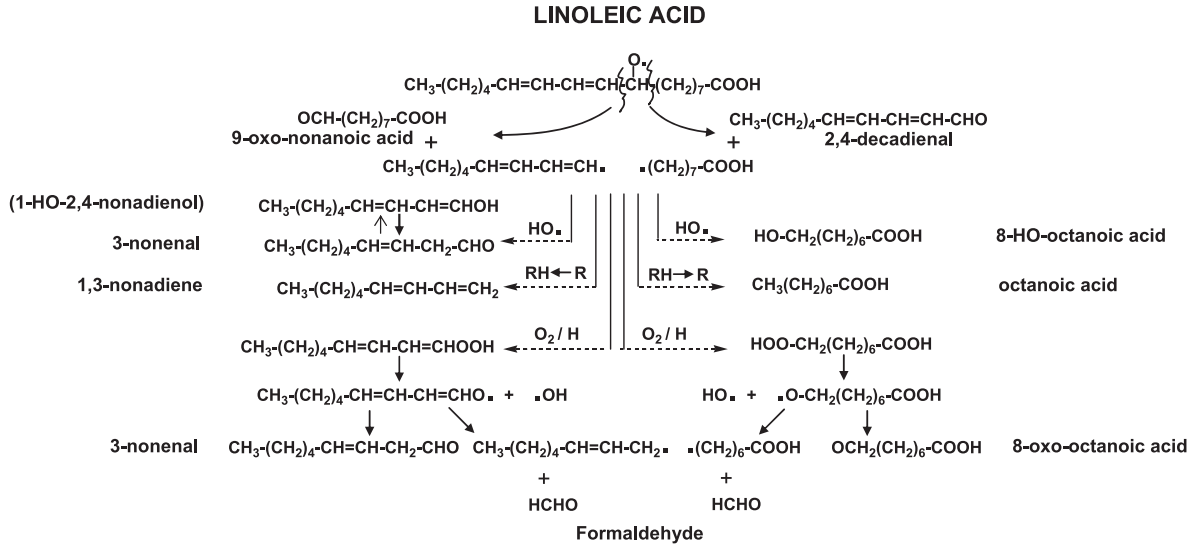
	β-scission		α-scission	
8–O• →	8-oxo-octanoic acid	+ decanal 1-decene (nonanol nonane nonanal formaldehyde)	2-undecenal	+ 7-HO-heptanoic acid heptanoic acid 7-oxo-heptanoic acid (6-HO-hexanoic acid hexanoic acid 6-oxo-hexanoic acid formaldehyde)
10–O• →	10-oxo-8-decenoic acid	+ octanol octane octanal (heptanol heptane heptanal formaldehyde)	nonanal	+ 9-oxo-nonanoic acid 8-nonenoic acid (octanol octane octanal formaldehyde)
11–O• →	11-oxo-9-undecenoic acid	+ heptanol heptane heptanal (hexanol hexane hexanal formaldehyde)	octanal	+ 10-oxo-decanoic acid 9-decenoic acid (nonanol nonane nonanal formaldehyde)

FIGURE 11.5 Scission pathways for oleic acid. *(From Schaich, 2005b; used with permission.)*

$$LOO^{\bullet} + TH \longrightarrow LOOH + T^{\bullet} \xrightarrow{O_2} TOO^{\bullet} \xrightarrow{RH} TOOH \tag{11.16}$$

$$LOO^{\bullet} + \text{-C=C-C=C-} \longrightarrow \text{-C=C-}\overset{\bullet}{\underset{OOL}{C}}\text{-C-} \longrightarrow \text{additional oxidation} \tag{11.17}$$

Perhaps the most noticeable co-oxidations involve proteins. Abstractable hydrogens are available on side-chain amino (Reaction 11.18) and thiol groups (Reaction 11.19), so histidine, lysine, arginine, and cysteine are prime



Following the same fragmentation pattern –

β-scission

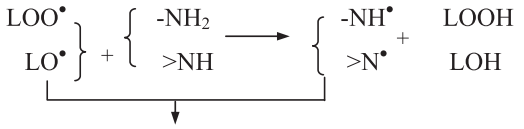
13–O• → 13-oxo-9,11-tridecadienoic acid
+ pentanol
pentane
pentanal
butanol
butane
butanal
formaldehyde

α-scission

hexanal + 12-oxo-9-dodecenoic acid
9,11-dodecadinoic acid
11-HO-9-undecenoic acid
9-undecenoic acid
11-oxo-9-undecenoic acid
formaldehyde

FIGURE 11.6 Scission pathways for linoleic acid. *(From Schaich, 2005b; used with permission.)*

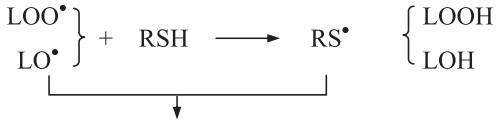
targets for hydrogen abstraction from proteins (Karel, 1975; Schaich and Karel, 1975, 1976; Yong and Karel, 1978, 1979; Schaich, 1980a). Radical addition reactions have also been reported (Reactions 11.18a and 11.19a) (Gardner and Weisleder, 1976; Gardner *et al.*, 1977, 1985).



(11.18)

-N-OOL, -N-OL adducts
>N-OOL, >N-OL adducts

(11.18a)

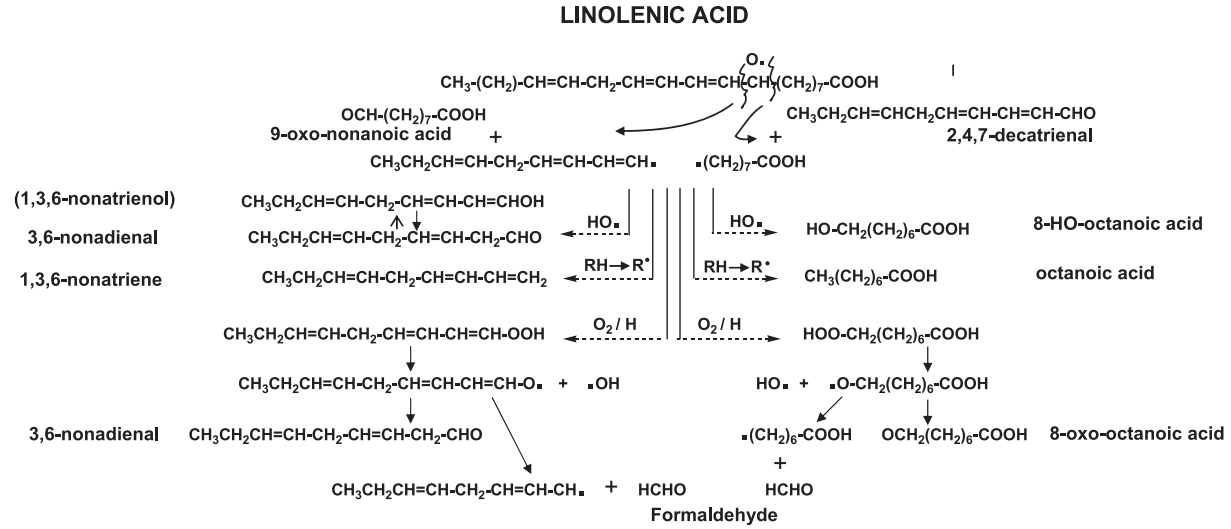


(11.19)

RS-OOL, RS-OL, RS-epoxy-L adducts

(11.19a)

Co-oxidation reactions should not be ignored in consideration of lipid oxidation kinetics, mechanisms, and overall effects in foods and biological systems. A critical issue is that while co-oxidation reactions terminate lipid oxidation chains they act as antioxidants at the same time, and lipid oxidation measured by hydroperoxides or downstream products slows. Co-oxidation products also limit extractability of lipids for analysis and often remove lipids from



Following the same fragmentation pattern –

	β-scission	α-scission
16–O• →	16-oxo-9,12,14-hexadecatrienoic acid + ethane	propanal + 15-oxo-9,12-pentadecadienoic acid 9,12,14-pentadecatrienoic acid (14-HO-9,12-tetradecadienoic acid 9,12-tetradecadienoic acid 14-oxo-9,12-tetradecadienoic acid formaldehyde)
13–O• →	13-oxo-9,11-tridecadienoic acid + 2-pentenol 2-pentene 2-pentenal (butanal butene formaldehyde)	3-hexenal + 12-oxo-9-dodecenoic acid 9,11-dodecadienoic acid (11-HO-9-undecenoic acid 9-undecedoic acid 11-oxo-9-undecenoic acid formaldehyde)
12–O• →	12-oxo-9-dodecenoic acid + 3-hexenal 3-hexene (2-pentenol 2-pentene 2-pentenol formaldehyde)	2,4-heptadienal + 11-HO-9-undecenoic acid 9-undecenoic acid (11-oxo-9-undecenoic acid 10-oxo-decanoic acid 9-decenoic acid formaldehyde)

FIGURE 11.7 Scission pathways for linolenic acid. *(From Schaich, 2005b; used with permission.)*

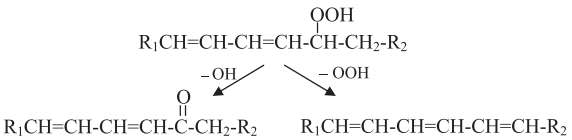
product streams normally analyzed. As a result, lipid oxidation in complex systems is probably always underestimated — sometimes severely — unless co-oxidation products are also measured. In foods, for example, that means monitoring at least oxidation in proteins and pigment bleaching. Sensitive target molecules need to be identified for each food product to tailor analyses and fully account for lipid oxidation.

Effects of co-oxidations will be considered in more detail in Section V.

d. Group Eliminations

Group eliminations are a minor form of termination but it is important to recognize the reaction because it accounts for some lipid oxidation products that are not easily formed by other mechanisms. The most common eliminations are HO^- and HOO^-, which can be eliminated from LOOH, yielding an internal carbonyl (ketone) (a) and a desaturated

product with an additional double bond (b) (Reaction 11.20) (Terao *et al.*, 1975; Bothe *et al.*, 1978). The specific product formed is determined by the hydroperoxide position on the fatty acid chain.



(11.20)

Formation of the ketone, the dominant product, prevents hydroperoxide decomposition to reactive alkoxyl radicals. Addition of a double bond to a polyunsaturated fatty acid forms a very reactive conjugated triene which is highly susceptible to radical addition and a variety of other secondary oxidations. Thus, while terminating a single radical chain, this product may still contribute to additional chains, adding to the complexity of lipid oxidation.

B. Reaction Mechanisms: Multiple Reaction Pathways

The chain reaction scheme shown in Figure 11.1 has been the accepted explanation of lipid oxidation for more than 50 years, yet it does not accurately account for the wide array of products observed in oxidizing lipids or for kinetics of the total process. Indeed, many published results are inconsistent with the chain reaction as written. In particular, the reaction scheme as drawn predicts that products should be generated and observed in strict sequence, i.e. hydroperoxides form first and their decomposition leads to a cascade of unspecified products. In some studies, products follow this pattern very generally, but under many conditions the predicted products form simultaneously with hydroperoxides or do not occur at all. Strategies for accurately analyzing lipid oxidation and for designing antioxidants require a clear understanding and tracking of all the oxidation processes active in a food. Thus, it is important to begin considering the multiple alternative pathways of both peroxyl and alkoxyl radicals that can be active in addition to and in competition with classical hydrogen abstraction in lipid oxidation. Figure 11.8 shows an integrated scheme for lipid oxidation which incorporates the classical free radical chain, shown flowing vertically down the center, but adds important side-reactions of $LOO^{\bullet}$, LOOH, $LO^{\bullet}$, and secondary radicals that compete with hydrogen abstraction in propagation and alter termination processes (Schaich, 2005b). This scheme has been

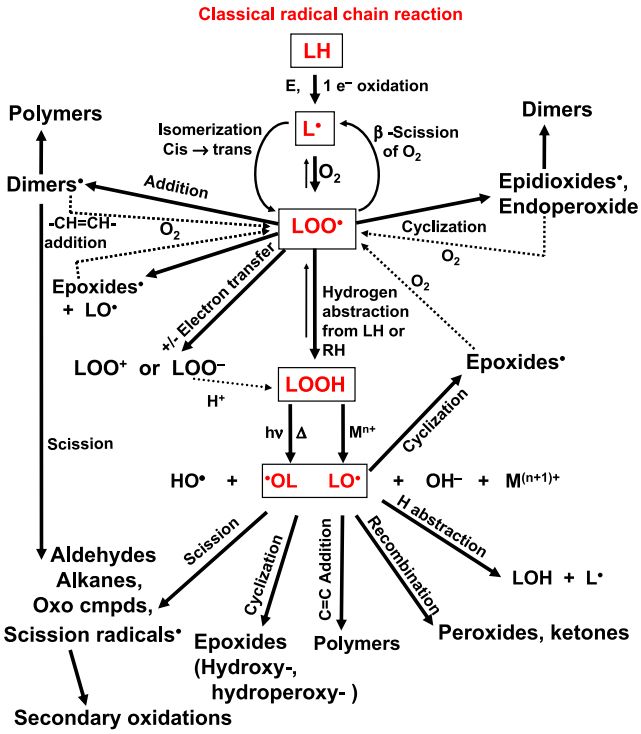


FIGURE 11.8 Integrated scheme for lipid oxidation showing alternative reactions that compete with classical hydrogen abstraction (vertical reactions in center). *(From Schaich, 2005b; used with permission.)*

compiled from extensive data in the literature and has not yet been proven per se, but it shows how thinking about lipid oxidation needs to be broadened in order to account for kinetics and products observed under different oxidation conditions and to meet the challenges of stabilizing foods with high polyunsaturated fatty acids, including *n*-3 fatty acids.

The major consequences of multiple reaction pathways are:

- Multiple $LOO^{\bullet}$ and $LO^{\bullet}$ reaction pathways occur simultaneously and in competition with each other. Mixtures of products can be formed at all stages, not just at the end of chains. Competing alternative reactions of $LOO^{\bullet}$ and $LO^{\bullet}$ alter propagation sequences and thus change kinetics, dominant products, detectability, and effects of lipid oxidation dramatically and in complex ways.
- Traditional lipid oxidation assays which monitor only one product may greatly underestimate lipid oxidation or miss it altogether if alternative pathways are more active than the one measured. This is particularly true for hydroperoxides and single volatile compounds such as hexanal. A combination of product analyses is needed to fingerprint all alternative pathways and accurately measure the full extent of lipid oxidation.
- All changes caused by lipid oxidation (flavors, odors, browning, texture alterations, color loss, etc.) derive from specific lipid oxidation products. Shifts away from H abstraction mean that aldehydes, epoxides, dimers, or other products are formed in place of alcohols, and this alters food qualities and stability patterns markedly. Understanding how lipid oxidation affects food properties thus requires analysis of a full range of lipid oxidation products.
- Toxicity and safety issues: shifts from volatile scission products to non-volatile internal rearrangement and addition products reduce off-odors, which is desirable. However, off-odors are the consumer's first clue that foods are rancid and should not be eaten. Epoxides are more toxic and react more rapidly with DNA and proteins, while dimers have reduced digestibility and some evidence of toxicity. These products have no sensory warning signals for consumers.

A brief description of propagation processes and products formed in alternative pathways and the conditions under which specific pathways are favored is provided as follows.

1. Hydrogen Abstraction

Hydrogen abstraction is the best known chain carrying reaction cited in all lipid oxidation schemes and is the ultimate reaction even with alternative pathways. Hydrogen abstraction by peroxyl radicals yields hydroperoxides (Reactions 3 and 4, Figure 11.1), the first stable product of lipid oxidation and the product most often measured to determine the extent of lipid oxidation. Hydrogen abstraction by alkoxyl radicals (Reactions 8a and 10, Figure 11.1) produces alcohols or hydroxylipids. However, alcohols are usually minor products, while they should be the only products if hydrogen abstraction is the only reaction occurring. Thus, there must be other reaction competing pathways that produce the aldehydes, epoxides, and other products most commonly observed in lipid oxidation.

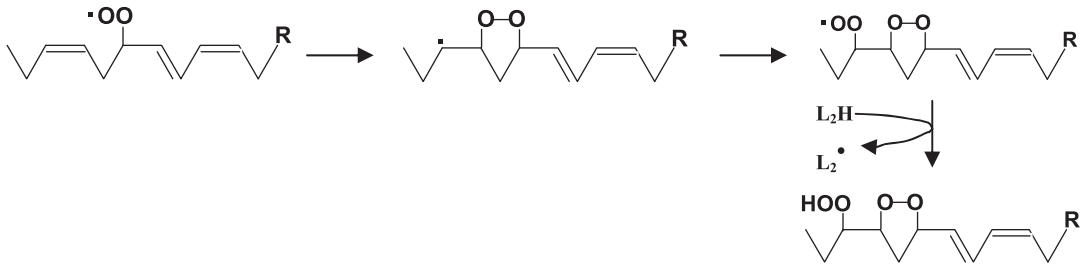
Hydrogen abstractions from other lipid molecules by $LOO^{\bullet}$ and $LO^{\bullet}$ are favored under conditions providing close contact between lipid chains without competition from other H sources, e.g. in pure lipids, in highly polyunsaturated fatty acids that have bent chain conformations that align multiple bis-allylic hydrogens, in the lipid interior of membranes, in aprotic solvents that do not compete with lipids, in low-viscosity media that facilitate molecular movement, and at elevated temperatures that contribute activation energy (Ingold, 1969a; Kim *et al.*, 1993; Schaich, 2005b). In solvents, H abstraction is favored at moderate lipid concentrations where enough substrate is present to supply hydrogens. However, at low lipid concentrations cyclization or scission dominates, while at high concentrations radical additions and recombinations become more important (Schaich, 2005b). These differences are important to understand when designing model systems for experiments or when interpreting lipid oxidation patterns in different food systems.

When abstractable hydrogens are limited in quantity or accessibility, the following alternative reactions divert the radical stream.

2. Internal Rearrangement, or Cyclization

Lipid free radicals are very short lived. If a compound with abstractable hydrogen is not nearby, the free electron looks for another avenue to find a pairing electron. One option is to add to the first carbon of an adjacent double bond, generating epidioxides with peroxyl radical rearrangements (Reaction 11.21) and epoxides with alkoxyl radical

cyclization (Reaction 11.22) and leaving a new free radical on the distal carbon of the double bond. Note that, with two oxygen atoms, peroxyl radicals add to a double bond two carbons away while alkoxyl radicals add to an immediately adjacent double bond.



(11.21)

(11.22)

As with all lipid alkyl radicals, addition of oxygen generates new peroxyl radicals now at a new position, and these go on to abstract hydrogens from neighboring lipid molecules. This propagates the radical chain while forming hydroperoxy epidioxide and epoxide products (Chan *et al.*, 1980).

Although internal rearrangement can occur from any position, for both $LOO^{\bullet}$ and $LO^{\bullet}$ there is a stronger tendency to cyclize from internal positions, probably because the $-O^{\bullet}$ is oriented more advantageously. Internal hydroperoxides are found in higher concentrations in fatty acids with four or more double bonds and in photosensitized oxidations (see Section IV, D, 1), so cyclic products epidioxides and epoxides are correspondingly higher. Indeed, the levels and positional distribution of internal cyclic products are characteristic markers distinguishing autoxidation from photosensitized oxidation.

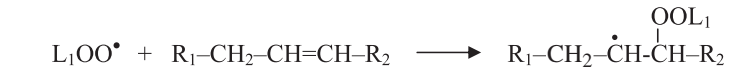
Internal cyclization reactions are reasonably fast (Kochi, 1962; Porter, 1990). Their reaction rates – $k(LOO^{\bullet})$ ~10^3 s^{-1} and $k(LO^{\bullet})$~10^5 s^{-1} – are comparable to hydrogen abstraction, especially in fatty acids with three or more double bonds, so reaction conditions will determine the dominant pathway in oils and foods.

Cyclization is favored when oxygen is limited (Van Sickle *et al.*, 1967) and abstractable hydrogens are not available, e.g. in aprotic solvents (Kochi, 1962) and at low lipid concentrations (Van Sickle *et al.*, 1967). When lipids are dissolved in aprotic solvents such as chloroform or acetonitrile at low concentrations and stored at room temperature or moderately elevated temperatures, as in typical lipid oxidation experiments, external abstractable hydrogens are absent or limited so cyclization can be the only active reaction (Haynes and Vonwiller, 1990). In oils or pure lipids, abstractable hydrogens are both readily available and close by, so abstraction competes with cyclization to generate mixed products whose proportions vary with temperature and extent of oxidation. Cyclization percentages ranging from 30 to 100% in oils or fatty acids have been reported (Schaich, 2005b). Cyclization is also favored by moderately elevated temperatures (e.g. 40°C) (Bors *et al.*, 1984) and lipid chain orientation (Wu *et al.*, 1977). Indeed, epoxides have been reported as the dominant lipid oxidation products in surface monolayers (e.g. oil sprayed or coated onto food surfaces) and in membrane interiors. Propagation via internal rearrangements decreases with temperature because epidioxy and epoxy peroxyl radicals have an increasing tendency to dimerize rather than abstract hydrogens (Schaich, 2005b).

