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Functional properties of protein isolates from beach pea (*Lathyrus maritimus* L.)

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Abstract

Protein isolates of beach pea were prepared using sodium hydroxide (NaOH) and sodium hexametaphosphate (SHMP). Functional properties of the isolates so prepared were investigated and compared with those of other pea samples. Protein isolate of beach pea, prepared via NaOH extraction, had a protein content of 86.6%, while SHMP-extracted isolates contained 85.1% protein. Corresponding values for NaOH- and SHMP-extracted green pea and grass pea were 90.6, 89.9, 90.6 and 88.3%, respectively. Sulphur-containing amino acids were more prevalent in SHMP-extracted beach pea and green pea, while they were higher in NaOH-extracted grass pea. Tryptophan content was higher in NaOH-extracted than SHMP-extracted isolates. The predicted biological value and protein efficiency ratio of beach pea protein isolates indicated the high quality of products so prepared. Beach pea protein isolates exhibited a minimum solubility at pH 4.5. The pH and NaCl concentration effectively changed the functional properties of protein isolates. Beach pea protein isolates (NaOH- and SHMP-extracted) had in-vitro digestibility of 80.6 to 82.6% for pepsin-trypsin and 78.6 to 79.2% for pepsin-pancreatin.

1. Introduction

The use of plant proteins in the formulation of new food products, or in conventional foods, has been the focus of much research in recent years. In order to develop plant proteins for use as food ingredients, their physicochemical and functional properties must be evaluated. Because of inadequate supplies and shortage of food proteins, there has been a constant search for unconventional legumes as new protein sources for use in both functional food ingredients and nutritional supplements (Morrow, 1991; Onweluzo, Obanu, & Onuoha, 1994). Onweluzo, Obanu, and Onuoha have demonstrated the potential application of lesser known leguminous seed fractions as functional food ingredients for the developing countries.

The seeds of beach pea (*Lathyrus maritimus* L.) offer an unexploited source of protein. This relatively unknown leguminous plant grows along the sandy and gravel shorelines of Newfoundland, Nova Scotia, Quebec, and Ontario in Canada and is also found along the shorelines of Arctic and sub-Arctic regions from Greenland to Siberia and Japan (Fernald, 1950; Talbot & Talbot, 1994). Unlike other legumes, beach pea has not been cultivated and information about its nutritional value is scarce. Recently, Chavan, Shahidi, Bal, and McKenzie (1999) reported that beach pea contains a higher amount of crude protein and amino acids than green pea and grass pea. It was also reported that beach pea proteins are superior in sulphur-containing amino acids and nutritional value over those of green pea and grass pea (Chavan et al., 1999).

In addition to providing essential amino acids, the ultimate success of utilizing any seed protein as a food ingredient depends largely on its functional properties (Aluko & Yada, 1995; Nwanekezi, Obanu, & Onuoha, 1994). The present study reports on the response of beach pea proteins to changes in pH and NaCl concentrations, as measured by physicochemical and functional properties. Changes in pH and NaCl concentration often occur during the processing of foods; therefore, it is important to study the effects of

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changes in these factors on the structure–function relationships of food proteins. Thus, functional properties of beach pea protein isolates were determined as reported in this paper.

2. Materials and methods

2.1. Materials

The mature pods of beach pea (*L. maritimus* L.) were collected from Bellevue Beach, Salmon Cove and Sandy Cove in September–October 1995 and 1997. The grains and pod shells were separated manually. The total fresh weight and recovery of seeds and pod shells were recorded immediately after harvesting and separating, prior to drying, grinding and storing for chemical analyses and processing. Seeds of green pea (*Pisum sativum* L.) and grass pea (*Lathyrus sativus* L., var. code No. X850002), used in certain experiments for comparative purposes, were obtained from the Crop Science and Plant Ecology Department, University of Saskatchewan and Agriculture and Agri-Food Canada Research Centre, Morden, Manitoba, respectively. Pea seeds were first ground using a Moulinex coffee grinder (Black and Decker Canada Inc., Brockville, ON; 60 mesh) and then subjected to protein isolation. Food-grade samples of sodium hexametaphosphate [SHMP, $(NaPO_3)_n$] were obtained from Albright and Wilson Americas (Toronto, ON).

2.2. Methods

2.2.1. Preparation of protein isolates

2.2.1.1. Extraction of proteins and preparation of isolates. Pea meals (25–50 g) were added to distilled water or a sodium hexametaphosphate (SHMP, 2.8%, w/v) solution, at a meal to solvent ratio (R) of 1:5 (w/v). The mixture was stirred with a magnetic stirrer for 10 min and the pH of the solution was then adjusted to 9.0 using 1 M HCl or NaOH and stirring continued for another 30 min at room temperature. Each extract was separated by centrifugation at $4000\times\boldsymbol{g}$ for 20 min. The residues were re-extracted two more times with the same solvent under similar conditions. The extracts were combined and proteins precipitated by adjusting the pH to 4.5 with 1 M HCl, followed by separation by centrifugation at $4000\times\boldsymbol{g}$ for 20 min. The precipitate was redispersed in 100 ml distilled water at pH 9.0 and reprecipitated at pH 4.5. After separation of proteins by centrifugation, the precipitate was washed twice with distilled water (R = 1:2). The precipitated protein was re-suspended in distilled water and the pH was adjusted to 7.0 with 1 M NaOH prior to freeze-drying. The freeze-dried protein isolates were stored in air-tight glass containers at room temperature for further analyses. All extractions were carried out in triplicate. Sufficient quantities of protein isolates were prepared as outlined in Fig. 1.

