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Lipoxygenase and Flavor of Soybean Protein Products

Walter J. Wolf

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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Table I. Flavor Detection Level of Soy Flours in Bread

Workers	Detection level of soy, %
Haas (1934)	>0.4
Finney *et al.* (1950)	<4
Ofelt *et al.* (1952)	>5
Ehle and Jansen (1965)	>4
Cotton (1974)	2

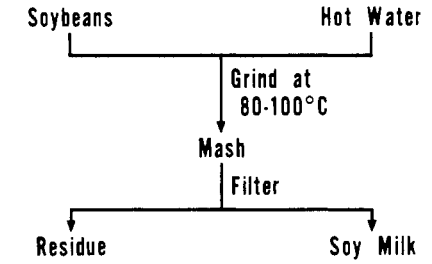

Figure 1. Modified soy milk process to rapidly inactivate lipoxygenase and avoid flavor development (Wilkens *et al.*, 1967).

flours and lowest for those containing ethanol-extracted flour.

Two years later, Ofelt *et al.* (1952) reported that a large untrained taste panel could not detect a flavor difference between bread containing 4% nonfat milk solids and 5% defatted soy flour. They pointed out that early in their development soy flours were variable in quality and uniformity and that confusion resulted when different workers evaluated the flours available in the market place.

In spite of the limited success of early baking studies, the work has continued as soy flours have been improved in quality and uniformity. Nonetheless, recent studies (Table I) indicate that the flavors of soy flours can still be detected at levels of 2-4%. Early workers also noted that baking formulas and procedures had to be adjusted when soy flour was added to bread. Recent work at Kansas State University demonstrated that full-fat soy flour can be used in bread at levels up to 24% provided that dough conditioners such as sodium stearoyl-2 lactylate are incorporated into the dough (Tsen and Hoover, 1973). Organoleptic studies of such breads have not been reported yet.

Soy flour with lipoxygenase activity is still used as a bleaching agent in bread in the United States and England. Almost all bread baked in England has enzyme active soy flour added to it (Pringle, 1974; Wood, 1967). In addition to bleaching the pigments, lipoxygenase is also claimed to cause oxidation of the gluten proteins (Logan and Learmonth, 1955) with the result that a finer crumb is obtained (Wood, 1967).

In the United States enzyme active soy flour preparations are designed for use at the 1% level (wheat flour basis). In continuous bread baking it was found desirable to incubate the lipoxygenase preparation with cottonseed or soybean oil and then use the peroxidized oil in the dough formula. The peroxidized oil is claimed to improve the flavor of the bread (Kleinschmidt *et al.*, 1963).

Early Approaches to the Soy Flavor Problem. The flavor problem of raw soybeans and soy flours was recognized before Haas and Bohn's patent application in 1928. For example in 1924, Berczeller, a Hungarian chemist, stated emphatically that soybeans "in their natural state are very evil-tasting," notwithstanding the fact that soybeans and most other plantstuffs are not normally eaten in the raw form. Berczeller patented a process consisting of steaming whole beans for 10–12 min under conditions to minimize absorption of water followed by removing the moisture in a vacuum. The dried beans were then dehulled and ground. This is typical of many empirical attempts to eliminate the flavors characteristic of raw soybeans (Bailey *et al.*, 1935); heat treatment is a common feature of many patents that have been issued. In the last two decades the soy industry has improved the flavor of soy flours by carefully controlled moist heat treatment sometimes referred to as toasting. Toasting eliminates much of the flavor found in raw soy flours but in turn develops a nutty or toasted flavor and darkens the product. Another process used commercially consists of chemical treatment with calcium chloride and hydrogen peroxide (Paulsen, 1963).

It was not generally realized that lipoxygenase may be responsible for the flavor problem, as well as for the bleaching effects of raw soy flours in breadmaking, until the 1960's when several laboratories began evaluating soybeans as a source of edible protein to supply the demand for more protein in developing countries.

Lipoxygenase and Flavor of Soy Milk. Workers at the New York State Experiment Station in Geneva did an extensive study of the ancient Oriental process of making soy milk. In this process, soybeans are soaked in water overnight, ground with added water, heated, and then filtered to remove the insoluble portion. Initial studies at Geneva indicated that flavor of the resulting soy milk would limit acceptability (Hand *et al.*, 1964). Subsequently this group found that the off-flavor of soy milk was accompanied by the formation of a complex mixture of volatile compounds (Wilkens *et al.*, 1967). The volatile compounds formed only when the milk was prepared from whole (undefatted) beans; water extracts of defatted beans contained few volatile constituents. Soy milk prepared in the absence of oxygen likewise contained few volatile compounds and lacked the rancid and beany odor of regular soy milk. It was postulated that generation of off-flavors resulted from reaction of lipoxygenase with the fat.

