

Lipid Oxidation in Full-Fat and Defatted Soybean Flakes as Related to Soybean Flavor¹

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ABSTRACT

Extracting 99.8% of the oil from full-fat soybean flakes with pentane-hexane removed none of their green-beany, bitter flavor. This oil, with paraffinlike, vegetable-oil flavor, did not develop any further flavor on storage. Almost all the flavor and residual lipids from defatted soybean flakes were extracted by hexane-absolute ethanol azeotrope (79:21). The oil and azeotrope extracts had thiobarbituric acid (TBA) numbers of 5.8 and 34, respectively, which were computed from absorbance readings at 532 μ . The oil and azeotrope extracts contributed about 10% to the TBA number of full-fat flakes. In both full-fat and defatted flakes, n-hexanal, acetaldehyde, and acetone represented the major volatile carbonyl compounds. These were characterized as 2,4-dinitrophenylhydrazones. No malonaldehyde derivative was obtained from the flakes. From defatted flakes, 3.6 p.p.m. carbonyl compounds were vacuum-stripped, but removal of this amount did not affect the original soybean flavor. Consequently, flavor of flakes could not be correlated with degree of oxidation as measured by TBA and volatile carbonyl compound analyses. Trace amounts of hydroperoxides appeared in lipid extracts from defatted flakes. When a soybean lipid extract, possessing similar composition to commercial soybean phosphatides, was UV-irradiated, the TBA number decreased from 67 to 33 and the flavor changed from that of paraffin and vegetable oil to that associated with rubber. Similar irradiation of commercial phosphatides generated TBA-reactive substances and bitter rancid flavors not characteristic of soybeans.

Soy flour products have been available in the United States for about 30 years. During this time, defatted soy flour, edible grade, has been greatly improved in nutritive value, physical characteristics, and functionality. Even though there has been extensive research on soy flavor and many patents for improving or reducing flavor have been issued, this factor still affects the acceptance and in many ways regulates the use of soy products in foods. To study aspects of this factor, Teeter and co-workers (1) investigated the carbonyl compounds in steam distillates of soybean meal extracts. Fujimaki et al. (2) and Janicek and Hrdlicka (3) identified some volatile carbonyl compounds in whole soybeans and in defatted soy flour. All these workers concluded that carbonyl compounds may contribute to the distinctive flavor of soybeans. Honig et al. (4) showed that almost all the flavor was removed with the residual lipids from defatted soybean flakes.

Since oxidative degradation of lipids, during either storage or processing, is a common cause of objectionable flavors in foods, we investigated the extent of lipid oxidation in full-fat and defatted soybean flakes and correlated lipid oxidation, by chemical assay and taste tests, with the flavor of soybeans.

MATERIALS AND METHODS

Preparation of Soybean Flakes

Certified, seed-grade, Amsoy soybeans (1965 crop) were used to prepare full-fat and defatted flakes. Soybeans of 8 to 10% moisture were cracked into six to eight

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parts, dehulled in a Eureka seed cleaner, tempered to 12% moisture, and flaked through smooth rolls set to produce flakes about 0.008 in. thick. The flakes were defatted by extraction with redistilled pentane-hexane (Skellysolve F, b.p. 33° to 57°C.) at room temperature. A Soxhlet apparatus was used in which the solvent from a separate distilling flask was condensed and then allowed to flow through the full-fat flakes. The miscella was siphoned back into the distilling flask, and the process was maintained for 6 hr. Residual pentane-hexane in the extracted flakes was evaporated at room temperature into the air current of a laboratory hood for 16 to 24 hr. The defatted flakes contained 9 to 10% moisture and 0.16% residual oil as determined by AOCS Official Methods BC2-49 and BC3-49 (5), respectively. Both toasted full-fat and defatted flakes were prepared by steaming for 30 min. at 100°C. and atmospheric pressure in a preheated autoclave.

