Relationships of Sensory Characteristics and Gas Chromatographic Profiles of Soybean Protein Products

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ABSTRACT

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To survey the present state of soy protein processing, commercial samples were evaluated for odor and flavor and analyzed by gas chromatography for volatile constituents and by microbiological procedures for molds, aerobic bacteria, and coliforms. Sensory tests showed flavor score ranges of 5.5–6.3 for flours, 4.4–5.9 for concentrates, 5.1–6.9 for isolates, and 5.3–6.7 for textured flours. Some objectionable flavor characteristics of raw soy were retained, and detrimental off-flavors were generated during processing. Major volatile constituents were residual solvents (ethanol and hexane) and hexanal. Significant statistical correlations exist between total volatiles and flavor scores of soy concentrates and isolates. Microbiological test results were typical of standard profiles. Direct gas chromatographic analysis was useful to screen soy products of known history for residual solvent and for content of volatile components related to oxidative deterioration.

A bibliography on the production, properties, and food uses of soybean proteins (Wolf 1974) contained 11 references on soy protein flavor. The literature on soy continues to emphasize flavor as the factor that limits the use of soybean protein products (Cowan et al 1973, Dutton 1978, Rackis et al 1979, Sessa 1979). Kalbrener et al (1971) surveyed 19 commercial soy products including concentrates, isolates, and flours for odor and flavor characteristics. The ranges in scores for the three types of products were 4.2–6.7 for flours, 5.6–7.0 for concentrates, and 5.9–6.4 for isolates. The authors concluded that most of the samples evaluated showed flavor improvement relative to raw, defatted soy flour, but that a truly bland soy product was not yet commercially available. Much research has been undertaken since that study; attempts to solve the soy flavor problem include: extracting with hexane-alcohol azeotropes (Honig et al 1976, Rackis et al 1973); wet-milling with ethanol (Eldridge et al 1977); extracting defatted soy flour with ethanol, methanol, and isopropyl alcohol (Baker et al 1979); soaking of soybeans in acidifed water to prevent off-flavor development (Kon et al 1970); and determining the contribution of oxidized phosphatidylcholines to the bitter taste of soy (Sessa et al 1976). Some of the soy protein research was aimed at analyzing soy products for volatile compounds. Maga (1978), Lovegren et al (1979), and Heydanek and McGorrin (1981a) identified volatiles in cereals and dried legumes before processing. Rackis et al (1972) developed flavor profiles of maturing soybeans, and Honig and Rackis (1975) later identified the volatile constituents of maturing soybeans. The effects of processing and storage on volatiles development in cereals and oilseeds have been reported by Legendre et al (1978), Rayner et al (1978), Honig et al (1979), and Heydanek and McGorrin (1981b).

Objectives of this study were to survey current soy protein processing, to develop a flavor profile for each soy product type, to determine the volatile compounds present in processed soy proteins, and to correlate flavor data with content of volatile components.

MATERIALS AND METHODS

Samples of commercial soy protein products were obtained from 10 manufacturers in the United States and Europe. The products included six concentrates, two textured concentrates, nine isolates, 12 flours, and 10 textured flours. None of the products contained additives such as coloring and flavoring agents, vitamins, or minerals. The flours had been hexane-defatted and toasted (moist heat processing) to varying degrees, except for one raw sample. The nitrogen solubility index (NSI) was determined according to AOCS official methods (1965). The concentrates were produced by either alcohol or dilute acid washing.

Sensory Analysis

A 15-member trained panel experienced in testing soybean protein products evaluated the samples for odor and flavor. The products were tested as 2% dispersions in carbon-filtered tap water at room temperature. The individual odors and flavors of the samples were rated on a 0–3 scale (0 = no odor and/or flavor, 1 = weak, 2 = moderate, and 3 = strong intensity). Odor-intensity values (OIV) and flavor-intensity values (FIV), which are weighed averages, were calculated by the following formula:

$$\text{OIV or FIV} = \frac{1 \times \text{No. weak responses} + 2 \times \text{No. moderate responses} + 3 \times \text{No. strong responses}}{\text{No. of testers}}$$

Overall scores were based on a 10-point scale, with 10 as bland (excellent quality) and 1 as strong (poor quality). Balanced incomplete block designs were used as testing patterns for the five types of products (Cochran and Cox 1957). Each product type was tested separately with a wheat flour control. The testers evaluated three samples at each panel sitting; each sample received a total of 18 scores that were used to calculate an overall mean score.

