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Role of Lipoxygenase and Lipid Oxidation in Quality of Oilseeds

Allen J. St. Angelo,* James C. Kuck, and Robert L. Ory

Lipoxygenase is a prime suspect for catalyzing lipid oxidation in raw peanuts but it is destroyed in the roasting process. After roasting, however, lipid oxidation is catalyzed primarily by nonenzymic catalysts. Examination of several fresh samples of commercial peanut butters showed that the initial peroxide contents differed. This suggested that the samples were already in different stages of peroxidation and demonstrated the need for controlling oxidation in the peanuts before roasting and processing. We found that minor constituents such as metals, metalloproteins, and salts are possible catalysts of lipid oxidation. When ascorbyl palmitate, citric acid, or ethylenediaminetetraacetic acid were added to the peanut butter before storing, oxidation was decreased or completely controlled. Some natural compounds were also examined as possible inhibitors of lipoxygenase activity in raw peanuts.

Today, one of the major concerns of consumers is a growing awareness of the nutritional composition of foods. This concern has focused on the peanut because of its potential value as an economic source of high protein. In addition to containing 25–30% protein, peanuts are high in phosphorus and B vitamins, low in carbohydrates, and exceptionally high in oil (ca. 50%). Furthermore, the oil is an excellent source of unsaturated fatty acids (80%), of which 20–30% is polyunsaturated.

While peanuts are used in many food products, including candies, cookies, ice cream, and breakfast cereals, more than half of the peanuts produced in the United States are consumed as peanut butter. The protein in peanut butter makes it a good supplementary food in menu planning. Also, it ranks very high on the list of food energy sources. In the United States, peanut butter is second only to dry beans as the most economical source of protein (USDA, 1977). Addition of peanut butter can also improve the flavor of baked goods.

The causes of lipid oxidation in vegetable oils are numerous and involve such factors as light, air, high temperature, enzymes, microorganisms, trace metals, and the presence of free fatty acids. Because of the high percentage of unsaturated fatty acids, peanuts are susceptible to oxidative rancidity, staling, and loss of desirable flavors or odors. Therefore, they present a storage problem if high quality in the final products is to be retained. Many of these reactions are catalyzed by metals or enzymes, particularly lipoxygenase. This enzyme has been isolated and characterized in raw peanuts by several workers (Siddiqi and Tappel, 1957; St. Angelo and Ory, 1972; Sanders et al., 1975). The hydroperoxide products formed by the action of lipoxygenase on polyunsaturated fatty acids or triglycerides can be decomposed into acids, ketones, aldehydes, or other substances that form during processing or storage. These degradative products can then react with amino acids and proteins to impair flavor and/or lower nutritive value of peanuts or food products in which they are incorporated. Recently, Kuck et al. (1978) has shown that oxidized linoleic acid can react with lysine and threonine to form new reaction products.

In our laboratory, we have been concerned with preserving the desirable characteristics and high quality of peanuts and peanut products. This paper will discuss the functions of lipoxygenase and other constituents as related to quality and storage of oilseeds, particularly raw peanuts, and the ultimate effect of these functions on roasted products.

MATERIALS AND METHODS

Peanuts and peanut butter samples were obtained from commercial suppliers. Rapeseeds were a gift of Dr. R. Ohlson, A/B Karlshamns Oljefabriker, Sweden. Soybean lipoxygenase was purchased from Sigma Chemical Co., St. Louis, MO. Erucic acid was purchased from Eastman Chemical Co., Rochester, NY.

Peanuts were water blanched or spin blanched commercially and sent to us immediately after blanching, along with comparable unblanched peanuts. Procedures for fractionation of oil and proteins from raw or roasted peanuts or peanut butter were similar to those used previously (St. Angelo and Ory, 1975b; St. Angelo et al., 1977b).

Lipoxygenase activity was calculated either from initial rates of oxygen uptake, assuming an initial dissolved oxygen concentration of 240 nmol/L at 25 °C, or from formation of conjugated diene hydroperoxide (CDHP) as

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measured by absorbance readings at 234 nm as previously described (St. Angelo and Ory, 1972). Oxygen uptake was measured with an oxygen electrode (Rank Bros., Cambridge, England) equipped with a Houston recorder, Model 3000. Absorbance was measured with a Gilford spectrophotometer, Model 250, and recorder, Model 6050.

