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Protein classification of beach pea (*Lathyrus maritimus* L.)

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Abstract

Protein fractionation of whole seed, cotyledons and hulls of beach pea (*Lathyrus maritimus* L.) was carried out. Surface topography of pea protein isolates and protein fractions, as well as their polyacrylamide gel electrophoresis (PAGE), was studied. The nitrogen solubility of beach pea seed meal was minimum at pH 4.5. Globulin was the major protein fraction present and its content in whole seeds (57%), cotyledons (62%) and hulls (24%) of beach pea was lower than those of other common pea cultivars; the same was true for its albumin content. However, glutelin content in beach pea seed and its parts (cotyledons and hulls) was higher than those of other pea cultivars. The albumin fraction contained the highest amount of total sulphur-containing amino acids followed by glutelin, globulin and prolamine. The amount of sulphur-containing amino acids in beach pea protein fractions was higher than in other peas. Predicted biological value of albumin and glutelin fractions of beach pea was also higher than green pea and grass pea. Beach pea seed protein fractions showed different UV spectra from other pea cultivars. Protein isolate and protein fractions of beach pea seeds showed similar topographical characteristics to those of other peas. Major polypeptide bands in the range of 35–47 kDa in the protein isolate, as well as protein fractions for beach pea, were detected by PAGE.

Keywords: Protein classifications; Beach pea; *Lathyrus maritimus*

1. Introduction

Beach pea (*Lathyrus maritimus* L.) is a relatively unknown leguminous plant which 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 is not yet cultivated on a commercial scale, possibly due to lack of knowledge and appreciation of its nutritional value. Recently, we have reported the compositional characteristics and physicochemical properties of beach pea seeds and plant parts (Chavan, Shahidi, Bal, & McKenzie, 1999a, b; Shahidi, Chavan, Bal, & McKenzie, 1999).

In general, the protein quality of legumes suffers from low levels of essential amino acids, namely methionine, cysteine and tryptophan (Bressani, 1972; FAO, 1970). The proteins present in legume seeds can be broadly classified into metabolic proteins, which are involved in normal cellular activities, and storage proteins which are synthesized during seed development. Of the storage proteins, globulin constitutes a major proportion of the legume seed proteins and its limitations in human and monogastric nutrition are well known (Millerd, 1975). The amino acid composition of food crops can be altered, either by varying the relative proportions of embryo and endosperm, or by changing the relative proportions of metabolic and storage proteins. Pant, Nair, Singh, and Koshti (1974) separated the seed flour of 28 species of non-edible legumes into fractions such as albumin, globulin, prolamine, glutelin, and non-protein nitrogen. Fractionation and amino acid composition of bean cotyledon protein revealed that the alkali-soluble fraction had the highest proportion of methionine (Yu & Bliss, 1978). Information on the distribution of seed protein fractions and amino acids of beach pea is lacking in the existing literature. Therefore,

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the objective of this work was to study the classification of beach pea and related pea proteins, their amino acid composition and nutritional quality.

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. Samples were then dried, ground and stored for chemical analyses and processing. Seeds of green pea (*Pisum sativum* L.) and grass pea (*Lathyrus sativus* L., var. code No. X850002), used for comparison in certain experiments, 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 solvent extraction for separation of different types of proteins.

2.2. Separation of protein classes based on their solubility characteristics

Protein classes of beach pea were separated according to their solubility using a modified version of the Osborne classification procedure as described by Lund and Sandstrom (1943). Dried and defatted pea samples (approximately 2–3 g) were dispersed into 25 ml of distilled water and extracted over a 15 min period at room temperature ($25\pm1^{\circ}C$) using a Gyrotory shaking water bath (Gyrotory water bath shaker Model G76, New Brunswick Scientific Co., Inc. New Brunswick, NJ). The suspension was then centrifuged at $4000\times\boldsymbol{g}$ for 10 min and the supernatant was recovered and saved. The residues were re-extracted twice more with the same solvent and recovered supernatants were combined and designated as the water-soluble fraction. The residue was then extracted successively with 5% (w/v) NaCl, 70% (v/v) ethanol at 65°C in a shaking water bath, and 0.2% (w/v) NaOH in a similar manner as for the water-soluble fraction; respective soluble fractions were collected separately. The total nitrogen contents of the supernatants collected and the residue left after sequential extractions were analysed for the protein content using the Kjeldahl method of analysis. The content of each protein fraction was calculated as the percentage of the total nitrogen content (as sum of nitrogen contents of all fractions, including the residue) of the meal.