Volatile Component Analyses

The soy products were analyzed by gas chromatography (GC) using a modified direct technique for volatile components (Fore and Dupuy 1972, Honig et al 1979, Rayner et al 1978). A Packard 7400 instrument with a flame-ionization detector was fitted with a 3-ft long, ⅛ in. i.d. glass column packed with Porapak P (Supelco, Inc., Bellefonte, PA). Flow rates were: air, 380 ml/min; hydrogen, 40 ml/min; and helium carrier gas, 40 ml/min. Inlet temperature was 120°C, and the detector temperature was 230°C. A 100-mg sample, layered between glass wool, was packed into a 7 cm × 4 mm i.d. glass precolumn, which was then placed in the heated injection port. *n*-Butanol was chosen as an internal standard because its retention time did not overlap any of the volatile compounds detected in the samples. The internal standard, 1 μl of 0.1% *n*-butanol in water, and 50 μl of distilled, deionized water were injected onto the precolumn before it was connected to the Porapak P column. Peaks were identified by comparing retention times with those of reference compounds. Quantitations were achieved by standardizing peak areas with a known amount of *n*-butanol and calculating the response factors for 1 ppm of each compound relative to 1 ppm of *n*-butanol. Replicate tests of each sample were run.

[1]The mention of firm names or trade products does not imply their endorsement or recommendation by the U.S. Department of Agriculture over other firms or similar products not mentioned.

Microbiological Analyses

Standard microbiological methods were used to determine total molds, aerobic bacteria, and coliforms on the dry soy protein products (Bothast et al 1974).

Statistical Analyses

The sensory and GC data were analyzed by a one-way analysis of variance. Correlation coefficients were calculated, and multiple regression analysis (Snedecor 1956) and cluster analysis (Hartigan 1975) were also performed.

RESULTS AND DISCUSSION

The flavor of soybean products comes from three sources: the bean itself, processing, and deterioration during storage (Dutton 1978). The range of flavor descriptions and corresponding intensity values for samples we tested indicate that processing conditions for commercial soy products are responsible for generating flavors not typical of raw soybeans. The profile of the raw bean includes a strong grassy/beany flavor and a weak, bitter taste (Rackis et al 1972). Therefore, to improve flavor quality in a particular soy product, it is necessary to determine at what point of manufacture specific flavors are removed, generated, or intensified. It should then be possible to improve the sensory quality without sacrificing nutritional and functional properties.

Sensory and Volatile Component Data

All soy products examined in this study retained, to varying degrees, the original grassy/beany flavor and bitter taste of the raw, mature soybean (Table I). The intensity values for grassy/beany and bitter in the raw bean were 2.7 and 1.0, respectively, on the 0–3.0 scale for description intensity. Processing significantly decreased the grassy/beany flavor to 0.6 or less in all soy product types, and the bitter taste was reduced slightly, to 0.6 or less.

The range of flavor scores for the soy products was from 4.1 for a concentrate to 6.9 for an isolate. The wheat flour tested as a control received an average odor and flavor score of 8. A raw, defatted soy flour tested with the flour group received an average score of 3.4. Neither of these two flours was included in calculating the averages. The mean flavor scores for the five product types show a significant difference between types, with the concentrates rating the lowest and the isolates the highest. All product types were described as having cereal and grassy/beany flavors and bitter taste. One-way analysis of variance showed that intensities of both bitter and cereal characteristics were not significantly different among the product types, but the grassy/beany characteristic was significantly stronger in the flour than in the textured flour and isolates. The highest intensities of toasted flavor were in the textured concentrate and textured flour samples, whereas the isolate samples had no toasted flavor. The concentrates and isolates had the highest amount of other off-flavors. The off-flavor descriptions were a combination of flavors that did not occur often enough to list separately. Typical off-flavor descriptions included: soapy, cardboardy, metallic, woody, sulfur, and rancid.

Chromatograms of soy protein products obtained in this study by a direct GC method were similar to those reported by Rayner et al (1978). Fourteen predominant peaks were quantitated and correlated with sensory data. Five of the seven peaks eluting between 55 and 110°C were identified as methanol, acetaldehyde, ethanol, acetone, and hexane. The group of volatile compounds eluting after the butanol standard at 115°C included pentanal, hexanal, heptanal, 2-heptanol, and 2-pentylfuran plus two unidentified peaks.