Two methods for correcting the flavor problem were examined: (a) removal of the volatile compounds from soy milk prepared by the conventional process and (b) modification of the process to prevent formation of volatile constituents. The latter approach was found to be most feasible. The process was modified by grinding the unsoaked, dehulled beans in water at temperatures of 80–100° and holding the temperatures for 10 min (Figure 1). The rationale for this modification was that lipoxygenase is inactivated immediately and that oxidation of the oil and subsequent development of flavor compounds are prevented.

Lipoxygenase and Flavor of Full-Fat Flour. At this same time investigators at the Northern Regional Research Laboratory in Peoria did an extensive study on the production of full-fat soy flours and also concluded that inactivation of lipoxygenase is a key step in the preparation of good flavored full-fat soy flours (Mustakas *et al.*, 1969). They reported formation of hydroperoxides during water soaking of whole beans and a rapid development of peroxides and rancid odors when soybean meats (dehulled, cracked beans) were wetted to raise the moisture content to 25%. Dry heat at 100° or steaming of dehulled beans inactivated lipoxygenase and on subsequent grinding a full-fat flour of good flavor was obtained. Moreover, such a flour did not become rancid on subsequent storage whereas flour without sufficient heat treatment did.

This preliminary study led to the development of a pilot process in which full-fat flour was prepared by extrusion cooking (Mustakas *et al.*, 1970). The steps (Figure 2) include cracking and dehulling of the beans followed by a dry-heat preconditioning step in which lipoxygenase and other enzymes are inactivated. After cooling, the meats are tempered to 15–20% moisture and extrusion cooked. The extruded material is then dried, cooled, and milled into a flour. Under optimum extrusion conditions the re-

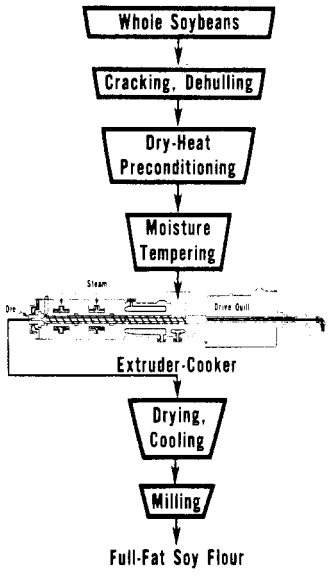

Figure 2. Outline of process for preparing full-fat soy flour by extrusion cooking (Mustakas *et al.*, 1970).

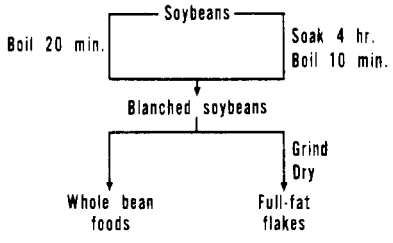

Figure 3. Blanching process for inactivating soybean lipoxygenase in preparation of whole soybean products (Nelson *et al.*, 1971).

sulting flour scored well in flavor tests and had good storage stability characteristics.

Flavor of Whole Soybean Foods. Another contribution to the development of soy-based foods of good flavor has come from the University of Illinois. This work was concerned with the development of products in which the entire soybean is used (Nelson *et al.*, 1971). This group feels that the flavor compounds are absent in the intact bean but that disruption or damage of the cotyledon tissue results in development of "painty" or "beany" flavor. They report that it is essential to inactivate lipoxygenase *in situ* by blanching in boiling water (Figure 3). If the beans are hydrated to about 50% moisture (4-hr soaking), a 10-min blanch is sufficient whereas unsoaked beans require a 20-min blanch. About a dozen prototype foods containing blanched beans were developed.

The Illinois group has also described a flaked product made from whole soybeans (Shemer *et al.*, 1973). The soaked beans were blanched for 20 min, mashed, and then drum-dried to yield an undehulled, full-fat flake.