Extraction of Lipids from Defatted Flakes

Defatted flakes (110 g., 9 to 10% moisture, and 0.16% residual oil) were mixed with absolute ethanol (110 ml.) and extracted four times in 1 hr. with peroxide-free, anhydrous diethyl ether (about 130 ml. each pass) in a Soxhlet extractor; the residual flakes were then re-extracted for 6 hr. with an azeotropic mixture of hexane-absolute ethanol (79:21). Lipids in the ether and azeotrope extracts amounted to 1% for each. Defatted flakes were also extracted directly in a Soxhlet apparatus with the hexane-absolute ethanol azeotropic mixture to give the lipid in 3% yield. The lipids that are extracted with diethyl ether and the azeotropic mixture of hexane-absolute ethanol represent a combination of residual oil as determined by the AOCS Official Method BC3-49 (5) and difficultly extractable phosphatides that require solvents more polar than pentane-hexane to effect their extraction (4).

Thiobarbituric Acid Assay

The distillation method of Tarladgis et al. (6), as modified by Fhee and Watts (7), was used to determine the extent of lipid oxidation in soybean flakes and in various lipid extracts by measuring absorbance of the thiobarbituric acid (TBA) reaction product at 532 μ . Absorbance values for the meal distillates reacted with TBA were converted to mg. malonaldehyde per 1,000 g. of sample. These values are reported as "TBA number." The results were expressed in terms of malonaldehyde to facilitate the calculation of the distribution of TBA-reactive substances in flakes and extracts of these flakes. Defatted flakes or lipid fractions (1 to 4 g.) were blended with 50 ml. distilled water for 2 min. at full speed in a Virtis "45" homogenizer. This slurry was quantitatively transferred into a Kjeldahl flask by washing with an additional 45 ml. distilled water, and acidified to pH 1.1 to 1.2 by addition of 5 ml. hydrochloric acid (1:2). Boiling chips and 0.5 ml. of Dow Corning Antifoam AF were added to prevent bumping and foaming. This mixture was then distilled until 50 ml. of distillate was collected, in 13 to 14 min. An aliquot of the distillate (5 ml.) was reacted with TBA for 35 min. and analyzed at 532 μ on a Beckman DU spectrophotometer. For TBA analysis of full-fat flakes, dilute acid was added before blending to inactivate lipid-oxidizing enzymes (7).

A standard curve was made for malonaldehyde prepared from 1,1,3,3-tetraethoxypropane (TEP) (K & K Laboratories, Inc., Plainview, N. Y.). Dilutions from a fresh stock solution of TEP were acidified to pH 1.2 before reaction with TBA. This standard curve served to determine the recovery of

malonaldehyde (69 to 72%) in the 13- to 14-min. distillates from hydrolyses of TEP. Assuming the recovery values for malonaldehyde from TEP- and TBA-reactive substances from soybeans to be the same, malonaldehyde recovery values were used to plot the corrected standard curve shown in Fig. 1. To determine whether constituents in the flakes will bind malonaldehyde generated from TEP during blending or distillation, measured amounts of TEP were blended with raw, defatted flakes and the homogenate was then treated according to the TBA procedure. All the expected amount of malonaldehyde formed from TEP was recovered in the distillate.

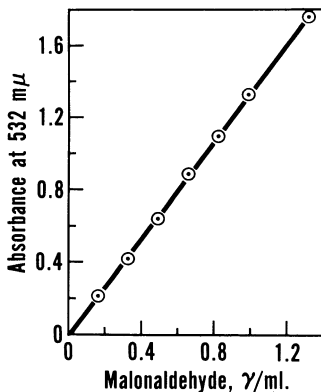


Fig. 1. Relation between absorbance and concentration of malonaldehyde corrected for distillation losses.

