Preparation and Properties of Protein Isolate from Hexane–Acetic Acid Treated Commercial Soybean Meal

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Protein isolates were prepared from commercial soy meal treated with hexane containing 3% acetic acid at 28 and 58 °C. The sensory, physicochemical, and functional characteristics of the spray-dried isolates were determined. The yield of isolate on the protein basis for control meal was 66%, while that of isolate from treated meals decreased by 12–18.2%. The trypsin inhibitor activity (52 TIU/mg of protein) of isolates was reduced significantly compared to that of its corresponding meal (138 TIU/mg of protein). Sensory evaluation studies showed a 66% reduction in beany flavor in treated isolates. In gel electrophoresis, the isolate from treated meal at 58 °C showed dissociation and aggregation, and fluorescence measurements indicated quenching due to unfolding of the protein molecule. At pH 7.0, the nitrogen solubility of isolate from the meal treated at 58 °C was higher (95%) compared to that of other isolates (82–85%). Higher fat absorption values for both treated isolates but superior emulsifying properties only in isolate from meal treated at 58 °C were observed. However, there was no change in foaming capacity values. Use of higher temperature (58 °C) for extraction had a beneficial effect in terms of improved functionalities.

Keywords: *Commercial soybean meal; hexane–acetic acid; protein isolate; beany flavor removal; functionality*

INTRODUCTION

The current production of soybean in India has reached 2.3 million tonnes, of which 1.5 million tonnes of defatted soymeal is available for export and domestic use. Since soy proteins possess a wide range of physical and functional properties, they find application in a variety of foods which include meat, poultry, seafood, and dairy products (Kolar et al., 1985). However, many soy protein preparations are characterized by undesirable flavor, and this has been a major constraint limiting their widespread acceptance (MacLeod and Ames, 1988). These off-flavors are mainly generated by the action of lipoxygenases which act on polyunsaturated fatty acids to form hydroperoxides which subsequently degrade to form secondary products such as aldehydes, ketones, alcohols, and acids that contribute to off-flavors (Wolf, 1975; Sessa, 1979; MacLeod and Ames, 1988).

Aqueous ethanol, 2-propanol, and azeotropic mixtures of hexane and alcohols have been used as extraction solvents to obtain soy meal, protein concentrates, and isolates devoid of undesirable flavors (Johnson and Lusas, 1983). Recently, we have reported from this laboratory the effect of extraction of soybean with hexane containing acetic acid on the quality of oil (Bhagya and Srinivas, 1992) and the physicochemical and sensory characteristics of soy meal (Srinivas et al., 1992). The objective of the present study was to prepare a protein isolate from commercial defatted soy meal after treatment with hexane containing acetic acid and study its physicochemical and functional properties. Since no information is available, we report in this paper the results of such study.

MATERIALS AND METHODS

Materials. Defatted soybean flakes, commercially processed by solvent extraction using hexane followed by desolventization by steam, were obtained from Premier Proteins Ltd., Madhya Pradesh, India.

Acrylamide, bis(acrylamide), β-mercaptoethanol, pepsin, and pancreatin were from Sigma Chemical Co. *N,N,N′,N′*-Tetramethylethylenediamine was from Koch Light Laboratories Ltd. and Sepharose 6B from Pharmacia Fine Chemicals. All other chemicals used were of analytical reagent grade.

Methods. *Secondary Extraction of Soy Flakes with Hexane–Acetic Acid.* Commercial defatted soy flakes were further treated with hexane containing 3% acetic acid (v/v) at room temperature (28 °C) using a glass column with a stopper. Extraction was carried out at a flake to solvent ratio of (1:3 w/v) for 18 h with renewal of solvent every 6 h. Also, defatted soy flakes (35 g) were further extracted in a Soxhlet apparatus with hexane containing 3% acetic acid for 3 h (Srinivas et al., 1992). The temperature in the extractor was 58 °C. The flakes were desolventized in a vacuum drier at 50 °C and powdered to pass through a 60 mesh sieve (Srinivas et al., 1992). Flour prepared from soy flakes without further extraction served as control.