Shelf life was measured by determining the increase in CDHP values, which were shown to be proportional to peroxide values obtained in storage studies in peanuts and peanut butter (St. Angelo et al., 1975). The method depends on the initial oxidation of a polyunsaturated fatty acid containing methylene-interrupted double bonds. Upon formation of the hydroperoxide, the nonconjugated moiety is converted to a conjugated diene, which can be measured at 234 nm.

Peanut samples, prepared for microscopy by sputter coating with a 200–300 Å thick layer of gold–palladium alloy, were examined in an International Scientific Instruments Super II scanning electron microscope.

RESULTS AND DISCUSSION

In earlier studies, we investigated the effect of storing several varieties of peanuts, both raw and roasted. Results showed that (a) raw and roasted peanuts will oxidize even when stored in the cold and (b) roasted peanuts oxidize faster than their raw counterparts (St. Angelo and Ory, 1975a). Examination of the data from raw peanuts suggested that some peanuts had already undergone peroxidation prior to storage, whereas others (apparently undamaged) had not. The history of these groups of peanuts was not known because they were obtained from several suppliers. However, our results, along with the knowledge that peanuts undergo digging, windrowing (drying), curing, combining, shelling, blanching, packaging, shipping, and storing, indicate that they are susceptible to damage in each of these steps. Thus, quality of the final product can be affected by the care that is taken during handling and processing. With proper treatment, peanuts should retain their high food-grade quality; but with neglect, they can quickly become inedible, rancid, stale, moldy, or insect infested.

Peanut Butter. The most important product made from peanuts in the United States is peanut butter, which utilizes more than 50% of the peanut crop. Peanuts processed for this purpose are subjected to oxidative changes and become progressively stale and rancid because of their high fat content. Owing to the problems related to fat oxidation, extending the shelf-life and retaining the desirable flavor are a major concern to the industry. St. Angelo et al. (1977a) have shown that reactions that take place in raw peanuts prior to processing will affect the quality of the peanut butter. An examination of several fresh peanut butters obtained directly from commercial suppliers immediately after manufacturing showed that the CDHP values varied from 3.2–15.5. These results indicated that peanut quality differed before roasting and stressed the importance of quality control in regard to peroxide content of the raw stock.

Several constituents of peanuts were investigated as possible catalysts of fatty acid oxidation in peanut butter. Metalloproteins and iron and copper salts were the major catalysts, but citric acid, ethylenediaminetetraacetic acid (EDTA), and ascorbyl palmitate retarded or reduced their catalytic effect. Sodium chloride did not catalyze peroxidation when added in aqueous solution, but did have a very slight oxidative effect when added in a peanut oil emulsion. Peanut oil itself increased oxidation, possibly by providing extra substrate. Water acted either as a prooxidant or antioxidant, depending on the concentration

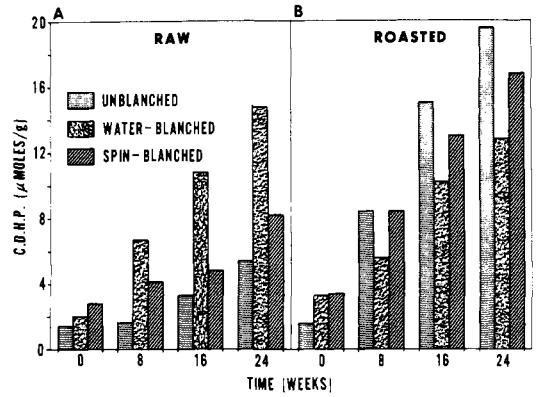

Figure 1. Shelf life stability of raw (A) and roasted (B) peanuts stored at 25 °C. Values represent the difference between initial CDHP content and that found at the time of analysis.

(St. Angelo et al., 1973, 1977a; St. Angelo and Ory, 1973, 1975c).