Multiple regression equations were calculated between sensory data including overall odor and flavor scores plus descriptions (such as cereal/grain, grassy/beany, toasted, musty/stale, fermented, and "off") and the volatile components data including 14 individual peaks plus total volatiles. These calculations were done for all soy products combined. Correlation coefficient for flavor scores vs total volatile compounds was −0.58. Neither individual odor/flavor descriptions nor overall scores correlated significantly with individual GC peaks.

To determine if grouping GC peaks for comparison with sensory data would improve correlations, the volatile compounds eluting between 55 and 110°C, which mainly result from processing, were combined, as were those eluting between 115 and 180°C, which are usually from oxidative deterioration. Multiple regression analysis was then used on the two groups of volatile compounds plus total volatiles and the overall scores plus individual descriptions. Flavor score vs total volatile component correlations were significant only for the isolate and concentrate groups, with coefficients of −0.74 and −0.58, respectively. The isolates and concentrates had a wider range of flavor quality than did the textured flour and flour (excluding raw flour) groups, which could account for better correlations. Only the isolate group had significant correlations of total volatile compounds with individual descriptions ($r = 0.90$ for grassy and $r = 0.78$ for painty). Most of the soy samples had too small a range of description intensities for correlations with volatiles to be significant. Honig and Rackis (1975) found no direct correlation between changes in volatile components and beany and bitter characteristics in raw mature and immature soybeans. Wilkens and Lin (1970) concluded that the flavor of soy protein products is likely to be caused by a large mixture of compounds rather than a limited number. The results from our study confirm this conclusion.

Soy Flours

Sensory and volatile components data for the 12 soy flours are presented in Table II. The lowest-scoring soy flour (Sample A) was a raw hexane-defatted flour. The data for raw flour are included in the tables for information only and were not included in calculations. The other samples were toasted flours with a relatively

TABLE I
Sensory Data for Five Soy Product Types

Descriptions	Flours	Textured Flours	Concentrates	Textured Concentrates	Isolates	Significance[a]
			Flavor Scores[b]			
Means[c]	5.9 a	6.0 a	5.4 b	6.0 a	6.1 a	
Ranges	5.5–6.3	5.3–6.6	4.1–5.9	5.9–6.0	5.1–6.9	
			Flavor Intensity Values[d]			
Cereal	0.7	1.0	0.8	0.9	0.7	NS
Grassy/beany[c]	0.6 a	0.4 a	0.5 ab	0.5 ab	0.4 b	**
Bitter	0.6	0.6	0.4	0.4	0.4	NS
Toasted[c]	0.4 a	0.8 b	0.2 a	0.9 b	0 a	**
Fermented[c]	0 a	0 a	0.3 b	0 a	0 a	**
Off-flavors[c]	0.3 a	0.3 a	0.9 b	0.2 a	0.7 b	**

[a] NS = Not significant; ** = significance at $P = .99$.
[b] 10 = Bland, 1 = strong.
[c] Scores and intensity values with letters in common are not significantly different at 95% confidence level.
[d] 0 = None, 1 = weak, 2 = moderate, 3 = strong intensity.

TABLE II
Flours: Sensory and Volatile Constituent Data

Sample	Favor Score[a]	Flavor Intensity Values: Grassy/Beany	Toasted	NSI[b]	Volatile Constituents (ppm): 55–110°C	115–200°C
A	3.4	2.7	0	90	181	17
B	5.5	0.6	1.1	20	82	8
C	5.5	0.7	0.9	14	88	14
D	5.8	0.4	0.6	21	96	41
E	5.8	0.7	0	70	48	17
F	5.9	0.9	0.1	71	304	14
G	5.9	0.6	0.1	46	88	13
H	6.0	0.8	0.3	57	129	21
I	6.0	0.8	0.4	20	62	9
J	6.0	0.6	0.4	15	93	26
K	6.1	0.9	0.1	40	82	10
L	6.3	0.6	0.4	32	190	9
$\bar{X}$	...	...	...	...	121	17

[a] Least significant difference = 0.5.
[b] Nitrogen solubility index.

