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Functional properties of flours prepared from three Chinese indigenous legume seeds

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The functional properties of *Phaseolus angularis, Phaseolus calcaratus* and *Dolichos lablab* flours were investigated and compared with those of soybean flour. The minimum nitrogen solubilities of *P. angularis* and *P. calcaratus* flours were at pH 5 while that of *D. lablab* flour was at pH 4. Compared with soybean flour, *P. angularis, P calcaratus* and *D. lablab* flours exhibited lower foam capacities, water- and oil-holding capacities, but higher gelation capacities. The emulsifying activities and emulsion stabilities of all legume flours tested were pH-dependent with minimum values at pH 4. Their emulsion stabilities were greater than 80.2% from pH 2 to 10, except at pH 4. Foam capacities and stabilities were also pH-dependent, highest foam stabilities being at pH 4. © 1998 Published by Elsevier Science Ltd. All rights reserved

INTRODUCTION

There is a growing interest in the utilization of flours or fractions from different types of legumes (Gujska *et al.*, 1994). For efficient utilization and consumer acceptance of legume seed flours, studies on their desirable functional properties are important. Previous studies on the functional properties of flours have been focused mainly on popular legumes grown in the developed countries (Eke and Akobundu, 1993; Nwanekezi *et al.*, 1994). The search for the utilization of unconventional legumes as potential food ingredients is still continuing (Pandey and Sirvastava, 1990; Morrow, 1991; Onweluzo *et al.*, 1994).

Unlike soybean, cowpea, and other well-known legumes, the seeds of *P. angularis, P. calcaratus* and *D. lablab* (white bean), indigenous to China, are traditionally used as soup ingredients for therapeutic purposes such as ameliorating symptoms of dropsy, relieving diarrhoea and as a tonic for the viscera (Li, 1973). In order to develop applications of these three legumes in food formulations, this study was undertaken to assess their functional properties and to compare them with those of soybean flour.

MATERIALS AND METHODS

Preparation of legume flours

Mature seeds of *P. angularis, P. calcaratus* and *D. lablab*, and soybean (*Glycine max*), imported from mainland China, were chosen for functional property tests. Cleaned seeds of the four legume species were manually dehulled after soaking in distilled water at room temperature (24°C) (10 h for *P. angularis* and *P. calcaratus* seeds and 3 h for *D. lablab* seed and soybean). The cotyledons were freeze-dried and then ground in a cyclotec mill (Tecator, Hoganas, Sweden) to pass through a 0.5 mm screen. The flours were defatted with acetone according to the method reported by Aluko and Yada (1995).

Protein content

The protein content was calculated by multiplying the nitrogen content of the legume flour determined by a CHNS/O Analyzer (Perkin Elmer 2400, Connecticut, USA) with a factor of 6.25.

Bulk density and pH

The bulk density (g ml^{-1}) was determined according to the method of Onuma-Okezie and Bello (1988) using a 10 ml graduated cylinder. In addition, the pH was measured by using a 10% (w/v) dispersion of each flour.

Nitrogen solubility

Nitrogen solubility of the flours in water at 5% (w/v) was determined over a pH range from 2 to 10 according to the method of Beuchat *et al.* (1975). The suspensions were stirred at different pH at 24°C for 45 min and then centrifuged at 3000×g for 30 min. The supernatants

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were freeze-dried, weighed and analyzed for their nitrogen content with a CHNS/O Analyzer. Nitrogen solubilized was expressed as a percentage of the nitrogen content of the flour.

Gelation properties

The method of Carcea Bencini (1986) was used with slight modifications. Flour suspensions of 2 to 18% (w/v) were prepared in 100 ml distilled water by mixing in a 250 ml Waring Blendor at the 'Hi' speed for 2 min. The suspensions were poured into test tubes which were boiled in a water bath for 1 h, followed by rapid cooling under running cold tap water. The lowest concentration at which the gel formed did not collapse or slip from the inverted test tube was regarded as the Least Gelation Concentration (LGC).

Water- and oil-holding capacity

The method of Carcea Bencini (1986) was used with slight modifications. One gram of flour was mixed with 10 ml of distilled water or corn oil (Mazola, CPC International, USA) and centrifuged at 2200 × g for 30 min. The volume of the supernatant was measured. The water-holding capacity was expressed as the number of grams of water held by 1 g of flour. The oil-holding capacity was expressed as the number of ml of oil held by 1 g of flour.

Emulsifying activity and emulsion stability

According to the method by Sathe *et al.* (1983), 100 ml of 7% (w/v) flour suspension at a certain pH (ranged from 2 to 10) was homogenized at 11 000 rpm for 30 s using a Polytron homogenizer. One hundred ml of corn oil (Mazola, CPC International, USA) was then added, and homogenized for a further minute. The emulsions were centrifuged in 50 ml graduated centrifuge tubes at 1200 × g for 5 min and the volume of the remaining emulsion was measured. Emulsifying activity (EA) was calculated as follows: EA % = (volume of emulsified layer/volume of whole layer in centrifuge tube)×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: ES % = (volume of remaining emulsified layer/original emulsion volume)×100.

