Nonpolar-Volatile Lipids from Soy Protein Isolates and Hexane-Defatted Flakes

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ABSTRACT: Lipid extracts from two samples of commercial soy protein isolates (SPI) and two samples of commercial hexane-defatted flakes were fractionated by silicic acid-column chromatography. The material eluted with 100% chloroform was collected, further fractionated by silica solid-phase extractions, and analyzed by gas chromatography–mass spectroscopy by using mass spectra, retention times of authentic standards, and Kovats indices for identification. Thirty-eight compounds were identified and quantitated in the lipid fractions from soy protein isolates (SPI); 23 of these are reported for the first time as components of SPI. An additional 13 compounds are reported for the first time as components of hexane-defatted soybean flakes. The major classes of compounds reported for the first time associated with SPI include: butyl, methyl, and ethyl esters of fatty acids; phenols, diphenyls and phenyl esters; and abietic acid derivatives. Dehydroabietinal at 0.180 to 0.191 ppm of the protein isolates was the most abundant aldehyde in the SPI lipid extracts The third most abundant aldehyde found in SPI after dehydroabietinal and hexanal was 2-butyl-2-octenal (0.065 to 0.086 ppm). Dehydroabietic acid methyl ester was present in SPI (0.309 to 0.459 ppm). Dehydroabietene (0.628 ppm) and abietatriene (0.396 ppm) were tentatively identified in one sample of hexane-defatted flakes. JAOCS 74, 461-467 (1997).

KEY WORDS: Butyl esters, dehydroabietic acid methyl ester, dehydroabietinal, gas chromatography, hexane-defatted soybean flakes, lipids, mass spectroscopy, phenols, soy protein isolates, 2-butyl-2-octenal.

In previous investigations, soy protein isolates (SPI) were shown to contain from 3.9 to 5.9% total lipids by weight (1–3). Approximately 67% of these lipids were phospholipids, and approximately 5% appeared to be phytoglycolipids (4).

Headspace analyses of unheated and heated SPI by gas chromatography—mass spectroscopy (GC–MS) have been used to examine the volatile components associated with soy proteins (5,6) and hydrolyzed soy proteins (7). This method provides qualitative information about the broad range of volatile components of soy proteins. Because of the impact that associated lipids can have on the sensory and functional

attributes of proteins, this investigation was undertaken to provide qualitative and quantitative information on the nonpolar lipids associated with SPI and hexane-defatted flakes.

EXPERIMENTAL PROCEDURES

Protein products. SPI designated as Pro Fam 970 (referred to as samples A and B to distinguish the different code dates) were obtained from Archer Daniels Midland (ADM) (Decatur, IL). Hexane-defatted soybean flakes (typical for the commercial preparation of SPI) were obtained from ADM and Protein Technologies International (PTI) (St. Louis, MO).

Chemicals. Dehydroabietinal was synthesized by the method of Carrau et al. (8). Abietic acid methyl ester and dehydroabietic acid methyl ester were prepared by refluxing 0.5 g of 85% abietic acid (Sigma Chemical Co., St. Louis, MO) in 50 mL methanol with 2% concentrated sulfuric acid for 3 h. The reaction products were extracted into 100 mL hexane, which was washed twice with 50 mL water and dried over sodium sulfate. The reaction mixture was composed primarily of a mixture of methyl abietate and methyl dehydroabietate. These two components were purified in a Microsorb Silica column 5 μ m, 4.6×250 mm with a 5 μ m, 4.6×15 mm guard column (Rainin Instrument Co. Inc., Woburn, MA). The separation was accomplished with a linear solvent gradient of 0.1 to 4% 2-propanol in hexane from 0 to 20 min at 0.5 mL/ min. Elution of methyl abietate and methyl dehydroabietate was detected at 276 nm at 5.27 and 6.35 min, respectively. The procedure of Casnati et al. (9) was used to prepare 2-butyl-2-octenal. The method of Rosevear and Wilshire (10) was used to prepare 2-methyl-4-(1,1,3,3tetramethylbutyl)-phenol. Other chemicals used as standards were purchased from either Sigma Chemical Co. or Aldrich (Milwaukee, WI).

