Thermal and Surface Active Properties of Citric Acid-Extracted and Alkali-Extracted Proteins from *Phaseolus* Beans

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Acid-extracted crystalline protein isolates and alkali-extracted amorphous proteins from four *Phaseolus* beans were investigated for thermal and surface properties. Differential scanning calorimetry (DSC) analysis of crystalline isolates gave denaturation enthalpy (ΔH) values ranging from 12.4 to 31.0 J/g; for amorphous isolates of the same beans ΔH ranged from 10.3 to 11.9 J/g, suggesting that the crystalline proteins were less denatured than the corresponding amorphous isolates. Differences in protein content, nitrogen solubility index (NSI), surface hydrophobicity (S_0), and foam expansion were observed between the alkali- and acid-extracted isolates. The alkali-extracted isolates showed protein contents ranging from 69.62 to 81.61%, NSI of 24.23–66.75%, S_0 of 2128–17 000 FI%$^{-1}$, and foam expansions of 30.0–49.0%. The acid-extracted isolates showed higher protein contents (75.84–96.09%) and NSI (52.19–92.37%) but much lower S_0 (1966–7479 FI%$^{-1}$) and low foam expansion (3.0–26.0%). Surface tension of crystalline and amorphous proteins solutions ranged from 54.8 to 58.5 and from 54.2 to 56.3 mN/m, respectively. However, the rate of decay of tension in the crystalline isolates was lower than that of the corresponding amorphous isolates. Regression analysis revealed that protein surface activity was dependent on protein content and surface hydrophobicity.

INTRODUCTION

Seed storage proteins play a major role in the world's food supply by being a principal protein source. Forecasts have indicated increased utilization of vegetable proteins in the diets of developed nations, and this has resulted in increased attention toward functional and nutritional properties of the proteins. Other factors, such as the abundance and relative case of extraction, sometimes in the form of discrete microstructures (crystals, micelles), have prompted the utilization of plant proteins as model systems for research directed at protein structure and biosynthesis (Wright, 1987; Kinsella, 1981).

Isoelectric precipitation involving the extraction of ground, dried seeds with neutral of slightly alkaline solutions followed by acid precipitation (Paredes-Lopez et al., 1991; Fan and Sosulski, 1974) is a commonly used recovery method with legume seeds; this procedure gives protein isolates which have amorphous microstructure (Alli and Baker, 1980). Murray et al. (1981) described a process by which proteins obtained from a dilute salt extract of legume seed exhibited a micellar structure. A procedure for the preparation from legume seeds of soluble proteins having a variety of crystalline structures has also been described (Alli and Baker, 1980; Melnychyn, 1969). Comparative studies on the crystalline and noncrystalline preparations have shown distinctive differences between the two types of microstructures; these include differences in subunit structure of *Phaseolus lunatus* proteins as indicated by sodium dodecyl sulfate–polyacrylamide disc gel electrophoresis (Alli and Baker, 1983), size exclusion chromatography (Musakhanian and Alli, 1987), and ion-exchange chromatography (Alli et al., 1990) as well as differences in sugar composition of the carbohydrate moiety of the protein preparations (Musakhanian and Alli, 1990). In a recent study on biological properties, Li et al. (1989) demonstrated that the proteins with crystalline microstructures exhibited lower trypsin inhibitory activities than corresponding proteins with noncrystalline microstructures. Paredes-Lopez et al. (1991) also reported certain differences between micellar proteins and isoelectric proteins prepared from chickpea.

It is now recognized that differences in molecular structure of proteins are related to differences in biological properties as well as functional properties. Graham and Phillips (1979a,b) demonstrated structure–function relationshps of proteins by studying surface properties of proteins of distinctly different molecular structure. There are several papers on the relationship between other molecular properties of proteins and their air–water surface activity (Cherry and McWatters, 1981; Halling, 1981; Kinsella, 1982). Arntfield and Murray (1981) investigated the use of differential scanning calorimetry as an indicator of thermal denaturation of several plant proteins.

In the present study, legume seed proteins which are extracted with citric acid solutions and sodium hydroxide solutions and which have previously been shown (Alli and Baker, 1980) to have crystalline and noncrystalline (amorphous) microstructures were examined for surface active properties (surface tension, foaming) and for certain molecular characteristics (hydrophobicity, thermal stability).

