Functional Properties of Pea Globulin Fractions[1]

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ABSTRACT

Protein was extracted from green pea flour using neutral buffer containing 0.5*M* NaCl. Globulins were precipitated by a combination of dilution and adjustment to pH 4.5 and further fractionated into legumin and vicilin-rich fractions by differential solubility at pH 4.8. Both vicilin and legumin preparations were 80% soluble at pH 3, but the vicilin preparation was markedly more soluble than legumin at pH 7 (96 vs. 73%). The vicilin preparation produced more stable foams and emulsions, whereas the legumin gave greater foam expansion and emulsion capacities. Surface hydrophobicity and foaming capacity of both preparations were markedly increased by heating neutral solutions 5 min at 90° C.

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Field peas (*Pisum sativum*) have been investigated as sources of protein concentrates and isolates (Anonymous 1974, Sumner et al 1981, Gueguen 1983). Pea seeds contain 20–30% protein, the majority of which are storage proteins. The two major storage proteins, legumin and vicilin, are classical globulins and represent 65–80% of the total buffer-extractable protein (Schroeder 1982). Legumin contains more essential sulfur-containing amino acids than vicilin (Derbyshire et al 1976), and it has been suggested that increasing the proportion of legumin to vicilin is a means of improving the nutritional quality of pea proteins (Casey and Short 1981).

Even though it may have all prerequisites to be nutritionally superior, a protein will have no effect on human nutrition unless it has the functional properties necessary for its successful incorporation into food systems (Ryan 1977). Pea legumin and vicilin have several similarities to soy glycinin and conglycinin, respectively (Derbyshire et al 1976). For example, they have comparable molecular weights, amino acid compositions, and subunit structures (Derbyshire et al 1976). Soybean glycinin and conglycinin have been shown to differ in their functionality (Saio et al 1969, Hermansson 1978, Aoki et al 1980).

The objective of this study was to investigate functional properties of legumin, vicilin, and mixed globulin fractions extracted from green peas.

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MATERIALS AND METHODS

Preparation of Pea Globulins

Whole dry green peas (*Pisum sativum*) designated B-160 strain provided by Dumas Seed Co., Pullman, WA, were ground into flour by a single pass through a Udy mill (Udy Corp., Boulder, CO) equipped with a 0.5-mm screen operating at 12,800 rpm.

Protein fractions were prepared by a method adapted from Scholz et al (1974), Thomson et al (1978) and Daniellson (1949a) as shown in Figure 1. Important features of this scheme are the resolubilization of the pH 4.5 protein precipitate with extracting buffer to produce salt extract II and separation of legumin and vicilin fractions by dialysis of salt extract II against pH 4.8 McIlvaines buffer containing 0.2*M* NaCl. The protein fractions were freeze-dried and stored until needed in screw-cap glass vials in a desiccator over anhydrous $CaSO_4$ at −20° C to prevent solubility changes (Culbertson 1984).

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Electrophoresis was performed using the Tris glycine buffer system of Laemmli (1970), an 8–16% linear gradient of polyacrylamide for the resolving gel (O'Farrell 1975), and a 4% acrylamide stacking gel. Separated proteins were visualized using 0.2% Coomassie Brilliant Blue R-250, in 9% acetic acid/45% methanol.

Chemical Composition

Nitrogen content. Total nitrogen content of freeze-dried samples was determined using the AOAC micro-Kjeldahl procedure (method 42.014, 1980). A nitrogen-to-protein conversion factor of 5.52 (Holt and Sosulski 1979) was used.

Carbohydrate content. Total carbohydrate in the protein fractions was estimated according to the phenol-sulfuric acid

method (Dubois et al 1956). A standard curve was constructed using glucose.

Lipid content. The crude fat content of pH 4.5 freeze-dried pellet (precipitate from salt extract I) and mixed globulin preparation was determined gravimetrically using water-saturated isobutanol as extractant.

