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Gas Chromatographic and Mass Spectral Analyses of Soybean Milk Volatiles

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Gas chromatographic and mass spectral techniques were employed in the isolation and identification of the volatile components of whole-fat soybean milk. Forty-one compounds were positively identified, and an additional 13 compounds were tentatively identified. Hexanal was the major volatile component. Their precursors and the presence in other soybean derivatives are discussed. The typical green-beany flavor of soy can probably be ascribed to a mixture of many compounds.

In the production of soy-based food products, the problem of off-flavor development is often a deterrent to product acceptability. One such product, whole-fat soybean milk, develops beany-rancid flavors during its production (Wilkens *et al.*, 1967).

Soybean milk has been prepared for hundreds of years in the Orient by a traditional method of soaking beans in water for several hours, followed in order by grinding with water, filtration, and cooking for about 30 min. (Smith, 1949).

There were a considerable number of reports on the studies of volatile compounds from raw soybean (Badenhop and Wilkens, 1969; Janiček and Hrdlička, 1964; Mattick and Hand, 1969), soybean flour (Arai *et al.*, 1966a,b; Arai *et al.*, 1967; Fujimaki *et al.*, 1965; Teeter *et al.*, 1955), and soybean oils (Chang *et al.*, 1961; Gaddis *et al.*, 1961; Grosch and Schwencke, 1969; Hill and Hammond, 1965; Hoffmann, 1961a,b; Kawahara and Dutton, 1952; Martin *et al.*, 1948; Mookherjee and Chang, 1963; Mustakas *et al.*, 1969; Schepartz and Daubert, 1950; Schmalfuss and Treu, 1927; Smouse and Chang, 1967; Stapf and Daubert, 1950; Von Pezold, 1959). This paper reports the isolation and identification of volatile compounds from whole-fat soybean milk.

MATERIALS AND METHODS

Sample Preparation. Whole soybeans, variety Harasoy 63, were soaked in 40° C distilled water for 2 hr to double the original bean weight. Two parts of the soaked beans were ground with nine parts of 25° C distilled water in a Model CB-5 Waring Blendor for 5 min to achieve the final product temperature of 45 to 50° C. A total of 110 l. of sample was prepared for distillation.

The volatiles were distilled from 1.1-l. batches with a rotary flash evaporator system under reduced pressure (about 320 mm Hg). The forming tendency was partially reduced through the addition of Antifoam AF which had previously been stripped of volatiles. A constant temperature water bath at 80° C supplied heat input for the distillation. The volatiles were trapped in a series condenser system with 15, 0, and −76° C interfaces, as shown in Figure 1. Approximately 250 ml of distillate was collected from each batch.

The volatiles were extracted from 11-l. distillates of the 0° C condenser trap with 500 ml redistilled CS_2 in a continuous liquid-liquid extractor. The −76° C trap distillate was extracted with CS_2 by repeated separatory flask extractions. After cautiously distilling off most of the solvent in a nitrogen atmosphere, the 200 to 300 mg residue was obtained from the combined CS_2 extract.

Gas Chromatography. Components of the CS_2 extract were fractionated with a Varian Aerograph Model 1520B gas chromatograph on a 10-ft × 1/4 in. o.d. aluminum column packed with 20% SE-30 on 60 to 70 mesh Anakrom AB at a helium flow rate of 60 ml per min. The temperature was programmed from 70° to 275° C at 1° C per min. Eighteen fractions were collected in 16-in. × 2-mm i.d. glass tubes with dry-ice coolants. Each fraction was rechromatographed on a 10-ft × 1/4-in. o.d. aluminum column packed with 25% Carbowax 20M on 60-70 mesh Anakrom AB at a helium flow rate of 60 ml per min. The temperature was programmed from 50° to 250° C at 1° C per min. The subfractions were rechromatographed on a 10-ft × 1/8-in. o.d. stainless steel column packed with 25% Carbowax on 60 70 mesh Anakrom AB at a helium flow rate of 30 ml per min with temperature programming from 50 to 250° C at 1° C per min for relative retention time data against a series of ethyl ester standards.