MATERIALS AND METHODS

Materials. Crystalline and noncrystalline (amorphous) protein isolates were prepared from *Phaseolus vulgaris* beans (white kidney and navy beans) and from *P. lunatus* beans (baby lima and large lima beans) using procedures which have been described previously (Alli and Baker, 1980). The crystalline proteins were extracted with citric acid solutions and recovered by refrigeration; the amorphous proteins were extracted with sodium hydroxide solution and recovered by isoelectric precipitation. Soybean isolate (Frank E. Dempsey and Sons Ltd., Lachine, PQ) and bovine (Sigma Chemical Co., St. Louis, MO) were purchased.

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Protein Content of Isolates. The proteins were analyzed for total nitrogen with the Labconco Rapid III Kjeldahl system (Labconco Corp., Kansas City, MO), using a modified procedure of the official method for automated Kjeldahl analysis (section 7.021–7.024; AOAC, 1980).

Differential Scanning Calorimetry. Thermal denaturation of protein isolates was monitored by differential scanning calorimetry (DSC) with a Du Pont 9900 thermal analyzer equipped with a Du Pont 910 cell base and a pressure DSC cell. The thermal analyzer was calibrated with indium. A pressure of 1400 kPa with N_2 was used for all calorimetric experiments to eliminate pan failure due to moisture loss at temperatures above 100 °C. Samples (8–12 μL) of protein solutions (30% w/v in water) were sealed in hermetic pans, and thermal grease (Dow-Corning, Midland, MI) was used to improve the thermal contact between pans and the thermocouple detectors. Data were collected at 0.4-s intervals (10 °C/min heating rate) and analyzed [transition enthalpy, ΔH (J/g), and temperature, T_m] using the Du Pont software analysis program.

Nitrogen Solubility Index. The nitrogen solubility index (NSI) was determined according to the method of the American Association for Cereal Chemists (AACC, 1983; Method 46-23), with slight modifications. A volume of sodium dihydrogen phosphate (0.02 M, pH 7.0) was added to the protein isolates to prepare 0.1% (w/v) dispersions. The dispersions were agitated (1 h) at ambient temperature (25 °C) and filtered (Whatman No. 1 paper), and the filtrate (10 mL) was analyzed for nitrogen using the Labconco Rapid III Kjeldahl system; antifoaming agent (2 drops) was added to the filtrates prior to digestion to prevent excessive foaming. The NSI was calculated as percent nitrogen present in the filtration, and the factor 6.25 was used for conversion to protein.

Surface Hydrophobicity. The surface hydrophobicity of the proteins was determined using the fluorescent probe *cis*-parinaric acid (CPA) method of Townsend and Nakai (1983) as modified by Closs et al. (1990). Fifteen microliters of *cis*-parinaric acid [3.6×10^{-3} M in absolute ethanol containing 10 μg/μL butylated hydroxyanisole (BHA)] was added to a 2-mL aliquot of a 0.015% solution (w/v) containing 0.001% sodium dodecyl sulfate (SDS). The surface hydrophobicity (S_0) was measured 3 min after addition of CPA on a Perkin-Elmer fluorescence spectrometer (Model 3000) at an excitation wavelength of 325 nm and an emission wavelength of 420 nm and expressed as

$$S_0 = \frac{\text{(sample fluorescence)} - \text{(blank fluorescence)}}{\text{protein concentration (\% w/v)}}$$

Foaming Properties. Foaming properties were determined using a modification of the procedure described by Patel et al. (1988). Sodium dihydrogen phosphate buffer (25 mL; 0.02 M, pH 7.0) was placed in a 50-mL beaker and stirred (magnetic stirrer, 3.0-cm polypropylene stirring bar) to give a deep vortex. A protein sample (0.125 g) was added gradually, and the mixture was stirred for a further 30 min. The protein dispersion (25 mL, 0.5% w/v) was then transferred to the stainless steel bowl of the Virtis (Model 23) homogenizer (Virtis Co, Gardiner, NY), and whipped at 7000 rpm for 5 min. The whipped dispersion was transferred quickly into a graduated cylinder (50 mL). Air pockets in the cylinder were removed by holding the base of the cylinder with one hand and the top of the cylinder with the other hand and making two quick downward shakes. The total volume of foam, including any drained liquid, was measured 2 min after whipping. Foam expansion (% FE) was calculated using (Patel et al., 1988)

$$\%\ \text{FE} = \frac{\text{(total vol of foam + liquid)} - \text{(25 mL)}}{\text{25 mL}} \times 100$$

Samples of soy protein isolate and bovine serum albumin, treated in exactly the same manner, were used as reference proteins.

