Flavor Problems of Vegetable Food Proteins

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ABSTRACT

Flavor is a major factor that limits the use of many vegetable proteins in foods. In high quality whole cereal grains, flavor and flavor stability present little or no problem; but when some cereals are further processed into protein concentrates and isolates, objectionable flavors can arise from oxidative deterioration of unsaturated fatty esters in protein-bound lipids. However, degermed wheat and corn flours (endosperm products) have little or no flavor. Raw legumes and oilseeds enriched with respect to lipoxygenases and other metallo-proteins possess lipid-derived, objectionable flavor compounds. Lipoxygenase-mediated conversion of lipids to lipohydroperoxides and their subsequent degradation form volatile and nonvolatile constituents responsible for off-flavors. n-Hexanal, 3-*cis*-hexenal, n-pentylfuran, 2(1-pentenyl)furan, and ethyl vinyl ketone are major contributors to grassy-beany and green flavors. Higher 2,4-alkadienals have oxidized painty, rancid flavors sometimes noted in residual lipids. Geosmin, an oxygenated hydrocarbon, is responsible for the musty, moldy, earthy flavor of dry beans. This compound may contribute to similar flavors noted in soy and corn protein isolates. Thermally degraded phenolic acids account for some of the objectionable cooked odors of soy products that have been subjected to high temperature treatment such as retorting, autoclaving, and sterilization. Oxidized phosphatidylcholine most likely accounts for the bitter taste of soy products. Oxygenated fatty acids, including the bitter-tasting trihydroxy octadecenoic acids, have been identified in the bitter phosphatidylcholines isolated from soybeans. Oxidized lipids appear to be associatted with the bitter, astringent, and rancid flavors of protein isolates prepared from wet-milled corn germ flour. Grassy-beany, bitter flavor compounds preexist in the maturing soybean and are also generated during processing. In some legumes development of off-flavors can be readily controlled by rapid inactivation of lipoxygenase with heat, alcohol, or acid treatment. Legume powders of acceptable flavor quality can be prepared by wet-milling whole seeds in aqueous alcohols. Extraction of meals with hydrogen bond-breaking solvents, such as alcohols or azeotropic mixtures of hexane and alcohol, effectively removes protein-bound lipids to yield concentrates with greatly improved flavors. Soy protein concentrates approaching the blandness of wheat flour have been prepared by a combination of azeotrope extraction and steaming. Similar processes can also be used to greatly improve flavor scores of corn germ protein isolates. Based on our present knowledge about the identity of off-flavor constituents and how they are derived, much progress has been made to effectively remove or modify them. These developments should result in new emerging technology that would be applicable to the manufacture of highly acceptable protein products from various vegetable sources.

INTRODUCTION

Learning from experience, man has established which foods are nutritionally beneficial and essentially free of adverse physiological effects. By continuous consumption from generation to generation, such foodstuffs become classified as "traditional" foods. Once established, flavor characteristics then become a secondary property by which these foods are recognized and enjoyed. Recognition of acceptable sensory qualities involve taste, odor, color, texture, and other factors. Therefore, the successful introduction of a new protein food becomes especially difficult if its sensory qualities are different. Acceptance may require a long period of consumption so that one can acquire a taste for the new protein food. Otherwise, agents that impart acceptable sensory properties must be added to mask objectionable flavors of the new protein food. Processors using this masking approach have been unsuccessful in overcoming the objectionable flavor characteristics of soy protein products in spite of diligent effort.

Here, we describe the particular flavor problems associated with some vegetable proteins, identify some of the characteristics of the flavor principles and their precursors, and discuss extraction processes designed to improve organoleptic qualities. Advances in flavor improvement have been made; however, the achievements are much more modest than technological achievements, i.e., the transformation of vegetable proteins into food products with functional characteristics of animal protein.

In this review, identification of factors responsible for off-flavor production and removal of them is limited primarily to soybeans, peanuts, peas, beans, and corn germ flour and isolates for two major reasons: (a) recognition that flavor limits their use in food systems and (b) existence of acceptability and formal flavor panel data that define and quantitate the nature of the off-flavors. Sensory evaluations are still needed to define more definitively flavor characteristics of other vegetable protein resources – cottonseed, rapeseed, sunflower – since unacceptable flavor principles are known to be present.

RECOGNITION THAT A FLAVOR PROBLEM EXISTS

Various groups and representatives of industry point out that flavor is the most important factor limiting the use of soy protein in foods (1-4). Johnson (4) reports that 1974 production of edible soy protein products was 1.13 billion pounds and predicts a potential increase to 3.7 billion pounds by 1985. Part of this potential market includes soy milk type beverages in which flavor acceptability is of concern. When added to bread to increase protein content for nutritional purposes, enzyme active soy flour results in flavor problems at levels above the 2% required to improve dough characteristics (5), but properly treated soy flour may be used satisfactorily at significantly higher levels in

TABLE I

Organoleptic Evaluation of Soy Protein Products (13)

Products	Odor		Flavor	
	Score[a]	Description	Score[a]	Description
Soy flours A-G	5.8-7.5	NP,[b] beany, corn meal vanilla, CW[c]	4.2-6.7	Beany, green beany, bitter, raw beany, toasted
Raw flour[d]	5.8	Beany	4.1	Raw beany, beany, bitter, green beany
Concentrates A-E	6.4-7.4	Beany, CW, NP, musty, stale, toasted, corn meal	5.6-7.0	Beany, bitter, astringent
Isolates A-F	6.8-7.7	CW, musty, beany, corn meal, spoiled	5.9-6.4	Beany, bitter, chalky, cardboard, astringent, toasted, nutty, cereal

[a] 10 bland; 9-7, weak; 6-5, moderate; 3-4, strong; 1-2, very strong.

[b] NP - none predominant, several responses.

[c] CW = odor similar to combination of high protein oat cereal and singed wool in water.

[d] Laboratory prepared raw, defatted soy flour.