Gas Chromatography–Mass Spectrometry. The subfractions were rechromatographed under conditions similar to those used for retention time data. The effluent was passed into a heated (200° C) helium separator (Watson and Biemann, 1964) interfaced at the inlet of a Model 12-101A Bendix Time-of-Flight mass spectrometer. The elution of compounds was observed through the use of a Bendix total output integrator and a Textronix Type 545B oscilloscope. The scan rate was set to give a 5-sec scan between *m/e* 40 and 150.

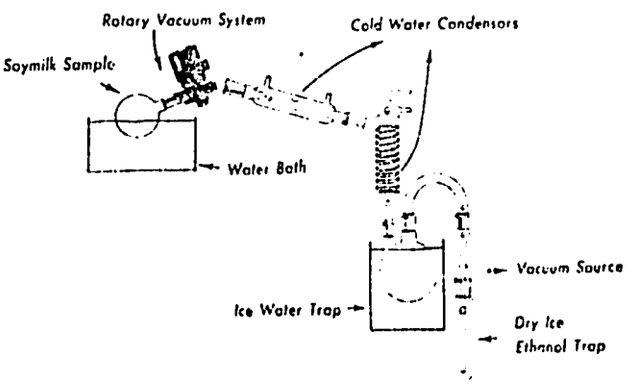

Figure 1. Vacuum distillation system for collection of soybean milk volatiles

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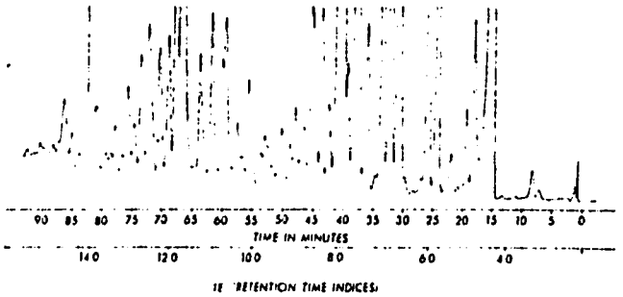

Figure 2. Gas chromatogram of the volatile components from soybean milk

Programmed at 2° C per min from 50° C to 240° C on a 10-ft × 1/8-in. o.d. Carbowax 20M column

RESULTS AND DISCUSSION

It is recognized that the lipids in the soybean milk in this investigation were subjected to more extreme oxidizing conditions than normally would be encountered in soybean milk. However, the collected distillate possessed a characteristic odor of original soybean milk.

The gas chromatographic separation of the total volatile distillate in the Carbowax column is shown in Figure 2. Peak identifications were designated positive if mass spectral and retention time data matched known standards analyzed under identical instrument conditions. Relative retention time indices (I_E) of the unknown compounds were calculated relative to a series of internal ethyl ester standards, where the carbon chain length of the acid function represents a whole number (ethyl hexanoate = 6.00) (van den Dool and Kratz, 1963). Where standard compounds were not available for comparison, tentative identifications were assigned if reference literature spectra were available or the I_E values could be interpolated through homologous series plots.

Approximately 80 peaks were evidenced by the CBW × 20M gas chromatogram of the whole sample, as shown in Figure 2. Many of these consisted of two or more compounds and were separated by first collecting cuts on the nonpolar column and rechromatographing on the Carbowax column. Many of the smaller components remain unidentified because of mass spectral sensitivity limitation combined with overlapping peaks and background, especially at the higher temperature region of the glc program.

