Functional Properties of Acid and Salt Extracted Proteins of Yellow Peas (*Pisum sativum* L. Miranda)†

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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 evaluated. Nitrogen solubility index of AP was the highest (8.9% at pH 5.0 to 100% at pH 10.0–12.0), followed by MAP (3.4% at pH 5.0 to 97.3% at pH 12.0) and CAP (3.5% at pH 6.0 to 88.7% at pH 12.0). Maximum fat adsorptions of AP, MAP, and CAP were 534 (T = 30.3 °C, P = 8.9), 522 (T = 18.3 °C, P = 10.0), and 510% (T = 13.9 °C, P = 10.3), respectively, with T being a significant variable for AP and CAP and T and P for MAP. Least gelation concentrations for AP and MAP or CAP were 18 and 15%, respectively, with significant effect of P and T for AP. Emulsion capacities of AP, MAP, and CAP were 62, 66, and 58 mL/g of proteinate, respectively. AP had the most stable emulsion.

INTRODUCTION

The composition of seed protein extracts, such as nitrogen content and amino acid compositions and their conformation, affects the functional properties of the protein (Elizalde et al., 1991; Kinsella, 1979; Okezie and Bello, 1988). Tests have also been used to assist in predicting the protein usefulness in food products, such as those evaluating soy protein added to beef patties. Soy protein gave better yield and nutrient retention than beef patties (Rice et al., 1989). Tests have determined that soy protein used in rice flour extrusion gave better protein solubility of texturized products (Noguchi et al., 1981). Extracted protein and solid in soy milk have been reported to affect yield and texture of tofu (Shen et al., 1991).

Since plant protein provides not only nutritional quality but also desirable characteristics, there is a need to know and evaluate both qualities, so that it can be used effectively in the food product development. Some of the many other important criteria to develop viable texturized protein products are that the protein should have optimum fat adsorption, emulsification properties, gelation, water absorption, and binding (Kinsella, 1979).

Characteristics of protein extracted from yellow pea flour at different pH (P) and temperature (T) regimes have been reported (Soetrisno and Holmes, 1992). There were similar nitrogen content, amino acid composition, and molecular weight patterns among the three proteinates. This study evaluated isolates extracted at 13 temperature–pH combinations for their nitrogen solubility, least gelation concentration (LGC), fat adsorption (FA), emulsion capacity (EC), and stability (ES).

MATERIALS AND METHODS

Coagulated proteinate was prepared by acid (AP), magnesium (MAP), and calcium (CAP) precipitation from extracts of yellow pea (*Pisum sativum* L. Miranda) flour (Soetrisno and Holmes, 1992), according to central composite rotatable design (CCRD) with temperature (°C, T) and pH (P) as independent variables.

Nitrogen Solubility Index (NSI). The nitrogen solubility index of a 1% (w/v) proteinate solution in the range pH 2.0–12.0 was determined according to the method of Thompson (1977), by measuring the nitrogen content (micro-Kjeldahl; AOAC, 1990) in the filtrate after centrifugation compared to that in the total solution.

Table I. Quadratic Regression Model Coefficients for Nitrogen Solubility Index of Acid Proteinate for Substitution into Equation,[a] with Temperature and pH as Independent Variables

	A (constant)	B_1	B_2	B_{11}	B_{22}	B_{12}
	Nitrogen Solubility Index (NSI), %					
at pH 2[b,c]	356.278	−2.287	−49.972	−0.069	1.517	0.650
at pH 3[b]	189.178	−4.214	−12.345	−0.054	−0.487	0.778
at pH 4[b]	−48.933	−6.619	24.985	−0.032	−2.238	1.010
at pH 5[b]	123.342	−0.926	−25.983	−0.007	1.346	0.132
at pH 6[d]	−54.404	−3.186	43.677	−0.095	−3.846	0.728
at pH 7[b,c]	141.465	−1.999	−6.372	−0.058	−0.655	0.578
at pH 8[b–d]	197.127	−0.373	−20.018	−0.067	0.177	0.440
at pH 9[c,d]	189.781	−2.383	−12.476	−0.063	−0.483	0.642
at pH 10[d]	323.313	−0.836	−48.202	−0.095	1.716	0.628
at pH 11[b,d]	205.918	0.603	−25.284	−0.070	0.801	0.362
at pH 12[b]	178.058	−3.224	−8.768	−0.012	−0.169	0.445

