Protein Yields and Characteristics from Acid and Salt Coagulations of Yellow Pea (*Pisum sativum* L. Miranda) Flour Extractions[†]

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Acid proteinate (AP), magnesium proteinate (MAP), and calcium proteinate (CAP) extracted from yellow pea flour at different pH (*P*) and temperature (°C, *T*) levels were analyzed. The yield (grams per 100 g of flour) of AP (maximum 20.2%; T = 32.1 °C, P = 9.8) was significantly affected ($P < 0.10$) by different combinations of temperature and pH during extraction, while the yield of CAP was only affected ($P < 0.001$) by pH (max = 19.0%; T = 27.0 °C, P = 10.8). MAP (max = 16.8%) was not affected by either factor. There were no significant differences in nitrogen content (70.7% protein) for the three proteinates due to temperature and pH variables. Hydrophilic amino acid contents of AP, MAP, and CAP were 64.2, 61.8, and 62.1%, respectively. Cysteine and methionine contents were the lowest for all proteinates. Gel electrophoresis of all proteinates showed similar molecular weight subunit patterns, ranging from 17 000 to 84 000 with disulfide bond generally at MW of 63 000–>100 000 for all proteinates.

INTRODUCTION

Increased utilization of legume protein by the food industry, especially soybean protein (Kinsella, 1979), has increased research on the utilization of legume or seed proteins in foods (Kim et al., 1990; Gebre-Egziabher and Sumner, 1983; Thompson, 1977; Rhee et al., 1972). Legume or seed protein is used as an ingredient primarily to increase nutritional quality and provide a variety of functional properties, including desirable structure, texture, flavor, and color characteristics in formulated food products.

Knowledge of protein structure and its molecular size in different legume or seed cultivars will bring about an understanding of the protein properties. This will permit manipulation of these properties for food product development (Leterme et al., 1990; Kim et al., 1990; Wang and Damodaran, 1990; Cumming et al., 1973). Nutritional and functional qualities of a protein are largely determined by its amino acid content and nitrogen solubility (Kinsella, 1979).

A new variety of yellow pea (*Pisum sativum* L. Miranda) has been successfully grown and yields a nutrient quality comparable to those of soybean when used in animal rations (England et al., 1986; Savage et al., 1986). In Oregon, approximately 15 million pounds are produced annually and at relatively low price (J. Carnes, International Seeds, Inc., Halsey, OR, 1988, personal communication). If the protein in these yellow peas could be characterized and developed into a viable product as an isolate, it would be of economic benefit to the farmer.

The objective of this study was to investigate the conditions needed for maximum yield and to characterize yellow pea protein extracted by acid and salt coagulations.

MATERIALS AND METHODS

Yellow peas (*P. sativum* L. Miranda) of sample grade US. No. 1 (based on USDA standards) were grown in 1989 and provided by International Seeds, Inc., Halsey, OR. Thirty-five kilograms of yellow peas was ground into medium particles and stored at 3 °C.

Table I. Visual Display of the Temperature–pH Combinations Used for the Central Composite Rotatable Design with Two Independent Variables[a,b]

design points		−1.207	−1.000	0.000	1.000	1.207
	temp, °C	pH				
		7.14	8.00	9.00	10.00	10.86
1.414	10			*		
1	15		*		*	
				*		
0	25	*		***		*
				*		
−1	35		*		*	
−1.414	40			*		

[a] Temperature–pH combination for each of coagulant types (acid, 0.54%; $CaCl_2 \cdot 2H_2O$, 0.54%; $MgSO_4$ anhydrous). [b] Asterisks represent treatment of protein extraction for designated temperature (°C, *T*) and pH (*P*).

Table II. Proximate Composition of Yellow Pea Flour and Calcium Content of Proteinates[a]

	per 100 g		per 100 g
energy, cal	349.0	ash: flour, g	2.8
protein (N × 5.7), g	19.5	AP, g	6.3 (Ca = 0.03 g)
carbohydrate, g	64.2	MAP, g	4.6 (Ca = 0.04 g)
fat, g	1.5	CAP, g	8.7 (Ca = 3.04 g)
moisture, g	12.0		

[a] AP is acid proteinate, MAP is magnesium proteinate, and CAP is calcium proteinate.