Surface Tension. Surface tensions were measured using the Fisher surface tensiometer (Model 20). Seven numbered tensiometer glass dishes (no. 1–7) containing sodium dihydrogen phosphate buffer (18 mL, 0.02 M, pH 7.0) and 2 mL of 0.01% protein solutions were carefully injected by syringe at the bottom of each dish. The dishes were covered with a glass dish and allowed to stand undisturbed. The surface tension of the solution in dish 1 was measured 5 min after addition of the protein solution. Surface tension measurements of the solutions in dishes 2–7 were taken 10, 15, 25, 60, 120, and 180 min, respectively, after addition of protein solutions. The initial or zero-time surface tension measurement was made on the sodium dihydrogen phosphate buffer (0.02 M, pH 7.0) alone.

Statistical Analysis. Data collected from this study were analyzed using the Statistical Analysis System (SAS) on McGill University's system for interacting computers (MUSIC). The Statgraphics software package (version 3.0) was used for the generation of regression curves.

Table I. Differential Scanning Calorimetric Analysis of *Phaseolus* Bean Protein Isolates (30% w/v in Water)

isolate[a]	T_d[b] ± SD,[c] °C	ΔH[d] ± SD,[c] J/g	$T_{1/2}$,[e] °C	ΔH_v,[f] J/g
WKB (a)	97.6 ± 0.3	10.3 ± 0.4	5.91	0.071
WKB (b)	95.4 ± 0.1	28.7 ± 1.4	5.45	0.067
WKB (s)	92.0 ± 0.1	22.8 ± 1.6	6.36	0.071
NB (a)	94.4 ± 0.1	11.5 ± 0.2	4.77	0.105
NB (b)	102.5 ± 0.3	31.0 ± 0.7	4.55	0.080
NB (s)	97.4 ± 0.1	25.7 ± 0.1	5.00	0.785
BLB (a)	94.9 ± 0.1	11.9 ± 0.3	5.00	0.056
BLB (b)	91.7 ± 0.1	20.6 ± 0.8	5.45	0.028
BLB (s)	93.5 ± 0.1	12.8 ± 0.7	5.00	0.110
LLB (a)	ND[g]	ND[g]	ND[g]	ND[g]
LLB (b)	92.7 ± 0.2	20.5 ± 0.3	5.45	0.032
LLB (s)	95.4 ± 0.3	12.4 ± 1.5	4.55	0.161

[a] WKB, white kidney bean; NB, navy bean; BLB, baby lima bean; LLB, large lima bean. a, amorphous; b, bipyramidal crystalline; s, spheroidal crystalline. [b] Maximum denaturation temperature. [c] Standard deviation of means from triplicate determinations. [d] Enthalpy of denaturation. [e] Peak width at half-height. [f] van't Hoff enthalpy of denaturation. [g] Not detected.

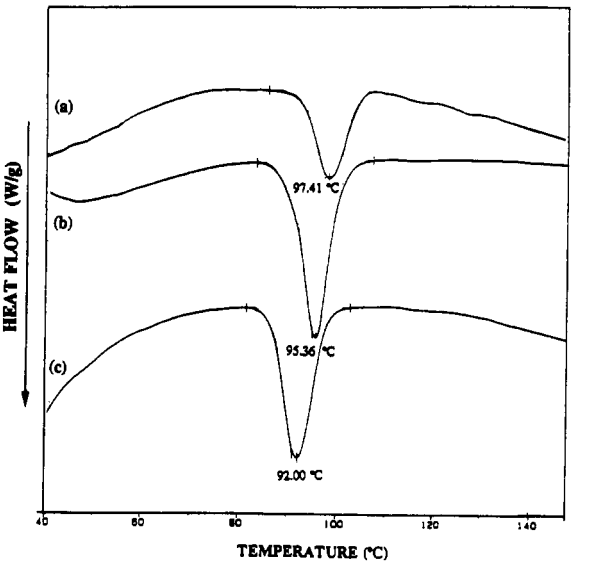

Figure 1. Differential scanning calorimetry thermograms of white kidney bean protein isolates: (a) amorphous; (b) bipyramidal; (c) spheroidal microstructures.