Separation and Analysis of Carbonyl Compounds from Soybean Flakes

To obtain carbonyl compounds from full-fat flakes, 4-g. samples were blended, acidified, and distilled, according to the procedure used for TBA analysis (6), directly into 50 ml. of 0.2% 2,4-dinitrophenylhydrazine (2,4-DNPH) in 2N hydrochloric acid. The solution was cooled in a refrigerator, and the precipitate was recovered by filtration. The distillation and collection procedures were repeated on fresh samples until a sufficient amount of precipitate was collected for further analyses. The composite precipitate (3 mg.), dissolved in 0.3 ml. of chloroform, was chromatographed on a magnesia-Celite (1:1) column according to the procedure of Schwartz et al. (8). Magnesia 2665, with an iodine number of 18.2, and Celite 545 (Fischer Scientific Co., Fair Lawn, N. J.) were used. After elution with chloroform, chloroform-methanol (1:1), methanol, and formamide were used successively to elute derivatives more tightly bound. Effluents were collected in 8-ml. fractions and analyzed at 340 $m\mu$. Fractions that comprised a peak were pooled, desolventized, and dried on a rotary evaporator at 40°C. The fraction that eluted with formamide was extracted into chloroform and then dried.

Volatile carbonyl compounds were obtained from defatted soybean flakes by the following procedure: About 500 g. of flakes were swept with nitrogen gas and vacuum-stripped for 8 hr. at 60°C. and 75 mm. of mercury. Volatile components were condensed in a series of traps. The first two were cooled with salt-ice and the third with solid carbon dioxide-ethanol. Trap 1 condensed almost all the water vapor; in trap 2, containing 0.2% 2,4-DNPH in 2N hydrochloric acid, volatile

carbonyl compounds were converted to their respective 2,4-DNPH derivatives; and trap 3 condensed those compounds (including carbonyls) passing through the previous traps. The 2,4-DNPH solution was then added to trap 3. The 2,4-DNPH derivatives from traps 2 and 3 were combined, collected on tared sintered-glass funnels, washed with 2N hydrochloric acid, dried in a desiccator, and weighed.

The carbonyl constituents of full-fat and defatted flakes, as 2,4-DNPH derivatives, were identified and characterized by chromatography and spectral analysis. The 2,4-DNPH derivatives of the steam-distillable carbonyl compounds from full-fat flakes were fractionated on magnesia-Celite columns and analyzed by descending paper chromatography on Schleicher and Schuell 589 Blue Ribbon paper with N,N-dimethylformamide-saturated decahydronaphthalene as the developing solvent. The chromatograms were air-dried and observed under visible and ultraviolet light (366 m μ) before being sprayed with 10% alcoholic potassium hydroxide. Spectral analyses of the column fractions in chloroform and in ethanolic sodium hydroxide were made according to the procedure of Jones et al. (9). Authentic 2,4-DNPH derivatives of various carbonyl compounds were used as references.

The 2,4-DNPH derivatives of volatile carbonyl compounds of defatted flakes were chromatographed on Silica Gel G thin-layer plates prepared by the method of Stahl (10). The chromatograms were developed with benzene-petroleum ether (75:25) and sprayed with 10% alcoholic potassium hydroxide. To quantitate the 2,4-DNPH derivatives, the untreated chromatographed samples were scraped from the plate, eluted with chloroform, centrifuged, and analyzed with a Beckman DK2A spectrophotometer. Corrections for experimental losses were based on results from similar treatments of known 2,4-DNPH derivatives.

Analysis of Lipid Extracts for Hydroperoxides

Oil-free soybean phosphatides (Central Soya Co., Inc., Chicago, Ill.) and ether and azeotrope extracts of defatted soybean flakes were analyzed for hydroperoxide by the thin-layer chromatographic technique of Oette (11). The oil-free soybean phosphatides and solvent extracts of defatted flakes were spotted on two thin-layer plates. One plate was subjected to ultraviolet irradiation at 12 cm. from a source of ultraviolet light (254 m μ) as detailed in the next section. Chloroform-methanol-water (75:25:4.2) was the developing solvent. Hydroperoxides were detected by spraying the developed chromatograms with 5% potassium iodide solution followed by 1% starch in 1% acetic acid.

