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Nutritional Quality, Fatty Acids, Amino Acids and Functional Characteristics of Bael (*Aegle marmelos* L.) Seed Protein Concentrate

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ABSTRACT

Studies were carried out on bael (*Aegle marmelos* L.) seed protein concentrate (BSPC) to evaluate the proximate, mineral and amino acid composition, nitrogen extractability and functional properties. The protein content was found to be 70.8 g/100 g BSPC. Calcium and phosphorus were observed in major quantities. The bael seed meal (BSM) lipid is found to be rich in unsaturated fatty acids (75%). Essential amino acids occurred in good quantities in BSPC. Nitrogen extractability of BSPC in water was found to be higher at 1 : 40 (w/v) ratio and an extraction time of 40 min. Minimum nitrogen extractability (14 g/100 g protein) and maximum extractability (97 g/100 g protein) were observed at pH 4 and 12, respectively. In the presence of sodium chloride (0.1 and 0.5 M), the nitrogen extractability was found to be increased between pH 4 - 10. Protein precipitability was maximum (90 g/100 g protein) at pH 5.5. SDS-PAGE of BSM and BSPC showed different polypeptides with molecular weights from 205 kDa to 12 kDa. The lower water holding capacity and higher oil holding capacity, foam capacity and foam stability were noted in BSPC. The sorption isotherm studies showed that the BSPC was non-hygroscopic in nature.

Key words: bael seed, nutritional quality, fatty acid profile, functional characteristics, mineral content, amino acid composition, nitrogen extractability

INTRODUCTION

Although most protein concentrates and isolates have been traditionally prepared from oilseeds such as soybean, peanut, sesame and sunflower seeds, other leguminous proteins have also been used in the food industry because of their functional characteristics. The publications related to functional characteristics of commercial seeds/products include northern bean, winged bean, linseed flour, cowpea flour, faba bean⁽¹⁻⁵⁾, Bengal gram, pumpkin seed⁽⁶⁻⁷⁾, beach pea protein isolate, sunflower meal, sesame seed, almond seed protein concentrate, sesame seed protein, erythrina seed flour, soy flour, peanut protein concentrate, and cashew nut⁽⁸⁻¹⁶⁾.

Bael (*Aegle marmelos* L.) is a dry-land plant belonging to the family *Rutaceae*, which grows wild in India. Physico-chemical characteristics of bael fruit pulp and preparation of beverages using sugar-milk and tamarind was reported⁽¹⁷⁾. Shilpa and Rajyalakshmi⁽¹⁸⁾ studied the preparation of blended bael fruit beverage and evaluated its storage stability

at room temperature for two months. Flavor components of bael fruit⁽¹⁹⁾, chemical composition of bael seed with protein content as high as 70% in de-oiled seed meal⁽²⁰⁾ were also reported. The fixed oil content of 18 g/100 g in the bael seed was reported⁽²¹⁾. The oil was reported to be used as a purgative⁽²²⁾.

Though bael seed is rich in protein, literature on the utilization and characterization of the seed protein is limited. Hence, the present investigation was undertaken to prepare the bael seed meal (BSM) and bael seed protein concentrate (BSPC) and evaluate the proximate composition, mineral content, amino acid composition and functional properties of the seed proteins. The studies will be helpful in the commercial utilization of bael seed in preparation of various protein rich food products.

MATERIALS AND METHODS

I. Materials

Bael fruits were procured from different forest sources

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in and around Hyderabad, India. Refined sunflower oil was purchased from a departmental store. Chemicals and solvents used for the study were of laboratory grade (98 - 99% purity) from Sd. Fine Chemicals Company limited, Mumbai, India. Protein markers for SDS-PAGE were from Sigma Chemicals Co., St. Louis, USA.

II. Preparation of Bael Seed Meal (BSM) and Bael Seed Protein Concentrate (BSPC)

The seeds were manually separated from pulp and dried in a cabinet tray drier (Chemida make, Mumbai, India) at $45 \pm 2^\circ\text{C}$. A representative sample of the dried seeds was collected through a sample divider (Prem Engineering Commercial Corporation, Delhi, India) and one part of it was ground to pass through a BS30 mesh sieve (500μ) in a laboratory grinder (Sumeet Food Processor, Nasik, India) to obtain BSM. The remaining part of the dried seeds was coarse ground and winnowed to separate cotyledons. The cotyledons were ground and defatted with hexane at room temperature ($28 \pm 2^\circ\text{C}$). The defatted meal was dried in a tray drier at $45 \pm 2^\circ\text{C}$, ground to pass through a BS60 mesh sieve (240μ) to obtain BSPC. The products were packed in polyethylene pouches and stored at room temperature for further experimental work.