Amino acid analysis. The amino acid analysis was performed by the Washington State University Bioanalytical Laboratory. Amino acids were determined from hydrolysates obtained by heating approximately 20 mg of protein samples in double-distilled 6*N* HCl in evacuated and sealed ampules at 110°C for 24 hr. Cystine and methionine were analyzed after performic acid oxidation and HCl hydrolysis following the method of Moore (1963). After removal of solvents from hydrolysates by vacuum evaporation, the residues were dissolved in 2*N* sodium citrate buffer, pH 2.2. Data were expressed in mol %.

Tyrosine and tryptophan concentrations were determined according to the spectrophotometric method of Edelhoch (1967), with slight modification. Guanidine hydrochloride was obtained from Aldrich Chemical Company, Inc. (Milwaukee, WI) and used without further purification. To 9–10 mg of protein sample, guanidine hydrochloride (6*M*) solution in 0.02*M* phosphate buffer, pH 6.5, was added to give a protein concentration of 1 mg/ml. After complete dissolution, the absorbance of each sample was read at 280 and 300 nm. The protein solution (3 ml) was adjusted to pH greater than 11.5 by addition of 10 μl of 4*N* KOH and the absorption read at 300 nm. The molar concentration of tyrosine and tryptophan was estimated using the equations of Edelhoch (1967).

Functional Properties

The functional properties of protein fractions in 50 m*M* acetate buffer (pH 4 and 5) or 50 m*M* phosphate buffer (pH 3, 6, and 7) were studied. All buffer solutions were adjusted to an ionic strength of 0.1 using KCl.

Protein solubility. The solubility of protein fractions was determined by sonicating 50-mg samples in 10 ml buffer for 2 min using an Aerograph ultrasonic cleaner (Branson Instruments, Inc., Stamford, CT). The protein solutions were centrifuged for 10 min at 1,300 rpm in an International model HN centrifuge (International Equipment Co., Needham Heights, MA). The amount of soluble protein was determined in 1.0-ml aliquots of the supernatant using a biuret method (Gornall et al 1949). Bovine serum albumin (Sigma Chemical Co., St. Louis, MO) was used to prepare a standard curve.

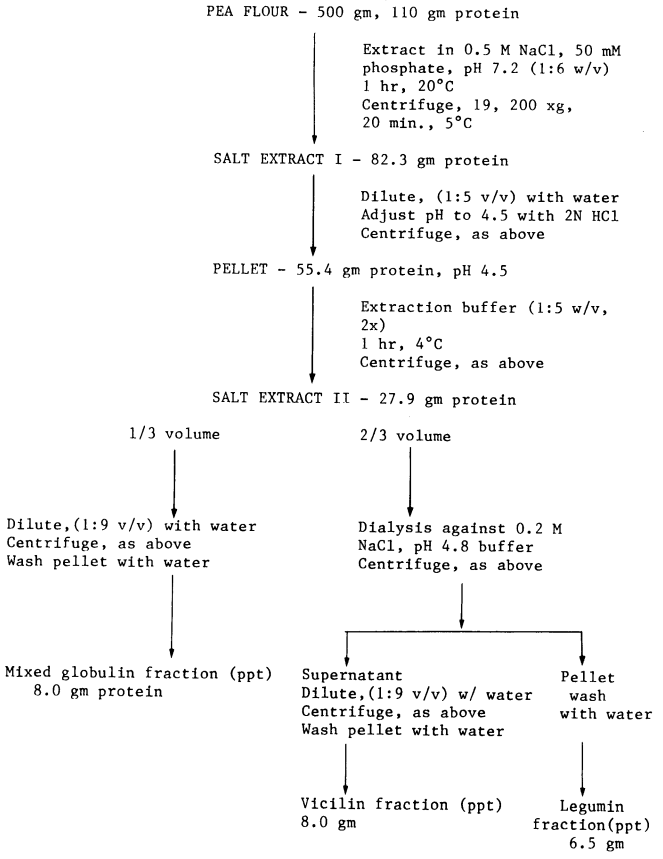

Fig. 1. Procedures used to fractionate globulins from green peas.

