Decomposition of Linoleic Acid Hydroperoxides. Enzymic Reactions Compared with Nonenzymic

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In biological tissue, linoleic and linolenic acid hydroperoxides are decomposed primarily by the following pathways: (1) reduction (or nucleophilic reactions), (2) isomerization by linoleic acid-hydroperoxide isomerase, (3) epoxidation by flour-water suspensions, (4) vinyl ether formation, (5) anaerobic lipoxygenase reactions, and (6) production of volatile aldehydes. In these reactions enzymes are probably involved. Decomposition of fatty acid hydroperoxides (or their esters) by nonenzymic reactions occurs by both heterolytic and homolytic mechanisms. Although heterolytic reactions are not restricted to production of the corresponding hydroxy fatty acid from the hydroperoxide, reportedly it is the major reaction. Homolytic decompositions are much more complex, and the products often depend upon the conditions used. Presumably hydroperoxides homolytically decompose mainly through formation of peroxy or alkoxy radicals. Recently studies on systems which should promote alkoxy radical formation indicated that the major reaction pathways are: (1) dimer formation from termination reactions, (2) loss or gain of a hydrogen radical, (3) cyclization of the alkoxy radical to the α unsaturation, and (4) 1,4 addition to the conjugated diene.

Lipid oxidation confronts oil chemists with a multifaceted problem. If not inhibited, polyunsaturated fats or oils readily peroxidize and cause food and edible oil spoilage. Among the undesirable characteristics of peroxidized oils are off-flavors, toxicity, and diminished nutrient content (especially tocopherols and carotenoids). In biological systems, lipid hydroperoxides are known to damage biomembranes, initiate further autoxidations, and react with proteins and smaller metabolites. Hypothetically, lipid peroxidation may be a significant factor in aging.

Lipid hydroperoxides are the initial products of autoxidation, and their decomposition results in a variety of secondary products, such as volatiles, polymers, and oxygenated compounds. Hydroperoxide decomposition often proceeds by a homolytic mechanism. In foods the main catalysts of homolysis are transition metal ions or metalloproteins. In biologically active material, hydroperoxides are often degraded by enzymes, which act by largely little-known mechanisms (homolytic *vs.* heterolytic).

Although hydroperoxides are the initial products of lipid oxidation, most researchers have oxidized oils or other fatty esters to study secondary products. In this review the extensive literature covering oil oxidation has been largely ignored because it has been the subject of many reviews in the past. With few exceptions, literature dealing with the decomposition products of preformed, isolated linoleic acid hydroperoxides (LOOH) or linolenic acid hydroperoxides (LnOOH) and their methyl esters (MLOOH and MLnOOH) has been concentrated on. Decomposition by both enzymic and nonenzymic means is concentrated on also. Only when the hydroperoxides are directly decomposed can the resultant compounds be studied as "primary" rather than "secondary" products. Even these direct reactions with pure lipid hydroperoxides yield certain products that appear to be secondary. Recent work of this nature is emerging that promises to give new insight into understanding the complex reactions of lipid oxidation and rancidity.

DECOMPOSITION BY ENZYMES

Living organisms are often exposed to varying amounts of lipid hydroperoxides. These hydroperoxides are generated by both enzymic and nonenzymic means. Contrary to early reports of lipoxygenase (EC 1.13.1.13) activity in animal tissue, peroxidation of lipids occurs nonenzymically in animal tissue being initiated largely by hemoproteins. In many plants the presence of lipoxygenase seems to predetermine lipid hydroperoxide formation especially if cellular integrity is disrupted mechanically. Despite the wide distribution of lipoxygenase among a range of plant families, no physiological role for hydroperoxide formation has yet been discovered.

The harmful effects that lipid hydroperoxides have on organisms probably account for the existence of enzymes to dispose of them. In animals the enzyme glutathione:hydrogen-peroxide oxidoreductase (EC 1.11.1.9, GSH-peroxidase) has been identified as the system acting on hydroperoxides. Since the initial studies of the enzymic decomposition of LOOH in soybean homogenates (Blain and Styles, 1959; Blain and Barr, 1961; Gini and Koch, 1961), many enzymic systems and their products have been characterized in plant systems. Often in plant extracts more than one type of reaction competes for the available hydroperoxide substrate. Other complications arise from two positional isomers of the substrate, 9-hydroperoxy-*trans*-10,*cis*-12-octadecadienoic acid (9-LOOH) and 13-hydroperoxy-*cis*-9,*trans*-11-octadecadienoic acid (13-LOOH). The corresponding hydroperoxides of linolenic acid are: 9-hydroperoxy-*trans*-10,*cis*-12,*cis*-15-octadecatrienoic acid (9-LnOOH) and 13-hydroperoxy-*cis*-9,*trans*-11,*cis*-15-octadecatrienoic acid (13-LnOOH). Lipoxygenase isoenzymes specifically form either one positional isomer or varying ratios of two positional isomers depending upon the source. At least one hydroperoxide-decomposing enzyme is specific for only one isomeric LOOH. To simplify discussion of the plant hydroperoxide-decomposing enzymes, a brief introductory section on positional specificity of lipoxygenases is included.

Enzymes acting on LOOH or LnOOH can be classified into six characteristic reaction categories as follows: (1) reduction (or nucleophilic reactions), (2) isomerization by LOOH-isomerase, (3) epoxidation by flour-water suspensions, (4) vinyl ether production, (5) anaerobic lipoxygenase reactions, and (6) production of volatile aldehydes. These reactions are summarized in Figure 1. Because LOOH and LnOOH decompose easily, some of these reactions may be nonenzymic.

Lipoxygenase Specificity. Lipoxygenase enzymes from different sources vary in their specificity for the position of oxygenation, C-9 *vs.* C-13. There is also steric specificity of the oxygenation, which is, at present as a rule, L at

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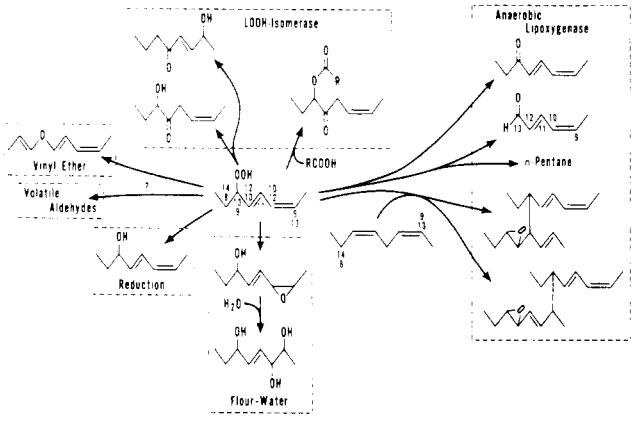


Figure 1. Enzymic decompositions of linoleic acid hydroperoxide (LOOH). Carbon numbers of linoleic acid, tridecadienoic acid, and the two isomers of LOOH are indicated as a guide to formation of isomeric products.