III. Proximate Composition, Total Polyphenols and Mineral Content

Proximate components of BSM and BSPC were assayed by standard methods⁽²³⁾. The protein content of the sample was calculated by multiplying the nitrogen content by the factor of 6.25⁽²⁴⁾. The carbohydrate content in samples was estimated by difference (by subtracting the percentages of moisture, ash, fat, protein and fibre from 100) and the calorific values were computed by the addition of values obtained by multiplying percentages of carbohydrates and proteins with a factor of 4 and percent fat content by a factor of 9^(25,26). The bulk density of BSM and BSPC was measured by filling the samples in a 100 mL graduated measuring cylinder and expressed as weight in grams per volume occupied in cc. The total polyphenol content was determined by using Folin-Ciocalteu reagent and expressed as mg of gallic acid equivalent/100 g sample⁽²⁷⁾. Minerals such as copper, iron, magnesium, potassium and zinc were measured in a Shimadzu AA 6701 F, Atomic Absorption Flame Emission Spectrophotometer (Shimadzu Ltd, Japan) by AOAC⁽²³⁾ (1995) method. The calcium and phosphorous contents were determined by the methods described by Ranganna⁽²⁸⁾.

All the analyses were carried out at room temperature ($28 \pm 2^\circ\text{C}$) in triplicate, and average values were reported with standard deviation.

IV. Fatty Acid Composition of Lipid by Gas Chromatography (GC) and Gas Chromatograph -Mass Spectrometry (GC-MS)

The BSM lipid was extracted by using chloroform and methanol in the ratio of 2 : 1 at room temperature. Fatty acid methyl esters (FAMES) were prepared by saponification and methylation using 2% sulfuric acid in methanol. The esters were extracted into ethyl acetate, washed, dried over anhydrous sodium sulfate and concentrated for quantification in GC and GC-MS.

The GC analyses were performed with an Agilent 6850 gas chromatograph equipped with a flame ionization detector (FID). A DB-225 capillary column (30 m, 0.25 mm i.d.) was used and the column temperature was maintained at 160°C for 2 min, increased to 180°C at $6^\circ\text{C}/\text{min}$, maintained for 2 min at 180°C , then further increased to 230°C at $4^\circ\text{C}/\text{min}$ and maintained for 10 min at 230°C . The carrier gas was nitrogen at a flow rate of 1.5 mL/min. The injector and detector temperatures were 230°C and 250°C , respectively, and the split ratio was 50 : 1.

The GC-MS analyses were performed with an Agilent (Palo Alto, USA) 6890N gas chromatograph on a HP-5 MS capillary column (30 m \times 0.25 mm i.d.; 0.25 μm and connected to an Agilent 5973 mass spectrometer operating in the EI mode (70 eV; m/z 50 - 550; source temperature 230°C ; quadrupole temperature 150°C). The column temperature was maintained at 200°C for 2 min, increased to 300°C at $4^\circ\text{C}/\text{min}$, and maintained for 20 min at 300°C . The carrier gas was helium at a flow rate of 1.0 mL/min. The inlet temperature was maintained at 300°C and the split ratio was 50 : 1. Structural assignments were based on interpretation of mass spectrometric fragmentations by comparison of retention times and fragmentation pattern of authentic compounds from the Wiley and NIST libraries.

V. Amino Acid Composition

The amino acid profile was determined by analyzing the hydrolyzed samples on Biochrom Automatic amino acid analyzer (Biochrom 30, England). The vacuum tubes were purged with nitrogen to displace air. BSPC equivalent to 5 mg protein was placed in 20 mL vacuum hydrolysis tube, to which 6 N HCl (10 mL) was added. The tubes were evacuated and sealed under vacuum. The sample was allowed to hydrolyze in a Lab model oven (Dalal, Mumbai, India) at $110 \pm 2^\circ\text{C}$ for 24 h. The hydrolyzed reaction mass was cooled, volume was made up to 25 mL and filtered. An aliquot of 5 mL was evaporated under vacuum to dryness and the residue was mixed with 2.5 mL of loading buffer (pH 2.2).