Flavor of Defatted Soybean Products. Full-fat flour, soy milk, and whole soybean products previously discussed are used to only a limited extent in the United States (Wolf and Cowan, 1971). Rather, the bulk of the soy protein forms—defatted grits and flours, concentrates, and isolates—is made from defatted flakes. The soybean processing industry initially developed an efficient and economical process to remove the oil from soybeans, and the defattted flakes were a by-product that is now an essential ingredient for animal feeds. In the last 25 years small but increasing amounts of defatted flakes have been converted into edible products. It is debatable, however, whether this is the best process for making edible protein products of high quality.

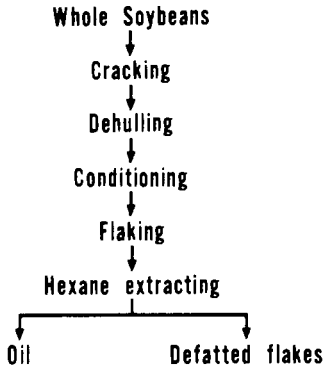

Figure 4. Outline of process for converting soybeans into oil and defatted flakes.

Table II. Thiobarbituric Acid (TBA) Reactive Substances in Soybean[a] and Pea[b] Samples

Sample	TBA no.[c] for homogenizing medium: Water	Acid
Full-fat soy flakes	83.8	10.5
Defatted soy flakes	13.5	11.6
Raw peas	9.3	0.8–1.0

[a] Sessa *et al.* (1969). [b] Rhee and Watts (1966). [c] Milligrams of malonaldehyde per kilogram of sample.

Table III. Summary of Odor and Flavor Scores for Soybean Protein Products[a]

Product	Score[b]: Odor	Flavor
Defatted flours	5.8–7.5	4.2–6.7
Concentrates	6.4–7.4	5.6–7.0
Isolates	6.8–7.7	5.9–6.4

[a] Kalbrener *et al.* (1971). [b] Scale of 1–10 where 10 is bland and 1 is strong odor or flavor.

The multistep process for conversion of soybeans into oil and defatted flakes is outlined in Figure 4. The cracking step is carried out at 8–10% moisture. It is generally assumed that enzymatic activity is minimal at this low moisture level when the cellular structure is disrupted but data supporting this assumption are scant. After cracking, the loosened hulls are separated and the cracked meats are then tempered with steam to raise the moisture to 11–12% to ensure plasticity during flaking. The steaming step is conducive to enzymatic reactions because of the large surface area exposed to the air, high local moisture levels when the steam condenses, and the resulting rise in temperature.

Laboratory-prepared full-fat and defatted flakes have been assayed for lipid oxidation by the thiobarbituric acid (TBA) method (Table II). Full-fat flakes homogenized in water had a very high TBA number presumably because of lipoxygenase activity during homogenization. When lipoxygenase activity during homogenization was inhibited by carrying out this step in acid solution, the TBA number for full-fat flakes was much lower but still about the same as for raw peas homogenized in water. Defatted flakes also gave TBA numbers of the same magnitude as raw peas ground in water. In raw peas a TBA number of 9.3 was considered to indicate extensive oxidation of lipids (Rhee and Watts, 1966). Further work is needed on the extent of lipid oxidation during processing and determina-

Table IV. Compounds That May Contribute to Flavor of Soybean Products

Compounds	Source	Odor–taste	Reference
Volatile carbonyl compounds	Defatted meal	Green beany	Fujimaki *et al.* (1965)
Volatile carbonyl compounds	Full-fat and defatted flakes	Green beany	Sessa *et al.* (1969)
Phenolic acids	Defatted flour	Sour, bitter, astringent	Arai *et al.* (1966a)
Volatile fatty acids	Full-fat meal		Arai *et al.* (1966b)
Volatile amines	Full-fat meal	Fishy	Arai *et al.* (1966b)
Volatile alcohols and esters	Full-fat meal	Green beany	Arai *et al.* (1967)
Ethyl vinyl ketone	Soaked beans	Green beany	Mattick and Hand (1969)
1-Octen-3-ol	Soaked beans	Mushroom-like	Badenhop and Wilkens (1969)
Volatile aldehydes, ketones, alcohols, and others	Soy milk	Rancid, beany, grassy	Wilkens and Lin (1970)

tion of the importance of such oxidation to flavor of defatted flakes.

