Effect of Processing and Storage Conditions on Lipid Composition of Soy Products[1]

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ABSTRACT

Of the original lipids in soybeans, about 12% remained in defatted flakes and 2.5% in isolated protein produced under commercial conditions. At these levels, residual lipids likely contribute directly to soy flavor. The majority of the lipids were triglycerides; but free fatty acids were found in relatively high concentrations, especially in the defatted flakes. Fatty acid analysis by gas-liquid chromatography demonstrated that with increased processing the level of unsaturated fatty acids was reduced. Likewise, prolonged room-temperature storage resulted in decreased unsaturated fatty acid levels.

Lipid degradation by both enzymatic and autoxidative means has been associated with the development of off-flavors in numerous food products. Wilkens et al. (1) investigated the off-flavors associated with soybean milk and concluded that they are directly attributable to oxidation of polyunsaturated lipids by lipoxygenase action. In a similar study, Hand (2) found 40% less polyunsaturated fatty acids in soybean milk prepared by cold- than by hot-extraction procedures. Fat-splitting enzymes play an important role in producing bitterness in stored soybeans (3). Mattick and Hand (4) reported that ethyl vinyl ketone, which has a green-bean-like flavor, is formed in soy milk prepared by cold-water extraction. Arai and co-workers (5) isolated a volatile fatty acid fraction from raw soybeans which had a weak but characteristic odor. Sessa et al. (6) concluded that some lipid degradation occurred in the preparation of defatted flakes and that characteristic soybean flavor is closely associated with certain lipids. Fujimaki and co-workers (7) also believe that soy-flavor problems are associated with lipid-decomposition products. They stated that, during processing, chemical and physical factors such as decomposition of natural antioxidants; higher content of metal catalysts; and increased surface area, atmospheric oxygen, and humidity can accelerate lipid degradation. Honig et al. (8) fractionated and tasted milligram amounts of the free and bound lipids associated with defatted flakes and concluded that the quantity of volatile lipid-degradation products and their associated flavors was small. However, they did postulate that nonvolatile lipid fractions could contribute significantly to flavor.

We followed changes in lipid composition of soy products as affected by processing and storage.

MATERIALS AND METHODS

Sample Procurement

Defatted soy flakes and a protein isolate (Promine D, Central Soya Co.,

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Chicago) were prepared commercially from a given lot of raw soybeans. Upon receipt, a portion of each sample was placed in 4°C. storage. Another series of samples was stored at 22°C. for 12 months. After 6 months, half of the samples stored at 4°C. were placed in 22°C. storage for 6 more months, and half left at 4°C. Before being evaluated, all samples were frozen. Both raw soybeans and defatted flakes, while frozen, were ground through a Wiley mill equipped with a 30-mesh screen. The soy-protein isolate was evaluated without further grinding.

Free and Free-Plus-Bound Lipid Extraction and Resolution

Free lipids are defined as those soluble in diethyl ether; free-plus-bound lipids, as those extractable in 79 parts hexane and 21 parts ethanol (8). Varying amounts of soy products were extracted as lipid material present varied. In all cases, a 5:1 solvent-to-product ratio was maintained. Extractions were performed at room temperature by mixing for 5 min. at high speed in an explosion-proof blender and using compressed nitrogen gas to flush the mixing bowl. The solution was vacuum-filtered until the residue was dry. This filtration was aided by flushing nitrogen gas over the filtration flask. The lipid-solvent mixture was evaporated to a syrup at 40°C. with a rotary vacuum evaporator. The residues were made up to 5 ml. with redistilled chloroform, sealed in vials, and refrigerated until used.

Thin-layer chromatography (TLC) plates were prepared with Silica Gel G, 250 μ thick. The plates were air-dried for 2 hr. and activated before use by heating at 130°C. for 1 hr. The polar solvent system was composed of chloroform:methanol:water (65:24:4, v./v./v.);the nonpolar system, of petroleum ether:diethyl ether:acetic acid (90:10:1, v./v./v.) (8). A 50% sulfuric acid spray was used for detection of components. The plates were sprayed and then charred for 5 min. at 150°C. Lipid classes were tentatively identified by relative R_f values for known lipid compounds separated under the same conditions. Relative proportions of lipid classes separated by TLC were measured by a Photovolt Model 52C densitometer attached to a Varicord Model 42B variable response recorder. Free and free-plus-bound lipid fractions were quantified by weighing the solvent-free lipid extracts from a known weight of soy material.