2.3. Determination of protein and soluble nitrogen

Approximately 0.5 g of pea meals were dispersed in distilled water (1:100, w/v) and pH of the dispersion was adjusted between 2.0 and 12.0 using a 1 M solution of HCl or NaOH. The dispersions were shaken at 200 rpm in an orbital shaker for 30 min and pH values were recorded after mixing. Samples were centrifuged at $3500\times\boldsymbol{g}$ for 20 min and nitrogen content of the supernatant (extract) was determined by Kjeldahl analysis (AOAC, 1990). The content of soluble nitrogen was expressed as the percent ratio of nitrogen in the supernatant to that in the meal.

The pH of the remaining supernatant was adjusted to 4.5 ± 0.1. It was then centrifuged at $4000\times\boldsymbol{g}$ for 20 min and nitrogen content of an aliquot of it was determined by Kjeldahl analysis (AOAC, 1990). The difference in the soluble nitrogen content of supernatants before and after pH adjustment to 4.5 ± 0.1 was considered as protein nitrogen content and expressed as percentage of protein nitrogen recovered from total nitrogen of the meal. Volume of aliquots recovered for nitrogen determination was considered in the calculations. Percentages of soluble nitrogen and protein nitrogen were calculated using the formula given below.

$$\text{Soluble nitrogen}\% = \frac{\text{mg of nitrogen}_{\text{extract}}}{\text{mg of nitrogen}_{\text{sample}}}\times 100$$

$$\text{Protein nitrogen}\% = \frac{\text{mg of nitrogen}_{\text{extract}} - \text{mg of nitrogen}_{\text{supernatant}}}{\text{mg of nitrogen}_{\text{sample}}}\times 100$$

2.4. 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 subsequently 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 analyzer (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, Frenczi, and Kovac (1974). Cysteine and methionine were determined after performic acid oxidation prior to hydrolysis in 6 M HCl, and were measured as cysteic acid and methionine sulphone, respectively (Blackburn, 1978).

2.5. Evaluation of nutritional parameters of beach pea proteins

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:

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

(b) Amino acid score

$$= \frac{\text{mg of amino acid per test protein}}{\begin{array}{c}\text{mg of amino acid per g of} \\ \text{FAO/WHO standard pattern}\end{array}} \times 100$$

Essential amino acid (g amino acid/16 g N) pattern of the FAO/WHO standard protein is Ile = 4.00, Leu = 7.04, Lys = 5.44, Met + Cys = 3.52, Phe + Tyr = 6.08, Thr = 4.00, Trp = 0.96 and Val = 4.96.

(c) Predicted biological value (BV)

The following regression equation (Mørup & Olesen, 1976) was used for prediction of BV.

$$\text{BV} = 10^{2.15} \times q_{\text{Lys}}^{0.41} \times q_{\text{Phe+Tyr}}^{0.60} \times q_{\text{Met+Cys}}^{0.77} \times q_{\text{Thr}}^{2.4} \times q_{\text{Trp}}^{0.21}$$

where,

$$q = \frac{{}^{a_i}\text{sample}}{{}^{a_i}\text{reference}} \quad \text{for } a_i\text{sample} \leqslant a_i\text{reference}$$

$$q = \frac{{}^{a_i}\text{reference}}{{}^{a_i}\text{sample}} \quad \text{for } a_i\text{sample} \geqslant a_i\text{reference}$$

$$a_i = \begin{array}{l}\text{mg of the amino acid per g of total} \\ \text{essential amino acid}\end{array}$$

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

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

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

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

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

2.6. Separation of different protein fractions and polyacrylamide gel electrophoresis

2.6.1. Separation of protein fractions

Defatted and dried pea samples (1 g) were dispersed, while stirring with a magnetic stirrer, into 25 ml of distilled water and extracted over a 30 min period at room temperature (22°C). The suspension was then centrifuged at 4000×***g*** for 20 min and the resultant supernatant recovered. The residues were re-extracted three more times with the same solvent and recovered supernatants were combined and designated as the water-soluble fraction. The residue was then extracted successively with a 0.5 M solution of sodium chloride in a 0.01 M phosphate buffer (pH 7.0), 70% (v/v) ethanol at 65°C in a shaking water bath, and 0.1 M sodium hydroxide to separate the total seed proteins into albumin, globulin, prolamine and glutelin fractions, respectively. Proteins from respective soluble fractions were precipitated by isoelectric precipitation at pH 4.5, using 1 M HCl or NaOH and separated by centrifugation at 12,000×***g*** for 20 min (Sorvall Superspeed RC2-B, Automatic Refrigerated Centrifuge, Newtown, CT). The precipitate was then washed with distilled water at pH 4.5, redispersed in distilled water, neutralized at pH 7.0, and subsequently freeze dried. These freeze-dried fractions were used for determination of total crude protein and amino acid composition.