[a] $Y = A + B_1T + B_2P + B_{11}TT + B_{22}PP + B_{12}TP$. Quadratic regression equation for 13 pH temperature proteinate (AP) treatments. Raw data are available (Soetrisno, 1991). [b] CCRD is suitable for predicting the response. [c] pH (P) is significant variable. [d] Temperature (T)(°C) is significant variable.

Table II. Quadratic Regression Model Coefficients for Nitrogen Solubility Index of Magnesium Proteinate for Substitution into Equation,[a] with Temperature and pH as Independent Variables[b]

	A (constant)	B_1	B_2	B_{11}	B_{22}	B_{12}
	Nitrogen Solubility Index (NSI), %					
at pH 2[b,c]	−116.047	2.078	42.600	−0.042	−2.747	0.025
at pH 3[b,c]	−52.353	5.288	19.767	−0.042	−1.048	0.395
at pH 4[b,c]	−508.290	19.540	73.338	−0.064	−2.019	−1.790
at pH 5[b]	−8.123	−0.878	5.339	−0.007	−0.504	0.130
at pH 6[b,c]	63.357	−0.147	−15.645	−0.005	0.929	0.045
at pH 7[d]	190.451	−1.428	−35.233	−0.021	0.245	1.588
at pH 8[b]	−107.630	−4.437	45.621	0.003	−3.169	0.392
at pH 9[b]	−174.171	0.169	58.901	−0.004	−3.756	0.028
at pH 10[c]	−146.605	−3.572	59.535	−0.008	−4.096	0.420
at pH 11	592.034	−6.205	−96.684	−0.013	4.175	0.775
at pH 12[b]	212.546	−1.651	−23.981	0.002	1.051	0.210

[a] $Y = A + B_1T + B_2P + B_{11}TT + B_{12}PP + B_{12}TP$. Quadratic regression equation for 13 pH temperature proteinate (MAP) treatments. Raw data are available (Soetrisno, 1991). [b] CCRD is suitable for predicting the response. [c] pH is significant variable. [d] Temperature (T)(°C) is significant variable.

