Functional Properties of Protein Concentrates from Three Chinese Indigenous Legume Seeds

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The functional properties of the protein concentrates (PCs) from *Phaseolus angularis*, *Phaseolus calcaratus*, and *Dolichos lablab* seeds were investigated and compared with those of soybean PC. The minimum nitrogen solubilities of *P. angularis* and *P. calcaratus* PCs were at pH 5, while that of *D. lablab* PC was at pH 4. These three PCs had lower viscosities, similar emulsifying activities and emulsion stabilities, and higher water- and oil-holding capacities and foam capacities than soybean PC. Their minimum emulsifying activities, minimum foam capacities, and maximum foam stabilities were at pH 4. Moreover, emulsion stabilities of these three PCs were high (above 93.2%) over the pH range of 2−10.

Keywords: *Phaseolus angularis; Phaseolus calcaratus; Dolichos lablab; functional property; protein concentrate*

INTRODUCTION

Because of inadequate supplies and shortage of food proteins, there has been a constant search of unconventional legumes as new protein sources for use as both functional food ingredients and nutritional supplements (Tjahjadi et al., 1988; Pandey and Sirvastava, 1990; Morrow, 1991; Onweluzo et al., 1994). Onweluzo et al. (1994) have reported the potential application of some lesser known legume seed fractions from the developing countries as functional food ingredients. The seeds of *Phaseolus angularis*, *Phaseolus calcaratus*, and *Dolichos lablab* (white bean), three indigenous Chinese legume seeds, are less exploited as protein sources in China. These seeds are traditionally used as a soup ingredient for therapeutic purposes such as ameliorating symptoms of dropsy, relieving diarrhea, and tonic to the viscera (Li, 1973).

In addition to providing essential amino acids, the ultimate success of utilizing any seed protein as food ingredients depends largely upon its desirable functional properties (Nwanekezi et al., 1994; Aluko and Yada, 1995). In the following study, the functional properties of these three seed proteins were evaluated and compared with those of soybean protein.

MATERIALS AND METHODS

Preparation of Protein Concentrates. Fully grown seeds of *P. angularis*, *P. calcaratus*, *D. lablab*, and soybean, imported from mainland China, were chosen for testing of their functional properties. Cleaned seeds of the four legume species were ground in a cyclotec mill (Tecator, Hoganas, Sweden) to pass through a 0.5 mm screen. The four meals were defatted with acetone according to the method reported by Aluko and Yada (1995). These defatted meals were stirred in 15 volumes of water for 15 min, and the pH was adjusted to 9.5 before another 1 h stirring. These suspensions were centrifuged at 5500*g* for 20 min. The pH of the supernatants was adjusted to their isoelectric points, which were 4 for *D. lablab* and soybean proteins and 5 for *P. angularis* and *P. calcaratus* proteins. The precipitates were redispersed in distilled water, and the pH was adjusted to 7 prior to freeze-drying.

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Protein Content. Protein content of the PCs was calculated by multiplying the nitrogen content determined by a CHNS/O elemental analyzer (Perkin Elmer 2400, Connecticut) by a factor of 6.25.

Nitrogen Solubility. Nitrogen solubilities of the proteins at 2% (w/v) were determined by the method of Beuchat et al. (1975) over a pH range from 2 to 10. The dispersions were stirred at different pHs at 24 °C for 45 min and then centrifuged at 3000*g* for 30 min. The supernatants were freeze-dried, weighed, and analyzed for nitrogen content with a CHNS/O elemental analyzer. Nitrogen solubilized is expressed as percent of the nitrogen content of the protein sample.

Water- and Oil-Holding Capacity. The method of Carcea Bencini (1986) was used with slight modifications. One gram of protein samples was stirred in 10 mL of distilled water or corn oil (Mazola, CPC International) and then centrifuged at 2200*g* for 30 min. The volume of the supernatant was measured. The water-holding capacity is expressed as the number of grams of water held by 1 g of protein sample. The oil-holding capacity is expressed as the number of grams of oil held by 1 g of protein sample. Density of the oil was found to be 0.92 g/mL.

