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Biochemical Aspects of Lipid-Derived Flavors in Legumes

David J. Sessa

Lipoxygenase-mediated conversion of polyunsaturated fatty acids to aldehydes and alcohols is a major contributor to the off-flavors in legume protein products. Numerous volatile compounds produced by action of either purified pea or soybean lipoxygenases on linoleic acid and linolenic acids include 2-*n*-pentylfuran and 3-*cis*-hexenal, both of which reportedly contribute to the green-beany flavor of soybeans. Higher alka-2,4-dienals, described as tasting oxidized, cardboardlike, oily, and painty, have also been generated by enzymic oxidations. A novel 5-substituted-2-furaldehyde from linolenic acid decomposition is released from bitter tasting soy phospholipids. Nonvolatile oxygenated fatty acids are also generated in model systems with soybean lipoxygenase and linoleic acid or its hydroperoxide in the presence of electron donors. Similar fatty acids can also arise from action of cysteine–Fe^{3+} on linoleic acid hydroperoxides, are found on bitter-tasting soy phosphatidylcholines (SPC), and are produced by soy lipoxygenases acting on purified SPC substrates. Development of off-flavors can be controlled by inactivation of lipoxygenase with heat, acid, alcohol, or antioxidants.

Lipoxygenase, an iron-bearing protein, not only catalyzes the hydroperoxidation of polyunsaturated fatty acids but also like peroxidase, an iron porphyrin enzyme that catalyzes degradation of fatty acid hydroperoxide in the presence of a hydrogen donor, or cytochrome *c*, can cause homolytic cleavage of hydroperoxy groups via one electron transfer. The resulting oxygenated products are similar to those synthesized by reacting fatty hydroperoxides with a chemical catalyst: cysteine and ferric chloride (Gardner and Sessa, 1976).

Many of these products and also the volatile compounds arising from fatty acid decomposition contribute to either the desirable, fresh-vegetable flavors associated with normal metabolism of the growing plant or the undesirable, derived, off-flavors that occur after harvesting and during storage or processing. Production of off-flavors is a problem associated with legumes such as green peas, lentils, and soybeans. With soybeans, in particular, flavor is one of the major deterrents to its greater usage in food products (Hammond and Call, 1972). The predominant beany and bitter flavors of raw, full-fat, and defatted soy flours remain detectable in most commercially manufactured soy flours, concentrates, and isolates (Kalbrener et al., 1971). Oxidized soy phosphatidylcholine (SPC) has been shown to be a bitter principle in soybeans (Sessa et al., 1976).

Current reviews on lipoxygenase and peroxidase and their relationship to food quality include Axelrod (1974), Burnette (1977), Eriksson (1975), Eskin et al. (1977), Grosch, (1972), Sessa and Rackis (1977), Varoquaux and Avisse (1975), Veldink et al. (1977), and Wolf (1975). In this review not only is the literature on the biogenesis of lipid-derived flavors in legumes discussed with emphasis on pea seeds (*Pisum sativum*) and soybeans (*Glycine max*) but also the action of soy lipoxygenase with purified SPC is elaborated on and a new lipid oxidation product, a 5-substituted-2-furaldehyde derived from linolenic acid, is reported. Action of soy lipoxygenase on intact soy phospholipids has been alluded to (Brockmann and Acker, 1976, 1977; Morrison and Panpaprai, 1975; Sessa et al., 1974) but has not been proven.

BIOCATALYTIC OXIDATION OF POLYUNSATURATED FATTY ACIDS

Lipid Oxidizing Potential in Legumes. The hemoproteins (peroxidase, catalase, and cytochrome) in higher plants accelerate the autoxidation of polyunsaturated fatty acids since they, as well as lipoxygenase, have a relatively low activation energy of 12–20 kJ mol^{-1} (Eriksson, 1975). Pinsky et al. (1971) demonstrated that the food legumes of the genera *Vicia*, *Glycine*, *Phaseolus*, and *Pisum* all have uniformly high lipoxygenase activity. Eriksson (1967) showed that for peas 5–8% of the total lipoxygenase content is located in the skin, 80% in the outer tissue and 12% in the inner tissue of the cotyledon. Oxygen diffusion into the fresh pea was sufficient for catalyzing the aerobic reaction as long as there was contact between enzyme and substrate. Lipoxygenase activity, based on enzyme units/gram dry weight, was found to be highest in the pea center and lowest in the skin.