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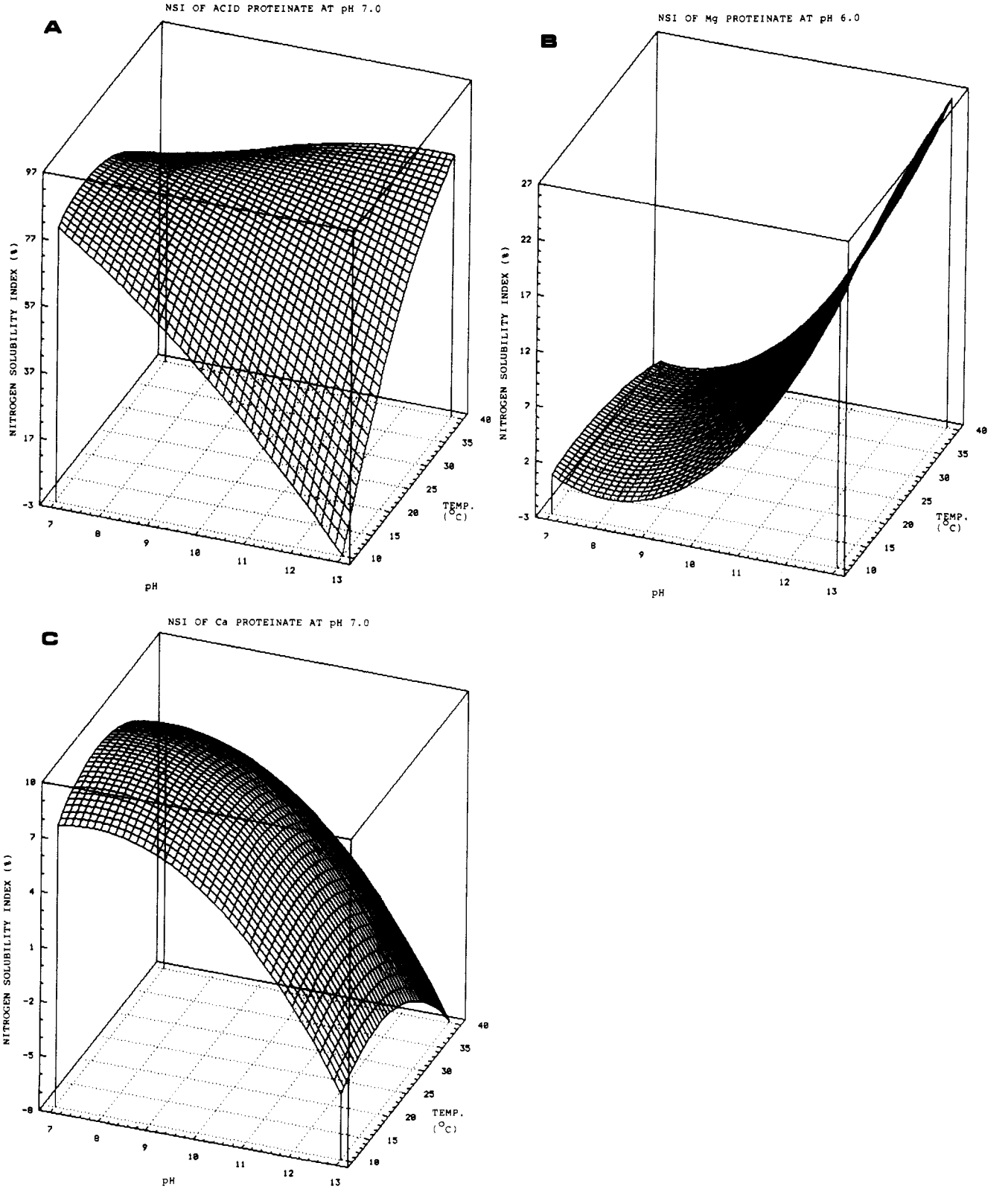

Figure 1. Three-dimensional response surface models of the nitrogen solubility index of acid (A), magnesium (B), and calcium (C) proteinates from yellow peas at pH 6.0 or 7.0.

Least Gelation Concentration (LGC). Samples from 13 different extractions were tested for LGC. The modified method of Tjahjadi et al. (1988) was used. Protein concentrations used were 20, 18, 16, and 14% for AP and 15, 13, 11, and 9% for MAP or CAP. The LGC was determined as the lowest concentration of the proteinate that gave a gel that did not fall or run when the test tube was inverted.

Fat Adsorption (FA). The modified method of Tjahjadi et al. (1988) was applied using 0.5-g sample with 3 mL of soybean oil added.

Emulsion Capacity (EC). The method was modified from that of Okezie and Bello (1988). An aliquot of 0.5 g of proteinate was dissolved in 12.5 mL of 3% NaCl with 30 mL of soybean oil added while mixing. The emulsion was transferred into a 50-mL centrifuge tube and held in a water bath (80 °C, 15 min) and then centrifuged at 3000 rpm for 30 min. The volume of oil separated was used to calculate the EC.