TABLE II

Flavor of Full-Fat Soy Flour: Effect of Steaming (17)

Steaming, min	Flavor score[a]	Flavor description				
0	1.5	Beany	Bitter	Green		
3	4.5	Beany	Bitter	nutty	Sweet	Toasted
10	6.0	Beany	Nutty	Bitter	Toasted	Sweet
20	6.3	Beany	Nutty	Bitter	Toasted	Sweet
40	6.1	Beany	Nutty	Bitter	Toasted	Sweet

[a] See Table I.

TABLE III

Beany and Bitter Flavor Thresholds of Soybean Products (13)

Sample[b]	Thresholds[a]		
	Sample detection, %	Beany, %	Bitter, %
Flour H	0.005	0.033	0.04
Concentrate D	0.04	0.16	0.20
Isolate E	0.06	1.25	>3.0
Isolate F	0.06	0.20	2.00

[a] Percent sample in charcoal-filtered water.

[b] See Table I for corresponding flavor scores.

bread. The beany character of soybean milk is unacceptable to many people; however, *Lactobacillus* fermentation is effective in masking or modifying beany flavor (6,7).

Several studies on beef patties containing soy protein products (8-11) indicate up to 20% hydrated textured soy products would be acceptable to the consumer based on various palatability characteristics. More recently, Twigg et al. (12) reported that flavor scores of patties containing 30% textured soy protein or soy protein concentrates were significantly lower (scores of 3.6-4.8) compared to a score of 6.0 for all-beef patties. Flavor scores were based on a 9-point scale where 1 = poorest; 9 = best. Different preparations of soy protein varied widely in flavor score in a standard taste panel. A consumer panel gave higher scores.

An organoleptic evaluation in 1971 of 18 commercial soy flours, concentrates, and isolates which represented most commercial methods of processing at that time confirmed that these products are not bland (13). Results are given in Table I. When evaluated by a 17-member taste panel, 2% dispersions of the samples in charcoal-filtered tap water had odor scores ranging from 5.8 to 7.7; and flavor scores, from 4.2 to 7.0. Scores were rated on a 10-point scale where 10 is bland and 1 is strong.

Flavor scores of the products do not show a great reduction in flavor intensity when flours are processed into concentrates and isolates, in spite of the removal of nonprotein constituents. However, differences exist between flavor descriptions of flours, concentrates, and isolates. This indicates that extraction of the original beany, bitter, and green flavor components of raw flour was incomplete and that off-flavors are created during processing, depending upon the conditions of manufacture. Similar results were obtained by Rutkowski (14), except that different descriptive terms were used to describe the off-flavors in soy protein products.

Some tasters will record such terms as grassy, beany, green, and green pea for the "beaniness" off-flavor of raw soybeans, but most often the tasters will use the terms interchangeably (15). In a different taste panel exercise (16), members were tested for their taste acuity using lima bean extract (0.002-0.25%) as a standard for beany and 3-hexenal (0.025-0.2%) for grassy. Most panel members were unable to consistenly differentiate the two flavors; hence, category grassy-beany could be a proper category to use in taste panel evaluations.

Table II lists some of the predominant characteristics of

TABLE IV

Lipid-Oxidizing Activity of Various Leguminosae (22)

Genus	Common name	Oxygen consumption^{a}
Vicia	Broad bean	840
Pisum	Pea	1769
Glycine max	Soybean	4150
Phaseolus	Yellow bean	6480

^{a}μl per 10 g fresh tissue per min determined by polarography.

TABLE V

Thiobarbituric Acid (TBA) Relative Substances in Peas (23) and Soybean Products (25,26)

	TBA Number in homogenized media^{a}	
Legume sample	Water	Dilute acid (pH 1.1)
Green pea	9.3	0.9
Mature soybeans	12.0	1.1
Full-fat flakes, raw	83.8	11.1
Full-fat flakes, toasted	7.7	3.3
Defatted flakes, raw	11.6	9.7

^{a}mg Malonaldehyde/kg sample.

TABLE VI

Formation of Thiobarbituric Acid (TBA) Reactive Substances Formed During Processing of Soybeans Into Oil and Meal (25)

Sample	Moisture content, %	TBA number^{a}
Cracked soybeans^{b}	10	2
Full-fat flakes^{c}	14	10.5
Defatted flakes	8-10	11.6
Crude oil	---	5.8
Defatted flakes		
Ether extract	---	66.9
Azeotrope extract	---	34.00

^{a}mg Malonaldehyde/kg sample.

^{b}Tempering to 22% moisture, TBA = 3 (26).

^{c}Rehydrating flakes, TBA increased to 15 (26).

raw and steam-treated full-fat flours. The flavors in raw flour are: beany, bitter, green, in order of decreasing intensity. Results are the same with raw defatted soy flour. At a dilution of one part raw soy flour in 500 parts of wheat flour, the panel was able to correctly identify all samples that contained raw soy flour (17). Sample-detection thresholds of raw soy flour, a concentrate, and two isolates are given in Table III.

The threshold level is the concentration of sample where the panel could detect any flavor other than water. Raw defatted soy flour (flour H) was detectable at a level one-tenth the level of the concentrate and two isolates. The threshold levels of beany and bitter flavors are somewhat higher than the sample-detection thresholds. The threshold levels indicate that the objectionable beany and bitter components must be present in very low concentrations in the commercial products and that they are quite intense to be detectable at such low levels.

The present situation is also compounded by subjective reactions, i.e., consumer attitudes of "substitutes" vs. the real thing. When soy-based extenders for meat were being offered in 1970, meat prices were soaring in the United States and the consumer was eager to buy alternatives. Many supermarkets began offering soy-ground beef blends for 15 to 25 cents a pound less than for regular hamburger. Such blends captured more than a quarter of the ground meat market. But when beef prices fell in 1973, demand for meat blends went down even though a small cost savings was still possible. Therefore, consumer acceptability is dependent upon preference as well as favorable price differentials.