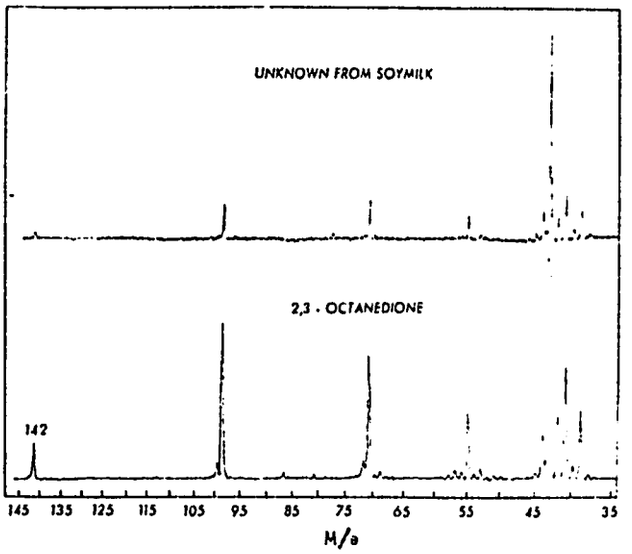

Figure 4. Mass spectra of 2,3-octanedione

Definite identification of 41 compounds was obtained, and tentative identifications were assigned to an additional 13 compounds, as shown in Table I. The compounds not previously identified in soy-derived products are marked with asterisks. There were no definite reasons to suspect that some of the identified compounds represent artifacts or contaminant, but such a possibility cannot be excluded.

Figures 3 through 6 are the mass spectra of the compounds not reported previously in soy-derived products. The upper spectra in each figure was from soymilk, the lower spectra was from the corresponding authentic compound. Only previously unpublished mass spectra are shown here.

Many compounds shown in Table I arose from the lipids in the soybean milk by mechanisms involving autoxidation, hydrolysis, decarboxylation, and dehydration.

Acetaldehyde can arise from both linoleic and linolenic acids (Gaddis *et al.*, 1961). Pentanal, hexanal, 2-heptanal, 2-octenal, 2,4-nonadienal, 2,4-decadienal, and pentanol were shown to arise from linoleic acid by the catalytic action of lipoxygenase in soybean (Grosch and Schwencke, 1969). In addition to the compounds mentioned above, 2-heptanone,

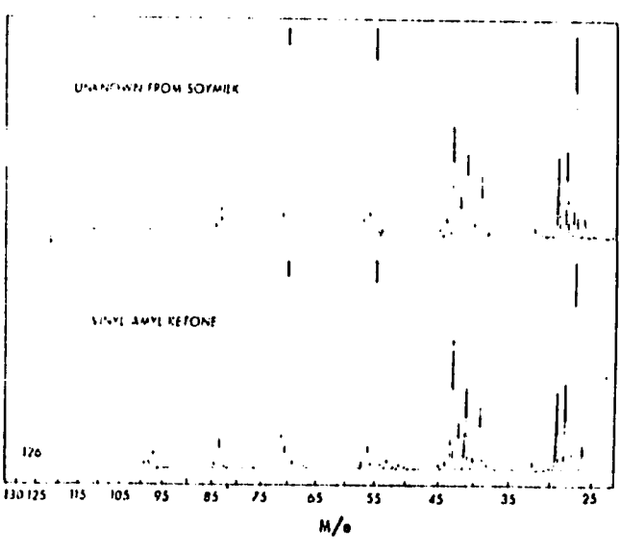

Figure 3. Mass spectra of amyl vinyl ketone

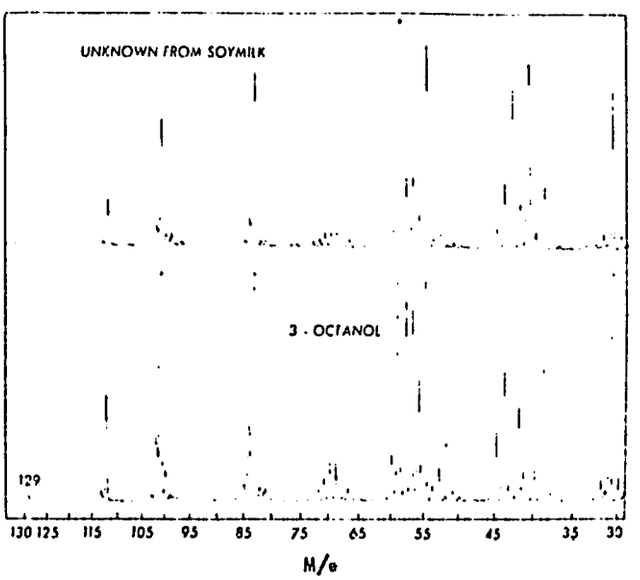

Figure 5. Mass spectra of 3-octanol

Table I. Volatile Compounds of Soy Milk Identified by Gas Chromatography and Mass Spectrometry