Emulsion Stability (ES). The method of Okezie and Bello (1988) was used except that a 0.5-g sample was homogenized with 12.5 mL of distilled water with addition of 25 mL of soybean

Table III. Quadratic Regression Model Coefficients for Nitrogen Solubility Index of Calcium Proteinate for Substitution into Equation,[a] with Temperature (T) and pH (P) as Independent Variables[b]

	A (constant)	B_1	B_2	B_{11}	B_{22}	B_{12}
	Nitrogen Solubility Index (NSI), %					
at pH 2	116.581	1.432	-12.955	-0.046	0.377	0.072
at pH 3[c]	-201.170	3.207	52.356	-0.066	-3.144	-0.042
at pH 4[c]	-344.563	4.467	68.128	-0.061	-3.421	-0.192
at pH 5	196.389	-2.735	-36.538	0.002	1.694	0.278
at pH 6[c]	-66.530	0.711	12.691	0.003	-0.562	-0.090
at pH 7[c]	-22.069	0.548	6.647	-0.012	-0.428	-0.005
at pH 8[c]	60.923	0.556	-11.768	-0.006	0.593	-0.020
at pH 9	213.318	1.545	-44.607	-0.027	2.326	-0.038
at pH 10	315.657	2.935	-71.742	-0.005	4.263	-0.280
at pH 11[c]	111.515	1.368	-10.244	-0.065	-0.131	0.235
at pH 12[c]	416.553	-4.770	-65.072	-0.030	2.669	0.720

[a] $Y = A + B_1T + B_2P + B_{11}TT + B_{22}PP + B_{12}TP$. Quadratic regression equation for 13 pH temperature proteinate (CAP) treatments. Raw data are available (Soetrisno, 1991). [b] Temperature is in °C. [c] CCRD is suitable for predicting the response.

Table IV. Quadratic Regression Model Coefficients for Least Gelation Concentration and Fat Adsorption of AP, MAP, and CAP for Substitution into Equation,[a] with Temperature and pH as Independent Variables

	A (constant)	B_1	B_2	B_{11}	B_{22}	B_{12}
	Least Gelation Concentration (LGC), %					
AP[d,e]	-62.900	2.110	11.880	-0.004	-0.380	-0.200
MAP	-1.770	0.520	2.020	-0.001	-0.030	-0.050
CAP[b]	15.000	0.000	0.000	0.000	0.000	0.000
	Fat Adsorption (FA), %					
AP[c,e]	-1950.800	59.850	356.150	-0.510	-14.480	-3.280
MPA[c-e]	-1521.460	78.120	198.320	-0.420	-1.290	-6.210
CAP[c,e]	105.300	38.610	-5.570	-0.200	4.730	-3.730

[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] Stationary point of the RSA is in flatness. [c] CCRD is suitable for predicting the response. [d] pH (P) is significant variable. [e] Temperature (T) (°C) is significant variable.

Table V. Quadratic Regression Model Coefficients for Emulsion Capacity and Total Volume of Emulsion for Substitution into Equation,[a] with Temperature and pH as Independent Variables

	A (constant)	B_1	B_2	B_{11}	B_{22}	B_{12}
	Emulsion Capacity (EC), mL of Oil/g of Proteinate					
AP[c,d]	-20.938	2.128	10.507	-0.005	-0.195	0.252
MAP[c]	-547.476	6.070	136.068	-0.084	-0.245	8.212
CAP	-820.565	9.474	176.069	-0.189	0.100	-10.510
	Total Volume, mL, at 0 h					
AP[b]	1.306	1.664	12.854	-0.006	-0.150	-0.533
MAP[b]	96.216	0.222	-4.623	-0.005	0.000	0.238
CAP[b]	97.738	-0.339	-4.241	-0.002	0.050	0.176

[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) is significant variable. [d] Temperature (T) (°C) is significant variable.

oil. The emulsion was transferred into a 50-mL centrifuge tube, and total volume, total oil, and liquid separated during standing (25 ± 2 °C) were recorded at 0, 0.5, 2, and 6 h.