RESULTS AND DISCUSSION

Thermal Properties of Protein Isolates. Table I shows the peak denaturation temperature (T_d), specific enthalpy of denaturation (ΔH), width at half-peak height ($T_{1/2}$), and van't Hoff enthalpy of denaturation (ΔH_v) of the crystalline (citric acid-extracted) and amorphous (alkali-extracted) proteins prepared from the beans. The DSC thermograms obtained with the proteins prepared from white kidney bean only are shown in Figure 1. The T_d values of the proteins ranged from 91.7 to 102 °C and are somewhat higher than the peak denaturation temperatures reported for dry bean globulins (T_d = 90.16 °C) and pure glycoprotein II of *P. vulgaris* (T_d = 89.36 °C) by Wright and Boulter (1980) but comparable to those reported by Sosulski et al. (1985) for the air-classified

Table II. Protein Content, Solubility, Surface Hydrophobicity, and Foam Expansion Data of *Phaseolus* Protein Isolates[a]

isolate[b]	protein content, %	nitrogen solubility index (NSI), %	surface hydrophobicity (S_0), FI %$^{-1}$	foam expansion, %
WKB (a)	72.8 (0.78) A	65.7	17000 (619) A	40.0 (0) A
WKB (b)	95.7 (1.12) B	89.6	7479 (361) B	21.0 (1.41) B
WKB (s)	91.3 (0.51) C	85.0	1966 (49) C	8.0 (0) C
NB (a)	76.0 (1.15) A	66.8	12103 (218) A	40.0 (5.66) A
NB (b)	96.1 (1.16) B	91.1	4618 (125) B	8.0 (0) B
NB (s)	88.2 (0.67) C	82.4	3291 (627) C	3.0 (1.41) C
BLB (a)	81.6 (1.44) A	63.8	15875 (106) A	49.0 (1.41) A
BLB (b)	87.9 (3.81) B	76.3	2687 (144) B	26.0 (2.83) B
BLB (s)	84.0 (1.06) AB	57.1	2893 (144) B	25.0 (4.24) B
LLB (a)	69.6 (0.49) A	24.2	2128 (143) A	30.0 (2.83) A
LLB (b)	85.5 (0.78) B	92.4	3098 (130) B	22.0 (2.83) A
LLB (s)	75.8 (3.84) C	52.2	3456 (104) B	22.0 (2.83) A
BSA	97.3 (0.33)	97.27	30422 (511)	54 (2.83)
soy protein	87.3 (0.49)	65.04	3523 (563)	44 (1.00)

[a] Results are means (standard deviations) of triplicate determinations, except for % NSI which were from duplicate determinations. For each bean, mean values with the same letters (A–C) are not significantly different ($P < 0.10$). [b] WKB, white kidney bean; NB, navy bean; BLB, baby lima bean; LLB, large lima bean; BSA, bovine serum albumin. a, amorphous; b, bipyramidal crystalline; s, spheroidal crystalline.

protein fractions from navy bean and lima bean meal. The thermogram from the amorphous protein from large lima bean showed no endothermic heat flow, suggesting that this protein was completely denaturated.

Table II also shows that the enthalpy of denaturation values of the amorphous proteins (alkali-extracted) were lower than those of the citric acid-extracted proteins (bipyramidal crystalline microstructures) from the corresponding beans. The ΔH for amorphous isolates ranged from 10.3 J/g for white kidney bean to 11.9 J/g for baby lima bean. The proteins which showed bipyramidal crystalline microstructures had ΔH values which ranged from 20.5 to 31.0 J/g with significantly higher values for the *P. vulgaris* beans (white kidney, navy) when compared with *P. lunatus* (baby lima, large lima) beans. The ΔH values of the crystaline isolates from white kidney bean and navy bean were comparable to the ΔH value of 28.03 J/g reported for pure glycoprotein II of *P. vulgaris* (Wright and Boulter, 1980). Reports by others (Arntfield and Murray, 1981; Paredes-Lopez et al., 1991) have shown that micellar proteins prepared by extraction with neutral salt solution and precipitated with cold water gave higher T_d and ΔH values than isoelectric proteins prepared from the same beans; this is similar to our results, which show differences between the crystalline and amorphous proteins of the same beans. On the basis of the sharpness of the endothermic peaks (small half-peak widths, $T_{1/2}$), it can be suggested that the proteins are highly cooperative; i.e., intramolecular bonds rupture within a narrow range of temperatures (Harwalker and Ma, 1987). Privalov (1979) suggested that an indication of how closely the denaturation reactions of proteins approximate a two-state process could be obtained by comparing the calorimetric enthalpies of denaturation (ΔH) to the van't Hoff enthalpies of denaturation (ΔH_v) which were calculated using the formula proposed by Wright (1982); only one endothermal peak was present for each of the isolates, and the ΔH_v values were found to be considerably lower than the ΔH values, suggesting the presence of multiple domains in the protein isolates.