Lipid extraction and fractionation. Lipids were extracted by a modification of the method of Bligh and Dyer (11) as previously described (12). Approximately 20 g SPI was extracted twice, each time with 200 mL of a nonacidified solvent mixture. The lipids obtained from two separate extractions were combined and brought to near dryness in a rotary evaporator at 50°C with 0.7 kg/cm² vacuum and suspended in 10 mL chloroform. The lipid extract in chloroform was

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loaded onto a 1.8 × 30 cm column of silicic acid, 100–200 mesh (Bio-Rad, Hercules, CA), preconditioned with chloroform. The first 200 mL of eluent were collected and brought to near dryness in a rotary evaporator at 50°C with 0.7 kg/cm² vacuum. This material was suspended in 3 mL carbon tetrachloride.

Approximately 100 mg of the lipid fraction in carbon tetrachloride was loaded onto a silica solid-phase extraction cartridge (Waters Chromatography, Milford, MA), preconditioned with carbon tetrachloride, and separated into three fractions with successive elutions with (i) 5 mL carbon tetrachloride, (ii) 3 mL carbon tetrachloride/benzene (1:2), and (iii) 3 mL benzene. All solvents were evaporated under a stream of dry nitrogen at 50°C. Resulting lipid fractions were suspended in hexane and stored in a freezer at –15°C. All surfaces contacted with the samples were either glass or Teflon that were rinsed first with methanol/chloroform (2:1, vol/vol), then with chloroform. A reagent blank was performed on the extraction and fractionation process.

Gas chromatography-mass spectrometry (GC-MS). GC-MS was done with a Hewlett-Packard Model G1800A GCD System (Wilmington, DE), equipped with an electronionization detector (EID), a Model G1030A Chemstation controller, and an HP-1 capillary column (30 m \times 0.25 mm i.d. with 0.1 µm film thickness). A 1-min splitless injection was used. The column temperature was held at 50°C for 2 min, then raised at 5°C/min to 300°C, at which it was held for 3 min. High-purity helium was the carrier gas at 1 mL/min flow rate. The EID was set to detect in the mass range of 10 to 425 m/z. The ionization voltage was 70 eV. Response factors were taken as 1.0 compared to an internal standard of phenanthrene, and relative compositions were calculated on the basis of peak area. All determinations were performed in duplicate. Identification of lipid compounds was accomplished by comparison of mass spectra and retention times to authentic standards, where possible. Compounds without authentic standards were identified by their Kovat's indices and comparison to the National Institute of Standards and Tests (NIST) spectral library. Kovats indices were calculated against C_7 – C_{25} paraffins as references. Some identified compounds were further evaluated with the selective ion mode (SIM) of the mass spectral detector. The total lipid extracts were diluted to approximately 50 μg/μL with methylene chloride, and 1 μL was injected onto the same GC column. The SIM method was prepared by the automatic SIM setup feature specific for each compound examined. The more volatile compounds ($R_t \le 20$ min) were quantitated by injecting a portion of the unfractionated lipid extract (with a mass range of 10–425) to prevent loss during the fractionation procedures.

Thin-layer chromatography (TLC). TLC was accomplished on a 250- μ m layer of activated silica gel G (Alltech Associates, Inc., Deerfield, IL). The plate was developed in one direction with carbon tetrachloride (13). Lipids were visualized by iodine vapors, by spraying with a 0.05% aqueous solution of Rhodamine 6G, and by spraying with 4.2 M $\rm H_2SO_4$, followed by charring at 130°C. $\rm R_f$ values of unknown peaks were compared to standards of mixed paraffins ($\rm C_7$ – $\rm C_{25}$) and cholesteryl oleate (Aldrich).