Statistical Design and Analysis. A two-factor central composite rotatable design (CCRD) (Cochran and Cox, 1957) was employed to determine the interaction of P and T combinations during protein extraction on characteristic measures, as described in Soetrisno and Holmes (1992). Temperature (°C, T) and pH (P) were independent (X) variables, and the results obtained from NSI, LGC, FA, EC, and ES measurements were dependent (Y) variables. Statistical Analysis System (SAS Institute Inc., Cary, NC) and Statgraphics (Statistical Graphic Co., Rockville, MD) programs were used to generate ANOVA,

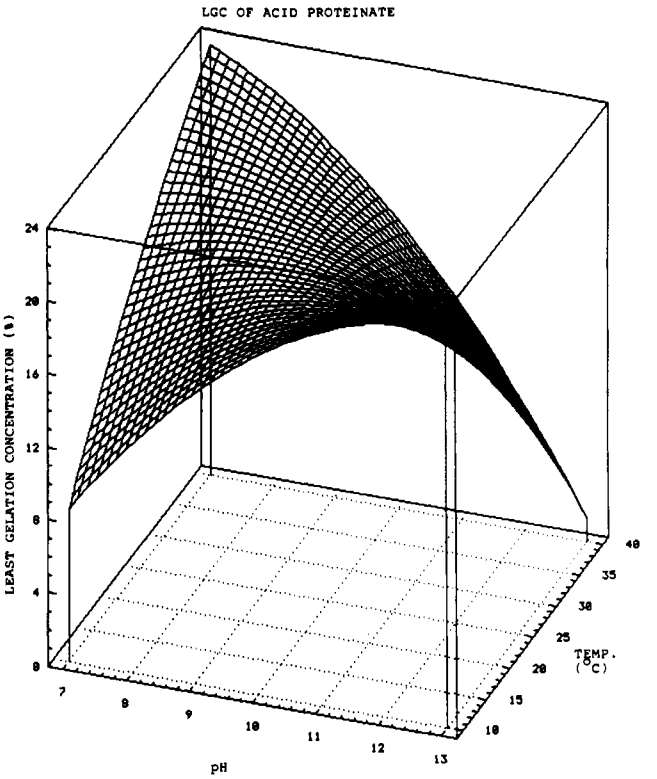

Figure 2. Three-dimensional response surface model of least gelation concentration of acid proteinate from yellow peas.

parameter estimates, response surface analysis (RSA), canonical analysis, and ridge maximum and minimum of the responses. Differences were considered statistically significant at $P < 0.10$. All measurements were done in duplicate. The following model of quadratic polynomial regression was assumed for evaluating the individual Y variables:

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

RESULTS AND DISCUSSION

Nitrogen Solubility Index. The solubility of a protein product is often dependent on the coagulant used to obtain it. However, there was no significant effect of T–P combination treatments during extraction on the NSI of AP or MAP (Tables I and II), compared to that of CAP (Table III). Figure 1 shows the RSA of NSI profiles. NSIs of AP and MAP proteinate were significantly affected by the treatments during extraction. Acid coagulation caused higher NSI of proteinate compared to Mg or Ca coagulation, which was 8.9% at pH 5.0 to 100% at pH 12.0 for AP, compared to 3.4% at pH 5.0 to 97.3% at pH 12.0 and 3.5% at pH 5.0 to 88.7% at pH 12.0, respectively, for MAP and CAP. Similar results had been reported (Hsu et al., 1982; Voutsinas et al., 1983) on NSI of yellow pea protein isolate, with the lowest NSI at pH 4.0–6.0 but higher than the NSI reported by Sumner et al. (1981) on pea protein isolate from a similar preparation.

In the case of AP, NSIs at pH 7, 8, and 11 were high when the treatment during extraction was done at combinations of low T–low P, high T–high P, or high T–low P. NSI at pH 2 was high at low T–low P or high T–high P treatment combination only.