LIPID-DERIVED OFF-FLAVORS IN LEGUMES

Lipoxygenase (linoleate/oxygen oxidoreductase, E.C. 1.13:11.12) catalyzes the hydroperoxidation of polyunsaturated fatty acids and esters containing a 1,4-pentadiene system. The mechanism of lipoxygenase catalysis by which hydroperoxides are formed and then subsequently degraded to form a variety of secondary products has been reviewed (18,19). This enzyme is widely distributed in a great variety of plants and its presence in animal tissue has been shown recently (18).

Lipoxygenase has a very important role in enhancing food quality as well as creating deleterious effects (19,20). In the form of enzyme active soy flour at a level of about 0.5 to 1.5% (flour weight basis), lipoxygenase contributes several functional properties in baking technology. Lipoxygenase is an important physiological factor in the biosynthesis of ethylene and fruit ripening, and it is an important beneficial factor in the development of the characteristic flavor of many fruits and vegetables. In addition to these beneficial effects, lipoxygenase is also responsible for the deterioration of food quality and for the generation of objectionable flavors in many foods. The production of off-flavors is a particular problem with raw legumes such as soybeans, peas, lentils, fababeans, and many others in the genus Leguminosae.

LIPID OXIDIZING POTENTIAL OF LEGUMES

The likelihood of enyme-catalyzed oxidation of polyunsaturated acids in legumes is great since they possess a high degree of lipid unsaturation in both the oil and phospholipids. The legumes contain several lipoxygenase isoenzymes with considerable activity, capable of oxidizing unsaturated fatty acids in free and ester-bound form (19,20).

Sessa (21) reported that soy lipoxygenase type 1, which normally acts only on free fatty acid, can oxidize ester-bound unsaturated fatty acids on the anionic form of soy phosphatidylcholine. An active peroxidase capable of utilizing linolenic hydroperoxide is present in soybeans (15). Practically all of the Leguminosae are on the top of the list in terms of lipd-oxidizing activity (22,23). The lipid-oxidizing potential of various legumes is given in Table IV.

Legumes are particularly susceptible to oxidative degradation by enzymatic and nonenzymatic reactions (24). Degradation occurs following the breakdown of the cell wall and is still evident after cooking. The result is the development of various off-flavors in peas, lentils, fababeans, and soybeans (19,24). Based on an analysis of thiobarbituric acid (TBA) reactive substances, Sessa et al. (25) and Rackis et al. (26) demonstrated that processing whole soybeans into oil and meal increased oxidative degradation of unsaturated lipids. Data are summarized in Table V. When samples are aerobically homogenized in water, the resultant TBA numbers are higher than those of samples blended in acid solutions because of higher lipoxygenase activity in nonacid medium. Toasting inactivates the enzymes and lowers TBA numbers and diminishes differences between water and acid media. Analysis of peas showed similar results (23). When the full-fat soy flakes were hexane defatted, most of the lipid substrate was extracted and lower TBA numbers were noted.

Table VI contains data that show the extent of lipid oxdiation which may occur during processing of soybeans into defatted flakes. Whole soybeans, which have been cracked into 6-8 pieces and then dehulled, have a TBA number of 2. When the cracked beans were tempered to 14% moisture and formed into thin flakes after passage through smooth rolls, a TBA number of 10.5 for these flakes represented a five-fold increase. After extracting

TABLE VII

Flavors Derived from Purified Linoleic (LOHP) and Linolenic (LNHP) Acid Hydroperoxides (16)

Flavor characteristic	Tasters' response, %		
	LOHP, 50 ppm	LNHP, 10 ppm	Soy flour, 0.25%
Grassy-beany	80	90	94
Bitter	16	19	30
Astringent	19	19	34
Raw vegetable	20	16	---
Musty-stale	35	---	8
Rancid oil	44	5	3

99.8% of the oil from full-fat flakes with pentane/hexane, the change in TBA number was slight. In spite of an apparent increase in lipid oxidation, as measured by TBA assay, the flavor remained the same as in the original whole soybeans: grassy-beany and bitter. Flavors described as hydrocarbon, rancid, and painty are noted in full-fat soy products that have been subjected to aerobic blending in water. Rancid flavors in underblanched frozen raw vegetables, notably peas, and ground-stored fababeans were cited in recent reviews (19,24).

Taste panel evidence has been reported that either hydroperoxides arising from lipoxygenase action on linoleic and linolenic acids or their degradation products lead to soybean-like flavors (16). The flavors are listed in Table VII. Painty-fishy flavors were present in crude hydroperoxide mixtures of these two fatty acids.

Enzymes, heat, light, and a number of metal catalysts including metallo proteins such as hematin compounds degrade these fatty acids. The hydroperoxides produced initially then decompose by a complex series of reactions (18,19) into a large number of volatile and nonvolatile compounds. Oxygenated fatty acids, with epoxy, oxo, and hydroxyl functional groups resulting from homolytic decomposition of hydroperoxides, have been identified (27,28). Their contribution to off-flavors has yet to be determined.

KEY FLAVOR COMPOUNDS IN LEGUMES

Volatile Flavor Constituents

In growing plant tissue, lipid enzymes and flavor precursors are compartmentalized in intact cells. Normal metabolic activity is governed by regular control mechanism. During storage and processing, cells deteriorate and allow enzyme-substrate interaction, with the result that a large number of flavor compounds can be produced by the interaction of oxidized lipids with proteins and carbohydrates. On heating of the foodstuffs, the concentrations of volatile compounds are increased and a large number of new ones are formed. Qvist and von Sydow (29) identified over 100 compounds in rapeseed protein preparations representing aliphatic hydrocabons, alcohols, aldehydes, ketones, nitriles, furan derivatives, and sulphur-containing constituents. Determinations were also made of the absolute concentrations of about 70 of them judged to be of potential importance in terms of the aroma of rapeseed protein. A comparison of the volatiles from soy protein (30) with those from heated beef (31) revealed a number of similarities and dissimilarities.