2.2.1.2. Chemical analysis. Moisture, crude protein, lipid, ash, crude fibre and carbohydrate contents (by difference) of samples were determined by standard methods of analysis (AOAC, 1990). Soluble sugars were extracted into 80% (v/v) ethanol according to the procedure of Cerning and Guilhot (1973) and their contents were determined using, essentially, a modified method of Nelson (1944). The phenolics from beach pea were isolated as described by Shahidi and Naczk (1989). One gram of sample was extracted three times with 10 ml of 70% (v/v) aqueous acetone at room temperature for 30 min using a Polytron PT 3000 homogenizer (Brinkman Instruments, Rexdale, ON) for 1 min, at 10,000 rpm. The slurry was centrifuged at $5000\times\boldsymbol{g}$ for 10 min, the supernatants were collected, combined and evaporated to dryness at 30°C under vacuum. The extracted phenolics were then dissolved in 25 ml of methanol, centrifuged again and the total content of phenolics in methanol was determined colorimetrically according to the method of Swain and Hillis (1959). To 0.5 ml of methanolic solution of phenolics, 0.5 ml Folin-Denis reagent, 1 ml saturated solution of sodium carbonate and 8 ml water were added and mixed well. Absorbance was read at 725 nm after 30 min standing at room temperature and the content of phenolics was calculated as percent *trans*-sinapic acid equivalents, on a dry weight basis. Condensed tannins were determined as described by Naczk, Shahidi, and Sullivan (1992).

2.2.1.3. Non-protein nitrogen. The content of non-protein nitrogen (NPN) was determined by the method of Bhatty and Finlayson (1973) as modified by Naczk, Diosady, and Rubin (1985). One gram of meal was shaken with 40 ml of a 10% solution of trichloroacetic acid (TCA) at 20°C for 1 h using a wrist-action shaker (Burrel, Pittsburgh, PA). The insoluble residue was removed by centrifugation at $5000\times\boldsymbol{g}$ for 10 min and subsequently treated three more times with 15 ml of a 10% (w/v) TCA solution. The supernatant was collected, as before, and made up to 100 ml with distilled water; an aliquot of it was taken for determination of soluble nitrogen using the Kjeldahl procedure (AOAC, 1990).

2.2.1.4. Total amino acids. Total amino acids were determined as described by Shahidi, Naczk, Hall, and Synowiecki (1992). Samples were freezed-dried and then hydrolysed for 24 h at 110°C with 6 M HCl (Blackburn, 1978). The HCl was removed under vacuum, and dried samples were reconstituted using a lithium citrate buffer at pH 2.2. The hydrolysed amino acids were then determined using a Beckman 121 MB amino acid ana-

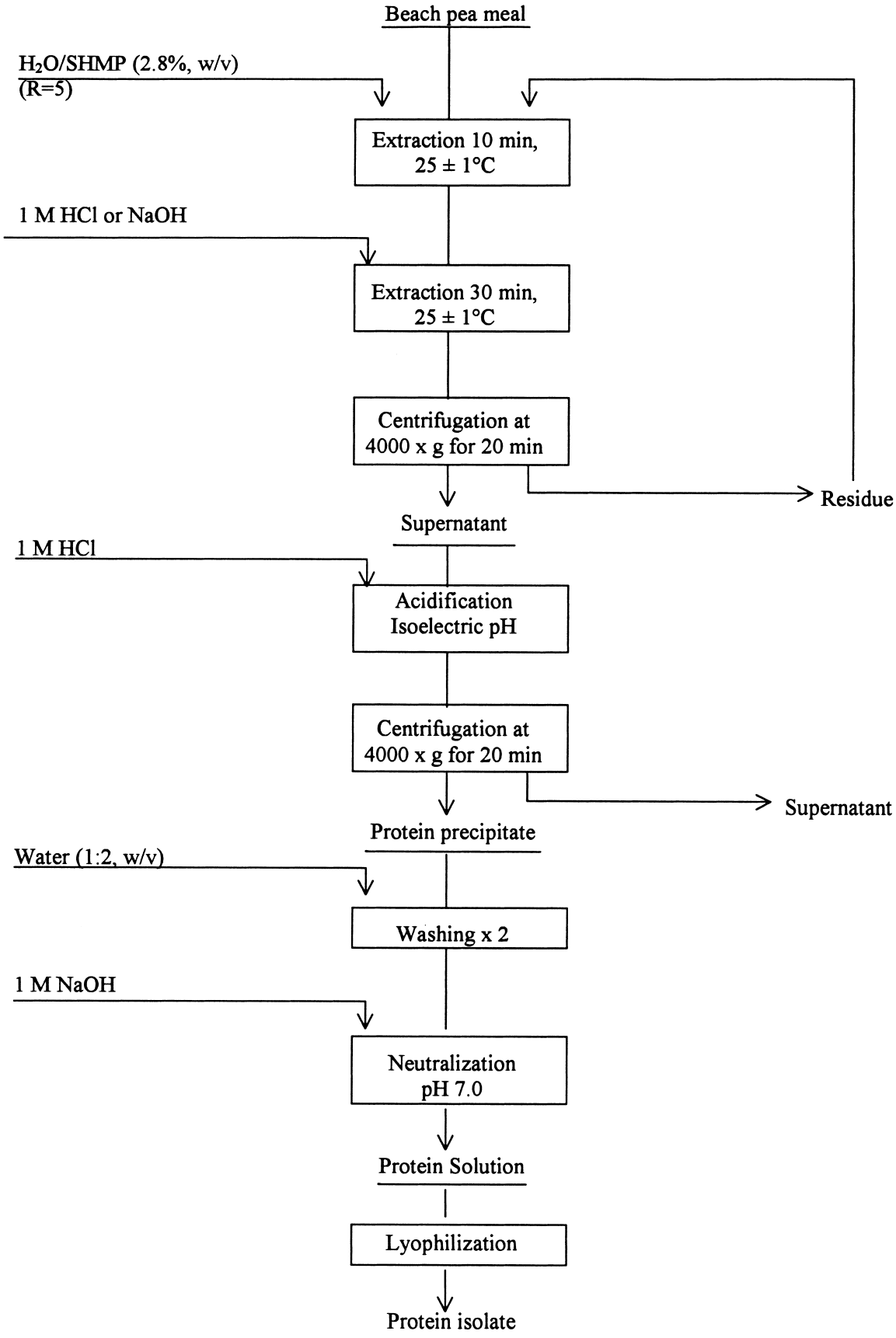

Fig. 1. Flow chart for preparation of protein isolates from beach pea.