One final comment about cyclic lipid oxidation products: they are highly reactive and thus are often difficult to detect. Epidioxide-$OO^{\bullet}$ radicals are particularly prone to dimerization with even moderate heat (Neff *et al.*, 1988) and the dimers often decompose still further. Epoxides react extremely rapidly with proteins in complex systems, and thus disappear from lipid extracts. Thus, it is often necessary to look for footprints of epidioxides and epoxides to determine their contributions to lipid oxidation in foods or biological tissues.

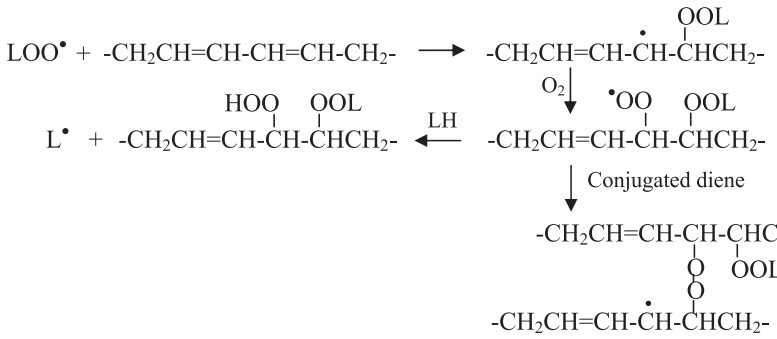
3. Addition

Lipid peroxyl radicals have a strong attraction to double bonds and add to them with relative ease, transferring the unpaired electron to the second carbon of the double bond:



(11.23)

This third propagation pathway has two possible endpoints. The most obvious is formation of dimers and polymers when the peroxyl group remains intact. This reaction is favored by addition to conjugated double bonds (Reaction series 11.24); the chain carrier is a new peroxyl radical formed at the second carbon of the double bond. This peroxyl radical can abstract hydrogens, forming new hydroperoxides (Reaction 11.24a), or can add to additional double bonds (Reaction 11.24b), leading eventually to development of long polymers and increased viscosity characteristic of very oxidized oils (Witting *et al.*, 1957; Privett, 1959; Sims and Hoffman, 1962). Contributions of $LOO^{\bullet}$ dimerizations increase with oxidation because doubly allylic abstractable hydrogens have been removed during initial reactions and the double bond system has been shifted to a conjugated system.

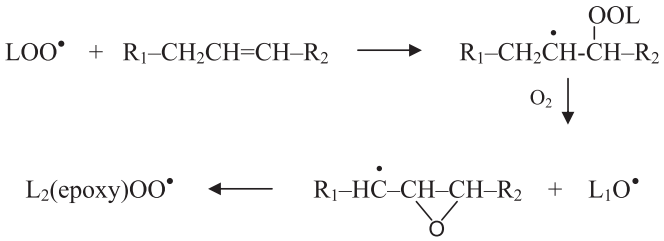


(11.24)

(11.24a)

(11.24b)

The reaction is different when $LOO^{\bullet}$ adds to isolated or non-conjugated double bonds, as in oleic acid or unoxidized linoleic acid, respectively (Reaction 11.25) (Schaich, 2005b). In this case, the peroxyl adduct decomposes, forming an epoxide and allylic radical and releasing an alkoxyl radical (Reaction 11.25a). The chain is propagated by both the alkoxyl radical and the new epoxyperoxyl radical formed in the presence of oxygen (Reaction 11.25a). This is a true branching reaction since two new propagating radicals ($LO^{\bullet}$ and epoxy$OO^{\bullet}$) with increased reactivities are generated from the initial $LOO^{\bullet}$.



(11.25)

(11.25a)

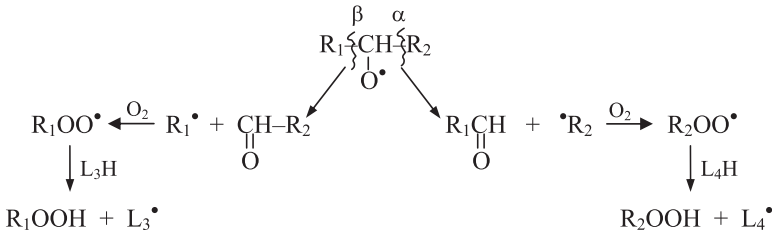
$LO^{\bullet}$ radicals do add to double bonds (Reaction 11.26), but not readily, because they are strong hydrogen abstractors and very rapidly cyclize to epoxides (Schaich, 2005b). Addition of $LO^{\bullet}$ is favored by absence of allylic hydrogens and by conjugation, conditions which only hold after oxidation is reasonably well established. Hence, propagation by $LO^{\bullet}$ addition is most active in catalyzing chain branching in secondary stages of oxidation. In addition, $LO^{\bullet}$ adds only to *cis* double bonds (Walling and Thaler, 1961), in contrast to $LOO^{\bullet}$, which can add to either *cis* or *trans* double bonds.

(11.26)

4. Scission

Scission of alkoxyl radicals has already been introduced as a termination process (Reaction 11.14, Section III, A, 3, b), but scission reactions are also important in propagation. Some of the radicals arising from α and β scissions rearrange internally to non-radical products (mostly aldehydes), but most of them add oxygen to form peroxyl radicals and then abstract hydrogens to propagate the radical chain (Reaction 11.27). Note that the peroxyl radicals and resulting hydroperoxides in this case are in terminal positions rather than the midchain products formed via

hydrogen abstraction. In addition, unsaturated fragments, particularly those containing conjugated dienes, are still susceptible to oxidation and their subsequent reactions also contribute to chain branching.

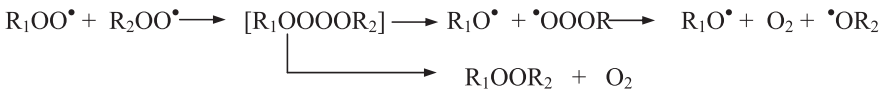


(11.27)

Scission is favored over H abstraction in the presence of water or strong proton donors (e.g. polar protic solvents such as alcohols) that provide the protons necessary to stabilize the scission products. However, too much water shifts propagation to termination because protons are drawn from non-lipid sources, stopping radical chains, and increased hydrolysis yields tertiary lipid oxidation products. Scission also increases markedly with temperature since thermal energy facilitates covalent bond rupture.

5. Disproportionation

Disproportionation occurs when two identical radicals join, then decompose to produce two or more different radicals. In lipid oxidation, disproportionation occurs when peroxyl radicals accumulate to high enough concentrations that they begin to react, forming tetroxide intermediates. These then decompose to form two alkoxyl radicals that are 1000 times more reactive as hydrogen abstractors than the original peroxyl radicals (Reaction 11.28) (Thomas, 1965; Lindsay *et al.*, 1973), and subsequent reactions of $RO^{\bullet}$ lead to greatly accelerated oxidation in true propagation reactions (Traylor and Russell, 1965). In an alternative slower reaction, the tetroxide decomposes to release oxygen and form a peroxide (R_1OOR_2) as a termination reaction (Reaction 11.28a).



(11.28)

(11.28a)

Note that in both reactions, oxygen is released back into the headspace or sample to provide fuel to maintain the radical chain. Hence, it can be an important means of prolonging lipid oxidation even when oxygen becomes limiting in samples. Paradoxically, release of oxygen can also confound analyses of lipid oxidation by oxygen consumption, making it appear that oxidation has slowed when it is just shifting gears from $LOO^{\bullet}$ to $LO^{\bullet}$ mediation. Recycling of oxygen may explain in part why oxygen consumption appears to slow as oxidation progresses, as will be discussed in Subsection C, next.

Favored by high oxygen that increases $ROO^{\bullet}$, disproportionation is most important in secondary stages of oxidation or during thermal oxidation. It occurs only in lipids oxidized as pure oils or in aprotic solvents. In polar solvents such as alcohols, or aqueous systems such as emulsions, $ROO^{\bullet}$ decomposition increases dramatically, the preferred reaction of $ROO^{\bullet}$ shifts to direct release of oxygen ($ROO^{\bullet} \rightarrow R^{\bullet}$), and disproportionation becomes a termination, rather than a propagation process (Walling *et al.*, 1970; Heijman *et al.*, 1985). Increasing solvent or system viscosity also favors termination over propagation by slowing radical movement and inhibiting Reaction 11.19 (Hiatt and Traylor, 1965).

C. Progression and Kinetics of Lipid Oxidation

1. Progression of Lipid Oxidation

One of the most challenging aspects of lipid oxidation is that it is such an ill-defined reaction. Most chemical reactions have a known beginning and fixed end products. However, the precursors of lipid oxidation are always present in foods and rates of reaction as well as reaction pathways and products change over time. Hence, lipid oxidation is a cumulative mix of reactions that create a dynamic and constantly changing process.

Hydroperoxides (LOOH) are the first stable products and they break down to keep the oxidation going, so lipid oxidation is often described in terms of hydroperoxide reactions. Three rate periods are customarily defined (Labuza, 1971):

- Induction period: very low levels of oxidation, undetectable formation of LOOH (and other products).
- Monomolecular rate period: initial stages of oxidation up to ~1% oxidation, LOOH accumulate slowly and decompose as single, isolated molecules (Reaction 11.29); $LOO^{\bullet}$ is the main chain carrier in early stages; both $LOO^{\bullet}$ and $LO^{\bullet}$ become chain carriers as oxidation becomes established.

$$LOOH \longrightarrow LO^{\bullet} + {}^{\bullet}OH \text{ (UV light, heat) or } LO^{\bullet} + {}^{-}OH \text{ (metals)} \tag{11.29}$$

- Bimolecular rate period: later stages of lipid oxidation up to ~7−15% oxidation, LOOH accumulate rapidly to high levels and begin to decompose via LOOH or $LOO^{\bullet}$ + LOOH interactions, as was shown in Reactions 11.2 and 11.3, respectively. However, $LO^{\bullet}$ reactions are much faster and more specific, so $LO^{\bullet}$ becomes the dominant chain carrier and controls the directions of reactions throughout the bimolecular rate periods.

These three rate periods are integrated conceptually with the progression of individual reactions in initiation, propagation, and termination of lipid oxidation in Figure 11.9. Included in the figure are the intermediates or products used to measure the extent of lipid oxidation in foods and biological materials.

Oxygen consumption detects the earliest events in lipid oxidation and reflects the rate of initiation; just as importantly, it is independent of products and pathways so is a true reflection of early oxidation. Oxygen consumption continues throughout oxidation, but its practical use ends when oxygen becomes limiting in the headspace or when peroxyl radicals accumulate to levels that disproportionately release oxygen back into the headspace.

Conjugated dienes, the first chemical change in lipids, and the corresponding hydroperoxides are also early indicators of lipid oxidation and they remain useful indicators at least until hydroperoxides begin to decompose bimolecularly. Because oxygen can add to and come off fatty acids repeatedly during the induction period until a hydrogen donor is available, development of hydroperoxides may lag behind conjugated dienes; and conjugated dienes may last longer than hydroperoxides because they remain intact in many products. However, both conjugated dienes and hydroperoxides decompose as lipid oxidation progresses, so while they are useful to follow the progress of

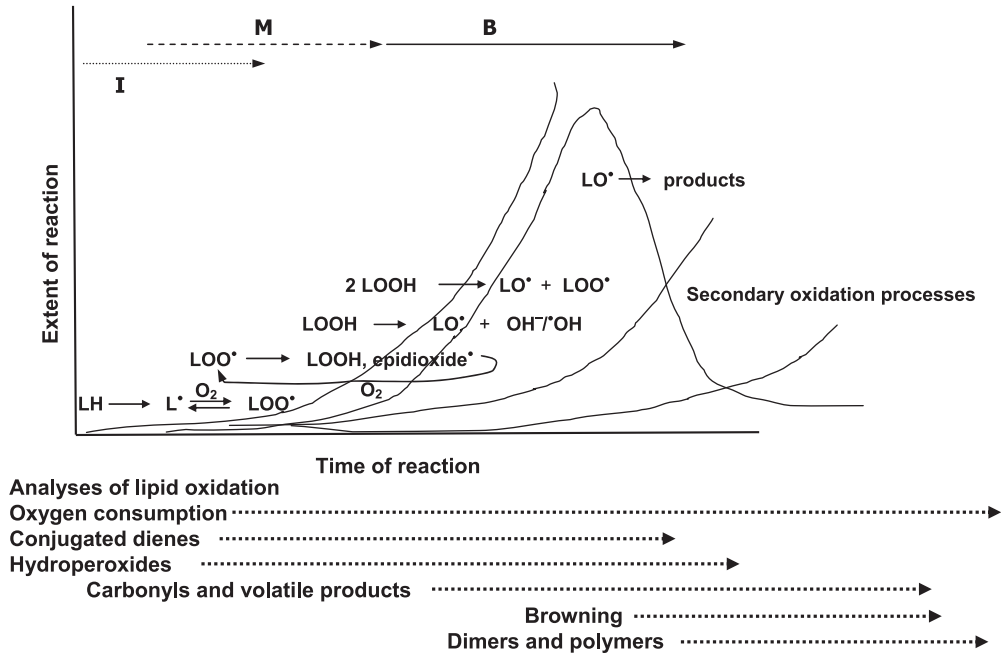


FIGURE 11.9 Diagrammatic representation of changes in dominant reactions and products over the course of lipid oxidation as traditionally described. Three separate rate periods are usually designated: induction period (I), monomolecular rate period (M), and bimolecular rate period (B). Shown below the graph are intermediates and products used to measure lipid oxidation at different stages. However, expectations that secondary products develop only after hydroperoxide decomposition are not valid in all systems. Multiple products should be measured at all points for accurate assessment of oxidation extent.

reactions in the early stages and continuously over time, neither of these measures can accurately measure the extent of oxidation in later stages or in isolated samples.

Secondary products such as aldehydes, epoxides, other volatiles, browning, and dimers/polymers were traditionally expected to develop only after decomposition of hydroperoxides, as shown in Figure 11.9. Indeed, many papers have reported this pattern. However, late detection may have resulted from insensitive methodologies and long times between sampling. Recent studies using more sensitive contemporary gas and high-pressure liquid chromatographies with mass spectrometry detection, as well as new chemical analyses accurate at nanomolar levels, have found secondary products formed in parallel with hydroperoxides, as would be predicted from the integrated reaction scheme in Figure 11.5. Thus, the graph in Figure 11.9 should be taken as a useful guide only. More research with multiple simultaneous product analyses will be needed to determine whether the graph is accurate as drawn, and how the various product curves change with different foods and different reaction conditions. The safest approach is always to follow lipid oxidation by simultaneously assaying multiple products from different stages and pathways to provide an accurate picture of the extent and progress of oxidation.

2. Kinetics of Lipid Oxidation

Some consideration of the kinetics of lipid oxidation needs to be included to provide a basis for understanding the driving forces underlying lipid oxidation as a process. Rate equations must be developed to account for conditions in each individual system, so they can become quite complicated and are different in every reference. To illustrate the principles, only very general approaches will be presented here.

At low pO_2 and during early oxidation (monomolecular LOOH decomposition), the reaction is directly dependent on oxygen and the rate of initiation. Thus, the overall rate of lipid oxidation may be expressed simplistically as:

$$\text{Rate of oxidation (low } pO_2) = k_o(k_i/2k_{t1})^{1/2}[\text{LH}]\,[O_2] \tag{11.30}$$

where rate constants refer to the corresponding oxygenation, initiation, and termination reactions in Figure 11.1. The overall rate thus reflects the balance between initiation reactions (k_i) and alkyl radical ($L^\bullet$) termination (k_t), primarily by recombination. Assuming that initiators are ubiquitous so $L^\bullet$ are constantly being generated, the rate-limiting step at low oxygen concentrations is formation of the peroxyl radicals necessary for propagation; this process depends on the presence and diffusion of oxygen to radical sites.

At moderate to high pO_2, sufficient oxygen is present to add to every $L^\bullet$ formed, so the rate dependence shifts from oxygen to the concentration of available oxidizable lipids and propagation processes involving LOOH:

$$\text{Rate of oxidation (non-limiting } pO_2) = k_p(k_i/2k_{t3})^{1/2}[\text{LOOH}]^{1/2}\,[\text{LH}] \tag{11.31}$$

Under these conditions, the overall rate is proportional to the available lipid (total amount and degree of unsaturation) plus propagating LOOH concentrations, and it is controlled by the balance between initiation, propagation, and peroxyl radical recombination. The rate-limiting step is hydrogen abstraction to form and accumulate LOOH. Thus, although they are not directly accounted for in rate equations or by measurement, factors such as light, heat, and metal contaminants that increase decomposition of LOOH and enhance chain branching, or conversely interfere with chain propagation (e.g. antioxidants or co-oxidation targets such as proteins), also strongly influence reaction rates.

In the monomolecular rate period, oxidation kinetics can be described by Equation 11.30 or 11.31, or by combining rate constants, more generally as:

$$\text{Oxidation rate} = -dO_2/dt = K_M\,[\text{ROOH}]^{1/2} \tag{11.32}$$

where K_M is the monomolecular rate constant. K_M can readily be determined by plotting the square root of the peroxide values versus time in early oxidation; the slope is $K_M/2$. Typical values are [10^{-2} to 10^{-3} moles oxidized/mole lipid]$^{1/2}$ per hour (Labuza, 1971).

However, in the bimolecular rate period, lipids are consumed rapidly to form hydroperoxides that subsequently decompose in pairs, and oxidation now becomes more complicated. Adding bimolecular LOOH decomposition, the generalized rate now becomes proportional to [LOOH]^2:

$$\text{Rate of oxidation} = \underbrace{\frac{k_p k_i^{1/2}}{(2k_{t1\text{-}3})^{1/2}}}_{\text{overall bimolecular rate constant } K_B}[\text{LH}]\,[\text{LOOH}] \tag{11.33}$$

In this case, both LH and LOOH are rapidly changing. A semilog plot of [LOOH]/[unreacted LH] versus reaction time will generate a curve in which the final slope equals K_B. The early and final slopes then differentiate monomolecular from bimolecular rate periods for a given system.

IV. WHICH FACTORS CONTROL LIPID OXIDATION?

The discussion of mechanisms above remains only a collection of theoretical reactions until they are connected to real systems. Foods and biological systems are much more complicated in their oxidation than the reactions described above because they are structurally complex with many catalysts, oxidation targets, and antioxidants; and all of these factors interact to influence the final kinetics, pathways, and extent of lipid oxidation. This chapter will outline the most important factors that influence lipid oxidation as a guide to evaluating each food or biological system and identifying what pro-oxidant and antioxidant factors are active. Effects of lipid composition, physical structure of the product; presence of catalysts, antioxidants, and sensitive co-oxidation targets; and environmental factors must all be considered when deciding how to formulate and process a product for stability, analyze for lipid oxidation, and finally to develop effective stabilization strategies. The latter will be discussed in Section VI.

Factors affecting lipid oxidation can be divided into five general categories (Table 11.1): nature of the lipids, surfaces, presence of other pro-oxidant and antioxidant compounds, and the environment and solvent system. Only major effects will be discussed here as an introduction.

A. Nature of Lipids

1. Degree of Unsaturation

The number of highly abstractable doubly allylic hydrogens increases with the number of double bonds, so it is not surprising that oxidizability of fatty acids in oils increases the degree of unsaturation (Table 11.2). In particular, the presence of fatty acids with three or more double bonds − linolenic in vegetable oils, arachidonic acid in animal fats, and docosahexaenoic and eicosapentaenoic acids in fish oils − markedly sensitizes oils to rapid oxidation. However, the degree of increase is not proportional to the number of double bonds because each double bond puts a bend in a fatty acid chain. Difficulty in aligning chains so that allylic hydrogens are in close juxtaposition decreases the rate of hydrogen abstraction. Also, with four or more double bonds, the acyl chains bend back on themselves, so that opposite halves of the molecule align and facilitate intramolecular hydrogen abstractions in competition with intermolecular radical transfers. Each additional double bond thus adds progressively less to the oxidation rate because multiple oxidations within molecules increase, reducing the total number of lipid molecules affected.

2. Free Fatty Acids versus Esters versus Triacylglycerols

Both pro-oxidant and antioxidant effects of fatty acids have been reported, mediated primarily through the acid group, so specific effects of free fatty acids will probably reflect the composition and environment of the food or reaction system where they are produced.