Viscosity. Viscosities of protein solutions (1%, 3%, 5%, 7%, and 10%, w/v) at pH 7 were studied at 22 °C (Carcea Bencini, 1986). The viscosity was determined with a Brookfield LV viscometer (Brookfield Engineering Lab., Stoughton) at 100 rpm using spindle number 2.

Emulsifying Activity and Emulsion Stability. The method used was according to Sathe et al. (1983) with the following modifications. One hundred milliliters of 2% (w/v) protein suspension at different pHs (ranged from 2 to 10) was homogenized at 11 000 rpm for 30 s using a Polytron homogenizer. One hundred milliliters of corn oil (Mazola, CPC International) was then added and homogenized for another 1 min. The emulsions were centrifuged in 50 mL graduated centrifuged tubes at 1200*g* for 5 min, and the volume of the emulsion left was measured. Emulsifying activity (EA) was calculated as follows:

$$\text{EA \%} = \text{vol of emulsified layer/vol of whole layer in centrifuge tube} \times 100$$

To determine the emulsion stability (ES), emulsions prepared by the above procedures were heated at 80 °C for 30 min, cooled to room temperature, and centrifuged at 1200*g* for 5 min. ES was calculated as follows:

$$\text{ES \%} = \text{vol of remaining emulsified layer/original emulsion volume} \times 100$$

S0021-8561(97)00047-2 CCC: $14.00

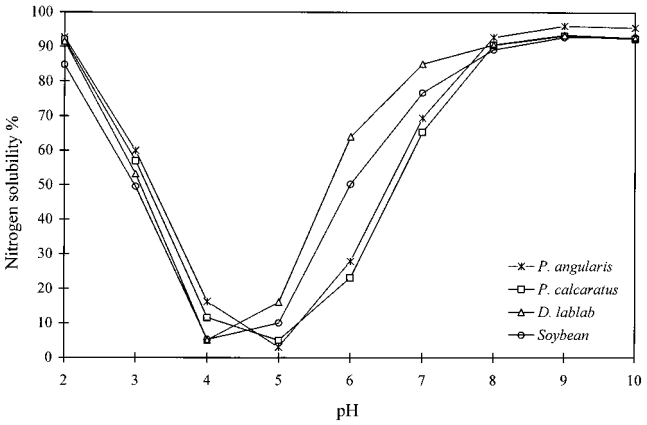

Figure 1. Effect of pH on the nitrogen solubilities (%) of *P. angularis*, *P. calcaratus*, *D. lablab*, and soybean protein concentrates.

Foam Capacity and Foam Stability. The method of Nath and Narasinga Rao (1981) was used with slight modifications. One hundred milliliters of 1.5% (w/v) protein suspension was whipped at low speed in a Waring blendor for 5 min, and their foam volumes were recorded after 30 s. Foam capacity (FC) is expressed as percent increase in foam volume measured at 30 s, and foam stability (FS) was determined by the increase of foam volume after standing for 5, 30, and 120 min. Both FC and FS were determined as a function of pH.

Statistical Analysis. In this study, all measurements were done in triplicate except the tests of nitrogen solubility which were done in duplicate. Data collected were analyzed by one-way analysis of variance and the Tukey test (Ott, 1988).