In comparison with peas, whole soybeans not only have higher lipoxygenase activity (Pinsky et al., 1971) but also larger amounts of lipids, with a higher percentage of

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Table I. Properties of Lipoxygenase Isoenzymes from Soybean and Pea[a]

	soybean			pea	
type[b]	1	2	3	2	3
$M_r \times 10^4$	10.2	10.0	10.0	7.2-7.8	10.6
pH opt.	8.5-9.5	6.6	6.5-7.0	6.3	7.0-7.2
pI	5.68	6.25	6.15	6.00-6.15	n.d.[c]
Ca^{2+} activity	no	yes	inhibition	inhibition	inhibition
L[d] activity	yes	yes	yes	yes	yes
MeL[d] activity	no	yes	yes	yes	yes
β-carotene/L activity	+[c]	++[c]	++	++	n.d.

[a] The values compiled in this table were obtained from numerous literature sources. [b] Isoenzyme type 1, Theorell enzyme, was absent in fresh green peas and stored pea seed. [c] +, low; ++, moderate; n.d., not determined. [d] L, linoleic acid; MeL, methyl linoleate.

polyunsaturated fatty acids (Sessa and Rackis, 1977). With mature whole soybeans, the low moisture level limits oxygen diffusion into the center of the cotyledon compared to fresh immature peas with their higher moisture content. However, lipoxygenase activity can occur anaerobically if preformed hydroperoxide is present (Garssen et al., 1971).

The whole soybean is usually processed to a full-fat grit or flake for easy extraction of oil. Once the cell structure of the soybean is broken down during processing, normal metabolic reaction can run rampant since the cell's control mechanisms are no longer effective. Sessa et al. (1969) and Rackis et al. (1970) demonstrated that processing whole soybeans into oil and meal did, indeed, increase the oxidative deterioration of unsaturated lipids based on results of thiobarbituric acid (TBA) analysis of the processed products. A ninefold increase in TBA number occurred when full-fat flakes were blended aerobically in water than when blended in acid to inactivate lipoxygenase. A similar increase in TBA number was recorded for green peas (Rhee and Watts, 1966).

Lipid oxidation potential of peas and soybeans appears to be great based on the hemoprotein's availability to lipid substrate and its low activation energy.

Action of Lipoxygenase Isoenzymes on Phospholipids. Lipoxygenase isoenzymes from peas and soybeans with properties characterized and enumerated in Table I can act either on free polyunsaturated fatty acids or on ester bound forms such as methyl ester or triglyceride. According to Haydar et al. (1975), pea lipoxygenases do not catalyze oxidation of glyco- and phospholipids. They established that phospholipase A_2 action is prerequisite to oxidation of phospholipids by pea lipoxygenase.

Sessa et al. (1974) alluded to the fact that crude soy lipoxygenase catalyzed the oxidation of SPC. According to Morrison and Panpaprai (1975), phosphatidylcholine in doughs containing soy flour was oxidized by soy lipoxygenase. Brockmann and Acker (1976) found that crude soy lipoxygenase will oxidize SPC in dry surroundings. Bitter-tasting phosphatidylcholines isolated from defatted soy flakes were found to contain oxygenated fatty acids (Sessa et al., 1977). These oxygenated fatty acids were thought to be derived from polyunsaturated fatty acid hydroperoxides generated by lipoxygenase.