It has long been recognized that free fatty acids often oxidize more slowly than their esters (Table 11.2). This is due in large part to non-radical decomposition of hydroperoxides by the acid groups (Figure 11.10). The acidic H^+ contributed from ionized fatty acids adds to the −OOH of the hydroperoxide and induces nucleophilic rearrangement resulting in scission of the chain on either side of the peroxyl carbon. Products can be two aldehydes, as shown in Figure 11.10, or an aldehyde plus an alcohol (Uri, 1961; Scott, 1965). LOOH decomposition can also be induced by hydrogen bonding between undissociated acids (−COOH) and −OOH (Sliwiok *et al.*, 1974). These reactions prevent chain branching and slow overall oxidation.

Carboxylic acids are also excellent metal complexers and they are surface active. These two activities can be complementary or antagonistic depending on the system. Complexation blocks electron transfer orbitals in metals and usually reduces redox potential, which should decrease oxidation rates. Fatty acids can move metals to interfaces, away from double bond targets in bulk oils, but if hydroperoxides and other metal targets concentrate at the interface, oxidation can be greatly accelerated. Moving in the opposite direction, fatty acids can pick up metals at interfaces with aqueous phases and carry them into bulk oils where their polarity attracts metals strongly to polar double bonds to initiate oxidation. Because the effects of free fatty acids are unpredictable, the safest guideline is to limit their addition or prevent their formation as much as possible.

TABLE 11.1 Factors Influencing the Rate and Course of Lipid Oxidation

Nature of lipids	Degree of unsaturation	
	Free fatty acids vs esters vs TAGs	
	trans vs *cis*	
	Conjugation	
	Phospholipids	
Surface effects	Monolayer dispersion	
	Emulsions	
	Bulk lipids	
Presence of catalysts	Pro-oxidants	Preformed hydroperoxides
		Metals
		Porphyrins, e.g. chlorophyll
		Heme compounds
	Enzymes	Lipoxygenase, cyclooxygenase, xanthine oxidase
	Amino acids	
	Ascorbic acid (low concentrations)	
Presence of inhibitors	Antioxidants (endogenous and added)	
	Polyphenols	
	Metal chelators and complexers	
	Synergists	
	Amino acids	
	Enzymes	Glutathione peroxidase
	Interceptors	Proteins
		DNA
		Vitamins
		Pigments
Environment and solvent system	Temperature	
	Light	
	Oxygen pressures	
	Water	
	pH	
	Solvent	
	Packaging	

TAGs: triacylglycerols.
Modified from Schaich (2005a).

Without a free carboxyl group to induce LOOH decomposition, oxidation increases with the number of double bonds in esters and acylglycerols, and with the degree of esterification in acylglycerols (Table 11.3) (Cosgrove *et al.*, 1987). Triacylglycerols oxidize more rapidly than free fatty acids, but considerably more slowly than free esters, because the irregular organization and orientation of fatty acyl chains in triacylglycerols prevent close alignment and thus

TABLE 11.2 Effects of Degree of Unsaturation on Relative Oxidizability of Fatty Acids

	Relative Rate of Oxidation	
Fatty Acid	Fatty Acid	Ester
18:1	1	1
18:2	28	41
18:3	77	98
20:4		195

Extracted from Schaich (2005b), data from Scott (1963); used with permission.

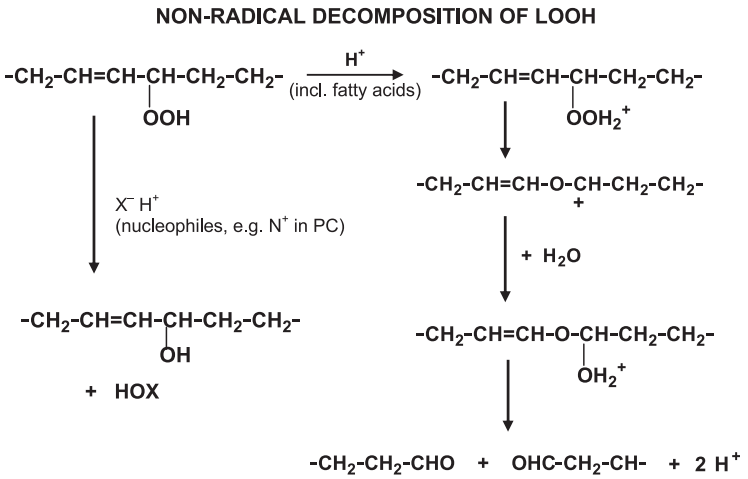


FIGURE 11.10 Non-radical decomposition of lipid hydroperoxides by acids and nucleophiles. These pathways account in part for lower rates of oxidation for fatty acids and phospholipids, particularly phosphatidylcholine, respectively. *(Figure from Schaich, 2005a; redrawn from O'Brien, 1969; used with permission.)*

TABLE 11.3 Lipid Oxidizability

	Oxidizability
	$(M^{-1/2}\ s^{-1/2} \times 10^2)$
Free fatty acids	2.03
Monoacylglycerol	2.83
Diacylglycerol	5.89
Triacylglycerol	7.98

inhibit hydrogen atom transfer between chains (Carless and Nixon, 1960), and limit access of initiators to double bonds. The arrangement of fatty acids in triacylglycerol crystals also influences oxidation: concentrating unsaturated fatty acids at *sn*-2 stabilizes triacylglycerols, while unsaturation at *sn*-1 and 3 enhances oxidation and randomization of triacylglycerols decreases oxidation (Raghuveer and Hammond, 1967). A symmetrical SUS or USU structure favors β-crystal structures, where the close association between chains thus facilitates radical transfers. In contrast, randomization shifts chains to an α-structure with less organization, and this interferes with radical transfer.

3. Trans *versus* cis *Isomers*

Lessons learned in organic chemistry apply equally well to lipid oxidation. Allylic hydrogens in *cis* double bonds are more exposed and accessible for abstraction in *cis* fatty acids than in their *trans* isomers. Thus, *cis* fatty acids oxidize more readily than their *trans* counterparts (Sargis and Subbaiah, 2003). This reactivity pattern explains in part why lipid oxidation is initially rapid then slows over time, i.e. reactive *cis* double bonds that migrate when a lipid radical is formed convert to the less reactive *trans* isomers, as was explained in Section III, A, 2, b. Decreased *trans* reactivity also has distinct consequences for secondary oxidations and for the *in vivo* action of dietary *trans* fatty acids (Pokorny *et al.*, 1976b).

4. Conjugation

Conjugation of fatty acids has very interesting effects on lipid oxidation. Normal non-conjugated double bonds tend to disrupt acyl chain associations. In contrast, conjugation of double bonds straightens the acyl chain and allows closer association of that region between chains which should facilitate hydrogen abstractions. However, the requisite doubly allylic hydrogens are missing in conjugated systems so they react more slowly in initial oxidation. On the other hand, as was considered in the sections on alternative reactions, conjugated systems facilitate both internal rearrangements and radical additions, so can exert critical directing effects on reaction pathways and product distributions in secondary stages of oxidation. Effects of conjugation can be missed or completely misinterpreted if only peroxide values are measured.

5. Phospholipids

Although the acyl chains of phospholipids follow the same oxidation pattern as triacylglycerols and fatty acids/esters, i.e. increasing oxidation with increasing unsaturation of component fatty acids, the oxidative behavior of phospholipids in oils as contaminants or as emulsifiers in multiphase products is complicated. Other phospholipid components participate in the reaction, and the fatty acid composition of phospholipids relative to other system lipids also affects reactivity. Hence, phospholipids can be either pro-oxidants or antioxidants, depending on the system and concentration (Nwosu *et al.*, 1997).

a. Pro-oxidant Effects

Fatty acids in phospholipids are highly unsaturated, often containing arachidonic acid in animal tissues, and the fatty acid acyl chains of phospholipids are highly oriented in membranes and at interfaces, which facilitates electron transfer via the aligned double bonds (Weenan and Porter, 1982). Both of these factors contribute to very rapid oxidation of phospholipids, especially phosphatidylethanolamine, which has the highest unsaturation. In mixed systems, phospholipids are preferentially oxidized and the radicals generated can seed oxidation of triacylglycerols (Nwosu *et al.*, 1997). Phospholipids bind large amounts of water (e.g. each molecule of phosphatidylcholine binds 37 molecules of water), and their hydration water allows them to mobilize catalysts. Phospholipids also complex and activate metals (Nwosu *et al.*, 1997), moving them from aqueous phases into closer contact with lipid phases.

b. Antioxidant Effects

In mixed systems, phospholipids exert a 'sparing' effect on triacylglycerols in oil phases. Exposed to catalysts at interfaces (Bishov *et al.*, 1960), phospholipids are preferentially oxidized in order of unsaturation (Sugino *et al.*, 1997). This could be pro-oxidant, but the association of phospholipids into bilayers or mesophases isolates lipid radicals and limits interaction with the oil phase. High viscosity in bilayers slows migration of catalysts, radical transfers, and chain propagation, while it accelerates terminations (Barclay and Ingold, 1981). Phospholipids also act as co-solvents for antioxidants, increasing the accessibility of tocopherol and other antioxidants to chain-initiating radicals in aqueous microenvironments (Koga and Terao, 1995).

However, the major antioxidant effect of phospholipids by far is from non-radical decomposition of LOOH by the nucleophilic choline group [$-N^+(CH_3)_3$] of lecithin (Figure 11.11, left reaction), which interrupts the radical chain and blocks further reactions (O'Brien, 1969; Corliss and Dugan, 1971). This action does not stop initiation, though, so there can be a build-up of conjugated dienes or products from alternative pathways that are not detected if lipid oxidation in systems containing phospholipids is measured only by peroxide values.

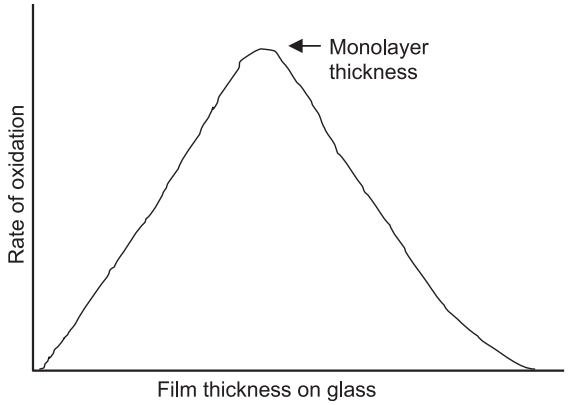


FIGURE 11.11 Effects of film thickness on oxidation of lipids spread on a surface. Maximum oxidation occurs at film thickness approximating a molecular monolayer of lipids. *(Figure from Schaich, 2005a; redrawn from Koch, 1962; used with permission.)*

Such mixed effects argue again that lipid oxidation should always be followed by assays of multiple products from different stages and pathways to provide an accurate picture of the extent and progress of oxidation.

c. Mixed Effects

Because phospholipids bind both metals and water, they can be either (or both) pro-oxidants or antioxidants depending on the system. Although effects on oxidation have been measured, few details about metal binding by phospholipids are yet available. Speculative explanations are that when the metal ligand orbitals are filled by phospholipids, when the redox potential is decreased out of the range of any reducing agents present, or when the phospholipids move metals away from reactive sites, the actions inhibit oxidation (Chen and Nawar, 1991; Yoshida *et al.*, 1991). However, metal binding can accelerate oxidation if the metal redox potential is lowered in a system with LOOH and the metal and LOOH are both concentrated at interfaces. The metal then catalyzes a cascade of LOOH decomposition and generation of $LO^{\bullet}$. Water binding by the phosphate group of phospholipids may be protective by stabilizing LOOH and metals (preventing decomposition and electron transfer, respectively) or may be destructive by activating electron transfer from metals or facilitating contact between metals and LOOH. The specific effects are determined ultimately by the specific nature of the food or reaction system, which is one reason why so many different effects of phospholipids have been reported.

B. Surface Effects

Lipid oxidation is obvious in bulk oils, but foods are complex with multiple phases and complex structure. How does the form of lipid and its organization in a food matrix affect oxidation? All food matrices have surfaces, both solid and liquid, which govern lipid exposure and molecular organization as well as contact with pro-oxidants and antioxidants. Some authorities even argue that all oxidation takes place at surfaces or interfaces, although this view is controversial. It is well established that lipid oxidation rates increase with surface area (Bishov *et al.*, 1960). For lipids dispersed on a solid surface such as a dehydrated food matrix or surfaces of crackers or snack foods, maximum oxidation occurs with dispersal at just about a molecular monolayer of lipid over the solid matrix (Koch, 1962) (Figure 11.11). Monolayer coverage is the point of maximum surface area and oxygen exposure for a lipid film. Lower coverage separates lipid molecules so radical transfers do not easily occur, while multiple layers force lipid molecules to compete for radical transfers, enhance side-reactions that reduce efficiency of the primary radical chain, and increase the thickness through which oxygen must diffuse. As bulk oil volume increases further, oxygen diffusion and interfacial character become the controlling factors, and the oxidation rate declines (Mikula and Khayat, 1985).

Films and surfaces alter oxidation pathways as well as kinetics. Spreading lipid in films in low concentrations orients chains but restricts migrational mobility of hydrogens, so abstraction reactions are greatly impeded. This favors radical recombination over hydrogen abstraction as the initial reaction, and rearrangements of $LOO^{\bullet}$ and LOOH to hydroxyepoxides, endoperoxides, and similar products (Reaction 11.34) rather than decomposition to $LO^{\bullet}$ as secondary reactions (Slawson *et al.*, 1973). As a result, fewer products are found than in emulsions or bulk

oil systems, and they are mostly internal cyclization products (Leermakers *et al.*, 1966; Porter *et al.*, 1971; Wu *et al.*, 1978).

(11.34)

Lipid oxidation is arguably considered to be fastest in emulsions which have the greatest surface area of lipids, greatest diffusion of oxygen, and greatest interface with the aqueous phase containing multiple sources of protons and catalysts (Table 11.4). A categorical statement about whether emulsions oxidize more quickly than spread surface films cannot be made since individual systems vary tremendously and relative oxidation rates are affected by many factors in addition to surfaces. Certainly, there is more lipid to sustain oxidation in emulsions, while LH supply in films is limited, so secondary oxidation can be more extensive in emulsions. Even so, initial oxidations in some films can be faster than in emulsions. For the purpose of this discussion, it is sufficient to note that any system with high surface exposure, whether solid or liquid, has a very high potential for rapid lipid oxidation.

Lipid oxidation in emulsions differs from dry film systems in that modulating effects of water, emulsifying agents, and the nature of the interface compete with dispersion and surface area enhancement of lipid oxidation. Hence, the design and even preparation method for the emulsion can have marked pro-oxidant and antioxidant effects on its oxidative stability. In general, decreasing droplet size (increased surface area) and increasing proportions of water and concentrations of emulsifying agents (Hyde, 1968) enhance initiation at the oil droplet surface (Labuza, 1971). However, the type of emulsifier has been recently shown to exert a major control over oxidation by altering or facilitating specific molecular associations. With lecithin as an emulsifier, for example, apparent oxidation rates in emulsions can be lower than on spread surfaces owing to non-radical decomposition of LOOH as described above. The reverse may be true if a negatively charged emulsifier is used and binds redox metals at oil−water interfaces. Also, emulsifiers, like antioxidants, appear to exhibit a kind of 'polar paradox' where polar and non-polar agents preferentially stabilize phases of the opposite polarity.

Contrary to what might be expected, lipid oxidation is slowest in bulk oil phases where oxygen solubility is high but diffusion limited, and surface area is usually low relative to volume. In the triacylglycerols of oils, lipid hydrocarbon chains are only loosely associated without orientation, making radical transfer relatively inefficient. Hydroperoxides that are necessary for propagation migrate to entrained water microdroplets and less hydrophobic interfaces or self-associate within the oil phase. When the hydroperoxides decompose, propagating $LO^{\bullet}$ are released away from target lipid chains with abstractable hydrogens. When high local concentrations of radicals are generated in oil phases, extensive polymerization is favored in preference to hydrogen abstraction and chain propagation (Treibs, 1948). This is one reason why viscosity increases occur in oxidizing food oils almost as rapidly as production of off-odors and flavors.

TABLE 11.4 Effects of Surface on Overall Rate Constants for Lipid Oxidation During the Monomolecular (K_M) and Bimolecular (K_B) Rate Periods

Methyl Linoleate System	K_M ($\times 10^{-3}$)	K_B ($\times 10^{-2}$)
Bulk	13	6.4
Filter paper − wet	20	6.8
Filter paper − dry	7	4.6
Emulsion	32	8.5
TAG dispersed on cellulose	1.7	0.8

TAG: triacylglycerol.
Data from Labuza (1971); extracted from Schaich (2006); used with permission.

C. Initiators and Catalysts

1. Preformed Free Radicals

Lipid oxidation is so constantly present in foods that it often seems to initiate itself. However, it must be remembered that, in foods and biological systems, many reactions occur that produce radicals. Even in trace quantities these radicals can initiate lipid oxidation by abstracting hydrogens and generating the first $L^{\bullet}$:

$$LH + R^{\bullet}, RO^{\bullet}, ROO^{\bullet} \longrightarrow L^{\bullet} + RH, ROH, ROOH \quad (11.35)$$

Free radicals that most commonly initiate lipid oxidation chains and their reactivity with oleic, linoleic, and linolenic acids are shown in Table 11.5. Hydroxyl radical ($HO^{\bullet}$), the most strongly oxidizing radical known, is produced by reduction of hydrogen peroxide that is produced by enzymes in living tissues and by metals in foods. It is so reactive that it abstracts hydrogens rather non-selectively from acyl chains (Patterson and Hasegawa, 1978; Heijman *et al.*, 1985). However, also because it is so reactive, it does not migrate, so must be generated very close to its target.

The perhydroxyl radical, $HOO^{\bullet}$, is also generated by decomposition of hydroperoxide, as well as from protonation of superoxide anion, $O_2^{-\bullet}$, the reduction product of oxygen. Its reactions are slower than $HO^{\bullet}$, but competitive with organic peroxyl radicals (Bielski *et al.*, 1985). Particularly when generated in enzyme reactions, $HOO^{\bullet}$ can be an effective initiator of lipid oxidation; $O_2^{-\bullet}$, however, reacts only with lipid hydroperoxides and does not react with unoxidized lipids (Bielski *et al.*, 1983) (Table 11.5).

As has been noted in previous sections, organic peroxyl radicals abstract hydrogens several orders of magnitude more slowly than alkoxyl radicals (1−200 vs 10^6−10^7 l/mol/s), but even so, $ROO^{\bullet}$ and $RO^{\bullet}$ arising from oxidation of non-lipid molecules in the reaction system very effectively start oxidation chain reactions in lipids.

One final comment: remember that these radicals are needed only to start the lipid oxidation chain reaction, so only trace levels are needed. Once started, the lipid oxidation chain is autocatalytic. The presence of any non-lipid radicals then competes with lipid radicals and may either augment lipid oxidation or have no effect.

2. Metals

Transition metals such as iron, copper, and cobalt are perhaps the most active initiators of lipid oxidation in foods because they are so ubiquitous; metals are also important, but less dominant, in intact plant or animal tissues where

TABLE 11.5 Hydrogen Abstraction Rates of Common Radicals that Initiate Lipid Oxidation

	Rate Constant (l/mol/s)				Radical	
Fatty Acid	18:1	18:2	18:3	20:4	Half-life (s)	References
Radical						
$HO^{\bullet\bullet}$ (allylic H)	~10^9	9.0×10^9	7.3×10^9	~10^{10}	10^{-9}	Patterson and Hasegawa (1978), Hasegawa and Patterson (1978)
(non-allylic H)	4×10^2	3.4×10^3	7.0×10^3	1.0×10^4		Patterson and Hasegawa (1978), Hasegawa and Patterson (1978)
$RO^{\bullet}$	3.3×10^6	8.8×10^6	1.3×10^7	2.0×10^7	10^{-6}	Erben-Russ *et al.* (1987)
$ROO^{\bullet}$	1.1	6×10^1	1.2×10^2	1.8×10^2	10	Hasegawa and Patterson (1978)
$O_2^{-\bullet}$	No reaction	No reaction	< 1	< 1		Bielski *et al.* (1985), Gebicki and Bielski (1981)
(MLOOH)		7.4×10^3				Bielski *et al.* (1983)
$HOO^{\bullet}$	No reaction	1.1×10^3	1.7×10^3	3.1×10^3		Bielski *et al.* (1985)
$O^{-\bullet}$	7.5×10^2	9.7×10^3	1.2×10^4	1.9×10^4		Hasegawa and Patterson (1978)

Adapted from Schaich (2005b).

compartmentalization and metal complexers in cells slow the access of metals to lipids. Both metal valences are active (Schaich, 1992). Oxidized metals are stronger direct initiators, forming initial radicals by withdrawing an electron from the double bonds (Reaction 11.36). In contrast, reduced metals tend to be indirect initiators; they react preferentially with oxygen to form complexes (Reaction 11.37) or reduced oxygen species (Reactions 11.38 and 11.39) that can then react more efficiently with lipids. Reducing metals also decompose trace levels of unreactive hydroperoxides to reactive alkoxyl radicals that initiate free radical chains (Reactions 11.40 and 11.41).