Protein Extraction. Proteins of yellow peas were extracted according to the temperature, pH (Table I), and coagulation treatments. The protein extraction procedure is reported in Figure 1. Yields were calculated from total weight of freeze-dried products per 100 g of yellow pea flour. Protein samples were powdered and kept in glass bottles and refrigerated (3 °C) until needed for protein characterizations.

Proximate Analysis. A composite sample of yellow pea flour was analyzed for proximate composition by Columbia Laboratories Inc. (Corbett, OR). Calcium was analyzed by ashing 2 g of combined proteinate samples at 525 °C for 24 h, dissolving in 3 mL of 3 N HCl solution, and diluting to 25 mL with redistilled water. The calcium concentration was determined using a Perkin-Elmer 2380 atomic absorption spectrometer.

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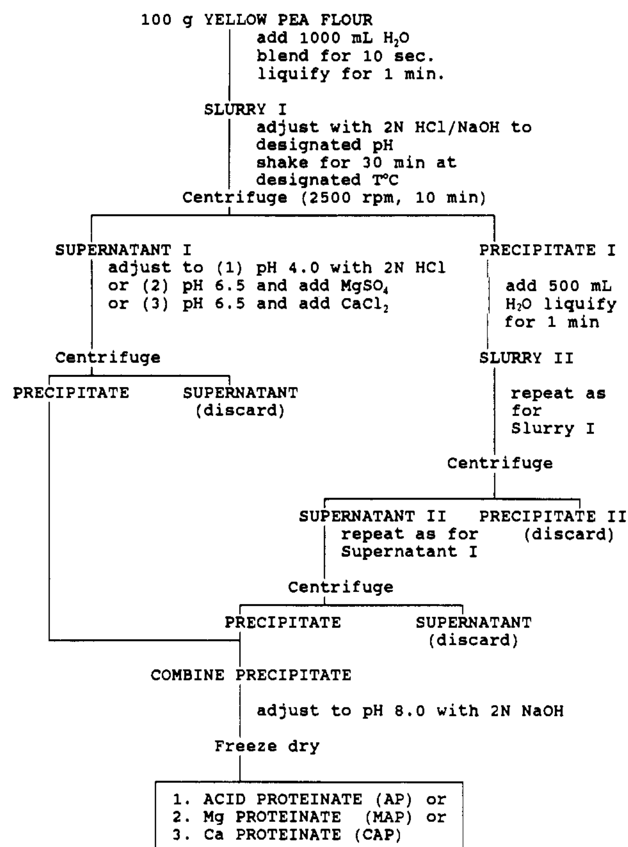

Figure 1. Extraction process of yellow pea protein using acid (pH 4.0), $MgSO_4$ anhydrous, or $CaCl_2·2H_2O$ as coagulant.

Table III. Protein Content and Recovery from Extraction of Yellow Pea Flour

proteinate[a]	yield, g/100 g	protein,[b] %	protein recovery,[c] %
AP	20.2	71.2	73.8
MAP	16.8	72.4	62.4
CAP	19.0	69.5	67.7

[a] AP is acid proteinate, MAP is magnesium proteinate, and CAP is calcium proteinate over temperature–pH (10–40 °C; pH 7.14–10.86) combinations. [b] % N × 5.7. [c] Total protein in the yield compared to that in 100 g of yellow pea flour.

Table IV. Quadratic Regression Model Coefficients for Yield and N Content of Three Proteinates for Substitution with Independent Variables of *T* and *P* into Equation[a]

	A (constant)	B_1	B_2	B_{11}	B_{22}	B_{12}
			Yield, %			
AP[b–d]	−29.954	−1.41	14.835	−0.016	−1.162	0.248
MAP	48.249	0.370	−9.099	−0.0003	0.569	−0.032
CAP[b,c]	38.572	−0.087	−6.372	−0.00002	0.410	0.012
			N Content, %			
AP	11.292	−0.21	0.896	0.001	−0.08	0.016
MAP[b]	7.122	0.214	0.818	0.0002	−0.018	−0.029
CAP[b]	−2.111	0.154	2.61	−0.0005	−0.114	−0.017

[a] $Y = A + B_1T + B_2P + B_{11}TT + B_{22}PP + B_{12}TP$. AP is acid proteinate, MAP is magnesium proteinate, and CAP is calcium proteinate. Raw data are available (Soetrisno, 1991). [b] CCRD is suitable for predicting the response. [c] pH (*P*) (pH 7.14–10.86) is significant variable. [d] Temperature (*T*) (°C) (10–40 °C) is significant variable.