C-13 and D at C-9 for all lipoxygenases. Linoleic and linolenic acids usually behave identically in their oxidation specificity, but this section is restricted to oxidation of linoleic acid.

Christopher *et al.* (1972) examined the specificity of lipoxygenase isoenzymes, types 1 and 2, from soybeans. Under pure O_2 at 0° and pH 9, type 1 oxidized linoleic acid to 92% 13-LOOH and 8% 9-LOOH; however, at other conditions, 13-LOOH can drop as low as 48%. Type 2 specificity fluctuates somewhat with conditions, but it averages about equal ratios of 9- and 13-LOOH. Partially purified preparations of soybean lipoxygenase, presumably a mixture of isoenzymes, yielded isomeric mixtures that varied from 70% (Hamberg and Samuelsson, 1965) to 100% 13-LOOH (Dolev *et al.*, 1967).

Many other lipoxygenases, such as partially purified soybean lipoxygenase, oxidize predominantly at C-13. Some of these lipoxygenases are: flaxseed, 74–94% 13-LOOH (Zimmerman and Vick, 1970a); *Chlorella pyrenoidosa, ca.* 80% 13-LOOH (Zimmerman and Vick, 1973); *Dimorphotheca sinuata,* 92–100% 13-LOOH (Gardner *et al.*, 1973); and peanut, 100% 13-LOOH (St. Angelo *et al.*, 1972). A highly purified lipoxygenase from alfalfa yielded a 50:50 mixture of 9- and 13-LOOH (Chang *et al.*, 1971) and thus behaved like soybean lipoxygenase, type 2.

Those lipoxygenases oxidizing predominantly C-9 are: potato, 95% 9-LOOH (Galliard and Phillips, 1971); corn, 83–88% 9-LOOH (Gardner and Weisleder, 1970); and wheat, barley (Graveland *et al.*, 1972), and oats (Heimann *et al.*, 1973a), mostly 9-LOOH. Further work with corn lipoxygenase oxidations at pH 7 revealed that the 7–9% 13-LOOH produced had racemic steric configuration at C-13 (Hamberg, 1971). Apparently, 13-LOOH was formed by autoxidation. However, at pH 9 corn lipoxygenase produces 85% 13-LOOH (Veldink *et al.*, 1972), a specificity which implies that at pH 9 the 13 isomer is not due to autoxidation.

Reduction. Systems that reduce LOOH to hydroxyoctadecadienoic acid (LOH) have been characterized in plants and animals.

The enzyme from animal tissue, GSH-peroxidase, has been researched extensively. Christophersen (1966) discovered that GSH prevented peroxidation of endogenous lipids in liver homogenates with GSH being oxidized to the disulfide, GSSG. Little and O'Brien (1968) further characterized this reaction and identified it as being mediated by the enzyme, GSH-peroxidase. One equivalent of LOOH or ethyl linolenate hydroperoxide oxidized two molar equivalents of GSH. LOOH and LnOOH were reduced, respectively, to LOH and the corresponding hydroxy acid (LnOH) of LnOOH (Christophersen, 1968, 1969). The mitochondria and supernatant fraction of homogenates contained almost all the GSH-peroxidase (Little and O'Brien, 1968; Flohé and Schlegel, 1971).

Reduction of LOOH and related fatty acid hydroperoxides by plant systems has been observed in a number of species: wheat flour (Graveland, 1970a, 1973b), barley (Graveland *et al.*, 1972), rye and maize (Graveland, 1973a), oats (Graveland, 1973a; Heimann *et al.*, 1973a; Heimann and Schreier, 1970, 1971), potato tubers (Galliard and Phillips, 1972), and *Dimorphotheca sinuata* (Gardner *et al.*, 1973). Some workers have implied that LOOH or LnOOH was reduced by agents already present in plant extracts. Graveland (1970a, 1973a) postulated that thiol groups may mediate the reduction. Others have implicated enzymes. A "lipoperoxidase" in oats utilized electron donors, such as *p*-phenylenediamine and other peroxidase reagents, to reduce LOOH to LOH (Schreier and Heimann, 1971; Heimann and Schreier, 1971; Heimann *et al.*, 1972).

The oat lipoperoxidase was chromatographically inseparable from lipoxygenase activity (Heimann *et al.*, 1973b). They proposed a bifunctional lipoxygenase-lipoperoxidase complex to account for the data. In soybeans, a similar enzyme, "guaiacol-LOOH-oxidoreductase," has been studied (Schormüller *et al.*, 1968; Grosch *et al.*, 1971, 1972). The product(s) of the reaction was not characterized, but presumably the system in soybean is similar to the one described for oats. Also, like the oat system, soybean guaiacol-LOOH-oxidoreductase was found inseparable from lipoxygenase activity (Grosch *et al.*, 1972). This observation was confirmed by Heimann *et al.* (1973b).

LOOH-Isomerase. An LOOH-isomerase activity was found in flaxseed extracts by Zimmerman (1966) and Zimmerman and Vick (1970b). They reported the formation of 12-oxo-13-hydroxy-*cis*-9-octadecenoic and 10-oxo-9-hydroxy-*cis*-12-octadecenoic acids (α-ketols) from LOOH. Since the substrate was predominantly 13-LOOH, the major product was 12-oxo-13-hydroxy-*cis*-9-octadecenoic acid. Production of 10-oxo-9-hydroxy-*cis*-12-octadecenoic acid from 9-LOOH was reportedly minor. In contrast, Veldink *et al.* (1968, 1970a) recorded that flaxseed LOOH-isomerase did not catalyze the isomerization of 9-LOOH, but that only 13-LOOH was converted to 12-oxo-13-hydroxy-*cis*-9-octadecenoic acid. LOOH-isomerase from corn germ was capable of utilizing both isomeric forms of LOOH and of yielding two positional isomers of the α-ketol (Gardner, 1970). However, only 10-oxo-9-hydroxy-*cis*-12-octadecenoic acid was formed when linoleic acid was added to corn germ extracts, because of the specificity for oxidation of C-9 by corn lipoxygenase.

Veldink *et al.* (1970b) helped elucidate the mechanism of flaxseed LOOH-isomerase action. Using 13-LOOH with the hydroperoxy group labeled with ^{18}O, they proved that only the 12-oxo group of the product was labeled. Thus the unlabeled 13-hydroxyl at the original position of the hydroperoxy group may have come from the solvent. A cyclic oxygen intermediate was proposed to account for the transfer of ^{18}O (Veldink *et al.*, 1970b; Vliegenthart *et al.*, 1972).