Direct:

$$RCH{=}CHR + M^{(n+1)+} \longrightarrow \overset{+}{R}CH{-}\overset{\bullet}{C}HR + M^{n+} \xrightarrow{LH} \left[L^{\bullet} + RH \right] \tag{11.36}$$

Indirect:
(a) formation of active metal−oxygen complexes:

$$M^{n+} + O_2 \longrightarrow [M^{(n+1)+}\ldots O_2^{-\bullet}] \xrightarrow{LH} L^{\bullet} + M^{n+} + HO_2^{\bullet} \xrightarrow{L'H} L'^{\bullet} + H_2O_2 \tag{11.37}$$

(b) autoxidation of reduced metals (active at moderate to high pO_2):

$$Fe^{2+} + O_2 \longrightarrow Fe^{3+} + O_2^{-\bullet} \overset{H}{\rightleftharpoons} HOO^{\bullet} \xrightarrow{L} L^{\bullet} + H_2O_2 \tag{11.38}$$

$$O_2^{-\bullet} + HOO^{\bullet} \longrightarrow H_2O_2 \xrightarrow{Fe^{2+}} Fe^{3+} + HO^{-} + {}^{\bullet}OH \xrightarrow{LH} H_2O + L^{\bullet} \tag{11.39}$$

(c) reduction or oxidation of hydroperoxides (ROOH, H_2O_2, LOOH) (dominates with low metal, substrate, and oxygen concentrations):

$$ROOH + M^{n+} \xrightarrow{fast} RO^{\bullet} + {}^{-}OH \tag{11.40}$$

$$ROOH + M^{(n+1)+} \xrightarrow{slow} ROO^{\bullet} + H^{+} \tag{11.41}$$

Decomposition of hydrogen peroxide and miscellaneous organic peroxides by metals provides a major source of radicals for initiating for lipid oxidation. However, hydroperoxide decomposition by metals is just as important in accelerating chain propagation and branching in later stages of oxidation when higher concentrations of LOOH accumulate. This critical influence on both initiation and propagation of lipid oxidation provides solid arguments for why metals are generally considered the most important lipid oxidation catalysts in foods and also in biological systems.

Although oxidized and reduced metals are each active alone, their catalytic effectiveness is amplified tremendously when redox cycling occurs (Schaich, 1992). The combination of oxidized and reduced states of metals, particularly iron, creates a powerful factory for continuous cycling of oxygen and active catalysts, while generating new lipid radicals, as shown in Reaction Series 11.42. This can involve cycling either hydroperoxide oxidation and reduction (Reaction 11.42), or combining oxidation of double bonds to generate $L^{\bullet}$ with reduction of LOOH to generate $LO^{\bullet}$ (Reaction 11.42a). Rates of cycling can be accelerated by several orders of magnitude in the presence of reducing agents, particularly ascorbic acid, that maintain the faster reacting lower valence state in metals.

Cycling:

Cycling

$$\mathbf{Fe^{2+}} + \begin{Bmatrix} ROOH \\ LOOH \end{Bmatrix} \xrightarrow{fast} \mathbf{Fe^{3+}} + \begin{Bmatrix} RO^{\bullet} + OH^{-} \\ LO^{\bullet} + OH^{-} \end{Bmatrix} \xrightarrow{LH} \begin{Bmatrix} ROH + L^{\bullet} \\ LOH + L^{\bullet} \end{Bmatrix}$$
$$\downarrow ROOH$$
$$\mathbf{Fe^{2+}} + ROO^{\bullet} + H^{+} \xrightarrow{LH} ROOH + L^{\bullet} \tag{11.42}$$

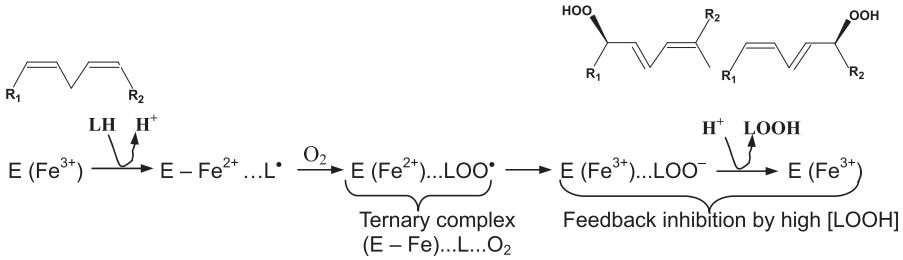
$$LH + M^{(n+1)+} \longrightarrow L^{\bullet} + H^{+} + M^{n+} \xrightarrow[LH]{O_2} LOOH + L^{\bullet}$$
$$LOOH + M^{n+} \longrightarrow LO^{\bullet} + OH^{-} + M^{(n+1)} \tag{11.42a}$$
$$\uparrow AH \rightarrow$$

3. Enzymes

Enzymes are a major class of lipid oxidation initiators in unprocessed tissues, including postharvest plants and grains and postslaughter meats. Normally separated from their substrates in plant and animal cells, enzymes are brought into contact with their lipid substrates when food preparation and processing disrupts cell organization. Lipoxygenase reacts directly with lipids to form hydroperoxides without radicals, while other enzymes catalyze oxidations that generate radicals capable of initiating lipid oxidation. Their basic reactions are described below.

a. Lipoxygenase

Lipoxygenases are iron metallo enzymes that catalyze the aerobic oxidation of fatty acids with *cis*-1,4-pentadiene structures to generate conjugated LOOHs without releasing lipid free radicals (Reaction 11.43). To do this, the enzyme binds the diene section of the fatty acid in its active site and, via the iron also bound there (Aoshima *et al.*, 1977), removes the bisallylic hydrogen (Egmond *et al.*, 1973; deGroot *et al.*, 1975). The acyl chain rotates, oxygen bound to a separate site on the enzyme reacts with the free radical, and H^+ is donated from histidine residues in the active site to complete the LOOH before it is released. The resulting conjugated dienes are always *trans*, *cis* relative to the hydroperoxide (Egmond *et al.*, 1972).



(11.43)

Lipoxygenase action by itself does not initiate lipid oxidation. However, lipoxygenase acts as a kind of engine for producing reservoirs of LOOH that later decompose to generate initiating $LO^{\bullet}$ and $^{\bullet}OH$ radicals by light and heat, $LO^{\bullet}$/$LOO^{\bullet}$ by metals, or $LO^{\bullet}$ in secondary reactions of the enzyme itself (Aoshima *et al.*, 1981). Very low LOOH levels produced in plant or animal tissues may provide the 'invisible' initiators that make lipid oxidation sometimes appear spontaneous. Under some conditions (e.g. cold and dark, as in frozen unblanched materials), LOOH produced by lipoxygenases can accumulate to relatively high levels, then lead to a cascade of rapid oxidation when LOOH decomposes.

The rate and specificity of lipoxygenase oxidations depend on the type and concentration of fatty acids, oxygen concentration, pH, and enzyme source and isozyme form. The preferred substrate is linoleic acid; linolenic and arachidonic acids are also oxidized but more slowly (Gardner, 1986). There are three different isozyme forms of lipoxygenase that differ in their specificity for C9 and C13 on linoleic acid, their pH optima, and their phase preference for reactions:

- LPOx-1: optimum pH = 9, prefers charged substrates, (e.g. $LOO^{\bullet}$), has no phase specificity, so reacts equally in organic and aqueous phases
- LPOx-2: optimum pH ~6.8; prefers un-ionized acids, esters, and organic phases; reactions are depressed in emulsions; strong co-oxidation capabilities
- LPOx-3: optimum pH ~6.8, prefers emulsions; strong co-oxidation capabilities.

In addition to these general preferences, isozymes from different materials add their own specificities or eccentricities. Examples are shown in Table 11.6. It should also be noted that at pH ~6, lipoxygenases have some hydroperoxidase activity wherein they catalyze the decomposition of LOOH to LOH (Reaction 11.44). This action contributes to formation of some characteristic flavor compounds in plant tissues (e.g. cucumber).

$$\text{Hydroperoxidase activity of LPOx:} \quad \text{LOOH} \xrightarrow{\text{LPOx}} \text{LOH} + \text{H}_2\text{O} \tag{11.44}$$

Lipoxygenases are found in low levels in all biological materials. Relatively high lipoxygenase levels are found in grains, peanuts, tomatoes, potatoes, soybeans and other legumes, beans, peas, and muscle tissue.

TABLE 11.6 Reaction Characteristics of Lipoxygenases from Various Plant Sources

Plant	pH Optimum	Position Preference	Reference
Soybean (1)	8.5–9.0	13-OOH	Hamberg and Samuelsson (1967)
	7.0	13-OOH:9-OOH 2:9	
	6.5	9-OOH	
Soybean (2)	6.1	13-OOH:9-OOH 80:20	Fukushige *et al.* (2005)
Soybean (3)	6.5	13-OOH:9-OOH 40:60	
Corn	6.6	Mostly 9-OOH (10t, 12c)	Gardner and Weisleder (1970)
		Small amount of 13-OOH (9t, 11t)	
	9.0	13-OOH:9-OOH 85:15	Veldink *et al.* (1972)
Wheat (1)	5.7–6.5	13-OOH:9-OOH 15:85	Grosch *et al.* (1976)
Peanut (1)	6.0	100% 13-OOH	Belitz and Grosch (1986)
	6.2	84% 13-OOH (9c, 11t)	Pattee and Singleton (1979)
		16% 9-OOH (10t, 12c)	
	8.3	10% as much product formed	
		13-OOH:9-OOH 2:1	
		Less 13-OOH at low pO_2	
Tomato (1)	5.5	13-OOH:9-OOH 95:5	Belitz and Grosch (1986)
Peas (2)	6.5	13-OOH:9-OOH 50:50	Belitz and Grosch (1986)

Numbers in parentheses refer to the isozyme form.

b. Xanthine oxidase

Xanthine oxidase is an enzyme with two flavin adenine dinucleotide molecules, two molybdenum atoms, and eight iron atoms in its active site. It catalyzes the oxidation of hypoxanthine to xanthine, with the simultaneous reduction of oxygen to superoxide anion, $O_2^{-\bullet}$. $O_2^{-\bullet}$ dismutates to form hydrogen peroxide, which decomposes to reactive hydroxyl radicals that initiate lipid oxidation (Kellogg and Fridovich, 1975; Porter and Lehman, 1982; Thomas *et al.*, 1982). This reaction is particularly important in initiating lipid oxidation in dairy products.

$$\text{hypoxanthine} + O_2 \longleftrightarrow \text{xanthine} + O_2^{-\bullet} \tag{11.45}$$

$$\text{xanthine} + O_2 \longleftrightarrow \text{uric acid} + O_2^{-\bullet} \tag{11.45a}$$

$$2\,O_2^{-\bullet} + H_2O \longrightarrow 2\,O_2^{-\bullet}/HOO^{\bullet} \longrightarrow 2\,H_2O_2 \xrightarrow{M^+} 2\,HO^{\bullet} + 2\,HO^- \tag{11.45b}$$

M^+ is a reducing metal.

4. Hemes

The ability of heme proteins such as hematin, hemoglobin, myoglobin and metmyoglobin, catalases and peroxidases to catalyze lipid oxidation much more rapidly than free iron was first reported nearly 90 years ago. The pioneering work of Watts (Watts, 1954, 1962; Younathon and Watts, 1959) and Tappel (1953a, b, 1955, 1961, 1962, 1995) in the

1950s and 1960s established the effects clearly, but the mechanisms of heme action remained controversial. Several characteristics or requirements for heme catalysis, however, were identified (Schaich, 1980a, 1992):

- the porphyrin–Fe structure is required (not just iron)
- not all heme proteins show the same catalytic activity, due in part to differences in degree of exposure and/or accessibility of the hematin structure
- Fe^{3+}-hemes are most active even without oxygen; Fe^{2+}-hemes require oxygen for catalysis
- no change of heme iron valence is involved in catalysis
- catalysis reverses to inhibition at high heme levels.

From these observations, three fundamental mechanisms were proposed: (1) fundamental electron transfer from ferric hemes (Watts, 1954, 1962; Younathon and Watts, 1959); (2) hemes form complexes with hydroperoxides, and alkoxyl radicals generated in subsequent decomposition of the complex then increase rates of chain propagation (Tappel, 1953a, b, 1955, 1961, 1962, 1995); and (3) free inorganic iron released from hemes, rather than the hemes themselves, catalyzed lipid oxidation (Love and Pearson, 1974). While each of these theories addresses some characteristics, none of them is completely consistent with kinetics, structure–activity patterns, product mixes, and solvent effects of heme catalysis (Schaich, 1980a).

Modern analytical techniques have now revealed that heme catalysis involves generation of ferryl iron – Fe(IV)=O or Fe(IV)-OH – in complexes of the hemes with hydroperoxides or oxygen (Rao *et al.*, 1994; Nam *et al.*, 2000a, 2003). Active proteins bind fatty acids in a heme pocket, with the hydrocarbon end inside and the double bonds oriented towards the heme iron (Rao *et al.*, 1994; Nam *et al.*, 2000a, 2003). Ferric heme iron combines with a hydroperoxide or oxygen to form Fe(IV), which is a very strong oxidant, equivalent to $HO^{\bullet}$ in reaction rates but more selective owing to a lower redox potential (Traylor and Xu, 1990). Ferryl iron then rapidly abstracts H from the doubly allylic C11 of linoleic acid (Rao *et al.*, 1994) or even more rapidly from hydroperoxides (Traylor and Xu, 1990), in contrast to the very slow oxidation of hydroperoxides by non-heme Fe^{3+}:

$$(P^{\bullet+})Fe^{4+}(O) + LOOH \xrightarrow{\text{very fast}} LOO^{\bullet} + Fe^{3+}OH + H_2O \tag{11.46}$$

Fe^{4+} states are maintained by shuttling redox electrons between states in the apoprotein without involving the iron center.

$$(P^{\bullet+})Fe^{4+}(O) \rightleftharpoons (P)Fe^{4+}(OH) \tag{11.47}$$

Thus, electrons can be shuttled easily between two reactive states without the loss of oxidizing power or reduction of Fe^{4+} to less reactive Fe^{3+} (Nam *et al.*, 2000b). In this way, the hemes become a kind of engine for continuously producing cascades of radicals and rapidly catalyzing lipid oxidation.

Figure 11.12 shows a general representation of the current understanding of ferric heme-mediated formation and reaction of Fe^{4+} (Schaich, 2005b). The three reaction streams support three unique characteristics differentiating heme catalysis from other oxidants: a cascade of radical propagations and extremely rapid oxidation rates plus high proportions of epoxides and hydroxylated products (Dix and Marnett, 1983, 1985; Nam *et al.*, 2000a, b).

Fe^{2+}-hemes generate ferryl complexes but more slowly than ferric hemes. They either use hydroperoxides as oxygen sources or react directly with oxygen to form ferryl iron.

$$(P)Fe^{2+}(H_2O) + \tfrac{1}{2}\,O_2 \longrightarrow Fe^{4+}(O) + H_2O \tag{11.48}$$

This ability to use oxygen directly provides constant replenishment of $Fe^{4+}(O)$ in longer reactions. Fe^{2+} hemes may also contribute to delayed catalysis by regenerating more rapidly reacting Fe^{3+} hemes after some peroxyl radicals have been formed (Bruice *et al.*, 1988).

$$ROO^{\bullet} + Mb(Fe^{II})\,O_2 \longrightarrow ROOH + Mb(Fe^{III}) + O_2 \tag{11.49}$$

Altogether, these characteristics make Fe^{2+}-hemes more important in maintaining oxidation over long periods during storage, in contrast to the rapid immediate action of Fe^{3+}-hemes. Mechanisms of heme catalyses of lipid oxidation have been reviewed in detail by Schaich (2005b); heme catalyses of lipid oxidation in foods has been reviewed by Baron and Andersen (2002).

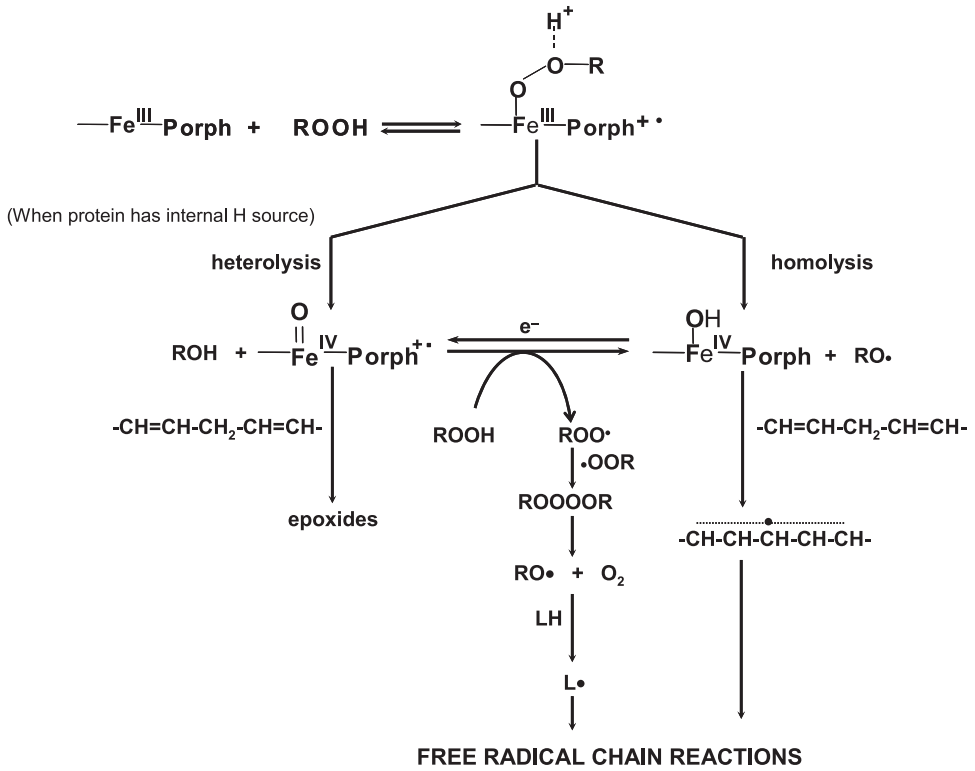


FIGURE 11.12 Formation and reaction pathways of ferryl iron in catalysis of lipid oxidation. *(Figure from Schaich, 2005a; data from Nam* et al.*, 2000a, b; used with permission.)*

5. Chlorophyll and Other Pigments

With extended conjugated double-bond systems and/or activated carbonyls, chlorophyll and many other pigments act as light gatherers in plants. They retain that ability even outside the plant system, so when present in foods or as contaminants in oils, they act as powerful photosensitizers. Photosensitizers absorb low-energy visible light which is normally inert, are bumped into excited states, then transform the excitation energy to reactive species, either free radicals or singlet oxygen, $^1O_2$, which then react rapidly with lipids. This process will be explained in detail in the next section.

Chlorophyll is probably the most notorious oxidation catalyst among pigments because it can oxidize lipids by both free radicals and singlet oxygen (Figure 11.13); even traces of chlorophyll remaining in oils or lipid extracts

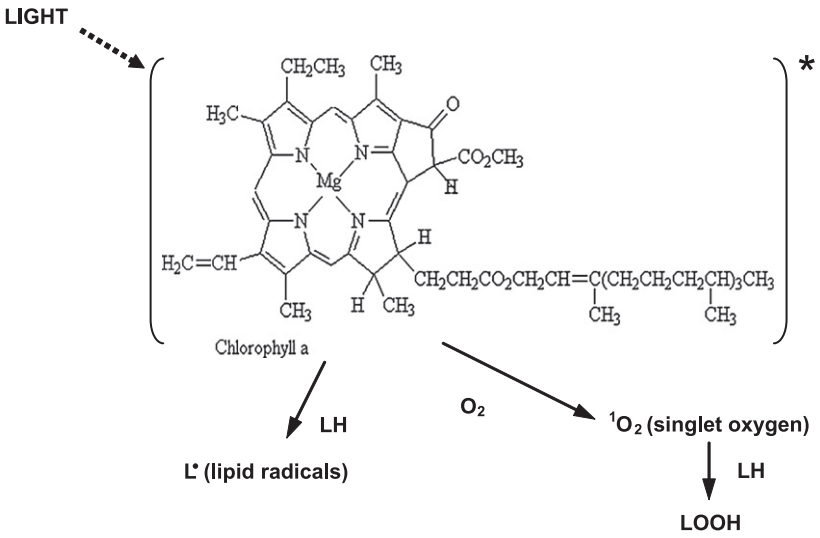


FIGURE 11.13 Multiple modes of photosensitization active with chlorophyll.

rapidly set off lipid oxidation. Many synthetic food colors are known to be active photosensitizers (Umehara *et al.*, 1979), and it is likely that other natural pigments similarly influence lipid oxidation.