It is possible that the observed thermal properties of the proteins of the two types of proteins (acid-extracted, alkali-extracted) could be related to the differences between the effects of acidic conditions and basic conditions used for extraction of the proteins. Although the alkaline conditions used to extract the amorphous proteins can be expected to cause disruption of covalent bonds, the subsequent isoelectric precipitation step is considered to cause denaturation which could account for the lower ΔH (Murray et al., 1985; Ma and Harwalker, 1988; Paderes-Lopez et al., 1991).

Nitrogen Solubility Index (NSI). The NSI values of the protein solutions (0.1% w/v) of the *Phaseolus* protein isolates are shown in Table II. Except for the amorphous proteins from large lima bean, which appeared to be completely denatured on the basis of thermal analysis and which showed an NSI of 24.2%, the proteins showed NSI values ranging from 63.8 to 92.4%. The bipyramidal crystalline isolates of both *Phaseolus* beans showed the highest degree of solubility (average NSI = 87.3%) followed by the spheroidal proteins (average NSI = 69.2%), and the amorphous proteins showed the lowest NSI (average NSI = 55.12%). The NSI values of the acid-extracted bipyramidal and spheroidal proteins from *P. lunatus* were markedly different when compared with the NSI of the corresponding proteins from *P. vulgaris*. This suggests the compounding effect of genetic variations on the solubility properties of the proteins in addition to differences arising from the protein preparation procedures. Previous workers (Matsudomi et al., 1985; Kato et al., 1981) have reported that the solubility of protein isolates was dependent on the state of denaturation of the isolates. To determine whether this is the case with the proteins which demonstrated differences in enthalpy of denaturation, a statistical correlation between ΔH and NSI was carried out; regression analysis gave a statistically significant correlation of $r = 0.867$ ($P < 0.001$). Kilara and Mangino (1991) reported a similar relationship between NSI and enthalpy of denaturation of whey protein concentrate and suggested that enthalpy of denaturation may serve as a predictor for solubility of proteins. This could be an explanation of the observation that the amorphous protein from large lima bean which showed no endothermic heat flow also showed a very low NSI of 24.2%.

Surface Active Properties. Results from measurements of surface hydrophobicity and foam expansion of the proteins are given in Table II. Published studies on protein functionality often attempt to correlate protein solubility, protein surface hydrophobicity, thermal denaturation, foaming, and emulsifying characteristics; although there are papers which indicate that there is a good relationship between protein solubility and certain functional attributes (O'Neill et al., 1990; Kitabatake and Doi, 1988; Nakai, 1983; Kinsella, 1976), there are several other papers which suggest that solubility may not be the

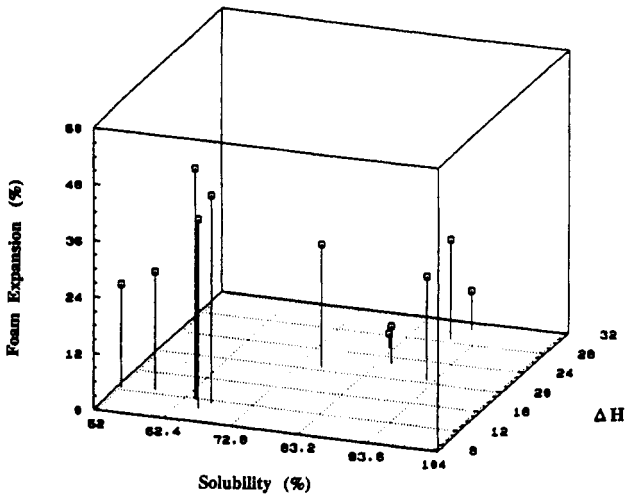

Figure 2. Three-dimensional plot of foam expansion, enthalpy of denaturation, and solubility of 12 *Phaseolus* protein isolates.