Analytical. Protein isolates and defatted flakes were analyzed for moisture with three replications by drying at 130°C for 1 h. Nitrogen was determined with three replications by the MacroN Nitrogen Analyzer (Foss Heraeus Analysensysteme GmbH, Donaustr, Germany), and values were multiplied by 5.71 to calculate protein (14). Values were reported as weight percentages on a dry basis.

RESULTS AND DISCUSSION

Composition data for the commercial SPI and hexane-defatted flakes are presented in Table 1. The total lipid and protein contents of these samples were similar to the commercial isolates and defatted flakes previously investigated (2). SPI lipids, eluted from the silica solid-phase cartridge with 100% $CC1_4$, accounted for $0.423 \pm 0.016\%$ and $0.402 \pm 0.009\%$ by weight of the total SPI on a dry basis. This material produced a single spot by TLC that corresponds to a hydrocarbon standard. GC-MS analysis of this material confirmed that much of it was composed of hydrocarbons; however, most of this material was not adequately resolved by GC regardless of the amount injected per separation. TLC analysis of the corresponding fraction from the hexane-defatted flakes demonstrated that it was composed primarily of hydrocarbons, along with a substantial amount of sterol esters. The wt% of these fractions on a total defatted flakes dry basis was 0.005 ± 0.001% (ADM) and $0.004 \pm 0.002\%$ (PTI). This dramatic difference in hydrocarbon content between the defatted flakes and the SPI suggests an unexpected synthesis or degradation of pre-existing defatted-flake components during SPI processing or subsequent storage, or possibly contamination during SPI processing.

TABLE 1
Composition of Soy Protein Products

Component (%)	ADM ProFam 970 "A"	ADM ProFam 970 "B"	ADM defatted flakes	PTI defatted flakes
Moisture	4.3 (0.09) ^a	1.0 (0.03)	3.9 (0.06)	1.1 (0.06)
Protein $(N \times 5.71)^b$	80.4 (1.67)	81.0 (0.31)	46.8 (0.01)	48.3 (0.53)
Total lipids ^b	3.7 (0.03)	3.9 (0.14)	1.32 (0.04)	1.72 (0.04)

^aFigures in parentheses are standard errors; ADM, Archer Daniels Midland (Decatur, IL) PTI, Protein Technologies International (St. Louis, MO).

^b% by weight dry basis.

Compounds in the lipid fraction, eluted from a silica solidphase cartridge with CCl₄/benzene and benzene, and some of the hydrocarbons from the CCl₄ fraction, are reported in Table 2. Thirty-eight compounds were identified in the lipid fractions from commercial SPI, 23 of these being reported for the first time as components associated with soy protein. An additional 13 compounds were reported for the first time as components associated with hexane-defatted soybean flakes (Table 3). The major classes of compounds associated with SPI reported for the first time include butyl, methyl, and ethyl esters of fatty acids; phenols, diphenyls, and phenyl esters; and abietic acid derivatives.

The esters of hexadecanoic and octadecanoic acid were the predominant fatty acid esters in both SPI and defatted flakes. With the exception of a small amount of 9-octadecenoic ethyl ester in the hexane-defatted flakes from Protein Technologies International (PTI), only the saturated fatty acids occurred as the butyl and ethyl esters.

Dehydroabietinal was the most abundant aldehyde in the lipid extracts of the commercial SPI, and 2-butyl-2-octenal

TABLE 2.

Components of Soybean Protein Isolates (SPI)