One interesting point about pigments is that they have the potential to be both pro-oxidants and antioxidants. With extended regions of conjugated double bonds, carotenoids act as $^1O_2$ quenchers, offering multiple double bonds for formation of hydroperoxides in preference to reactions with lipids. Thus, at low concentrations carotenoids, especially β-carotene, are antioxidants (see also Section VI). However, carotenoid alkoxyl and peroxyl radicals are reactive, and at high concentrations these pigments become pro-oxidants. It is likely that other pigments act in the same manner, so the safest approach is to limit pigment concentrations in lipid-containing foods.

D. Environment and Solvent System

1. Light

a. Ultraviolet Light

Direct initiation of lipid oxidation by light is not a favorable reaction because light energy at wavelengths above 200 nm is insufficient to break bonds and also provide activation energy for ionization and then push the molecular fragments apart (Table 11.7).

$$\mathrm{LH} \xrightarrow{h\nu} \mathrm{L}^{\bullet} + {}^{\bullet}\mathrm{H} \quad \text{or} \quad \mathrm{L_a}^{\bullet} + {}^{\bullet}\mathrm{L_b} \tag{11.50}$$

The principal UV-absorbing groups of lipids are carbonyls, double bonds, and peroxide O—O bonds. Of these, only O—O bonds have bond energies accessible to UV light. Thus, catalysis of lipid oxidation by UV light is mediated through homolytic scission of any preformed hydroperoxides to generate the true initiators — $LO^{\bullet}$, $HO^{\bullet}$, and $RO^{\bullet}$ — that abstract hydrogens from lipid molecules to begin the free radical chain reaction. What makes UV light so important as an initiator is that it generates two reactive radicals and consequently two lipid oxidation chains for each hydroperoxide decomposition, and perhaps more importantly, it generates a very strongly oxidizing hydroxyl radical, $HO^{\bullet}$, instead of a hydroxide ion.

$$\left.\begin{matrix}\mathrm{ROOH}\\ \mathrm{HOOH}\\ \mathrm{LOOH}\end{matrix}\right\} \xrightarrow{h\nu\ (uv)} \left\{\begin{matrix}\mathrm{RO}^{\bullet} + {}^{\bullet}\mathrm{OH}\\ \mathrm{HO}^{\bullet} + {}^{\bullet}\mathrm{OH}\\ \mathrm{LO}^{\bullet} + {}^{\bullet}\mathrm{OH}\end{matrix}\right\} \xrightarrow{2\,\mathrm{L_2H}} \begin{matrix}\mathrm{ROH} + \mathrm{H_2O} & (11.51)\\ 2\,\mathrm{L_2}^{\bullet} + 2\,\mathrm{H_2O} & (11.51a)\\ \mathrm{LOH} + \mathrm{H_2O} & (11.51b)\end{matrix}$$

TABLE 11.7 Photon Energies (E_p) Available in Light at Various Wavelengths in Comparison to Bond Dissociation Energies (BDEs) of Important Functional Groups

Bond	BDE (kJ/mol)[a]	Wavelength (nm)	E_p (kJ)[b]
C=C	612	200	596
O—H	463	230	518
C—H	412	260	458
C—O	360	290	411
C—C	348	320	372
C—N	305	350	341
O—O	157	410	291

[a] *Hecht (2000)*
[b] *Atkins and Beran (1990), Kamiya* et al. *(1963).*

ROOH is any organic hydroperoxide, HOOH is hydrogen peroxide, LOOH is a lipid hydroperoxide.

Light is always around unless deliberately excluded in dark or packaging. Altogether, these factors make UV light one of the most powerful catalysts of lipid oxidation. Hence, consideration of light effects is critical when handling and processing lipids in any form: as reagents, in oils, or in foods.

b. Visible Light

Visible light (> 400 nm) lacks the energy to produce radicals directly, but it can initiate lipid oxidation indirectly through photosensitizers (Table 11.8), molecules that absorb low-level light energy, and transform it to chemical energy sufficient to drive reactions. In this process, called photosensitization, a sensitizer molecule ^1S absorbs light and is excited to a high energy state $^3S^*$, then transfers the excitation energy either to molecular bonds in lipids to form free radicals directly (Type 1) (Foote, 1976) or to oxygen to form singlet oxygen $^1O_2$, which then adds to double bonds of unsaturated fatty acids (Type 2) (Murray, 1979):

Type 1 sensitization (free radical) $\longrightarrow$ $L^\bullet$ (e^- transfer reaction)

$$^1S \xrightarrow{h\nu} {}^3S^* \begin{cases} \xrightarrow[-H^+]{LH} {}^\bullet SH + {}^\bullet L \xrightarrow{O_2} {}^0S + LOO^\bullet \xrightarrow{H} LOOH \\ \xrightarrow[-e^-]{L} (S^{-\bullet} + L^{+\bullet}) \text{ or } (S^{+\bullet} + L^{-\bullet}) \xrightarrow[H^+]{O_2} {}^0S + LOOH \end{cases} \tag{11.52}$$

Type 2 sensitization ($^1O_2$, singlet oxygen) $\longrightarrow$ LOOH (no free radicals produced)

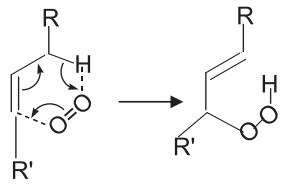
$$^1S \xrightarrow{h\nu} {}^3S^* \begin{cases} \xrightarrow{^3O_2} {}^1S + {}^1O_2 \xrightarrow{LH} {}^0S + LOOH \\ \xrightarrow{^3O_2} S\text{-}O_2^* \begin{cases} \rightarrow {}^1S + {}^1O_2 \xrightarrow{LH} LOOH \\ \rightarrow {}^0S + LOO^\bullet \xrightarrow{H^+} LOOH \end{cases} \end{cases} \tag{11.53}$$

The dominant reaction depends on the photosensitizer. Some sensitizers (such as chlorophyll) can act by either radical or singlet oxygen reactions (Sastry and Lakshminarayana, 1971; Terao and Matsushita, 1977b) with the dominant mechanisms determined by reaction conditions and solvent; other sensitizers act by only one mechanism, radical or singlet oxygen (Murray, 1979) (Table 11.8).

TABLE 11.8 Photosensitizers Found in Foods or Used Commonly in Food Chemistry Research

Type 1 – Free Radical	Type 2 – Singlet Oxygen
Chlorophyll, pheophytin	Chlorophyll
Hemes – protoporphyrin	Hemes
– myoglobin	Erythrosine
– hemoglobin	Rose bengal
– flavins (especially riboflavin)	Flavins
Xanthenes	Methylene blue
Anthracenes	Proflavine
Anthroquinones	Eosin
Crystal violet	Food dyes (?)
Food dyes	

Type 1 photosensitization generates radical chain reactions that are indistinguishable from normal autoxidations, while Type 2 photosensitization is direct oxygenation in which singlet oxygen forms hydroperoxides 1500 times faster than with normal triplet oxygen (Rawls and Van Santen, 1970). In a concerted 'ene' reaction, $^1O_2$ attaches to either carbon of a double bond and adds an allylic proton to form a hydroperoxide directly in a cage reaction without generating radicals:



(11.54)

An important distinguishing characteristic of singlet oxygen oxidation is that it shows no preference for a particular carbon in the double bond, so yields comparable amounts of hydroperoxides at both internal and external positions for isolated or non-conjugated double bonds in unsaturated fatty acids (Terao and Matsushita, 1977a). (Remember that autoxidation generates hydroperoxides primarily at external positions.) $^1O_2$ addition to conjugated double bonds (e.g. natural conjugated linoleic acid or oxidized linoleic acid) yields cyclic endoperoxides in high concentrations:

(11.55)

When exposed to UV light, heat, or metals, these hydroperoxides then decompose in a cascade of radicals that can lead to explosive oxidation. What makes singlet oxygen sensitization so damaging as an initiator of lipid oxidation is that the rate of hydroperoxide formation depends primarily on the concentration of the sensitizer, not oxygen, and the sensitizers can act in parallel rather than in series. Consequently, high concentrations of hydroperoxides can be generated in a short time.

Light catalysis of lipid oxidation can easily be detected in kinetic patterns and product distributions (Table 11.9). In early stages of autoxidation, formation of $LOO^{\bullet}$ depends on oxygen, and an induction period with very low or no hydroperoxides is characteristic. Antioxidant strategies are designed to extend the induction period as long as possible. In contrast, photosensitized oxidations depend on concentrations of sensitizers, are independent of pO_2 except under very limited oxygen, and show no induction period, accumulating high levels of hydroperoxides immediately. Eliminating light during handling and by packaging thus has dramatic immediate effects on photosensitized oxidations, but will only affect autoxidations after LOOH accumulation. Similarly, as was discussed in Section IV, A, 1, oxidation rates increase geometrically with numbers of double bonds in autoxidation owing to abstraction sensitivity of doubly allylic hydrogens. However, photosensitized oxidation rates are directly proportional to the number of double bonds since $^1O_2$ shows no preference in bond addition (Terao and Matsushita, 1977a).

Increased proportions of non-conjugated and internal 10- and 12-hydroperoxides, as well as cyclic products, can also be used diagnostically to differentiate photosensitization from autoxidation (Table 11.9). Finding these products is a clear indication of light effects, so protection of the product from light during processing and with packaging will be critical for stabilization. It is important to note also that internal and cyclic hydroperoxides decompose into different secondary products than those resulting from autoxidation, so 'light-struck' oils and foods will have altered flavors, odors, and co-oxidations of other molecules compared to 'rancid' products. Cyclic products, in particular, are thought to be important precursors of light-induced off-flavors (Neff *et al.*, 1982). Flavor reversion in soybean oil is a light-induced oxidation (Chang *et al.*, 1966).

2. Heat

When considering heat effects on lipid oxidation, storage temperatures are always the first targets. However, heat-induced changes are much more pronounced at the high temperatures used in processing than at typical transportation and storage temperatures. Even relatively small increases in processing or storage temperatures can dramatically

TABLE 11.9 Comparison of Kinetics and Products of Free Radical Autoxidation and Type 1 Photosensitization versus Singlet Oxygen Type 2 Photosensitized Oxidation

	Free Radical – Type 1 Autoxidation	$^1O_2$ – Type 2 Photosensitization
Mechanism	Conventional free radical chain reaction	'ene' reaction, concerted addition of $^1O_2$, no free radicals involved
		Radicals generated by LOOH decomposition
Kinetics	Induction period present	No induction period
	Dependent on pO_2	Independent of O_2 (when O_2 not limiting)
		Dependent on sensitizer concentration
	Rate not proportional to number of double bonds	Rate directly proportional to number of double bonds
Relative reactivity	18:1 ~1	18:1 ~1
	18:2 17	18:2 2
	18:3 25	18:3 3
Products	Conjugated LOOHs	Both non-conjugated and conjugated LOOHs
	External 9, 13, 16 positions dominant	LOOHs at all positions
	Scission products all from external LOOHs	High proportion of cyclics, endoperoxides, epoxides, dihydroperoxides, and trihydroperoxides at internal positions
		Scission products from internal LOOHs, altered scission position preferences

Data from Foote (1968, 1976), Kearns and Khan (1969), Foote *et al.* (1970), Murray (1979), Neff (1980), Neff and Frankel (1984), Neff *et al.* (1982), Frankel *et al.* (1982), Frankel (1985, 1987), Pryor (1978), Umehara *et al.* (1979). Adapted from Schaich (2005a); used with permission.

shorten shelf-life. Thus, control of temperature effects on lipid oxidation must be addressed at every stage of food production, handling, and storage.

Heat has three major effects on lipid oxidation:

- Especially at low to moderate temperatures, heat accelerates lipid oxidation by increasing the rate of LOOH decomposition and initiation of secondary chains, so faster oxidation kinetics is where heat effects are most clearly observed (Labuza, 1971; Marcuse and Fredriksson, 1968).
- Heat mediates shifts in dominant reaction pathways and resulting products:
  - hydrogen abstraction from LOH and LOOH in preference to LH (Frankel, 1982)
  - less scission, altered scission patterns (e.g. hexanal to decadienal from linoleic acid), and increased di 90% dimers and polymers (Pokorny *et al.*, 1976a)
  - increased dimerization and polymerization, changes in types of dimers formed: more C–O–C and C–C dimers and fewer C–O–O–C cross-links (Frankel, 1984, 1991)
  - increased *trans* isomers (Piretti *et al.*, 1978).
- At high temperatures, e.g. frying processes, > 150°C, thermal energy induces scissions of acyl chains to provide a base of radicals that underlie all molecular degradation and initiate chains of autoxidation when air is present (Nawar, 1969, 1986).

In the interest of space, only the first of these effects will be discussed here.

a. Thermal Decomposition of Hydroperoxides at Low to Moderate Temperatures

The effects of low to moderate heat on LOOH decomposition are rather straightforward. In any system, heat gives the greatest boost to reactions that have high activation energies. Of all the steps in lipid oxidation, only

TABLE 11.10 Activation Energies for the Various Reaction Steps in Lipid Autoxidation

Reaction	Activation Energies (E_a) (kcal/mole)
($L^\bullet + O_2$)	0
k_p ($LOO^\bullet + LH$)	~5–15
k_t (2 $ROO^\bullet$)	~4
k_t (2 $R^\bullet$)	5
k_t ($R^\bullet + ROO^\bullet$)	1
* k_{d3} (monomolecular) LOOH →	31
* k_{d3} (bimolecular) 2 LOOH →	50 (uncatalyzed system)

Data from Labuza (1971) and Marcuse and Fredriksson (1968).

hydroperoxide decomposition has energy hurdles, requiring 31 and 50 for monomolecular and bimolecular LOOH decomposition, respectively (Table 11.10). Thus, at the low to moderate temperatures used in most handling, storage, and some processing, heat acts primarily by breaking the O–O bonds in traces of organic or lipid hydroperoxides preformed by metals, lipoxygenases, photosensitizers, or other reactions. The reactive $RO^\bullet$, $LO^\bullet$, and $^\bullet OH$ radicals generated then abstract hydrogens from neighboring lipids to form $L^\bullet$ and initiate radical chains. As oxidation progresses and appreciable levels of authentic LOOH accumulate, the major effect of heat shifts to acceleration of propagation rather than initiation (Marcuse and Fredriksson, 1968; Labuza, 1971).

3. Water

No discussion of lipid oxidation is complete without consideration of moisture effects. As shown in the classical graph of degradation processes versus water activity (Figure 11.14), lipid oxidation is the only degradation reaction in foods that cannot be stopped by sufficient removal or binding of water. Lipid oxidation is rapid at high moistures and water activity, as are all reactions (region D), but unlike other degradation reactions, lipid oxidation is just as rapid in very dry systems (region A). Lipid oxidation is lowest (region B) when only a monolayer of water molecules is bound to macromolecules in foods.

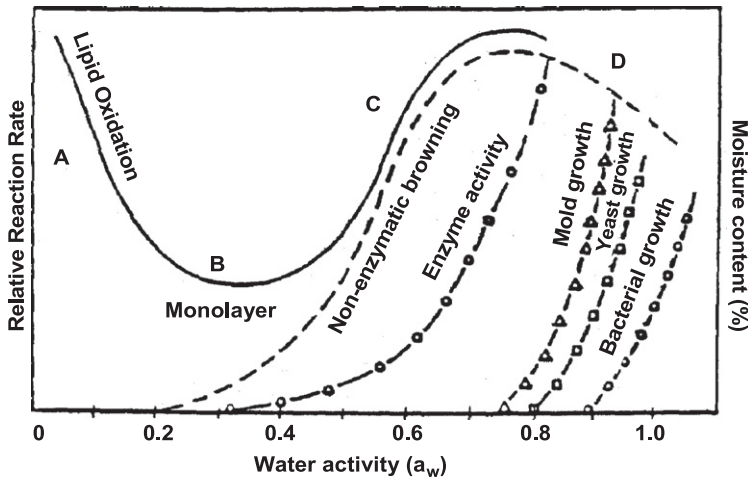


FIGURE 11.14 Classical graph of effects of water activity on chemical reactions in foods. *(Adapted from Karel, 1980.)*

Water has both pro-oxidant and antioxidant effects on lipid oxidation, as shown by the rate changes between regions on the graph. This biphasic action may be explained as follows (Labuza, 1971; Karel, 1980):

- A, Very dry systems − high oxidation: molecular sites of oxidation are bare, providing facile access of oxygen to lipids, metals are unhydrated and reactive, hydroperoxides are uncomplexed and decomposed readily, the food matrix is open and porous, allowing free flow of oxygen.
- A → B, Water binding to molecules provides surface protection, decreasing oxidation: water hydrogen bonding to LOOH retards decomposition, hydration of metals inhibits electron transfers and shifts redox potentials, water binding to surfaces of the macromolecular matrix retards oxygen diffusion, water attachment to potentially reactive sites excludes oxygen.
- B, Monolayer value − lowest oxidation: conceptual single layer of water molecules bound to molecular surface protects reactive sites from oxygen and catalysts but is insufficient for mobilization.
- B → C, Intermediate moisture region − increasing oxidation: hydration of molecules increases molecular mobility and reactivity; multilayers of water accumulating on molecular surfaces dissolve catalysts and increase their diffusion and interaction with lipids, and mobilize and activate metals.
- C → D, Fluid water region − high but decreasing oxidation: water dilutes metals and other catalysts and reactants, emulsion formation separates lipid phases from most catalysts, high moisture promotes non-enzymatic browning, products are antioxidants.

Figure 11.14 shows clearly that the moisture content and water activity (a_w) strongly influence the rate of oxidation of products with unsaturated fatty acids, over and above all other catalysts present. Although foods seldom move from one extreme moisture level to the other during storage, they do constantly dry out or absorb moisture to moderate extents during storage, and each moisture change can significantly alter the kinetics and products of lipid oxidation. For example, a dry product stabilized at the monolayer a_w can rapidly oxidize when it either loses or gains moisture and changes a_w. Intermediate moisture foods are moderately reactive in oxidation, but they can be stabilized by dehydration or their shelf-life can be severely shortened by gaining moisture and increasing a_w. Consequently, moisture content and degree of water binding by system molecules (a_w) must be carefully controlled by formulation and packaging in order to stabilize foods and other materials against lipid oxidation.

4. Oxygen

Oxygen is obviously a major player in lipid oxidation, forming peroxyl radicals by addition to $L^\bullet$ radicals, so it is logical to expect that oxidation is dependent on and proportional to the amount of oxygen available. However, oxygen addition is diffusion controlled and almost instantaneous, so it is not the rate-limiting step in lipid oxidation. The key issue in sustaining oxidation is that enough oxygen must be present to react with all the $L^\bullet$ formed. When $O_2 < LH$, oxidation does not progress at full steam, and the rate of oxidation increases rapidly with increasing pO_2 as long as oxygen is limiting. For most lipid oxidations, formation of $LOO^\bullet$ depends on O_2 only up to ~10 mm pO_2. However, when $O_2 > LH$, the excess oxygen is wasted and has no additional effect on kinetics.

There are several important points to recognize about oxygen contributions to lipid oxidation:

- Oxidation rate is determined by the rate of $L^\bullet$ formation by initiators, not by oxygen concentration (De Groot and Noll, 1987). Consequently, stopping initiation can be just as important as limiting oxygen for control of lipid oxidation.
- Oxygen often influences formation of $L^\bullet$ as much as oxygen addition to these radicals. For example, Fe^{2+} requires oxygen to oxidize to Fe^{3+} and produce $O_2^{-\bullet}$ and radicals deriving from it, so the presence or absence of a metal chelator can dramatically affect apparent oxygen dependence of subsequent lipid oxidation over and above oxygen reactions with fatty acids.
- At higher pO_2, oxygen has little effect on oxidation rate, but it controls the course of oxidation and degree of oxygenation of products. Oxygen alters the balance between alternative reaction paths (e.g. internal rearrangement versus abstraction) and determines the proportion of oxygenated recombination products formed, as was discussed in Termination Processes (Section III, A, 3).
- Oxygen has different effects on initiation and propagation, so the oxygen dependence observed varies with the products measured.

- Oxygen catalysis of lipid oxidation is proportional to exposed surface area in both liquid and solid foods. Reaction of lipids with oxygen in the atmosphere is faster than oxygen diffusion in oils.
- The effects of oxygen on oxidation decrease at elevated temperatures where increased thermal initiation can become greater than the solubility of oxygen.