2.6.2. Scanning electron microscopy (SEM)

Structural morphology of protein fractions was studied using SEM. Samples of freeze dried protein isolates were mounted on circular aluminium stubs with double sticky tape, and then coated with 20 nm of gold using Edwards S150A sputter coater, examined and photographed in a Hitachi (S-570) scanning electron microscope (Hitachi S-570 Scanning Electron Microscope Hitachi, Ltd. Tokyo, Japan) at an accelerating potential of 20 kV.

2.6.3. Polyacrylamide gel electrophoresis (PAGE)

Protein fractions, separated as described in the above section, and protein isolates (using sodium hydroxide and sodium hexametaphosphate (SHMP) extraction procedures (Chavan, Shahidi, Bal, & McKenzie, 2000) were used for PAGE studies.

For comparison, non-denatured proteins present in protein isolates were prepared by extracting the meal with water (pH adjusted to 9.0 with 1M sodium hydroxide) and SHMP (2.8%, w/v) at a meal to solvent ratio of 1:5 (w/v) at pH 9.0. The extracted protein was precipitated at pH 4.5 and then redissolved in distilled water, adjusted to pH 7.0 and extensively dialyzed against 10 changes of distilled water at 4°C for 72 h. The dialyzed extract was centrifuged at 12,000×***g*** for 20 min and then freeze dried prior to use for non-denaturing PAGE (NPAGE) studies.

NPAGE was performed on 12% (w/v) vertical polyacrylamide Bio-Rad gels (13.5 cm, length and 2 mm thick) at pH 6.8 using a 20 mM Tris-glycine buffer containing 1% bromophenol blue and 0.01% sodium azide. Protein samples (100 μg) were loaded onto each well and electrophoresed (Electrophoresis Apparatus Bio-Rad Protean™, Bio-Rad Laboratories, Hercules, CA) at a constant current of 80 volts for stacking gels and 180 volts for resolving gels supplied by a Pharmacia electrophoresis constant power supply unit (ECPS 2000/300, Pharmacia Fine Chemicals, Uppsala, Sweden).

SDS-PAGE was carried out on Bio-Rad gels composed of stacking gel (4%, w/v, 1.5 cm) and resolving gel (12%, w/v, 12 cm) using 20 mM Tris-glycine buffer containing 0.1% SDS and 0.01% sodium azide at pH 6.8. Protein samples were dissolved in 65 mM Tris-HCl (pH 6.8) containing 10% (w/v) SDS and 1% of bromophenol blue. Reduction of disulphide bridges was performed by the addition of a small quantity of dithiothreitol (DTT, 0.8M) at 100°C for 3 min. Protein samples (100 μg) were loaded onto each well and electrophoresis was conducted as described for NPAGE studies. The molecular weight markers used were myosin (205 kDa), β-galactosidase (116 kDa), phosphorylase b (97.4 kDa), bovine serum albumin (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (29 kDa), and α-lactalbumin (14.2 kDa).

Following electrophoresis, gels were stained with Coomassie Brilliant Blue R-250 in acetic acid-water-methanol (1:4:5, v/v/v) and destained in a mixture of methanol-acetic acid-water (1:1.5:17.5, v/v/v) until a desired background colour was obtained. The gels were fixed and stored in a 7% (v/v) acetic acid solution.

2.7. Statistical analysis

All experiments were replicated at least three times. Mean values and standard deviations were reported when and where necessary. Analysis of variance (ANOVA) was performed and differences in mean values 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

The relative proportions of protein fractions of beach pea, separated according to their solubility in different solvent systems, are presented in Table 1. The water-soluble fraction of total nitrogen for beach pea was 43.0% which is similar to the 44.0 and 44.8% for green and grass peas, respectively, but the salt-soluble fraction was 2–3% higher than other pea cultivars tested. The alkali-soluble fraction of total nitrogen was approximately 2% lower than other peas, but the alcohol-soluble fraction (prolamine) was similar to other pea samples. The residual nitrogen fraction is due to the presence of other proteins which might be complexed with phenolic compounds, including tannins, and could remain in the residue.