Components of Soybean Protein Isolates (SPI)							
Retention		Kovat's	ProFam	ProFam			
time (min)	Compound ^a	index	SPI "A" ^b	SPI "B" ^b			
7.23	Toluene ^c	n/a	0.177	0.230			
7.76	2-Hexanone ^c	n/a	0.037	0.105			
7.94	$Hexanal^c$	n/a	0.048	0.128			
16.48	Limonene ^c	1029	0.018	0.040			
17.84	*Hexachloroethane c	1070	0.177	0.161			
18.29	Nonanal ^c	1084	0.057	0.057			
20.31	*Benzoic acid, ethyl ester	1150	0.013	0.009			
21.06	2-Decanone	1175	0.012	0.017			
21.40	*2,4,6-Trimethyl phenol ^c	1184	0.103	n.d.			
21.45	Decanal	1186	0.013	0.038			
21.87	Dodecane	1202	0.048	0.049			
22.35	*2-Propenoic acid, 6-methylheptyl ester	1219	0.017	0.029			
23.57	*Indole ^c	1260	0.029	0.042			
24.35	*2,4,6-Trimethylbenzaldehyde	1286	0.029	n.d.			
24.53	2,4-Decadienal ^c	1293	0.022	0.036			
25.62	*2-Butyl-4-methyl phenol ^c	1333	0.012	0.009			
25.67	*2,4,6-Trichlorophenol	1335	n.d.	0.005			
26.37	*Skatole ^c	1362	0.020	0.003			
26.39	*2-Butyl-2-octenal ^c	1363	0.065	0.086			
27.46	Tetradecane	1403	0.065	0.069			
28.93	*2,6-Di- <i>tert</i> -butyl-1,4-benzoquinone ^c	1459	0.003	0.062			
29.43	?	1478	0.879	0.678			
29.77	*2,4-Dibutylphenol ^c	1491	0.053	0.057			
30.83	2,4-Dibatyiphenoi	1535	0.371	0.037			
32.26	2-Pentadecanone ^c	1596	0.058	0.044			
32.40	*Diphenylamine ^c	1602	n.d.	0.044			
32.62		1612	0.030	0.020 n.d.			
	*Benzophenone ?						
35.44	·	1735	0.261	n.d.			
36.83	Octadecane ?	1801	0.463	0.183			
38.44	•	1877	0.180	0.153			
39.11	Hexadecanoic acid, methyl ester ^c	1909	0.322	0.701			
40.45	*Hexadecanoic acid, ethyl ester ^c	1979	n.d.	0.218			
41.02	*?	2009	0.162	0.111			
42.33	9,12-Octadecenoic acid, methyl ester ^c	2077	0.165	0.314			
42.52	9-Octadecenoic acid, methyl ester ^c	2087	0.272	0.312			
42.97	Octadecenoic acid, methyl ester ^c	2111	n.d.	0.108			
44.06	*Hexadecanoic acid, butyl ester ^c	2174	0.972	0.623			
44.18	*Octadecanoic acid, ethyl ester ^c	2181	n.d.	0.121			
44.65	?	2207	0.616	1.361			
45.66	*Dehydroabietinal ^c	2265	0.191	0.180			
46.60	*2-Propenoic acid, 3-(4-methoxyphenyl)-, 2-octyl ester	2320	n.d.	0.107			
46.84	*Dehydroabietic acid, methyl ester ^c	2335	0.459	0.309			
47.47	*Octadecanoic acid, butyl ester ^c	2374	0.804	0.539			
47.78	*Abietic acid, methyl ester c	2393	n.d.	0.100			
52.59	*(1,1-Dimethylethyl) phenyl phosphoric acid, diphenyl ester	n/a	n.d.	0.276			

^aCompounds preceded by an asterisk represent compounds identified in SPI for the first time.

 $^{^{}b}$ mg/kg of SPI on dry basis; n.d., not detected; n/a, not applicable.

^cCompounds confirmed by authentic standards. See Table 1 for company source locations.