Emulsion capacity. Emulsion capacity of protein fractions was determined by the method of Webb et al (1970) as modified by Eisele (1980). Emulsion capacity experiments were run at ambient temperature (20°C) and data expressed in milliliters of oil emulsified per milligram of protein.

Emulsion stability. The emulsion stability was determined by the method of Yatsumatsu et al (1972) as modified by Johnson and Brekke (1983). The quantity of protein required to emulsify 25 ml of oil was determined from emulsion capacity data. Protein suspensions of 5 mg/ml of pH 3, 4, or 7 buffer were diluted with corresponding buffer to give the calculated amount of protein necessary. Emulsion stability was expressed using the following equation:

$$\% \text{ Emulsion stability} = \frac{\text{Height of emulsified layer}}{\text{Total height of tube contents}} \times 100 .$$

Foaming properties. The foaming properties were measured using a water-jacketed apparatus similar to that of Waniska and Kinsella (1979). The temperature of water in the column jacket was 18 ± 1°C.

Protein solutions to be foamed (0.5 mg/ml) were prepared by diluting the supernatant from the pH 3, 4, and 7 protein solubility assays. Air was sparged through 15 ml of protein solution for 1 min at a rate of 60 ml/min. The volume of foam at the end of the sparging period was taken as foam capacity and the time required for half of the volume of liquid uptake in the foam to be drained as a measure of stability (Culbertson 1984). Foam expansion was expressed using the following equation:

$$\text{Foam expansion} = \frac{\text{Volume of foam}}{\text{Volume of liquid uptake in foam}} .$$

The volume of liquid in the foam was measured as the difference between the starting 15 ml solution and the residual volume of liquid after 1 min of air sparging.

Surface Hydrophobicity

The *cis*-parinaric acid fluorescent probe method of Kato and Nakai (1980) with slight modification was used to measure surface hydrophobicity (S_0) of protein fractions. Ten microliters of 3.3 m*M* ethanolic *cis*-parinaric acid (Molecular Probes, Inc., Junction City, OR) were added to one set of test tubes. The relative fluorescent intensity (RFI) at 415 nm was recorded after excitation of the *cis*-parinaric acid conjugates at 325 nm in an Aminco-Bowman spectrofluorometer J4-8961 (American Instrument Co., Silver Springs, MD) using a slit width of 0.5 mm. The fluorometer was standardized by adjusting the reading to 50% relative fluorescent intensity when 10 μl of *cis*-parinaric acid solution was added to 2 ml of *n*-heptane.

The net fluorescence intensity was determined by subtracting the fluorescence reading of each sample without the *cis*-parinaric acid from the reading with *cis*-parinaric acid. The initial slope of RFI versus protein concentration was determined by linear regression analysis and designated surface hydrophobicity (S_0). Proteins soluble in 50 m*M* phosphate buffer pH 3 and 7 were diluted with the corresponding buffer so that the final protein concentrations ranged from 0.25 to 1.0 mg/ml.

To study the effect of heating on surface hydrophobicity and foaming characteristics, 15 ml of 1 mg/ml protein solutions, pH 7, were heated in a 90°C water bath for 5 min and cooled rapidly under running tap water for 5 min before serial dilutions ranging from 0.1 to 0.4 mg/ml. Surface hydrophobicity was determined as

outlined above. The heat-treated solutions at 0.5 mg protein/ml (1:1, v/v) were also subjected to the foaming test as previously described.

Statistical Analysis

Wherever applicable, significance was analyzed with the *t* test described by Steel and Torrie (1960).

