Secondary Extraction of Soybeans Using Hexane–Acetic Acid: Effect on Beany Flavor Removal and Physicochemical Properties

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Sensory and physicochemical characteristics of soy meal obtained after secondary extraction of soy flakes using hexane containing 3 or 5% acetic acid were compared with those of hexane-extracted meal. Sensory evaluation study using the threshold dilution technique showed a 55–63% reduction of beany flavor in treated meals. Hexane containing 5% acetic acid treatment showed total inactivation of lipoxygenase (L-1 and L-2 + L-3), but no effect on trypsin inhibitor activity. Compared to hexane-extracted meal, the nitrogen solubility index of hexane containing 3 and 5% acetic acid extracted meal showed reductions of 19 and 39%, respectively. In vitro digestibility increased in treated sample (81.7%) as compared to that of hexane-extracted meal (76.7%). Gel filtration, polyacrylamide gel electrophoresis, and fluorescence emission spectrum indicated the dissociation of proteins into lower molecular weight protein fractions in treated meals; the higher the concentration of acetic acid in hexane, the greater was the extent of dissociation.

INTRODUCTION

Of 1.8 million tons of current production of soybean in India, nearly 1 million tons of defatted soy meal is available, and most of it is exported. Although the number of soy-based industries incorporating soybean in different food formulations has increased, consumer acceptability of various products, especially soy milk, is limited due to the presence of beany, bitter, and astringent flavors. Considerable evidence has accumulated in the literature implicating lipoxygenase-mediated conversion of lipids to lipohydroperoxides and their subsequent degradation to form volatile and nonvolatile constituents responsible for off-flavors (Wolf, 1975; Sessa and Rackis, 1977; Sessa, 1979; Moll et al., 1979; MacLeod and Ames, 1988). Various treatments such as hot water grinding of soybeans, extrusion cooking, blanching, and grinding at low pH, have been shown to inactivate lipoxygenase, resulting in soy flour of superior quality (Wolf, 1975). Hexane partially extracts phosphatides, but residual lipids, especially oxidized phosphatidyl choline, have been claimed to cause bitter and beany flavors in soybean and its products (Sessa et al., 1974, 1976). Aqueous ethanol, 2-propanol, and azeotropic mixtures of hexane and alcohols have been used for secondary extraction of residual lipids from hexane-extracted meal to improve flavor and odor (Johnson and Lusas, 1983). We have recently reported some of the quality characteristics of primary hexane–acetic acid extracted soy oil and advocated the use of hexane–acetic acid as a secondary extraction solvent rather than a primary one, in view of some quality changes observed in the primary extracted oil (Bhagya and Srinivas, 1991). There is no information available in the literature on the effect of such extraction on the quality of soy meal. In this paper, the sensory and physicochemical characteristics of soy meal obtained after secondary extraction of soy flakes using hexane–acetic acid solvent are reported.

MATERIALS AND METHODS

Soybean seeds of the yellow variety Clark 63 were obtained from M/s Shakthi Soya Ltd., Coimbatore, India. The seeds were cleaned, cracked, and dehulled according to the method described by Shamanthaka Sastry et al. (1969). The dehulled seeds were equilibrated to 12% moisture and passed through flaking rolls to obtain flakes of 0.3-mm thickness and dried to 5% moisture at 40 °C.

Secondary Extraction of Soy Flakes with Hexane–Acetic Acid. Soy flakes (35 g) were initially defatted with food grade hexane in a Soxhlet extractor. Secondary extraction was carried out with hexane containing 3 or 5% acetic acid (v/v) for 3 h. The solvent to flake ratio in the extractor was 1.5:1. The temperature in the Soxhlet extractor was 58 °C, which is the boiling point of the hexane–acetic acid azeotropic mixture (Hensarling and Jacks, 1983). The flakes were desolventized in a vacuum drier at 50 °C and powdered to pass through a 60-mesh sieve.

Proximate Analysis. Moisture, ether extractives, protein (N × 5.71), and total ash contents were determined according to AOAC (1984) methods. Fiber content was estimated according to the neutral detergent fiber method of analysis (Goering and Vansoest, 1970). Carbohydrate was calculated by difference.