Compound	I_E Relative Retention Time Indices: Unknown from Soy Milk	I_E Relative Retention Time Indices: Authentic Compound	Mass Spectral Identification	Reference for Mass Spectra	G.C. Peak Identification Comment
Aldehydes					
Acetaldehyde	0.4	0.44	+	A	P
Isobutanal[a]	1.00	1.00	+	A	P
Butanal	2.00	2.00	+	A	P
Pentanal	3.12	3.16	+	A	P
Hexanal	4.44	4.43	+	A	P
2-Pentenal	4.82	4.76		...	T
Heptanal	5.43	5.52	+	A	P
2-Hexenal	5.80	5.82	+	A	P
Octanal	6.59	6.58	+	A	P
2-Heptenal	6.86	6.82		...	T
2,4-Hexadienal[a]	7.56	7.62	+	A	P
Nonanal	7.62	7.62	+	A	P
2-Octenal	8.02	8.00	+	A	P
2,4-Heptadienal	8.52	8.58	+	A	P
2-Nonenal	8.99	9.02	+	A	P
2-Decenal	10.00	10.00	+	A	P
2,4-Nonadienal	10.52	...		(Cornu and Massot, 1966)	T
2,4-Decadienal	11.78	11.67	+	A	P
Acetals					
1,1-Diethoxyethane[a]	2.00	2.00	+	(Cornu and Massot, 1966)	T
1,1-Diethoxypropane[a]	3.00	3.00	+	(Cornu and Massot, 1966)	T
1,1-Diethoxyhexane[a]	6.00	6.00	+	(Cornu and Massot, 1966)	T
1,1-Diethoxynonane[a]	9.00	9.00	+	(Cornu and Massot, 1966)	T
Esters					
n-Hexylacetate[a]	6.40	6.37	+	A	P
Pentyl hexanoate[a]	8.85	8.76	+	A	P
Sulfur Compound					
Benzothiazole[a]	13.05	13.02	+	A	P
Hydrocarbons					
Pentane[a]	0.60	0.44	+	(Cornu and Massot, 1966)	T
1,4-Pentadiene[a]	0.70	0.70	+	A	P
Ethylcyclohexene[a]	2.79	2.95		...	T
Decane	3.47	3.39		A	P
3-Methyldecane[a]	4.29	4.38		...	T
Aromatic Compounds					
Benzene	2.64	2.59	+	A	P
n-Propylbenzene[a]	5.80	5.74	+	A	P
n-Butylbenzene[a]	6.84	6.80	+	A	P
Benzaldehyde	8.86	8.84	+	A	P
Ketones					
Ethyl vinyl ketone	3.76	3.67	+	A	P
2,3-Pentanedione[a]	4.18	4.04	+	A	P
2-Heptanone	5.45	5.41	+	A	P
3-Octanone	6.21	6.19	+	A	P
2-Octanone	6.53	6.51	+	A	P
Amyl vinyl ketone[a]	6.70	6.69	+	A	P
2,3-Octanedione[a]	6.92	6.90	+	A	P
Alcohols					
Ethanol	2.34	2.34	+	A	P
1-Penten-3-ol	5.06	...	+	(Bondarovich *et al.*, 1967)	T
2-Hexanol[a]	5.83	5.66	+	(Cornu and Massot, 1966)	T
1-Pentanol	6.00	6.00	+	A	P
1-Hexanol	7.06	7.00	+	A	P
3-Octanol[a]	7.51	7.52	+	A	P
1-Octen-3-ol	7.98	7.98	+	A	P
1-Octanol[a]	9.09	9.09	+	A	P
2-Octen-1-ol[a]	9.57	...	+	(Cornu and Massot, 1966)	T
1-Nonanol[a]	10.00	10.02	+	A	P
Others					
1-Pentylfuran	6.00	6.02	+	A	P
Hexanoic acid	12.46	12.50	+	A	P
γ-Nonalactone[a]	13.80	13.71	+	A	P

[a] Previously not reported in soybean-derived products. P = positive; T = tentative; A = authentic compound.

2-octanone, 1-octene-3-ol, and 2-pentyl furan can also arise from linoleic acid (Smouse and Chang, 1967).

As is known by a large number of reports, octanal, nonanal, 2-nonenal, and 2-decenal can arise from oleic acid. 2-Pentenal, 2,4-heptadienal, ethanol, and 1-penten-3-ol can arise from linolenic acid.