TABLE 3.
Components of PTI and ADM Hexane-Defatted Flakes

Retention		Kovat's		
time (min)	Compound ^a	index	PTI^b	ADM^b
7.25	Toluene ^c	n/a	0.718	0.087
7.65	2-Hexanone ^c	n/a	0.491	0.309
7.91	Hexanal c	n/a	0.141	0.451
17.83	*Hexachloroethane c	1070	0.278	0.051
18.29	Nonanal ^c	1084	0.024	0.115
20.29	*Benzoic acid, ethyl ester	1150	0.005	n.d.
21.48	Decanal	1186	0.018	n.d.
21.88	Dodecane ^c	1200	0.034	0.119
22.22	*2-Propenoic acid, 2-methylheptyl ester	1215	0.016	n.d.
22.61	*p-Mentha-6,8-dien-2-one	1228	n.d.	0.012
23.55	Indole ^c	1260	0.007	n.d.
24.14	*Octanoic acid, ethyl ester	1289	0.007	n.d.
24.46	Dodecanal	1290	0.012	n.d.
24.53	2,4-Decadienal ^c	1293	0.005	0.062
25.90	2-Undecanal	1344	0.018	n.d.
26.39	*2-Butyl-2-octenal ^c	1363	0.127	0.012
27.46	Tetradecane ^c	1400	0.042	0.158
28.34	*Caryophyllene	1437	0.030	0.075
29.02	*2,6-Di- <i>tert</i> -butyl-1,4-benzoguinone ^c	1462	0.034	0.024
29.41	?	1478	0.401	0.213
29.75	*2,4-Dibutyl phenol ^c	1491	n.d.	0.010
29.84	*o-Hydroxybiphenyl	1494	0.015	0.013
30.02	*2,6-Di- <i>tert</i> -butyl-4-methyl-phenol (BHT) ^c	1501	0.128	0.013
30.80	2,0-Di-tert-butyr-4-methyr-phenol (bi 11)	1535	0.128	0.177
32.60	: Benzophenone	1612	0.013	0.016
35.02	*2,3-Dihydro-1,1,3-trimethyl-3-phenyl-1H-indene	1716	n.d.	0.704
36.92	Octadecane ^c	1800	n.d.	0.704
37.36	*2,4-Diphenol-4-methyl-2-pentene	1826	0.193	0.100
37.53	*6,10,14-trimethyl-2-pentadecanone ^c	1836	0.195	0.055
38.98	Nonadecane ^c	1900	0.106	0.033
39.09	Hexadecanoic acid, methyl ester ^c	1909	0.100	0.330
40.46	*Hexadecanoic acid, rietry ester*	1979	0.036	0.075
41.01	*?	2009	0.036	n.d.
41.40	*Dehydroabietene	2029	0.628	n.d.
42.24	*Abietatriene	2072	0.396	n.d.
42.37	9,12-Octadecenoic acid, methyl ester ^c	2072	0.140	n.d.
42.52	9-Octadecenoic acid, methyl ester ^c	2087	0.194	0.075
43.01	Octadecanoic acid, methyl ester ^c	2111	0.058	0.073
43.65	*9-Octadecenoic acid, therify ester *9-Octadecenoic acid, ethyl ester *	2150	0.038	n.d.
44.07	*Hexadecanoic acid, butyl ester ^c	2174	0.719	0.147
44.19	• •	2181	0.017	0.147
44.19	*Octadecanoic acid, ethyl ester ^c ?	2101	0.017	0.055
		2265	0.049	
45.60	*Dehydroabietinal ^c			n.d.
46.22	*9,17-Octadecadienal	2297	0.069	n.d.
47.33	*Phosphoric acid, triphenyl ester ^c	2366	n.d.	0.216
47.47	*Octadecanoic acid, butyl ester ^c	2374	0.594	0.111
52.61	*(1,1-Dimethylethyl)phenyl phosphoric acid, diphenyl ester	n/a	n.d.	0.218

^aCompounds preceded by an asterisk represent compounds identified in hexane-defatted flakes for the first time.

was the third most abundant after hexanal. Neither dehydroabietinal, 2-butyl-2-octenal, nor the other abietates have been previously reported as components of soybeans or soy protein products. The only reported natural sources of abietates and dehydroabietates are in the resins of coniferous trees and shrubs (15). Abietic acid is a tricyclic diterpene that remains as the major component of rosin after turpentine is removed from the sap of conifers. Abietatriene was tentatively identified in the lipid extract from the PTI defatted flakes (R $_{\rm t}$ of 42.24 min). Its mass spectrum is shown in Figure 1A. It differs from dehydroabietinal and dehydroabietic acid (Scheme 1) only in its methyl group located at C $_{18}$ instead of a carbonyl group or carboxyl group, respectively. The mass spectrum of the compound from the PTI flakes that eluted at 41.40

^bmg/kg of flakes on dry basis; n.d., not detected; n/a, not applicable.