RESULTS AND DISCUSSION

Protein Content and Nitrogen Solubility. The protein contents of *P. angularis*, *P. calcaratus*, *D. lablab*, and soybean protein concentrates (PCs) obtained were 79.6%, 78.0%, 85.0%, and 78.7% (w/w, dry weight), respectively. The moisture contents of the four freeze-dried legume PCs were approximately 4% by weight. Figure 1 shows the variations of nitrogen solubility on different pH conditions. The minimum nitrogen solubilities of *P. angularis* and *P. calcaratus* PCs were 3.33% and 5.00%, respectively, at pH 5, while those of *D. lablab* and soybean PCs were 5.08% and 5.26%, respectively, at pH 4. Minimum nitrogen solubility around pH 4 and 5 was also observed in the PCs of some other legumes such as black gram (Sathe et al., 1983), lupin seed (Sathe et al., 1982a), faba bean, and field pea (Sosulski and McCurdy, 1987). On either side of pH 4 and 5, there was a sharp increase in the solubility for all four PCs. At pH 2 about 84.8−92.8% of the nitrogen was soluble, and about 92.4−95.6% of the nitrogen was soluble at pH 10. Over the pH range from 4 to 8, *D. lablab* PC had a markedly higher solubility than the other PCs (Figure 1). Generally, variations in nitrogen solubilities among different legume proteins have been noted (Coffmann and Garcia, 1977). In Figure 1, the profiles of the nitrogen solubility against pH of *P. angularis*, *P. calcaratus*, and *D. lablab* PCs compared favorably with that of the reference soybean PC. The legume PCs studied all showed good solubility in both the acidic and alkaline pH regions which was an important characteristic in food formulations (Idouraine et al., 1991).

Water- and Oil-Holding Capacity. Table 1 shows that the water-holding capacity (WHC) of *P. calcaratus* PC is the highest (5.28 g/g) while those of *P. angularis* and *D. lablab* PCs (5.05 and 5.08 g/g, respectively) are not significantly different. The WHCs of these three PCs were significantly higher ($p < 0.05$) than that of soybean PC (3.46 g/g). The WHCs of PCs from some other legume seeds such as *Phaseolus mungo* L., *Phaseolus vulgaris* L., and sunflower seed were 5.90, 5.93, and 3.90 g/g, respectively (Sathe et al., 1983; Sathe and Salunkhe, 1981; Sosulski and Fleming, 1977). Basically, the differences in WHC among these legume PCs might be attributed to the different protein conformations and the variations in the number and nature of the water-binding sites on protein molecules (Chou and Morr, 1979).

In Table 1, the oil-holding capacities (OHCs) of *P. calcaratus* and *D. lablab* PCs (4.71 and 4.77 g/g, respectively) were significantly higher ($p < 0.05$) than that of *P. angularis* PC (4.38 g/g). The OHCs of PCs from some other legume seeds such as black gram, defatted lupin seed, and winged bean were reported to be 3.48, 3.89, and 4.01 g/g, respectively (Sathe et al., 1983, 1982a,b). Table 1 shows that the OHCs of *P. angularis*, *P. calcaratus*, and *D. lablab* PCs were significantly higher ($p < 0.05$) than that of the reference soybean PC (3.06 g/g). The higher OHCs of these three PCs might be attributed to a higher level of nonpolar side chains in their molecules. Our results suggested that *P. angularis*, *P. calcaratus*, and *D. lablab* PCs had both good WHC and OHC.

Viscosity. The results of viscosity measurement of *P. angularis*, *P. calcaratus*, *D. lablab*, and soybean protein solutions at different concentrations are presented in Table 1. A significant increase ($p < 0.05$) in viscosity with increasing PC solid concentrations was observed in all four PCs. Concentration dependence of viscosity was also reported for sunflower protein (Fleming et al., 1974) and *P. vulgaris* L. protein (Sathe and Salunkhe, 1981). At concentrations varying from 1% to 10% (w/v), the viscosity of *D. lablab* protein solution was higher than those of *P. angularis* and *P. calcaratus* protein solutions. However, the reference soybean protein solution had a higher viscosity than all these three protein solutions at each corresponding concentration. Therefore, the viscosities shown in Table 1 appeared to be a function of not only the solid concentration but also the type of proteins. It was suggested that even in beans of the same genus, their protein conformational characteristics might differ substantially enough