Several reviews (19,24,32,33) have summarized the major volatile compounds derived from lipid oxidation that may be associated with the off-flavors of soy products. The compounds most likely responsible for the green, beany, or grassy odors and flavor of peas and soybeans are given in Table VIII. The odor and flavor threshold values of these compounds when tasted in water or milk were even lower than those given in Table VIII. For example, Kinsella (39) reports a three-fold difference in flavor threshold values of 3-*cis*-hexenal in oil vs. milk (i.e., 0.15 ppm vs. 0.05 ppm) and a 25-fold difference for 2-*trans*-hexenal with 2.5 ppm detected in oil vs. 0.1 ppm in skim milk.

If we assume that the compounds listed in Table VIII all contribute to the grassy-beany, green flavor of raw soybeans, the formation of even small amounts of these by lipid oxidation during processing of soybeans into oil and meal (25) could greatly affect flavor, since the grassy-beany flavor threshold of raw defatted soy flour in water is 330 ppm (Table III).

Soy protein shows a distinct affinity for certain flavor compounds, and specific soy protein components bind grassy-beany, bitter, astringent soy flavor components. This has prompted Sessa and Rackis (24) to postulate that denaturing protein to irreversibly bind flavor constituents could reduce the intensity of some off-flavors.

Lipoxygenase-mediated conversion of lipids to hydroperoxides and subsequent formation of short chain aldehydes contribute to the desirable flavor of many fruits; yet, it is the appearance of many of these same volatile compounds that produced rancid off-flavors in legumes. *Cis*-3-hexenal, n-hexanal, and *trans*-2-hexenal are important contributors to fresh tomato flavor, but at higher concentrations, rancid flavors resulted (40).

How to determine which of the many organic compounds in a complex mixture of volatiles are responsible for the characteristic aroma and flavor of foodstuffs can be a complicated exercise (41). Analysis of blueberry essence was used to illustrate that linalool and *cis*-3-hexenal in the correct combination are essential to the characteristic flavor, but by themselves are totally unrelated to blueberry flavor. As a result, the volatile compounds responsible for the grassy-beany flavor of legumes (see Table VIII) may actually represent a more complex mixture of constituents that are derived from precursors other than primarily lipids.

Maga (42) has summarized the volatile compounds identified to date in seven cereal grains. His review demonstrates that a large number of volatile compounds were

TABLE VIII

Compounds Contributing to the Green Vegetable Flavors of Raw Peas and Soybeans

Compound	Flavor description	Threshold (ppm in oil)		Reference
		Odor	Taste	
n-Hexanal	Green grassy	0.32	0.15	34
3-*cis*-Hexenal	Green beany	0.11	0.11	35
n-Pentyl furan	Beany	2	1-10	35
Cis-2-(1-pentenyl) furan		8	8	36
Trans-2-(1-pentenyl) furan		2	2	36
Ethyl vinyl ketone	Green beany	5 (milk)		37
Contributors[a]				
n-pentanol; n-hexanol and n-heptanol				38

[a] Postulated to contribute or enhance the grassy-beany flavor of raw soybeans.

TABLE IX

Bitterness of Autoxidized Soy Phosphatidylcholine (44)

Concentration,[a] ppm	Bitter response, %[b]
250	100
100	63
50	44
30	40
10	0

[a] In charcoal-filtered tap water.

[b] Percent of tasters giving a positive response.

identified in raw rice and an intermediate number in wheat; raw corn had the lowest number of volatiles. Numerous volatiles are present in the relatively bland cereals; however, except for hexenal the cereals do not contain the compounds responsible for the grassy-beany flavors of legumes that are listed in Table VIII.

Nonvolatile Flavor Compounds

In general, free fatty acids (above C:12) normally found in plant lipids do not have much flavor other than that described as candlelike or soapy (43). A bitter taste is not usually associated with lipids or their oxidation products. However, an intensely bitter taste developed when oil-free soy phosphatides were irradiated with ultraviolet light (25). Purified soy phosphatidylcholine (SPC) oxidized in an aqueous suspension developed a bitter taste, whereas hydrogenated soy PC similarly treated did not (44).

From the data shown in Table IX, the bitter threshold level of oxidized SPC was calculated to be about 60 ppm. Three oxidized PCs designated SPC-A, SPC-B, and lyso-SPC were isoalted from the residual lipids of hexane-defatted soy flakes (45). A seven-member taste panel found that 0.05% suspensions of SPC-B and lyso-SPC were strongly bitter, whereas SPC-A was rated weakly bitter (see Table X). The extent of bitterness was related to the extent of oxidation of the unsaturated fatty acids. Based on the bitter values given in Tables IX and X and the fact that defatted soy flakes contain at least 0.08% oxidized PC, it was concluded that oxidized phospholipids may be largely responsible for the bitter taste of raw soybeans and products prepared from them.

In soy flakes the other phospholipids, phosphatidylethanolamine and phosphatidylinositol, do not appear to have a bitter taste. Whether oxidized phospholipids in other legumes taste bitter remains to be determined. Oxygenated fatty acids isolated from lipoxygenase-catalyzed oxidation of linoleic acid taste bitter (46). Taste threshold of the various unsaturated tri-and tetrahydroxy derivatives are in the range of 200-1500 ppm. The presence of a double bond in the trihydroxy acids enhances the bitter taste three-fold. Most likely, the free fatty acids in defatted soy flakes contribute little to the bitterness of soy products because only trace levels are present. Oxidized SPC contains a complex mixture of oxygenated fatty acids (28). So, it may be that the content of esterified polyhydroxy fatty acid may account for the range in bitterness of the various SPCs that were taste tested (see Tables IX, X). However, whether soybeans contain free oxygenated fatty acids has not been determined.

The triglycerides in soybeans undergo considerable transformation in composition during maturation, and yet the characteristic grassy-beany flavor does not vary in intensity (15). This would indicate that minimal oxidation of the highly unsaturated lipids by lipoxygenase may be enough to initiate formation of this flavor. On the other hand, bitterness increases three- to four-fold, particularly in the latter stages of maturity. There is a significant correlation between lipoxygenase activity and the formation of a bitter flavor. Sessa (21) has shown that lipoxygenase Type 1 isoenzyme can oxidize the anionic form of SPC. Analysis of the fatty acids in the intact SPC revealed the presence of several oxygenated forms, including trihydroyxyoctadecenoic acid, which is bitter (46). A similar reaction in the intact soybean could lead to the preexistence of a bitter phospholipid in mature whole soybeans.