^cCompounds confirmed by authentic standards. See Table 1 for company source locations.

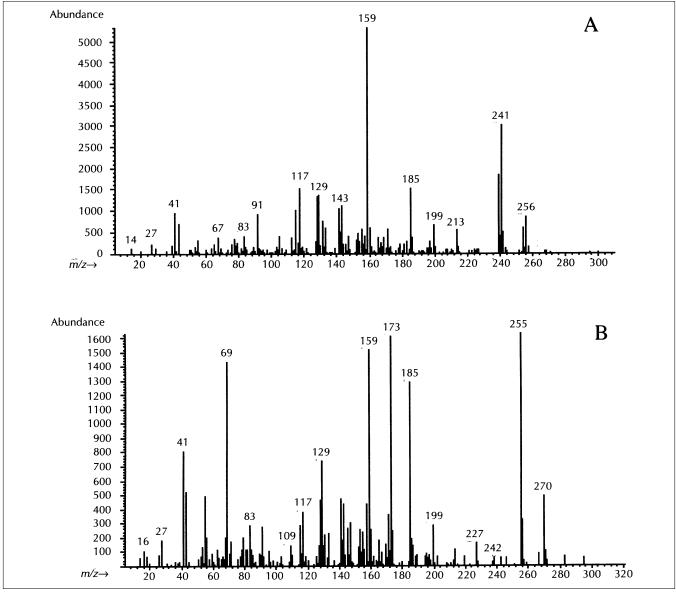


FIG. 1. Mass spectra of compounds from PTI defatted flakes (A) eluted at 41.40 min, tentatively identified as dehydroabietene, and (B) eluted at 42.24 min, tentatively identified as abietatriene.

min is similar to the spectrum reported for dehydroabietene (Fig. 1B) (16). It differs from abietatriene only in a hydrogen in place of a methyl group at C_{18} .

Only a small amount of the dehydroabietinal was found in the PTI defatted flakes (R_t of 46.6 min, Table 3), and none was found in the defatted flakes provided by ADM. Neither dehydroabietic acid methyl ester nor abietic acid methyl ester was found in defatted flakes. This indicates that dehydroabietinal, dehydroabietic acid methyl ester, and abietic acid methyl ester were synthesized during or shortly after processing the commercial SPI. Funk and Croteau (17) proposed that the biosynthesis of abietic acid in conifers occurs by the enzymatic conversion of abietadiene \rightarrow abietadienol \rightarrow abietadienal \rightarrow abietic acid. A similar reaction pathway, beginning with abietatriene and leading to the formation of dehydroabi-

etinal and dehydroabietic acid methyl ester, may be occurring in soybeans.

This lipid fraction from SPI contained four phenols and three phenyl compounds. The lipids from defatted flakes contained an additional three types of phenyl compounds and two additional phenols, including butylated hydroxytoluene (BHT). BHT is a common phenolic compound added to a wide variety of foods for its antioxidant properties. Many of the other phenols in this lipid fraction have closely related structures, including 2-butyl-4-methyl phenol, 2,4-dibutyl phenol, and 2,4,6-trimethyl phenol. If these compounds have flavor thresholds and characteristics similar to other nonpolar phenols (e.g., astringency at 0.005 ppm for *p*-cresol) (18), they may contribute to the undesirable flavor characteristics of many soy products. Di-*tert*-butyl benzoquinone, which is

reported to be an oxidation product of phenolic antioxidants (19) and various other phenols, including 2,4- and 2,6-*tert*-butyl phenols (15), was found in all samples.

The compound from Pro Fam 970 SPI "A" that eluted at 35.44 min was initially identified as 2-methyl-4(1,1,3,3-tetramethylbutyl) phenol. The standard for this compound was prepared, and its elution time did not match that of the sample. This unknown material may be one of several possible isomers of methyl octylphenol.