Soy lipoxygenases, commercial type 1 (Sigma Chemical Co., St. Louis, MO), and an ammonium sulfate precipitate from soy (Restrepo et al., 1973) were reacted with various substrates including linoleic acid, trilinolein, and SPC (Sessa, unpublished data). Partially purified lipoxygenase—an ammonium sulfate precipitate, acting either

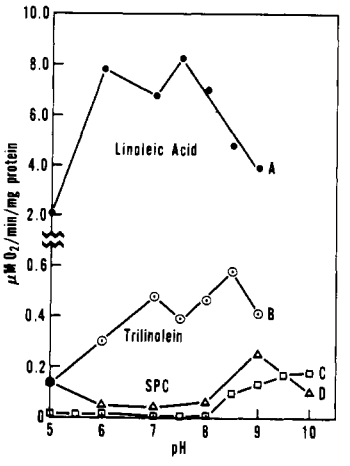

Figure 1. Lipoxygenase activity as a function of pH and substrate. (A, B, C) Oxygen uptake with partially purified soy lipoxygenases in precipitate from 50% saturation with $(NH_4)_2SO_4$. (D) Oxygen uptake with commercial soy lipoxygenase, type 1. Uptake recorded with a Gilson Oxygraph.

on linoleic acid or trilinolein substrates—had greatly diminished oxygen uptake activity above pH 8.5, whereas, on SPC substrate, activity increased (Figure 1). With commercial lipoxygenase type 1 optimum activity with SPC occurred at pH 9.0. Heat-denatured enzyme (15 min, boiling water bath) gave no oxygen uptake activity over entire pH range under the conditions of the assay. No oxygen uptake occurred for SPC and lipoxygenase at pHs 7.0–8.5, the pH region where optimum uptake occurred by action of partially purified lipoxygenase on linoleic acid and trilinolein substrates. Phosphatidylcholine, which exists as a zwitterion in the pH range of about 4–8.5, becomes an anion above pH 8.5. Bild et al. (1977), who tested the effects of substrate polarity on action of soybean lipoxygenase isoenzymes, found that linoleyl sulfate, negatively charged at both pH 9.0 and 6.8, is well suited for lipoxygenase 1 but is inactive with lipoxygenase isoenzymes 2 and 3. Apparently we are observing a similar charge effect when lipoxygenase 1 is acting on the anionic form of SPC at pH 9.0. A possible explanation of the in situ reaction of lipoxygenases on membrane phospholipids at neutral pH is that an excess of negative charge exists in whole mitochondrial phospholipids even though phosphatidylcholine and phosphatidylethanolamine account for 70% of the phospholipids according to Green and Fleischer (1963). Conceivably, lipoxygenases can interact with membrane phospholipids in situ. Further research on action of lipoxygenases on membrane phospholipids when bound to protein is needed to prove this point.

TLC analysis of the SPC after enzyme action at pH 9.0 showed no evidence for free fatty acids. Therefore, the enzyme preparation used in the present study did not contain phospholipase A_2, and the high pH did not saponify the fatty acids. GLC analysis of the fatty acids from intact SPC showed a 2.3% reduction in content of polyunsaturated fatty acids. TLC analysis of these acids showed that a complex mixture of oxygenated fatty acids similar to those reported by Sessa et al. (1977) were generated by this enzyme action.

Cooxidation of Substrates via Lipoxygenase and Polyunsaturated Fatty Acids. In the presence of substrate fatty acid and product, lipoxygenase is capable of oxidizing a large number of substances including chlorophyll and carotenoid pigments, cholesterol, cytochrome *c*, 1,3-diphenylisobenzofuran, and thiols in dough. Chlorophyll is degraded by a coupled reaction of lip-

oxygenase from peas, soybeans, or other legume seeds, with an unsaturated fatty acid and a heat-labile factor (Holden, 1965; Buckle and Edwards, 1970). The heat-labile factor is thought to be hydroperoxide isomerase, which converts the hydroperoxide to a keto hydroxy compound (Zimmerman and Vick, 1970). On the other hand, Imamura and Shimizu (1974) found no evidence for the participation of hydroperoxide isomerase for the bleaching of chlorophyll. Carotene can be cooxidized by hydroperoxides generated from action of soybean lipoxygenase isoenzymes, particularly types 2 and 3, on linoleic acid or linoleyl sulfate (Weber et al., 1974; Weber and Grosch, 1976; Ikediobi and Snyder, 1977; Grosch et al., 1977). Arens et al. (1973) isolated a lipoxygenase isoenzyme, type 2, from peas which oxidized carotene in the presence of linoleic acid and O_2.