Sensory Evaluation Studies. To quantify the beany flavor present in soybean, the dilution technique was used. The flavor components were extracted with 0.75% NaCl solution for 1 h at room temperature (~28 °C). The suspension was centrifuged at 4000 rpm for 20 min. The clear supernatant solution was used to prepare further dilutions with 0.75% NaCl solution and used in the threshold test. Ten trained panelists from the scientific staff of the institute, familiar with beany flavor, were asked to identify the beany notes in the diluted extracts. The recognition threshold was used to quantify the beany flavor present in the samples. For the assessment of recognition threshold, the ascending concentration series method (Indian Standards, 1983) was used. The ascending series for each sample consisted of five dilutions around the approximate threshold determined by a small panel and a blank (0.75% NaCl solution). Of these dilutions, the panelists evaluated samples only up to the level at which they could identify the beany flavor. Between evaluations, water and 0.75% NaCl solution were used for palate clearance.

Nitrogen Solubility Index (NSI). One gram of sample was suspended in 30 mL of distilled water, and the pH of the suspension was adjusted to 7.0 or 9.0 and shaken for 1 h. The volume was made up to 50 mL with water, and the contents were centrifuged at 6000 rpm for 20 min. Nitrogen content in a known aliquot of the supernatant was determined according to the Kjeldahl method, and the values are expressed as percent of the total nitrogen present in the samples.

Trypsin Inhibitor Activity. This was determined according to the procedure of Kakade et al. (1974) using benzoyl-DL-arginine *p*-nitroanilide hydrochloride as substrate and 2× crystallized bovine trypsin. Results are expressed as trypsin inhibitor units (TIU) per milligram of flour.

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Table I. Proximate Analysis of Hexane and Hexane Containing 3 or 5% Acetic Acid Extracted Soy Flour[a]

constituents, %	hexane	hexane containing 3% acetic acid	hexane containing 5% acetic acid
protein (N × 5.71)	48.1	49.1	49.0
ether extractives	0.4	tr	tr
fiber	6.7	6.8	7.0
ash	7.7	7.6	7.7
carbohydrate (by diff)	37.1	36.5	36.3

[a] Values are expressed on moisture-free basis and averages of two independent determinations.

Lipoxygenase Activity. The lipoxygenase activity, in 0.2 M sodium phosphate buffer (pH 6.5) extracts of soy meal, was determined according to the procedure of Axelrod et al. (1981). The activities of lipoxygenases L-1 and L-2 + L-3 were determined at pH 9.0 and 6.5, respectively, using linoleic acid as substrate and measuring the change of absorbance at 234 nm.

In Vitro Digestibility. The method of Akeson and Stahman (1964) using pepsin–pancreatine enzyme systems was employed. The digested protein correlated to the total protein was expressed as percent digestibility value.

Gel Filtration. One gram of sample was extracted in 10 mL of 0.1 M phosphate buffer (pH 7.5) in 0.4 M NaCl containing 0.01 M β-mercaptoethanol for 1 h and centrifuged at 10 000 rpm for 20 min. The clear supernatant was extensively dialyzed against the same buffer. The dialyzed extract (85 mg of protein equivalent) was loaded on to a column of Sepharose 6B (1.5 × 98 cm) which had been equilibrated with the buffer and eluted with the same buffer at a flow rate of 25 mL/h. Fractions of 3 mL were collected in an LKB automatic fraction collector, and the absorbance of each fraction was measured at 280 nm.

Polyacrylamide Gel Electrophoresis (PAGE). Electrophoresis was performed using 7.5% gels in 0.02 M phosphate buffer (pH 7.5) containing 0.01 M β-mercaptoethanol. One hundred micrograms of protein equivalent in the same buffer was applied to each gel, and electrophoresis was run at a constant current of 3 mA/tube. The gels were stained with 0.5% amido black in 7.5% acetic acid for 30 min and destained with 7.5% acetic acid.

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS–PAGE). The various fractions obtained after gel filtration of total proteins from control and treated soy meal were pooled and concentrated, and slab SDS gel electrophoresis was performed according to the procedure of Laemmli (1970) using 12.5% gels. About 50 μg of protein was applied to each gel slot, and electrophoresis was performed at a constant current of 8 mA/slot. After the electrophoresis, the gel slab was removed and fixed in a solution of methanol, acetic acid, and water (5:10:85 v/v/v) stained with 0.1% Coomassie Blue R250, and destained with the same solvent.