Nitrogen Determination. N determination was done using the micro-Kjeldahl method (AOAC, 1990), on all 13 treatments for the AP, MAP, and CAP. The results are presented as grams of protein per 100 g of sample, with a conversion factor nitrogen to protein of 5.7. Protein recovery was calculated on the basis of total protein recovered compared to that in 100 g of yellow pea flour.

Table V. Amino Acid Compositions of Three Proteinates[a] from Yellow Pea Flour (Micrograms of Amino Acid per Milligram of Nitrogen)

	AP	MAP	CAP
hydrophilic	(64.2%)	(61.8%)	(62.1%)
Ser	232.1	326.4	350.1
Thr	172.8	242.2	266.4
Tyr	122.3	179.0	180.0
Asp + Asn	547.0	742.4	798.6
Glu + Gln	858.1	1167.7	1252.0
Lys	287.2	377.7	400.2
Arg	440.2	645.2	686.6
His	113.7	158.6	175.3
hydrophobic	(35.8%)	(38.2%)	(37.9%)
Cys	17.3	35.0	21.6
Met	18.7	28.9	30.7
Pro	102.4	323.0	322.6
Gly	195.8	285.6	308.4
Ala	212.9	308.2	336.2
Val	261.6	371.1	399.3
Leu	368.6	511.2	547.0
Ile	215.5	294.2	317.5
Phe	152.9	212.5	229.1
total	4319.1	6208.9	6621.6

[a] AP is acid proteinate, MAP is magnesium proteinate, and CAP is calcium proteinate. Amino acid score: AP = 0.34; MAP = 0.60; CAP = 0.49 (FAO/WHO/UNU, 1985).

Amino Acid Composition. The composition was determined for AP, MAP, and CAP extractions at pH 9 and 25 °C, at the Center for Gene Research (Oregon State University, Corvallis, OR). Samples were hydrolyzed in 6 N HCl plus 1.0% phenol before being injected onto a high-performance liquid chromatograph.

Electrophoresis. Electrophoresis of the proteinate used sodium dodecyl sulfate–polyacrylamide gels (SDS–PAGE) according to the procedure of Laemmli (1970). A protein concentration of 1.25 mg/mL, with 20 μL of sample solution per well, was used. Protein markers of known molecular weights (MW-SDS-70L, Sigma Chemical Co., St. Louis, MO) were used with bromophenol blue as the tracking dye.

SDS–PAGE for all samples was done with and without addition of 2-mercaptoethanol (2-ME) as a reducing agent, using 13 and 12% acrylamide gels, respectively. Additionally, undenatured (without addition of SDS) samples were also run to reveal the molecular weight pattern of protein polymers. Electrophoresis was performed at a constant current of 40 or 35 mA/2 gels for unreduced or reduced samples, respectively. Staining was done in 0.125% Coomassie brilliant blue R-250, 50% methanol, and 10% acetic acid, for 4 h. Destaining was done in 50% methanol and 10% acetic acid, for 2 h, and then continued in 5% methanol and 7% acetic acid, for 6 h. All assays were done at 25 ± 2 °C. Molecular weights of protein subunits were estimated from the plot of log MW vs the ratio of the distance traveled in comparison with the tracking dye. Gels were stored in a 7% glacial acetic acid solution until photographs were taken.