Table 1. Water- and Oil-Holding Capacities and Viscosities of *P. angularis, P. calcaratus, D. lablab,* and Soybean Protein Concentrates

protein concentrate	WHC (g/g)	OHC (g/g)	viscosity (cP) 1%[a]	3%	5%	7%	10%
P. angularis	5.05^{w}	4.38^{w}	2.08^{mw}	2.88^{nw}	4.23^{ow}	6.48^{pw}	12.3^{qw}
P. calcaratus	5.28^{x}	4.71^{x}	2.18^{mx}	3.10^{nw}	4.23^{ow}	6.13^{px}	10.2^{qx}
D. lablab	5.08^{w}	4.77^{x}	2.40^{mx}	3.70^{nx}	5.68^{ox}	7.65^{py}	12.5^{qw}
soybean	3.46^{y}	3.06^{y}	4.05^{my}	5.85^{ny}	8.60^{oy}	12.2^{pz}	23.7^{qy}

[a] Concentration (w/v). w−z: Values in the same column with different superscripts are significantly different (Tukey, $p < 0.05$). m−q: Values in the same row with different superscripts are significantly different (Tukey, $p < 0.05$).

Table 2. Effect of pH on the Foam Stabilities (%) of *P. angularis, P. calcaratus, D. lablab,* and Soybean Protein Concentrates

protein concentrate	pH 2			pH 4			pH 6			pH 8			pH 10		
	5[a]	30	120	5	30	120	5	30	120	5	30	120	5	30	120
P. angularis	128[b]	106[c]	76.0[d]	78.0[e]	70.0[f]	66.0[f]	88.0[h]	80.0[i]	70.0[j]	124[k]	106[l]	74.0[m]	140[n]	121[o]	92.0[p]
P. calcaratus	120[b]	102[c]	64.0[d]	78.0[e]	74.0[f]	73.0[f]	80.0[h]	76.0[hi]	74.0[i]	120[k]	108[l]	60.0[m]	120[n]	116[n]	60.0[o]
D. lablab	134[b]	128[c]	108[d]	60.0[e]	57.0[ef]	54.0[f]	85.6[h]	80.0[i]	74.0[j]	132[k]	120[l]	92.0[m]	140[n]	132[o]	110[p]
soybean	96.6[b]	80.0[c]	60.0[d]	54.0[e]	50.0[f]	44.0[f]	70.0[h]	54.0[i]	43.0[j]	88.0[k]	70.0[l]	56.0[m]	94.0[n]	71.0[o]	58.0[p]

[a] The value in this row represents time in minutes. b−p: Values of FS % in the same row with different superscripts are significantly different (Tukey, $p < 0.05$); b−d for pH 2, e, f for pH 4, h−j for pH 6, k−m for pH 8, n−p for pH 10).

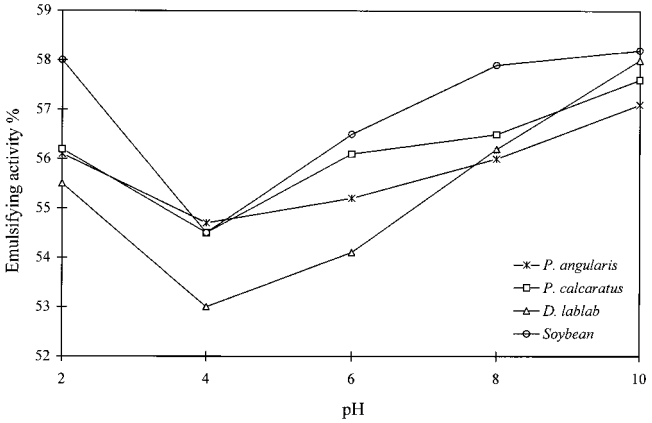

Figure 2. Effect of pH on the emulsifying activities (%) of *P. angularis, P. calcaratus, D. lablab,* and soybean protein concentrates.