In addition to producing α-ketols, corn germ extracts form γ-ketols from LOOH (Gardner, 1970). 10-Oxo-13-hydroxy-*trans*-11-octadecenoic and 12-oxo-9-hydroxy-*trans*-10-octadecenoic acids (γ-ketols) were derived from 9-LOOH and 13-LOOH, respectively. Significantly an oxo group is formed α to the original hydroperoxy group in both the α- and γ-ketols. It is not known whether the same LOOH-isomerase mediates the formation of both α- and γ-ketols.

Esselman and Clagett (1974) reported a "lipohydroperoxidase" from alfalfa seedlings that produced γ-ketols from LOOH, but did not produce α-ketols. By use of the ^{18}O label they proved that both the oxo and hydroxy groups of the γ-ketol were derived from the hydroperoxy moiety. This enzyme, in contrast to the γ-ketol producing

enzyme from corn germ, appeared to be forming 13-hydroxy-10-oxo-*trans*-11-octadecenoic and 9-hydroxy-12-oxo-*trans*-10-octadecenoic acids from 13-LOOH and 9-LOOH, respectively. More work is required to establish whether the enzyme from corn germ is substantially different from the alfalfa enzyme in mechanism of action.

An acylated α-ketol was produced in corn germ extracts during the oxidation of linoleic acid (Gardner, 1970). The product 9-(*cis*-9,*cis*-12-octadecadienoyl)-10-oxo-*cis*-12-octadecenoic acid essentially consisted of linoleic acid esterified to the hydroxyl of the α-ketol. It was proved that the α-ketol plus linoleic acid did not result in the acylated α-ketol, but that linoleic acid plus LOOH was required. This observation is consistent with the conclusion that the hydroperoxy group may be replaced not only by solvent, but by an available acyl nucleophile, linoleic acid, during the reaction. This hypothesis was strengthened by Christianson and Gardner (1975) when they showed that α-ketols with groups other than hydroxyl could be produced with corn LOOH-isomerase. Instead of the hydroxyl, either *S*-ethyl, methoxy, or oleyl groups could be formed in the presence of either ethyl mercaptan, methanol, or oleic acid, respectively.

LOOH-isomerase has been observed in many plant extracts. Oxidations by the following plant extracts have yielded either α-ketol or α- and γ-ketols: barley, α- and γ-ketols (Graveland *et al.*, 1972); wheat flour, α-ketol (Graveland, 1973b); wheat germ, α-ketol (Zimmerman and Vick, 1970b); and rye flour, unresolved α- or γ-ketols (Graveland, 1973a). Although LOOH-isomerase has been reported in soybeans, mung beans (Zimmerman and Vick, 1970b), and peanuts (St. Angelo and Ory, 1972), no products of the reaction were characterized.

LOOH-isomerase appears to be localized both in soluble and particulate fractions of seed extracts. Zimmerman and Vick (1970b) demonstrated that it was largely in the soluble fraction of flaxseed extracts. Also, LOOH-isomerase was bound to particulates in extracts of flax, watermelon, and sunflower seedlings, cauliflower buds (Zimmerman and Vick, 1972; Zimmerman *et al.*, 1974), and corn germ (Gardner and Christianson, 1972).

Epoxidation by Flour-Water Suspensions. Graveland (1970a,b) uncovered an oxidation reaction of linoleic acid in wheat flour-water suspensions. In this system lipoxygenase is adsorbed on gluten and, through action of the complex, linoleic acid becomes oxidized to a mixture of 9-hydroxy-*cis*-12,13-epoxy-*trans*-10-octadecenoic and 13-hydroxy-*cis*-9,10-epoxy-*trans*-11-octadecenoic acids. Presumably these fatty acids are produced through a LOOH intermediate, but when preformed LOOH was added to flour-water suspensions or to dough, the hydroxyepoxyoctadecenoic acids did not form. Thus a lipoxygenase-mediated intermediate similar but not identical with LOOH may be responsible. The hydroxyepoxyoctadecenoic acids are readily hydrolyzed by a water-soluble factor from flour into the corresponding 9,12,13-trihydroxy-*trans*-10-octadecenoic and 9,10,13-trihydroxy-*trans*-11-octadecenoic acids (Graveland, 1970a).

In 1970(a), Graveland reported that linoleic acid oxidation formed 13-hydroxy-*cis*-9,10-epoxy-*trans*-11-octadecenoic and 9-hydroxy-*cis*-12,13-epoxy-*trans*-10-octadecenoic acids in a 15:85 ratio. He also found that isomeric LOOH produced in the flour-water suspensions were 9-LOOH and 13-LOOH in a 15:85 ratio. Although Graveland did not speculate on the mechanism, his data implied that 9-hydroxy-*cis*-12,13-epoxy-*trans*-10-octadecenoic acid was derived from 13-LOOH and 13-hydroxy-*cis*-9,10-epoxy-*trans*-11-octadecenoic acid was from 9-LOOH. However, in a subsequent communication, Graveland *et al.* (1972) reported oxidation of linoleic acid in wheat flour suspensions resulted in predominantly 9-LOOH instead of the 13-LOOH reported previously; thus a different pathway of epoxidation may be involved. The possibility that the epoxide was derived instead from oxygen addition to the *cis*-olefin γ,δ to the hydroperoxy group was strengthened by both Graveland (1973b) and Heimann *et al.* (1973a). When linolenic acid was oxidized by wheat flour-water suspensions (Graveland, 1973b), the main products were 9-LnOOH and 9,12,13-trihydroxy-*trans*-10,*cis*-15-octadecadienoic acid (the solvolysis product of 9-hydroxy-12,13-epoxy-*trans*-10,*cis*-15-octadecadienoic acid). To explain the reaction products, he proposed a mechanism of olefin epoxidation γ,δ to the hydroperoxy group. Similarly, in oat extracts the predominant products of linoleic acid oxidation are 9-LOOH, 9-hydroxy-*cis*-12,13-epoxy-*trans*-10-octadecenoic, and 9,12,13-trihydroxy-*trans*-10-octadecenoic acids (Heimann *et al.*, 1973a).