Teng and Smith (1976) thought that the cooxidation of cholestenol with soy lipoxygenase and ethyl linoleate to form cholestenone and a mixture of epimeric oxocholestene hydroperoxides proceeded via a radical mechanism. Whereas, cooxidations that involve bleaching of the heme absorption in cytochrome c (Finazzi Agrò et al., 1972) and conversion of 1,3-diphenylisobenzofuran to 1,2-dibenzoylbenzene (Chan, 1971) are postulated to occur via singlet oxygen mechanism.

Changes in rheological properties of mechanically developed doughs from baker's grade flour are affected by soybean lipoxygenase, possibly through coupled oxidation of protein SH groups by lipoxygenase (Frazier et al., 1973). Other cooxidation studies involving either lipoxygenase or peroxidase include conversion of violaxanthin to xanthoxin (Firn and Friend, 1972) and the transformation of a trihydroxychalcone to dihydroflavonol and an aurone. The latter occurs via peroxidase from garbanzo seedlings, *Cicer arietinum* (Wong and Wilson, 1976).

To test whether formation of relatively high amounts of oxygenated fatty acids on intact SPC (Sessa et al., 1977) results from coupled reaction involving lipoxygenase 1 and linoleic acid, purified enzyme (Christopher et al., 1970) was reacted with linoleic acid in the presence of SPC at pH 7.0, 20 °C. Under these conditions, the isolated enzyme showed activity with linoleic acid but none with SPC alone. The only oxygen uptake noted with the combination of linoleic acid and SPC was that utilized by linoleic acid alone. Upon GLC and TLC analysis of transesterified fatty acids from intact SPC, no oxygenated compounds were evident.

The low oxygen uptake noted for action of lipoxygenase 1 on SPC polyunsaturated fatty acids, the small conversion of polyunsaturated fatty acids to oxygenated compounds, and the negative results from the cooxidation study indicate that some other type of oxidation is involved to account for the high content of oxygenated fatty acids in bitter-tasting, isolated SPC (Sessa et al., 1977).

LIPOXYGENASE AND FLAVOR BIOGENESIS

Volatile Compounds. Linoleic and linolenic acid hydroperoxides or their breakdown products when tested in dilute solutions are grassy/beany, musty/stale, bitter, and astringent, closely resembling those flavors elicited by raw soy flour (Kalbrener et al., 1974). The volatile products generated by soy lipoxygenase isoenzyme on pure linoleic and linolenic acids, given in Table II, consist of saturated and unsaturated aldehydes, a furan and a ketone (Grosch, 1967; Grosch and Schwencke, 1969; Grosch and Laskawy, 1975; Heimann et al., 1975; Leu, 1974), several of which reportedly contribute to the grassy/beany character. The aldehyde can arise from decomposition of alkoxy radicals generated from action of lipoxygenase on linoleic acid. Mechanisms for generating some of the primary decomposition products from linoleic and linolenic acid hydroperoxides are shown in Figures 2 and 3.