small flavor score range of 5.5–6.3. A least significant difference (LSD) between flavor scores of 0.5 was calculated. The raw defatted soy flour with an NSI of 90 retained the same intensities of grassy/beany and bitter as the raw bean, but it also had a weak, astringent note. Heat treatment of the soy flours significantly decreased the grassy/beany flavor, but cereal/grain and toasted characteristics still were evident. The toasted flavor developed with heat treatment of the flour and textured flour products. The intensity of the toasted flavor correlated significantly with the NSI of soy flours, with a coefficient of −0.74 (Fig. 1). The correlation coefficient of grassy/beany vs NSI was 0.49. The cereal flavor may either develop with heating or may exist naturally in the bean and be masked by the grassy/beany flavor. Overtoasting caused the flours to be rated low, as was noted with the grassy/beany flavor. The 0.6 difference in flavor scores between sample B and sample K is probably due to the amount of toasting given to the sample B as indicated by the differences in NSI values and toasted flavor descriptions. Generation of other off-flavors is not a problem in soy flours. Musty/stale is the most predominant off-flavor. The most common residual solvent in the flours was hexane, but a few samples also contained ethanol. The amount of residual solvent did not affect the flavor scores significantly, although sample F, which had the highest content of volatile compounds in the 55–110°C range, was given the highest number of off-flavor descriptions. The most commonly observed aldehyde eluting above 115°C was hexanal at an average of 6 ppm, but it did reach 26 ppm for flour sample D. No significant correlations were obtained between sensory and volatile component data for the flours, partly because products with similar flavor evaluations had dissimilar volatile compound profiles. Cluster analysis of the flour data grouped samples A and H for common GC patterns, but panel members reported distinctly different flavor scores and descriptions.

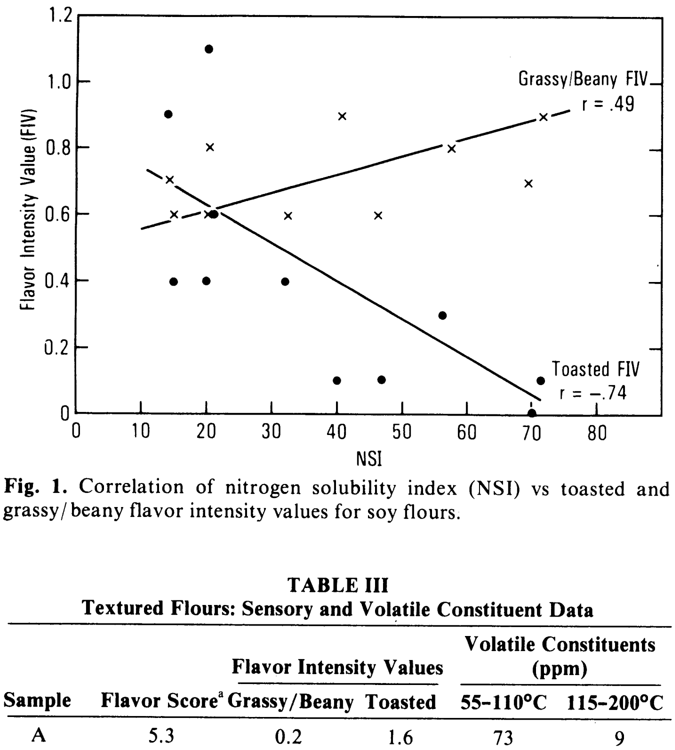

Fig. 1. Correlation of nitrogen solubility index (NSI) vs toasted and grassy/beany flavor intensity values for soy flours.

TABLE III
Textured Flours: Sensory and Volatile Constituent Data

Sample	Flavor Score[a]	Flavor Intensity Values		Volatile Constituents (ppm)	
		Grassy/Beany	Toasted	55–110°C	115–200°C
A	5.3	0.2	1.6	73	9
B	5.5	0.4	1.0	46	8
C	5.5	0.4	1.1	37	6
D	5.6	0	1.2	48	7
E	5.8	0.6	0.9	76	24
F	6.1	0.6	0.3	59	8
G	6.3	0.5	0.7	28	5
H	6.4	0.6	0.3	56	9
I	6.4	0.4	0.8	46	4
J	6.6	0.7	0.3	65	13
$\bar{X}$	...	...	...	53	9

[a]Least significant difference = 0.5.