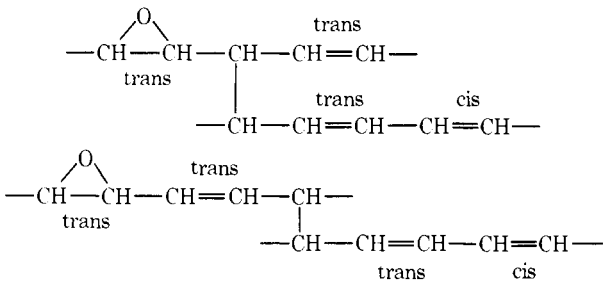
Cereal flour-water suspensions other than wheat also produce trihydroxyoctadecenoic acids so that a similar reaction sequence of epoxidation, followed by epoxide ring opening, evidently is operative. Some of these flours were: barley (Graveland *et al.*, 1972) and rye, oat, and maize (Graveland, 1973a). Because all these flours also produce other fatty acid products, varying LOOH-isomerase and reducing activities are indicated as well. In addition, other fatty acids were formed that did not fit into any pathway of LOOH decomposition now known. These were: 9,10-dihydroxy-*cis*-12-octadecenoic; 12,13-dihydroxy-*cis*-9-octadecenoic; 13-oxo-9,10-dihydroxy-*trans*-11-octadecenoic; 9-oxo-12,13-dihydroxy-*trans*-10-octadecenoic (Graveland *et al.*, 1972; Graveland, 1973a); 9,12-dihydroxy-*trans*-10-octadecenoic; and 10,13-dihydroxy-*trans*-11-octadecenoic acids (Graveland, 1973a).

Vinyl Ether Production. A novel enzyme, at present unique to potato tubers, converted 9-LOOH into 9-(nona-1′,3′-dienoxy)non-8-enoic acid (Galliard and Phillips, 1972). The geometry of this acid was fully characterized as 9-(*trans*-1′,*cis*-3′-nonadienyloxy)-*trans*-8-nonenoic acid, as well as the corresponding product from 9-LnOOH, 9-(*trans*-1′,*cis*-3′,*cis*-6′-nonatrienyloxy)-*trans*-8-nonenoic acid (Galliard *et al.*, 1973). The 13-LOOH isomer did not serve as a substrate for the enzyme (Galliard and Matthew, 1975). These vinyl ether fatty acids are easily degraded into C_8 and C_9 carbonyl fragments by an enzyme or Fe(II) ions (Galliard *et al.*, 1974).

Anaerobic Lipoxygenase Reactions. In 1962, Vioque and Holman identified 9-oxooctadeca-10,12-dienoic and 13-oxooctadeca-9,11-dienoic acids from a mixture of products resulting from oxidation of linoleic acid by soybean lipoxygenase. Garssen and his coworkers (1971) showed that oxooctadecadienoic acid formation requires anaerobic conditions, linoleic acid, LOOH, and native lipoxygenase. Only LOOH, not linoleic acid, is converted to oxooctadecadienoic acids. Although not directly demonstrated, presumably 13-LOOH was converted into 13-oxooctadeca-9,11-dienoic acid. Other compounds being derived from 13-LOOH under anaerobic conditions were cleavage products: 13-oxo-*cis*-9,*trans*-11-tridecadienoic acid, 13-oxo-*trans*-9,*trans*-11-tridecadienoic acid, and *n*-pentane. In pentane formation, soybean lipoxygenase, type 1, is much more active than the isoenzyme, type 2 (Johns *et al.*, 1973).

Further studies of the anaerobic reaction revealed dimeric octadecadienoic acids, as well as oxygenated dimers (Garssen *et al.*, 1972). Being formed exclusively from linoleic acid were dimers with the following linkages: C-11–C-13′ or –C-9′ and C-13–C-13′ or –C-9′. Spectral evidence indicated that those fatty acids dimerized at C-11 were *cis*-9,*cis*-12-dienes and those dimerized at C-9 or C-13 were conjugated *cis,trans*-dienes. Oxygenated dimers, a result of allowing one molecule of linoleic acid and one molecule of LOOH to react, were postulated from available data to be composed of the following partial structures.

The observed dimeric products resulting from the anaerobic reaction were indicative of a free-radical reaction.



DeGroot *et al.* (1973) detected radicals by electron spin resonance spectroscopy using 2-methyl-2-nitrosopropanol as a scavenger. The radical trapped proved to be at C-13 or C-9, or both.

Production of Volatile Aldehydes. Although the presence of volatile aldehydes in macerated plant tissue has been known for some time, recent research has indicated that lipoxygenase may initiate aldehyde formation. The hypothesis is not new since Nye and Spoehr (1943) were the first to suggest lipoxygenase as the cause of hexenal formation in macerated leaves. Tressl and Drawert (1973) have published a review on the production of volatile aldehydes in fruits.

Hexenal is formed from linolenic acid in leaves (Major and Thomas, 1972; Hatanaka and Harada, 1973; Saijyo and Takeo, 1972) and in fruits or vegetables (Grosch and Schwarz, 1971; Grosch, 1968; Kazeniac and Hall, 1970). The initial aldehyde formed from linolenic acid is *cis*-3-hexenal, which is in turn converted into *trans*-2-hexenal (Kazeniac and Hall, 1970; Hatanaka and Harada, 1973).

Other aldehydes reported to originate from linolenic acid are: acetaldehyde, propanal, crotonaldehyde, pent-2-enal, hepta-*trans*-2,*cis*-4-dienal (Arens *et al.*, 1973), and hexanal (Jadhav *et al.*, 1972).

Hexanal is produced mainly from linoleic acid (Saijyo and Takeo, 1972; Grosch and Schwarz, 1971). When linoleic acid was oxidized by either a partially purified soybean lipoxygenase (Grosch and Schwencke, 1969) or a highly purified pea lipoxygenase (Arens *et al.*, 1973), not only hexanal was formed, but pentanal, hept-2-enal, oct-2-enal, nona-2,4-dienal, and deca-2,4-dienal. Also, non-*trans*-2-enal reportedly originates from precursor, linoleic acid (Grosch and Schwarz, 1971).

Very few of these investigators used preformed LOOH or LnOOH in their studies on the origin of volatile aldehydes even though some suggested an LOOH or LnOOH intermediate. Tressl and Drawert (1973) used radiolabeled LOOH in the production of labeled volatile aldehydes. Such experiments seem obvious and necessary. Kalbrener *et al.* (1974) indicated that LOOH and LnOOH from lipoxygenase oxidation may easily decompose to volatile aldehydes even after their isolation by column chromatography. In this work LOOH and LnOOH gave grassy-beany taste at 50 and 10 ppm, respectively. However, it is not known whether all aldehyde formation in plant material is derived from fatty acid hydroperoxide. At least two investigators do not believe hydroperoxides are the intermediates (Grosch and Schwarz, 1971; Major and Thomas, 1972).