MAP had significantly higher NSIs at pH 2 and 3, when extraction was done at low pH–all T combination treatments. The minimum solubility at pH 6 was increased when the extraction was done at high pH–all T combination treatments. CAP had a wide pH range of minimum solubility.

Table VI. Quadratic Regression Model Coefficients for Emulsion Stability for Substitution into Equation,[a] with Temperature and pH as Independent Variables

	A (constant)	B_1	B_2	B_{11}	B_{22}	B_{12}
			Retained Oil, mL/g of Proteinate			
after 0 and 0.5 h						
AP,[b] MAP,[b] CAP[b]	50.000	0.000	0.000	0.000	0.000	0.000
after 2 h						
AP[b]	50.000	0.000	0.000	0.000	0.000	0.000
MAP[d]	200.334	-3.171	-24.461	0.011	0.310	0.849
CAP	73.808	-1.048	-1.734	-0.005	0.150	-0.163
after 6 h						
AP	48.770	0.047	0.165	0.000	-0.005	-0.003
MAP[c]	51.216	-0.485	-8.556	-0.016	0.175	0.534
CAP	239.256	-4.949	-28.360	0.002	0.575	0.670
			Retained Water, mL/g of Proteinate			
after 0 h						
AP,[b] MAP,[b] CAP[b]	25.000	0.000	0.000	0.000	0.000	0.000
after 0.5 h						
AP[b]	25.000	0.000	0.000	0.000	0.000	0.000
MAP	16.185	0.045	2.053	-0.001	0.000	-0.124
CAP[d]	13.316	0.081	2.592	0.000	-0.100	-0.142
after 2 h						
AP[b]	25.000	0.000	0.000	0.000	0.000	0.000
MAP[d]	-24.194	0.419	10.700	0.000	-0.050	0.573
CAP[d]	-21.735	0.325	10.369	0.000	-0.040	-0.567
after 6 h						
AP[b]	25.000	0.000	0.000	0.000	0.000	0.000
MAP[c-e]	-53.666	1.107	15.901	0.001	-0.135	-0.776
CAP[d,e]	-127.473	1.160	33.163	-0.002	-0.125	-1.801

[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] Stationary point of the RSA is in flatness. [c] CCRD is suitable for predicting the response. [d] pH (P) is significant variable. [e] Temperature (T) (°C) is significant variable.