Blanching. For uses of peanuts in products other than peanut butter, the first step after shelling is the removal of skins. The mechanical removal of skins (testae) is known as blanching. There are several procedures for blanching peanuts: dry, spin, alkali, water, and hydrogen peroxide blanching. Various temperatures are used in each of these procedures, but generally the range is from 49 to 138 °C. The different processes can affect texture. For example, water-blanched peanuts are crisp, whereas spin-blanched peanuts are softer (Reeve, 1962). Peanuts are selectively blanched depending upon the intended use. Since peanuts are so susceptible to oxidation, the choice of blanching procedure before they are stored can be extremely important.

The shelf life of water-blanched peanuts has been reported to be longer than for unblanched nuts; that of spin-blanched peanuts almost as long as water-blanched. Alkali-blanched peanuts keep longer than those treated with either hot water or hot air (Woodroof, 1973). Recently, St. Angelo et al. (1977b) compared the effects of water and spin blanching on oxidative stability of the same peanuts before and after roasting. The shelf life of water-blanched raw peanuts was the shortest and was most stable for the unblanched (control) and spin-blanched raw peanuts. The opposite results were found for roasted peanuts; water-blanched peanuts had the longest shelf life when compared to roasted, unblanched, and spin-blanched peanuts. This observation is illustrated in Figure 1A for raw peanuts stored for 24 weeks at 25 °C in sealed glass jars. The unblanched (stored unblanched, but hand-blanched immediately prior to analysis) and spin-blanched peanuts had the longest shelf life when compared to the water-blanched raw peanuts. The CDHP content of the unblanched peanuts increased from 1.4 to 5.8, whereas that of the spin-blanched samples increased from 2.8 to 8.2. The CDHP value for the water-blanched, raw peanut samples increased from 2 to 15 during the 24 weeks of storage, a 7.5-fold increase in oxidation. Spin-blanched peanuts had the lowest rate increase, 2.9, compared to 4.1 for the unblanched controls.

Samples of the same peanuts (unblanched, water-blanched, and spin-blanched) were roasted, stored for 24 weeks at 25 °C in sealed glass jars, and then analyzed for CDHP content. Results show (Figure 1B) that water-blanched peanuts had the longest shelf life, whereas unblanched, roasted peanuts were the least stable. After 24 weeks of storage, the CDHP value for the water-blanched peanuts increased from 3.4 to 12.8 (a 3.8-fold

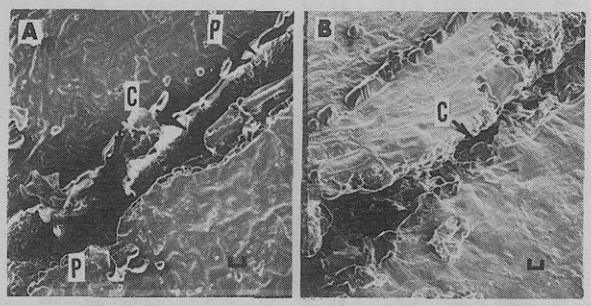

Figure 2. Photomicrographs of whole raw peanuts: A, spin-blanched; B, water-blanched; C, cuts made by razor blades; P, indicates protein bodies lining the walls of the cuts; marker represents 10 μm.

increase). The CDHP content of unblanched peanuts increased from 1.6 to 19.6, which represented a 12.3-fold increase in oxidation. The spin-blanched, roasted peanuts had an intermediate rate of oxidation, increasing from 3.3 to 16.7 (5.1-fold). These results are the inverse of those found in raw peanuts (Figure 1A), that water-blanched peanuts were the least stable.

As observed in Figure 1, unblanched, roasted peanuts peroxidize faster than unblanched raw peanuts. This phenomenon was also observed in raw and roasted unblanched peanuts stored for 1 year at 4 °C (St. Angelo and Ory, 1975a). Also, the rate of oxidation in raw, water-blanched peanuts is almost twice as fast as in the corresponding roasted peanuts. However, the opposite is seen for spin-blanched peanuts—when roasted, these peanuts oxidize twice as fast as the equivalent raw peanuts.