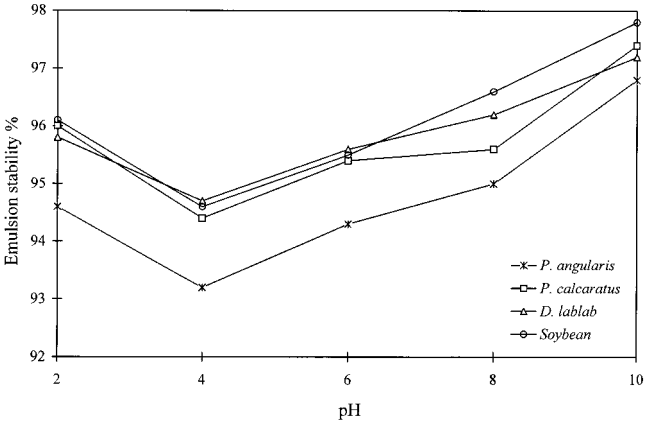

Figure 3. Effect of pH on the emulsion stabilities (%) of *P. angularis, P. calcaratus, D. lablab,* and soybean protein concentrates.

to affect their specific viscosities, which were conformation dependent (Sathe et al., 1983; Idouraine et al., 1991).

Emulsifying Properties. The profiles of emulsifying activities (EAs) against pH of *P. angularis, P. calcaratus, D. lablab,* and soybean PCs (Figure 2) were similar to each other, and it was found that they had a minimum EA (53.0−54.7%) at pH 4 with EAs increasing on either side of pH 4. The EAs of these four PCs at pH 2 ranged from 55.5% to 58.0%, and those at pH 10 ranged from 57.1% to 58.2%. The V-shaped pattern of the profiles (Figure 2) might account for the variations of the hydrophilic−lipophilic balance of the proteins along the pH gradient from 2 to 10. Similar observations on the pH dependence of EAs of legume proteins have been reported (Nath and Narasinga Rao, 1981; Sathe et al., 1982a). Moreover, the relationships between EA and pH for *P. angularis, P. calcaratus,* and *D. lablab* PCs (Figure 2) were similar to those between their nitrogen solubilities and pH (Figure 1). This was in agreement with the general correlation between EA and nitrogen solubility found in previous studies (Crenwelge et al., 1974; Hung and Zayas, 1991).

Similar to the results of EA (Figure 2), the emulsion stabilities (ESs) of *P. angularis, P. calcaratus,* and *D. lablab* PCs were pH dependent with a V-shaped pattern (Figure 3). The profiles of ES against pH of *P. angularis, P. calcaratus,* and *D. lablab* PCs compared favorably with that of soybean PC used in this study. At pH ranges of 2−10, the ES of *P. angularis* PC was slightly lower than those of the others, and all four PCs had their ESs higher than 93.2% (Figure 3). Hung and Zayas (1991) suggested that various factors including pH, droplet size, net charge, interfacial tension, viscosity, and protein conformation could affect the values of ES. In this case, the high ES, which involved heating of proteins at 80 °C for 30 min, might be attributed to the dissociation of some proteins, and the resulting subunits formed had more hydrophobic groups which

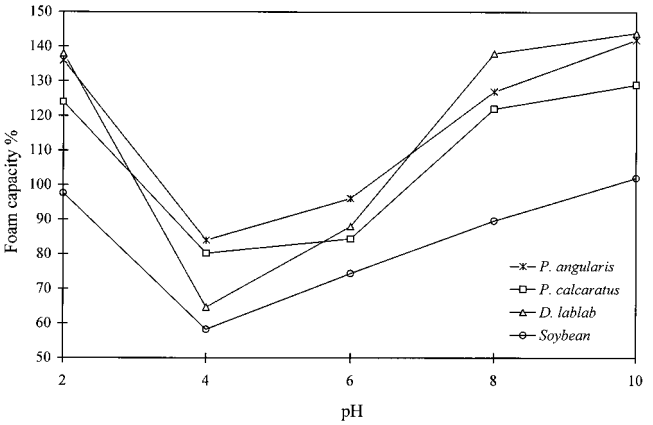

Figure 4. Effect of pH on the foam capacities (%) of *P. angularis, P. calcaratus, D. lablab,* and soybean protein concentrates.

interacted more strongly with the lipid phase (Mahajan and Dua, 1995).