Preparation of Protein Isolate. One kilogram of control and treated meals was dispersed in water in the ratio of 1:10 (w/v). The pH of the dispersion was adjusted to 8 with 2 N NaOH, and the mixture was stirred for 1 h at room temperature. The insoluble residue was removed by centrifugation at 12 000 rpm (Westfalia Separator, AG, Oelde, Germany, type LWA 205), and from the supernatant, proteins were precipitated at pH 4.5 using 2 N HCl. The isoelectric protein was centrifuged and washed with water. The wet protein isolate was dispersed in water and neutralized with sodium hydroxide and spray-dried. (Laboratory Model BE 1216, Bowen Engineering Inc., Somerville, NJ; gravity feed and automization with air at a pressure of 15 psi, inlet temperature 180 °C, and outlet temperature 115 °C.) For spray-drying, the slurry was adjusted to 15% solids with water. The spray-dried protein isolate was used for further studies.

Proximate Analysis. Moisture, protein (N × 6.25), ether extractives, and ash content were determined according to the method of AOAC (1984). Fiber content in soy meal was estimated according to neutral detergent fiber method (Goering and Van Soest, 1970), and carbohydrate was by difference.

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×

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crystallized bovine trypsin. Results are expressed as trypsin inhibitor units (TIU) per milligram of protein.

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) using linoleic acid as substrate and measuring the change of absorbance at 234 nm.

In Vitro Digestibility. This was determined according to the method of Akeson and Stahman (1964) using pepsin–pancreatin enzymes. The digested protein correlated to the total protein was expressed as percent digestibility value.

Nitrogen Solubility Index (NSI). The NSI was determined according to the procedure outlined in AOCS (1985) as described earlier (Srinivas et al., 1992).

Sensory Evaluation Studies. The beany flavor present in different samples was quantitated using the dilution technique (Indian Standards, 1983) as outlined earlier (Srinivas et al., 1992).

Gel Filtration. Protein isolate (0.5 g) 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. Protein solution equivalent to 75 mg of protein was loaded onto a column of Sepharose 6B (1.5 × 94 cm) that had been equilibrated with the buffer and eluted with the same buffer at a flow rate of 25 mL/h. Fractions of 4 mL were collected using an LKB automatic fraction collector, and the absorbance of each fraction was measured at 280 nm in a Shimadzu UV 150-02 double-beam spectrophotometer.

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. Protein solution (100 μg) was applied to the gel, and the 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 and destained with the same solvent.

Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis (SDS–PAGE). This was performed according to the procedure described by Laemmli (1970) using 12.5% slab gels. About 80 μg of protein was applied to each slot, and electrophoresis was performed at a constant current of 8 mA/slot. After electrophoresis, the gel slab was removed and fixed in a solution of methanol, acetic acid, and water (5:10:85 v/v), stained with 0.1% Coomassie Blue R 250, and destained with the same solvent.

Fluorescence Spectrum. The fluorescence emission spectrum was recorded in the range of 300–400 nm after excitation at 280 nm using a Shimadzu RF-5000 spectrofluorometer at 25 °C. Protein solution in 0.1 M phosphate buffer (pH 7.5) in 0.4 M NaCl containing 0.01 M β-mercaptoethanol having an absorbance of 0.1 at 280 nm was used. The temperature of the cell was maintained with a constant temperature circulating water bath. Both excitation and emission slits were 5 nm.

Nitrogen Solubility vs pH. Nitrogen solubility as a function of pH was determined by suspending 0.5 g of protein isolate in 20 mL of water, and the pH of the suspension was adjusted to the desired value with 1 N HCl or 1 N NaOH. The suspension was then shaken for 1 h using a rotary shaker at room temperature and centrifuged at 5000 rpm for 20 min. The pH of the clear supernatant was noted. The solubilized nitrogen was estimated by the Kjeldahl method, and the values are expressed as percent of the total nitrogen in the material.

Fat Absorption Capacity (FAC). This was determined according to the method of Sosulski et al. (1976) using 2 g of isolate and refined groundnut oil. The values are expressed as the amount of oil (milliliters) absorbed by 100 g of protein.

Emulsification Capacity (EC), Emulsion Activity (EA), and Emulsion Stability (ES). The method of Beuchat et al. (1975) was used for EC measurements at room temperature. Isolate equivalent to 1 g of protein and 23 mL of water was blended for 30 s in a Waring blender at low speed. After complete dispersion, refined groundnut oil was added from a buret at a rate of 0.4 mL/s and blending continued until phase separation was observed visually. EC is expressed as milliliters of oil emulsified by gram of protein. EA and ES were determined according to the method of Yatsumatsu et al. (1972).