Fluorescence Spectrum. The fluorescence emission spectrum was recorded in the range 300–400 nm after excitation at 280 nm using an Aminco Bowman spectrofluorometer. Protein solution in 0.1 M phosphate buffer in 0.4 M NaCl (pH 7.5) containing 0.01 M β-mercaptoethanol, having an absorbance of 0.1 at 280 nm, was used.

RESULTS AND DISCUSSION

The proximate analysis of hexane and hexane–acetic acid treated soy meal is given in Table I. The contents of protein, total ash, fiber, and carbohydrate did not show any variations among different samples. The value for ether extractives of hexane-extracted meal was 0.4% compared to traces in hexane–acetic acid extracted meal.

Sensory Evaluation Studies. In the present investigation, sensory evaluation was conducted on sample extracts made with 0.75% NaCl solution. This modification was found to be more suitable and sensitive than the earlier methods of using 2% dispersion of the flour in water and expressing the results on a 10-point intensity scoring scale, 10 being bland and 1 strong (Warner and Baker, 1984; Frankel et al., 1988). As far as we are aware, the dilution threshold method of ascending concentration series used here for the quantification of beany flavor present in soy meal has not been reported. Madhavi et al. (1989) have used this method for the quantification of nutty flavor present in processed groundnut meal. The results of the sensory analysis are given in Table II. In the case of hexane-extracted soy meal, the beany note was recognized at a dilution of 1480 mL/g of meal, while for the hexane containing 3 and 5% acetic acid extracted meals, the dilution for recognition came down to 662 and 540 mL/g meal, respectively. The results show that secondary extraction of soy flakes with hexane–acetic acid removed the beany flavor to an extent of 55–63%. Honig et al. (1976) have reported that extraction of defatted soy flakes with hexane alcohol azeotrope resulted in removal of residual lipids and flavor components and obtained products with improved flavor.

Table II. Recognition Threshold Level for the Detection of Beany Flavor in Soy Meal Extracted with Hexane–Acetic Acid

sample	mean threshold, mL/g of meal	SD[a]	beany flavor removed, %
hexane	1480	1.24	0
hexane contg 3% acetic acid	662	1.80	55.2
hexane contg 5% acetic acid	540	1.92	63.5

[a] The statistical analysis was carried out after transformation of data into logarithms; the mean and standard deviations are values obtained by back transformation.

Table III. Nitrogen Solubility Index (NSI), Trypsin Inhibitor Activity, Lipoxygenase Activity, and in Vitro Digestibility of Hexane-Extracted and Hexane–Acetic Acid Extracted Soy Meal[a]

sample	NSI, % pH 7.0	NSI, % pH 9.0	trypsin inhibitor activity, TIU/mg of meal	lipoxygenase activity, units/mL of extract L-1	lipoxygenase activity, units/mL of extract L-2 + L-3	in vitro digestibility, %
hexane	71.0	89.0	110	10.8	27.2	76.7
hexane contg 3% acetic acid	57.5	72.0	106	0	14.4	76.7
hexane contg 5% acetic acid	43.2	55.5	110	0	0	81.7

[a] Values are averages of two independent determinations.

Nitrogen Solubility Index. At pH 7.0, the NSI of hexane-extracted meal was 71%, and the NSIs of hexane containing 3 and 5% acetic acid extracted meal were 57.6 and 38.7%, respectively (Table III); the reductions were 19 and 39%, respectively. This reduction of NSI values could be due to denaturation of proteins by acetic acid. At pH 9.0, although the NSI values were higher, the extent of reduction in NSI of treated meals was similar to that at pH 7.0. Hensarling et al. (1974) have reported that the solubility of cottonseed proteins in 0.02 N NaOH was not affected up to 4% concentration of acetic acid in hexane.

Trypsin Inhibitor Activity (TIU). The TIU of control and treated meals is given in Table III. The value for hexane-extracted meal was 110 TIU/mg of meal, and no reduction in the values after treatment was observed. Extraction of soy flakes with hexane–alcohol azeotrope did not result in reduction of trypsin inhibitor activity (Honig et al., 1976).