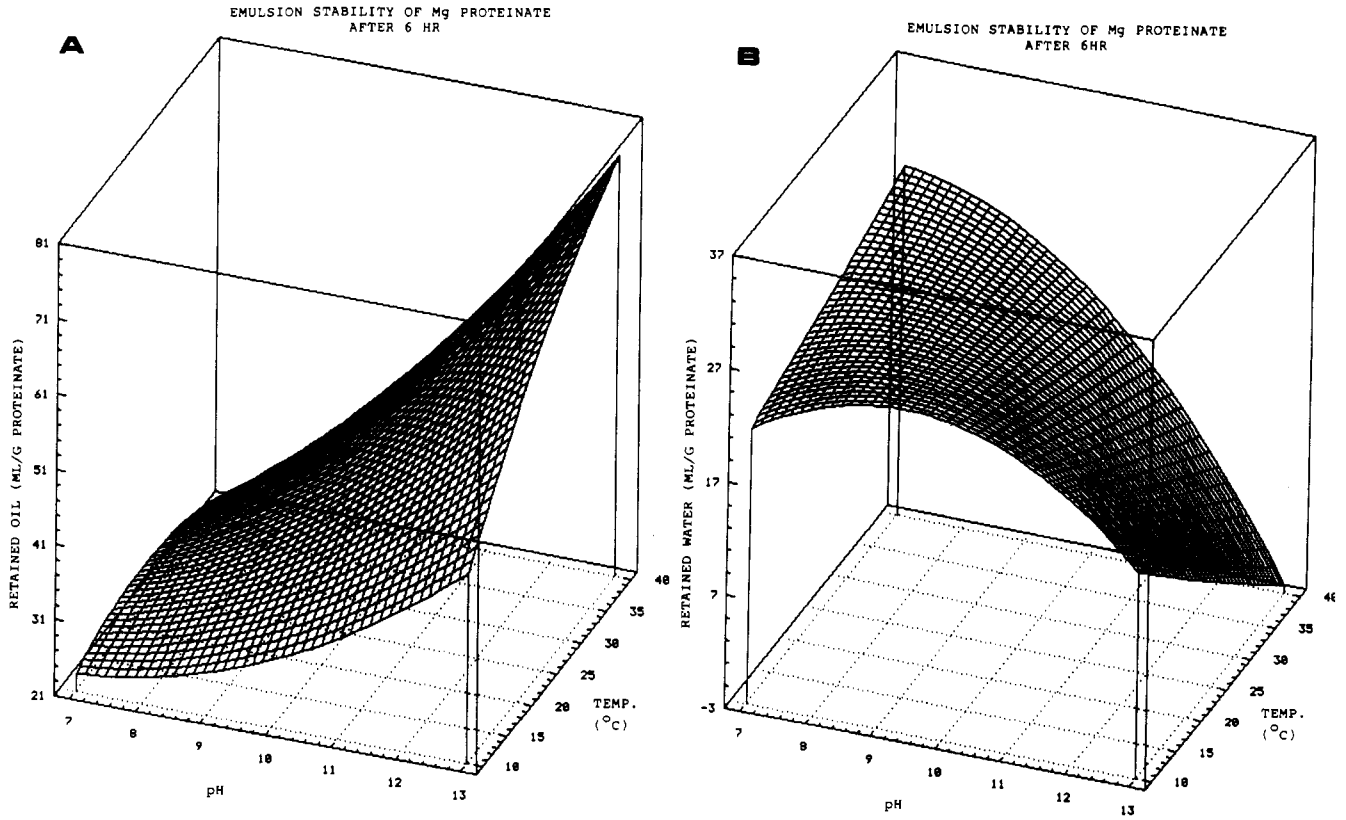

Figure 3. Three-dimensional response surface models of emulsion stability of magnesium proteinate from yellow peas as measured by retained oil (A) and retained water (B) after 6 h.

However, the practical significance of this may be limited. For example, 100% solubility for acid-coagulated proteinate was at a pH 10–12. Rarely are foods at this pH. The more typical pH range was used to present the functional properties. In the range pH 5–7, the acid (Figure 1A), magnesium (Figure 1B), and calcium (Figure 1C) proteinates had NSIs of 8.9–81.3, 3.4–17.2, and 3.5–9.2%, respectively. The lower NSIs for MAP and CAP were probably due to salting-out denaturation during protein coagulation.

Least Gelation Concentration. There were very distinct differences among LGCs of AP, MAP, and CAP. Table IV data show the regression coefficients were zero for CAP. The MAP and CAP could form a strong gel at

all concentrations tested; only the amount of water held within the matrix gel made the difference. MAP and CAP from almost all extraction treatments had 15% LGC, in which all water was held within the gel system, except MAP extracted at T = 15 °C and P = 8.0 which had 13% LGC. RSA of both MAP and CAP showed flat surfaces (not shown in figure), due to no effect on LGC value of P or T during extraction. The same concentration of divalent ions in all proteinates probably played the chief role in water imbibing activity.

LGC of acid proteinate was significantly ($P < 0.001$) affected by the P and T levels during protein extraction. It was 18.0% (T = 22.7 °C, P = 9.6) (Table IV; Figure 2) as the least concentration to form a gel. Low P–high T and high P–low T combinations gave higher LGC values.