RESULTS AND DISCUSSION

Isolation and Purification of Pea Globulin Fractions

The total nitrogen content of pea flour was 3.99%, corresponding to 22% total protein. Nearly 75% of the flour protein was extracted in pH 7.2 buffered saline solution, and 67% of the solubilized protein was pelleted upon dilution and acidification of the extract (Fig. 1).

Soybean protein isolated by acid precipitation at pH 4.5 is not completely soluble in neutral saline solutions, and conglycinin and glycinin protein fractions contribute substantially to this loss of salt solubility (Nash et al 1971, Lilliford and Wright 1981). In our study, a reextraction of the pH 4.5 pellet was carried out to remove insoluble protein. Upon resuspension of the pellet in pH 7.2 buffered saline, approximately 50% of the protein failed to redissolve. The insoluble protein had a similar electrophoretic pattern to that of the supernatant (data not shown). Gueguen (1983) found that pea protein isolate extracted at pH 7 and precipitated at pH 5.3 contained 6.2% lipids. Our pH 4.5 pellet contained 6% lipid. The isolation procedure used in this study is relatively mild and is expected to yield "native proteins" with fewer lipids than in the pH 4.5 pellet. In fact, the lipid content of our mixed globulin fraction was 0.1%.

SDS Polyacrylamide Gel Electrophoresis

The electrophoretic patterns of the protein fractions are shown in Figure 2. Two major bands of approximately 40,000 and 20,000 mol wt in the legumin pattern are not found in the vicilin. Subunits of 40,000 and 20,000 mol wt have been reported to be the components of pea legumin (Casey 1979, Davey and Dudman 1979, Krishna et al 1979, Hurkman and Beevers 1980). Hurkman and Beevers (1980) also reported a 93,000 mol wt polypeptide in legumin which may correspond to the most cathodic (largest molecular weight) polypeptide in the legumin fraction. The band at approximately 70,000 mol wt corresponds to convicilin, a third storage protein in peas, often found in vicilin preparations (Davey and Dudman 1979, Croy et al 1980b) but also as a contaminant in legumin fractions (Casey 1979, Hurkman and Beevers 1980). Major subunits of 50,000, 30,000–35,000, and 19,000 mol wt along with minor lower molecular weight polypeptides have been reported for pea vicilin (Davey and Dudman 1979, Croy et al 1980a, Gatehouse et al 1981). Subunits of approximately 14,000, 30,000–35,000, and 50,000 mol wt are enriched in the vicilin fraction. However, these and several other polypeptides are also found in the legumin fraction. The cross-contamination of the legumin and vicilin fraction prepared by isoelectric precipitation is common (Jackson et al 1969, Davey and Dudman 1979, Hurkman and Beevers 1980).

Chemical Composition

The mixed globulin, legumin, and vicilin fractions contained 17.52, 18.0, and 16.74% nitrogen, respectively. In their review of storage proteins of legume seeds, Derbyshire et al (1976) reported a nitrogen content of 18.04% for pea legumin and 17.40% for vicilin. The nitrogen-to-protein conversion factor of 5.52 determined by Holt and Sosulski (1979) corresponds to 18.11% N in a mixture of pea proteins.

The mixed globulin fraction contained 1.15% carbohydrate, the legumin fraction 0.82%, and the vicilin fraction 1.01%. Davey and Dudman (1979) extracted storage proteins from protein bodies from mature pea seeds and found that legumin contained 0.86% carbohydrate, whereas the average carbohydrate content of four vicilin fractions was 1.06%. We have no explanation for the mixed globulin preparation containing a greater carbohydrate content than vicilin. Electrophoresis did not indicate any polypeptides unique to the mixed globulin fraction. This suggests that a carbohydrate-rich protein was not lost in the fractionation.

Amino acid composition. The mixed globulin, legumin, and vicilin preparations are rich in aspartic and glutamic acids (Table I). Legumin contains more methionine and cystine than the vicilin preparation, but lower levels of isoleucine, phenylalanine, and lysine. The amino acid profiles generally agree with earlier data (Grant and Lawrence 1964, Jackson et al 1969, Casey and Short 1981).