NONENZYMIC REACTIONS

During food storage the nonenzymic decomposition of lipid hydroperoxides could be of much greater significance than decomposition by enzymes. In heat-processed foods and edible oils, enzyme activity is usually negligible, and autoxidation becomes the primary cause of lipid peroxidation. The accompanying rancid off-flavors are due to further decompositions of hydroperoxides. In the absence of antioxidants or decomposing reagents, hydroperoxides accumulate and initiate further lipid oxidation and other free-radical reactions.

The most common route of lipid hydroperoxide decomposition is believed to be by a free-radical mechanism involving homolytic cleavage of the hydroperoxy group. Among the catalysts or conditions that promote homolytic decompositions of hydroperoxides are heat, photolysis, metal ions or their complexes, metalloproteins, and many other agents that promote free radicals. Transition metal ions and metalloproteins effectively catalyze homolytic reactions of hydroperoxides as outlined by Ingold (1962)

$$M^{n+} + ROOH \longrightarrow M^{(n+1)+} + RO\cdot + OH^-$$

$$M^{(n+1)+} + ROOH \longrightarrow M^{n+} + RO_2\cdot + H^+$$

Depending upon the conditions, metal ions and their complexes can act with dual antioxidant-prooxidant properties because of their ability to decompose hydroperoxides as well as to initiate free radicals.

Heterolytic reactions also may affect lipid hydroperoxide decompositions significantly because numerous nucleophiles attack the hydroperoxy group effectively.

Homolytic. Thermal decomposition of MLOOH yielded mostly dimer (Williamson, 1953; Frankel *et al.*, 1960). According to Frankel and his associates, when MLOOH was decomposed neat at 210°, the products were 82% dimer, monomers with decreased conjugated diene content, and 8–10% volatiles, half of which were water. They concluded the dimers were linked by a carbon–carbon bond and contained the following functional groups (mole/mole of dimer): 1 hydroxyl, 0.5 nonester carbonyl, 2 double bonds, and perhaps some epoxide or intramolecular peroxide. These workers postulated that the homolytic cleavage of the hydroperoxy group to alkoxy and hydroxyl radicals was responsible for the observed reaction. Because the yield of dimer was directly proportional to the MLOOH content in reactions composed of mixtures of MLOOH and methyl linoleate, they believed that only MLOOH participated in polymerization reactions. Mounts *et al.* (1970) countered this hypothesis with work showing that dimer was produced from one molecule of methyl linoleate and one molecule of MLOOH in mixed thermal (210°) reactions. However, these results obtained with a ^{14}C radiotracer may have been dependent on the low percentage of MLOOH (5–6%) relative to methyl linoleate in the reaction. A homolytic mechanism was proposed by Evans *et al.* (1967) to explain the thermal decomposition of pure 13-LOOH to *n*-pentane at 260°. This fragment undoubtedly originates from cleavage between C-13 and -14.

At low temperatures, MLOOH decomposed at a greatly diminished rate (Johnston *et al.*, 1961). As evidenced by a loss in conjugated diene and peroxide, MLOOH was destroyed at 4° over a period of days either under N_2 or O_2. Products were primarily dimer and scission acids. Under O_2 there was a simultaneous uptake of O_2 and peroxide loss. Privett and Nickell (1956) also observed an oxidation of MLOOH that did not have any detectable lag period. Because the rate of oxygen uptake could be reduced significantly by dilution with methyl myristate, oxidation must depend on a free-radical chain. The products were polymer with isolated trans and conjugated trans,trans unsaturation and monomer with enhanced trans,trans conjugation. Kanazawa *et al.* (1973) reported that the oxidation of LOOH followed first-order kinetics with respect to LOOH or oxygen. One mole of LOOH absorbed 1 mol or more of oxygen.

Metal ions and metalloproteins readily decompose hydroperoxides by a homolytic route. Maier and Tappel (1959a) studied the decomposition of LOOH by hematin, hemoprotein, and various metalloprotoporphyrins in aqueous solution. The reaction catalyzed by hematin was studied in more detail (Maier and Tappel, 1959b). That hematin catalyzed formation of an alkoxy radical from MLOOH was proved by trapping the radical with hydroquinone to yield MLOH as a product. Without hydroquinone there was a 62% loss in diene conjugation, and the

resultant product mixture was analyzed to have the following functionality: epoxide, hydroxyl, carbonyl, and double bonds with isolated trans, conjugated cis,trans, and trans,trans configuration. In studies of LOOH decomposition by metal ions and hemoproteins, O'Brien (1969) found a variety of products having thin-layer chromatographic (tlc) mobilities less than the mobility of LOOH, and noted that these products resembled those produced by thermal decomposition (60°) of LOOH. In order of effectiveness in LOOH degradations were: hematin and hemoproteins > Fe(II) > Fe(III) > Cu(II). Decomposition by metal ions in their higher valency state was stimulated by electron donors, such as ascorbate and cysteine, presumably because they kept the metal ions at their lowest valence, favoring alkoxy radical formation.

Products from the decomposition of LOOH by Fe(II) and Fe(III)-cysteine were identified by Gardner *et al.* (1974). In the FE(III)-cysteine reaction a catalytic concentration of Fe(III) (10^{-5} *M*) was kept reduced by a large excess of cysteine. Under these conditions in aqueous ethanol, LOOH decomposed into nine detectable monomeric fatty acids, each of which was a mixture of two or more positional isomers. Positional isomers were obtained primarily because of the isomeric hydroperoxides used (79% 13-LOOH and 21% 9-LOOH). The fatty acids and the percentage of positional isomers were: (I) 74% 13-oxo-*trans,-trans-* (and *cis,trans-*) 9,11-octadecadienoic and 26% 9-oxo-*trans,trans-* (and *cis,trans-*) 10,12-octadecadienoic acids; (II) 78% 9-oxo-*trans*-12,13-epoxy-*trans*-10-octadecenoic and 22% 13-oxo-*trans*-9,10-epoxy-*trans*-11-octadecenoic acids; (III) 49% 9-oxo-*cis*-12,13-epoxy-*trans*-10-octadecenoic and 51% 13-oxo-*cis*-9,10-epoxy-*trans*-11-octadecenoic acids; (IV) 13-hydroxy-9,11-octadecadienoic and 9-hydroxy-10,12-octadecadienoic acids; (V) 88% 11-hydroxy-*trans*-12,13-epoxy-*cis*-9-octadecenoic and 12% 11-hydroxy-*trans*-9,10-epoxy-*cis*-12-octadecenoic acids; (VI) 71% 11-hydroxy-*trans*-12,13-epoxy-*trans*-9-octadecenoic and 29% 11-hydroxy-*trans*-9,10-epoxy-*trans*-12-octadecenoic acids; (VII) 13-oxo-9-hydroxy-*trans*-10-octadecenoic and 9-oxo-13-hydroxy-*trans*-11-octadecenoic acids; (VIII) isomeric mixtures of 9,12,13-dihydroxyethoxy-*trans*-10-octadecenoic and 9,10,13-dihydroxyethoxy-*trans*-11-octadecenoic acids; and (IX) 60% 9,12,13-trihydroxy-*trans*-10-octadecenoic and 40% 9,10,13-trihydroxy-*trans*-11-octadecenoic acids. Due to the position of the functional groups, certain reaction pathways were probably operative. In Figure 2 a possible mechanism for the degradation of LOOH by Fe(III)-cysteine is proposed.