TABLE X

Bitterness of Oxidized Soy Phosphatidylcholines SPC) Isolated from Defatted Soy Flakes (45)

Compound	Concentration tasted, %	BIV[a]
SPC-A	0.05	0.9
SPC-A[b]	0.05	2.4
SPC-B	0.05	3.0
	0.01	1.2
Lyso-SPC	0.05	3.0
	0.025	1.8
	0.01	0.8

[a] Bitter intensity value based on the scoring system 0, none; 1, weak; 2, moderate; 3, strong.

[b] From defatted flakes stored 1 year at 4 C.

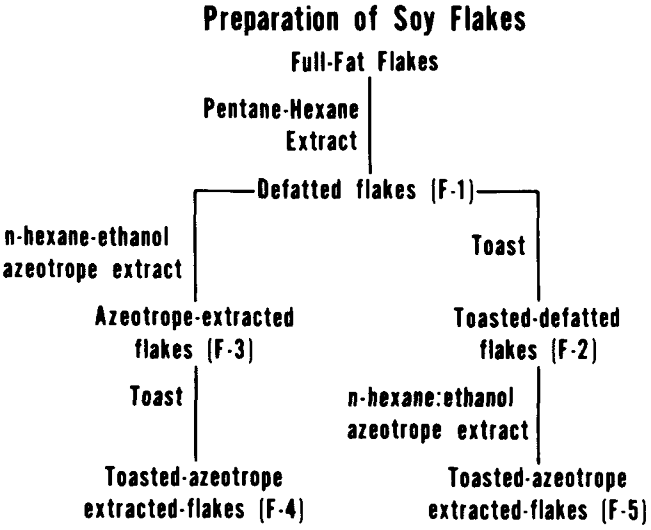

FIG. 1. Formation of the cooked soybean odor by thermal degradation of phenolic acids (49).

Compounds formed by the interaction of aldehydes with soy phospholipid develop a bitter taste (47). In model systems consisting of nonanal and nonhydrogenated phosphatidylethanolamine, a bitter taste developed. However, whether this occurs in foodstuffs remains to be determined.

Nonlipid Precursors of Off-Flavors in Legumes

Musty is a frequent description of flavor characteristics in commercial soy protein products (see Table I). Geosmin, an oxygenated hydrocarbon, has been identified as being mainly responsible for the musty, moldy, earthy odor of off-flavored dry white navy beans (48). Buttery et al. (48) speculate that the possible source of geosmin in the beans could result from the microbial action of *Actinomycetes* either on the beans themselves or in the water supply used during the growing of the beans.

When wet defatted soybean meal is sterilized, it develops a characteristic unpleasant cooked odor that constitutes one of the major barriers to consumer acceptance of high temperature processed soy products. The cooked soybean odor is also characteristic of canned, textextured soy products that have been retorted. According to Greuell (49), the cooked soybean odor is produced during thermal decarboxylation of the phenolic acids, p-coumaric and ferulic, as shown in Fig. 1. Both precursors can be extracted with aqueous alcohol. The extracted

TABLE XI

Subjective and Objective Flavor Analyses of Soy Protein Products

Taste panel score	GC-predicted score[a]
7.9	8.3
7.6	8.0
7.6	7.2
7.4	5.9
7.4	6.6
7.2	7.6
6.8	7.1
6.8	5.6
6.7	7.2
6.7	5.9
6.5	6.8
6.3	5.7
6.2	6.1
6.0	6.1
5.2	5.6
4.0	4.6
3.8	4.8
3.5	4.5

[a] Derived from the regression equation of flavor scores on log of hexanal content (54).

soybean meal does not produce the odor when it is heated.

OBJECTIVE ANALYSIS OF FLAVOR

Stepwise regression analysis has been used to show that chromatographic data can be used with good accuracy for the determination of the sensory quality of soy sauce (50). Sensory qualities were linearly related to gas chromatographic profiles, so that only a few peaks need to be isolated for the identification of the compounds that are most closely related to the flavor of the product. With similar analysis it may be possible to observe changes in flavor quality and to use such analyses as a quality control measurement. In a recent symposium (51), instrumental analysis of flavor compounds to assess flavor stability of fats and oils was discussed. The reports indicated that very good correlation of volatiles and flavor panel scores can be achieved and that major volatiles responsible for the quality of the vegetables can be identified. Warner et al. (52) were able to show that flavor scores of vegetable oils can be predicted from pentanal and hexanal contents, with correlation coefficients ranging from -0.60 to -0.96. Correlation coefficients of total volatiles in oil were lower than for hexanal or pentanal. This emphasizes the point that objective-subjective correlations need to be established between each taste panel and each instrumental method.

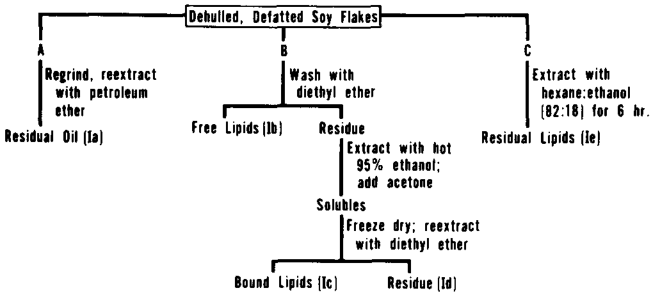

FIG. 2. Extraction of various lipid fractions from raw, dehulled, defatted, soy flakes (57).