An aliquot of 20 μL was injected into automatic amino acid analyzer (Biochrom 30, England). All the amino acids were detected after post column derivatization with ninhydrin reagent. Eluates were spectrophotometrically monitored at 570 nm and the concentrations of the unknown samples were determined by comparing with standard peak areas (Agilent amino acid standard kit, USA).

Cysteine and methionine contents in BSPC were determined after converting into cysteic acid and methionine sulfone respectively, by oxidizing the sample with performic acid (9 : 1 mixture of 80% formic acid and 30% hydrogen

peroxide) for 18 h at room temperature by the method of Moore⁽²⁹⁾.

VI. Functional Properties of BSM and BSPC

The water and oil holding capacities of BSM and BSPC were determined by dispersing 1 g of sample in about 10 mL of distilled water or sunflower oil as per a method described earlier⁽³⁰⁾. Foaming capacity (FC) and foam stability (FS) of BSM and BSPC were measured by generating the foam by dispersing 2 g of sample in 100 mL distilled water as per a standard method⁽³¹⁾. FC and FS of a standard (BSA, Fraction V, Sigma Aldrich, St. Louis, MO, USA) were determined by applying similar conditions for comparison with the BSPC. The emulsifying capacity (EC) of BSPC was determined according to the method of Beuchat *et al.*⁽³²⁾. EC is expressed as mL of oil required per g sample. EC was also determined for a standard BSA for comparison. All the experiments were carried out at room temperature ($28 \pm 2^\circ\text{C}$) in triplicate and mean values \pm standard deviation (SD) were reported.

VII. Protein Solubility Studies

Studies related to protein characteristics such as nitrogen extractability, protein precipitability and buffer capacity were restricted to BSPC as per the methodology reported by Jyothirmayi *et al.*⁽¹³⁾. Nitrogen extractability of BSPC was investigated in water, 0.1 and 0.5 M NaCl solutions. The ratio of BSPC to water in the range of 1 : 10 to 1 : 60 (w/v) was used for 1 h extraction. Subsequently, the extraction time was reduced to the range from 10 to 60 min, the pH was varied from 2 to 12 by using 0.5 M HCl or 0.5 M NaOH solution, and the supernatants were analyzed for nitrogen content and expressed as the percent nitrogen extractability.

Protein precipitability of BSPC was carried out by the reported method using six grams of BSPC dispersed in distilled water⁽³³⁾. Buffer capacity of BSPC was carried out as per the method described earlier by dispersing one gram of sample in 40 mL distilled water⁽¹³⁾.

VIII. Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE of BSM and BSPC was conducted and individual polypeptides were separated and identified⁽³⁴⁾. SDS-PAGE was performed using 4% stacking gel and 10% separating gel on a Mini Slab Gel Electrophoresis Unit (Balaji Scientific Services, Chennai, India) with loading of 25 μL . The individual peptides were identified by using a wide range protein molecular weight marker (Sigma Chemicals Co., St. Louis, USA).

IX. Equilibrium Moisture Content-Relative Humidity (EMC-RH) Studies

Moisture sorption isotherm of BSPC at room temperature was plotted as per Ranganna⁽²⁸⁾. The sample of 5 g each

was taken in glass petri dishes and exposed to RH conditions of 10, 30, 50, 70, 90 and 100% using appropriate concentrations of sulfuric acid. Moisture sorption isotherm was plotted for the EMC and the corresponding equilibrium relative humidity (ERH).

RESULTS AND DISCUSSION

Our study was the first to report on nutritional quality, fatty acids, amino acids and functional characteristics of bael seed protein concentrate. The yield of BSPC was 24.5 ± 1.40 g/100 g of bael seed. Chemical and nutritional contents of BSM and BSPC are presented in Table 1. The results show that BSPC possesses higher protein content (70.8%) than BSM (34.7%), which is comparable with soy protein concentrate (69.1%)⁽³⁵⁾. The fat content in BSM was comparable with reported value (39.11%) for jojoba seed⁽³⁶⁾.