The average molar concentrations of tyrosine were similar for mixed globulin, legumin, and vicilin fractions (Table I). Legumin

TABLE I
Amino Acid Composition[a] of Pea Globulin Fractions (mol %)

	Pea Fractions		
Amino Acid	Legumin	Mixed Globulin	Vicilin
Aspartic acid	11.75	12.36	13.66
Threonine	3.13	2.89	2.88
Serine	5.52	6.04	6.91
Glutamic acid	22.05	22.78	18.73
Proline	4.90	4.57	4.55
Alanine	5.52	4.80	5.16
Cystine[b]	0.84	0.68	0.45
Valine	5.21	5.02	5.30
Methionine[b]	0.62	0.39	0.21
Isoleucine	3.85	4.31	5.11
Leucine	7.88	8.81	10.16
Phenylalanine	3.59	4.13	4.90
Lysine	5.63	6.64	7.98
Histidine	2.25	1.86	1.63
Arginine	8.52	6.97	6.04
Tyrosine[c]	1.44	1.51	1.52
Tryptophan[c]	1.31	0.88	0.42

[a] Values are means of duplicate determinations.
[b] Performic acid oxidized prior to acid hydrolysis.
[c] Molar concentration ($\times 10^4$) in 1 mg protein/ml guanidine hydrochloride solution.

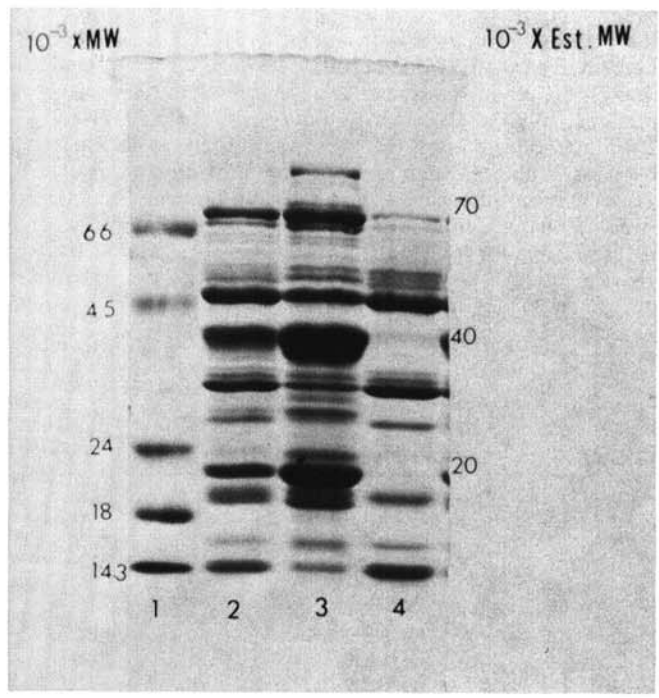

Fig. 2. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of molecular weight standards (well 1), mixed globulins (well 2), legumin (well 3), and vicilin (well 4) preparations. Molecular weight standards: bovine serum albumin (66,000 mol wt), ovalbumin (45,000 mol wt), trypsinogen (24,000 mol wt), lactoglobulin (13,000 mol wt), and lysozyme (13,400 mol wt). Anode at bottom. Migration is from top to bottom.

contained three times more tryptophan than vicilin. The ratio of tyrosine to tryptophan in legumin was 1.1:1 and 3.6:1 in vicilin. Danielsson (1949b) reported tyrosine-to-tryptophan ratios of 4:1 and 10:1 for legumin and vicilin, respectively. The discrepancy in the ratios may result from differences in purification procedures and methods of tyrosine and tryptophan determination. A strong denaturing agent guanidine hydrochloride (6*M*) was used in this study whereas Danielsson (1949b) used an alkaline treatment (0.1*N* NaOH) which has been shown to be less quantitative (Edelhoch 1967).