Fat Adsorption (FA). Acid proteinate gave maximum FA = 534% (T = 30.3 °C, P = 8.9), which is more than twice the value previously reported (Sumner et al., 1981). There was a significant ($P < 0.01$) effect of T level during extraction on the FA (Table IV). The lower T had significantly lowered adsorption ability. The FAs were 522 (T = 18.3 °C, P = 10.0) and 510% (T = 13.9 °C, P = 10.3) for MAP and CAP, respectively, with significant ($P < 0.10$) effects of T and P for MAP and of T only for CAP. Extreme combinations, low P–low T and high P–high T, caused decreased FA for MAP, but only the high P–high T combination caused decreased FA for CAP. These high FA values were probably due to denaturation of protein during extraction and/or salt coagulation, which brings about the exposure of lipophilic or hydrophobic residues, resulting in increased lipid–protein complexes (Kinsella, 1979).

Emulsion Capacity (EC). There were significant ($P < 0.10$) increases in EC with decreasing T or increasing P during extraction of AP (Table V). The maximum EC value was 62 mL/g of proteinate at T = 17.7 °C and P = 10.6. Only decreasing P significantly ($P < 0.10$) increased the EC of MAP, with maximum value of 66 mL/g (T = 24.6 °C, P = 7.9). The EC of CAP was not affected by either factor during extraction; its maximum value was 58 mL/g (T = 27.4 °C, P = 8.5). All EC values were higher than the value reported by Sumner et al. (1981). This is mostly due to the ability of proteinates to bind fat, in addition to the higher concentration of protein in the dispersion (Mine et al., 1991). The pH of the dispersion was 7.5–8.0, as opposed to the acidic pH that had been reported to give highest emulsion activity.

Emulsion Stability (ES). ES as measured by volume changes during standing had maximums of 79, 78, and 76 mL/g of proteinate for AP, MAP, and CAP emulsions, respectively. A slight decrease in volume was observed after 6 h for MAP, and there was no change for the other two (Table V). Initial volume before homogenization was 75 mL. Emulsion of AP was very stable for more than 6 h (Table VI) with very thick mayonnaise-like texture, while the emulsions of MAP and CAP were stable up to 0.5 h only as measured by retained water, with thick dressing-like viscosity. These results were expected as AP formed a more viscous solution than MAP and CAP during protein dispersion, which indicated that AP has higher ability to imbibe water.

Retained water in MAP or CAP emulsion after 2 h of standing (Table VI) was affected by both increasing T ($P < 0.10$) and decreasing P ($P < 0.001$), with the same maximum volume of 26 mL/g of proteinate, at T = 36.1 °C and P = 7.8 for MAP and at T = 37.4 °C and P = 8.0 for CAP. When the stability was measured by retained oil, AP emulsion was stable for up to 6 h, while MAP and CAP emulsions were stable for up to 2 h (Table VI). Retained oil in MAP and CAP emulsions was not significantly affected by either factor during extraction. Maximum volumes of retained oil in AP, MAP, and CAP emulsions were 50, 49, and 55 mL, respectively, after 6 h of standing. Figure 3 shows the differences on response surface model for emulsion stability of MAP when measured by retained oil and retained water after 6 h of standing.

Descriptive Evaluation. Although objective color measurements were not taken, there were consistent differences in the proteinates. The dry acid-coagulated proteinate was shiny white with fluffy particles. Mg-coagulated proteinate was white with denser particles, while Ca-coagulated proteinate was creamy white with the densest particles.

Summary. The functional properties of proteinates may be used to predict the application of these proteins in food systems. Laboratory-scale extractions of yellow pea flour recovered more than 62% of its protein. Although acid or salt coagulation gave similar nitrogen content, their solubilities, gelation properties, and emulsion stabilities were different. Acid proteinate was easily dispersed and had high solubility in low pH, making it possible to incorporate into beverages or soups; together with salt proteinates, it can also be used as animal protein replacer or extender. On the basis of these data of functional properties, all three proteinates have marketability, if food product development is explored further.

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