Flavor Evaluation and TBA Analysis of UV-Irradiated Lipid Extracts

A commercial sample of oil-free soybean phosphatides was irradiated by ultraviolet light to initiate oxidation. A high-intensity, short-wave-length (254 m μ) ultraviolet light source (Mineralight model R-51, Ultraviolet Products, Inc., San Gabriel, Calif.) was placed at distances of 4 and 12 cm. above the sample which was irradiated for 1 to 6 hr. The flavors of unirradiated and irradiated samples were evaluated organoleptically by us. TBA-reactive substances formed during irradiation were determined by the TBA procedure. A diethyl ether extract of defatted soybean flakes was irradiated and analyzed similarly.

RESULTS AND DISCUSSION

Identification of Carbonyl Compounds in Soybean Flakes

The major carbonyl compounds steam-distilled from raw, full-fat soybean flakes are shown in Table I. Identified as their 2,4-DNPH derivatives were: acetone, n-hexanal, acetaldehyde, and 2-heptenal. Identification of column fraction 5 as a dicarbonyl compound was based on the blue color produced by treatment of its 2,4-DNPH derivative with 10% alcoholic potassium hydroxide and the absorption spectrum of this derivative (12). A dicarbonyl compound whose 2,4-DNPH derivative possessed similar properties was isolated as a volatile cleavage product of autoxidized methyl linolenate and was postulated to be hexene-3-dial-1,6 (13).

A bis(2,4-DNPH) derivative did not form when acidified TEP reacted with 2,4-DNPH. To prepare and identify the product of the reaction of malonaldehyde with 2,4-DNPH, an aqueous solution of TEP was acidified to pH 1.2 and distilled directly into 0.2% 2,4-DNPH in 2N hydrochloric acid. The precipitate was collected and the mixture was separated on magnesia-Celite columns (9). Effluents were monitored at 340 $m\mu$. The major peak was eluted with 15% chloroform in redistilled n-hexane; a second peak was eluted with chloroform. The component of the second peak gave R_f 0.75 on paper chromatography, turned red-brown with 10% alcoholic potassium hydroxide spray, and showed maximum absorbance at 355 $m\mu$ (CHCl_3) which shifted to 434 $m\mu$ in ethanolic sodium hydroxide. Effluents of the major peak were pooled, and evaporation of the solvent produced pale-yellow crystals, m.p. 106° to 107°C.; their maximum absorbance at 310 $m\mu$ (CHCl_3) shifted to 296 $m\mu$ in ethanolic sodium hydroxide. From the results of infrared and nuclear magnetic resonance spectroscopy, the crystalline material is identified as 2,4-dinitrophenylpyrazole. Kwon (14) suggested that malonaldehyde, when reacted with 2,4-DNPH, might form a pyrazole, but he was not successful in isolating and characterizing the reaction products. When concentrations of TEP were used to generate a TBA reaction product equivalent to that obtained from full-fat soybean flakes, we were unable to detect any 2,4-dinitrophenylpyrazole by our present method. These results indicate that our present method for detecting the 2,4-dinitrophenylpyrazole may not be sensitive enough to determine malonaldehyde at the low concentration existing in the soybean flakes.

TABLE I. CHARACTERISTICS OF MAJOR 2,4-DNPH^a DERIVATIVES FROM DISTILLATE OF FULL-FAT SOYBEAN FLAKES

Column Fraction ^b	R_f^c	Color with KOH	$\lambda_{\text{max.}}$		Identification as 2,4-DNPH Derivative
			CHCl_3	EtOH + NaOH	
			$m\mu$	$m\mu$	
1	0.72	Gray-brown	362	431	Acetone
2	0.87	Brown	358	432	n-Hexanal
3	0.58	Brown	355	434	Acetaldehyde
4	0.85	Brown	375	455	2-Heptenal
5	0.05	Blue	404, 440	562	A dicarbonyl compound

^a2,4-Dinitrophenylhydrazine.

^bColumn chromatography on magnesia-Celite.

^cDescending paper chromatography with decahydronaphthalene saturated with N,N-dimethylformamide.

