Effects of Residual Solvents and Storage on Flavor of Hexane/Ethanol Azeotrope Extracted Soy Products

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Hexane/alcohol azeotrope extraction (HEAE) effectively removes objectionable flavors of defatted soy flakes. Volatiles from soy protein products were analyzed by gas chromatography (GC) and by GC–mass spectrometry. Levels of 2–3 ppm ethanal, hexanal, and pentane were found in whole mature soybeans as well as 5–20 ppm each of methanol, ethanol, and acetone. Levels of 25 ppm hexanal and 293 ppm pentane found in pentane–hexane defatted flakes were reduced 4- to 150-fold, respectively, after azeotrope extraction. Air-dried HEAE flakes had from 2000 to 6000 ppm of residual hexane and ethanol and had lower flavor scores than when residual solvent values were reduced to 160 ppm by various techniques. Storage at 38 and 25 °C for 6 and 12 months, respectively, resulted in reduced amounts of residual solvents and increased flavor scores of some untoasted azeotrope-extracted products over those of samples stored at −18 °C.

Use of edible soy protein products has a potential of increasing from an estimated 1130 million lb in 1974 to 3700 million by 1985 (Johnson, 1976). Objectionable flavor is the major factor that will limit the use of such soy products.

Hexane/ethanol azeotrope extraction (HEAE) effectively removed objectionable flavors of defatted soy flakes and, with toasting, resulted in flavor scores of soy products comparable to those of wheat flour and casein (Honig et al., 1976). Extraction for 1 h removes less material than extraction for 6 h, but similar flavor scores have been obtained (Eldridge et al., 1971).

The effectiveness of alcoholic solvent extraction in improving flavor scores has been attributed to removal of residual lipids, reduction of enzymatic activity, and modification of other components (Sessa and Rackis, 1977). Flavor scores of soy products including hexane/ethanol reextracted flakes have been correlated with hexanal level, but this was complicated by high residual levels of ethanol and hexane solvents (Rayner et al., 1978).

Toasting also inactivates lipoxygenase as well as other enzymes and together with azeotrope extraction may reduce flavor formation as well as more effectively remove flavor volatiles (Rackis et al., 1975).

It was of interest to know what changes in volatile levels occurred before and after azeotrope extraction; whether solvents were adequately removed by air-drying; or if residual solvent still affected flavor and at what levels. Volatile levels in products from pentane/hexane defatted azeotrope-extracted flakes were determined by gas chromatography (GC) and compared with values from flakes defatted only with pentane/hexane as well as with amounts found in whole soybeans and dehulled full-fat soy flakes. We also examined the effects of storage at −18, 25, and 38 °C on flavor scores of products from toasted and untoasted azeotrope-extracted soy flakes. Flakes extracted for 1 h were compared with a sample extracted for 6 h to see if less complete extraction might affect stability. Changes in residual solvent during storage were also examined.

MATERIALS AND METHODS

Sample Storage. The following soybean products prepared in the laboratory as previously described (Honig

et al., 1976) were stored in closed glass containers in the dark: Pentane/hexane defatted flakes reextracted with hexane/ethanol azeotrope (82:18, v/v) at 56 °C for 1 or 6 h; toasted (T), defatted, HEAE flakes; toasted protein concentrate prepared by water extraction of defatted, HEAE flakes at pH 4.5 and then toasted; protein isolate from toasted pentane/hexane defatted, HEAE flakes, prepared by water extraction at pH 7.2, 75 °C, followed by precipitation of protein in the extract at pH 4.5. Toasting refers to treatment with steam at 100 °C for 10 min.

One series of samples was aged by storing for 6 months at 38 °C, then stored at −18 °C for 6 months until analyzed by the taste panel. A second series was aged at 25 °C for 12 months, then at −18 °C for about 6 months until tasted. A third series of samples from the same preparations were held from the beginning at −18 °C as reference samples.

Odor and Flavor Evaluation. Taste panel evaluations of samples were carried out as previously described (Honig et al., 1976). Two percent dispersions of an aged sample and a reference sample in charcoal-filtered tap water at room temperature were tasted in random order by each of the 15–20 experienced tasters at each session. Odor and flavor scores were based on a 10-point scoring system (Kalbrener et al., 1974) where 1 is very strong, 5 to 6 moderate, and 10 is bland. Panel members also described and rated the intensity of odors and flavors. The intensity value of individual descriptions was then calculated as

Table I. GC/MS Identified Volatiles from Full-Fat or Solvent-Extracted Soy Flours

component	RRT[a]	elution temp, °C
methanol	0.40	52
ethanal	0.64	62
ethanol	1.00	88
acetone	1.20	102
pentane	1.53	133
chloroform	1.69[b,c]	155
methylcyclopentane	1.74[b]	163
hexane	1.77	170
pyrrole	1.84[b,c]	176
toluene	2.05[d]	185
methyl ketone	2.08[b,c]	185
hexanal	2.11	185
1-hexanol	2.32	185

[a] Retention time on Porapak-Q programmed as described relative to ethanol. [b] Not detected in full-fat flakes. [c] Not detected in pentane/hexane defatted flakes. [d] Not detected in azeotrope-extracted flakes.