primary requirement for good protein functionality such as emulsification and foam expansion (Paredes-Lopez et al., 1991; Kella et al., 1989; Aoki et al., 1980). Several authors have suggested that charge frequency (Bigelow, 1967), hydrophobicity (Townsend and Nakai, 1983; Kato et al., 1981) and surface activity (Phillips, 1981; Graham and Phillips, 1979a,b) may have greater influence on protein functionality than solubility. Table II shows that, except for the amorphous alkali-extracted protein from large lima bean (which, as mentioned before, shows anomalous behavior), the amorphous proteins (alkali-extracted) had much higher surface hydrophobicity than the crystalline and spheroidal proteins (acid-extracted) from the corresponding beans. The bipyramidal crystalline proteins of both *P. vulgaris* beans (WKB and NB) showed higher surface hydrophobicities than the corresponding spheroidal proteins, while the spheroidal proteins extracted from *P. lunatus* beans showed somewhat similar surface hydrophobicities when compared with the bipyramidal proteins extracted from the same beans; again, this suggests genetic variations of similar proteins from the two species of beans. Linear regression analysis demonstrated that surface hydrophobicity was correlated very poorly with enthalpy of denaturation ($r = -0.524$; $P < 0.01$) and with NSI protein solubility ($r = -0.328$; $P > 0.25$).

The foam expansion measurements (Table II) indicate higher foaming capacities for the amorphous proteins (30–49%) of both *Phaseolus* beans than either the bipyramidal (8–26%) or spheroidal (3–25%) proteins. The degree of foam expansion was poorly correlated ($r = -0.564$; $P < 0.10$) with the % NSI. However, foam expansion showed a negative linear correlation with enthalpy of denaturation ($r = -0.780$; $P < 0.005$) and a linear correlation ($r = 0.813$; $P < 0.005$) wioth surface hydrophobicity of the protein isolates. A three-dimensional plot of protein foam expansion vs enthalpy of denaturation vs solubility (Figure 2) shows that optimum foam expansion can be obtained from the proteins with low enthalpy of denaturation (10.3–11.9 J/g) and moderate NSI (63.8–66.8%). Paredes-Lopez et al. (1991) also reported that isoelectric precipitated chickpea proteins, which showed lower solubilities and enthalpies of denaturation when compared with corresponding micellar proteins, showed higher foam capacities; results obtained by Nakai and Townsend (1983) suggest that moderate protein dispersibility but high hydrophobicity is associated with high foam capacity. To obtain an expansion for the relationship between the foam expansion of the protein isolates and the various predictor variables (i.e., ΔH, solubility, and surface hydrophobicity) of these isolates, without influencing the effect of any one variable with others of significance, backward stepwise regression was applied to the foam expansions of all protein isolates, as functions of ΔH, solubility, and surface hydrophobicity. Using the SAS program to perform the analysis on this model, an R^2 of 0.834 ($P < 0.001$) was obtained. Of the three predictor variables, ΔH and surface hydrophobicity were found to have a significant effect on foam capacities of the proteins.