Lipoxygenase Activity. Soybean contains three lipoxygenase isozymes, namely L-1, L-2, and L-3. The activity of L-1 and L-2 + L-3 of control and treated meal

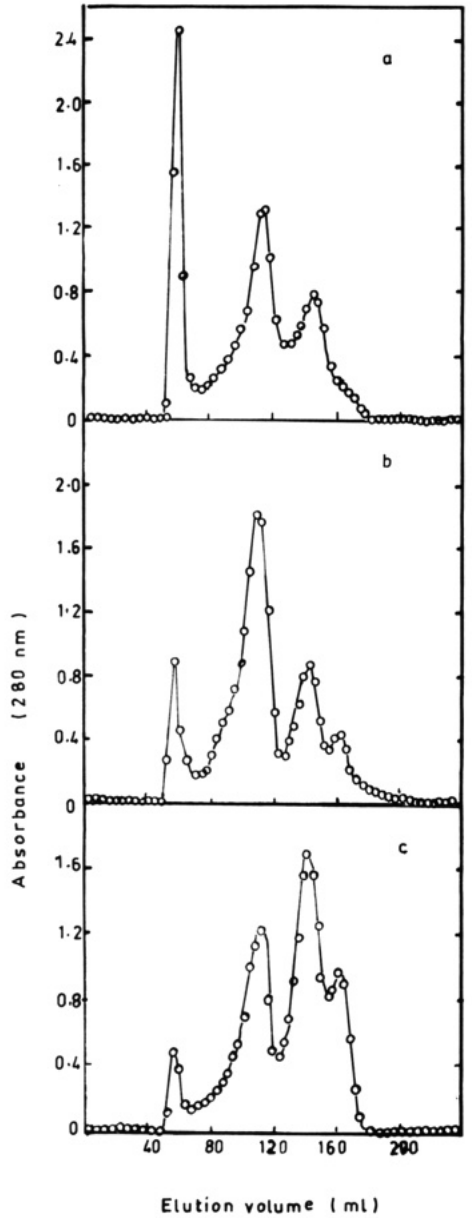

Figure 1. Gel filtration pattern of total proteins on Sepharose 6B-100 (1.5 × 98 cm): (a) hexane-extracted soy meal; (b) hexane containing 3% acetic acid extracted soy meal; (c) hexane containing 5% acetic acid extracted soy meal.

is given in Table III. In control soy meal, the activity of L-1 was 10.8 units/mL of extract and that of L-2 + L-3, 27.2 units/mL of extract, whereas in meals extracted with hexane containing 3% acetic acid, the activity of L-1 was absent and only 50% of the original activity of L-2 + L-3 remained. However, as the concentration of acetic acid in hexane was increased to 5%, no activity of L-2 + L-3 was detected. Although lipoxygenase activity is destroyed by the treatment, sensory evaluation studies showed that only 55–63% of beany odor could be removed by hexane–acetic acid treatment (Table II). It is possible that the secondary extraction solvents do not completely extract the flavor principles generated by the action of lipoxygenase as soon as the seed is crushed or other components responsible for beany flavor are present in the seed. The flavor components in soybean have been shown to be generated by enzyme action as soon as the seed structure is disrupted (Eldridge et al., 1977). Aqueous ethanol extraction of soy flakes (Eldridge et al., 1977) and soaking the intact soybean in aqueous ethanol (Eldridge et al., 1977; Borhan and Snyder, 1979) have been shown to inactivate the lipoxygenase system.

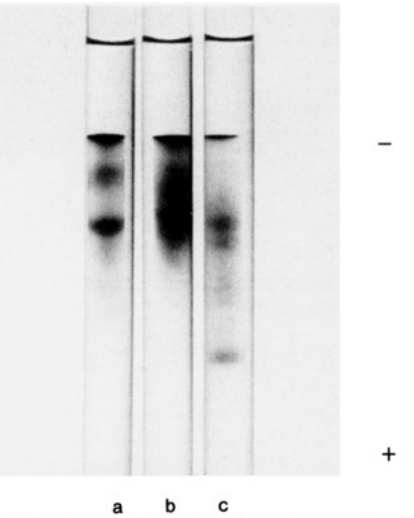

Figure 2. Gel electrophoresis of total proteins in 0.02 M phosphate buffer (pH 7.5) containing 0.01 M β-mercaptoethanol: (a) hexane-extracted soy meal; (b) hexane containing 3% acetic acid extracted soy meal; (c) hexane containing 5% acetic acid extracted soy meal.