The analysis of minerals showed that considerable amounts of calcium, phosphorus and iron were present in BSPC (Table 2). The values were comparable to those of legume and oil seeds⁽³⁷⁾. The BSPC possessed higher values

Table 1. Chemical and functional properties of bael (*Aegle marmelos*) seed^a

Parameter	BSM	BSPC
Moisture (g/100 g)	3.7 ± 0.1	6.1 ± 0.1
Total ash (g/100 g)	7.7 ± 0.1	5.2 ± 0.3
Crude fat (g/100 g)	37.6 ± 1.2	ND
Crude protein (g/100 g)	34.7 ± 0.4	70.8 ± 0.0
Crude fibre (g/100 g)	4.7 ± 0.3	1.8 ± 0.1
Carbohydrates ^b (g/100 g)	11.6 ± 0.5	16.0 ± 0.7
Energy (kcal/100 g)	523.0 ± 0.6	347.0 ± 0.4
Bulk density (g/cc)	0.5 ± 0.0	0.3 ± 0.1
Water holding capacity (g/100 g)	158.0 ± 0.4	139.0 ± 0.9
Oil holding capacity (g/100 g)	126.0 ± 0.4	156.0 ± 0.5
Foam capacity (increase in volume, mL)	4.0 ± 0.4	52.0 ± 0.9 (148 mL for BSA)
Foam stability (volume in mL after 30 min)	3.0 ± 0.6	48.0 ± 0.7 (112 mL for BSA)
Foam stability (volume in mL after 60 min)	2.0 ± 0.6	30.0 ± 0.4 (100 mL for BSA)
Foam stability (volume in mL after 90 min)	2.0 ± 0.5	24.0 ± 0.6 (60 mL for BSA)
Emulsification capacity (mL/g sample)	ND	18.5 ± 0.9 (53 mL/g BSA)

^a Values are means of triplicate analyses with \pm SD.

BSM - Bael seed meal.

BSPC - Bael seed protein concentrate.

ND - Not determined.

^b Carbohydrates were calculated by difference⁽²⁵⁾.

BSA - Bovine serum albumin.

of protein and minerals thereby indicating its equivalence or superiority over legume or oils seed proteins, which could help its utilization in supplementing or replacing legume or oil seed proteins in processed foods. Table 2 shows that BSPC was found to possess higher polyphenol content (745 mg/100 g) than BSM (367 mg/100 g).

The major fatty acids of BSM were oleic acid (30.7%), linoleic acid (24.3%) and palmitic acid (20.4%) followed by linolenic acid (19.9%) and stearic acid (4%) (Table 3).

Amino acid composition of the BSPC was presented in Table 4. Equal amount of tyrosine (3.3 g/100 g), higher amount of cysteine (1.54 g/100 g) and lower amounts of other essential amino acids were observed in BSPC when compared to soy protein concentrate reported in literature⁽³⁵⁾. In the present study, it was found that 100 g BSPC protein contains high amounts of leucine (5.97 g) and isoleucine (3.17 g). Very high amounts of aspartic acid, glutamic acid, proline and arginine were found in BSPC. The ratio of essential to non-essential amino acids in BSPC was 0.37 which was almost comparable to that reported (0.42) for defatted sesame seed meal⁽³⁸⁾. Ildiko *et al.*⁽³⁹⁾ reported that the mustard seeds proteins were well-balanced in amino

acid composition. Radha *et al.*⁽⁴⁰⁾ obtained a product with balanced amino acid composition by blending soy, sesame and peanut flours. Mustard is rich in glutamic and aspartic acids which is similar to other vegetable proteins. They reported that sulfur-containing amino acids and lysine were high compared to other vegetable proteins. More than 31% of total amino acid content was essential amino acids. These findings on amino acid composition strengthen BSPC for exploring its use in food formulations with cereals, where the concentration of cysteine is generally low.

The functional properties data were shown in Table 1 for BSM and BSPC. The data of BSM showed higher value of bulk density as compared to that of BSPC due to the presence of hull and higher particle size. Similar trend for bulk density was also observed in melon seed flour⁽⁴¹⁾. Higher water absorption values of protein indicate the swelling ability and property of dissociation for exposing additional binding sites. Water absorption is influenced by several factors such as number of hydration positions, physical environment, pH, solvent, presence of lipids and carbohydrates⁽⁴²⁾. The hulls would contribute for higher water absorption. Since BSM has higher hull content (37 g/100 g), it showed a higher water absorption capacity of 158 g/100 g material in contrast to 139 g/100 g for BSPC. The BSM absorbed less oil which might be due to the presence of fixed oil. These values were comparable to that of peanut flour and raw horse