Experimental Design and Statistical Analysis. The experimental design used was a two-factor central composite rotatable design (CCRD) (Cochran and Cox, 1957) (Table I), to predict maximum yields during protein extractions and optimum characteristic measurements. Temperature (*T*) and pH (*P*) were independent (*X*) variables. The results obtained from extractions and protein characterizations were dependent (*Y*) variables. Statistical Analysis System (SAS Institute Inc., Cary, NC) and Statgraphics (Statistical Graphic Co., Rockville, MD) programs were used to generate the ANOVA, parameter estimates, response surface analysis, canonical analysis, and ridge maximum of the responses. Differences were considered statistically significant at $P \leq 0.10$. The following model of quadratic polynomial regression was assumed for evaluating the individual *Y* variables:

$$Y = A + B_1T + B_2P + B_{11}TT + B_{22}PP + B_{12}TP$$

Quadratic models were used to plot three-dimensional response surfaces. Response surface analysis facilitated an understanding

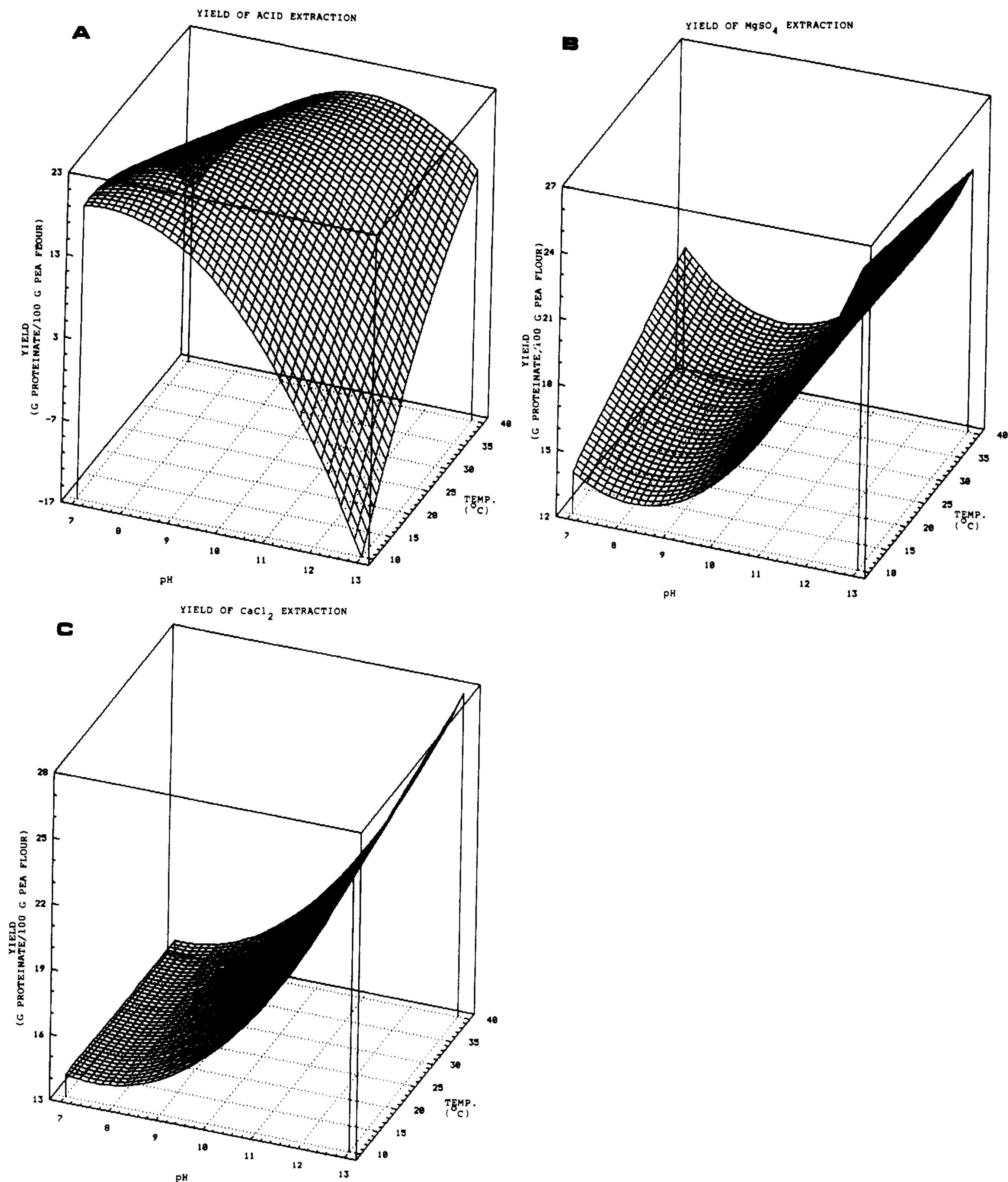

Figure 2. Three-dimensional response surface models of the yield from acid (A), magnesium (B), and calcium (C) extractions of protein from yellow pea flour.

of the nature of responses obtained by graphically indicating maxima, minima, or saddles. All measurements were done in duplicate.