TABLE II. CONTENT OF VOLATILE CARBONYL COMPOUNDS FROM DEFATTED SOYBEAN FLAKES

2,4-DNPH Derivative	λ_{max}	Content in Soybean Flakes ^a
	m μ	p.p.m.
n-Hexanal	356	3.2
Acetone	362	0.25
Acetaldehyde	354	0.11

^aVacuum distillation, 60°C., 8 hr., and 75 mm. Hg.

As shown in Table II, defatted flakes contained three of the five carbonyl components of full-fat flakes. These carbonyl compounds, arranged in order of decreasing concentration, are n-hexanal, acetone, and acetaldehyde. Both raw full-fat and defatted soybean flakes have similar flavor characteristics in spite of differences in the number and kind of their carbonyl constituents. Removal of the carbonyl compounds from defatted flakes by vacuum stripping (see "Materials and Methods") did not affect the original flavor. Taste tests suggested that the volatile carbonyl compounds at the levels stripped from the flakes do not contribute significantly to the distinctive soybean flavor. Except for the dicarbonyl component, the major carbonyl compounds identified in this study correspond to those reported by Fujimaki et al. (2). Our unidentified 2,4-DNPH derivatives are probably the minor compounds which were reported by Fujimaki et al. (2): methanal, 2-pentanone, 2-hexanone, and 2,4-decadienal.

Lipid Oxidation in Soybean Flakes and Extracts as Measured by the TBA Assay

Results of TBA assay of full-fat and defatted flakes and of defatted flakes after extraction of residual lipids, all three raw and toasted, are shown in Table III. Raw,

TABLE III. EFFECT OF ACID AND TOASTING ON FORMATION OF TBA-REACTIVE SUBSTANCES IN RAW SOYBEAN FLAKES

Sample	TBA Number ^a at 532 m μ		
	None ^b	Acid ^b	Toasting ^b
Full-fat	83.8	10.5	8.0
Defatted	13.5	11.6	3.3
Defatted, ether-extracted	11.6	10.3	... ^c
Defatted, azeotrope-extracted	9.5	9.5	3.3

^aThiobarbituric acid number, expressed as mg. malonaldehyde per 1,000 g. of sample.

^bTreatment before blending for assay.

^cNot determined.

full-fat flakes, which contain 20% oil, gave a TBA number of 83.8 and have a strong rancid odor when blended without acid. This high TBA number and the development of a strong odor were attributed to the action of lipoxidase (7) on soybean oil in full-fat flakes. When blended with acid to inactivate the lipoxidase, raw, full-fat flakes have a TBA number of 10.5 corresponding to the other samples. All toasted samples have TBA values lower than 10.5.

There were no significant changes in TBA numbers of the various soybean samples during storage. TBA numbers of raw, full-fat flakes stored for 1 month at

5°C. did not change. TBA numbers of both raw defatted and azeotrope-extracted flakes did not change more than three units during storage for 7 months at room temperature or at 5°C.

To ascertain the relative distribution of TBA-reactive substances in various soybean fractions, full-fat flakes were processed into soybean oil, defatted flakes, and residual lipids. TBA numbers were determined for all samples, and then these numbers were converted into "TBA values" based on the yield of each fraction (Table IV).

TABLE IV. RELATIVE DISTRIBUTION OF TBA-REACTIVE SUBSTANCES (AS MALONALDEHYDE) IN SOYBEAN SAMPLES

Sample	TBA Number ^a	Yield	TBA Value ^b	Total Apparent Malonaldehyde
		%		%
Full-fat flakes	10.5	...	10.5	100
Defatted flakes	11.6	80	9.3	88.6
Oil	5.8	20	1.2	11.4
Defatted flakes	11.6	...	11.6	100
Defatted, ether-extracted flakes	10.3	99	10.2	87.9
Ether extract	66.9	1	0.7	6.0
Defatted, azeotrope-extracted flakes	9.5	97	9.2	79.3
Azeotrope extract	34.0	3	1.0	8.6

^aMalonaldehyde, mg. per 1,000 g. of sample, at 532 m μ .