Table II. Volatile Products from Action of Lipoxygenase on Polyunsaturated Fatty Acids[a]

linoleic acid + pea (P) or soy (S) lipoxygenase	→ *n*-C_2-C_7-anals (P; S)
	n-prop-1-enal (P; S)
	2-C_4-C_6-enals (P)
	2-C_7- and -C_8-enals (P; S)
	2,4-C_9- and -C_{10}-dienals (P; S)
	2-*n*-pentylfuran (P; S)
linolenic acid + soy lipoxygenase	→ *n*-C_2- and -C_3-anals
	2-C_5- and -C_6-enals
	2,6-nonadienal
	2,4-heptadienal
	3,5-octadien-2-one
	2,4,6-nonatrienal

[a] From Grosch (1967); Grosch and Laskawy (1975); Grosch and Schwencke (1969); Heimann et al. (1975); Leu (1974).

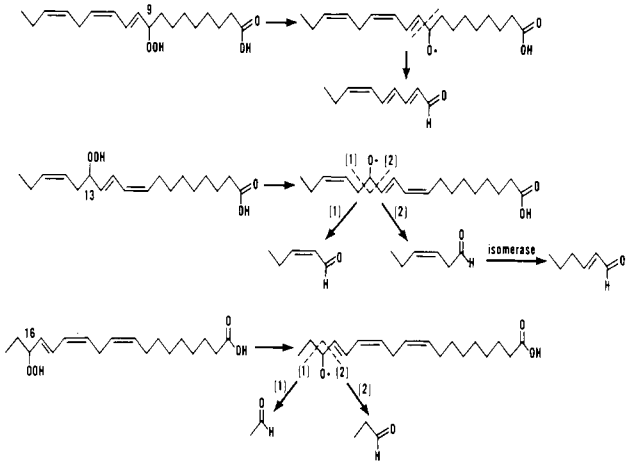

Figure 2. Formation of volatile aldehydes via decomposition of linolenic acid hydroperoxides.

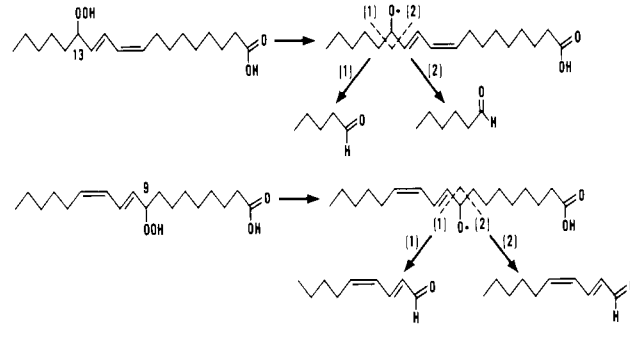

Figure 3. Formation of volatile aldehydes via decomposition of linoleic acid hydroperoxides.

Soy lipoxygenase isoenzymes types 2 and 3 generate a greater quantity and a greater number of different volatile carbonyl compounds than isoenzyme type 1 (Grosch and Laskawy, 1975; Fischer and Grosch, 1977). These volatile carbonyl compounds may arise via peroxyl radicals (Grosch and Schwencke, 1969) or by secondary reactions of radicals from labile polar peroxides (Morita and Fujimaki, 1973). Grosch et al. (1974) showed that linolenic acid oxidized by singlet oxygen formed propanal, 3-*cis*-hexenal, and 2-*trans*,6-*cis*-nonadienal via 1,2 addition of singlet oxygen to the double bonds of linolenic acid and subsequent cleavage of the dioxetanes formed. Recently, Veldink et al. (1977), who investigated the quenching of chemilu-

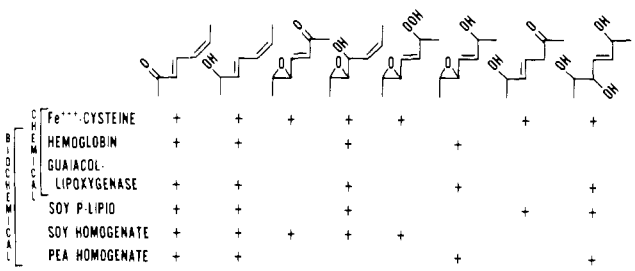

Figure 4. Comparison of products from the degradation of linoleic acid hydroperoxide by various chemical and biological systems; see Gardner and Sessa (1976).