The percentages of protein nitrogen and soluble nitrogen of beach pea, under different pH conditions are presented in Fig. 1A, B, respectively; the results are compared with those of green and grass peas. Approximately 50% of the total nitrogen content of pea seeds examined was soluble at pH 6–7. The nitrogen solubility of pea seeds was lowest at pH 4.5 and increased below and above this pH; the maximum solubility was observed at pH 10 and above. These results are similar to those of green gram (Krishnamurthy & Rama Rao, 1976), yellow pea (Hsu, Leung, Morad, Finney, & Leung, 1982) and moth bean (Borhade, Kadam, & Salunkhe, 1984). Prinyawiwatkul, Beuchat, McWatters,

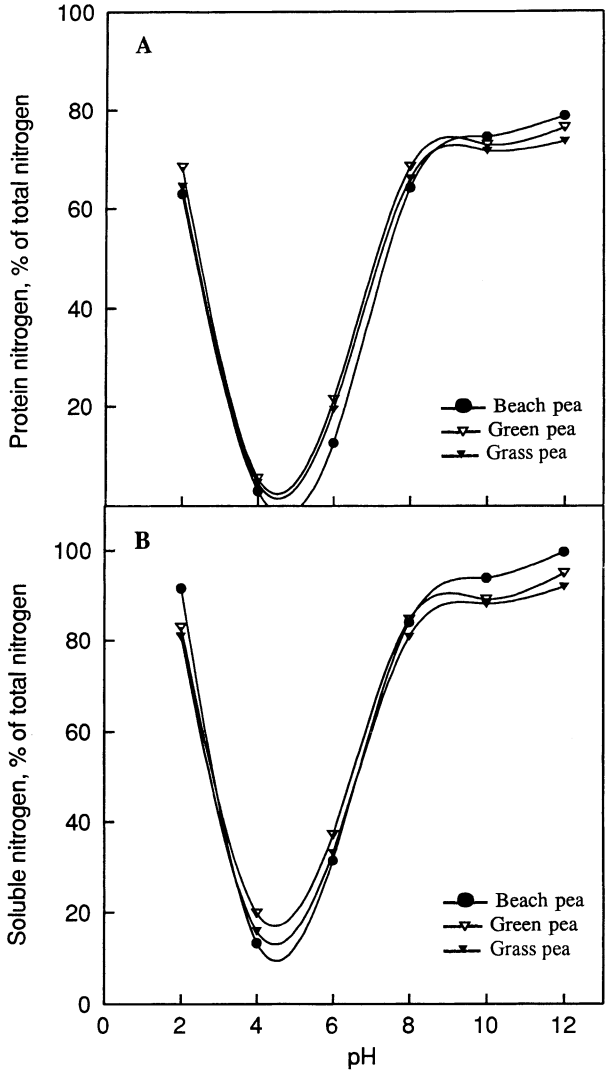

Fig. 1. Percentage of (A) protein nitrogen and (B) soluble nitrogen of beach pea as affected by pH of the extraction medium. Results are compared with those of green and grass peas.

and Phillips (1997) reported that the solubility of cowpea proteins was minimum at pH 4.0, but increased in both the acidic and alkaline regions. Most legumes have a protein solubility of about 10% or less at their isoelectric pH (Sefa-Dedeh & Stanley, 1979). Padmashree, Vijayalakshmi, and Puttaraj (1987) and Sosulski, Kasirye-Alemu, and Sumner (1987) have shown that solubility of cowpea protein at pH 4.0 ranges from 17 to 40%. Similar results for nitrogen solubility of *Phaseolus angalaris*, *Phaseolus calcaratus*, and *Phaseolus lablab* legume seeds were shown by Chau, Cheung, and Wong (1997). Taha (1987) reported that pigeonpea proteins exhibit a maximum precipitation (92%) at pH 4.4 when extracted with a 0.05M NaOH solution. The solubility of proteins or their extraction at the isoelectric point is generally at its lowest due to the overall neutral charge of protein molecules.

The separation of different protein fractions from beach pea was carried out using a number of solvents, namely water, salt, alcohol and alkali; their distribution in seeds, as well as cotyledons, hulls and residues is presented in Table 2. Globulin was the major protein of cotyledons of beach pea samples examined. Meanwhile, the contents of albumin and globulin in beach pea seeds, its cotyledons and hulls were lower than those of green and grass peas (results not shown). However, glutelin contents in beach pea seeds, cotyledons and hulls were higher than those of green pea and grass pea (results not shown). Hulls are the major storage sites of glutelin and non-protein nitrogen (as compared to other components) but contain a much smaller proportion of albumin and globulin. Similar results are reported in the literature for chickpea, pigeonpea, kidney bean, Great Northern bean and black gram (Singh & Jambunathan, 1982; Singh, Jambunathan, & Gurtu, 1981; Sathe, Deshpande, & Salunkhe, 1984).