Taste panel studies on commercial defatted flours, concentrates, and isolates were reported in 1971 (Kalbrener *et al.*) and are summarized in Table III. Samples were evaluated as 2% dispersions in water and scored on a scale of 1 to 10 where 10 is bland and 1 is a strong odor or flavor. A raw defatted flour prepared in the laboratory received an odor score of 5.8, a flavor score of 4.1, and was described as beany and bitter. Commercial flours generally were rated higher than raw flour but ranged from 4.2 to 6.7 in flavor scores. Concentrates ranged from 5.6 to 7.0 while isolates scored from 5.9 to 6.4 in flavor. It was generally agreed that bitter and beany flavors persist in most of the products although at reduced levels as compared to a raw, defatted flour. The flours that scored highest had undoubtedly been processed by moist heat treatment because they had low protein solubilities and received flavor descriptions of nutty and toasted. An inverse relationship was found between flavor score and solubility of the proteins in the samples. Raw flours had high protein solubility and a low flavor score while the samples with highest flavor scores had very low protein solubilities.

Identification of Flavor Components in Soybean Products. While work on improving flavor of full-fat and defatted soybean products was proceeding as described earlier, studies were also underway to identify the compounds responsible for the flavors encountered in these products. Because this work was recently reviewed elsewhere (Cowan *et al.*, 1973) it is only summarized here (Table IV). A large variety of compounds has been isolated but it is not yet possible to attribute the flavor of soy products to definite compounds among those identified to date.

Except for the phenolic acids and volatile amines many of the compounds identified thus far are known to be products of lipid oxidation. If lipoxygenase is allowed to remain active and conditions are favorable (moisture, oxygen, and substrate availability), the major initial reaction will be formation of hydroperoxides. The hydroperoxides in turn can undergo a large number of enzymatic and nonenzymatic transformations to give a wide variety of compounds (Gardner, 1975). An example of this situation is found in the preparation of soy milk by the traditional process (grinding soaked soybeans in water at room temperature). Wilkens and Lin (1970) isolated the volatile fraction from soy milk and found it to be extremely complex. They detected about 80 peaks in the gas chromatogram of this fraction, identified 41 compounds, and made tentative structure assignments to another 13 compounds. Hexanal constituted about 25% of the total volatile fraction obtained from soy milk. Other compounds isolated were hexanol, 2-hexenal, ethyl vinyl ketone, and 2-pentylfuran all of which have grassy, beany odors. It therefore seems likely that the flavor of soy protein products is caused by a large mixture rather than a very limited number of compounds.

Grosch and Schwencke (1969) incubated linoleic acid with partially purified soybean lipoxygenase and oxygen for 3 hr at 15° and isolated about a 3% yield of the following aldehydes (mole per cent in parentheses): *n*-pentanal (5); *n*-hexanal (45); *n*-hept-2-enal (10); *n*-oct-2-enal (5); *n*-nona-2,4-dienal (5); *n*-deca-2,4-dienal (26); unidentified (4). Here again hexanal is the major volatile carbonyl compound formed. These results, therefore, indicate that aldehydes isolated from soy products are derived from the lipids.

Kalbrener *et al.* (1974) have presented additional evidence that hydroperoxides of linoleic and linolenic acids can decompose to form flavor compounds. The pure acids were incubated aerobically with lipoxygenase (Theorell preparation) and the resulting hydroperoxides were then purified by silicic acid chromatography. The purified hydroperoxides were dispersed in water and submitted to a taste panel which also tasted a 0.25% dispersion of raw, defatted soy flour as a control. This control was scored 5.6 (on a 10-point scale described earlier). To obtain comparable scores for the linolenic and linoleic hydroperoxides it was necessary to dilute them to 10 and 50 ppm, respectively. Obviously the hydroperoxides are potent sources of flavor. The predominant description given to both hydroperoxide solutions was grassy–beany followed by bitter, astringent, and raw vegetable flavor. Flavor descriptions for the soy flour were like those of the hydroperoxides except for the raw vegetable flavors. This result suggests that the hydroperoxides do not give flavors that are identical with those of soy flours but the flavors are similar especially with respect to the grassy–beany notes.

Arai *et al.* (1970) and Gremli (1974) have shown that hexanal and other volatile aldehydes as well as ketones are bound to soy protein. Hexanol and hexanal bound to denatured soy protein are not extractable with hexane (Arai *et al.*, 1970). Apparently, once these compounds are formed it is difficult to reduce them to acceptable levels; special solvent mixtures are needed to extract them as discussed later.