Previously, it had been shown that regression analysis of the log of the hexanal content correlates with taste panel results of vegetable oils (53). Table XI shows a direct comparison between the taste panel scores of 18 soy protein products and those derived from the regression equation of the log of the hexanal content as analyzed by direct gas chromatography (54). The correlation coefficient for the regression was -0.84, which is highly significant at the 1% level. For some of the samples of soy flours, the differences in flavor scores between taste panel analysis and those derived by gas chromatographic data were greater than the standard error of about 0.67 for typical taste panel results for these same flours (55). The deviation in flavor scores in Table XI varied from 0.1 to 1.5 units. Part of the deviation could be attributed to the lowering of flavor scores by high residual levels of the solvents, ethanol, pentane, and hexane, that were used in the processing of these products (unpublished data).

PROCESSES TO IMPROVE FLAVOR

Extraction of Lipids

Based on nonaqueous extraction procedures diagrammed in Fig. 2, the lipids in hexane-defatted soy flakes can be divided into three groups: residual oil, free lipids, and bound lipids. By official designation (56), the residual oil fraction (1a) refers to substances extracted by petroleum ether under conditions of AOCS test method Bc 3-19. We define free lipids (1b) as those extracted by nonpolar lipid solvents, such as diethyl ether. Bound lipids (1c) refers to the additional extraction of lipids with more polar solvents, such as hexane/ethanol (82:18 v/v) at 56 C (55) or 95% ethanol. Bound lipids are strongly associated with protein and the efficiency of extraction depends, to a great extent, upon the ability of solvents to disrupt hydrogen bonds (57).

Yields and flavor characteristics of the various lipid fractions are given in Table XII. Defatted flakes prepared in the laboratory contain about 0.2% residual oil, whereas in commercially prepared, defatted soy flakes content can range from 0.5 to 1.5%. Residual oil content of soy protein

TABLE XII

Yields and Flavor Characteristics of Soy Lipid Fractions from Defatted Soy Flakes (57)

Lipid fraction[a]	Yield, g/100 g flakes	Flavor characteristics
Residual oil (1a)	0.16-0.22	Bland, oily, waxy
Free lipids (1b)	0.9-1.1	Bland, oily, waxy
Bound lipids (1c)	1.9-2.2	Hydrocarbon, oily, biting, throat-catching, lingering after-taste
Residue (1d)	2.1-2.4	Intensely bitter, mealy, sweet
Total residual lipids (1e)	2.5-3.0	Hydrocarbon, oily, biting, throat-catching, lingering after-taste

[a] See Figure 2 for identification.

TABLE XIII

Odor and Flavor Scores of Toasted, Azeotrope-Extracted, Defatted Soy Flakes (55)

Soy sample[b]	Scores[a] Odor	Flavor
F-1	5.2	4.0
F-2	7.4	6.6
F-3	7.8	7.4
F-4	8.1	7.8
F-5	8.5	7.4
Wheat flour	8.3	8.1
LSD[c]	---	0.67

[a] See Table I.

[b] See Figure 3 for details.

[c] LSD = Least significant difference at the 95% confidence level.

TABLE XIV

Lipoxygenase and Peroxidase Activity in Processed Soy Flakes (62)

Processing conditions	Lipoxygenase μ moles O_2/min/g	Peroxidase[a]
Hexane-defatted, raw[b]	249	1875
Azeotrope-extracted (3 hr)[c]	1.8	1585
Azeotrope-extracted (6 hr)	0.4	2098
Hexane-defatted, toasted[d]	0.5	244
Azeotrope-extracted (6 hr), and toasted	0.3	70

[a] Absorption units/g at pH 5.0.

[b] Room Temperature.

[c] Azeotrope extraction at 56 C.

[d] Toasting-live steam at 100 C for 15 min.

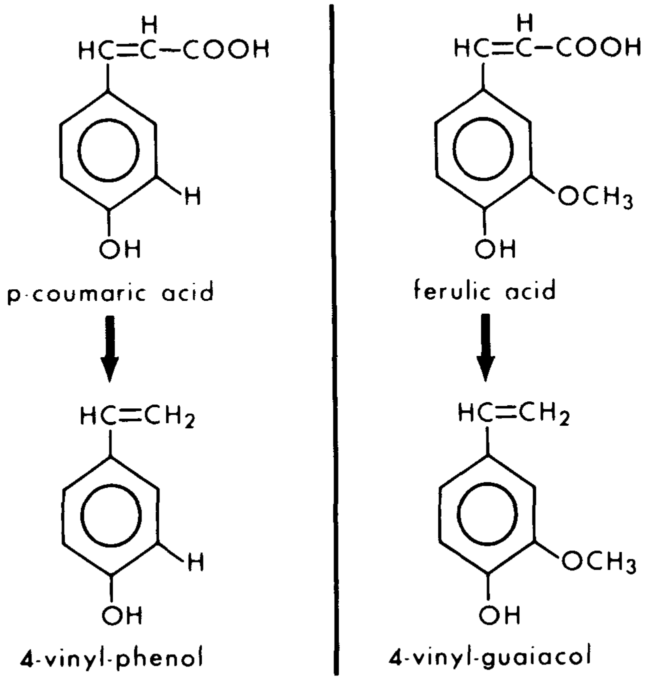

FIG. 3. Hexane/ethanol azeotrope (82:18 v/v) process for improving the flavor of hexane-defatted soy flours, a 6 hr extraction at 56 C, then toasting with live steam at 100 C for 10 min (55).

concentrates varies between 0.3 and 1.2%, while soy protein isolates usually contain 0.3% or less (58,59). Although the four lipid fractions are similar in composition, their flavor characteristics differ widely (57). Oxidized phospholipids are present in the bound lipid fraction.

Extraction of Off-Flavors

Various azeotrope mixtures have been evaluated for their effectiveness in improving organoleptic qualities of raw, pentane-hexane defatted soy flakes (60). Hexane-methanol, hexane-ethanol, and hexane-isopropanol removed the following percentages of the starting material after 6 hr–7.5, 2.0, 2.6%, respectively; the corresponding flavor scores did not correlate with the amount of material removed: 6.1 to 6.2; 7.0 to 7.2; and 5.0 to 5.4, respectively. The original raw flour had flavor scores of about 4.0 based on a 10-point scale.