Preparation of Methyl Esters

The lipid material extracted from 25 g. of soy product with 125 ml. of 79 parts redistilled hexane and 21 parts redistilled absolute ethanol was used to prepare methyl esters. A 0.5-ml. portion of the solvent-free lipid extract, along with 100 ml. of 1% sulfuric acid in redistilled absolute methanol and a boiling chip, was placed in a 250-ml. round-bottom flask equipped with a water condenser. The mixture was refluxed 2 hr., cooled, and diluted with an equal volume of cold distilled water. The mixture was saturated with sodium chloride before extraction of the methyl esters with three 10-ml. portions of redistilled petroleum ether. The petroleum-ether extracts were combined and saturated with anhydrous sodium sulfate.

Gas-Liquid Chromatography Conditions

A Hewlett-Packard Model 5750 dual-column flame detector unit was used. It was equipped with a stainless-steel column (8 ft. × 1/8 in. O.D.) packed with 60/80 mesh Chromosorb G AW-DMCS, which was coated with 7.5% HI-EFF 1 BP (diethylene glycol succinate). Nitrogen at a flow rate of approximately 40 ml. per

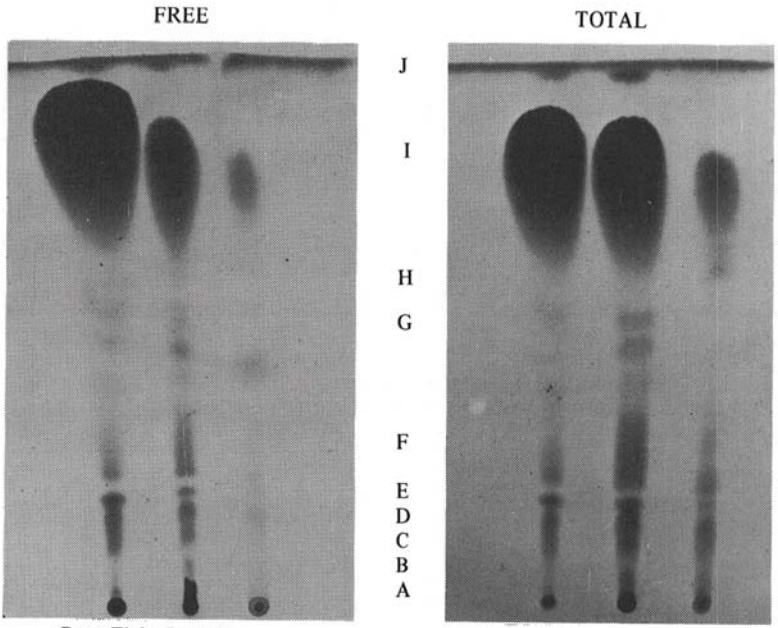
TABLE I. LIPIDS EXTRACTED WITH VARIOUS SOLVENTS FROM INDICATED SOY PRODUCTS

	Bean %	Flake %	Protein %
Free lipid (diethyl ether)	24.28	2.34	0.57
Free-plus-bound lipid (79 parts hexane, 21 parts ethanol)	25.41	2.98	0.63
AACC (petroleum ether)[a]	19.3	0.81	Trace
Bound lipids by difference (free-plus-bound less free lipid)[b]	4.45	21.47	9.52

[a]AACC method 30-26 (9).

[b]Expressed as percent of total (free-plus-bound) lipid.

min. was used as a carrier. The temperatures of the system were as follows: column, 185°C., isothermal; detector, 270°C.; and injector, 235°C. Specific fatty acids were tentatively identified using relative retention times of fatty acid methyl esters of increasing chain length, and comparative retention times of commercially prepared fatty acid methyl esters separated under the same chromatographic conditions.



Bean-Flake Protein

A. Polar Lipids
B. Unknown
C. Monoglycerides
D. Diglycerides
E. Free Sterols

Bean-Flake Protein

F. Free Fatty Acids
G. Unknown
H. Unknown
I. Triglycerides
J. Hydrocarbons

Fig. 1. Thin-layer chromatograph plate showing the nonpolar and total lipid distribution in soy products.