Table 2. Mineral and polyphenol content of bael (*Aegle marmelos*) seed^a

Parameter, mg/100 g	BSM	BSPC	Reported values for other seed proteins
Calcium	62.0 ± 0.8	91.0 ± 0.6	56.0 (Bengal gram) ⁽³⁷⁾
Copper	0.6 ± 0.0	2.4 ± 0.4	
Iron	1.7 ± 0.2	4.8 ± 0.4	4.4 (Greengram) ⁽³⁷⁾
Magnesium	1.1 ± 0.3	1.0 ± 0.5	
Phosphorus	295.0 ± 1.3	394.0 ± 1.2	670.0 (Groundnut seeds) ⁽³⁷⁾
Potassium	4.1 ± 0.4	7.5 ± 0.6	
Zinc	1.8 ± 0.4	2.3 ± 0.3	
Total polyphenol	367.0 ± 0.7	745.0 ± 0.4	400.0 (French beans) ⁽⁵⁰⁾

^a Values are means of triplicate analyses with ± SD.

ND - Not detected.

Table 3. Fatty acid composition of BSM lipid

Fatty acid	Composition (%)
Palmitic acid (16 : 0)	20.4
Palmitoleic acid (16 : 1)	0.3
Stearic acid (18 : 0)	4.0
Oleic acid (18 : 1)	30.7
Linoleic acid (18 : 2)	24.3
Linolenic acid (18 : 3)	19.9
Eicosanoic acid (20 : 0)	0.3
Eicosamonoenoic acid (20 : 1)	0.1

^aValues are means of duplicate analyses.

Table 4. Amino acid composition of BSPC^a

Amino Acid	g/100g protein	Reported values for soy protein concentrate g/100g protein ⁽³⁵⁾
Alanine	3.29	
Arginine	10.46	
Aspartic acid (Aspartate + Asparagine)	7.54	
Cysteine	1.54	0.2
Glutamic acid (Glutamate + Glutamine)	17.90	
Glycine	4.18	
Histidine	1.71	
Isoleucine	3.17	5.1
Leucine	5.97	9.8
Lysine	1.97	5.9
Methionine	1.23	1.5
Phenylalanine	4.03	5.7
Proline	6.10	
Serine	3.44	
Threonine	2.61	3.5
Tyrosine	3.29	3.3
Valine	3.67	5.7

^aValues are means of duplicate analyses.

gram meal^(14,43). Lazos⁽⁷⁾ reported that the higher water and lower oil absorption capacities might be partly due to the presence of polar amino acids. Foaming capacity and foam stability of the foams are also important quality attributes for proteins, which make them suitable for use in various food preparations such as cakes and buns. The data generated for foam studies using standard bovine serum albumin (BSA) for comparison was also presented in parentheses (Table 1). The BSPC possessed high value of foaming capacity with greater stability when compared to BSM because of its higher protein content. Foam stability of BSPC was found to be 24 mL after 90 min of standing which may be due to the presence of lower molecular weight proteins and air-water and protein-protein interactions. The BSPC showed good foam capacity and stability, hence it could find applications in various food preparations such as ice-creams, cakes and pastries. Emulsification capacity of BSPC was found to be 18.5 mL /g sample which was comparable to that of legume flour. The emulsification capacity of 19.8 mL oil /g green gram flour was reported earlier⁽⁴⁴⁾. The emulsification capacity data generated for BSA were also presented in Table 1 for comparison. EC is a very important property if the protein concentrate is to be used in products such as salad dressings and mayonnaise.

Nitrogen extractability studies are useful in the preparation of fruit-based drinks. Such studies showed that the optimum BSPC to water ratio is 1 : 40 (w/v) for maximum nitrogen extractability at 30 min (Figures 1 and 2). These results are similar to protein solubility found in defatted flax seed flour⁽⁴⁵⁾. The effects of pH and sodium chloride concentration on nitrogen extractability were shown in Table 5. BSPC possessed maximum and minimum nitrogen