The SPI samples contained both indole and 3-methyl indole (skatole). Indole has been described as having an intense fecal odor in high concentrations, while contributing a pleasant odor in highly dilute solutions. Skatole has also been identified as a major contributor to "boar taint," the unpleasent odor in the fat of mature boars. Indole and skatole were shown to be predominantly responsible for the off-flavor of casein exposed to ultraviolet radiation (20). Indole, skatole, and *p*-cresol contribute to the off-flavor in potato chips (21).

The material eluted from the silica solid phase with benzene was composed primarily of free fatty acids. Because it is likely that not all fatty acids were eluted with this solvent, no attempt was made to quantitate them.

To minimize the possibility that certain volatile components were lost during the evaporation of solvents, these compounds were measured by injecting a portion of the entire CHCl₃ fraction from the silicic acid column. This was possible with the highly volatile material because there were relatively few co-eluting compounds from the GC. However, it was not possible with the less volatile components. Because the abietates are a class of compounds that have not been previously reported in soybeans, the mass spectrometer was set on SIM to search for the specific ions representative for each of these compounds. With this method, the entire CHCl₃ lipid extract was analyzed by GC–MS, and each reported abietate was confirmed at its appropriate elution time.

In addition to the identified components associated with the SPI and defatted flakes, there were several compounds that have not yet been identified. The mass spectra of the major unidentified compounds are, m/z (rel. int.):

 R_t 29.43: 205 (4), 204 (9), 203 (72), 202 (12), 188 (17), 170 (69), 160 (33), 146 (11), 128 (65), 118 (12), 114 (15), 89 (13), 86 (14), 75 (100), 72 (36), 58 (67), 57 (310), 56 (11), 55 (22), 47 (14), 44 (41), 42 (14), 41 (37), 30 (25), 29 (35), and 27 (13);

 R_t 30.83: 220 (1), 219 (5), 218 (12), 217 (82), 188 (57), 184 (19), 174 (11), 156 (34), 146 (22), 128 (92), 114 (12), 100 (21), 89 (25), 86 (18), 72 (58), 58 (16), 57 (84), 56 (14), 55 (35), 44 (43), 43 (10), 42 (23), 41 (57), 30 (67), 29 (100), and 27 (20);

R_t 35.44: 220 (1), 150 (10), 149 (100), 121 (22), 105 (13), 79 (4), 77 (5), and 43 (4);

R_t 38.44: 246 (1), 189 (2), 127 (12), 113 (11), 111 (14), 97 (25), 84 (12), 83 (37), 82 (15), 73 (39), 70 (22), 69 (40), 56 (20), 55 (100), 43 (33), and 41 (18);

 $R_{\rm t}$ 41.02: 210 (4), 106 (8), 105 (100), 77 (24), and 51 (8); $R_{\rm t}$ 44.65: 241 (16), 240 (100), 239 (40), 225 (9), 224 (10), 209 (18), 208 (12), 194 (16), 178 (10), 166 (11), 165 (40), 153 (15), 152 (18), 127 (5), 115 (7), 105 (3), 102 (4), 89 (4), and 76 (6). The compound eluting at 41.02 min was not benzil, based on the elution time of standard benzil.

In addition to the reported unknown compounds in these samples of defatted flakes and protein isolates, other unidentified compounds were present, most at less than 5 ppb. It was not practical to identify all of these minor components in this investigation.

This investigation presents a new class of compounds associated with soy protein products: the abietates. Because of their variety and the consistency with which they occur, it seems unlikely that these abietates are the result of contamination during harvest, storage, or processing. However, until further research can be conducted to determine the precise mechanism of their biosynthesis in soybeans, this possibility cannot be ruled out. The importance of these relatively minor components of soy protein products is yet to be determined. Minor compounds can often make a major contribution to the overall flavor characteristics of a product. Because elucidation of the compounds that contribute to the undesirable flavor and odor of soy protein products has not yet been achieved, the compounds presented here may prove to be significant contributors to the undesirable flavor characteristics of such products.

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