Foam Capacity (FC) and Foam Stability (FS). The FC and FS were determined according to the method of Lawhon et al. (1972). Isolate equivalent to 1 g of protein was dispersed in 100 mL of distilled water and blended for 5 min, and the contents along with the foam were poured into a 250 mL measuring cylinder; the foam volume was recorded after 30 s. FC is expressed as percentage increase in volume. After 30 min, the volume of foam was measured and expressed as FS.

Table 1. Composition of Control and Hexane–Acetic Acid Extracted Commercial Soy Meal[a]

constituent	control meal	hexane–3% acetic acid extracted meal	
		28 °C[b]	58 °C[c]
protein (N × 6.25) (%)	59.6 ± 0.4	59.2 ± 0.3	60.2 ± 0.5
ether extractives (%)	1.0 ± 0.1	0.5 ± 0.1	0.2 ± 0.0
ash (%)	6.9 ± 0.1	6.9 ± 0.3	7.0 ± 0.2
fiber (%)	5.6 ± 0.1	5.6 ± 0.2	5.2 ± 0.2
carbohydrate (by diff) (%)	26.9 ± 0.1	27.8 ± 0.2	27.4 ± 0.3
trypsin inhibitor activity (TIU/mg of protein)	138 ± 1.0	136 ± 1.0	138 ± 2.0
nitrogen solubility index (%)	43.8 ± 0.4	37.4 ± 0.5	34.2 ± 0.4

[a] Values are averages of three independent determinations and expressed on moisture free basis. Lipoxygenase activity was not detected in the meal. [b] Column extracted for 18 h at 28 °C. [c] Soxhlet extracted for 3 h at 58 °C.

RESULTS AND DISCUSSION

The proximate composition and trypsin inhibitor activity of commercial soy meal and hexane–acetic acid treated meals are shown in Table 1. The values for protein, ash, fiber, and carbohydrate did not show any variations among different meals. However, ether extractive values were considerably lower in treated meals. The trypsin inhibitor activity of commercial soy meal was 138 TIU/mg of protein, and no reduction in the values after treatment was observed. Lipoxygenase activity was not detected in the meal.

At pH 7.0, the NSI of commercial defatted soy meal was 43.8%, and the NSIs of hexane containing 3% acetic acid extracted meal at 28 or 58 °C for 3 h were 37.4 and 34.2%, respectively, the reductions being 14.6 and 21.9%, respectively (Table 1). Srinivas et al. (1992) have reported similar reductions in NSI values (due to possible denaturation of proteins) for acidic hexane extracted laboratory-processed soy meal.

Yield of Protein Isolates and Their Quality Characteristics. The percent yield of protein isolate from control meal was 66 on protein basis, while the yields of isolate obtained from meal treated at 28/58 °C were 58 and 54, respectively (Table 2), the reductions in yield being 12 and 18.2%, respectively. This reduction in yield is due to decreased NSI (14–22%) in treated meals (Table 1). The protein content of isolate obtained from meal treated with hexane containing 3% acetic acid at 58 °C was higher (96%) compared to the other two isolates (91%). Since the protein content of the isolate is affected by the amounts of associated and conjugated or complexed nonprotein constituents precipitating as impurities with the protein (Smith and Circle, 1972), it could be possible that treating soy meal with hexane containing acetic acid at higher temperature would have removed some of the nonprotein constituents. The trypsin inhibitor activity (52 TIU/mg of protein) of protein isolates was reduced significantly compared to that of its corresponding soy meal (138 TIU/mg of protein).

Table 2. Yield and Quality Characteristics of Protein Isolates[a]

parameter	isolate from control meal	protein isolate from hexane–3% acetic acid extracted meal	
		28 °C[b]	58 °C[c]
yield of isolate (%)[d]	66 ± 0.5	58 ± 0.5	54 ± 0.6
protein (N × 6.25)[e] (%)	91 ± 0.5	91 ± 0.8	96 ± 1.2
trypsin inhibitor activity (TIU/mg of protein)	52 ± 1.0	52 ± 1.0	51 ± 1.0
in vitro digestibility (%)	88.4 ± 0.5	91 ± 0.2	89.4 ± 0.5

[a] Values are averages of three independent determinations. Values are mean ± standard deviation. [b] Column extracted for 18 h at 28 °C. [c] Soxhlet extracted for 3 h at 58 °C. [d] On protein basis. [e] Values are expressed on moisture free basis.