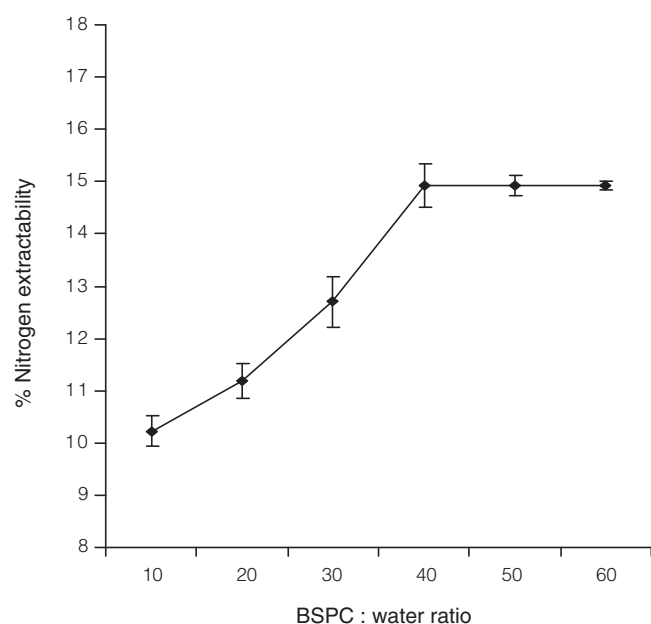


Figure 1. Effect of BSPC to water ratio on nitrogen extractability at 60 min, mean of triplicate analyses are presented and error bars show standard deviation.

extractability values of 97 ± 0.77 and 14 ± 0.95 g/100 g at pH 12 and 4 respectively. This could be due to the formation of smaller peptides/ hydrophobicity/ presence of more ionizable groups. The nitrogen extractability increased on either side of pH 4. It increased from 14 to 63 g/100 g as pH decreased from 4 to 2, and from 14 to 97 g/100 g as the pH increased from 4 to 12. The ionic sodium chloride increased extractability of nitrogen in both acidic and basic pH ranges which was reported to be an important requirement for food formulations⁽⁴⁶⁾. At pH 2 the nitrogen extractability was found to decrease by the addition of 0.1 and 0.5 molar NaCl. Similar extractability characteristics were found in tepary (*Phaseolus acutifolius*) seed, which was used for the preparation of fruit beverages⁽⁴⁷⁾. The extracted protein could be precipitated to the maximum extent (90 g/100 g) at pH 5.5 (Figure 3). On either side of pH 5.5 the precipitability of BSPC decreased. The protein precipitation was observed to

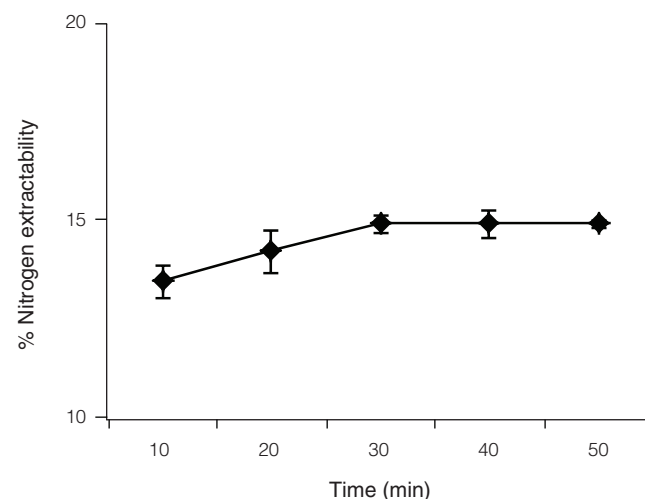


Figure 2. Effect of time on nitrogen extractability of BSPC at a BSPC to water ratio of 1 : 40, mean of triplicate analyses are presented and error bars show standard deviation.

Table 5. Effect of pH and salt on nitrogen extractability (g/100 g) of BSPC^a

pH	Water	0.1 M NaCl	0.5 M NaCl
2	63.98 ± 1.39	53.95 ± 1.24	46.19 ± 1.25
3	27.05 ± 1.16	34.28 ± 0.71	42.62 ± 1.02
4	14.51 ± 0.95	29.34 ± 1.25	39.23 ± 0.58
5	18.24 ± 0.76	32.16 ± 0.88	44.91 ± 1.92
6	19.62 ± 0.73	39.27 ± 0.87	72.69 ± 1.22
7	20.61 ± 0.68	49.52 ± 0.84	54.62 ± 1.08
8	34.38 ± 1.18	64.23 ± 0.69	76.01 ± 0.21
9	48.49 ± 0.34	68.28 ± 0.96	76.91 ± 1.69
10	70.02 ± 1.26	78.63 ± 0.51	88.58 ± 1.40
11	88.94 ± 1.63	92.02 ± 1.01	95.31 ± 0.62
12	97.86 ± 0.77	98.02 ± 0.48	99.34 ± 0.91