TABLE IV
Concentrates: Sensory and Volatile Constituent Data

Sample	Flavor Score[a]	Flavor Intensity Values			Volatile Constituents (ppm)	
		Cereal	Musty	"Off"	55–110°C	115–200°C
A	4.1	0.5	0.7	1.5	408	12
B	5.3	0.9	0.1	0.8	469	9
C	5.4	0.7	0.5	1.6	61	13
D	5.6	1.0	0.5	1.3	67	25
E	5.8	0.8	0.3	0.7	180	6
F	5.9	0.9	0.1	0.7	238	8
$\bar{X}$	...	...	...	...	237	12

[a]Least significant difference = 0.5.

Textured Soy Flours

Texturization of soy flour increased the cereal/grain and toasted flavors compared to those in defatted flours (Table I). Grassy/beany flavors decreased correspondingly, whereas the bitter taste remained at the same intensity level. Off-flavors were not common in textured soy flours, but a sulfur flavor was occasionally detected. The sulfur flavor likely is an artifact of the texturization process because it is not found in other soy products. The amount of volatile compounds in the textured flours was relatively low (Table III). Hexane was the major residual solvent. The total amount of volatile compounds eluting after 115°C was less than 14 ppm for all samples except one. Hexanal was the major volatile compound with an average of 2 ppm. Although the scores ranged from 5.3 to 6.6 and showed significant differences between products, the range in volatile compound content was small. No significant correlations were found between sensory characteristics and volatile components. Cluster analysis again grouped products because of similar volatile compound patterns (samples B, C, G, and I), but flavor scores and descriptions differed enough to result in low correlation coefficients.

Soy Protein Concentrates

The range in flavor scores was 1.8 for all soy protein concentrates but only 0.6 when the low-rated sample was omitted (Table IV). Sample A, with a score of 4.1, was not typical of concentrates currently produced because of the low score and the off-flavor descriptions of sour and woody. Comparison of concentrates with defatted flakes or flours revealed only slight variations in the basic flavor characteristics, but concentrates showed a large increase of off-flavors such as fermented, metallic, and spoiled that are not detected in the other forms of soy. The off-flavors in concentrates were common to both the alcohol- and the acid-washed products. Concentrates also had more total off-flavors than any of the other soy product types. These characteristics influenced the overall flavor and odor scores markedly and were the major reason why concentrates rated significantly lower than the other four soy product types. The main residual solvent in four of the six samples was ethanol. The other two samples had hexane as the major residual solvent. The amounts of volatile compounds eluting above 115°C were relatively low, with hexanal the major contributor at an average of 4 ppm. A correlation coefficient of −0.58 for flavor score vs total volatile compounds was statistically significant.

Two textured concentrates, which were evaluated in separate tests, had flavor characteristics similar to regular concentrates but differed slightly in intensity of grassy/beany and the amount of residual ethanol left in the product. Average flavor data are shown in Table I. The off- and fermented flavors typical of concentrates are significantly decreased with texturization. Hexanal is the major volatile eluting after 115°C. The results of the sensory and GC analyses of the textured concentrates were similar to those obtained for textured flour and granular concentrates, with the exception that the toasted flavor characteristic was lower in the granular form (unpublished data.)[2]

[2]K. Warner. 1980.

Soy Isolates

The isolates had the widest range of flavor scores (5.1–6.9) of the five product types tested (Table V). High intensity values for grassy/beany, bitter, and off-flavor descriptions, such as painty, soapy, and cardboardy, caused some of the isolates to be rated low. The isolates had more volatile compounds eluting after 115° C than any of the other product types. As with the other products, hexanal was the major aldehyde, followed by pentanal and 2-pentylfuran. Factors other than high levels of volatile components caused some of the samples to receive low scores. Sample B had relatively low levels of volatile compounds but a high intensity value for bitter, which is probably not due to volatile compounds (Sessa 1979). Significant correlation coefficients were obtained between total volatile components and grassy/beany (0.90) and painty (0.78) flavors. The overall flavor score correlated with total volatile constituents with a significant coefficient of −0.74.

Hexane and Hexanal Contents

To evaluate effects of soy processing and deterioration of products, the hexane and hexanal contents were determined in the soy samples (Table VI). The amount of hexane left in soy flour was somewhat dependent on the amount of heat treatment. A correlation coefficient of 0.67 was obtained between the NSI of flours and the hexane content. The additional processing given the textured flours, concentrates, and isolates reduced hexane levels significantly. Hexanal was responsible for 50% of the volatile compounds eluting above 115° C in isolate samples.