Table 3. Recognition Threshold Level for the Detection of Beany Flavor in Hexane–Acetic Acid Treated Soy Meal and Its Isolates

sample	mean threshold dilution (mL/g of sample)	SD[a]	beany flavor removed (%)
meal			
control	1939	1.13	0
hexane–acetic acid at 28 °C	970	1.20	50
hexane–acetic acid at 58 °C	949	1.10	51
isolate			
control	4650	1.31	0
hexane–actic acid at 28 °C	1543	1.21	67
hexane–acetic acid at 58 °C	1574	1.26	66

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

In vitro digestibility values showed no changes in control and treated isolates (Table 2).

Sensory Evaluation Studies. The data on the recognition threshold level for the detection of beany flavor in control and hexane–acetic acid treated meal and its corresponding isolates are given in Table 3. In control meal, the beany note was recognized at a dilution of 1939 mL/g of meal, while for treated meals, the dilution for recognition decreased to 949–970 mL/g of meal; the percent decrease in beany note was 50%. On the other hand, in the case of control isolate, the dilution for recognition was 4650 mL/g of isolate, and this came down to 1543–1547 mL/g of isolate in treated isolates, the reduction being around 66%. It is interesting to note that the beany flavor is concentrated in isolates as judged by the results that the beany flavor was recognizable at higher dilution. Undesirable soy flavor is the beany character carried by medium-chain aldehydes such as hexanal. It is desirable to remove them completely to produce products that are sufficiently bland for a wide range of applications. However, it is difficult to achieve this due to the binding of aldehydes to the soy protein (MacLeod and Ames, 1988). Soy protein isolate consists of two major protein fractions, 11S and 7S. It has been shown that the 7S fraction is mainly responsible for off-flavor binding (Damodaran and Kinsella, 1981).

Gel Filtration and Gel Electrophoresis. The gel filtration pattern of control and treated protein isolates is shown in Figure 1. The isolates showed three peaks eluting at V_e/V_0 of 1.0, 1.8, and 2.2, respectively. The relative proportions of different fractions obtained by the area measurement of enlarged tracings of each peak showed that for control isolate the proportions were 29, 55, and 16% for peaks 1, 2, and 3, respectively, while

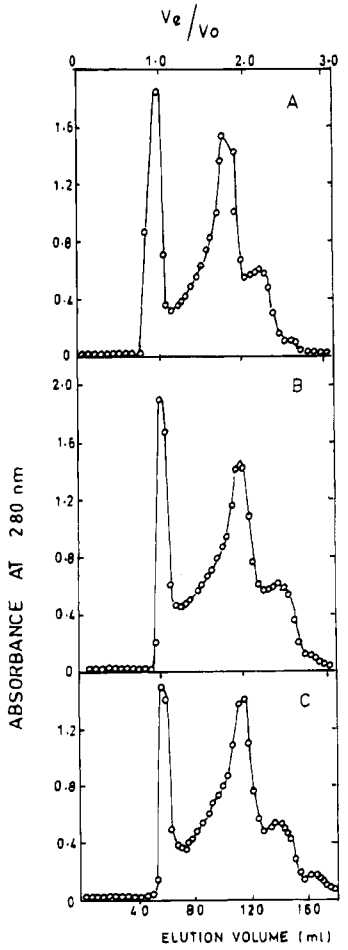

Figure 1. Gel filtration pattern of protein isolates on Sepharose 6B-100: (A) control meal; (B) hexane containing 3% acetic acid extracted meal at 28 °C for 18 h and (C) at 58 °C for 3 h.

for treated isolates the proportions were 21, 59, and 20%. Although the proportion of peak 1 is decreased and the other two peaks increased, it is not clear from gel filtration study alone that the proteins are dissociated. Our earlier study has shown that treating laboratory-processed soy meal with hexane–acetic acid solvent resulted in dissociation of proteins into lower molecular weight entities (Srinivas et al., 1992). Since in the present study we have used commercial soy meal that has been subjected to higher temperature during desolventization compared to laboratory-processed meal, the results are not strictly comparable.