The major flavor responses noted by the 15-member taste panel for the initial flakes and for the azeotrope-extracted flakes were similar– grassy-beany, bitter, astringent, cereal, and cardboardy. This similarity indicates that azeotrope extraction significantly improves flavor scores, but that extraction of flavor components or their precursors was incomplete. Nevertheless, flavor scores of 7.2 for hexane-ethanol azeotrope-extracted flours were significantly higher than scores of 4.2 to 6.6 for commercial flours (see Table I).

Combinations of extraction with toasting procedures yield even blander products, with scores approaching a value of 8 (55). Results are summarized in Table XIII. Schematic for the preparation of the flour samples is illustrated in Fig. 3.

Raw soy flakes (F-1, Table XIII) have a low flavor score. Toasting (live steam treatment) raw, defatted flakes for 10 min (sample F-2) greatly increased odor and flavor scores. Azeotrope extraction was a more effective process in improving flavor (sample F-3). Maximum scores were obtained with defatted flakes that were hexane-ethanol azeotrope-extracted and then toasted (sample F-4). The beneficial effect of post-toasting is attributed to the removal of residual solvents (61).

Over 99% of the lipoxygenase activity in raw soy flakes is destroyed during azeotrope extraction, whereas no destruction of peroxidase activity occurred (Table XIV). Toasting not only improved flavor scores, but also destroyed the trypsin inhibitors and other heat-labile antinutritional factors (62). When the azeotrope-extracted flakes were toasted, protein efficiency ratio of the flakes increased from about 1.0 to 2.2 on a basis of a value = 2.5 for casein (62).

Toasting of soy protein concentrates prepared from azeotrope-extracted flakes raised flavor scores to 7.9, which approach the blandness of wheat flour with a score of 8.1. A protein isolate from toasted azeotrope-extracted flakes scored 7.3 compared to 8.0 for sodium caseinate.

Improved flavor scores of toasted azeotrope-extracted flakes and of concentrates and isolates prepared therefrom were attributed to the ten-fold reduction in grassy-beany flavors and a two-fold decrease in the intensity of the bitter and astringent flavors (55). A process patent incorporates both hexane-ethanol and aqueous alcohol extraction to prepare soy flour and concentrates with flavor qualities higher than those prepared by present commercial practices (63).

Flavor scores of legumes can also be greatly improved by steeping or wet-milling whole seeds with aqueous alcohol (64). Flavor scores of the legumes treated with 50% ethanol are given in Table XV. Less than 1% of the original lipoxygenase activity remains after soaking the beans in 30 to 80% alcohol. It would be of practical importance to determine whether the improvement of flavor scores can be attributed to the direct inactivation of lipoxygenase or to irreversible binding of objectional flavors with alcohol-denatured protein.

Soy protein concentrates prepared in the laboratory by a combination of aqueous alcohol plus live steam treatment have flavor scores of 8.0 compared to scores of about 6.1

TABLE XV

Flavor Scores of Raw Legumes Treated with 50% Ethanol (64)

Legume	Flavor score[a] Untreated	Flavor score[a] Treated
Lima beans *(Phaseolus limensis)*	5.0	6.8
Soybeans	3.8	6.7
Peanuts	4.8	6.3
Split peas *(Pisum sativum)*	4.8	6.3
Black-eyed peas *(Vigna sinensis)*	4.6	6.1

[a] See Table I.

for toasted defatted flakes (17). In commercial operations, flavor problems are encountered with soy protein concentrates prepared by aqueous alcohol extraction and by other processes (13). Heretofore, the incomplete removal of objectionable flavor components from the defatted soybean flakes source material was believed to be responsible for the lack of a bland flavor. However, a recent patent (65) reveals a malodorous fraction in rectified alcohol that, upon reuse in processing of unextracted defatted soy flakes, imparts objectionable flavors to subsequently manufactured protein concentrates. The malodorous fraction can be removed from rectified alcohol by withdrawing a side-stream from the column-rectifying zone having a temperature range of ca. 180 to 200 F. By this invention, a soy protein concentrate, which normally was rated as being beany, bitter, and sour by a trained taste panel, was described as being relatively bland with slight cooked cereal flavor notes. As discussed earlier, commercial aqueous alcohol extraction also removes the precursors responsible for the disagreeable cooked soybean odor (49).

FLAVOR OF PEANUT PROTEIN PRODUCTS

In most countries where peanuts (groundnuts) are grown, they are extracted, the oil is refined for food uses, and the meal is sold for animal feed. In the United States, peanuts are used primarily in peanut butter, candy, confections, and salted-roasted nuts. Flavor problems that may arise in these applications are beyond the scope of this review. Here our main interest is in defatted peanut flours, since these flours contain significant amounts of residual oil and phospholipids that can give rise to oxidative deterioration.

Raw peanuts contain active enzymes that can adversely affect flavor. In roasted peanuts, metalloproteins can initiate the formation of off-flavors derived from lipid oxidation (66). Raw peanuts have a grassy flavor that can be eliminated by steaming; however, depending upon time and temperature, a bitter taste will develop (67). Aqueous alcohol treatment accelerates the destruction of the grassy flavor and results in the formation of a sweet taste, but no bitter flavors were detected in alcohol-treated peanuts (67).

Because of high residual lipid levels, oxidative deterioration reactions can lead to the production of objectionable off-flavors in some peanut flours (68). Edible grade defatted peanut flour and grits produced by a prepress, solvent extraction method, with a fat content of 1.5%, have been characterized as having a bland flavor (69). Cereal and snack foods fortified with 17% defatted peanut flour appear to have no adverse effect on organoleptic acceptability (70). A peanut-fortified corn-base cereal received a hedonic score of 6.1, which compared favorably with that for a commercial breakfast corn product. Meat patties containing peanut flour were rated equal to or better than similar patties containing equivalent amounts of soy flour (70).