In view of the various complexes of iron and other metal ions with cysteine, oxygen, hydroxyl radical, and hydroperoxide that are known or proposed (Maier and Tappel, 1959b; Sheng and Zajacek, 1970; Heaton and Uri, 1961; Gray, 1964), the Fe(II)-Fe(III) redox cycle and other reactions shown in Figure 2 probably oversimplify the actual catalysis. Also, it is not known if heterolytic reactions participate in the reaction. Thus, the proposed pathways in Figure 2 must be considered as a preliminary attempt to explain the data prior to further research in this area.

As can be seen, the major pathways of decomposition are postulated as: (1) loss or gain of a hydrogen radical yielding products I and IV; (2) cyclization of the alkoxy radical to the α-unsaturation yielding products II, V, VI, and to some extent III; and (3) 1,4 addition to the conjugated diene yielding products VIII and IX. The mechanism for formation of hydroxyepoxyoctadecenoic acid (Figure 2) is identical with the one proposed by Maier and Tappel (1959b) to explain the epoxide and hydroxyl groups found in the product mixture from decomposition of LOOH by hematin. Although they did not identify this structure, remarkable insight into the identity of the products may have been demonstrated. Formation of isomeric oxoepoxyoctadecenoic acids predominantly proceeds through the same type of epoxide formation, except it is believed the

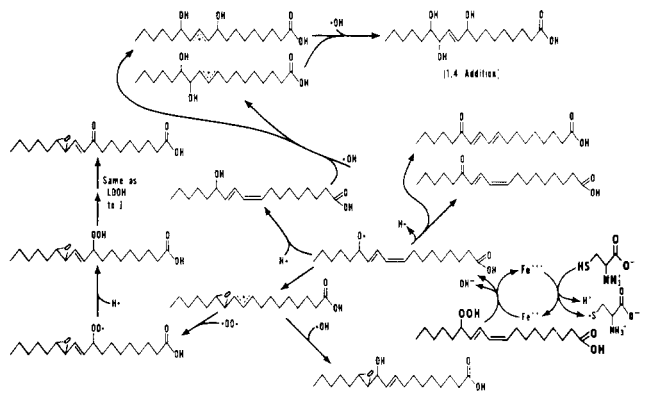


Figure 2. Proposed mechanism of product formation from decomposition of linoleic acid hydroperoxide by Fe(III)-cysteine (reactants are in boldface type).

oxo group is derived from secondary hydroperoxidation as shown in Figure 2. Because O_2 was required to produce the oxoepoxyoctadecenoic acids, the pathway shown is indicated. On the basis of the positional isomers found, a portion of the *cis*-epoxide, product III, may have been formed *via* a more traditional route of epoxide formation in the presence of hydroperoxide, *i.e.*, direct addition of peroxide oxygen across the double bond with retention of geometry. An example of such a reaction was studied by Sheng and Zajacek (1970), who oxidized olefins with organic hydroperoxides catalyzed by various metal ion complexes. For the most part, 1,4 addition to the conjugated diene seems to account for formation of trihydroxyoctadecenoic acid with some participation coming from 1,2 addition (γ,δ from the original hydroperoxy group). Presumably dihydroxyethoxyoctadecenoic acid formed by a similar pathway.

When equimolar Fe(II) was allowed to react with LOOH by Gardner *et al.* (1974), the products were not completely in harmony with the Fe(III)-cysteine reaction. The major product was a mixture of dimers with a calculated carbon number consistent with dimeric C-18 fatty acids containing oxygenated groups. Since the rate of the Fe(II)-catalyzed reaction was rapid, dimer formation may have been promoted by termination reactions due to a higher concentration of radicals. Monomeric fatty acids identified were similar to those found as products from decomposition with Fe(III)-cysteine. The monomers were: oxooctadeca-*trans,trans*-dienoic acid (15% yield), products IV, V and/or VI, VIII, and IX, as well as ethoxyoctadecadienoic and diethoxyhydroxyoctadecenoic acids.

When catalyzed by ionic iron, α-tocopherol, coenzymes Q, and related compounds are oxidized in the presence of LOOH, LnOOH, or their methyl esters while the hydroperoxides themselves are destroyed. Gruger and Tappel (1970a,b) studied the oxidation of α-tocopherol and coenzymes Q by MLnOOH and MLOOH catalyzed by Fe(III). As products of the reaction they identified the corresponding quinones of α-tocopherol and coenzymes Q but did not identify the fatty acid products. In a related study, Gardner *et al.* (1972) confirmed that α-tocopherol is oxidized to α-tocopherolquinone in the presence of oxygen, but under anaerobic conditions α-tocopherol or its model compound, 2,2,5,7,8-pentamethyl-6-hydroxychroman, added directly to LOOH or MLOOH. Besides the addition compounds, oxooctadeca-*trans,trans*-dienoic acid was isolated from the reaction mixture. Because the products were almost identical regardless of whether the reaction was catalyzed by Fe(III) or proflavine sensitized by visible light, the observed products were undoubtedly formed by free-radical mechanisms.