In gel electrophoresis (Figure 2) the isolate from control meal showed two major and two minor bands. In treated isolates the pattern was different, showing faster moving bands. Of the two major bands, the slow moving band at the top of the gel (Figure 2a) was the predominant one. In the treated samples, this band was absent. The intensity of the fast moving bands in the control increased after treatment (Figure 2b,c). The results thus indicate dissociation and aggregation of proteins. SDS–gel electrophoresis (figure not shown), however, showed no difference either in the mobility or in the intensity of bands among different isolates. Jacks et al. (1983) have reported that arachin exposed to acidic hexane resulted in dissociation as evidenced by increased mobility in nondenaturing gels but no corresponding change of migrational pattern in dissociating sodium dodecyl sulfate gels.

Fluorescence Spectrum. The fluorescence emission spectrum of control and treated isolates showed a

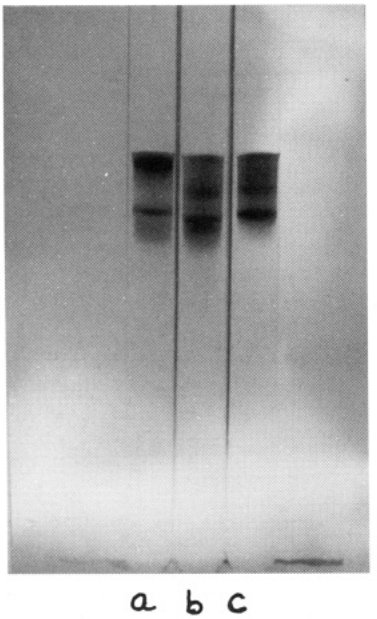

Figure 2. Gel electrophoresis of proteins in 0.02 M phosphate buffer (pH 7.5) containing 0.01 M β-mercaptoethanol: (a) control meal; (b) hexane containing 3% acetic acid extracted meal at 28 °C for 18 h and (c) at 58 °C for 3 h.

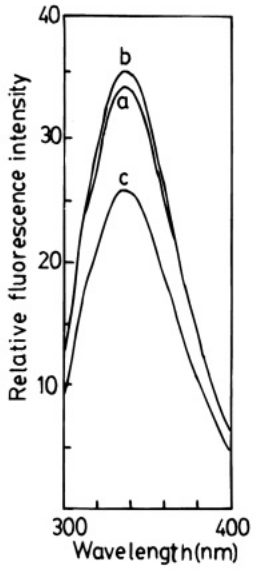

Figure 3. Fluorescence emission spectrum of proteins: (a) control meal; (b) hexane containing 3% acetic acid extracted meal at 28 °C for 18 h and (c) at 58 °C for 3 h.

maximum at 336 nm (Figure 3). Although the fluorescence intensity of isolate prepared from meal treated with hexane–acetic acid at 28 °C did not show significant difference compared to control, the isolate obtained from soy meal treated at 58 °C showed a reduction in the fluorescence intensity but no red shift in the emission maximum. Obviously, the temperature of extraction had an effect on the quenching of fluorescence. Generally, quenching and red shift in the emission maximum are suggestive of dissociation and denaturation (Edelhoch et al., 1967; Srinivas and Narasinga Rao, 1987) of protein molecules. The quenching of fluorescence in treated isolate (Figure 3c) is due to unfolding of the protein molecule, as a result of which the tryptophan residues experience a polar environment as a consequence of their exposure. An earlier study also showed similar quenching of fluorescence, when laboratory-processed soy meal was extracted with hexane containing different concentrations of acetic acid at 58 °C (Srinivas et al., 1992).