The various protein fractions of beach pea were analyzed for their amino acid composition and results are shown in Table 3. The albumin fraction contained the highest amount of sulphur-containing amino acids, followed by glutelin, globulin, and prolamine in beach pea; these are similar results to those of green and grass peas (results not shown), and the amounts of these amino acids were higher in beach pea than other pea samples examined. The contents of leucine, lysine, aspartic acid, glutamic acid and alanine were higher in the albumin fraction of beach pea seed proteins than in other fractions. Globulin was the major protein fraction with a lower proportion of sulphur-containing amino acids than those of albumin and glutelin. The total essential amino acids, the ratio of essential to total amino acids, amino acid score and BV of different protein fractions of beach pea are also shown in Table 3. These results are in agreement with literature values for chickpea, pigeonpea, red bean, mung bean and broad bean (Liang, An, & Wu, 1988; Singh & Jambunathan, 1982). It may also be concluded that the selection of cultivars in which the albumin and glutelin fractions are higher would result in improved methionine and cysteine content in the seeds of pea legumes. Predicted PER values for albumin, globulin, prolamine and glutelin fractions for beach pea were similar to or lower than those of other pea seed fractions (Table 4).

UV spectra of individual protein fractions from beach pea are shown in Fig. 2 and compared with those of green and grass peas. The absorption maxima of separated fractions occurred mostly in the range 260–282 nm. Beach pea seed protein fractions showed different spectra from those of green pea and grass pea (Fig. 2) with peak maxima at 282 (albumin), 260 (globule), 280 (prolanine) and 282 nm (glutelin). This difference in spectral data shows that beach pea protein fractions might contain different proportions of amino acids or may contain extracted phenolics and condensed tannins which could potentially change their UV spectra. Padhye (1979) studied the UV spectra of black gram proteins and found that albumins, globulins, prolamines and glutelins had similar UV spectral features.

The SEM structures of flours, protein isolates, albumins, globulins, prolamines and glutelins of beach pea

Table 1
Protein fractions of beach pea according to their solubility[a]

Protein fraction	Fraction of total nitrogen (%)
Water-soluble	43.0±1.23
Salt-soluble	41.0±1.03
Alcohol-soluble	4.50±0.08
Alkaline-soluble	6.35±0.10
Residue	5.07±0.32

[a] Results are means of four determinations, on a dry weight basis±standard deviation.

Table 2
Distribution (%) of protein fractions in different anatomical parts of beach pea seeds[a]

Sample	Albumin	Globulin	Prolamine	Glutelin	Residue
Whole seed	13.8±1.12a	57.2±2.14a	3.08±0.26a	19.1±1.07b	6.82±0.36b
Cotyledons	14.8±1.45a	61.9±2.82a	2.87±1.20a	18.9±2.11b	1.60±0.98c
Hulls	3.26±0.88b	23.7±1.94b	3.48±0.78a	35.9±2.52a	33.7±1.63a

[a] Results are means of three determinations, on a dry weight basis, and are expressed as percentage of total protein (%N×6.25). Means followed by different letters in each column are significantly ($P < 0.05$) different from one another.

Table 3
Total amino acid composition of seed protein fractions of beach pea (g/16 g N)[a]

Amino acid	Albumin	Globulin	Prolamine	Glutelin
Isoleucine	4.64±0.11a	4.09±0.13bc	4.09±0.12bc	4.06±0.13c
Leucine	8.48±0.12a	7.73±0.12bc	7.40±0.11d	7.57±0.15cd
Lysine	9.16±0.10a	7.06±0.13b	6.52±0.12cd	6.20±0.18d
Cysteine[b]	1.32±0.08a	0.93±0.06bc	0.77±0.10c	1.20±0.11a
Methionine[b]	1.20±0.04a	0.70±0.03c	0.43±0.08d	0.76±0.06bc
Total sulphur amino acids	2.52	1.63	1.20	1.96
Tyrosine	3.69±0.11a	3.30±0.10c	3.79±0.12a	3.33±0.12bc
Phenylalanine	5.41±0.09a	4.73±0.21cd	4.30±0.21d	4.76±0.15bc
Total aromatic amino acids	9.10	8.03	8.09	8.09
Threonine	4.41±0.13b	3.52±0.09d	5.15±0.14a	3.88±0.12c
Tryptophan[b]	0.80±0.05b	0.94±0.06a	0.65±0.05c	0.40±0.03d
Valine	5.53±0.16a	4.88±0.11bcd	4.67±0.13d	4.87±0.16cd
Histidine	2.59±0.03c	2.97±0.08a	2.62±0.10bc	3.08±0.10a
Total essential amino acids	47.23	40.85	40.39	40.11
Arginine	8.42±0.17b	8.73±0.21ab	7.51±0.15c	8.97±0.23a
Aspartic acid + Asparagine	12.1±0.18a	10.5±0.15c	11.6±0.24ab	11.21±0.28b
Glutamic acid + Glutamine	17.2±0.24a	16.4±0.23b	14.3±0.26c	17.14±0.30a
Serine	5.33±0.12a	4.78±0.13b	5.30±0.13a	5.13±0.11a
Proline	4.28±0.15a	4.05±0.10ab	3.70±0.18b	4.26±0.10a
Glycine	4.39±0.10c	4.00±0.11d	4.87±0.11a	4.46±0.13bcb
Alanine	5.11±0.13a	4.15±0.12bc	3.61±0.14d	4.00±0.20c
Total non-essential amino acids	56.8	52.6	50.92	55.17
E/T,%	45.4	43.71	44.23	42.10
Amino acid score	124	105.22	104.92	102.86
BV	51.0	39.0	26.77	52.21

[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. E/T, essential to total amino acids ratio; BV, biological value.
[b] Limiting amino acid.