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Table II. Volatiles of Whole Soybeans vs. Full-Fat and Solvent-Extracted Flours by Direct GC

flour	μg/g sample as is basis						
	methanol	ethanal	ethanol	acetone	pentane	hexane	hexanal
whole bean	19	2	23	4	3	4	2
full-fat	5	2	19	5	1	1	3
pentane/hexane defatted	30	9	123	90	293	181	25
HEAE[a]	12	6	6200	40	2	2200	7
alcohol treated, defatted[b]	30		1510		850	1210	

[a] Soxhlet reextraction of pentane/hexane defatted flakes with hexane/ethanol azeotrope (82:18, v/v). [b] Pentane/hexane defatted flour from 50% ethanol soaked beans (Eldridge et al., 1977).
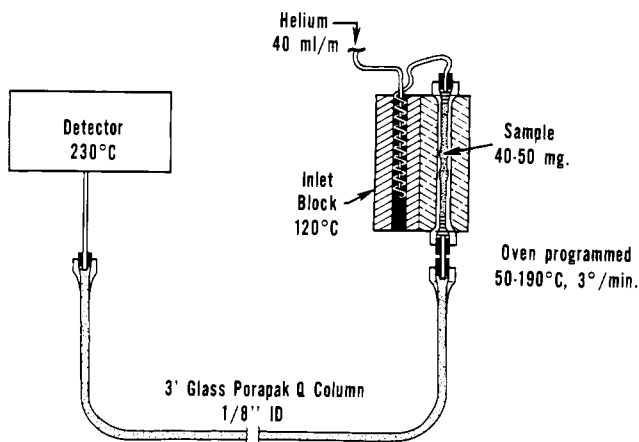

Figure 1. Diagram of direct sampling arrangement for GC analysis of soy volatiles on a Packard 7400 gas chromatograph equipped for on column injection.

Table III. Flavor Evaluation of Defatted HEAE Soy Products Prior to Aging

	flavor score	intensity value	
product[a]		grassy/beany	bitter
6-h HEAE flr.	6.0	1.1	0.5
1-h HEAE flr.	4.7	1.9	1.0
6-h HEAE flr.-T	6.9	0.8	0.4
1-h HEAE flr.-T	6.9	0.9	
protein concentrate	6.5	0.8	0.5
protein isolate	5.8		
wheat flour	7.7		

[a] HEAE = pentane/hexane defatted, hexane/ethanol azeotrope-extracted soy flakes. T = toasted.

reported by Rackis et al. (1972) based on a value of 1 for weak, 2 for moderate, and 3 for strong.

Analysis of Volatiles. Various samples of commercial or laboratory prepared soy products including products from HEAE flakes were analyzed for volatiles. Samples were ground in a Wiley Mill to pass a 40-mesh screen.

The direct GC sampling arrangement adapted from the procedures of Fore and Dupuy (1972) is illustrated in Figure 1. Samples of 40 or 50 mg are packed into a 7 cm by 3 mm i.d. glass precolumn between plugs of glass wool, and 80 μL of water is added on top. The precolumn is then placed in the inlet block of the chromatograph and connected by metal tubing through septa to the helium carrier gas and to the 84 cm by 3 mm i.d. separation column which is packed with Porapak P or Q. The heated carrier gas and water vapor sweep the low boiling volatiles from the sample directly onto the separation column. The chromatograph was a Packard 7400 series with on column injection and flame ionization detection (FID). Chromatographic conditions were: inlet, 120 °C; detector, 220 °C; oven-programmed from 50 to 200 °C at 5 °C/min; helium and hydrogen flow, 40 mL/min; air flow, 380 mL/min.

Table IV. Odor and Flavor Evaluation of HEAE Soy Products—Stored 6 Months at 38 °C[a]

product[b]	odor score		flavor score	
	ref[c]	aged	ref[c]	aged
6-h HEAE flr.	7.9	8.1	6.6	7.4[d]
6-h HEAE flr.-T	7.6	7.6	7.3	6.6
1-h HEAE flr.-T	8.0	8.0	6.8	6.1
protein concentrate	8.4	7.9	7.1	7.4
protein isolate	7.7	7.9	5.9	6.1

[a] Aged samples. [b] HEAE = hexane/ethanol azeotrope (82:18, v/v) Soxhlet extraction; T = toasting for 10 min at 100 °C. [c] Reference, original samples stored at −18 °C. [d] Significantly higher than reference score at 95% level.