The primary difference between the two methods of blanching is that water-blanched peanuts are sprayed with hot water for 90 s, whereas spin-blanched peanuts are not. In both procedures, the peanuts are slit with razor blades so finely adjusted that the surface is presumably cut slightly when the skins are being cut through. When the kernel surface is cut, many of the damaged cells containing protein or lipid release their contents onto the surface. In the water-blanching process, surface-released proteins or lipids are somehow fixed in the form of a glaze. The glaze is not observed in spin-blanched peanuts, which are not sprayed with hot water. According to Woodroof (1973), the glaze forms a protective shield against oxidation and mechanical injury. However, after observing this glaze, we used a scanning electron microscope to examine the surface of peanuts that were cut by the razor blades during blanching.

Under low magnification, the surface of hand-blanched control peanuts appeared to be rough, but the surface of water-blanched peanuts seemed smooth. Spin-blanched peanuts were more rough than smooth (St. Angelo et al., 1977b). Both the spin-blanched and water-blanched peanuts had slit marks similar in appearance and visible on the surface. The hand-blanched peanuts, naturally, did not have slit marks.

At a higher magnification of the slit walls on spin-blanched peanuts, subcellular particles were observed (Figure 2A). These particles were not found in the water-blanched peanuts (Figure 2B). Only aleurone grains (protein bodies) could positively be identified from the photographs. Presumably spherosomes (fat bodies) are also present since the two organelles are usually observed together in ultrastructural examination of oilseeds. These results suggest that the fat and protein may have been washed from the razor-made slits and could be responsible for the glaze observed on the surface of water-blanched peanuts.

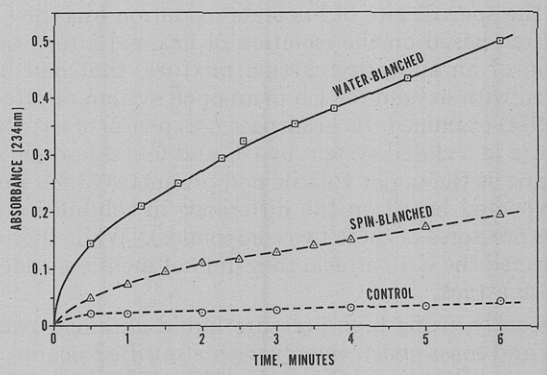

Figure 3. Effect of initial hexane attraction of stored raw whole peanuts: unblanched, spin-blanched, water-blanched.

To further examine the possibility that the glazed surface contains oxidized lipids, we extracted peanuts from each of the three blanching processes very carefully with 10 mL of hexane to remove only surface lipids. Portions were removed periodically and then examined for CDHP formation at 234 nm. Assuming that the surface lipids are the first to be extracted, then the extract obtained during the first several minutes should reflect a more accurate estimate of their oxidation state. These results (Figure 3) show that the initial readings from unblanched peanuts, taken after 30 s of extraction, were 0.021 compared to 0.049 for the spin-blanched peanuts and 0.146 for water-blanched peanuts. After incubating for 1 min, there was very little increase in absorption in extracts from the unblanched peanuts, that for the spin-blanched peanuts increased to 0.070, and there was a large increase to 0.208 for the water-blanched peanuts. After the first 5 min, the rates of oxidized lipid extractions were almost linear throughout the remaining 175 min of the tests. After 3 h of extraction, the final absorbance values plateaued at 0.342 for unblanched, 1.596 for spin-blanched, and 3.628 for water-blanched peanuts. These results suggest that there was more oxidized lipid on the surface of water-blanched peanuts than on the other two samples, or, at least, that the lipid was more readily extractable with hexane. Furthermore, this indicates that the glaze is probably associated with the protein and lipid that is removed from the slits then fixed to the surface and could be associated with the shortened shelf life of water-blanched raw peanuts.

Lipoxygenase. One of the primary catalysts of lipid oxidation in raw peanuts is lipoxygenase. Although the enzyme has been well studied in soybeans for several decades, investigations on peanut lipoxygenase are relatively new (Siddiqi and Tappel, 1957; St. Angelo and Ory, 1972; Sanders et al., 1975; St. Angelo and Kuck, 1977; Mitchell and Malphrus, 1977). Recently Sanders et al. (1975) isolated and purified three isozymes, two having a pH optimum of 6.2 and the third at 8.3. They reported the molecular weight of each isozyme to be 73 000, somewhat smaller than the 102 000 reported for soybeans (Theorell et al., 1947). Although soybean lipoxygenase contains one atom of iron per mole of enzyme (Chan, 1973; Roza and Francke, 1973), the peanut enzyme has not yet been shown to contain any metal.