The addition compounds between LOOH and 2,2,5,7,8-pentamethyl-6-hydroxychroman were isomeric and were identified as follows: 11-(2,2,5,7,8-pentamethyl-6-oxychro-

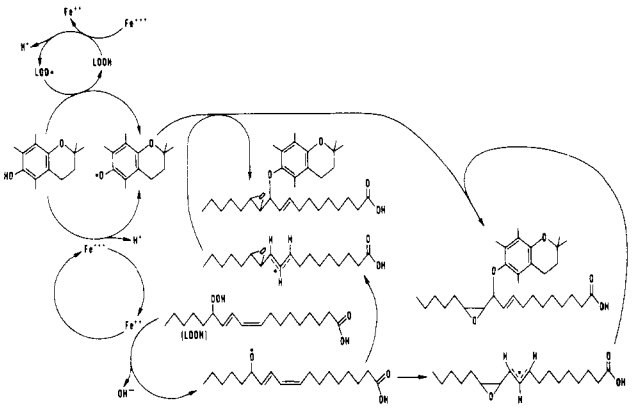


Figure 3. Proposed mechanism of addition between 2,2,5,7,8-pentamethyl-6-hydroxychroman and LOOH catalyzed by Fe(III) (from Gardner *et al.*, 1972).

man)-*trans*-12,13-epoxy-*trans*-9-octadecenoic, 11-(2,2,5,7,8-pentamethyl-6-oxychroman)-*cis*-12,13-epoxy-*trans*-9-octadecenoic and 11-(2,2,5,7,8-pentamethyl-6-oxychroman)-*cis*-9,10-epoxy-*trans*-12-octadecenoic acids. The two positional isomers, *i.e.*, 9,10-epoxide *vs.* 12,13-epoxide, were due to 9- and 13-LOOH used as reactants. A schematic showing the mechanism of how 13-LOOH might add pentamethylhydroxychroman has been proposed (Figure 3).

Heterolytic. In biological systems or foodstuffs, it is not known to what extent heterolytic reactions decompose lipid hydroperoxides. Since there are usually ample homolytic catalysts present, like transition metal ions, metalloproteins, or photosensitized chlorophyll, probably free-radical reactions are most significant. Lipid hydroperoxides are attacked readily by nucleophiles; heterolytic reactions, however, cannot be minimized.

According to O'Brien (1969), the nucleophiles he examined converted LOOH to a product he tentatively identified as LOH. The order of nucleophilic effectiveness was given as dimercaptopropanol > cysteine, ascorbate, thiourea, sulfite > thiosulfate, I^-, α-lipoic acid, methionine. Because saponification of MLOOH yielded LOH, hydroxide is also an effective nucleophile (Frankel *et al.*, 1961). Many reagents routinely reduce the hydroperoxy group quantitatively to hydroxyl, such as $NaBH_4$, $LiAlH_4$, H_2/Pd, and $SnCl_2$.

Hamberg and Gotthammar (1973) reported an anaerobic decomposition of 13-LOOH in aqueous ethanol at 100°. Although the thermal conditions would indicate a homolytic mechanism was possible, their data support an ionic mechanism. From a product mixture of at least eight components, one of the major products was identified as *threo*-11-hydroxy-12,13-epoxy-9-octadecenoic acid. Since the corresponding erythro isomer was absent, evidently the epoxide and hydroxyl added cis at the Δ-11 double bond (addition on the same side of the molecule). Cis addition could be explained by a homolytic mechanism if the hydroxyl radical of the hydroperoxy group would be trapped by a "solvent cage"; however, radioactive labeling proved that 90% of the hydroxyl was derived from the solvent. Tentative identification of 11-ethoxy-12,13-epoxy-9-octadecenoic acid from the product mixture also lent support to proof of solvent participation. Gardner *et al.*, (1972, 1974) proposed that similar compounds resulted from homolytic reactions, but they did not study the erythro–threo geometry of these compounds. Now research of this nature appears necessary.

DISCUSSION

Although all enzymic reactions are ultimately chemical processes, many LOOH decompositions by enzymes do not correlate with nonenzymic processes. The similarities

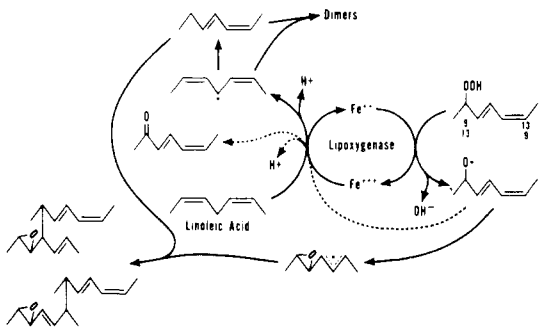


Figure 4. Mechanism proposed to explain products from anaerobic reactions of soybean lipoxygenase. Carbon numbers indicate the two isomeric forms of LOOH.

and contrasts between the two types of reactions are being given here to provide clues that may lead to a greater understanding of both.

The production of LOH or LnOH from LOOH or LnOOH, respectively, will not be elaborated upon since the enzymic *vs.* the nonenzymic reactions are probably similar and are not complex. Also, the potato enzyme that produces vinyl ethers will not be mentioned further since it appears uniquely enzymic and possibly unique to the potato.

Oxohydroxyoctadecenoic acids result from the action of both LOOH-isomerase (Zimmerman, 1966; Gardner, 1970) and the homolytic decomposition of LOOH (Gardner *et al.*, 1974). The enzymic reaction produces different isomeric forms of this fatty acid compared to the nonenzymic process (α- and γ-ketols *vs.* δ-ketol). In the enzymic formation of the α-ketol, 12-oxo-13-hydroxy-*cis*-9-octadecenoic acid, the oxygen has migrated from the original hydroperoxy group to the α unsaturation resulting in an oxo group at C-12 (Veldink *et al.*, 1970b). Although the mechanism of γ-ketol formation by corn germ LOOH-isomerase is not known, the position of the oxo group is the same as the α-ketol, *i.e.*, α to the carbon originally bearing the hydroperoxy group (Gardner, 1970). Formation of γ-ketols by alfalfa seedling extracts appears to be substantially different in mechanism since the result is transfer of one oxygen of the hydroperoxy group across three carbons to the oxo function while the other oxygen appears to be converted to the hydroxyl *in situ* (Esselman and Clagett, 1974). Unlike the enzymic products, the δ-ketols (13-oxo-9-hydroxy-*trans*-10-octadecenoic and 9-oxo-13-hydroxy-*trans*-11-octadecenoic acids) from homolytic decomposition of LOOH obviously have their oxo groups situated either at the carbon of the original hydroperoxy group or δ to it (Gardner *et al.*, 1974). Common to α-, γ- (corn germ), and δ-ketols is formation of a methylene α to the oxo group. This position of the methylene may indicate that a hydrogen radical has been transferred from the carbonyl-bearing carbon, but, of course, more research is needed to either support or reject this hypothesis and others concerning ketol formation.