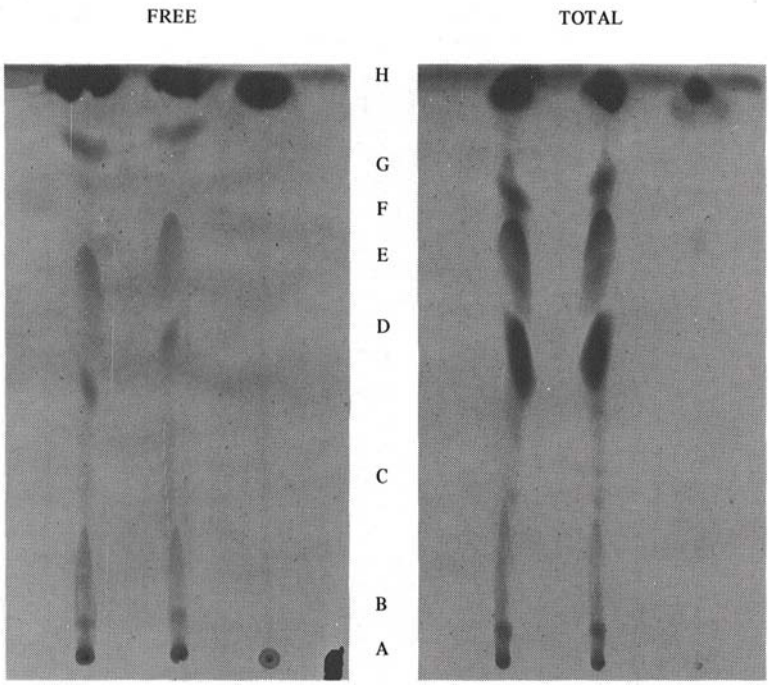


Fig. 2. Thin-layer chromatograph plates showing polar and total lipid distribution of soy products.

TABLE II. RELATIVE PROPORTIONS OF NONPOLAR FRACTIONS IN FREE AND FREE-PLUS-BOUND LIPIDS FOR INDICATED SOY PRODUCTS

Lipid Fraction	Free			Free-Plus-Bound		
	Bean %	Flake %	Protein %	Bean %	Flake %	Protein %
Polar lipids	2	2	1	2	3	1
Unknown	Trace	Trace	Trace	Trace	1	1
Monoglycerides	1	1	Trace	1	1	2
Diglycerides	1	1	2	1	1	2
Free sterols	1	1	Trace	2	2	1
Free fatty acids	1	2	Trace	1	5	2
Unknown	Trace	Trace	5	Trace	2	Trace
Unknown	Trace	Trace	Trace	Trace	2	Trace
Triglycerides	93	92	89	91	80	90
Hydrocarbons	1	1	3	2	3	1
	100	100	100	100	100	100

TABLE III. RELATIVE PROPORTIONS OF POLAR FRACTIONS IN FREE AND FREE-PLUS-BOUND LIPIDS FOR INDICATED SOY PRODUCTS

Fraction	Free			Free-Plus-Bound		
	Bean %	Flake %	Protein %	Bean %	Flake %	Protein %
Amino acids	2	2	1	2	2	1
Carbohydrates	1	1	Trace	2	2	Trace
Unknown	Trace	Trace	Trace	1	1	Trace
Lecithin	1	1	Trace	3	3	1
Cephalin	2	2	Trace	1	1	Trace
Steryl glucoside	1	1	Trace	2	2	Trace
Acylated steryl glucoside	1	1	Trace	1	1	Trace
Nonpolar lipids	92	92	99	88	88	98
	100	100	100	100	100	100

TABLE IV. EFFECT OF STORAGE ON MAJOR FATTY ACID COMPOSITION OF FREE-PLUS-BOUND LIPIDS IN INDICATED SOY PRODUCTS[a]

Fatty Acid	1[b]			2[c]			3[d]		
	B[e]	F[f]	P[g]	B	F	P	B	F	P
Palmitic	10.0	10.6	13.9	14.3	14.4	14.8	11.2	12.1	14.6
Stearic	7.4	8.8	11.6	9.4	10.3	13.3	6.4	11.9	14.1
Oleic	25.0	23.1	17.0	19.8	22.9	24.4	24.2	24.7	26.0
Linoleic	37.0	39.7	41.9	42.4	39.5	35.0	46.0	40.0	34.5
Linolenic	20.6	17.8	15.6	14.1	12.9	12.5	12.2	11.3	10.8

[a] % of total peak area.

[b] 1 = 12 months at 4°C.

[c] 2 = 6 months at 4°C. and 6 months at 22°C.

[d] 3 = 12 months at 22°C.

[e] B = Bean.

[f] F = Flake.

[g] P = Protein.

RESULTS

Percentages of free, free-plus-bound, and petroleum-ether-extractable lipids of raw soybeans, defatted flakes, and isolated soy proteinate are presented in Table I. Makeup of the free and free-plus-bound lipid fractions was observed with nonpolar and polar TLC. Nonpolar separation of the free and free-plus-bound lipids is shown in Fig. 1, and the polar separation of free and free-plus-bound lipids in Fig. 2.