lyzer (Beckman Instruments, Inc., Palo Alto, CA; Shahidi et al., 1992). Tryptophan was determined separately by hydrolysis of the sample under vacuum in 3 M mercaptoethanesulphonic acid at 110°C, as described by Penke, Ferenczi, and Kovacs (1974). Cysteine and methionine were determined after performic acid oxidation prior to hydrolysis in 6 M HCl, and measured as cysteic acid and methionine sulphone, respectively (Blackburn, 1978).

2.2.1.5. Evaluation of nutritional parameters. The amino acid composition of samples was used for calculation of the nutritional value of pea proteins as summarized below.

(a) The proportion of essential amino acids (E) to the total amino acids (T) of the protein:

$$\mathrm{E/T\%} = \frac{\begin{array}{c}\mathrm{Ile + Leu + Lys + Met + Cys + Phe + Tyr}\\ \mathrm{+Thr + Trp + Val + His}\end{array}}{\begin{array}{c}\mathrm{Ala + Asp + Arg + Gly + Glu + His + Ile}\\ \mathrm{+Leu + Lys + Met + Cys + \ Phe + Tyr}\\ \mathrm{+Pro + Ser + Thr + Trp + Val}\end{array}} \times 100$$

$$\text{(b) Amino acid score} = \frac{\text{mg of amino acid per g test protein}}{\text{mg of amino acid per g of FAO/WHO standard pattern}} \times 100$$

Essential amino acid (g amino acid/16 g N) pattern of the FAO/WHO standard protein is

$$\mathrm{Ile} = 4.00,\ \mathrm{Leu} = 7.04,\ \mathrm{Lys} = 5.44,\ \mathrm{Met + Cys} = 3.52,\ \mathrm{Phe + Tyr} = 6.08,\ \mathrm{Thr} = 4.00,\ \mathrm{Trp} = 0.96 \text{ and } \mathrm{Val} = 4.96.$$

(c) Predicted biological value (BV)

The following regression equation (Mørup & Olesen, 1976) was used for prediction of BV. $\mathrm{BV} = 10^{2.15} \times q_{\mathrm{Lys}}^{0.41} \times q_{\mathrm{Phe+Tyr}}^{0.60} \times q_{\mathrm{Met+Cys}}^{0.77} \times q_{\mathrm{Thr}}^{2.4} \times q_{\mathrm{Trp}}^{0.21}$

Where,

$$q = \frac{a_{\mathrm{i}}\ \text{sample}}{a_{\mathrm{i}}\ \text{reference}} \quad \text{for } a_{\mathrm{i}}\ \text{sample} \leqslant a_{\mathrm{i}}\ \text{reference}$$

$$q = \frac{a_{\mathrm{i}}\ \text{reference}}{a_{\mathrm{i}}\ \text{sample}} \quad \text{for } a_{\mathrm{i}}\ \text{sample} \geqslant a_{\mathrm{i}}\ \text{reference}$$

a_{i} = mg of the amino acid per g of total essential amino acids.

(d) The predicted protein efficiency ratio (PER) value:

The predicted PER values of different peas and plant parts as well as treated samples of peas were calculated from their amino acid composition based on three equations developed by Alsmeyer, Cunningham, and Happich (1974), as given below.

$$\mathrm{PER} = -0.684 + 0.456(\mathrm{LEU}) - 0.047(\mathrm{PRO}) \quad (1)$$

$$\mathrm{PER} = -0.468 + 0.454(\mathrm{LEU}) - 0.105(\mathrm{TYR}) \quad (2)$$

$$\mathrm{PER} = -1.816 + 0.435(\mathrm{MET}) + 0.780(\mathrm{LEU}) + 0.211(\mathrm{HIS}) - 0.944(\mathrm{TYR}) \quad (3)$$

2.3. Evaluation of functional properties of protein isolates

2.3.1. Water absorption capacity

Water absorption capacity was determined by a combination of the AACC (1995) method and those of Sosulski (1962) and Rutkowski and Kozlowska (1981). A 2-g sample was dispersed in 20 ml of distilled water. The contents were mixed for 30 s every 10 min using a glass rod and after mixing five times, centrifuged at 4000×***g*** for 20 min. The supernatant was carefully decanted; then the contents of the tube were allowed to drain at a 45° angle for 10 min and then weighed. The water absorption capacity of the protein isolate was expressed as percentage increase of the sample weight.

2.3.2. Whippability and foam stability

One hundred millilitres of a dispersion of protein isolates (1%, w/v) in distilled water were homogenized for 60 s using a Polytron (Brinkman PT 3000) homogenizer at 10,000 rpm. Immediately afterwards, the mixture was transferred into a 250-ml measuring cylinder and the foam volume recorded. The percentage ratio of the volume increase to that of the original volume of protein solution was calculated and expressed as foam capacity or whippability (Naczk et al., 1985). Foam stability was expressed (on the basis of 100 ml of a 1%, w/v dispersion) as the volume of the foam remaining after 0, 15, 30, and 60 min of quiescent period.

2.3.3. Fat-binding capacity

Fat-binding capacity of the protein isolates was determined by a turbidimetric method, as described by Voutsinas and Nakai (1983). To a lyophilized sample (40 mg), in a centrifuge tube, 1.5 ml of pure corn oil were added and the mixture was homogenized for 1 min at 8000 rpm using a Polytron homogenizer. The protein dispersion was centrifuged at 3020×***g*** for 20 min after holding for 30 min at room temperature. The free oil, separated after centrifugation, was pipetted off and 2 ml of distilled water were added to the contents of the tube.