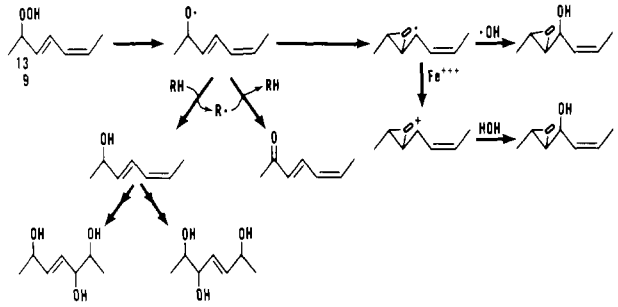

Figure 5. Postulated decomposition of linoleic acid hydroperoxides.

minescence by superoxide dismutase during lipoxygenase-catalyzed oxygenation of linoleic acid, concluded that superoxide is very important in the oxygenation process. Superoxide is a known source of singlet oxygen (Kasha and Khan, 1970). Singlet oxygen has been implicated in the above formation of aldehydes (Grosch et al., 1974). Role of lipoxygenase in formation of singlet oxygen has yet to be determined.

Several volatile compounds have been identified which possess flavors similar to those noted in raw peas and in raw or processed soy protein products. For example, *n*-hexanal tastes green/grassy, and hexenal, particularly 3-*cis*-hexenal, which readily isomerizes to 2-*trans*-hexenal on processing (Stone et al., 1975), possesses green/beany character (Hoffmann, 1961; Keppler, 1977). 2-*n*-Pentylfuran has a licorice-like flavor, but when added to a bland cottonseed oil it generates the green/beany note associated with soybean oil reversion flavor (Chang et al., 1966). Ho et al. (1978) reported on the synthesis of *cis*- and *trans*-2-(1-pentenyl)furan and found that these compounds, likewise possessing a licorice odor, contribute to the beany and grassy notes of reverted soybean oil.

Sessa and Plattner (1979) recently identified a 5-(pentenyl)-2-furaldehyde which they isolated from a bitter-tasting soy phospholipid fraction. They postulate its formation from a 9-hydroperoxide of linolenic acid. This furaldehyde also has a licorice-like odor. Its contribution to flavor in soy has yet to be determined.

Also, 2,6-nonadienal from linolenic acid (Figure 3) has a strawlike, grassy note (Hill and Hammond, 1965) and the alka-2,4-dienals (Table I) are described as tasting oxidized, cardboardy, oily and painty (Kinsella et al., 1967). Although only small amounts of these volatiles may be present in the food product, the unique flavor profile of a food usually results not only from the individual components but also from the interaction of many compounds present in different amounts. For example, 5 ppm linalool in beverage base tastes lemony, woody; 10 ppm *cis*-3-hexenal in beverage base has green, sharp flavor. Combination of the two, at that concentration, gives the beverage base a blueberry flavor (Parliment and Scarpellino, 1977).

Nonvolatile Compounds. Fatty acid hydroperoxides of soy and pea degrade to nonvolatile products similar to these isolated from model systems consisting of cysteine–Fe^{3+} catalyst, hemoglobin, or combination of an electron donor, guaiacol, with lipoxygenase (Gardner and Sessa, 1976). The oxygenated products derived from the hydroperoxides given in Figure 4 are probably generated by homolytic decomposition of the alkoxy radical as illustrated in Figure 5. Hydroxydiene can be produced by gain of a hydrogen radical, oxodiene by abstraction of a hydrogen radical on the same carbon atom, and epoxyene by interaction of an oxygen radical with a carbon atom adjacent to a double bond. The new radical generated in the formation of epoxyene then can interact with a hydroxyl radical or activated oxygen species to form a hydroxy epoxyene. Except for oxodiene and hydroxydiene, the degradation products in Figure 5 occur under aerobic conditions. A lipoperoxidase factor from soy is responsible for the breakdown of the 13- and 9-hydroperoxides (Blain and Styles, 1959; Blain and Barr, 1961; Gini and Koch, 1961; Grosch et al., 1972). Ishimaru and Yamazaki (1977) report that microsomes of pea seeds contain a new type enzyme, "peroxygenase", which catalyzes hydroxylation of indole, phenol, naphthol, and aniline in the presence of H_2O_2, linoleic acid hydroperoxide, cumene hydroperoxide, and *tert*-butyl hydroperoxide.