RESULTS AND DISCUSSION

Tables II and III present the proximate composition of yellow pea flour and calcium content of proteinates and protein recovery during extraction, respectively. Table IV presents parameter estimates for fitting the quadratic models for AP, MAP, and CAP on their extraction yields and nitrogen content.

There were significant differences ($P < 0.01$) with pH and temperature in extraction yields in AP and CAP but not in MAP. Nitrogen contents in all types of proteinate were not significantly different. Amino acid composition of three proteinates is in Table V.

Protein Extraction Yields. The yield of acid-coagulated proteinate was significantly affected by changes

in temperature and pH during extraction, as is represented in Figure 2A. The RSA has a hill shape with the maximum yield, 20.2%, at a treatment combination of $T = 31.6$ °C and $P = 9.8$. The yield of CAP was affected ($P < 0.001$) only by pH change. The RSA has a saddle shape (Figure 2C) with a maximum yield of 19.0% at a treatment combination of $T = 27.0$ °C and $P = 10.8$. On the other hand, MAP was not significantly affected by either factor. Its RSA has a saddle shape (Figure 2B), with a tendency to high yield with treatment combinations of high T and high P. Its optimum treatment combination was $T = 25.1$ °C and $P = 10.9$, which produced a yield of 16.8%. Significant positive relationships between pH and protein recovery were also reported by Shen et al. (1991) for soy milk coagulated with glucono-δ-lactone or $CaSO_4$.

Salt-coagulated proteinates had 6–16% lower yields compared to those of acid-coagulated proteinates (Table III). This might be because acid coagulation was done at pH 4.0, the lowest solubility of yellow pea protein according to preliminary work. Salt coagulations have a maximum yield at pH 6.5, since at this pH the carboxyl groups of aspartic and glutamic residues and the imidazole groups of histidine residues are partially deprotonated (Kroll, 1984). Those groups bind with calcium or magnesium ions and cause the protein to coagulate. Brooks and Morr (1984) reported that coagulation of soy protein extract at pH 4 caused precipitation of 11S and 7S proteins at pH 6.5.

According to Lu et al. (1980), the salt-coagulated protein is precipitated at a pH higher than its pI, as indicated by the pH of the whey, 6.5 for MAP and 5.5 for CAP, which explains the higher yield of acid-coagulated compared to salt-coagulated proteinates. Both acid and salt coagulations have higher yields than those reported by Gebre-Egziabher and Sumner (1983) or by Davis (1981) but lower than that reported by Naczk et al. (1986) or Sumner et al. (1981). These might be due to differences in variety and processing conditions, which caused precipitation of phytic acid and carbohydrates such as pectins and gums as has been reported (Shen et al., 1991; Thompson, 1987; Kantha et al., 1986; Brooks and Morr, 1984; McCurdy and Knipfel, 1990).

Nitrogen Content. There were maximum values of 71.2, 72.4, and 69.5% protein (Table III) for AP, MAP, and CAP, respectively. None of the coagulation methods produced significant changes in nitrogen content with changes of temperature and pH during extraction. This indicates that the protein being coagulated had similar purity.

As explained by Wang et al. (1983) and Aguilera and Garcia (1989), protein yield was significantly related to protein content in the extract solution. Nitrogen content of proteinate is about 3.7 times that in yellow pea flour. Lu et al. (1980) also reported no difference in protein content for soy protein curds that were coagulated by acid, glucono-δ-lactone, or calcium salts.