Hexanal comprised about 25 wt% of the volatile sample. Fujimaki *et al.* (1965) reported approximately 10 ppm of hexanal in raw soybean. With extremely low threshold (Day *et al.*, 1963), hexanal is probably one of the major compounds contributing to the disagreeable aroma of soybean milk. On exposure to air, hexanal could be oxidized to hexanoic acid (I_E = 12.46) which possesses a harsh, fetid odor. Hexanoic acid was found in defatted soyflour (Teeter *et al.*, 1955) and raw soybean (Arai *et al.*, 1966b).

Hexanol (I_E = 7.06), possessing a harsh, grassy odor at high concentration, was considered one of the alcohols which contributed to the green-beany odor of raw soybean (Arai *et al.*, 1967).

2-Hexenal (I_E = 5.80), known as leaf aldehyde, possesses a pronounced odor of green foliage. The authors were not able to detect the presence of 3-*cis*-hexenal, which was found in the oxidized soybean oil and described as possessing green-beany odors (Hoffmann, 1961). However, *t*-2-hexenal could be derived from *cis*-3-hexenal (Winter, 1962) during soybean milk preparation and volatile extraction.

Both 1-penten-3-ol (I_E = 5.06) and 1-octen-3-ol (I_E = 7.98) were reported in reverted soybean oil. 1-Octen-3-ol could be formed during the soaking of soybeans in water as pretreatment for soybean milk preparation. This compound has a moldy odor with the threshold between 0.5 and 1.0 ppm in soybean milk (Badenhop and Wilkens, 1969).

Ethyl vinyl ketone (I_E = 3.76) was reported as responsible for the green-beany odor of soybeans, and the threshold was estimated to be lower than 5 ppm in soybean milk (Mattick and Hand, 1969). Amyl vinyl ketone (I_E = 6.70) is identified in a soybean product for the first time. This compound has been found in oxidized milk fat (Keeney and Doan, 1951) and the decomposition product of corn oil (Krishnamurthy and Chang, 1967).

2-Pentyl furan (I_E = 6.00) was described as possessing a characteristic beany or grassy odor, and predominantly responsible for the reversion flavor of soybean oil (Smouse and Chang, 1967).

Not all of the compounds identified in this investigation possess a disagreeable odor. For example, it is well recognized that benzaldehyde possesses a cherry or almond-like aroma; octanol, nononol, and 1,1-diethoxypropane have an odor slightly reminiscent of roses; most of the aliphatic methyl ketones possess fruity aromas; 2,4-decadienal was reported as having potato chip-like odor (Mookherjee *et al.*, 1965); and γ-nonalactone possesses butter or coconut-like aroma. However, these compounds seem to occupy a very small portion of the total volatile flavor components of soybean milk.

The majority of the identified compounds possessed undesirable odors. Several compounds could be described as beany. No single peak in gas chromatograms could approximate the overall off-flavor of soybean milk.

Since no effort was made to relate the concentration of each identified compound occurring in the soybean milk, and probably a great number of compounds are still unidentified, the flavor reconstruction to form characteristic odors of soybean milk remains to be solved. However, it may be concluded that the typical green-beany flavor of soy can probably be ascribed to a mixture of many compounds.

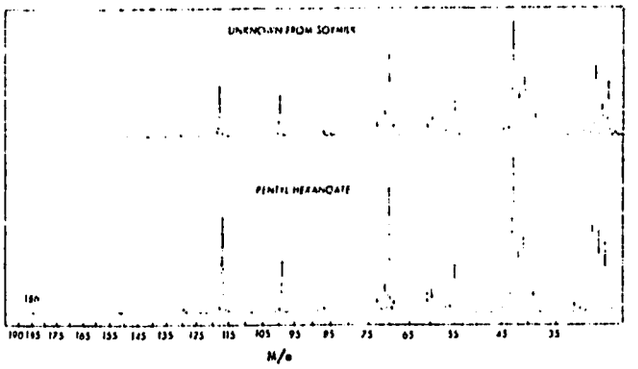

Figure 6. Mass spectra of pentyl hexanoate

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