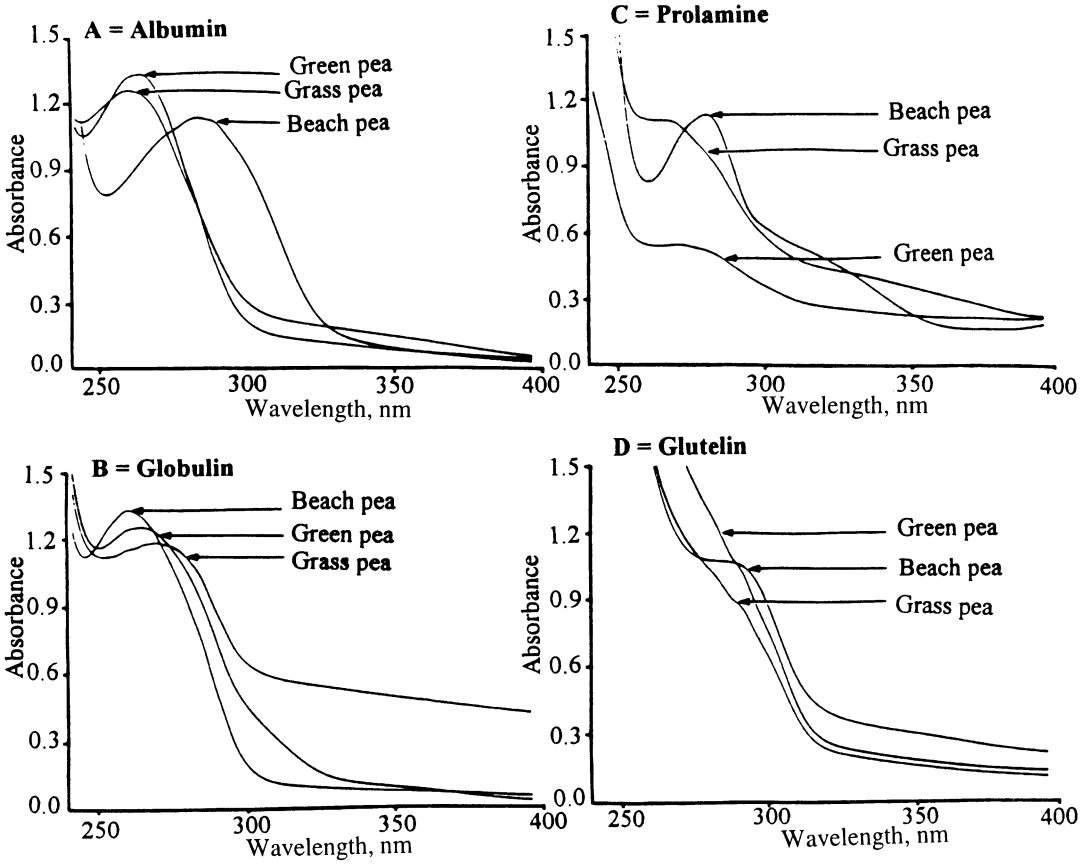

Fig. 2. UV spectra of beach pea protein fractions (A, Albumin; B, Globulin; C, Prolamine; D, Glutelin). Results are compared with those of green and grass peas.

are shown in Fig. 3. The SEM results indicate that albumins of beach pea had a very smooth and plate-like surface topography, while globulins were irregular in shape with rougher surfaces and large size particles. Prolamines and glutelins also were irregular in shape and with large particle size. However, prolamines showed higher porosity and looser structure than glutelins, even at a lower magnification. Pea flours and protein isolates, as expected, consisted of all these four protein fractions. However, their morphological characteristics resembled those of the fractions isolated (Fig. 3). The different topographical characteristics of protein fractions (albumin, globulin, prolamine and glutelin) may contribute to the overall physicochemical and functional properties of pea seed proteins. All protein fractions of beach pea seeds showed similar topographical characteristics when compared to those of green pea and grass pea protein fractions and protein isolates (results not shown). Thus, the present results lend further support to the findings of Sathe (1981), who employed SEM to study the surface structure of albumins and globulins from Great Northern bean and reported that albumins had rod-like structures, while globulins were irregular in shape.