Under anaerobic conditions, reaction of a mixture containing linoleic acid and L-13-hydroperoxylinoleic acid incubated with lipoxygenase type 1 generates formation either of pentane and an oxodienoic acid (Garssen et al., 1971) or of dimeric acids. The latter are formed by interaction of alkyl radicals or alkoxy and alkyl radicals (Garssen et al., 1972; Gardner, 1975).

Saturated fatty acids C_{12} and above generally have very little taste except for candle-like or, at alkaline pH, soapy flavors. Dimeric material derived from the interaction of methyl ester hydroperoxides has a tangy, bitter, persistent taste (Evans et al., 1960). Kalbrenner et al. (1974) reported that both linoleic and linolenic acid hydroperoxides or their decomposition products tasted bitter and astringent. Sessa et al. (1976) isolated bitter-tasting oxidized phosphatidylcholines from defatted soy flakes and attributed the bitter taste to a combination of chemical composition of oxygenated fatty acids and physical behavior of oxidized SPC, a surfactant, in water. The glyceryl phosphorylcholine portion of the molecule may promote contact of the slightly water-soluble oxygenated fatty acids with the bitter taste receptors on the tongue. Recently, Baur et al. (1977) found that fatty acids containing three or more hydroxyl groups taste bitter when dispersed with a tasteless emulsifying agent such as a sugar ester. The double bond in the trihydroxy acids enhances the bitter taste (Baur and Grosch, 1977). Sessa et al. (1977) identified many oxygenated fatty acids in addition to trihydroxy octadecenoic acids on bitter-tasting SPC. Whether or not these other oxygenated fatty acids taste bitter has yet to be determined.

ENZYME INHIBITION AND DIMINISHED FLAVOR DEVELOPMENT

Formation of volatile flavor compounds in peas and soybeans can be prevented by blanching. This process inactivates lipoxygenase and other enzymes that degrade lipids. With a model system of purified pea lipoxygenase tested under various combinations of time, temperature, and pH, Svensson and Eriksson (1972a) found that thermal inactivation followed first-order kinetics and that the enzyme had maximum thermal stability at pH 6.0. Oxygen

or hydrogen peroxide had no effect on heat inactivation of the enzyme (1972b). However, in the presence of linoleic acid or linoleic acid hydroperoxide, heat inactivation of pea lipoxygenase was accelerated over 2.5-fold after 1 min at 70 °C and pH 6.0. These authors attributed this destabilizing effect to an attack of substrate at a specific enzyme site.

Thermal inactivation of purified soy lipoxygenase also follows first-order kinetics. The purified enzyme is more susceptible to heat inactivation than are crude enzyme extracts. Enzyme in the meal showed greatest resistance to heat inactivation (Okubo et al., 1976). Wilkens et al. (1967) found that inactivation of soy lipoxygenase by grinding unsoaked, dehulled beans in hot water, 80–100 °C, gave diminished production of volatile compounds and hence less flavor in their soy milks. Similarly, Mustakas et al. (1969) improved the odor, flavor, and stability of full-fat soy flour by heat inactivation of soy lipoxygenase. This study led to development of a pilot process for preparing full-fat flour by extrusion cooking (Mustakas et al., 1970). In other blanching studies, undehulled, full-fat flakes (Shemer et al., 1973) and prototype foods produced from whole soybeans (Nelson et al., 1976) have good flavor qualities.