Figure 3 shows the surface tension decay curves of proteins isolated from white kidney bean (a), navy bean (b), baby lima bean (c), and large lima bean (d). The decay curves show an initial rapid decrease in surface tension. For the proteins prepared from the *P. vulgaris* beans, the initial rate of decay of the alkali-extracted amorphous protein was more rapid than those of the citric acid-extracted crystalline proteins. This difference was not observed with the proteins prepared from the *P. lunatus* beans, again suggesting genetic differences in the behavior of the proteins from the two species of beans. After the initial rapid decay, there was little change in surface tension. The surface tension of the amorphous proteins of the *P. vulgaris* beans was always lower than those of the crystalline proteins; this was not the case with the priotein from *P. lunatus*. The surface tension decay curves of the *Phaseolus* protein isolates were very similar to those reported by other workers using legumin and vicilin (Dagorn-Scaviner et al., 1986), myosin (O'Neill et al., 1990), soybean, ovalbumin, and BSA (Kitabatake and Doi, 1988; Song and Damodaran 1987), and casein (Closs et al., 1990); these studies also showed that diffusion to and adsorption at an air–water interface are controlled by a multitude of factors. Protein solubility, hydrophobicity, size, net charge, and composition, as well as degree of flexibility and therefore secondary structure, have been suggested as having some influence. Our results indicate a relationship between equilibrium surface tension, % NSI, and enthalpy of denaturation. Linear regression at the 95% confidence level showed that equilibrium surface tension correlated with both the degree of solubility ($r = 0.668$) and the enthalpy of denaturation ($r = 0.694$). The equilibrium surface tension of the protein solutions generally increased as NSI and ΔH increased. A negative curvilinear relationship ($r = -0.610$; $P < 0.05$) was found to exist between the equilibrium surface tension and the surface hydrophobicity of the protein isolates. MacRitchie (1978) suggested that the initial stages of protein adsorption at air–water interfaces are considered to be diffusion-controlled; once a protein film is formed at the interface, the subsequent rate of adsorption becomes dependent on the ability of the protein molecules to penetrate into the interface and rearrange. Unlike some papers (Closs et al., 1990), our results indicate that protein solubility, although important, was not the determining factor in either the rate of surface tension decay or the equilibrium surface tension of the protein solutions. The rate of surface tension decay during the initial stages of protein adsorption is generally greater for proteins which have a greater diffusion rate. The differences in diffusion rates of the proteins and thus initial surface tension decay are associated with compositional and hence molecular weight differences (Dagorn-Scaviner et al., 1986). Our work supports the findings of Song and Damodaran (1987) in that it suggests that the enthalpy of denaturation (ΔH) of the proteins indicated that the optimum ratio of ordered to disordered structure seems to be essential to cause greater change of surface tension per adsorbed molecule. The negative curvilinear relationship between the equilibrium surface