^a Values are means of triplicate analyses with ± SD.

be lower (42.72 g/100 g) at pH 2. These results are in agreement with a similar study reported by Smith *et al.*⁽⁴⁸⁾ on linseed meal, in which a maximum protein precipitation of 79 g/100 g was observed at pH 5.1. Buffer capacity of BSPC was presented in Figure 4. BSPC dispersed in distilled water had an initial pH of 6.4. Changes in pH of the dispersion

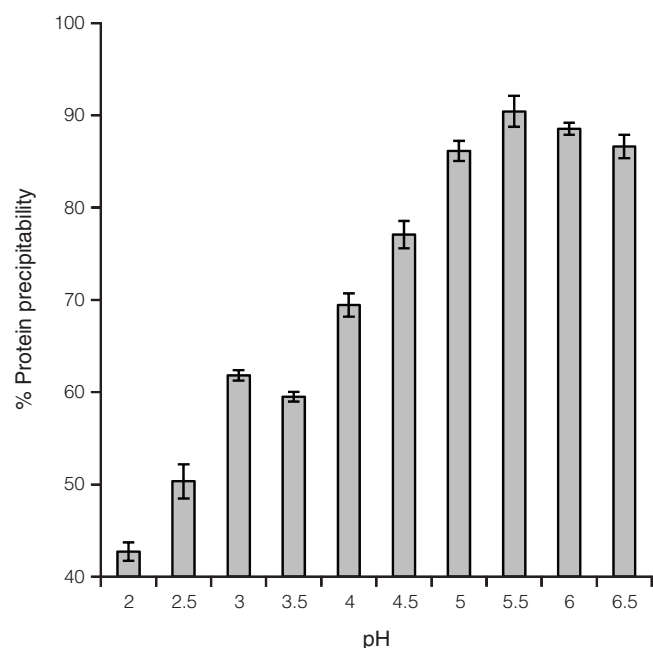


Figure 3. Protein precipitability of BSPC at different pH (2 - 6.5), mean of triplicate analyses are presented and error bars show standard deviation.

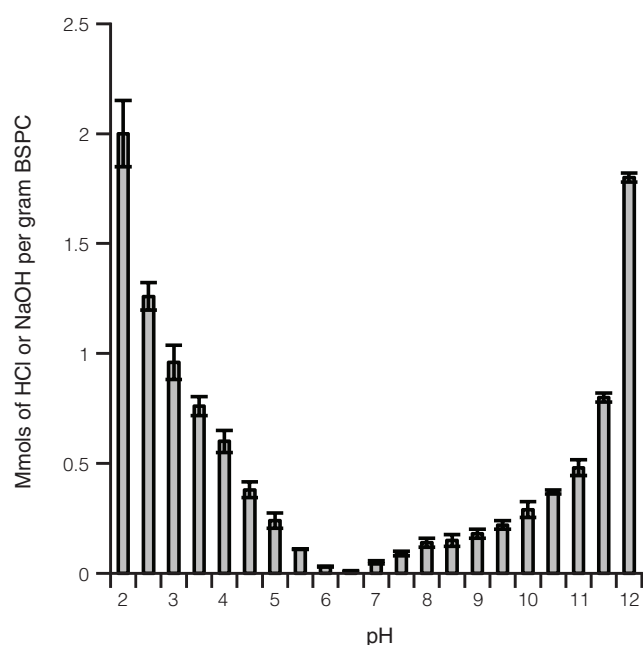


Figure 4. Buffer capacity of BSPC at different pH (2 - 12), mean of triplicate analyses are presented and error bars show standard deviation.

were brought by the addition of HCl or NaOH. At acidic pH, within the range about of 6.4 - 2.4, an average of 3.4 mmol of HCl was required per gram of BSPC to change the pH by one unit. Similarly, to bring about a change of one pH unit in the alkaline pH, an average of 0.82 mmol of NaOH was required per gram of BSPC. Higher buffer capacity of the BSPC was observed in alkaline medium than in acidic medium. It is suggested from the results of Figure 4 that for the preparation of protein isolates from BSPC, protein extraction at pH 12 would require 1.8 mmol of NaOH per gram BSPC. The buffer capacity studies are useful in the extraction of the protein from seed meal and protein concentrate. Initially the pH can be increased to get maximum protein solubility at pH 12, and later the pH can be brought down to 5.5 to get maximum precipitation of the protein, which can subsequently be used in various food formulations.