^bMalonaldehyde, mg., corrected on basis of yield from 1,000 g. full-fat or defatted flakes.

Pentane-hexane extraction of 1,000 g. full-fat flakes gave approximately 800 g. defatted flakes and 200 g. crude soybean oil. On the basis of these yields, defatted flakes and oil have TBA values of 9.3 and 1.2, respectively. Defatted flakes, therefore, retain 88.6% of the TBA-reactive substances present in the original full-fat flakes. The sum of the TBA values for defatted flakes and oil equals the TBA value for raw, full-fat flakes, indicating good recovery of TBA-reactive substances.

In the same manner, TBA values of the ether and azeotrope extracts were compared with defatted flakes and the results were similar. The ether- and azeotrope-extracted flakes retained almost all the TBA-reactive substances of the original defatted flakes. On the basis of the sum of the TBA values for azeotrope-extracted flakes and azeotrope extract, nearly 88% of the TBA-reactive substances was recovered. Most likely, some loss occurred during solvent removal.

Such analyses of the distribution of TBA-reactive substances in various fractions of soybeans indicate that these substances are primarily associated with the nonlipid fractions, presumably bound to protein. Extent of lipid oxidation was greater for both the ether and azeotrope extracts than for soybean oil, as indicated by TBA numbers of 66.9 and 34.0 for the extracts (compared to 5.8 for the oil).

TBA numbers in Table IV should reflect the extent of lipid oxidation in the extracts and residual flakes if the TBA assay is valid for our system; however, the extent of lipid oxidation did not correlate with our organoleptic evaluations. Soybean oil and the ether extracts, in spite of large differences in TBA number, had similar flavor characteristics. Both gave no objectionable odor and had paraffinlike,

vegetable-oil flavor. The azeotrope extract, on the other hand, gave an odor like that of linseed oil paint and had a flavor described as strongly hydrocarbonlike, bitter-biting, and astringent, with a lingering aftertaste that caused a throat-catching sensation. This extract had a TBA number between that of soybean oil and the ether extract. The full-fat, defatted, and ether-extracted raw flakes all had green-beany flavors and the azeotrope-extracted flakes, little flavor.

Effect of Interfering Substances on TBA Analysis

Since substances other than malonaldehyde will give a positive color reaction with TBA (15,16), spectral analyses of all TBA assays were made in the region 400 to 600 $m\mu$ on a Beckman DK2A recording spectrophotometer. Acetaldehyde, n-hexanal, acetone, furfural, and sucrose were steam-distilled under the acidic conditions of the TBA assay (6) to determine whether these compounds, present in soybeans, produce TBA-reactive substances that absorb at 532 $m\mu$. Spectra of the colored complexes produced by reaction of TBA with steam-distillates of TEP and defatted raw soybean flakes are shown in Fig. 2.

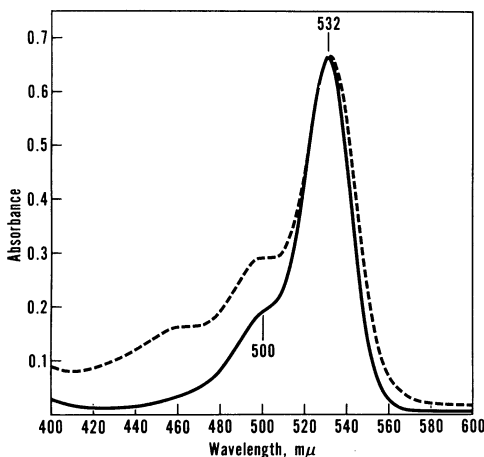


Fig. 2. Absorption spectra of the complex produced with thiobarbituric acid (TBA) reagent and distillates from: (—) acidified tetraethoxypropane standard solution; (-----) raw defatted soybean flakes.