Sessa and coworkers (1974) have recently shown that phosphatidylcholine may be another source of flavor in soybean protein products. They carefully purified phosphatidylcholine from commerical soybean lecithin and then allowed it to autoxidize in aqueous dispersion containing 1 ppm of copper ion for 18 days. A strong bitter taste developed but it is not yet known whether this oxidation can also be catalyzed by lipoxygenase.

Table V. Processes for Inactivating Lipoxygenase

Process	Reference
Grinding soybeans with hot water	Wilkens *et al.* (1967)
Dry heating–extrusion cooking	Mustakas *et al.* (1970)
Blanching	Nelson *et al.* (1971)
Grinding at low pH–cooking	Kon *et al.* (1970)

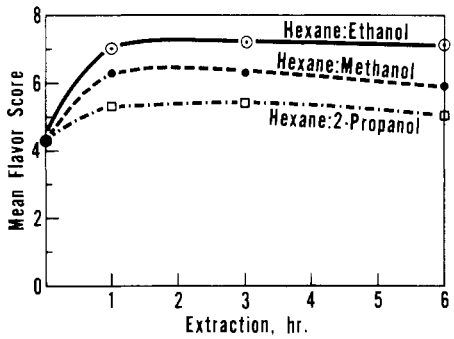

Figure 5. Effect of extraction time on flavor scores of soybean flakes extracted with hexane–alcohol azeotropic mixtures. Organoleptic evaluations were made on 2% water dispersions (Eldridge *et al.*, 1971).

Methods for Inactivating Lipoxygenase. If lipoxygenase is responsible for flavor development in soybean products, how can one inactivate it to prevent its action? In recent work several methods have been used to inactivate lipoxygenase during the processing of soybeans (Table V). The first three methods have already been discussed. The last one is based on the pH-activity relationship of lipoxygenase. Lipoxygenase is inhibited at low pH; consequently, if soybeans and other legumes are ground in acid the enzyme does not catalyze oxidation of the polyunsaturated fatty acids or triglycerides. Formation of volatile aldehydes and other compounds is thereby prevented. Kon *et al.* (1970) ground soybeans at low pH (3.85 or below), cooked the resulting acid slurry to denature lipoxygenase, and then neutralized the acid with sodium hydroxide. Neutralization results in the addition of about 0.5% salt which may be undesirable in some applications.

None of the processes listed in Table V appear to be in commercial use in the United States at this time although the second one (extrusion cooking) is being utilized in several installations overseas (Mustakas, 1974). Full-fat flour is produced in the United States and in England by steaming beans, drying, dehulling, and grinding (Pringle, 1974); this is essentially the process described by Berczeller (1924) and the blanching treatment proposed by Nelson *et al.* (1971).

The common theme to all processes proposed to inactivate lipoxygenase is heat. High temperatures and moisture, however, also denature and insolubilize the major proteins in soybeans. Soybeans treated by any of the above methods, therefore, have limited usefulness in the food industry. Food ingredients such as protein isolates and one type of protein concentrate cannot be prepared from heated soybeans because the protein is no longer soluble. More selective methods are needed for inactivating or inhibiting lipoxygenase without insolubilizing the bulk of the proteins.

Removal of Flavor from Defatted Soy Flakes. As discussed earlier, raw defatted flakes have objectionable beany, bitter, and astringent flavors. Commercially these flavors are generally decreased in intensity by steaming. This process has the same disadvantage as methods used to inactivate lipoxygenase in preventing the formation of flavors, namely, the insolubilization of the proteins. Protein solubility is essential for the preparation of isolates and is also a valuable functional property. Consequently, studies have been conducted on the removal of existing flavors from defatted flakes by other means than steaming.

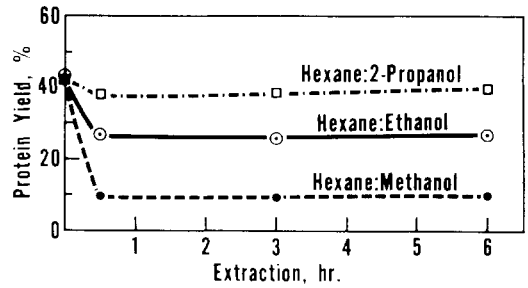

Figure 6. Effect of extraction time on protein yields from soybean flakes extracted with hexane–alcohol azeotropic mixtures (Eldridge *et al.*, 1971).