The effect of fortifying degermed cornmeal with defatted peanut flour has been evaluated with respect to nutrition, flavor, storage stability, and product application (71). Results are shown in Table XVI. The organoleptic characteristics were rated by 14 taste panelists. Mean flavor scores for the cornmeal-peanut flour blends (containing 15% peanut flour) range from 7.1 to 7.9 after storage for up to 12 months at 25 to 49 C. A control sample stored at -18 C had a score of 8.0. Comparable blends containing 15% defatted soy flour had scores of 6.6 to 7.8, with the control sample having a score of 8.0. Results of this study were in close agreement with similar tests with blends of degermed cornmeal containing varying levels of toasted, defatted soy flour (70). There appears to be little or no correlation between flavor scores of corn meal-soy flour or peanut flour blends with respect to peroxide value and free fatty acid content (71-73).

FLAVOR ASPECTS OF CEREAL PRODUCTS

Flavor and flavor stability present no problem with wheat flour, a wheat endosperm product. Wheat flour is commonly used in taste panel tests as a standard for a bland flavor. Degermed corn flour or meal, an endosperm product produced by dry-milling, usually has a mild flavor particularly desirable in snack foods and breakfast cereals. Most good quality whole cereal grains present little or no flavor problems, even though a suprisingly large number of volatile compounds are present (42).

Meal made from whole corn has a short shelf life because of the development of rancidity. Usually, this rancidity problem in whole grain can be readily controlled by proper inactivation of lipid oxidative enzymes. Both in the wet- and dry-milling industries, germ-rich streams are obtained that are a source of food oil. The upgrading of the oil-extracted residue from its use as an animal feed into a food-grade defatted corn germ flour has been commercialized (74,75).

There are no published taste panel data on corn germ flour produced from dry-mill corn germ. However, conditions of processing can have a large effect on the production of corn germ flour from wet-mill corn germ (76). Flour made from heat-dried germ had the flavor characteristics of a "good" soy flour; that is, weakly cereal-grain, bitter, and astringent. Flour made from air-dried wet-milled germ was judged to taste like raw soy flour with flavors similar to those given in Table II. Rancid painty flavors were also noted. Hexane-ethanol azeotrope extraction greatly im-

TABLE XVI

Flavor Scores of Stored Degermed Corn Meal Fortified with 15% Defatted Toasted Peanut or Soy Flour (71)[a]

	Temperature (months)[b]						
	-18 C	49 C		37 C		25 C	
Cereal-oilseed blend	(0)	(1)	(2)	(3)	(6)	(6)	(12)
Peanut flour	8.0	7.5	7.1	7.9	7.3	7.6	7.7
Soy flour	8.0	7.5	6.6	7.8	7.3	7.6	7.8

[a] Quality rating scale where 10 is excellent; 8, good; 6-7, less desirable; 4-5, objectionable.

[b] Least significant difference (5% confidence level) for comparison of scores is about 0.5.

TABLE XVII

Organoleptic Evaluation of Corn Germ Protein Isolates (78)

Sample	Odor			Flavor		
	Score[a]	Description	Intensity value[b]	Score[a]	Description	Intensity value[b]
Corn germ protein isolate, raw, untreated	6.5	Musty-stale Cereal-grain	0.3 0.5	4.4	Bitter Astringent Grassy-beany Musty-stale Cereal-grain	1.1 1.0 0.9 0.4 0.7
Corn germ protein isolate washed with 80% ethanol	7.3	Cereal-grain	0.3	5.8	Bitter Grassy-beany	0.5 0.5
Sodium caseinate	8.7	None predominant		8.1	Bitter Astringent Musty-stale Cereal-grain	0.3 0.1 0.2 0.1

[a] See Table I for scoring scale.

[b] Odor and flavors noted are panel averages based on a scale of 0 as absent, 1 as weak, 2 as moderate, and 3 as strong. A 0.3 unit difference is significant.

proved the flavor qualities because of the removal of bound lipid. Corn germ protein isolates prepared from hexane-defatted dry-milled corn germ contain little free lipid (0.35%) and 7-11% bound lipid (77,78). The bound lipid is most likely responsible for the undesirable organoleptic qualities of the isolate and for the development of oxidative rancidity that occurs during storage.

Comparative flavor scores of corn germ protein isolate and those further extracted with aqueous alcohol are summarized in Table XVII. The odor and flavor descriptions noted by the panelists were typical of soy protein products (13,55). Raw corn germ protein isolate is about the same as soy protein isolates prepared from raw defatted soy flakes. Flavor scores of alcohol-washed corn isolate were within the range of many commercial soy protein products, but they were considerably below a flavor score of 7.3 for soy isolates prepared from azeotrope-extracted soy flakes (55).

DISCUSSION

Model systems have been used to establish that residual lipids are indeed the precursors to the basic odors and flavors that limit the use of protein products prepared from food legumes, soybeans, peanuts, and corn germ isolates. Knowledge on the chemical and biochemical deteriorations of these lipids helps us to understand how off-flavors develop in these products. To develop even more effective methods to remove these lipids-derived flavor constituents or to prevent their formation, further knowledge is needed on the interaction of the degraded lipids with meal constituents such as protein and carbohydrates and on the off-flavors generated by this interaction. Basic to the importance of such knowledge is the finding that hydrogen bond-breaking lipid solvents are effective in removing protein-bound lipids that are responsible to a large extent for the bitter, astringent, and rancid flavors of protein products prepared from raw soybean meal and wet-milled corn germ flour.

Flavor scores of hexane/ethanol azeotrope-extracted soybean flakes and proteinates prepared from these extracted flakes are significantly higher than those prepared by present commercial practices. Even though much of the basic grassy-beany and bitter flavors of soy can be greatly diminished, under laboratory conditions, by a combination of solvent extraction and toasting, more research is needed to improve this extraction process. Flavor qualities of products manufactured on an industrial scale, utilizing azeotrope extraction, remain to be determined.

In commercial practices the creation of flavor notes such as musty-stale, cereal grain, and other nondescriptive odors and flavors can now become flavor problems. These additional flavor responses may not be lipid-derived. Stepwise regression analysis of the products at each processing step may be needed to establish the conditions by which they are formed. Perhaps only minor changes in such physical parameters as temperature, pressure, and pH may be all that is needed to eliminate these off-flavors.

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