Oil adhering to the sides of the tube was then removed with the help of a glass rod. Any oil trapped below the protein precipitate was removed by forcing it to the surface of the water. To the content of the tube, 1 ml of 0.1 M metaphosphoric acid [$(HPO_3)_n$, 35%, pH 2.1] was added, followed by centrifugation at 4200×***g*** for 15 min. The supernatant was pipetted off and the precipitate was washed with distilled water (3–4 ml) without dispersing it. Finally, the tube walls were cleaned with a cotton swab to remove any excess oil deposits. The protein precipitate was mixed well with 0.3 ml of distilled water and then 20 ml of the digestion medium (7 M urea in 50% H_2SO_4) were added to the mixture in 2-ml portions. The mixture was homogenized using a Polytron homogenizer for 30 s at 4000 rpm. The homogenate was held at room temperature for 30 min and the absorbance was then read at 600 nm using the digestion mixture as a blank. The aqueous supernatants, removed in the previous steps, were used for determination of lost protein during the handling of the protein precipitate as given by Lowry, Rosebrough, Farr, and Randall (1951).

The standard curve for determination of bound oil content was prepared as follows. To a series of tubes containing the proteins (40 mg), pure corn oil (0–100 mg; specific gravity of 0.89) were added followed by subsequent mixing with a glass rod. While mixing, 0.3 ml of distilled water and then 20 ml of digestion mixture were added to the tube. The mixture was homogenized at 4000 rpm for 30 s and held at room temperature for 30 min prior reading the absorbance value at 600 nm.

2.3.4. Emulsifying activity

Emulsifying activity of protein isolates was determined according to the modified method of Pearce and Kinsella (1978), as described by Wanasundara and Shahidi (1997). To protein dispersions (0.5%, w/v, 4 ml) in a Britton–Robinson Universal buffer (Britton, 1956) in the pH range of 2.0 to 12.0 and NaCl concentration of 0, 0.35 and 0.70 M, 4 ml of pure corn oil were added. The mixture was then homogenized for 1 min at 2000 rpm using a Polytron homogenizer. A 50-μl volume of the emulsion formed was immediately taken from the bottom of the container and diluted in 10 ml of the same buffer containing 0.10% (w/v) of sodium dodecylsuphate (SDS). Absorbances of the diluted samples were read at 500 nm using a diode array UV/VIS spectrophotometer (Hewlett Packard Canada, Ltd., Montreal, PQ) and recorded as emulsifying activity values.

2.3.5. Emulsion stability

Samples for determination of emulsion stability were prepared in a similar manner to those for determination of emulsifying activity. The absorbance at 500 nm was read as soon as the emulsion was formed, i.e. zero time. Subsequently, aliquots were removed at appropriate time intervals and absorbance values read at 500 nm. Emulsion stability was determined as the time, in min, required for absorbance at 500 nm to reach one half of that for the emulsion at zero time (i.e. half-life; Paulson & Tung, 1988).

2.3.6. Solubility

To study the effect of pH and salt concentration on solubility, 1% (w/v) protein dispersions were prepared by mixing 0.25 g of the isolate with 0, 2.0 or 4.0 ml of a 25.6% (w/v) NaCl solution in order to make a final NaCl concentration of 0.0, 0.35, or 0.70 M. The pH was then adjusted with 2 M NaOH or 2 M HCl, followed by addition of distilled water to reach a 25-ml volume. The protein dispersion was centrifuged at 4000×***g*** for 15 min. The protein content of the dispersion after centrifugation (supernatant) was determined using the Kjeldahl method (AOAC, 1990). The solubility was expressed as percent ratio of the protein content of the supernatant to that of the suspension.

2.4. In-vitro digestibility

In-vitro digestibility of protein isolates was determined using trypsin-pepsin and pepsin-pancreatin enzyme systems according to the method of Saunders, Connor, Booth, Bickoff, and Kohler (1973) with minor modifications. In a centrifuge tube, 1 g of protein material was suspended in 20 ml of 0.10 M HCl and mixed with 50 mg pepsin (from porcine stomach mucosa, 570 AU/mg solid) in 1 ml of 0.01 M HCl. The mixture was gently shaken at 37°C for 48 h and then centrifuged (4000×***g*** for 10 min). After removing the supernatant, solids were suspended in a solution made of 10 ml of water and 10 ml of a 0.10 M phosphate buffer (pH 8.0) containing 5 mg trypsin (from porcine pancreas, 1870 benzyl arginine ethyl ester (BAEE) units/mg solid). The mixture was gently shaken for 16 h at 23°C in a water bath shaker. The digested mixture was then centrifuged and TCA was added to the supernatant to reach a concentration of 8 M in the solution. The supernatant previously obtained from pepsin digestion was also treated in a similar manner. Precipitated proteins were removed by centrifugation at 10,000×***g*** for 25 min. The nitrogen content of the TCA-soluble matter nitrogen content of the supernatant was determined by Kjeldahl nitrogen analysis.

For pepsin-pancreatin digestion, 250 mg of the sample were suspended in 15 ml of 0.10 M HCl containing 1.5 mg of pepsin, followed by gentle shaking for 15 min at 37°C. The resultant solution was then neutralized with 0.50 M NaOH and treated with 4 mg pancreatin (from porcine pancreas, activity equivalent to 4× US Pharmacopeia) in 7.5 ml of phosphate buffer (0.10 M, pH 8.0). The mixture was shaken for 24 h at 37°C in a water bath shaker and the undigested solids were

separated by centrifugation, as given above. The supernatant was treated in a similar manner, as described earlier for trypsin-pepsin digestion; nitrogen content was then determined by Kjeldahl analysis. In vitro digestibility was expressed as percentage enzymatic digestion, as given below.

$$\text{Enzymatic digestion \%} = \frac{\text{Nitrogen (non-protein nitrogen) released by enzyme}}{\text{Total nitrogen content of undigested sample}} \times 100$$

2.5. Statistical analysis

All experiments were replicated at least three times. Mean values with standard deviations (S.D.) were reported when and where necessary. Analysis of variance (ANOVA) was performed and differences in mean values were determined using Tukey's studentized test at $P < 0.05$ and employing ANOVA and TUKEY'S procedures of Statistical Analytical System (SAS, 1990), respectively.