Microbiological Analyses

Analyses of bacteria, molds, and coliforms were either below or within the quantitative ranges of normal microbiological profiles of soy protein (Table VII). According to Hobbs and Greene (1976), the range for bacterial counts is 10^2–10^5 and 10^2–10^3 for coliforms. Coliforms were detected in three flours, one concentrate, and one textured flour. These samples also had high total aerobic bacteria counts. The genera of fungi found in the products were *Penicillium, Fusarium,* and *Aspergillus.*

CONCLUSIONS

Based on the sensory profiles of the soy products examined, the extraction of the original grassy/beany and bitter components of the raw soybean is incomplete under the manufacturing conditions now used. These methods also add off-flavors that are detrimental to the flavor quality of final products. Modification of certain processing parameters may be needed to further decrease undesirable flavors in soy protein products. Rackis et al (1973) reported that a combination of hexane/ethanol azeotrope extraction and toasting of soy flakes was more effective in diminishing grassy/beany and bitter characteristics than were more conventional methods.

Based on the volatile component analyses of the soy samples, use of a GC technique to evaluate the flavor of unaged samples with unknown history is not recommended. Although large amounts of residual solvent are left in some products and contribute to poor flavor quality, they do not correlate significantly with flavor characteristics of the soy products. Volatile compounds contributing to the cereal and toasted flavors of the soy were not

TABLE V
Isolates: Sensory and Volatile Constituent Data

Sample	Flavor Score[a]	Flavor Intensity Values			Volatile Constituent (ppm)	
		Grassy/Beany	Bitter	"Off"	55–110°C	115–200°C
A	5.1	0.8	0.3	0.5	153	99
B	5.3	0.3	1.4	0.5	34	23
C	5.4	0.6	0.3	0.5	93	97
D	5.8	0.5	0.3	0.7	66	41
E	6.0	0.4	0.3	0.6	28	23
F	6.6	0.3	0.4	0.5	45	17
G	6.7	0.3	0.2	0.5	24	15
H	6.8	0.4	0.2	0.3	21	17
I	6.9	0.3	0.3	0.4	47	19
$\bar{X}$	···	···	···	···	57	38

[a]Least significant difference = 0.5.

TABLE VI
Hexane and Hexanal Contents of Soy Products

Sample Code	Flour		Textured Flour		Concentrate		Isolate	
	Hexane	Hexanal	Hexane	Hexanal	Hexane	Hexanal	Hexane	Hexanal
A	87	8	6	3	4	2	15	45
B	15	2	3	1	3	3	1	15
C	21	5	1	1	4	6	4	45
D	12	26	2	2	5	11	7	22
E	16	7	11	5	19	2	1	12
F	73	6	3	2	27	3	1	9
G	30	6	1	1	···	···	2	6
H	42	7	10	3	···	···	1	7
I	4	3	1	1	···	···	1	11
J	31	7	14	6	···	···	···	···
K	30	5	···	···	···	···	···	···
L	65	4	···	···	···	···	···	···

Volatile Components (ppm)

TABLE VII
Microbiological Data for Five Soy Product Types[a]

Analyses	Flour	Textured Flour	Concentrate	Textured Concentrate	Isolate
Aerobic bacteria, g	1.9×10^2–6.1×10^4 (9×10^3)	0–2.4×10^4 (4×10^3)	2×10^1–2.4×10^3 (8.8×10^2)	2.5×10^1–7.1×10^3 (3.5×10^3)	0–4.4×10^3 (1×10^3)
Coliforms, g	0–690 (71)	0–58 (6)	0–108 (18)	0 (0)	0 (0)
Total molds, g	0–1.2×10^2 (1.4×10^1)	0–2.7×10^2 (4×10^1)	0 – 9.8×10^1 (2.7×10^1)	0 (0)	0–1×10^1 (2)

[a]Values in parentheses are mean values.

detected by this GC method. Few significant correlations were found between volatile carbonyl compounds and flavor data. This finding is compatible with the work of Sessa et al (1969), which showed that removal of carbonyls from defatted flakes did not change the flavor characteristics of the flakes; their conclusion was that these compounds contribute little to the overall soybean flavor. Therefore, direct GC analysis is only useful to screen soy products of known history for residual solvent and for volatile compounds indicative of oxidative deterioration.

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