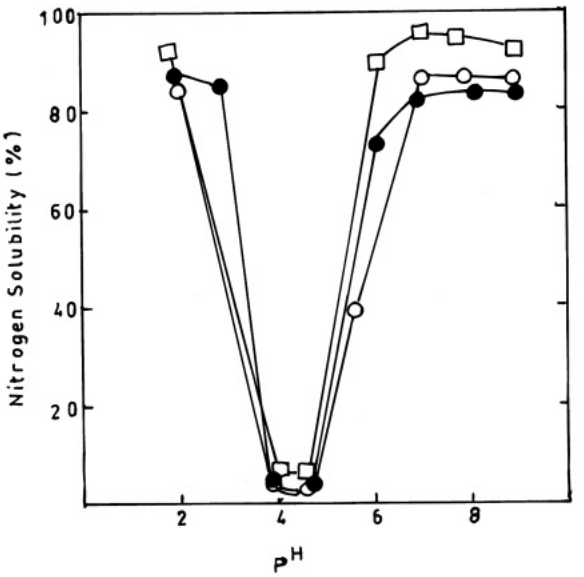

Figure 4. Nitrogen solubility vs pH profile of protein isolates: (○) control meal; (●) hexane containing 3% acetic acid extracted meal at 28 °C for 18 h and (□) at 58 °C for 3 h. The values are averages of three independent determinations.

Functional Properties of Protein Isolates. *(a) Nitrogen Solubility vs pH.* The nitrogen solubility vs pH profile for control and treated isolates, which is shown in Figure 4, exhibited a minimum solubility at around pH 4.5 (5%) and increased solubility at acidic and alkaline pHs. At pH 7.0, although the nitrogen solubilities of control and isolate obtained from treated meal at 28 °C were similar (82–85%), the nitrogen solubility of isolate obtained from treated meal at 58 °C was higher (95%). This increased solubility may be due to temperature-induced dissociation of certain oligomeric proteins in soy (Shen, 1976).

(b) Fat Absorption Capacity. The FAC values for treated isolates were higher (273–278 mL of oil/100 g of protein) compared to control which had a value of 190 mL of oil/100 g of protein (Table 4). The mechanism of oil absorption is not clearly understood, although various theories such as physical entrapment of oil and also involvement of surface area, size of the macromolecule, the charge, and the hydrophobicity have all been shown to affect the oil absorption (Kinsella, 1976; Kinsella et al., 1985). Thus, any change in any of the parameters cited above brought about by treatment would have affected the oil absorption.

(c) Emulsifying and Foaming Properties. The emulsifying properties such as EC, EA, and ES of control and treated isolates are shown in Table 4. The EC value (114 mL of oil/g of protein) for isolate obtained from meal treated at 58 °C was higher compared to those of control/28 °C treated isolates (98 mL of oil/g of protein). Similar trends of results were evident for EA and ES also. This increase in emulsifying properties can be due to higher nitrogen solubility of isolate obtained from meal treated at 58 °C (Figure 4). A close relationship between EC and nitrogen solubility has been reported for various oilseed proteins including soy proteins (Bhagya and Srinivasan, 1989; Kinsella, 1979). Emulsifying activity is also dependent on various intrinsic physical properties of the protein such as shape, hydrophobicity, flexibility, and surface charge (Shukla, 1982). There was no change in FC values between control and treated

Table 4. Oil Absorption Capacity and Emulsifying and Foaming Properties of Protein Isolates Prepared from Control and Treated Soy Meals[a]

isolate from	oil absorption capacity (mL of oil/ 100 g of protein)	emulsifying properties			foaming properties	
		EC (mL of oil/ g of protein)	EA (%)	ES (%)	FC	FS[b]
control meal	190 ± 3	98 ± 1.8	56 + 0.6	55 ± 0.8	160 ± 2	41 ± 2
hexane–acetic acid extracted meal at 28 °C	273 ± 5	98 ± 1.5	59 + 0.8	53 ± 0.6	162 ± 3	foam collapsed
hexane–acetic acid extracted meal at 58 °C	278 ± 4	114 ± 2.0	84 ± 1.0	72 ± 0.5	163 ± 2	foam collapsed

[a] Values are averages of three independent determinations ± standard deviation. [b] Determined at 30 min.

isolates, though the FS for treated isolates was poor; the foam collapsed at 30 min.

In conclusion, protein isolates were prepared from commercial soybean meal treated with hexane containing 3% acetic acid at 28 and 58 °C, and their properties were studied. The yield of isolates from treated meal was 12–18% lower compared to that of control. Most importantly, there was a 66% reduction in beany flavor in treated isolates. Isolate obtained from meal treated at 58 °C showed higher protein content and superior functional characteristics such as nitrogen solubility and emulsifying properties. Obviously, the use of a higher temperature (58 °C) for extraction had beneficial effects in terms of improved functionalities.

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