Figure 5 showed the SDS-PAGE profiles of SDS-soluble BSM and BSPC samples. In total, seven protein bands were observed, whereas three matched with the molecular markers, which were found to be 205, 97 and 45 kDa in both samples. Apart from these three, 29 and 12 kDa bands were also observed in the protein bands of BSPC, but not in BSM. The SDS-PAGE profiles indicated that the lower and higher molecular protein bands were present in the samples. Similar protein band trend was observed in hemp seed⁽⁴⁹⁾.

The BSPC had an initial moisture content of 6.48%, which equilibrated at 58% RH at room temperature (Figure 6). The critical moisture content for BSPC was found to be 8.76%, which equilibrated at 68% RH. The rapid moisture

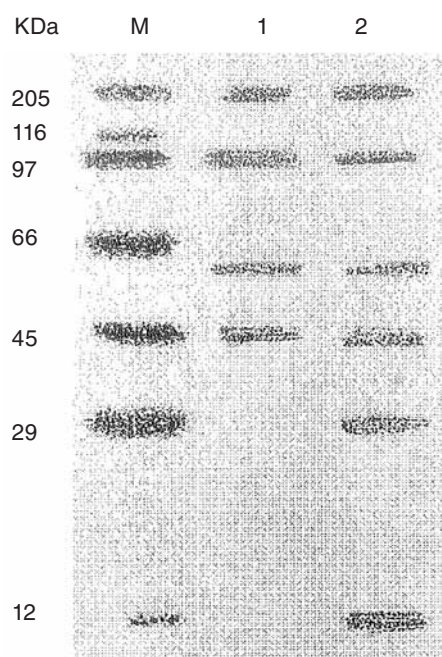


Figure 5. SDS-PAGE Pattern of bael seed. Lane M: Broad band standard molecular weight protein markers (myosin 205 kDa, galactosidase 116 kDa, phosphorylase B 97 kDa, bovine serum albumin 66 kDa, ovalbumin 45 kDa, carbonic anhydrase 29 kDa and cytochrome C 12 kDa), Lane 1: SDS-soluble BSM, Lane 2: SDS-soluble BSPC.

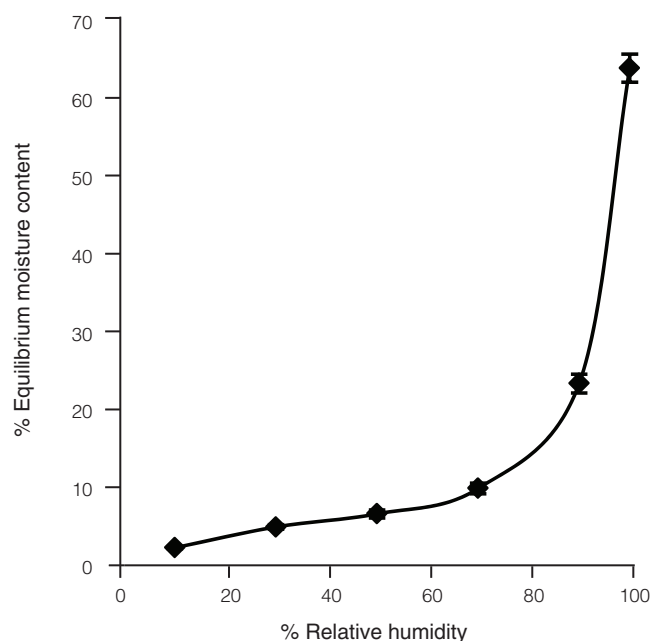


Figure 6. Experimental sorption isotherm of BSPPC, mean of triplicate analyses are presented and error bars show standard deviation.

gaining was observed above 70% RH. The ERH studies indicated that BSPPC was non-hygroscopic in nature, and hence could be stored in polyethylene pouches at room temperature.

CONCLUSIONS

This study is the first report on the preparation of a protein concentrate from bael seed and analysis of its functionality. The study indicated that the BSPPC was rich in protein and essential nutrients. Nitrogen extractability was found to be higher in alkaline pH of 12 with maximum precipitability at pH 5.5. The SDS-PAGE profile showed the presence of lower molecular protein bands in BSPPC. The chemical and functional properties of the seed protein concentrate were comparable to those of other oil seed meals. Hence, the BSPPC could be better utilized in the preparation of protein isolates and hydrolysates for further use in food industry.

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