3. Results and discussion

Protein isolates of beach pea were prepared using solvent extraction with NaOH or SHMP solutions. The NaOH-extracted beach pea protein isolate had 86.6% protein, while its SHMP-extracted counterpart contained 85.1% protein (Table 1). The yield of total protein in various protein isolates ranged from 67.9 to 77.3%, when compared with those for green pea and grass pea which varied between 59.4 and 67.0%. During SHMP extraction, it was noticed that precipitation of proteins at isoelectric point results in their coagulation and therefore adjustment of pH to about 4.5 (i.e. isoelectric point) was very difficult. This might be the reason for a lower protein content in SHMP-extracted protein isolates than in their NaOH-extracted counterparts. Incomplete recovery of proteins may, in part, be due to their loss during the washing process or their retention in the residue, due to complexation with other seed components. Deshpande and Campbell (1992) reported that grass pea protein isolates contained 83.3–92.1% protein, depending on the solvent used in their preparation. Sumner, Nielsen, and Youngs (1981) showed that the yield of protein in isolates ranged from 59 to 65% and the protein content of isolates ranged from 91 to 98% in different types of flours prepared from field pea. It was also noted that 8% protein remained in the residue. Ant'Anna, Vilela, and Gomes (1985) used different isolation conditions for protein isolate preparation from pigeonpea in order to alter their functional properties. These authors reported a protein yield of 49.7–63.6% in various isolates. Taha (1987) used 0.05 M NaOH for preparation of pigeonpea protein isolate and recovered 92% protein, as a precipitate, at pH 4.4, with a protein content of 88%. The present results are also similar to those of Sathe and Salunkhe (1981) who also prepared a protein isolate (92.4% protein) from Great Northern bean using a 0.5% Na_2CO_3 extraction solution.

Table 1
Chemical composition of beach pea protein isolates[a]

Constituent, %	NaOH-extracted	SHMP-extracted
Extract recovery	22.8±1.36a	26.5±1.63a
Moisture	2.35±0.01e	2.83±0.01a
Ash	5.99±0.08a	5.85±0.02b
Protein	86.6±1.34ab	85.1±1.50b
Lipid	3.20±0.01c	4.03±0.02a
Crude fibre	1.51±0.02b	1.83±0.03a
Soluble sugars	0.33±0.002f	0.39±0.001ef
Phenolics (mg/100 g)	76.4±0.01a	50.5±0.08b
Condensed tannins	ND	ND
NPN	ND	ND

[a] Results are means of three determinations, on a dry weight basis, ± standard deviation. Means followed by different letters in each row are significantly ($P < 0.05$) different from one another. NaOH, Sodium hydroxide. SHMP, Sodium hexametaphosphate. ND, Not detected. NPN, Non-protein nitrogen.

The ash content of beach pea protein isolate was considerably more than that of green and grass peas which ranged from 2.58 to 3.94%. Furthermore, pea protein isolates contained higher amounts of ash than the whole seeds, perhaps due to salt formation during protein precipitation at the isoelectric point. Lipid content in protein isolates was quite high as it is concentrated with the protein fraction. The content of crude fibre in beach pea protein isolates was 1.51–1.83%, much higher than those from green pea (0.50–0.93%) and grass pea (0.20–1.20%). These results are in agreement with those reported for mung bean, field pea, and green pea (Naczk, Rubin, & Shahidi, 1986; Sumner, Nielsen, & Young, 1981; Thompson, 1977). Soluble sugars in beach pea samples were lower for protein isolates than for their whole seeds. The protein isolates had a reduced content of phenolic compounds. Condensed tannins and non-protein nitrogen were not detected in beach pea protein isolates or those of green and field peas examined, thus indicating that water-soluble constituents such as sugars and phenolic compounds were largely removed under alkaline conditions employed for preparation of protein isolates.

Sulphur-containing amino acids were present in relatively higher quantities in SHMP than NaOH-extracted beach pea (Table 2). Tryptophan content was higher in NaOH than SHMP-extracted isolates. The percentage ratios of essential to total amino acids (E/T,%) for NaOH and SHMP protein isolates (beach pea 43.8 and

44.4, respectively) were well above 36%, which is considered adequate for an ideal protein (FAO/WHO, 1973). The present results demonstrate that isolates prepared are rich in lysine, leucine, glutamic and aspartic acids, but limiting in tryptophan, methionine and cysteine. Ant'Anna et al. (1985) have shown that the percent ratio of essential to total amino acids varies from 37.8 to 41.2 for pigeonpea protein isolates obtained under various isolation conditions. Ant'Anna et al. (1985) and Taha (1987) reported that, in all isolates, tryptophan and sulphur-containing amino acids (methionine and cysteine) were limiting. The present results show a slightly higher amount of total amino acids in beach pea protein isolates than those of mung bean protein isolates extracted by sodium hydroxide solubilization (Thompson, 1977). Distribution of amino acids in beach pea also indicates that beach pea may be used to complement cereal proteins which are low in lysine.

Robaidek (1983) reported that digestibility of proteins is a major factor in their quality assessment. Therefore, availability of total amino acids to the body is also a determining factor in protein quality determination. The predicted biological value of all protein isolates was lower (18–40) than those of the whole seed proteins (21–65), but the levels were higher in SHMP- than NaOH-extracted isolates. The predicted PER values (Table 2) were higher than literature values for seed proteins of cowpea (1.21), pigeonpea (1.82), and *L. sativus* (negative to 0.03; Salunkhe & Kadam, 1989) and green as well as grass pea in this study.