Malonaldehyde-TBA complex has a peak absorbance at 532 $m\mu$ with a slight inflection at 500 $m\mu$. The TBA reaction product from defatted soybean flakes has similar absorbance at 532 $m\mu$, more pronounced at 500 $m\mu$, and additional absorbance at 460 $m\mu$. n-Hexanal probably contributes to absorbance at 460 $m\mu$, since reaction of its distillate with TBA gives a yellow solution with maximum absorbance at 450 $m\mu$. Acetaldehyde distillate reacts with TBA to give a peak at 500 $m\mu$. It was expected that the acidic conditions used with the distillation procedure would degrade the carbohydrates of the meal to form furfural or other steam-distillable compounds that react with TBA. Furfural distillate reacts with TBA to give a major peak at 415 $m\mu$ and a minor one at 475 $m\mu$. However, the steam-distillate of a 1% sucrose solution, when reacted with TBA, gave no product that absorbs in the region 400 to 600 $m\mu$. Carbohydrate degradation products therefore contribute little to TBA numbers of the various soybean samples and to

the spectral characteristics of the TBA-reactive substances, because the spectra of defatted raw flakes show little absorbance at 415 and 440 $m\mu$. Acetone distillate did not react with TBA.

Distillates from soybean samples stored more than 7 months react with TBA to give solutions with more absorbance at 460 and 500 $m\mu$ than do TBA-reactive substances from freshly prepared flakes. Furfural and the carbonyl compounds, tentatively identified in defatted soybean flakes as 2,4-DNP derivatives (Table II), do not contribute to the absorbance at 532 $m\mu$. It would appear, therefore, that some compound that reacts like malonaldehyde accounts for the absorbance at 532 $m\mu$.

Hydroperoxides in Ether and Azeotrope Extracts

As shown in Fig. 3, ether and azeotrope extracts contained hydroperoxides. Purple spots characteristic of hydroperoxides emerged when the chromatograms were sprayed with potassium iodide-starch solutions. A commercial sample of oil-free soybean phosphatides was used for comparative purposes, since its composition and chromatographic behavior are similar to those of the lipid extracts from defatted soybean flakes (Fig. 3, A). The oil-free phosphatides (Fig. 3, B1) have no hydroperoxides present. Both the ether (Fig. 3, B2) and azeotrope extracts (Fig. 3, B3) contain small amounts of hydroperoxide in R_f region 0.2 to 0.5. When the oil-free phosphatides and extracts were ultraviolet-irradiated for 1 hr., hydroperoxides were formed and extensive trailing of components due to degradation occurred (Fig. 3, C). Hydroperoxides could be generated in oil-free soybean phosphatides after 5 min. of irradiation. However, there was no chromatographic evidence of hydroperoxides in a sample of oil-free soybean phosphatides refluxed for 6 hr. in hexane-absolute ethanol azeotropic mixture.

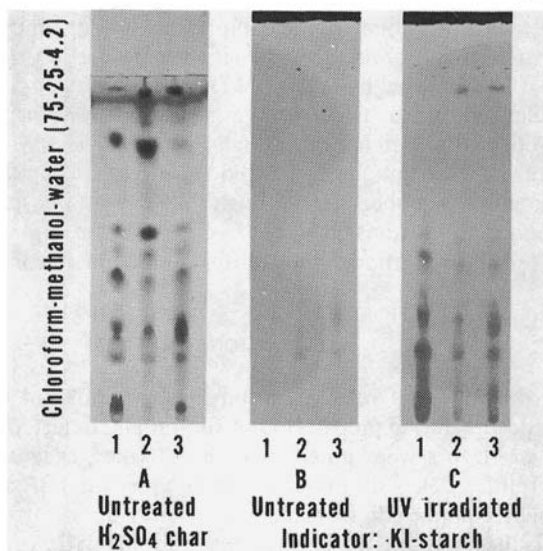


Fig. 3. Thin-layer chromatogram of: 1) oil-free soybean phosphatides, commercial source; 2) ether extract from defatted soybean flakes; and 3) hexane-ethanol (79:21) azeotrope extract from flakes extracted first with diethyl ether. A, unirradiated and visualized by spray with 50% H_2SO_4 followed by heat 30 min. at 130°C. B, unirradiated, and C, irradiated for 1 hr., 12 cm. from 254- $m\mu$ light source. Both B and C were visualized by spray with 5% KI followed by 1% starch in 1% HOAc. Solvent: Chloroform-methanol-water (75:25:4.2, by volume).