Relative proportions of the lipid classes separated by TLC and measured photodensiometrically for nonpolar and polar lipids are presented in Tables II and III.

The three basic soy products were evaluated for storage changes in fatty acid composition of free-plus-bound lipids by determining relative proportions of methyl esters by GLC. These data are presented in Table IV. The data in Table IV are condensed to represent total saturated and total unsaturated fatty acids, and plotted in Fig. 3. All analytical values are the average of duplicate determinations.

DISCUSSION

The efficiency of polar-solvent extraction in removing residual lipid material is evident from Table I. From a flavor standpoint, products extracted by the polar-solvent procedure were judged to be less "bitter" and "beany" than the other solvent-extracted products. It is also evident that bound-lipid concentration increased in the soy flakes and was higher in the protein isolate than in the raw soybean.

As reported by Honig et al. (8), the major lipid fraction was triglycerides. This was true of all three soy products evaluated. Little difference in composition appeared among free and free-plus-bound, polar and nonpolar, lipids of the three products.

Photodensiometric determinations of the nonpolar fractions revealed a higher proportion of free fatty acids in the free-plus-bound lipid fraction of defatted flakes. This would indicate that free fatty acids form while defatted flakes are being produced from raw soybeans, or that this increase is the result of selective extraction of triglycerides as compared to the fatty acids during commercial defatting. The apparent decrease in free fatty acids in the free and free-plus-bound lipid fractions of the isolated soy proteinate suggested that these substances were removed during further processing.

Decreasing proportions of several polar fractions were noted. Perhaps they

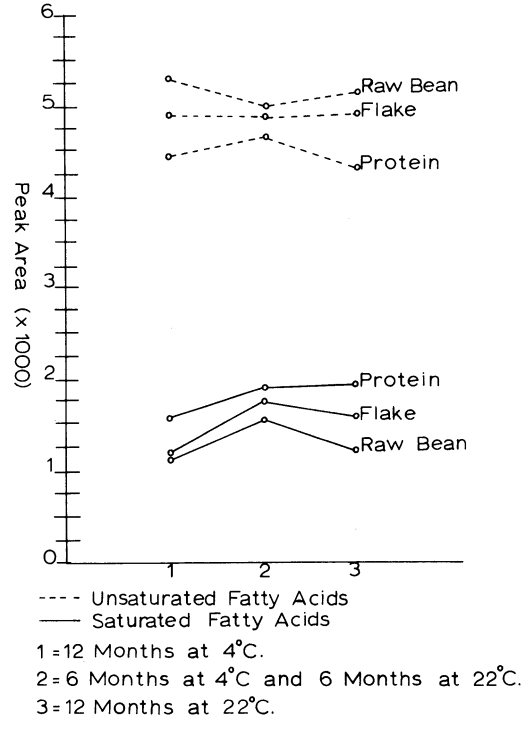


Fig. 3. Data as in Table IV.

reacted in processing to form additional or new flavor compounds in the isolated soy proteinate, or they were partially removed in further processing.

Honig et al. (8) concluded that residual lipid had little effect on soy flavor since purified lipid fractions have little flavor. However, considering possible synergistic effects of lipid classes and impurities, and that approximately 12% of the original lipid remained with the defatted flakes and approximately 2.5% in the isolated soy proteinate, it seems likely that residual lipids contribute directly to soy flavor.

As expected, linoleic acid was the predominant fatty acid in all soy products (Table IV). However, the linolenic acid level found in this study was higher than published values for typical soybean oil (8). The value in this study would be more typical of immature soybeans (10). Other possible explanations for these results include the fact that the samples were freshly prepared from a new crop of soybeans, thus little storage time initially elapsed before sampling was begun. Also, the method of grinding employed (in the frozen state) and the short time interval between grinding and solvent extraction could have minimized lipid degradation, thus giving a higher proportion of linolenic acid.

With processing, the total unsaturated fatty acid content decreased. Sessa et al. (6) reported that lipid degradation occurred during preparation of defatted flakes. This study demonstrates further lipid degradation occurring in processing isolated soy proteinate from defatted flakes. Figure 3 also clearly demonstrated that lipid decomposition of unsaturated fatty acids continues with storage, particularly during the first six months.

The work presented demonstrates that lipid changes occur throughout processing and storage of soy products. These changes can result in the formation and accumulation of products which can further contribute directly to soy flavor.

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