Foaming Properties. The foam capacities (FCs) of *P. angularis, P. calcaratus, D. lablab* and soybean PCs (Figure 4) were pH dependent and found to be lowest at pH 4 (84.0%, 80.2%, 64.6%, and 58.2%, respectively). The lowest FC was attributed to the protein behavior at their isoelectric points. On either side of pH 4, FC increased significantly and showed a V-shaped pattern. The FCs of *P. angularis, P. calcaratus,* and *D. lablab* PCs at pH 2 (ranged from 124% to 138%) and pH 10 (ranged from 129% to 144%) were much higher than those of the reference soybean PC which were only 97.6% and 102%, respectively. The higher FC at the above two pHs was due to the increased net charges on the protein, which weakened the hydrophobic interactions but increased the flexibility of the protein. This allowed the protein to diffuse more rapidly to the air−water interface to encapsulate air particles and then enhance the foam formation (Aluko and Yada, 1995). Along the entire pH range from 2 to 10, *P. angularis,*

P. calcaratus, and *D. lablab* PCs showed a better foamability than the reference soybean PC (Figure 4). The profiles of FC against pH for all four PCs were similar to that of their nitrogen solubilities against pH. A direct relationship between FC and nitrogen solubility for legume proteins has been suggested (Nath and Narasinga Rao, 1981; Kabirullah and Wills, 1988).

The results given in Figure 4 were equivalent to the foam stability (FS) at the first 30 s after foam formation, and the FS values after standing for 5, 30, and 120 min are presented in Table 2. At each pH tested, *P. angularis*, *P. calcaratus*, and *D. lablab* PCs were found to have higher FS values than the reference soybean PC after standing for 5, 30, and 120 min. The FSs of all four PCs decreased significantly ($p < 0.05$) as time increased (Table 2). The higher FSs of *P. angularis*, *P. calcaratus*, and *D. lablab* PCs might be due to the relatively higher surface activity of the soluble proteins in these three legumes than in that of soy protein (Onweluzo et al., 1994). *P. angularis*, *P. calcaratus*, and *D. lablab* PCs developed high initial foam volumes at both pH 2 and 10, but their FSs were all decreased significantly ($p < 0.05$) after standing for 120 min. On the other hand, the FSs observed at pH 4 for all four PCs did not change appreciably beyond 30 min as compared to those in other pH conditions which might be due to the more stable protein conformation at their isoelectric points (Yatsumatsu et al., 1972).

CONCLUSION

It was found that *P. angularis*, *P. calcaratus*, and *D. lablab* PCs had higher WHCs, OHCs, and FCs than soybean PC, and their EAs and ESs were comparable to those of soybean PC as well. Among *P. angularis*, *P. calcaratus*, and *D. lablab* PCs, no great differences were observed in their general functional properties. However, the comparisons made in this study are valid only for PCs prepared via isoelectric point precipitation and only for the precise conditions used. A change in any element of the preparation procedure could change the relative functional properties of the legume seed types under investigation. Nevertheless, our results suggest that PCs prepared from these three legumes could be used as ingredients in the preparation of comminuted sausage products (requiring high WHCs and OHCs), confections (such as nougats and marshmallow) and whipped toppings (requiring high FCs), and meat analogs (requiring high EAs and ESs). These legume PCs could be useful as whole or partial replacement of high-price materials such as egg albumen and casein. Since we only aimed at providing basic information on protein functionality based on simple model systems in this study, further investigations are necessary to understand the inherent complexity of protein functionality of these PCs in food systems.

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Received for review January 21, 1997. Revised manuscript received April 22, 1997. Accepted April 23, 1997.[⊗]

JF970047C

[⊗] Abstract published in *Advance ACS Abstracts*, June 15, 1997.