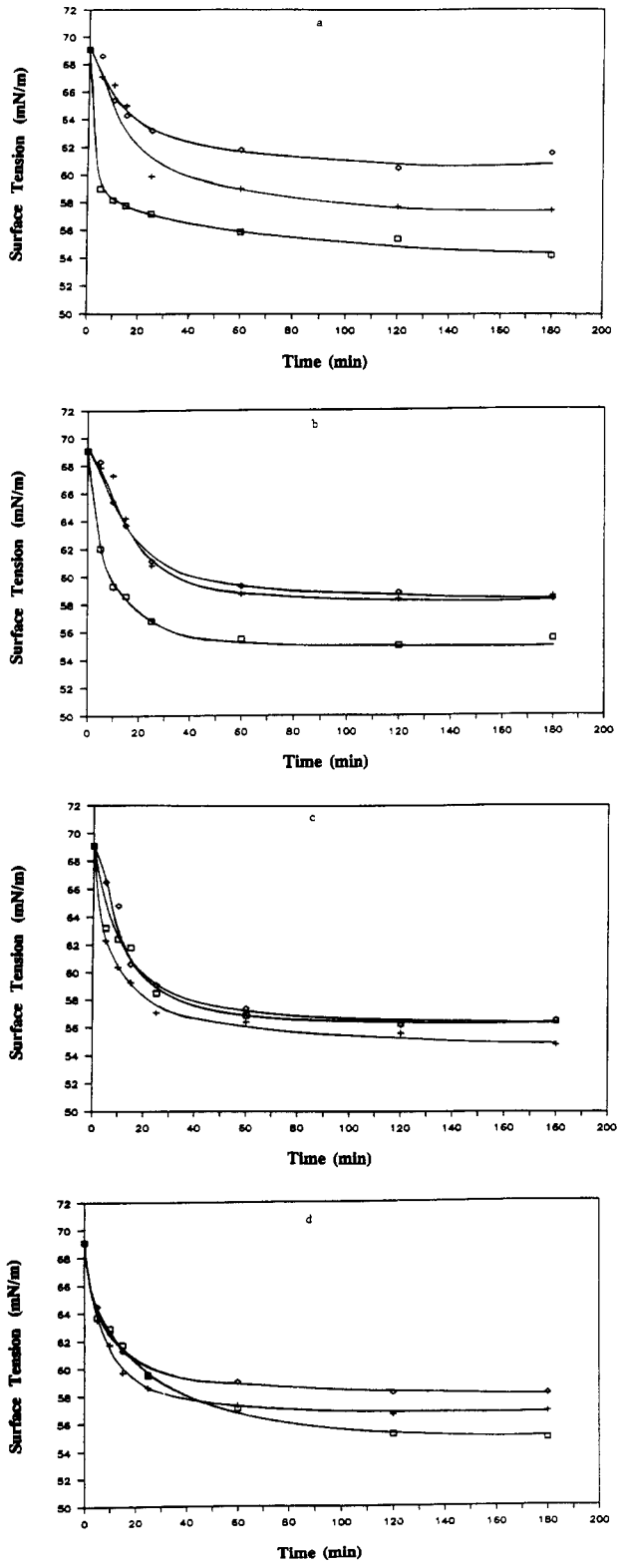

Figure 3. Surface tension decay curves of *Phaseolus* protein isolates: (a) white kidney bean; (b) navy bean; (c) baby lima bean; (d) large lima bean. (□) Amorphous; (+) bipyramidal; (◇) spheroidal.

tension and the surface hydrophobicity of the protein isolates obtained in the present study has been reported also by several authors (Kato et al., 1981; Kato and Nakai, 1979; Horiuchi et al., 1973). Although unfolding seems to be facilitated by the presence of hydrophobic domains at the surface regions (Kinsella, 1981), it is likely that certain protein molecules were able to maintain part of their structure, and thus a linear relationship was not obtained.

Foam expansion and surface tension of the protein solutions were found to be linearly related ($r = -0.743$; $P < 0.01$). This agrees with findings of other workers (Kato et al., 1981), who showed that protein solution with lower surface tension showed higher foam capacity. Kitabatake and Doi (1982), however, did not find a correlation between the foaming power of protein solutions and their equilibrium surface tension.

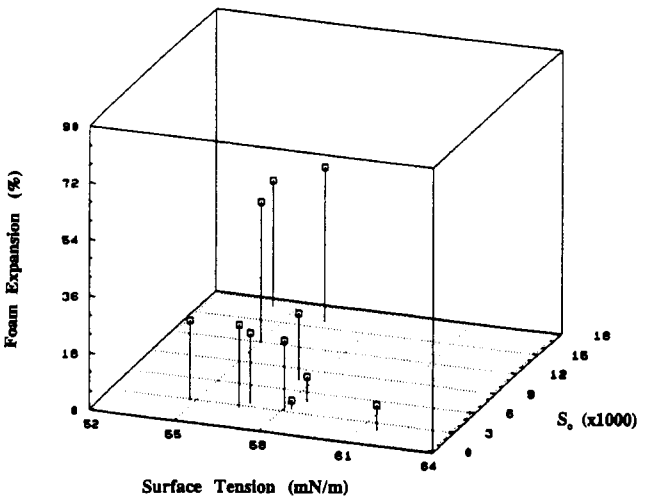

Figure 4. Three-dimensional plot of foam expansion, surface hydrophobicity (S_0), and surface tension of 12 *Phaseolus* protein isolates.

To determine the relationship between hydrophobicity, foam expansion, and surface tension, a three-dimensional plot was developed (Figure 4). The results demonstrate that optimum foam expansion can be obtained with proteins which have a low equilibrium surface tension (54.2–56.3 mN/m) and a relatively high surface hydrophobicity (>10 000 FI%$^{-1}$). Partially denatured proteins which showed low ΔH and relatively high surface hydrophobicity (S_0) showed greater surface-tension-lowering potential than the proteins which showed higher ΔH and lower S_0; this could be related to the protein film-forming potential at the air–buffer interface. This better film-forming potential can lead to better foam expansions.

Conclusion. The present work has demonstrated that *Phaseolus* protein isolates recovered by alkali extraction/isoelectric precipitation show differences in certain physicochemical and functional properties when compared to *Phaseolus* protein isolates recovered by acid extraction/refrigeration. In addition, proteins prepared from *P. vulgaris* beans showed distinct differences from corresponding proteins prepared from *P. lunatus* beans, suggesting interspecies variations. The functional properties of all isolated proteins were influenced by solubility and by surface hydrophobicity.

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