Table 2
Total amino acid composition of beach pea protein isolates extracted with sodium hydroxide (NaOH) and sodium hexametaphosphate(SHMP)[a]

Amino acid	NaOH-extracted	SHMP-extracted
Isoleucine	4.24±0.34a	4.30±0.12a
Leucine	7.95±0.96a	7.97±0.33a
Lysine	7.83±0.25a	7.03±0.10a
Cysteine[b]	0.87±0.05a	1.08±0.04a
Methionine[b]	1.05±0.03a	0.97±0.03ab
Total sulphur amino acids	1.92	2.05
Tyrosine	3.12±0.23a	3.37±0.90a
Phenylalanine	4.97±0.13a	4.94±0.15a
Total aromatic amino acids	8.09	8.31
Threonine	3.49±0.13a	3.46±0.67a
Tryptophan[b]	1.23±0.17a	0.84±0.17a
Valine	4.87±0.29a	4.87±0.23a
Histidine	2.56±0.04a	2.49±0.20a
Total essential amino acids	42.18	41.3
Arginine	7.86±0.37bc	7.78±0.16c
Aspartic acid + asparagine	12.6±0.42ab	12.4±0.38ab
Glutamic acid + glutamine	16.6±0.24a	15.1±0.98a
Serine	5.11±0.25a	4.82±0.29a
Proline	4.15±0.15a	3.98±0.43a
Glycine	3.88±0.68a	3.78±0.11a
Alanine	3.91±0.23a	3.88±0.12a
Total non-essential amino acids	54.1	51.7
E/T,%	43.8	44.4
Amino acid score	110	108
BV	36.5	40.13

[a] Results are mean values of triplicate determinations, ± standard deviation. Means followed by different letters in each row are significantly ($P < 0.05$) different from one another.

[b] Limiting amino acid. E/T, Essential to total amino acid ratio. BV, Biological value.

The water-binding properties of a protein isolate determine its degree of interaction with water, sometimes these are reported in the literature as water absorption. Beach pea protein isolates had water binding capacities of 257–288%, much lower than those of green and grass peas (263–311%). Water-binding capacity of proteins is a function of several parameters, including size, shape, steric factors, conformational characteristics, hydrophilic–hydrophobic balance of amino acids in the protein molecules as well as lipids, carbohydrates and tannins associated with proteins. Thermodynamic properties of the system (interfacial tension, energy of bonding), physicochemical environment (pH, ionic strength, vapour pressure, temperature, presence or absence of surfactants) and solubility of protein molecules, are among major factors responsible for the water-binding capacity of protein isolates (Chou & Morr, 1979). However, polar amino groups of protein molecules are the primary sites of protein–water interactions. Cationic, anionic and nonionic sites bind different amounts of water (Kuntz, 1971). The differences in water-binding capacities of protein isolates may be due to protein concentration and possibly their conformational characteristics. Sumner et al. (1981) reported that freeze-dried protein isolates from field pea had a water absorption capacity of 205%. Similar observations were reported for protein isolates of Great Northern bean (273%; Sathe and Salunkhe, 1981), *Pisum sativum* (260%; Johnson and Brekke, 1983) and Woodstone pea protein preparations (278–293%, Naczk, Rubin, & Shahidi, 1986).

The foaming property and foam stability of protein isolates are summarized in Table 3. The values represent percentage volume increases after whipping of 1% protein isolates in 100 ml water and foam stability is the volume of foam remaining after a specified time period as a percentage of the initial foam volume. Beach pea protein isolates exhibited increased foam volume (128–143%). This foam expansion was markedly lower than that for isolates. The low foaming capacity of beach pea protein isolates, compared to green pea (170–185%) and grass pea (151–175%), could be due to inadequate electrostatic repulsions, and hence, excessive protein–protein interactions to form aggregates that are detrimental to foam formation. Increase in foam expansion in certain protein isolates might be due to increased solubility, rapid unfolding at the air–water interface, limited intermolecular cohesion and flexibility of the protein surfactant molecules (Kinsella, Damodaran, & German,

1985). Lawhon, Carter, and Mattil (1972) also reported that constituents other than proteins may aid in the formation of whipped foam. As seen in Table 3, foams from beach pea protein isolates were reasonably stable after 30 and 60 min of standing. As the time of standing progressed, the foam volume was decreased. A similar trend was observed for cowpea (Aluko & Yada, 1997), soybean, and sunflower protein isolates (Lin, Humbert, & Sosulski, 1974). Kinsella et al. (1985) and Myers (1988) have suggested that, in foams, the ability to hold water in the protein film surrounding the air particle and presence of electrostatic repulsions are important for their stability.

Data on fat absorption show that beach pea protein isolates had oil absorption values ranging from 64 to 82 ml/100 g, respectively. Low fat-binding capacity of beach pea protein isolates suggests the presence of a large proportion of hydrophilic as compared to hydrophobic groups on the surface of the protein molecules. These results are comparable to those for Woodstone pea protein preparations (fat absorption of 90.1–94.5%; Naczk et al., 1986) and field pea protein isolates (fat absorption of 90 to 127%; Sumner et al., 1981). The mechanism of fat-binding by proteins is not fully understood, but it appears to be affected by lipid-protein complexes and protein content (Kinsella, 1979). Lin et al. (1974) have shown that the availability of lipophilic groups may also have an important role in contributing to higher binding of fat to proteins. However, low fat absorption may be desirable in some applications, such as Seviya and Chakali prepared by deep-fat frying of legume-based products.