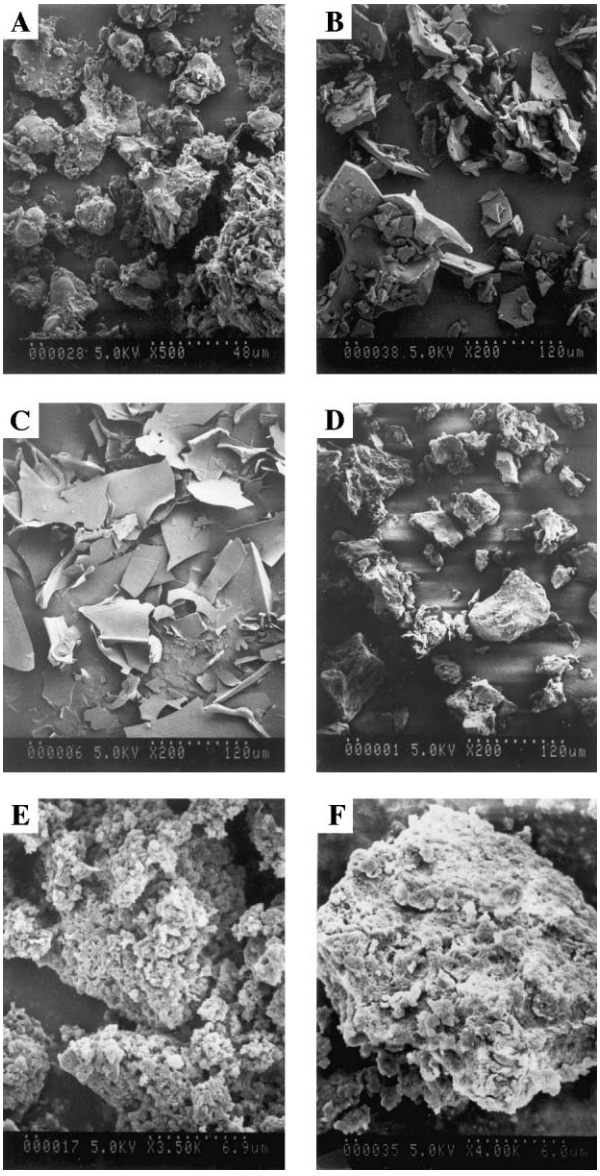

Fig. 3. Scanning electron micrograph of (A) beach pea flour and (B) protein isolate; (NaOH extracted): and protein fractions (C, albumin; D, globulin; E, prolamine; F, glutelin).

Electrophoretic patterns of NaOH- and SHMP-extracted and extensively dialysed protein isolates from beach pea in a NPAGE system are shown in Fig. 4A. Hames (1981) showed that NPAGE separates proteins based on their size and negative charge without having any denaturation effect. The major protein bands occured in the range of 30–45 kDa in non-denatured proteins of beach pea. Four of the bands were not observed in SHMP-extracted protein isolates from beach pea. In the case of green pea, after dialyzing, nine major bands were observed in the range of 29–97.4 kDa. In green pea, both NaOH- and SHMP-extracted samples showed similar protein bands. Grass pea dialysed protein isolates showed 14 intense bands in the NaOH-extract while low intensity bands were observed in the SHMP-extract. The major bands for grass pea were observed in the range of 29–30 and 40–95 kDa in both NaOH- and SHMP-extracted samples. In all cases, NaOH-extracted protein isolates, following extensive dialysis, showed very high intensity bands as compared with the SHMP-extracted protein isolates. After dialyzing protein isolates, the NPAGE of beach pea showed bands with much less intensity than those of green pea and grass pea protein isolates.

SDS-PAGE patterns of various protein isolates and protein fractions of beach pea (albumins, globulins, prolamines, and glutelins) are shown in Fig. 4B. Most polypeptides bind SDS in a constant ratio, such that they have essentially the same charge densities and migrate in the polyacrylamide gel according to their molecular weight. Beach pea protein isolates showed very few bands when compared to their green pea and grass pea counterparts. The major protein bands of beach pea protein isolates were observed at 35 and 47 kDa (Fig. 4B). The albumin fraction of beach pea showed 18 polypeptide bands with two major bands at

Table 4
Predicted protein efficiency ratio values of protein fractions of beach pea according to Eqs. (1)–(3)[a]

Sample	1	2	3
Albumin	2.98	2.99	2.38
Globulin	2.65	2.69	2.03
Prolamine	2.52	2.49	1.12
Glutelin	2.57	2.62	1.93

[a] Alsmeyer et al. (1974).