A low pH inhibits lipoxygenase in soybeans and other legumes, thereby minimizing formation of volatile compounds. Kon et al. (1970) prepared bland-tasting legume slurries free of lipoxygenase-induced off-odors and flavors by grinding and fractionating raw legume seeds under acid pH conditions 3.85 and below.

Mitsuda et al. (1967a) report the cysteine (under aerobic conditions) or hydrogen peroxide interacts with the catalytic site of lipoxygenase and induces irreversible inactivation of the enzyme. Chemical treatments of soy flour with calcium chloride and hydrogen peroxide (Paulsen, 1963) are used commercially to produce flour with improved flavors. Since cysteine reacts with linoleic acid hydroperoxide (Gardner et al., 1977), volatile flavor production should be diminished by promoting the interaction of these hydroperoxides, generated via lipoxygenase action, with cysteine.

Saturated monohydric alcohols inhibit lipoxygenase by hydrophobic bond formation between the alcohol and enzyme (Mitsuda et al., 1967b). Increased chain length of the alcohol causes increased degree of reversible inhibition. Flavor scores of legumes, including peas and soybeans, can be significantly improved by steeping or wet milling whole legumes with aqueous ethanol (Eldridge et al., 1977).

Siddiqi and Tappel (1956) showed that the antioxidants nordihydroguariaretic acid, propylgallate, and α-tocopherol each inhibited enzymic oxidation of linoleate with soybean and pea lipoxygenases. Yasumoto et al. (1970) tested the effects of several conventional antioxidants on lipoxygenase action. They found that inactivation of lipoxygenase took place in conjunction with autoxidation of the antioxidant. When we know the inactivation mechanism of antioxidants, methods correlating changes in sensory qualities with objective measurements, such as chemical change in the antioxidant or in the type of volatile products formed, can be developed. Eriksson and Svensson (1974) followed the effects of the antioxidant, *n*-propylgallate, on the formation of *n*-hexanal in enzyme catalyzed reactions directly from gas chromatograms.

Recently Naim et al. (1976) reported that the naturally occurring soybean isoflavones inhibit lipoxygenase activity. However, the amount present, in situ, is not enough to control enzymic oxidation during processing of soybeans. What is needed is a method to enable an endogenous or natural antioxidant to be released during cell disruption.

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Occurrence and Formation of Bitter-Tasting Trihydroxy Fatty Acids in Soybeans

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A quantitative method for the determination of the mixture of 9,12,13-trihydroxyoctadeca-10-enoic acid and 9,10,13-trihydroxyoctadeca-11-enoic acid (Tri-OH) in legumes is reported. Storage of a soybean flour at 22 °C increased the Tri-OH content from 0.03 to 0.05% in 3 months. Components of soybeans, glutathione, and horse radish peroxidase (HRP) were tested for their ability to form Tri-OH from linoleic acid hydroperoxides. Most effective was a protein fraction from soybeans containing lipoxygenase and peroxidase activities, followed by HRP and by proteins. In the latter case thiol groups are involved in the Tri-OH formation.

A bitter taste whose intensity increases during maturation contributes to the flavor of soybeans (Rackis et al., 1972). In stored soybeans Sessa et al. (1974, 1976) localized the bitter taste to one lysolecithin and two lecithin fractions, which together represented at least 0.08% of the defatted flour. The authors found that autoxidized soy lecithins are extremely bitter with thresholds in the range of 0.006%. Further experiments suggested that not the phosphocholine moiety of the lecithin molecule but a bound oxidized fatty acid is responsible for the bitter taste.

Enzymatic oxidation of linoleic acid by use of a protein preparation of soybeans with lipoxygenase and peroxidase activities generates fatty acids which after emulsification with a sugar ester taste bitter (Baur et al., 1977). The main bitter substance was identified as a mixture of 9,12,13-trihydroxyoctadec-*trans*-10-enoic acid and 9,10,13-trihydroxyoctadec-*trans*-11-enoic acid (Tri-OH refer to both isomers). The Tri-OH exhibits a bitter threshold of 0.6–0.9

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