Emulsifying activity (EA) of beach pea protein isolates (NaOH- and SHMP-extracted), measured as a function of pH and NaCl concentration, is shown in Fig. 2. Emulsion activity of all protein isolates increased as the pH and salt concentration increased. Sodium chloride, at 0.35 and 0.70 M, increased EA compared to the samples without it at the initial stage (pH 4–9), but afterwards decreased as pH increased. At higher pH and salt concentration, the EA of all protein isolates decreased. There was insignificant ($P > 0.05$) difference in emulsifying activity of NaOH- and SHMP-extracted protein isolates (Fig. 2). The effect of NaCl on EA may be due to its effect on protein adsorption at the oil-water (O/W) interface. Results of the present study, where EA first rose and then fell with addition of NaCl, are in accord with those of Waniska, Shetty, and Kinsella (1981) and Paulson and Tung (1988). The increase in EA with pH increase might suggest that droplet size decreased as the pH increased beyond the isoelectric point.

The effects of pH and salt concentration on emulsion stability (ES) are presented in Fig. 3. Increase in pH resulted in an increase in ES, but an increase in NaCl concentration in the medium gave lower ES values in all samples studied. There was insignificant ($P > 0.05$) difference between NaOH- and SHMP-extracted beach

Table 3
The predicted protein efficacy ratio (PER), foam expansion and foam stability of beach pea protein isolates[a]

Property	NaOH-extracted	SHMP-extracted
PER-1	2.75	2.76
PER-2	2.81	2.44
PER-3	2.80	2.17
Foam expansion	142.8±2.30a	128.4±3.98b
Foam stability %		
— 15 min	91.6±0.85a	90.6±1.36a
— 30 min	91.6±1.24a	89.1±1.45a
— 60 min	90.1±1.02a	87.5±1.83a

[a] PER values calculated according to Alsmeyer, Cunningham, and Happich (1974). NaOH, sodium hydroxide, SHMP, sodium hexametaphosphates. Results for foam properties are mean values of three determinations±standard deviations determined at pH = 7.0 and represent percentage value increase after whipping 100 ml of 1% (w/v) protein solution. Foam stability results indicate percentage of foam remaining after a given period of time.

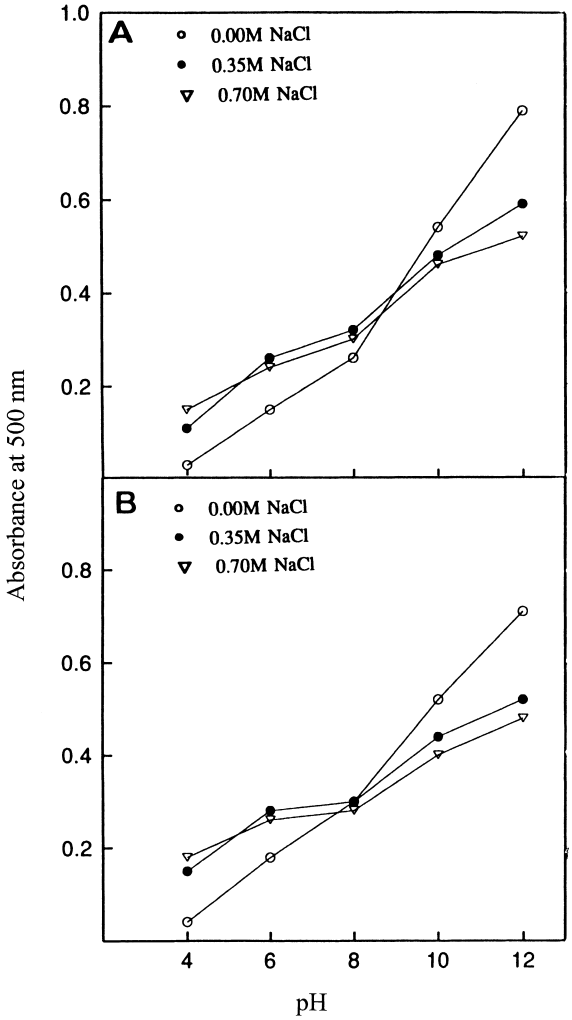

Fig. 2. Emulsifying activity of beach pea protein isolates (measured as absorbance at 500 nm) as a function of pH and NaCl concentration: (A) NaOH-extracted, (B) SHMP-extracted.

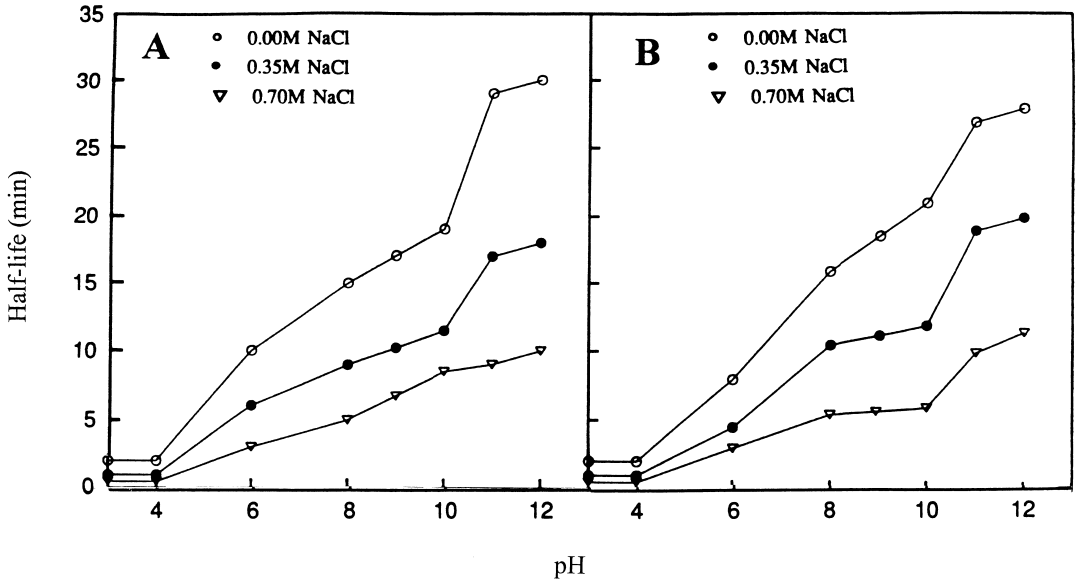

Fig. 3. Emulsion stability (time required to reduce the absorbance at 500 nm by 50%) of beach pea protein isolates: (A) NaOH-extracted, (B) SHMP-extracted as a function of pH and NaCl concentration.