Generation of Flavors in Lipid Extracts by Ultraviolet-Light Irradiation

Since oil-free soybean phosphatides and the ether extract of defatted flakes have little flavor and odor, we attempted to create flavors characteristic of the azeotrope extract by ultraviolet-light irradiation of the phosphatides. Flavor evaluations and TBA numbers of UV-irradiated, oil-free phosphatides are given in Table V. TBA numbers of the irradiated phosphatides increased linearly with time up to 4 hr. of irradiation, after which time the rate of increase slowed down.

TABLE V. TBA NUMBERS AND FLAVOR DESCRIPTIONS OF UV-IRRADIATED OIL-FREE PHOSPHATIDES

Irradiation ^a Time	TBA Number	Flavor Description
hr.		
0	5.5	Nutty, vegetable-oil, paraffinlike
1	12.8	Rancid, hydrocarbon
2	22.3	Stale, rancid
4	39.6	Rancid, slightly bitter
6	49.5	Disagreeable, bitter-biting
5 ^b	75.9	Intensely bitter-biting, choking

^aUltraviolet irradiation with 254-m μ light source 12 cm. above sample.

^bUltraviolet light source 4 cm. above sample.

Within 1 hr. of irradiation the nutty, vegetable-oil, paraffinlike flavors of the phosphatides changed to rancid and hydrocarbonlike. After 2 hr. of irradiation, a staleness masked the rancid flavor. After 4 hr., the sample still tasted rancid and also slightly bitter. More extensive irradiation yielded samples that produced disagreeable, bitter-biting, choking sensations. Except for the hydrocarbon flavor and bitter-biting sensation, none of the samples possessed either the odor or the flavor characteristics of the azeotrope extract. The formation of a bitter taste in oxidized lecithin was also noted by Weiss and Diemair (17).

Upon ultraviolet-light irradiation of the ether extract for 6 hr., its TBA number decreased from 66.9 to about half the original value. The flavor of this sample decreased in intensity with time, and changed from that of paraffin and vegetable oil to that associated with rubber. Even though the soybean phosphatides and ether extract have similar compositions, generate hydroperoxides, and have similar TBA numbers under certain conditions, they differ greatly in flavor upon ultraviolet irradiation.

CONCLUSIONS

On the basis of analyses of volatile carbonyl compounds and TBA assays, some lipid degradation occurs during preparation of full-fat and defatted flakes. However, no rancid odors or flavors were noted in soybean flakes, except when lipoxidase was activated in full-fat flakes during blending in water for TBA analysis. Removal of volatile carbonyl compounds from defatted flakes does not change the flavor characteristics of these flakes. Therefore, the odors and flavors associated with volatile carbonyl compounds contribute little to the over-all soybean flavor.

The residual lipids, extracted from defatted soybean flakes, contained hydroperoxides and more TBA-reactive substances than crude soybean oil as determined by TBA assay. Ultraviolet irradiation of the ether extract and soybean

phosphatides produced some flavors that were similar to those of the azeotrope extract. However, on the basis of other flavors present and over-all impression, these samples could be readily differentiated when tasted. Flavors due to oxidation of the residual lipids may arise during extraction of these lipids. If not removed completely from the flakes, the oxidized lipids can contribute to objectionable flavors in products prepared from these flakes. Extraction of the original soybean flavor is closely associated with removal of the difficulty extractable lipids. Hexane-absolute ethanol azeotropic mixture appears to be an excellent solvent for removing these lipids and the flavor. Preliminary taste tests on azeotrope-extracted flakes show them to possess little flavor. Further research is under way to establish the chemical entities responsible for soy flavor.

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