V. WHAT ARE THE CONSEQUENCES OF LIPID OXIDATION?

It is unlikely that lipid oxidation would have received so much attention as a reaction if it only produced off-odors and flavors. The problem with lipid oxidation is that even at extremely low levels, it broadcasts oxidation far beyond the lipid molecules immediately involved, so that nearly every aspect of chemistry and quality is affected. Some of the most important consequences are shown in Figure 11.15. All except those involving lipids alone result from co-oxidations of other macromolecules.

A. Direct Effects due to Changes in Lipids

1. Production of Off-Odors and Flavors, Flavor Fade

Lipid oxidation is known among non-scientists as 'rancidity' due to the characteristic sharp, oily off-odors that consumers smell when they open a jar of old peanut butter, a packet of potato chips, a bar of chocolate, a bottle of salad oil, or a bag of pet food. The sensory perception of rancidity also includes a variety of pungent off-flavors. These odors and flavors arise mostly from secondary lipid oxidation products, particularly aldehydes and ketones, which humans can detect at trace concentrations (parts per billion). The odors and flavors rapidly become objectionable as concentrations increase, so that most foods are rejected at less than 1% oxidation.

Some of the flavors and odors associated with oxidized lipids, along with the product responsible and levels of detection, are listed in Tables 11.11 and 11.12. One important point to note is that oxidation products are 10−10,000 times more detectable in water than in oil because the compounds become more volatile and have less competition for sensors on the tongue. In practical terms, this means that salad oil in a bottle may smell and taste rather innocuous but become inedible when combined with water and vinegar in salad dressings. Indeed, any food with appreciable moisture content will develop recognizable 'rancidity' faster than dry or oil-based products.

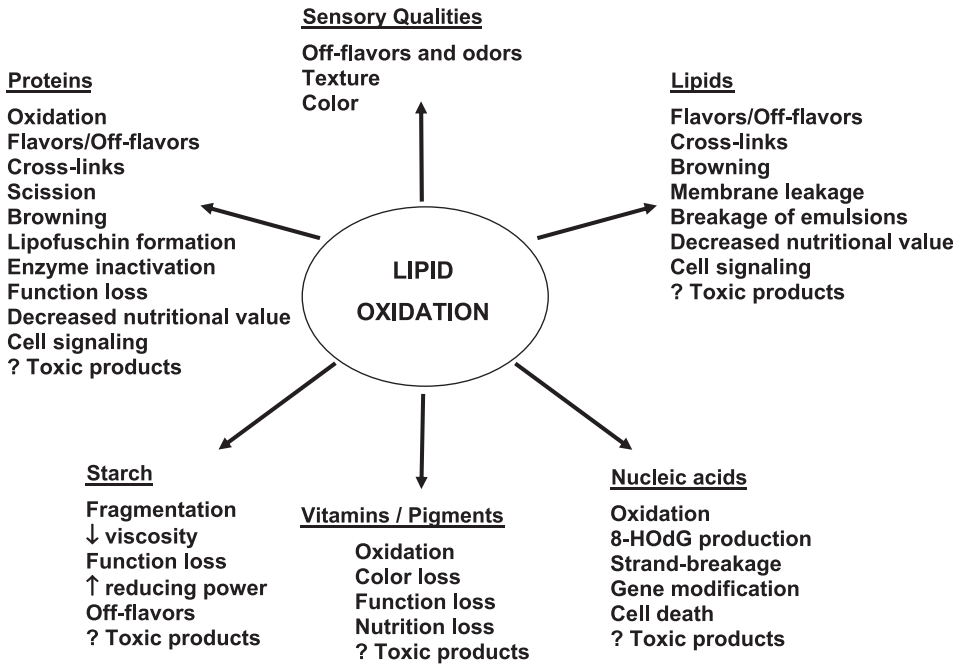


FIGURE 11.15 Consequences of lipid oxidation to sensory characteristics and chemical properties of foods.

TABLE 11.11 Off-flavors Produced by Various Lipid Oxidation Products

		Threshold Value (ppm)		
		In Oil		In Water
Compound[a]	Off-Flavor	Odor	Taste	Odor
Aldehydes				
Pentanal	Sharp, bitter almond	0.24	0.15	0.012
Hexanal	Green—fruity, bitter almond	0.32	0.08	0.008
Octanal	Fatty, soapy—fruity	0.32	0.04	0.0007
Nonanal	Tallowy, soapy—fruity	13.5	0.2	0.001
Decanal	Orange peels	6.7	0.7	0.0001
Nonenal (3c)	Green cucumber	0.25	0.03	
Nonenal (2t)	Tallowy, starch—glue	3.5	0.04	0.0008
Nonadienal (2t,4t)	Fatty, oily	2.5	0.46	
Nonadienal (2t,6c)	Cucumbers	0.01	0.0015	
Nonadienal (2t,6t)	Tallowy, green	0.21	0.018	
Decadienal (2t,4c)	Frying odor		0.02	
Decadienal (2t,4t)	Deep-fried	2.15	0.1	
Ketones and furans				
1-Pentene-3-one	Sharp, fishy		0.003	
1-Octen-3-one	Metallic			
1-Octen-3-ol	Moldy, mushroomy	0.077	0.0001	0.00009
2-Pentylfuran	Buttery, beany	2		
2-(1-Pentenyl)furan (*cis* and *trans*)	Licorice	2 to 6		

Data from Belitz and Grosch (1986).

A paradoxical flipside of this issue is that carbonyl products from lipid oxidation enter into Strecker degradation reactions with amino acids to generate some desirable and characteristic flavor products. Peanut butter, for example, tastes flat when fresh and unoxidized.

A phenomenon referred to as flavor fade also occurs. As lipid oxidation progresses, some of the characteristic fresh flavors are lost. It is not clear whether this occurs because flavor compounds are oxidized or because increasing levels of lipid aldehydes and other lipid oxidation products merely overwhelm the perception of native flavors.

2. Degradation of Membranes and Alteration of Lipid Structures

Lipid physical structures in foods depend largely on hydrophobic associations between acyl chains, but these are disrupted when lipids oxidize and either accumulate more polar products or cross-link and lose structural organization. Membranes become leaky or stiffen, either way losing functionality when component phospholipids oxidize in living tissues. In foods, the same changes in membranes lead to loss of turgor and degradation of texture. Oxidation of lipid phases in emulsions produces polar products that move to the interface and change surface tensions. At high enough product levels, emulsions will break. Oxidation affects lipid crystal structures as well. Introduction of polar groups disrupts chain packing in crystals and forces reorganization of the triacylglycerols to reduce repulsions. When this occurs in shortenings, margarines, or chocolate, invariably a shift to more dense β-crystal forms occurs, resulting

TABLE 11.12 Threshold Values of Major Volatiles in Oxidized Edible Oils

Compounds	Threshold Value Range (ppm)
Hydrocarbons	90–2150
Furans	2–27
Vinyl alcohols	0.5–3
1-Alkenes	0.02–9
2-Alkenals	0.04–2.5
Alkanals	0.04–1
2,4-Alkadienals	0.04–0.3
trans,cis-Alkadienals	0.02–0.06
Vinyl ketones	0.00002–0.007

Data from Frankel (1985).

in hardening, grittiness, and in the case of chocolate, an increased tendency to bloom (form white crystals on the surface).

B. Effects due to Interactions of Lipid Oxidation Intermediates and Products with Other Molecules, with Focus on Proteins

Although rancidity is a sensory condition generally attributed to lipids alone, most of the deleterious effects of lipid oxidation in foods listed in Figure 11.15 result not from lipid changes per se, but from reactions of lipid oxidation intermediate radicals and secondary products with other molecules. Many off-flavors attributed to lipids, e.g. warmed-over flavor in meats and some stale flavors, instead arise from co-oxidations. Thus, when tracking and measuring lipid oxidation in foods, it is just as important to follow the footprints of lipid oxidation in other molecules as to measure direct lipid oxidation products themselves. Unfortunately, these reactions are too often overlooked because they are not familiar and they are more difficult to analyze. Even more problematic is that, when co-oxidations occur, they have an antioxidant effect, removing lipid oxidation products from the analytical stream. As a result, lipid peroxide, carbonyl, and especially epoxide levels (and hence overall oxidation) may appear to be low or even negligible when the oxidation has actually occurred, perhaps extensively, and been transferred to other molecules.

One important factor that complicates sorting out co-oxidations is that nearly all lipid oxidation intermediates and products – especially radicals, hydroperoxides, aldehydes, and epoxides – react with proteins (Schaich, 2008), starches (Ishii *et al.*, 1976; Kawakishi *et al.*, 1983), pigments, DNA (Blair, 2001; Yang, 1993; Yang and Schaich, 1996), and other molecules. Radical reaction products are particularly difficult to track because they are usually oxidations rather than adducts, and they often transform or degrade further. New analytical methodologies that make it easier to detect individual modified amino acids may change this situation in the future. Carbonyls and epoxides form adducts that are more persistent and easier to detect, so co-oxidation has more often been attributed to reactions of secondary products than to radicals. The issue is not which reaction is right or wrong, because they all occur. Rather, it is critical to recognize that a continuum of damage caused by different lipid oxidation species develops and changes as oxidation (and co-oxidation) progresses. In early oxidation, radical co-oxidations dominate; with more extensive oxidation, reactions of epoxides and aldehydes become more important.

A general overview of how various lipid oxidation intermediates and products cause co-oxidation of proteins is presented here as a small introduction to an aspect of lipid oxidation that could fill an entire separate chapter.

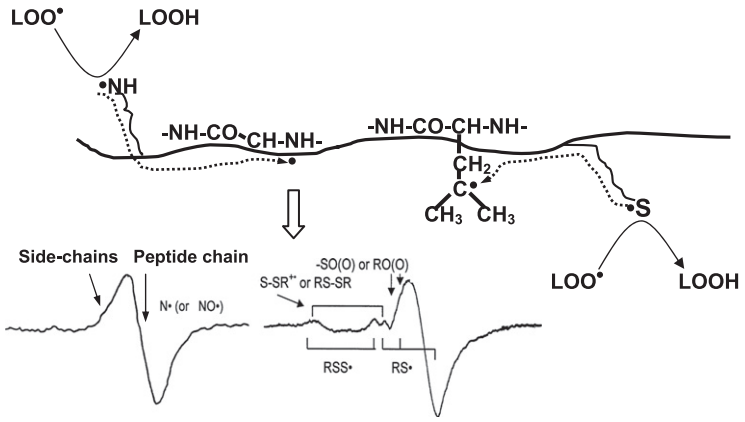


FIGURE 11.16 Free radical transfer from lipid peroxyl radicals to proteins. Hydrogen abstraction from His, Lys, Trp, Arg, and Cys side-chains generates $N^{\bullet}$ and $S^{\bullet}$ radicals. Overlapping unresolved spectra of multiple radical sites are evident in broad envelopes of electron paramagnetic resonance spectra. Radicals also become delocalized on nitrogens along the peptide backbone and can migrate to the side-chains of hydrophobic amino acids such as valine and leucine.

1. Reactions of Lipid Free Radicals

As noted above, proteins react with nearly every intermediate and product class of lipid oxidation — radicals, hydroperoxides, carbonyls, and epoxides — with variable consequences. Radical transfer from oxidizing lipids to proteins is the dominant process in the early stages of oxidation. The targets are any amino acid residues with readily abstractable hydrogens that can substitute for and compete with lipid acyl chains, i.e. those with amino or sulfhydryl groups on their side-chains (tryptophan, histidine, arginine, lysine, and cysteine), and the results are protein free radicals (Schaich and Karel, 1976; Schaich, 1980b). Free electrons that stay localized on side-chains create nitrogen- or sulfur-centered free radicals that give characteristic broad wings in electron paramagnetic resonance (EPR) spectra (Figure 11.16). Free electrons also migrate and become delocalized along the peptide backbone or on the β-carbon of hydrophobic amino acids such as valine. These radicals appear as narrower singlet EPR spectra. All these types of radicals may be present simultaneously. They remain quite stable in dry materials, often persisting for months to years.

Reactions of protein radicals parallel reactions of lipid radicals: addition of oxygen to form peroxyl radicals ($POO^{\bullet}$), abstraction of hydrogens to generate hydroperoxides (POOH), and decomposition of the POOH to yield protein alkoxyl radicals ($PO^{\bullet}$), and subsequent protein carbonyls (P—C=O) (Figure 11.17). Originally, protein radicals and hydroperoxides were thought to be relatively inactive. Current evidence, though, shows that they are indeed reactive, transferring radicals to other proteins (Soszylqski *et al.*, 1996), DNA (Gebicki and Gebicki, 1999), lipids (Gardner and Weisleder, 1976; Gardner *et al.*, 1977; Avdulov *et al.*, 1997), and potentially other molecules to broadcast and perpetuate oxidative damage.

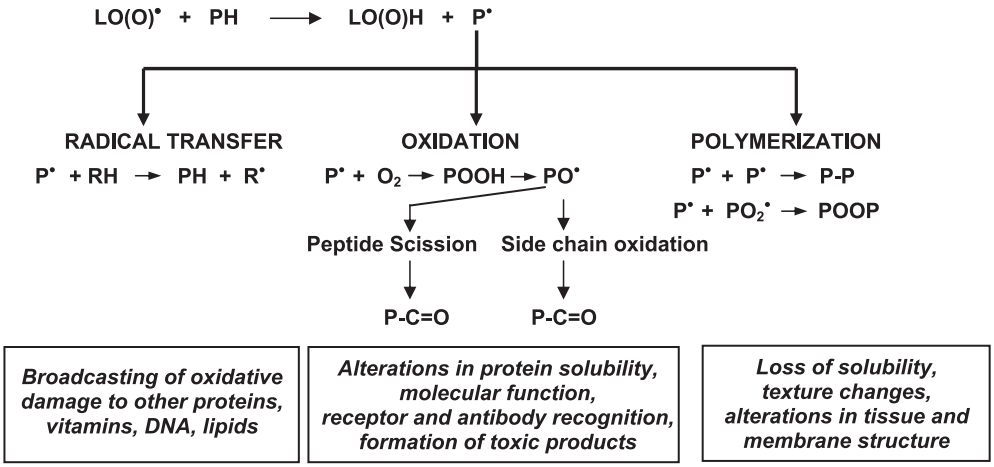


FIGURE 11.17 Pathways and consequences of free radical production in proteins. $P^{\bullet}$ is a protein radical on any α-carbon of the main peptide backbone or on an amino acid side-chain, and RH is any molecule with abstractable hydrogens. *(From Schaich, 2008; used with permission.)*

The formation of protein free radicals, hydroperoxides, and carbonyls sets the stage for a wide range of subsequent damaging reactions in foods, including alterations in protein conformation and solubility, loss of enzyme and other biological activity, scission and cross-linking that lead to marked texture degradation and loss of nutritional value. Amino acid targets are oxidized, transformed, and degraded. Many of the essential amino acids, as listed below, are among those most sensitive to damage by radical transfer from peroxidizing lipids (Schaich, 1980a, 2008):

Cysteine	Serine	Glycine
Tryptophan	Threonine	Alanine
Histidine		Valine
Lysine		Proline
Arginine		Leucine
Tyrosine		Isoleucine
Methionine		

In vivo, these same changes have disastrous consequences to physiological function and are intimately involved in the pathological processes that were noted in the Introduction.

2. Reactions of Lipid Hydroperoxides

Lipid hydroperoxides are not directly reactive themselves, but their ability to hydrogen bond to amine groups on proteins creates a reaction cage within which there is induced decomposition of LOOH and direct reaction of the resulting LO(O)$^{\bullet}$ with amino acid targets (Karel *et al.*, 1975; Schaich and Karel, 1976). The process, called molecule assisted homolysis, is very fast and may be metal independent or involve metals bound to proteins.

Without metals, lipid hydroperoxides bind at the surface of proteins, usually to amino or sulfhydryl groups on side-chains, and the nucleophilic atom induces hydroperoxide homolysis and radical transfer from lipid to protein:

$$\mathrm{LOOH + PH \longrightarrow [\,LO\text{-}\overbrace{OH\ldots H}\text{-}P\,] \longrightarrow LO^{\bullet} + P^{\bullet} + H_2O} \tag{11.56}$$

$$\mathrm{LOOH + R_3N \longrightarrow [\overbrace{R_3N\ldots HO}OR'] \longrightarrow R_3NO + LO^{\bullet}} \tag{11.57}$$

$$\mathrm{LOOH + 2\,RSH \longrightarrow RSSR + H_2O + LO^{\bullet}} \tag{11.58}$$

This concerted reaction may contribute to the sensitivity of histidine, arginine, lysine, tryptophan, cysteine, serine, and threonine to LOOH – all of which contain hydrogen bonding amino, carboxylic acid, and hydroxyl groups on their side-chains (Gardner, 1979; Schaich, 1980b). It also may be enhanced in lipid-bonding proteins such as bovine serum albumin where hydrophobic side-chains facilitate associations with lipids and bring reactive residues into close proximity with LOOH.

Metals bound to proteins can be even more reactive with lipids and more damaging overall than metals free in solution. In metalloproteins, LOOH binds at the metal ligand site and is reduced by the metal in a reaction cage; the LO$^{\bullet}$ generated oxidizes nearby amino acids, particularly histidine (Kowalik-Jankowska *et al.*, 2004) (Figure 11.18,

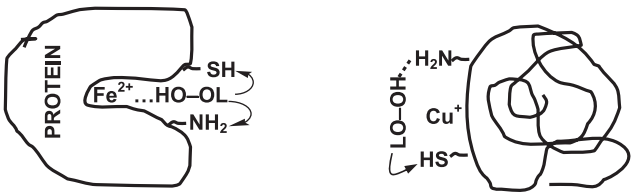


FIGURE 11.18 Metal-mediated radical transfer from LOOH to proteins. Lipid hydroperoxides bound in close proximity to metals in active sites or on protein surfaces are reduced in cage reactions and the resulting LO$^{\bullet}$ then abstracts a hydrogen from a susceptible amino acid nearby before release. *(From Schaich, 2008; used with permission.)*

left). In non-metalloproteins, metal-binding sites such as histidine, glutamic acid, or aspartic acid side-chains on protein surfaces also provide foci for metal-catalyzed reduction of LOOH in cage reactions (Figure 11.18, right).

Why is induced decomposition of LOOH important? With normal radical transfer by hydrogen abstraction, protein radicals and LOOH are generated simultaneously so there is a direct, measurable connection between initiation processes and lipid and protein oxidation products. Both LOOH and protein radicals appear at rates proportional to $L^{\bullet}$ and $LOO^{\bullet}$. However, since proteins supply the abstractable hydrogens for LOOH formation, the overall process is antioxidative: lipid free radical chains are stopped and fewer lipid molecules are oxidized.

$$LH \longrightarrow L^{\bullet} \xrightarrow{O_2} LOO^{\bullet} \xrightarrow{PH} \mathbf{LOOH} + \mathbf{P}^{\bullet} \tag{11.59}$$

In contrast, in concerted cage reactions, lipid molecules provide the abstractable hydrogens for LOOH formation, so LOOH is continually generated but then also removed as protein radicals form.

$$LH \longrightarrow L^{\bullet} \xrightarrow{O_2} LOO^{\bullet} \xrightarrow{LH} LOOH + LH \quad P^{\bullet} \tag{11.60}$$

$$LOOH + PH \longrightarrow [\,LO\text{-}OH\ldots\overbrace{H\text{-}P}\,] \longrightarrow \mathbf{LO}^{\bullet} + \mathbf{P}^{\bullet} + H_2O \tag{11.61}$$

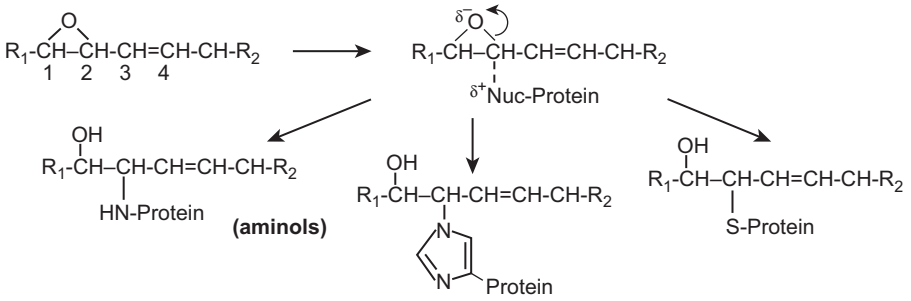
This is a pro-oxidant process since lipid radical chains are not intercepted, LOOH decomposition provides rapid, direct radical transfer to proteins, and $LO^{\bullet}$ are still released to propagate more radical chains. Of particular concern is that when lipid oxidation is followed by peroxide values alone, it can look like lipids are not oxidizing even while proteins are being extensively degraded, so lipids will be missed as the source of damage.