Table V. Odor and Flavor Evaluation of HEAE Soy Products—Stored 12 Months at 25 °C[a]

product[b]	odor score		flavor score	
	ref[c]	aged	ref[c]	aged
6-h HEAE flour	7.4	7.7	6.2	6.2
1-h HEAE flour	7.4	7.8	6.2	6.6
6-h HEAE flour-T	7.5	7.4	6.6	7.1
1-h HEAE flour-T	7.8	7.5	6.8	6.3
protein concentrate	7.1	6.9	6.6	6.2
protein isolate	7.3	7.6	6.4	6.2

[a] Aged sample. [b] HEAE = hexane/ethanol azeotrope (82:18, v/v) extraction on Soxhlet. T = toasted 10 min at 100 °C. [c] Reference = original sample stored at −18 °C.

Quantitation of the volatile components was obtained by integrating areas under the curves and dividing by the response of known standards. The quantities were then converted to micrograms of volatiles per gram of sample. Water blanks were also run to correct for background.

For GC/mass spectrometry (MS), 0.7–1.5 g of sample in a 12 cm by 6 mm o.d. glass tube was heated to 120 °C in the inlet block of the Packard GC and a coil of heating wire. Volatiles were swept by helium (35 mL/min) onto a 3-ft glass column packed with Porapak Q. The trapping column in the GC oven was cooled between −30 and −50 °C with dry ice. After 30 min, the sample was replaced with an empty precolumn and after the oven temperature reached 30 °C, it was programmed from 30 to 150 °C at 5 °C/min and then to 220 °C at 10 °C/min to separate the volatiles with the inlet at 120 °C and detector at 240 °C. The column effluent was split 1 to 6, with the smaller portion going to the FID and the other part to a silicone separator that excluded helium while passing the sample volatiles to the Nuclide 12-90 double focusing, magnetic scanning, mass spectrometer. The output of the spectrometer, which scanned between m/e 10 and m/e 450 every 9 s, was fed to a computer for later processing. Components were identified from mass spectra. Identification was confirmed by comparing GC elution temperatures with those of authentic standards.

RESULTS AND DISCUSSION

Volatile constituents detected in untoasted full-fat,

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Table VI. Residual Solvent Values of HEAE Soy Flours after Storage (μg/g)

sample flour	-18 °C storage		12 months at 25 °C		6 months at 38 °C	
	ethanol	hexane	ethanol	hexane	ethanol	hexane
6-h HEAE	1340	3400	76	1230	124	780
1-h HEAE	420	3490	90	1020	51	337
1-h HEAE-T	150	110	30	9	63	5

Table VII. Flavor Evaluation of Azeotrope-Extracted Flakes at Two Levels of Residual Solvent[a]

	odor			flavor		
	solvent level			solvent level		
	I	II		I	II	
	score[b]			score[b]		
	6.7	7.7		6.4	7.2	
description	intensity	value	description	intensity	value
alcohol	0.4	0.1	chemical	1.1	0.3
fruity/ester	0.8	0	sweet/fruity	0.6	0.2
grassy/beany	0.4	0.1	grassy/beany	0.5	0.4

[a] Level I: 1300 μg/g of ethanol, 3400 μg/g of hexane; level II: 32 μg/g of ethanol, 130 μg/g of hexane. [b] Significant difference at the 95% level.

pentane/hexane defatted, or HEAE meals are listed in Table I. Methanol through pentane, hexanal, and hexanol were found in full-fat as well as solvent-extracted meals and would appear to be natural constituents of soybeans. Chloroform was found only in the azeotrope-extracted meal and may have been a contaminant in the extraction solvent. Methyl cyclopentane and hexane are components of commercial solvents and were estimated to be the major volatiles under the hexane peak from defatted and HEAE flours in a 25:75 ratio based on ratios of parent ion intensities in the mass spectra of the two compounds. The substituted benzene tentatively identified as toluene was also present in full-fat and solvent-extracted flours and may be a natural constituent. A pyrrole and a methyl ketone were tentatively identified in the HEAE meal only.