Functional Properties

Protein solubility. Protein solubility of mixed globulin, legumin, and vicilin preparation is given in Table II. All three protein fractions had zero solubility at pH 5 and 6. Vose (1980) reported that pea protein isolate obtained through an ultrafiltration process had minimum solubility at pH 5. The minimum solubility of pea proteins generally occurs in the pH range 4–6 (Vose et al 1976, Johnson and Brekke 1983). The solubility at pH 4–6 noted by these authors may be due in part to albumins in the protein preparations. In this study, albumins were removed by using two precipitations and extensive water washing of precipitates. Sun and Hall (1975) noted no solubility of purified *Phaseolus vulgaris* globulin 1 (analagous to pea legumin) in the pH range 4–6 in the presence of 0.1*M* NaCl.

Mixed globulin and legumin fractions were more soluble at pH 3 than at pH 7. Legumin was the least soluble protein fraction at the three pHs tested. The observation that legumin is most soluble at pH 3 may be related to a dissociation into lower molecular weight subunits at acidic pH and low ionic strength (Derbyshire et al 1976).

Greater solubilities were observed in this study than those commonly observed with pea protein isolates (Gueguen 1983, Culbertson 1984). The isolation procedure used was expected to yield highly soluble native proteins. Schwenke et al (1983) reported that native faba bean and sunflower seed protein isolates were also highly soluble at pH 3 and 7.

Emulsifying Properties

The emulsifying properties of mixed globulin, legumin, and vicilin fractions are shown in Table II. The emulsifying properties of pea globulin fractions were studied at pH 3, 4, 5, 6, and 7. The three protein fractions did not stabilize emulsions at pH 5 and 6. In general, the emulsion capacities of legumin and mixed globulin fractions followed their pH-solubility profiles. Conversely, the vicilin fraction had its greatest emulsion capacity at pH 4, where it was least soluble. Despite relatively low solubility, legumin had a greater emulsion capacity at both pH 3 and 7 than vicilin or mixed globulin fractions. Both soluble protein and dispersed protein may be responsible for the emulsification capacities. McWatters and Holmes (1979) showed that large concentrations of soluble nitrogen were not necessarily related to maximum emulsifying capacities. Nakai (1983) reported that solubility, surface hydrophobicity, and molecular flexibility influence the emulsification behavior of globular proteins such as pea proteins that have extensive quarternary structure.

Emulsion stability values were similar for the three fractions at pH 4 and 7 (Table II). Similar emulsion stabilities indicate that the protein films were approaching a stable state in the emulsions. The higher emulsion stabilities at pH 4 (64–65%) versus pH 7 (58–59%) for legumin and mixed globulins may result from a stabilizing effect of undissolved particles of proteins in the pH 4 aqueous phase serving as a physical barrier to coalescence of oil droplets. The stability of vicilin emulsions at pH 3 resembled those at pH 4. Conversely, the emulsion stabilizing ability of mixed globulins and legumin at pH 3 was similar to that at pH 7. The high emulsion stability of vicilin at pH 3 may be caused by protein denaturation that enhances mutual cohesion to stabilize the interfacial membrane (Wang and Kinsella 1976).

Foaming Properties

Foaming tests were made at pH 3, 4, and 7. Foams with a very small bubble size were initially formed at pH 4. As more air was sparged into the protein solution, the density of foam decreased from top to bottom. The liquid was carried up into foams and drained rapidly when the sparging was discontinued. Consequently, numerical evaluation of foaming at pH 4 was not made. At pH 3, legumin and mixed globulins foam capacities were greater than that of the vicilin fraction (Table III); however, vicilin foams were more stable. All protein solutions foamed at pH 3, whereas foams formed at pH 7 were made up of large air bubbles that burst as they formed. The surface hydrophobicity of soluble protein at pH 3 was greater than at pH 7 (Table III). However, S_0 was not necessarily a good predictor of foaming capacity. For example, although the mixed globulin at pH 3 had a lower S_0 than legumin at pH 7, its foam capacity was greater. Heating the pH 7 protein solutions in a 90°C water bath for 5 min markedly increased surface hydrophobicity and improved foaming characteristics. The increased hydrophobicity indicated that unfolding of the protein molecules exposed buried hydrophobic regions. Voutsinas et al (1983) reported that the emulsion activity index (a measure of interfacial area per weight of protein) and surface hydrophobicity of pea protein concentrate increased upon heating for one minute at 80°C at pH 5.8.