Concerted LOOH reactions with proteins have been reported in buffered solutions of methyl linoleate bound to β-lactoglobulin (Hildago and Kinsella, 1989; Yuan *et al.*, 2007), lyophilized emulsions of methyl linoleate and lysozyme and other proteins (Schaich and Karel, 1975; Schaich, 1980a), HDL apoA1 and A2 reacted with cholesterol and phospholipid hydroperoxides (Garner *et al.*, 1998), low-density lipoprotein (LDL) and cytochrome *c* incubated with LOOH or phospholipid (PE) hydroperoxides (Fruebis *et al.*, 1992), and lupine conglutins reacted with LOOH (Fruebis *et al.*, 1992; Lqari *et al.*, 2003). However, it is likely that these reactions occur in most systems but are not recognized.

3. Reactions of Lipid Epoxides

Epoxides are probably the most underrated and understudied lipid-derived oxidant, in part because they are so reactive that it is not easy to track them analytically. It is especially difficult to connect epoxides to protein co-oxidations because epoxides react with proteins about 1000 times more rapidly than do aldehydes. This greatly reduces or even eliminates epoxides from the mix of products in oxidizing lipids, so protein damage detected is attributed to other lipid oxidation products. Thus, following footprints of epoxides in oxidized proteins may be more revealing than analyzing lipid epoxides alone.

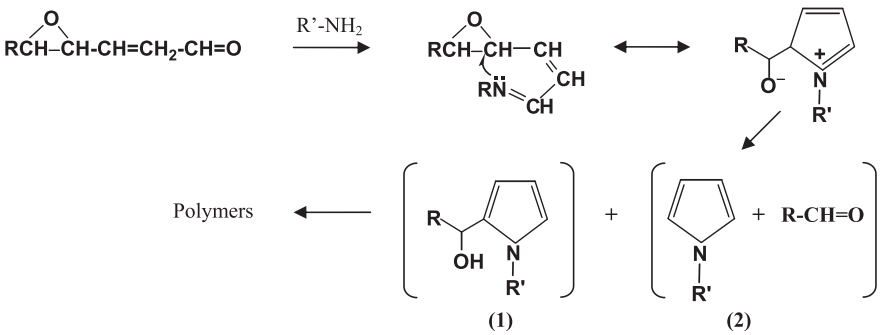
Epoxide reactions with proteins are strongly dependent on reaction conditions and the position of the epoxy group on the lipid acyl chain. When epoxy groups are next to double bonds, they readily hydrolyze (Lederer, 1996) and also undergo nucleophilic attack from amine groups on proteins (Ingold, 1969b). The fundamental process as it occurs between a linoleic acid epoxide and lysine, histidine, and cysteine (left to right) is shown in Reaction 11.62. The products, called aminols, have two key features: (a) a hydroxyl group at the β-carbon of the original epoxide, and (b) protein covalently linked to the α-carbon of the epoxide as shown below, or to the α-carbon on the opposite side of the double bond.



(11.62)

When water is present, the epoxide rings open but hydrolysis prevents addition of the protein (Lederer, 1996). Thus, these epoxide reactions with proteins are most important under anhydrous conditions, e.g. in dry foods and in hydrophobic interior regions of biomembranes and blood lipoproteins.

As lipid oxidation progresses, epoxide co-oxidations become more complex. Most importantly, the presence of both aldehyde and epoxide functional groups on the same lipid provides two sites for reaction and sets the stage for cyclization to form hydroxyalkyl pyrroles and furans (Reactions 11.63 and 11.64, respectively) (Zamora and Hidalgo, 1995, 2003, 2005; Zamora *et al.*, 1999; Hidalgo and Zamora, 2000) and also alkylpyridines (Reaction 11.65) (Zamora and Hidalgo, 2005; Zamora *et al.*, 2006). The reaction occurring in a given system is determined by the solvent, pH, temperature, and the nature of amine groups on the protein.



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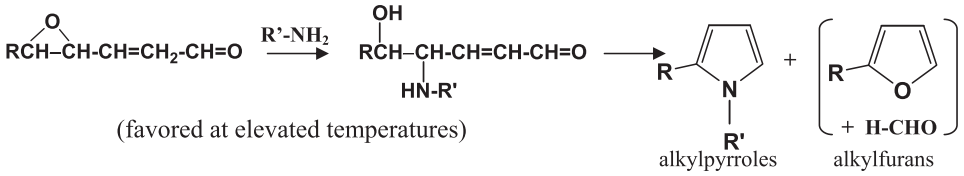

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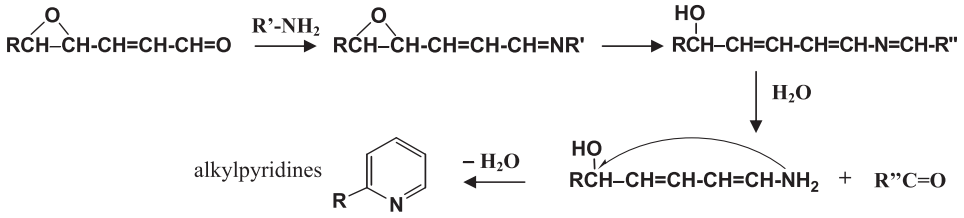


(11.65)

Detection of aminols is clearly diagnostic of epoxide co-oxidation of proteins. However, pyrroles and pyridines are also the major products of aldehyde co-oxidations of proteins, as will be explained in the next section, so it can be exceedingly difficult to distinguish epoxides from aldehyde reactions, except perhaps by kinetics. As a result, it is quite likely that reactions of epoxides with proteins have been overlooked and misinterpreted as aldehyde-mediated damage.

4. Reactions of Lipid Aldehydes

In the progression of co-oxidation, reactions of secondary products of lipid oxidation, particularly aldehydes, develop in later stages of lipid oxidation and introduce different types of protein oxidation products. Aldehydes form covalent adducts with proteins, whereas lipid radicals generally do not, and this has critical consequences for the properties that are modified in proteins. Except for cross-linking, damage done by free radicals remains largely 'invisible' at the molecular level. In contrast, reactions of aldehydes cause clearly perceivable changes such as production of flavors and odors, browning, and textural aggregation. Reactions of lipid aldehydes and other secondary products are responsible for the degradation that causes consumers to reject food products as inedible.

Aldehydes react with nucleophilic groups on proteins via Schiff bases, Michael addition, or a combination of both to form adducts, with three general outcomes:

- Linear adducts that change surface chemistry and protein recognition and potentially contribute to browning. This is the initial step for all aldehydes and is the dominant reaction between single, isolated carbonyl and amine groups.
- Cyclic products, especially dihydropyridines and pyrroles, as adducts or in cross-links. These contribute to browning, flavor and odor production, and loss of protein functionality. Cyclic products are the dominant products when multiple reactive groups are available, e.g. two amino groups on a terminal amino acid or two functional groups on an aldehyde (e.g. hydroxyl + aldehyde, two aldehydes, aldehydes in great excess over available amine).
- Protein cross-links, both intramolecular and intermolecular, that toughen textures, decrease solubility, and lead to phase separations. Cross-links require multiple reactive groups in the aldehyde, e.g. dialdehydes such as malonaldehyde and glutaraldehyde, or hydroxylalkenals such as 4-hydroxynonenal.

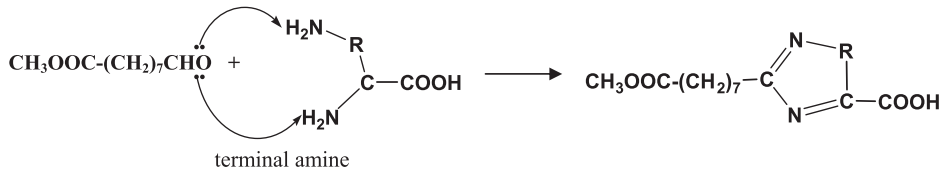
The exact reaction pathways that occur or dominate in a given system are influenced by the nature of the protein, the type of lipid oxidation products present, relative protein−aldehyde concentrations, pH, phase or solvent, oxygen tension, and other factors. The reactions described below provide some examples of the various products possible with different aldehydes. In real food systems, the products become much more complex than these simplified reactions.

a. Saturated Aldehydes

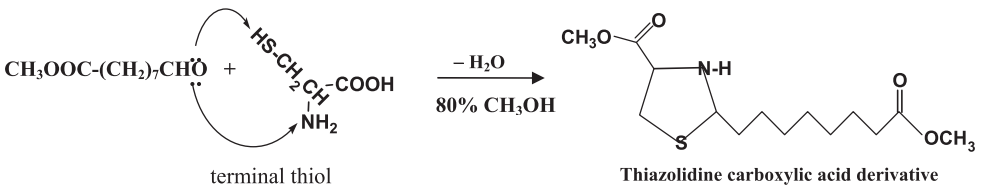
α and β scissions of lipid alkoxyl radicals release a variety of saturated aldehydes such as hexanal, nonanal, and 8-oxo-methyloctanoate. Although not very reactive, these aldehydes do add selectively to terminal amino acids via standard Schiff base reactions (McMurray, 2000):

$$R(CH_2)_n\text{-}C(H)=O \;+\; H_2N\text{-}CH(R)COOH \longrightarrow R\text{-}(CH_2)_n\text{-}C(H)=N\text{-}CH(R)COOH \qquad (11.66)$$

Since both peptide amine groups and side-chain amine and thiol groups are available, Reaction 11.66 is the first step and the remaining lone pair of electrons on the aldehydic oxygen undergoes a second Schiff base reaction with side-chain nucleophilic groups to generate heterocyclic products (Fenaille *et al.*, 2003):



(11.67)

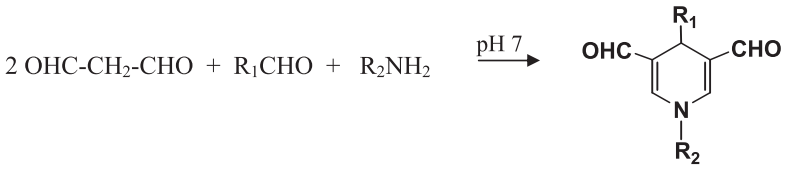


(11.68)

Dialdehydes such as malonaldehyde (MDA) are produced in downstream secondary oxidations of polyunsaturated fatty acids with three or more double bonds. Two aldehyde groups provide two sites for reaction with proteins. At low MDA levels, the results are usually cross-links:

$$\text{Protein } -NH_2 \leftarrow [O=C\text{-}CH_2\text{-}C=O] \rightarrow H_2N\text{-Protein} \qquad (11.69)$$

Under conditions of high lipid oxidation, two molecules of MDA combine with another aldehyde and an amino group to form pyridine derivatives (ring adducts on the protein (Freeman *et al.*, 2005; Kikugawa and Ido, 1984; Nair *et al.*, 1988):



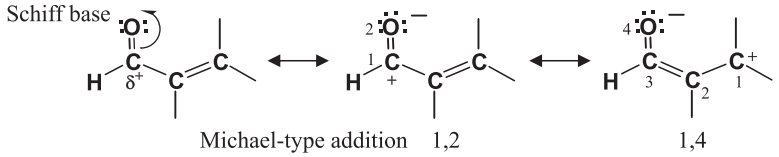
(11.70)

(1,4-dihydropyridine-3,5-dicarbaldehydes)

Dihydropyridines are important flavor precursors in foods (Buttery *et al.*, 1977; Suyama and Adachi, 1980; Maga, 1981).

b. Unsaturated Aldehydes

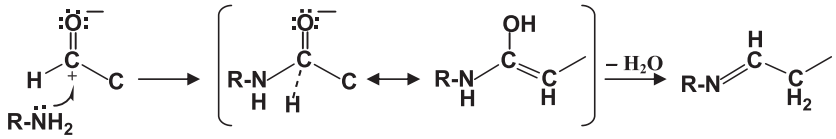
Unsaturated aldehydes, particularly 2-enals, are extraordinarily reactive compounds because they have three tautomeric forms in equilibrium,



(11.71)

and thus three potential reaction sites: Schiff base formation at the carbonyl and Michael-type 1,2 and 1,4 additions at the carbocations (Esterbauer *et al.*, 1991; Ege, 1999; McMurray, 2000). Thiols of cysteine, ε-amino groups of lysine, and imidazole nitrogens of histidine are the main protein targets (Esterbauer *et al.*, 1991; Petersen and Doorn, 2004).

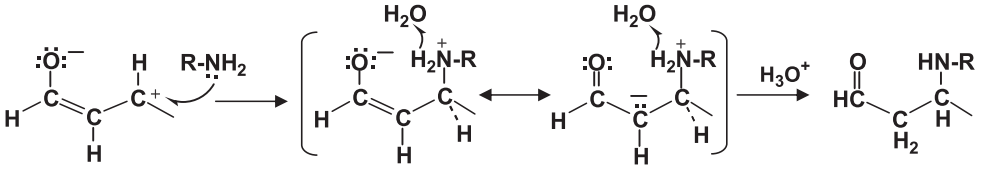
Direct (1,2) addition is comparable to the first step in Maillard browning reactions. The amine (or thiol) adds to the carbonyl carbon, and generates a carbinolamine intermediate that rearranges and dehydrates to a Schiff base as the final product (Ege, 1999; McMurray, 2000):



(11.72)

This is the main reaction in organic solvents and hydrophobic microenvironments; it is reversible and catalyzed by acid (Esterbauer *et al.*, 1991).

In aqueous solution or emulsions, the dominant reaction of lipid alkenals with amino acids is conjugated (1,4) addition. In this case, the amine adds to the carbocation formed at the β-carbon of the double bond initially in conjugation with the carbonyl:

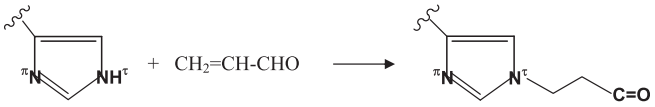


(11.73)

A key difference here is that the carbonyl group remains intact. Now complexed to the protein, this carbonyl is detectable in dinitrophenylhydrazine (DNPH) or antibody analyses for protein carbonyls (oxidation products). It can also form a Schiff base by reacting with an additional amine. This Michael addition−Schiff base protein−lipid aldehyde−protein complex is an important source of protein−lipid cross-links in more oxidized systems, and is the initial sequence found repeatedly in the formation of many complex adducts. Some of these will be described below.

Acrolein. Acrolein (CH_2=CH−CHO) is an unsaturated aldehyde formed by the oxidation of glycerol during frying, and it has been identified among secondary lipid oxidation products of arachidonic acid and *n*-3 polyunsaturated fatty acids *in vivo* (Uchida *et al.*, 1998a, b; Uchida, 1999; Kehrer and Biswal, 2000). Recently, this compound has received considerable attention as a carcinogen.

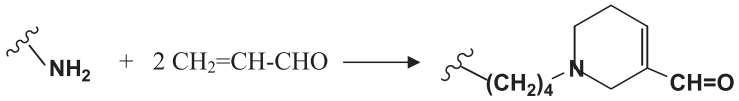
Acrolein is highly electrophilic so has very strong reactivity with nucleophiles such as thiols, the imidazole groups of histidine, and the ε-amino group of lysine via both Schiff base and Michael additions, and all these reactions contribute to its toxicity (Uchida *et al.*, 1998a; Esterbauer *et al.*, 1991). Michael addition is usually the preferred reaction because it is faster and the products are more stable (Esterbauer *et al.*, 1991; Uchida and Stadtman, 1992; Uchida *et al.*, 1998a, b). When aldehyde concentrations are low, the products are generally substituted propanals, as shown in Reactions 11.74 and 11.75.



(11.74)

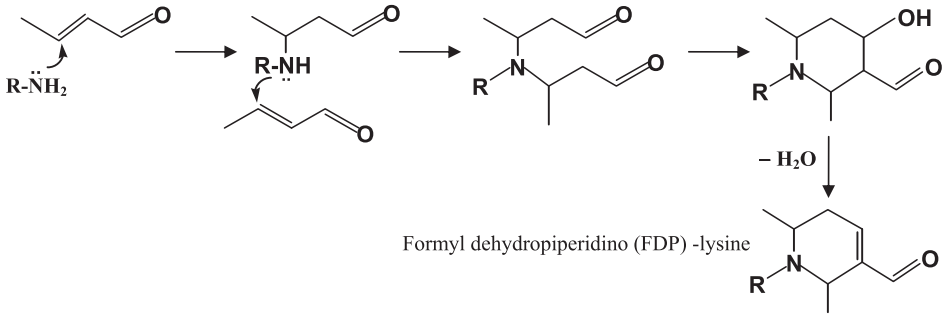
(11.75)

However, lysine adducts have a strong tendency to cyclize. When aldehydes are present in excess of the amines, lysine undergoes multiple additions with acrolein to generate the cyclic pyrrole derivatives shown in Reaction 11.76.



(11.76)

With long incubation time or high aldehyde concentrations, accumulated Michael adducts of acrolein cyclize, yielding N^{ϵ}-(2,5-dimethyl-3-formyl-3,4-dehydropiperidino) (FDP) structures by 1,4 addition (Ichihashi *et al.*, 2001).



(11.77)

FDP adducts retain a carbonyl function and thus are detected in carbonyl assays of oxidized proteins. Antibodies to FDP-lysine have detected acrolein adducts in oxidized LDL (Ichihashi *et al.*, 2001) and in bovine serum albumin co-oxidized with methyl arachidonate (Uchida *et al.*, 1998b).

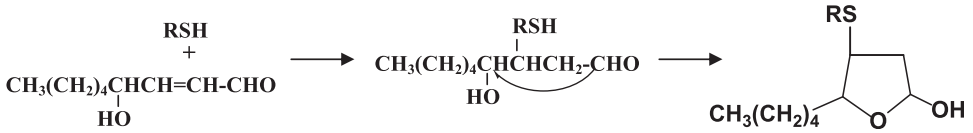
4-Hydroxy-2-alkenals and 4-oxo-2 alkenals. Oxidized alkenals such as 4-hydroxy-2-nonenal (HNE) and 4-oxo-2-nonenal (ONE) are the aldehydes most reactive with proteins (Esterbauer *et al.*, 1991).

4-Oxo-2-alkenals, also called γ-ketoaldehydes, may be formed independently but are actually tautomers of 4-HO-2-alkenals:

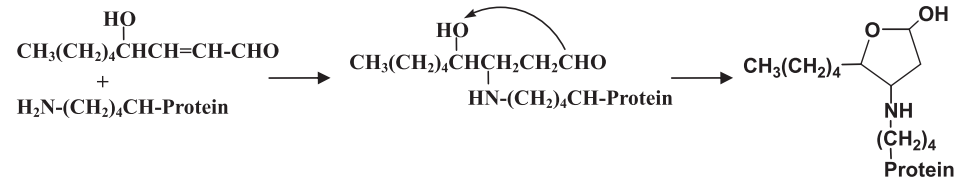
(11.78)

Under oxidizing conditions, reactions of these two oxidants are often difficult to distinguish except by kinetics (ONE reactions are orders of magnitude faster) (Lin *et al.*, 2005) and balance between pathways. Since both of these compounds have two reactive functional groups, their major reaction sequence is the same as that described above for dialdehydes. The first step is usually (1,4) Michael addition to nucleophilic amino acid side-chains (Uchida and Stadtman, 1992; Bruenner *et al.*, 1995; Schaur, 2003). HNE reacts with cysteine, histidine, or lysine; ONE reacts with these amino acids plus arginine. Subsequent reactions vary with the aldehyde, the specific amino acids present, relative concentrations of aldehyde and protein, and the reaction conditions.

The simplest reaction is cyclization of the aldehyde to form 2-hydroxy-furan adducts, shown below for cysteine and lysine (Uchida *et al.*, 1994, 1997; Uchida, 2003):



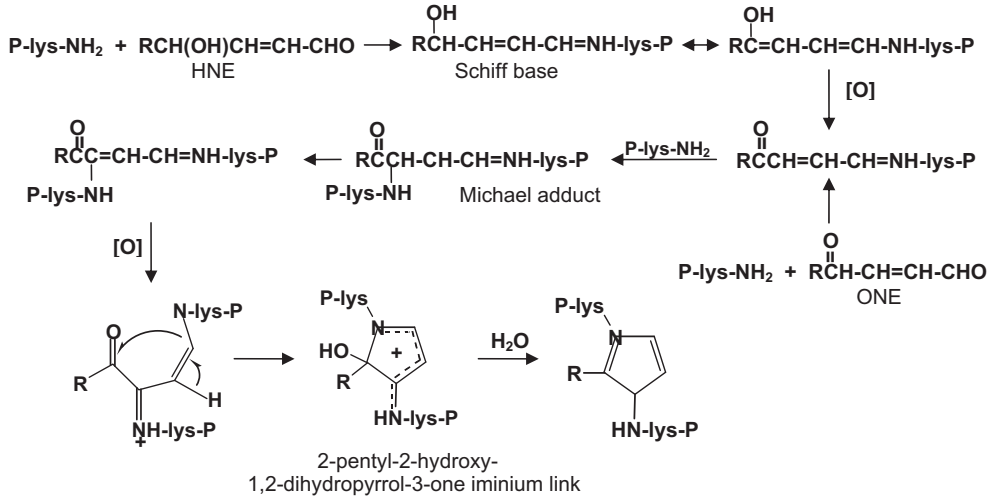
(11.79)



(11.80)

This reaction occurs when the amines and aldehydes are approximately equal in concentration, but the products hydrolyze readily so are not always found in product mixes.