Content of the major volatiles found in ground whole soybeans, full-fat, defatted, or HEAE flours appears in Table II. Except for methanol, similar amounts of these volatiles were found in both the ground whole soybeans and the full-fat flakes, suggesting that they may already be present at approximately these levels in the intact bean. Much higher levels of pentane and hexane in the pentane/hexane defatted flakes or of ethanol and hexane in the HEAE flakes should represent mostly solvent residue, whereas higher levels of other volatiles in these flakes over those from ground whole soybeans suggest formation during processing and storage. Formation of carbonyls, in particular, is associated with lipid oxidation in soybean flakes (Sessa et al., 1969). Azeotrope reextraction appears to reduce levels of pentane and hexanal over amounts found in pentane/hexane defatted flakes. Direct defatting with hexane/ethanol azeotrope also resulted in over 2000 μg/g of residual solvent. The alcohol-treated pentane/hexane defatted flour also has over 3500 μg/g of residual solvent ethanol, pentane, and hexane. Not only did the pentane/hexane not remove all the ethanol, but higher levels of pentane and hexane were found compared to the

defatted sample not treated with ethanol. This suggests that ethanol denatures protein or in some other manner increases the amount of pentane and hexane bound by the meal. Binding of volatiles to protein, lipid, or carbohydrate have been reported by Mohammadzadeh-K et al. (1969) and Maier (1972). Maier found that some acetone and ethanol were bound irreversibly under his conditions.

Storage Effects. Initial flavor scores and descriptions of HEAE soy products before storage are shown in Table III. All soybean samples scored lower than wheat flour, whereas the sample extracted for only 1 h with no toasting scored only 4.7 with moderate intensities of grassy/beany and bitter flavors. Pentane/hexane defatted flour usually scored around 4.0 with the same taste panelists.

Scores of samples aged by storage for 6 months at 38 °C are shown in Table IV along with scores of reference samples held at -18 °C. The flavor score of the aged untoasted 6-h HEAE flour was significantly higher than that of the reference sample. As will be shown later, the increase may be accounted for by loss of residual solvent; alternatively, flavor compounds may have become irreversibly bound to the protein (Sessa and Rackis, 1977). The apparent decreases in flavor score of the aged toasted flours were not statistically significant.

The values for samples stored at 25 °C for 12 months appear in Table V. No significant differences in odor or flavor scores were found between these aged samples and the reference samples.

In summary, no significant decrease in flavor score in any of the samples during storage was found.

Residual Solvent and Flavor. Changes in residual solvent levels of HEAE flours during storage are compared in Table VI. Storage temperature rather than length of storage appears to be the most important factor in reducing the ethanol and hexane content. Starting with a total residual solvent level of nearly 5000 μg/g the untoasted 6-h HEAE flour had less than 1000 μg/g of ethanol plus hexane after storage for 6 months at 38 °C. Toasting of the solvent extracted flakes reduced ethanol and hexane content to low levels which decreased further during storage at 25 and 38 °C.

As shown in Table IV, the 6-h HEAE flour after storage at 38 °C for 6 months had a flavor score of 7.4 compared to the score of 6.6 for the reference sample. The increase in odor and flavor scores of aged, untoasted flours compared to scores of the reference samples can be attributed to loss of solvent and other volatiles during storage. It was therefore deemed advisable to reduce residual solvent levels below 1000 ppm to obtain maximum flavor scores for HEAE flours. The exact levels at which solvent residues, including minor components, affect flavor remain to be determined.

The influence of residual solvents on flavor score is shown more directly in Table VII. Untoasted 6-h HEAE

Table VIII. Content of Residual Solvents and Other Volatiles in Commercial Soy Protein Products

product	micrograms/gram of sample				
	methanol	ethanol	acetone	pentane	hexane
toasted, defatted flours	5–24	18–115	9–46	19	90–410
protein concentrate	3	7	15	2	2
protein isolate	6	32	34		6

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Table IX. Volatile Levels in Azeotrope-Extracted Flours after Various Treatments (μg/g)

volatile component	air-dried	freeze-dried[a]	vacuum strip		toasted[d]
			no steam[b]	steam[c]	
methanol	3	34	5	7	5
ethanal	3			0.1	
ethanol	3600	124	32	23	19
acetone	11			4	16
hexane	2200	350	130	470	12
hexanal	4		2	5	3

[a] Freeze-dried twice at 0.7 mmHg after wetting to 35% moisture. [b] Flakes wetted to 35% moisture, then dried on rotary evaporator at 50 °C. [c] Stripped on rotary evaporator while bleeding in steam. [d] Steam heated at 100 °C for 10 min.