Amino Acids. Amino acid compositions of the three proteinates are presented in Table V. Although the amino acid composition was similar for all three proteinates with a hydrophilic:hydrophobic ratio of 60:40, the acid proteinate appeared to have a greater amount of nonprotein nitrogen than the MAP and CAP proteinates. All proteinates were limiting in cysteine and methionine (amino acid scores of 0.34 AP, 0.60 MAP, 0.49 CAP; FAO/WHO/UNU, 1985), as has been reported by others (Leterme et al., 1990). A similar distribution of amino acids also was reported by Okezie and Bello (1988) for winged bean protein isolate extracted at pH 10.0 and 12.0 and by Wang and Cavins (1989) on fractions obtained during tofu production.

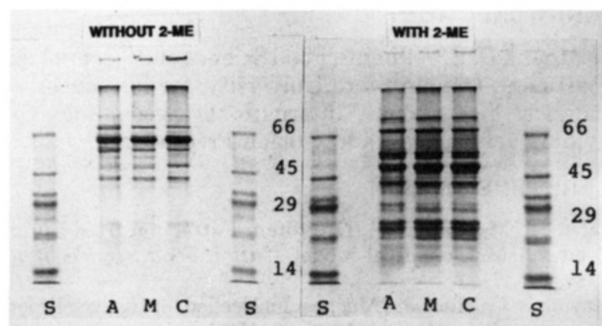

Figure 3. Electrophoresis patterns of acid (A), magnesium (M), and calcium (C) proteinates compared to that of molecular weight standard (S), without 2-mercaptoethanol (2-ME) addition gels (12%) and with 2-ME addition gels (13%).

Electrophoresis. SDS–PAGE photographs are reproduced in Figure 3. There were no apparent differences in electrophoresis patterns related to molecular weight (MW) subunits. All proteinates contained seven major and eight minor subunits with the highest MW = 84 000 and the lowest MW = 17 000. Hsu et al. (1982) reported 14 subunits ranging from 30 000 to 70 000 in dialyzed yellow pea protein isolate.

Electrophoretic patterns of undenatured samples (not shown in figure) revealed differences between protein polymers of AP and MAP or CAP samples. AP contained polymers that separated on the gel, although not clearly; MAP and CAP did not show any band separation at all, due to polymer sizes too large to enter the stacking gel. This could be the result of Mg or Ca bridging on the protein polymer.

Addition of 2-ME (Figure 3) to SDS-denatured protein gave a different molecular weight pattern compared to the one without 2-ME. Electrophoretic patterns of samples without the reducing agent showed high molecular weight subunits (>100 000) which could not enter the stacking gel (4% acrylamide); these apparently dissociated into smaller subunits with 2-ME treatment. The SDS–PAGE of unreduced samples resulted in the densest bands at subunits having MW = 63 000, which after treatment with reducing agent were at subunits having MW = 47 000 and 24 000–26 000. This indicates disulfide bonds are generally in the range 63 000–100 000.

Molecular weight patterns of undenatured proteinate samples indicated that forces, besides disulfide bonds, held the subunits together to form large protein polymers. These forces could be hydrophobic interactions, hydrogen bonds, electrostatic interactions, and/or salt bridges. The similarity in molecular weight pattern of subunits with AP, MAP, and CAP samples, regardless of temperature and pH treatment combinations during extraction, indicated similarity in the type of protein subunits that had been coagulated.

Data indicate that further exploration of yellow pea extracted proteinate is warranted. The coagulant used did not significantly influence yield or nitrogen content. Though there were no differences in the type of protein extracted, salt-coagulated proteinate contained calcium, 3.04% (w/w), which may be of value, particularly if dietary calcium is low in the general diet (Berner et al., 1990; Anonymous, 1991). Amino acid composition of all three proteinates showed a similar pattern, with a ratio of 60:40 for hydrophilic to hydrophobic amino acids. The information obtained by this study will facilitate the development of vegetable–protein foods with high calcium content.

ACKNOWLEDGMENT

We thank D. E. Williams, Food Science and Technology Department, Oregon State University, for his continual assistance; F. J. Lopes, International Seeds, Inc., for providing yellow peas; and Oregon Freeze-Dry.

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Received for review December 2, 1991. Accepted March 23, 1992.