Extraction with aqueous alcohol removes many of the objectionable flavors (Moser *et al.*, 1967) and is used commercially to prepare protein concentrates (Meyer, 1971). Appreciable protein denaturation also occurs by this procedure and the technique is not suited for making soy flours because the sugars dissolve and are removed.

Hexane defatted flakes are not free of lipids but the residual lipids can be extracted with a mixture of 20% hexane and 80% ethanol (Nielsen, 1960). Sessa *et al.* (1969) made the important observation that the residual lipids extracted with this solvent mixture have hydrocarbon-like, bitter, biting, and astringent flavors whereas the extracted flakes have little flavor. Further studies have confirmed and extended these findings (Eldridge *et al.*, 1971). Defatted flakes were extracted with azeotropic mixtures of hexane and methanol, ethanol, or 2-propanol and the flakes were evaluated by a taste panel after extraction for various times (Figure 5). Untreated flakes had a flavor score of 4.2–4.3 but after extraction with the hexane–alcohol mixtures the flavor scores increased. Maximum values were obtained after about 1 hr of extraction. Hexane–ethanol extraction gave the highest score—7.0–7.2. Flakes extracted with hexane–alcohol also yielded protein isolates of improved flavor. Isolates from unextracted (hexane defatted only) flakes had flavor scores of 5.0–5.2 whereas isolates from hexane–alcohol extracted flakes scored about 7. Although the alcohol used for extraction had no significant effect on the flavor score of the protein isolates, the alcohols denatured and insolubilized the proteins to varying extents. As a result, protein yields of isolates were decreased (Figure 6). The least insolubilization occurred with hexane–2-propanol and the most denaturation took place when hexane–methanol was used. Extraction with hexane–methanol has one other disadvantage: it is not selective in extracting the residual lipids and flavor compounds. In 6 hr of extraction, hexane–methanol removed nearly 8% of the meal solids whereas hexane–ethanol or hexane–2-propanol extracted only 2.0–2.5% of the solids.

Hexane–alcohol extraction shows promise as a means for extracting defatted soybean flakes to remove flavor compounds and thereby yield flours and grits of improved organoleptic quality for further processing into concentrates and isolates. A patent issued recently for preparation of soy protein concentrates incorporates an extraction step in which hexane–ethanol is used to remove the residual lipids from defatted meal before a final extraction with 40–70% aqueous ethanol to extract the soluble sugars and other minor constituents (Hayes and Simms, 1973).

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Effects of Lipoperoxides on Proteins in Raw and Processed Peanuts

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Oxidative degradation of unsaturated lipids in peanuts produces hydroperoxides and their subsequent breakdown products, acids, alcohols, aldehydes, and ketones. These compounds have been reported by others to damage proteins, enzymes, and amino acids. In the present investigation, lipid–protein interaction was examined in deoiled meals and in the proteins extracted from raw and roasted whole peanuts and peanut butter. Polyacrylamide electrophoresis was used as the principal technique to compare proteins before and after storage under conditions designed to promote peroxidation of lipids. Disc gels of deoiled residues from peanuts were stained for protein and lipid. The Sudan stains, which are used extensively for detecting liproproteins in mammalian tissues, were not sensitive enough to detect the small amount of lipid bound to peanut proteins, but Rhodamine 6G and Oil Red O were satisfactory. Details of these procedures and observations on the effects of peroxidized lipid–protein interactions on electrophoretic mobility and on solubility of various protein fractions are discussed.

Peroxidation of fatty acids has long been a concern to academia and food industry because lipid peroxides are involved in the development of rancidity in foods containing unsaturated fatty acids, the production of "off" odors and flavors, and the production of toxic or physiologically active compounds that can damage proteins, enzymes, and amino acids. Lipid peroxidation involves a free-radical mechanism, initiated by autoxidation, that can be catalyzed by either metalloproteins or enzymes, to form fatty acid hydroperoxides. Once initiated, the reaction is self-propagating, forming more hydroperoxide and more free radicals and/or breakdown products, depending upon the conditions. The products formed can complex with amino acids, proteins, or enzymes.

Lipid–protein complexes are believed to be held together either by electrostatic (ionic) attractions, as reported by Green and Fleicher (1963), by hydrogen bonding, van der Waals interactions, or hydrophobic interactions, considered by Némethy (1967) to be the main type of bonding between lipids and proteins *in vivo*. Covalent bonds between lipids and proteins in natural systems are uncom-

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