When amines are present in molar excess over aldehydes (e.g. in early oxidation), the reaction sequence changes. The first step here is Schiff base formation. The unreacted HO-then oxidizes to generate a second carbonyl (for HNE), which undergoes Michael addition to a second amine (usually lysine); the complex then oxidizes and cyclizes to form stable dihydropyrrol-iminium complexes (Sayre *et al.*, 1993, 1997; Xu *et al.*, 1999; Schaur, 2003; Zhang *et al.*, 2003):



(11.81)

The complexes formed by both HNE and ONE are very interesting products. The adducts themselves are fluorescent, and probably contributed to the fluorescent products originally detected by Tappel and co-workers (Chio and Tappel, 1969; Fletcher and Tappel, 1970; Fletcher *et al.*, 1973). The pyrroles are hydrolysable and the pyrrole -OH can react further, e.g. with another lysine, to form cyclic or acyclic mixed aminals; thus, the pyrroles may serve as precursors for development of flavor compounds during food processing and storage. Finally, the entire complex creates still another kind of peptide cross-link that contributes to hardening and toughening of textures in foods. These complexes also increase in Alzheimer's disease and may be involved in the tangling of β-amyloid chains (Sayre *et al.*, 1997).

5. Connecting Co-oxidation Reactions to Protein Damage

All lipid oxidation products react in some way with proteins, so there is really no time at which proteins are safe from the effects of co-oxidation. Rather, a continuum of co-oxidation damage develops in parallel with lipid oxidation as new lipid products are generated and shift the types of reactions that dominate. This section has focused on chemical details of the reactions to shift thinking about the processes from the global behaviors usually reported to the molecular level chemistry responsible. It is only by understanding the detailed chemistry that we can ever gain the ability to manipulate and control it to protect food quality.

Table 11.13 summarizes the connections between specific lipid oxidation products, their protein co-oxidation reactions, and the global protein behaviors affected. The overlap between pathway effects is clear, so measuring a single effect alone cannot determine what caused it. Neither is it sufficient to correlate protein changes with lipid oxidation products since, as has been noted several times, lipid oxidation products reacting with proteins are removed

TABLE 11.13 Protein Co-oxidation Products of Lipid Oxidation Species and Associated Effects on Protein Properties

Lipid Oxidation Product	Reaction	Products	Global Behaviors Affected
LOO$^{\bullet}$ and LO$^{\bullet}$ radicals	Radical	Radicals	Solubility changes, loss of enzyme activity
	Transfer	Amino acid oxidation products	Fragmentation, surface properties, texture
		Protein hydroperoxides, carbonyls	Amino acid destruction, loss of nutritional value
		Protein dimers	Cross-linking, solubility, antibody recognition, texture
LOOH	Cage reaction → radical transfer, LO$^{\bullet}$ released	Protein radicals, hydroperoxides, carbonyls	All effects of radicals but with enhanced oxidation rates
		Disulfide cross-links	
Epoxides	Nucleophilic	(Low oxidation) Aminol adducts	Altered surface properties, loss of antibody recognition
	Addition	(High oxidation) Hydroxylalkyl pyrroles	Loss of enzyme activity
		Hydroxylalkyl furans	Nutritional value, functional properties
		Alkylpyridines	
Saturated aldehydes	Schiff base	(Low oxidation) Schiff base linear adducts	Browning, loss of nutritional value, age pigments
		(High oxidation) Heterocyclic adducts	Flavors, functional properties, solubility, surface properties
Saturated dialdehydes	Schiff base, Michael addition	(Low oxidation) Schiff base cross-links	Browning, cross-linking, enzyme activity, nutritional value
		(High oxidation) Dihydropyridine adducts and cross-links	Texture, flavors, odors, functional properties
α,β-Unsaturated aldehydes	Michael addition, Schiff base	(Low oxidation) β-substituted alkanals, pyrroles	Browning, flavors, surface properties, age pigments
		(High oxidation) Methylpyridinium, dehydropiperidino adducts	Cross-linking, texture, enzyme activity, biological activity
Oxidized aldehydes, e.g.	Michael addition, Schiff base	Linear adducts, furans, pyrroles	Browning, fluorescence, texture hardening, flavors
4,5-Epoxy-2-alkenals		(High amine) Dihydropyrrol—iminium adducts, aminals	Cross-linking, texture, surface and functional properties
4-Hydroxy-2-alkenals			Enzyme activity, antibody recognition, nutritional value
Epoxyoxo acids, oxoacids		(High aldehyde) Epoxides; pyrrole, pyrrolidone, thiazolidine adducts	Browning, fluorescence, flavors, functional properties

from the analytical stream; these would then appear as negative correlations at best. In the past, direct determination of product structures was exceedingly difficult or impossible. However, modern mass spectrometry should now make identifying complex products almost routine. The reactions and product structures outlined in this section can thus provide guides for tracking footprints of lipid oxidation among protein co-oxidation products.

VI. HOW CAN LIPID OXIDATION BE CONTROLLED?

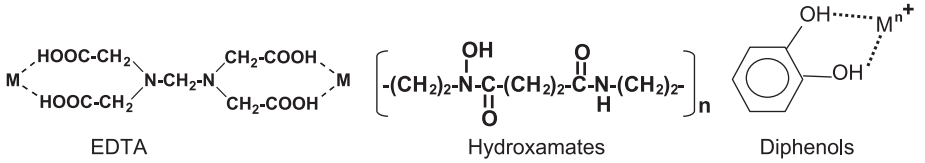
Since lipid oxidation causes so many problems, the overarching concern with lipid oxidation in foods is its prevention. Antioxidant approaches fall into three categories, all of which are necessary to stabilize products against oxidation. These can be applied individually but are usually combined to most effectively limit lipid oxidation:

- Type 1 antioxidants: agents that prevent initiation
- Type 2 antioxidants: compounds that quench radicals
- Type 3 antioxidants: environmental and processing factors.

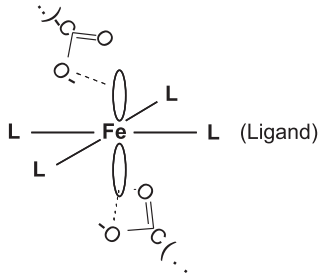
A. Type 1 Antioxidants: Agents that Prevent Free Radical Production

1. Metal Chelators and Complexers

For the most part, these are metals chelators (the most important of which is EDTA, ethylenediamine tetraacetic acid, shown below) and metal complexers (e.g. citric acid, polyphosphates, diamines, some amino acids, and to a lesser extent, ascorbic acid). Other common chelating groups include polycarboxylic acids, hydroxamates, and vicinal diphenols.



Two points need to be stressed about the actions of metal chelators and complexers. First, to be effective, chelators and complexers must have a structure that wraps around the metal and completely blocks all metal orbitals, preventing electron transfer, or be present at high enough concentrations that multiple molecules can act in concert to do the same.

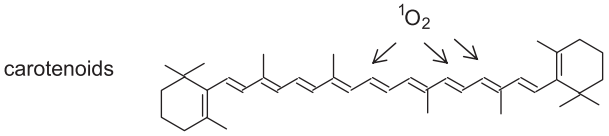


EDTA is an example of the first action, and citric acid is an example of the second. Compounds that incompletely block metal orbitals may act as synergists and slow electron flow but cannot stop initiation reactions. Most organic acids and phosphates are examples of synergistic action.

Secondly, chelators and complexers do not physically remove the metals from reaction; they merely block orbitals and reduce redox potential, making them less active than the parent metal as oxidizing agents. However, the complexes may become paradoxically more reactive in the total system if reducing agents with lower redox potential are also present to cycle the metal. An oxidized metal with low E^{o} is a poor catalyst, but reducing that metal generates a much more powerful reducing agent that will react very rapidly with oxygen and all hydroperoxides and be even more damaging than the parent metal. Thus, attention must be given to potential reducing agents in food systems when adding chelators.

2. Singlet Oxygen Scavengers, Particularly Carotenoids

As described in Section IV, D, 1, singlet oxygen adds across double bonds in lipids, particularly conjugated double bonds. With extended conjugated double-bond systems, carotenoids provide many sites that preferentially react with $^1O_2$ and spare fatty acids (Stahl and Sies, 2005). Only a few are noted in the structure below, but all are reactive with $^1O_2$.



Pyridoxine (vitamin B_6) and its derivatives are also efficient singlet oxygen scavengers (Bilski *et al.*, 2000). Initiation inhibitors are frequently overlooked as antioxidants, yet they serve a very critical role in controlling the radical load that must be overcome by free radical scavengers. The radical load is the cumulative total of radicals from all sources that must be quenched to effectively limit progression of lipid oxidation. The radical load determines the type, amount, and reactivity of antioxidant necessary to stabilize a given system. If the radical load is too high, no amount of radical chain quencher (classical antioxidants) can prevent lipid oxidation. However, neither can initiation inhibitors work alone. Some radicals from various sources are always produced and these must be quenched to prevent establishment of cycling chain reactions. Thus, Type 1 antioxidants are usually combined with Type 2 antioxidants for most efficient action.

B. Type 2 Antioxidants: Compounds that Quench Radicals

These are classical or primary antioxidants. Antioxidants (AH) quench free radicals by several mechanisms.

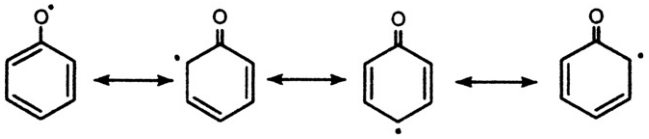
1. Hydrogen Atom Transfer

Hydrogen atom transfer (HAT) to quench radicals is the best known antioxidant action (Leopoldini *et al.*, 2004):

$$ROO^\bullet + AH \longrightarrow ROOH + A^\bullet \tag{11.82}$$

$$ROO^\bullet + AH_2 \longrightarrow ROOH + AH^\bullet \xrightarrow{R^\bullet} A + RH \tag{11.83}$$

For a compound to be an antioxidant, its radical $A^\bullet$ must be stable and unreactive or yield non-radical products so that it does not propagate radical chains. The most important antioxidant structures with H-transferring activity are phenolic compounds in which the $A^\bullet$ reactivity is reduced by resonance delocalization of the free electron over the aromatic ring.



The synthetic antioxidants BHA, BHT, propyl gallate, and TBHQ in commercial use in foods are all phenolic compounds, as are natural antioxidant tocopherols, flavonoids in fruits, and many herb and spice components, to mention just a few. The structures of some common phenolic antioxidants are shown in Figure 11.19.

There is currently great interest in replacing synthetic antioxidants with natural compounds. Some natural antioxidants are monophenols, while many have more than one phenolic group per ring (polyphenols). In general, antioxidant activity increases with the number of phenolic groups, although the position of the groups is also important. As noted above, diphenols and triphenols also complex metals (Afanas'ev *et al.*, 1989), so they have multiple mechanisms for inhibiting lipid oxidation and thus can be very powerful antioxidants. Many polyphenols in rosemary, oregano, and cranberries, for example, have this structure.

Additional compounds that inhibit lipid oxidation by HAT include aromatic amines, hydroxyl amines, thiophenols, aminophenols, ascorbic acid, glutathione, uric acid, carotenoids, amino acids, and proteins.

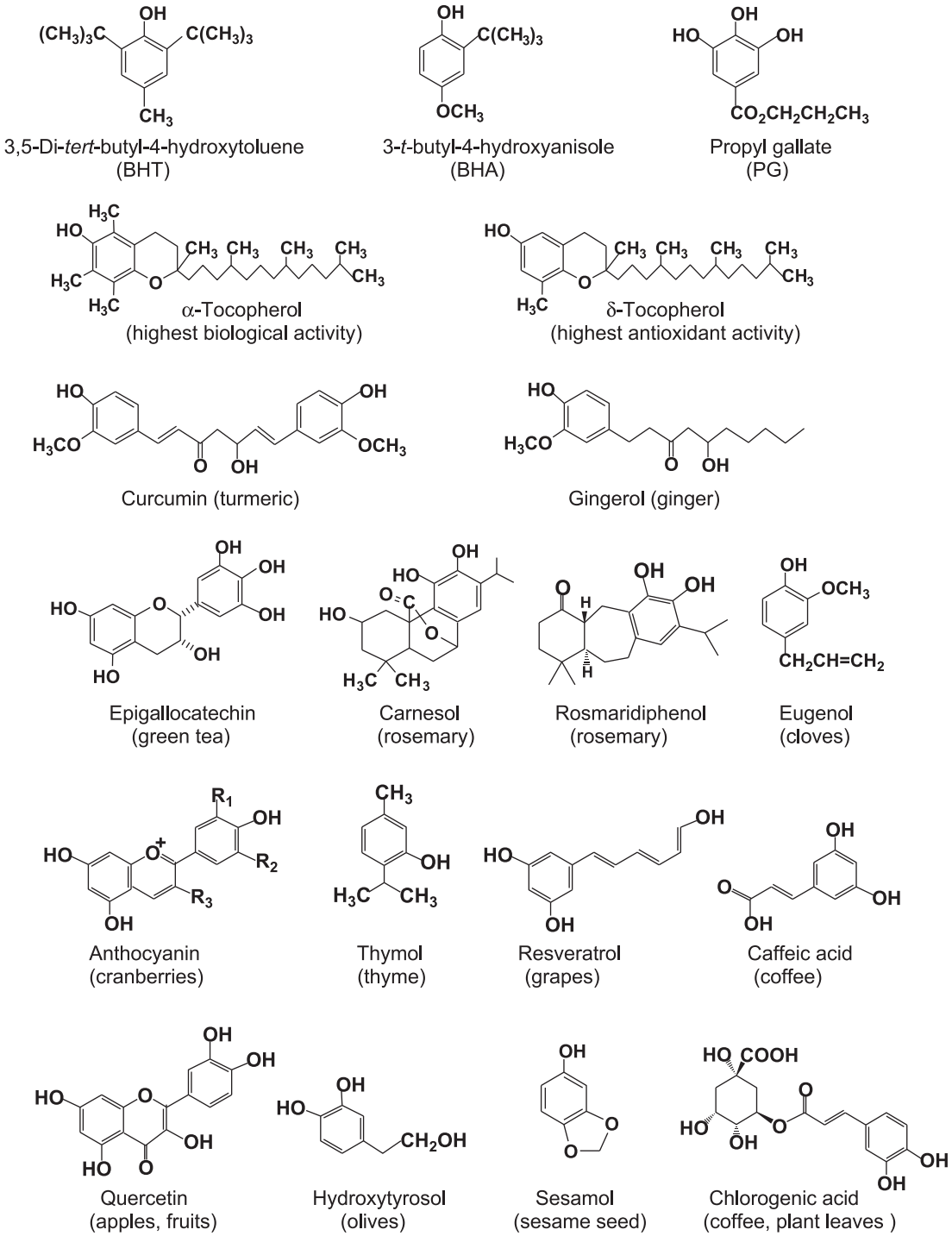


FIGURE 11.19 Structures of some common antioxidants. The top row shows synthetic antioxidants; the rest are antioxidants from natural sources (source in parentheses).

2. Reduction or Oxidation

Reactive radicals can be reduced or oxidized to unreactive ions (single electron transfer):

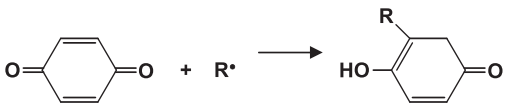
$$ROO^{\bullet} + ArOH \xrightarrow{+e^-} ROO^- + ArOH^{\bullet+} \tag{11.84}$$

$$ROO^{\bullet} + A \xrightarrow{-e^-} ROO^+ + A^- \tag{11.85}$$

Compounds with this capability include ascorbic acid and other reducing agents, some phenols and quinones, and high metal concentrations (Ganapathi *et al.*, 2000; Yen *et al.*, 2002; Leopoldini *et al.*, 2004). This antioxidant effect of high metal concentrations is frequently ignored or unrecognized.

3. Termination of Chains

Termination of chains can occur through direct reaction with $R^{\bullet}$, e.g. quinones, nitro compounds, and quinone imines:



(11.86)

4. Decomposition of Radicals or ROOH

Radicals or ROOH may be decomposed without generating new radicals. Compounds with this action include sulfides and disulfides (Kulich and Shelton, 1991), phosphates and thiophosphates, carboxylic acids (including phenolic acids and free fatty acids), enzymes (superoxide dismutase, glutathione peroxidase, catalase), amines (Fueno *et al.*, 1959), and phosphatidylcholines (O'Brien, 1969). In the reactions shown below, AcH denotes an acid and Ar denotes an aryl group.

Sulfides and disulfides:

$$LOOH + R_1SR_2 \longrightarrow LOH + R_1S(O)R_2 \xrightarrow{\text{heat}} R_1SOH + R_3CH{=}CH_2 \quad (11.87)$$

$$2\,LOOH + R_1SOH \longrightarrow 2\,LOH + R_1SO_3H \quad (11.88)$$

$$LOOH + R_1SSR_2 \longrightarrow LOH + R_1S(O)R_2 \longrightarrow R_1SSOH + R_3CH{=}CH_2 \quad (11.89)$$

$$2\,LOOH + R_1SSOH \longrightarrow 2\,LOH + R_1SSO_3H \quad (11.90)$$

$$LOO^{\bullet} + R_1SOH \longrightarrow LOOH + RSO^{\bullet} \longrightarrow \text{inactive products} \quad (11.91)$$

Acids and nucleophiles (e.g. N^+ in choline):

$$\text{Ar-COOH} + LOOH \longrightarrow \text{Ar-CHO} + LOH \quad (11.92)$$

$$\text{AcH...HOOL} \longrightarrow [\text{Ac}^-\text{H}^+\!\cdots\!\underset{\displaystyle H}{\underset{|}{O}}\text{OL}] \longrightarrow \text{AcOH} + LOH \quad (11.93)$$

$$X^-H^+ + LOOH \longrightarrow LOOH_2^+ \longrightarrow \beta\text{-scission aldehydes} \quad (11.94)$$

$$RH_2N: + LOO^{\bullet} \longrightarrow (LOO:)^- + RH_2N^{\bullet+} \quad (11.95)$$

$$\text{Ar-SOH} + 2\,ROOH \longrightarrow 2\,ROH + ArSO_3H \quad (11.96)$$

C. Type 3 Antioxidants: Environmental Factors that Limit Oxidation

Macroenvironmental factors such as refrigeration, blanching, dark, inert gas or vacuum, moisture content, and pH cannot stop lipid oxidation themselves but provide the microenvironments in which lipid oxidation must occur. When managed appropriately, they certainly slow the rate of lipid oxidation and they can also shift reaction pathways. Thus, they provide the very important functions of reducing the radical load so that primary antioxidants can be more effective, and controlling products to minimize detrimental effects to food quality. Indeed, if these environmental factors are not controlled, no amount of chelator or radical quencher can successfully hold lipid oxidation at levels

low enough to maintain sensory acceptability and safety of food products. Control of environmental factors is particularly important when multiple reaction mechanisms are active because they provide a means of directing the oxidation to or from specific pathways.

Environmental factors affect products from formulation through processing to storage. Formulation factors include moisture levels and pro-oxidant enzymes such as lipoxygenase and lipase that require blanching to inactivate. Processing environment factors that are important encompass everything from potential metal contamination from equipment and processing water to temperatures and light, and oxygen exposure during processing. Packaging and storage environments include the pO_2 inside the package and surrounding it, vacuum versus inert atmosphere versus air headspace, moisture inside the package and migrating into or out of it, temperature and light exposures, and so on.

Simplistically, the easiest approach to stabilizing foods against lipid oxidation might be to vacuum pack all foods in light-impermeable packages and then to freeze them. However, other considerations such as cost, protection of other food qualities, and storage requirements must be balanced against requirements for limiting lipid oxidation in each food product. In addition, real foods always have more than one catalyst and complex matrices so the catalytic factor effects described in this chapter are never as straightforward as when written in an equation on a page. Thus, in actual practice, it is usually necessary to creatively combine multiple antioxidant approaches and even multiple antioxidants to effectively block lipid oxidation in foods.

It is hoped that the information presented in this chapter will enable the reader to evaluate more fully how lipids are oxidized in individual systems, which factors are driving the oxidation, and which products are causing the most trouble. This information can then be applied to tailor combinations of antioxidant approaches to effectively and economically maintain sensory food quality while retaining the highest possible levels of nutritional quality and safety.

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