pea protein isolates in terms of emulsion stability. The low ES at low pH and salt concentration may be attributed to increased rate of interaction between the emulsified droplets, since net charge on the proteins is decreased by the presence of the chloride ions. As the pH and ionic strength were increased, the Coulombic repulsion increased between neighbouring droplets, coupled with increased hydration of the charged protein molecules, which may account for the higher ES obtained. These results are in agreement with literature values for cowpea (Aluko & Yada, 1997), canola (Paulson & Tung, 1988), and green pea protein isolates (Johnson & Brekke, 1983).

Solubility of protein isolates prepared by NaOH- or SHMP- extraction as a function of pH and NaCl concentration is presented in Fig. 4. The protein isolates in the absence of NaCl exhibited a gradual increase in solubility above and below their isoelectric points. Protein isolates of beach pea, as well as those of green and grass peas, exhibited minimum solubility at pH 4.5, at which they precipitated. Åman and Gillberg (1977) reported that in addition to protein, preparations may also contain RNA, acidic polysaccharides, phytic acid and acidic polyphenols which are extracted from the meal. The effect of NaCl concentration on protein isolates was to increase their solubility around the isoelectric point and decrease it in the higher acidic and alkaline regions. The decrease in solubility of protein isolates in beach pea was more when pH and salt concentration increased when compared to protein isolates from green pea and grass pea (results not shown). These results are similar to those obtained for green pea (Johnson & Brekke, 1983), sesame seed (Prakash & NarasingaRao, 1986), canola (Paulson & Tung, 1987),

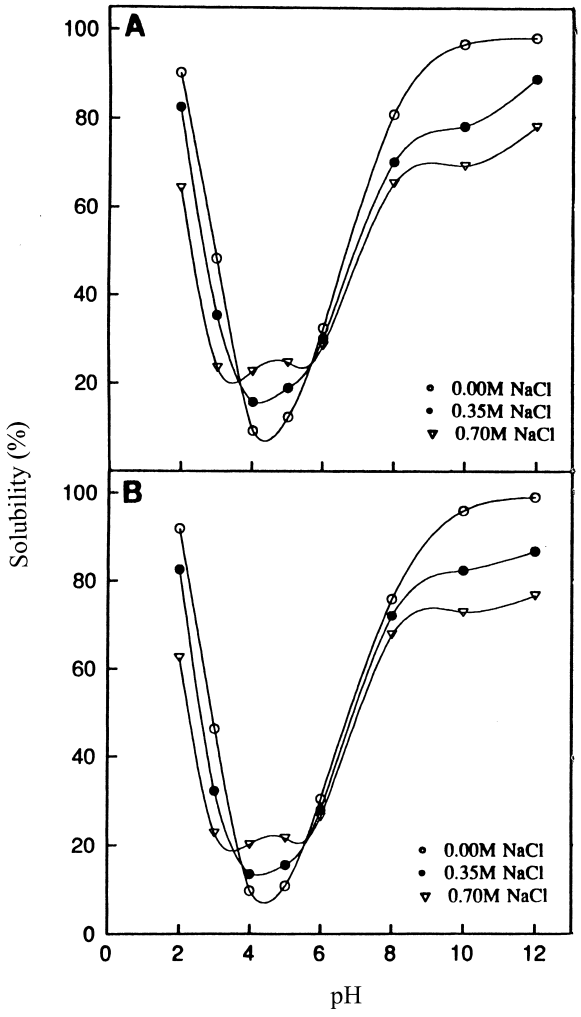

Fig. 4. Solubility of beach pea protein isolates as a function of pH and NaCl concentration: (A)NaOH-extracted, (B) SHMP-extracted.

soybean flour (McWatter & Holmes, 1979), and cowpea (Aluko & Yada, 1995, 1997).

In-vitro digestibility of beach pea with pepsin-trypsin and pepsin-pancreatin were evaluated. Beach pea protein isolates (NaOH- and SHMP-extracted) had digestibility values with pepsin-trypsin of 80.6 to 82.6% and with pepsin-pancreatin of 78.6–79.2%. SHMP-extracted protein isolates of all pea cultivars (including green and grass peas; not shown) showed lower digestibility with pepsin-trypsin or pepsin-pancreatin than NaOH-extracted isolates. Protein digestibility of isolates was significantly ($P \leqslant 0.05$) higher than those of the whole meals for cowpea (73%) and pigeonpeas (59%; Salunkhe & Kadam, 1989). Johnson and Brekke (1983) reported that in-vitro digestibility of green pea protein isolates with a multi-enzyme system was 84%. Le-Guen, Huisman, Gueguen, Beelen, and Verstegen (1995) studied digestibility of protein isolates in piglets from two varieties of pea (Finale and Frijaune) and reported that protein digestibility ranged from 83.7 to 85.4%. Similarly, Wanasundara and Shahidi (1997) have shown that flaxseed protein isolates had an in-vitro protein digestibility of 90% with pepsin-trypsin and pepsin-pancreatin enzymes.

4. Conclusions

Functional properties and in-vitro digestibility of beach pea protein isolates are similar to those of other pea protein isolates. Meanwhile, nutritional value (BV and PER) of beach pea protein isolates was better than isolates of green pea and grass pea. Thus, beach pea protein isolates may be used in the formulation of a wide range of new food products or as a possible replacement for animal proteins in conventional foods.

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