In Vitro Digestibility. There was no difference in the in vitro digestibility values in hexane and hexane containing 3% acetic acid treated meal (Table III). However, hexane containing 5% acetic acid treated meal showed a higher digestibility value (81.7%) compared to that of hexane-extracted meal (76.7%).

Gel Filtration. Gel filtration patterns of the total protein from hexane and hexane–acetic acid extracted meals are given in Figure 1. The hexane-extracted soy meal proteins showed three peaks eluting at V_e/V_0 values of 1.0, 2.0, and 2.5, respectively. The proteins from treated meal showed three peaks similar to those of the control and an additional peak (peak 4) eluting at V_e/V_0 value of 2.8. Although there was no difference in the V_e/V_0 values of control and treated meals, the relative proportions of different fractions obtained by the area measurement of enlarged tracings of each peak were different. The proportions of different protein fraction for hexane-extracted meal were 31, 43, and 26% for peaks 1, 2, and 3 respectively. For hexane containing 3% acetic acid extracted meal, the relative proportions, were 12, 57, 21 and 10% for peaks 1, 2, 3 and 4 respectively, whereas for hexane containing 5% acetic acid extracted meal, they were 7, 34, 43, and 16%. The results clearly show that in treated meal the proteins are dissociated into lower molecular weight protein fractions, the extent of dissociation being greater in hexane containing 5% acetic acid treated meal.

To quantify the contribution of different protein fractions in the association and dissociation behavior, the gel filtration peaks from control and treated protein fractions were analyzed by SDS–PAGE. Due to the overlapping of the electrophoretic bands from different gel filtration peaks, unequivocal conclusions could not be drawn in identifying the protein components involved in the aggregation and those undergoing dissociation in the treated samples. It may not be out of place to mention that the two major protein fractions of soy bean, namely 11S and 7S, are multimeric in nature (Arrese et al., 1991).

Polyacrylamide Gel Electrophoresis. The PAGE pattern of the total proteins from hexane-extracted soy flour consisted of five bands: two major and three minor bands (Figure 2). There was also a band at the top of the

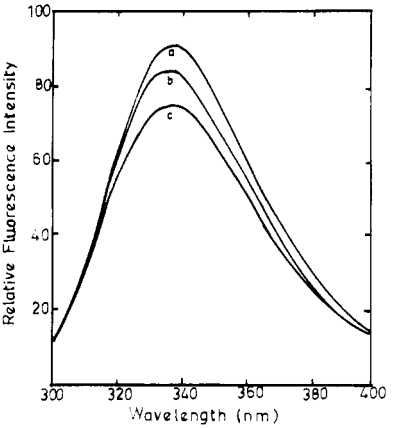

Figure 3. Fluorescence emission spectrum of total proteins: (a) hexane-extracted soy meal; (b) hexane containing 3% acetic acid extracted soy meal; (c) hexane containing 5% acetic acid extracted soy meal.

gel. In the case of proteins from hexane containing 3% acetic acid extracted soy meal, the major band appears to move faster and diffuse. On the other hand, the proteins from hexane containing 5% acetic acid extracted meal showed greater mobility and intensity of fast moving bands (Figure 2c). The results show that the high molecular weight proteins are dissociated into lower molecular weight fractions, conforming to the results of gel filtration studies (Figure 1).

Fluorescence Spectrum. The fluorescence emission spectrum of the proteins from control and treated samples showed a maximum at 336 nm (Figure 3). In proteins containing both tryptophan and tyrosine, the fluorescence spectrum of tryptophan alone is observed between 328 and 342 nm (Teale, 1960). The proteins from hexane–acetic acid extracted meal showed quenching of fluorescence proportional to the acetic acid concentration; the higher the acetic acid concentration (5%), the greater is the quenching of fluorescence. Generally, quenching and red shift in the emission maximum are suggestive of dissociation and denaturation of the protein molecule (Edelhoch et al., 1967; Srinivas and Narasinga Rao, 1987). In the present study, the proteins from treated meal showed only quenching but no red shift in the emission maximum. This suggests that the proteins are dissociated and the quenching is due to tryptophan groups experiencing polar environment as a consequence of their exposure from the interior of the protein molecule.

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Registry No. Acetic acid, 64-19-7; hexane, 110-54-3; soybean trypsin inhibitor, 9078-38-0; lipoxygenase, 9029-60-1.