The greater foam stability of vicilin compared to legumin may be explained by the fact that vicilin contains fewer cysteine residues, and therefore fewer potential disulfide bridges, allowing for a more flexible structure. Osborne and Campbell (1898) and Danielsson (1949a) noted that vicilin was sensitive to heat coagulation relative to legumin. Vicilin may form a cohesive interfacial film, resisting migration from the film into solution resulting in good foam stability. Legumin contains more sulfur amino acid than vicilin and would be expected to unfold more slowly than vicilin and give relatively low foam stability (Townsend and Nakai 1983).

TABLE II
Solubility and Emulsion Properties of Pea Protein Fractions

Variable	Mixed Globulin	Protein Fraction	
		Legumin	Vicilin
Solubility (%)			
pH 3	94.6 ± 0.56	83.0 ± 0.57	89.9 ± 2.5
pH 4	65.0 ± 0.57	51.6 ± 0.0	61.3 ± 5.23
pH 7	86.1 ± 1.27	73.6 ± 0.28	96.9 ± 1.55
Emulsion capacity (ml oil/mg protein)			
pH 3	1.88 ± 0.06	2.48 ± 0.0	1.43 ± 0.02
pH 4	1.18 ± 0.03	1.39 ± 0.03	1.67 ± 0.11
pH 7	1.56 ± 0.06	2.24 ± 0.0	1.22 ± 0.03
Emulsion stability (%)			
pH 3	59.95 ± 1.94 a[a]	58.32 ± 2.10 a	66.37 ± 2.32 b
pH 4	65.18 ± 3.78 a	64.28 ± 2.67 a	65.79 ± 1.83 a
pH 7	57.6 ± 4.31 a	56.57 ± 3.33 a	58.99 ± 1.47 a

[a] Means in the same row followed by the same letter are not significantly different ($P > 0.05$).

TABLE III
Protein Hydrophobicity and Foaming Characteristics of Protein Fractions at pH 3, pH 7, and pH 7 After Heating

	Mixed Globulin	Legumin	Vicilin
pH 3			
S_0	9.34	19.53	14.73
Foam capacity (ml)	35.4 b[a]	32.5 b	23.63 a
Foam stability (sec)	62.3 a	98.4 b	207.3 c
Foam expansion	5.25 a	5.90 c	5.67 b
pH 7[b]			
S_0	7.62	11.22	2.20
pH 7 (heated)			
S_0	61.05	79.55	51.5
Foam capacity (ml)	61.33 b	43.83 a	41.38 a
Foam stability (sec)	91.4 a	109.2 b	158.8 c
Foam expansion	5.93 b	7.17 c	4.66 a

[a] Means in same row with same superscript are not significantly different ($P > 0.05$).
[b] No foams were formed at pH 7.

CONCLUSION

The pea protein preparations in this study were quite soluble, in part because heat or pH extremes were not used during preparation and because the original protein precipitate was resolubilized, which reduced the lipid content of the final products.

Legumin and vicilin preparations had differing solubility, foaming, and emulsion properties. This suggests that for a particular application, a pea cultivar with a specific protein make-up (Schroeder 1982) might be appropriate as raw material for production of a protein isolate. Alternately, processing conditions could be changed to enrich the isolate for legumin or vicilin.

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