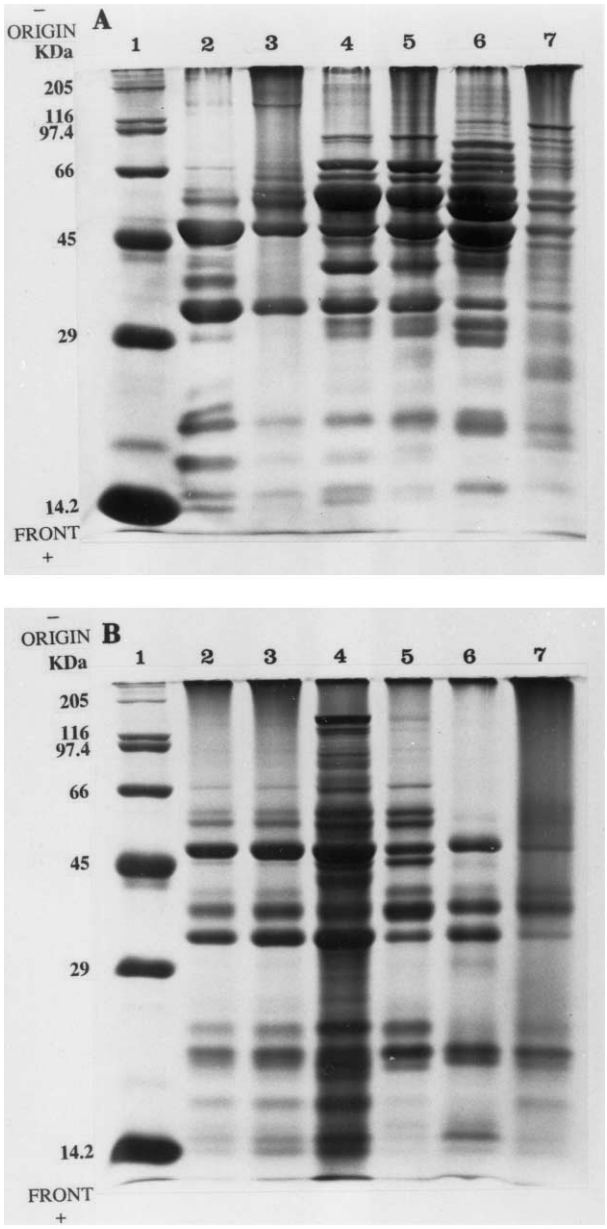

Fig. 4. (A) The NPAGE of pea proteins (1, molecular weight markers; 2, beach pea NaOH protein isolate; 3, beach pea SHMP protein isolate; 4, green pea NaOH protein isolate; 5, green pea SHMP protein isolate; 6, grass pea NaOH protein isolate; 7, grass pea SHMP protein isolate). (B) The SDS-PAGE of beach pea proteins (1, molecular weight markers; 2, NaOH protein isolate; 3, SHMP protein isolate; 4, albumin; 5, globulin; 6, prolamine; 7, glutelin).

35 and 47 kDa and others in the range of 14.4–117 kDa. The globulin fraction showed 11 polypeptides, while prolamine and glutelin each had six polypeptide bands. All major bands were observed in the range of 35–47 kDa in protein isolates as well as in protein fractions for beach pea (Fig. 4B). The results show that beach pea proteins are structurally very simple in comparison with green pea and grass pea which had complicated proteins with higher number of bands and intensities (results not shown). Deshpande and Campbell (1992) reported that grass pea was characterized by the presence of all three types of storage protein fractions generally associated with food legumes; the 11S legumin type (apparent molecular weight after dissociation gives 35–40 and 22–26 kDa), and two 7S, vicilin (subunit molecular mass 43–47 kDa) and convicilin (subunit molecular mass 64–66 kDa) types. Idouraine, Yensen, and Weber (1994) showed that SDS-PAGE of tepary bean in sodium phosphate buffer and salt fractions contained 37 and 27 polypeptides, respectively, with major bands at 29, 45 and 49 kDa. Similar results were reported by Utsumi, Yokoyama and Mori (1980) for vicia faba, and by Singh et al. (1981) for pigeonpea, as well as by Sathe and Salunkhe (1981) for Great Northern bean proteins.

4. Conclusions

Results of the present study demonstrate that beach pea albumin, globulin, prolamine and glutelin protein fractions were similar to those of other pea samples. Nutritional parameters such as predicted BV and PER of all protein fractions of beach pea were better than other peas and, thus, beach pea may potentially serve as an important chief source of unexploited protein. Albumin and glutelin fractions of beach pea contained a high amount of sulphur-containing amino acids; thus beach pea may be used for production of transgenic legumes and for improvement of other pea seeds.

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