Epoxyhydroxyoctadecenoic acid formation in flour-water suspensions (Graveland, 1973b) proceeded by a route different from nonenzymic reactions whether or not they are homolytic (Gardner *et al.*, 1974) or heterolytic (Hamberg and Gotthammar, 1973). In the nonenzymic reaction the hydroperoxy group is cyclized to the carbon α to it, and the hydroxyl adds β. By the homolytic mechanism (Figure 2) an epoxyene radical is intermediate; thus, a product with the hydroxyl γ to epoxide should have been found also, but was not. The enzymic product did have a hydroxyl γ to epoxide, but it formed by an entirely different mechanism compared to the nonenzymic process. Apparently the flour-water process promoted direct addition of hydroperoxide oxygen across the γ,δ cis unsaturation. As would be expected, the epoxide retained the

cis geometry of the olefin, whereas in the homolytic reaction the epoxides are primarily trans. Another similarity in products was noted between the flour-water system *vs.* homolytic process, *i.e.*, the formation of trihydroxyoctadecenoic acid, but their mode of formation apparently is not related. In flour-water suspensions, trihydroxyoctadecenoic acid is derived from solvolysis of epoxyhydroxyoctadecenoic acid, but in the homolytic reaction, it appears to be derived by 1,4 and 1,2 addition to the diene (Figure 2).

An anaerobic reaction of soybean lipoxygenase (Garssen *et al.*, 1971, 1972) formed products from LOOH and linoleic acid that correlated well with products from the homolytic decomposition of LOOH (Gardner *et al.*, 1974). Since iron has been discovered as a component part of soybean lipoxygenase (Chan, 1973; Roza and Francke, 1973), the iron may be related to the observed products of the anaerobic reaction. In Figure 4, a mechanism is proposed that shows lipoxygenase-bound iron which cycles electrons with linoleic acid and LOOH. Through this mechanism the known products of the anaerobic process possibly are explained. Although the redox mechanism probably oversimplifies the enzyme active site, it fits the available data. In native lipoxygenase the iron exists as Fe(III) (Pistorius and Axelrod, 1974). Inasmuch as cysteine (Roza and Francke, 1973) and thiol chelators (Pistorius and Axelrod, 1974) inhibit lipoxygenase, the rate-limiting step may be removal of a hydrogen radical from linoleic acid by the Fe(III) state of the enzyme. In harmony with the anaerobic mechanism proposed in Figure 4, the normal aerobic oxidation of linoleic acid by lipoxygenase may be

$$\underset{\text{(linoleic acid)}}{LH} + Fe^{3+} \xrightarrow{\text{slow}} L\cdot + H^+ + Fe^{2+}$$

$$O_2 + Fe^{2+} \xrightarrow{\text{fast}} \underset{\text{(superoxide)}}{\cdot O_2^-} + Fe^{3+}$$

$$\cdot O_2^- + H^+ \longrightarrow \cdot O_2H$$

$$HO_2\cdot + L\cdot \longrightarrow LOOH$$

Oxygen can induce a physical effect on lipoxygenase, *i.e.*, fluorescence enhancement (Finazzi-Agro *et al.*, 1973). Furthermore, the observation of singlet oxygen-like reactions in lipoxygenase oxidations has been reported (Chan, 1971). Superoxide is a known source of singlet oxygen (Kasha and Khan, 1970).

Almost all the common straight-chain volatiles produced either by biological systems or autoxidation are believed to be derived from cleavage either at the hydroperoxy carbon or at another carbon after secondary reactions. There are considerable similarities in the structures of the volatiles regardless of whether they originate from autoxidation or macerated tissue. Biological systems are more selective than autoxidation processes and, thus, usually result in a narrower range of volatiles. Although there is sparse information concerning volatile formation from pure lipid hydroperoxides, the literature is extensive on the subject of volatile production by autoxidizing triglycerides and other fatty esters.

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Lipoxygenase and Flavor of Soybean Protein Products

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Work of the last 10 years indicates that lipids are a major source of compounds responsible for objectionable flavors in soybean protein products. Lipoxygenase is an important factor in the generation of flavor compounds from the lipids when soybeans are processed under high moisture conditions as in the preparation of soy milk by the traditional process. Less certain is the significance of lipoxygenase action when soybeans are processed under low moisture conditions as in the commercial extraction of oil. However, the potency of the flavor compounds that can arise by decomposition of hydroperoxides generated by lipoxygenase suggests that very little oxidation may be needed to give rise to objectionable levels of flavor constituents. Consequently, lipoxygenase cannot be ruled out as a causative factor until further work clearly demonstrates that lipoxygenase catalysis is not occurring at low moisture levels. High temperature is the key step currently proposed for inactivation of lipoxygenase during processing of soybeans: (a) grinding with hot water; (b) dry heating-extrusion cooking; (c) blanching; and (d) grinding at low pH followed by cooking. Products from such processes have improved flavor, but may lack functionality because of poor protein solubility caused by heat treatment. An alternative approach is to extract the flavor components after they are formed with hexane–ethanol or hexane–2-propanol. Relatively little denaturation of the proteins occurs with these extraction solvents.

In 1928 Haas and Bohn applied for the first of a series of five consecutive patents issued in 1934 and assigned to J. R. Short Milling Co., Chicago, Ill. In their patent they described the use of ground soybeans as an agent for bleaching the carotene pigments of wheat flour during breadmaking (Haas and Bohn, 1934). A whiter bread crumb was obtained as a result of bleaching the flour pigments and their preparation was designed to replace the chemicals—nitrogen peroxide, chlorine, nitrogen trichloride, and benzoyl peroxide—then in use to bleach white flour. One method of preparing the bleaching agent involved grinding washed beans, removing the hulls, and mixing the resulting full-fat flour with four parts of corn flour (Haas, 1934). Between 0.75 and 2% of the soy-corn flour mixture was sufficient for bleaching.

The bleaching agent was heat-labile and required air or oxygen for reaction to occur and bleaching was rapid at 40–50° in the presence of moisture as in the mixing of bread dough. Haas and Bohn speculated that an enzyme caused the bleaching and subsequent work by others confirmed this speculation. The enzyme was named carotene oxidase although later studies indicated that a coupled reaction with unsaturated fats was involved (Holman and Bergstrom, 1951). The name lipoxidase originally introduced by Andre and Hou (1932) for an enzyme in soybeans that oxidized fat was then used, but now the preferred name is lipoxygenase (EC 1.13.1.13).

Flavor Problems with Soy Flour in Bread. Haas (1934) pointed out that addition of raw soy-corn flour at levels above 2% (0.4% soy flour) "provided an undesirable bean flavor" to bread. This fact has been confirmed by many workers since then (Table I) who were interested in adding soy flour at levels above that required for bleaching in order to increase the protein level as well as to correct the lysine imbalance in wheat proteins. For example, Finney *et al.* in 1950 reported that excellent bread could be baked with wheat–soy flour blends containing 4–8% soy flour (wheat flour basis) as judged by loaf volume and crumb grain. However, trained judges could detect soy flavor even at the 4% level. Finney and coworkers also reported the flavor strongest for breads containing full-fat

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