Table X. Comparison of Drying Treatment and Residual Solvent Content of Defatted HEAE Soy Flakes with Flavor Score and Yield of Protein Isolate

	flakes			
	residual solvent		isolate	
drying treatment	ethanol, μg/g	hexane, μg/g	flavor score	yield,[a] %
freeze-dried	123	350	6.8	26.5
air-dried	1930	4040	7.2	13.5

[a] Percent of starting flakes extracted at pH 7.2 and room temperature.

flakes with a level of about 4700 μg/g of ethanol plus hexane were compared with similar flakes in which residual solvent content was reduced to about 160 μg/g by wetting the flakes to 35% moisture and then vacuum stripping to dryness. With the reduction in solvent content, odor scores increased from 6.7 to 7.7 and flavor scores from 6.4 to 7.2, both significant increases. The intensities of solvent-type odor and flavor descriptions as seen in the table also decreased with loss of solvent. Fruity/ester odor decreased from 0.8 to 0 and chemical flavor from 1.1 to 0.3. Grassy/benzy odor intensity also appears to be influenced since it decreases from 0.4 to 0.1. Of these descriptions, only grassy/beany was usually mentioned in previous evaluations of soy samples, including untoasted HEAE flours (Honig et al., 1976).

Volatile constituents found in commercial soy protein products are listed in Table VIII. Three samples of toasted, defatted soy flours contained from 18 to 115 μg/g of ethanol and 90 to 410 μg/g of hexane along with low levels of methanol, acetone, and pentane. Low levels of all five volatiles found in the commercial products are naturally present in whole soybeans as seen in Table II. Some formation of acetone has been attributed to heating (Dupuy et al., 1975), which may account for higher levels of acetone in some toasted samples. Higher levels of eth-

anol and hexane may be attributed, in part at least, to residual solvent. Most soy protein products are prepared from flakes defatted with hexane solvent (Johnson, 1976). Levels of volatiles in a protein concentrate were similar to levels found in the whole beans, with somewhat higher amounts of ethanol and acetone in the isolate. The levels found were all well below levels at which effects on flavor were evident.

Table IX illustrates the effectiveness of various procedures for removing solvents. Freeze-drying of wetted flakes, vacuum stripping at 50 °C of wetted flakes or with bleeding in of steam were all effective in reducing residual solvents below 500 μg/g. Toasting with steam at 100 °C for 10 min reduces solvent levels to the lowest levels but also greatly reduces protein solubility (Honig et al., 1976).

Finally, in Table X, protein yield of an isolate from a flour in which solvent levels were reduced below 500 μg/g by freeze-drying is compared to the yield of an isolate from an air-dried azeotrope-extracted sample. After the freeze-drying, the yield was comparable to that obtained from raw pentane/hexane defatted flakes. Flavor scores are similar. Whether the residual solvent in the air-dried flakes reduced protein extractability requires further investigation.

LITERATURE CITED

Dupuy, H. P., Rayner, E. T., Fore, S. P., *J. Am. Oil Chem. Soc.* **52**, 354 (1975).
Eldridge, A. C., Kalbrener, J. E., Moser, Helen A., Honig, D. H., Rackis, J. J., Wolf, W. J., *Cereal Chem.* **48**, 640 (1971).
Eldridge, A. C., Warner, K., Wolf, W. J., *Cereal Chem.* **54**, 1229 (1977).
Fore, S. P., Dupuy, H. P., *J. Am. Oil Chem. Soc.* **49**, 129 (1972).
Honig, D. H., Warner, K., Rackis, J. J., *J. Food Sci.* **41**, 642 (1976).
Johnson, D. W., "World Soybean Research", Hill, L. D., Ed., Interstate Printers and Publishers, Inc., Danville, IL, 1976, pp 1014–1017.
Kalbrener, J. E., Warner, K., Eldridge, A. C., *Cereal Chem.* **51**, 406 (1974).
Maier, H. G., *Lebensm. Wiss. Technol.* **5**, 1 (1972).
Mohammadzadeh-K, A., Feeney, R. E., Smith, L. M., *Biochim. Biophys. Acta* **194**, 246 (1969).
Rackis, J. J., Honig, D. H., Sessa, D. J., *Cereal Chem.* **49**, 586 (1972).
Rackis, J. J., McGhee, J. E., Honig, D. H., *J. Am. Oil Chem. Soc.* **52**, 249A (1975).
Rayner, E. T., Wadsworth, J. I., Legendre, M. G., Dupuy, H. P., *J. Am. Oil Chem. Soc.* **55**, 454 (1978).
Sessa, D. J., Rackis, J. J., *J. Am. Oil. Chem. Soc.* **54**, 468 (1977).
Sessa, D. J., Honig, D. H., Rackis, J. J., *Cereal Chem.* **46**, 676 (1969).