In their current abstract, Pattee and Singleton (1978) stated that previous work on the hydroperoxide isomers of lipoxygenase had produced conflicting results. They alluded to our 1972 paper (St. Angelo et al., 1972), in which a direct gas chromatographic (GC) technique and a partially purified lipoxygenase preparation was used to examine volatile end products of the reaction. We reported

that the specific site of linoleate oxidation was the C-13 position, based on the isolation of hexanal after examination of an aqueous reaction mixture, that had been purged with oxygen for 1 h in an open system. Pattee et al. (1974) examined the headspace (gas phase) of a reaction mixture in a closed system by GC and found hexanal and pentane as the major volatile end products (which would be expected based on the difference in solubilities and vapor pressures of these two compounds). While they also confirmed the C-13 attack, they did not mention evidence for C-9 attack.

Recently, using high-pressure liquid chromatography (LC) and mass spectrometry with a purified peanut lipoxygenase, Pattee and Singleton (1976, 1978) isolated and identified four geometrical isomers of 9- and 13-hydroperoxides from linoleic acid. The LC method can resolve nonvolatiles as well as volatile materials. Thus, with this instrumentation, they were able to separate and identify the isomers in question (nonvolatiles), something that the GC method cannot do. Although the results obtained by the two different systems under diverse conditions cannot be directly compared, we are presently resolving these apparent discrepancies using the direct GC method.

In 1972, St. Angelo and Ory showed that inhibition was 59 and 55% when sulfhydryl reducing agents, 0.002 M 2,3-dimercaptoethanol and 0.001 M dithiotheritol (DTT) were incubated with peanut lipoxygenase for 1 h at 4 °C. At a concentration of 0.1 M, inhibition with DTT was 100%. *p*-Chloromercuribenzoate (0.001 M) did not inhibit the enzyme. These phenomena suggested that the inhibition might be due to a change in the tertiary structure of the protein. In 1973, Roza and Francke showed that cysteine partially inhibited soybean lipoxygenase. When added with an iron chelating agent, *o*-phenanthroline, total inhibition was achieved. Later, Pistorius and Axelrod (1974) showed a slight inhibition of the soybean enzyme by 2-mercaptoethanol and total inhibition when this compound was added in combination with *o*-phenanthroline. They concluded that the enzyme contained iron, which can be removed by the chelating agent, but only after the enzyme has been treated with a reducing agent. Also, they suggested that sulfhydryl-containing compounds could reduce a disulfide bond in the enzyme, thereby facilitating the chelating process. Stevens et al. (1970) suggested that soybean lipoxygenase contains two disulfide bonds.

In a separate study, we examined rapeseed for lipoxygenase. Rapeseed is one of the principal commercial oilseeds in the world. According to Franke and Frehse (1954), rapeseed high in polyunsaturated fatty acids did not have any lipoxygenase activity. Bronisz et al. (1958) found only slight activity in rapeseed extracts at pH 6.0. Appleqvist (1972) suggested that the enzyme was probably present in rapeseed, but could not be detected because of the presence of an inhibitor. We examined rapeseed by the same procedures and techniques used for measuring lipoxygenase activity in soybean and peanut, but we too were unable to confirm the presence of lipoxygenase in rapeseed.

In 1972, Christophorsen and Bremer reported that erucic acid inhibited fatty acid oxidation in mitochondria from rat heart and liver. Since erucic acid is the most abundant fatty acid in rapeseed (40%), we explored the possibility of erucic acid being a lipoxygenase inhibitor using peanut and soybean lipoxygenase and extracts of rapeseeds. Figure 4 compares lipoxygenase activities of the three oilseeds examined. As expected, the soybean enzyme is the most active, followed by the peanut enzyme. No

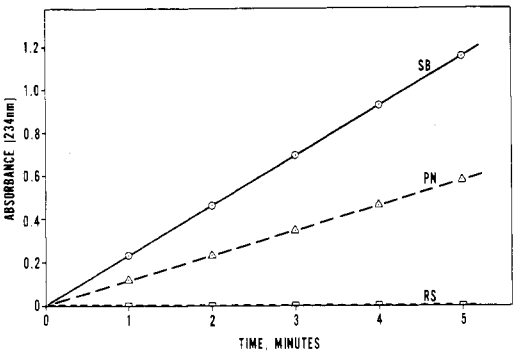

Figure 4. Relative lipoxygenase activity in soybean, peanut, and rapeseed. Each cuvette contained 0.8×10^{-4} M linoleic acid, 0.2 mL of enzyme extract (0.1 mg of soybean enzyme, or 20 mg of peanut enzyme preparation, or 20 mg of rapeseed soluble fraction) plus buffer to 3 mL total. Assay pH: soybean (SB), 7.6; peanut (PN), 6.0; rapeseed (RS), 6.0 and 7.6.

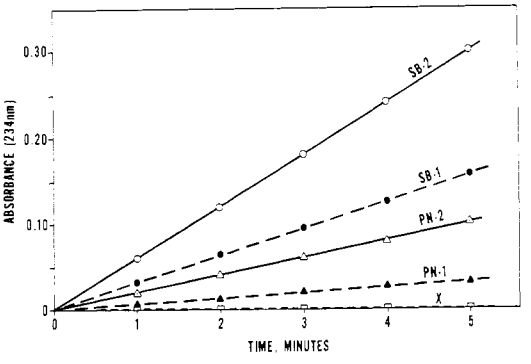

Figure 5. Effect of erucic acid on lipoxygenase activity in soybean and peanut. Conditions: SB-1, SB-2 (0.1 and 0.2 mL of soybean enzyme solution), PN-1, PN-2 (0.1 and 0.2 mL of peanut enzyme solution) were assayed without erucic acid, same as in Figure 4; for assays with erucic acid, enzyme solutions contained 0.1% (v/v) and were incubated for 1 h before addition of substrate. Assay conditions and pHs are the same as in Figure 4. Curve on horizontal axis (X) represents data obtained for soybean, peanut, and rapeseed preparations plus erucic acid.

activity was found in dehulled rapeseed endosperm tissue. Rapeseed hulls were also assayed for lipoxygenase activity, but without success. To ascertain whether rapeseed hulls contained an inhibitor, hull extracts were incubated with peanut lipoxygenase for 30 min, then assayed for activity. Since the enzyme was not affected, we concluded that the presence of an inhibitor in rapeseed hulls is not responsible for the lack of activity in rapeseed. In addition, when the resuspended precipitate and supernatant (obtained during the fractionation procedure) were added to either peanut lipoxygenase or soybean lipoxygenase, no inhibition was detected.

Action of Inhibitors on Lipoxygenase. Erucic acid (0.1%, v/v) was tested to determine its effect on the soybean and peanut lipoxygenases. A preliminary report was given earlier (Ory and St. Angelo, 1975). As seen in Figure 5, this acid inhibited both lipoxygenases. To explore the erucic acid effect further and to determine the minimum concentration of erucic acid required to inactivate soybean lipoxygenase, we conducted similar experiments with the oxygen electrode for measuring lipoxygenase activity. Figure 6 illustrates the effect of ethanol and ethanol solutions of erucic acid on soybean lipoxygenase at pH 8. The results show that 7.3 μmol of erucic acid is needed to completely inhibit soybean lipoxygenase under the conditions used. The clear circles show that ethanol, used as the solvent for erucic acid, has

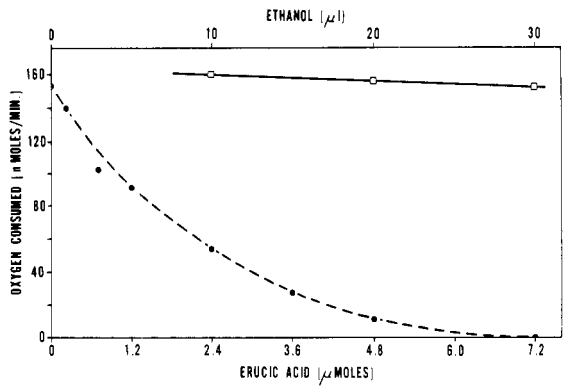

Figure 6. Response of soybean lipoxygenase to erucic acid and ethanol additions. Assay conditions: 20 μg of soybean lipoxygenase; 0.05 M phosphate buffer, pH 8.0; erucic acid/ethanol solution, total volume 2.0 mL, incubated for 5 min at 29 °C, then 0.5 μmol of linoleic acid/Tween-20/phosphate buffer was added to final volume of 3.0 mL. Activity assayed by oxygen utilization method described in the Methods Section; (●–●) with erucic acid/ethanol added; (O–O) with ethanol.

Table I. Effect of Peanut Tannin Preparation on Soybean Lipoxygenase

tannin added, mL	A_{480}, min^{-1} [a]	oxygen consumed min^{-1} 3 mL^{-1}, [b] nmol
0.0	0.52	256
0.2	0.52	
0.4	0.45	140
0.6	0.45	
0.8	0.37	95
1.0	0.25	
1.2		83

[a] Determined by ferric thiocyanate method of Koch et al. (1958). Enzyme concentration was 100 μg; linoleic acid, 0.7 μmol in 0.01 M Tris buffer, pH 7.6, 25 °C. [b] Determined by oxygen electrode method. Enzyme concentration was 400 μg; linoleic acid, 1.0 μmol in 0.05 M sodium phosphate, pH 8.4, 29 °C.

no inhibitory effect with the given concentrations.

Finally, the complete removal of lipids, including erucic acid, by acetone extraction of rapeseed still failed to produce an active lipoxygenase. These findings suggest that rapeseed may contain either an inhibited lipoxygenase or no enzyme in the quiescent state. Possibly germination may produce some activity.

In 1963, Narayanan et al. reported on the presence of an inhibitor of peanut lipoxygenase in extracts from peanut testae. Since phenols are known to be good cross-linking agents and can react with proteins, including enzymes, and because peanut skins are comprised of several pigments, peanut tannins were prepared by the method of Stansbury et al. (1950), then tested as a possible inhibitor by two methods. The first method was the ferric thiocyanate test described by Koch et al. (1958), and the second was the oxygen (uptake) electrode method. The CDHP method for measuring lipoxygenase activity was not used because of a strong absorption by the inhibitor preparation at 234 nm. The results of both methods (Table I) show that the tannin fraction inhibited soybean lipoxygenase. In the first method run at pH 7.6, 1.0 mL of the 0.1% aqueous solution gave about 50% inhibition, whereas at pH 8.4, 1.2 mL of the solution gave 67% inhibition with the oxygen electrode method.

According to Stansbury et al. (1950), the tannin fraction represents about 7% of the weight of the skins and belong to the catechol group of tannins. Its analysis was reported to be 61% carbon, 4% hydrogen, with a molecular weight of approximately 1500. The visible and ultraviolet absorption spectra of our tannin-inhibitor preparation showed maxima at 250 and 280 nm, in agreement with Stansbury et al. (1950) and very similar to the spectra of catechol.

Recently, Sanders (1977) reported that tannin-like compounds isolated from peanut fruit parts showed a close relationship with seed maturity and, furthermore, that tannins may offer some resistance to microbial attack. He also noted that the tannins were condensed or catechol-like, an observation similar to that of Stansbury et al. (1950).

To summarize these data on two types of naturally occurring inhibitors of lipoxygenase, one was identified as erucic acid, an unsaturated fatty acid having a 22-carbon chain length with a cis double bond at the C-13,14 position. The second inhibitor is a polyphenolic compound, probably catechol-like. Plant phenolics are very diverse compounds and can form several types of bonds with proteins, e.g., hydrogen, covalent, ionic, and hydrophobic (Loomis, 1974). The mechanism of inhibition and the type of binding to lipoxygenase by erucic acid and the peanut tannin inhibitor have not yet been determined.

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Biochemical Aspects of Lipid-Derived Flavors in Legumes

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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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