Foaming capacity and foam stability

The method of Nath and Narasinga Rao (1981) was used with slight modifications. One hundred ml of 2.5% (w/v) flour suspension was whipped at 'low' speed in a 250 ml Waring Blendor for 5 min, and foam volumes were recorded after 30 s. Foam capacity (FC) was expressed as percent increase in foam volume measured after 30 s, and foam stability (FS) was determined by measuring the FC 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 defatted *P. angularis*, *P. calcaratus* and *D. lablab*, and soybean flours were 30.4, 32.1, 30.7 and 52.8% (w/w, dry weight), respectively. In Fig. 1, the minimum nitrogen solubilities of *P. angularis* and *P. calcaratus* flours were 5.0 and 7.0%, respectively, at pH 5, while those of *D. lablab* and soybean flours were 10.0 and 12.0%, respectively, at pH 4. Similar isoelectric points were observed in some legume flours such as winged bean flour (Narayana and Narasinga Rao, 1982), chickpea flour (Carcea Bencini, 1986) and yam bean flour (Nwanekezi *et al.*, 1994). For all four legume flours used in this study, there was a sharp increase in their nitrogen solubilities on either side of pH 4 and 5. At pH 2 about 46.1 to 71.0% of nitrogen was soluble, and about 71.3 to 88.8% of nitrogen was soluble at pH 10. Generally, the nitrogen solubility profiles against pH of all four flours (Fig. 1) were similar to each other and in agreement with those of other seed flours reported previously (Narayana and Narasinga Rao, 1982; Shanmugasundaram and Venkataraman, 1989).

Bulk density and pH

Table 1 shows that *P. angularis*, *P. calcaratus* and *D. lablab* flours are significantly ($p < 0.05$) more dense than

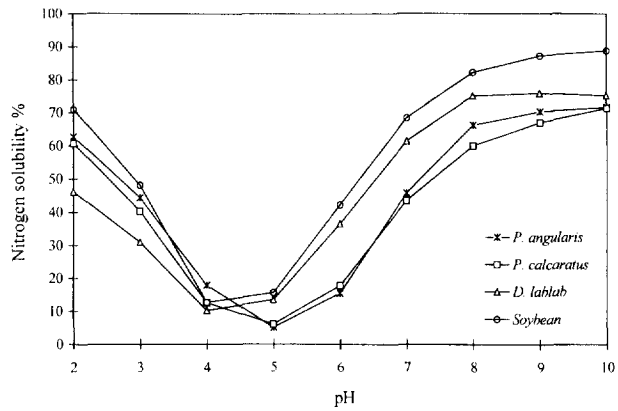

Fig. 1. Effect of pH on the nitrogen solubilities of *P. angularis*, *P. calcaratus*, *D. lablab* and soybean flours.

Table 1. Bulk densities, pH values, least gelation concentrations, water- and oil-holding capacities of *P. angularis*, *P. calcaratus*, *D. lablab* and soybean flours

Flours	Bulk density (g ml^{-1})	pH value	LGC (%)	WHC (g g^{-1})	OHC (ml g^{-1})
P. angularis	0.62^{x}	6.60	12.0	1.46^{x}	1.40^{x}
P. calcaratus	0.59^{y}	6.53	13.0	1.47^{x}	1.28^{xy}
D. lablab	0.64^{x}	6.59	10.0	1.04^{y}	1.19^{y}
Soybean	0.47^{z}	6.65	17.0	1.80^{z}	1.93^{z}

$^{x-z}$ Values in the same column with different superscripts are significantly different (Tukey, $p < 0.05$).

soybean flour with *P. angularis* and *D. lablab* flours having the highest bulk density. Basically, the bulk density of soybean flour in this study was comparable to that reported by Carcea Bencini (1986).

The pH values of the *P. angularis*, *P. calcaratus*, *D. lablab* and soybean flour dispersions (10%, w/v) were found to range from 6.53 to 6.65 (Table 1). The acidity of all these flours was in general agreement with the pH values of many legume flours including soybean flour (Sosulski *et al.*, 1976).

Gelation

Least Gelation Concentrations (LGCs) of *P. angularis*, *P. calcaratus* and *D. lablab* flours, as shown in Table 1, were comparable to those of *Phaseolus vulgaris* L. flour (10.0 to 14.0%) (Deshpande *et al.*, 1982) and black gram flour (12.0%) (Sathe *et al.*, 1983), but lower than that of the reference soybean flour. Sathe *et al.* (1982) have associated the variations in gelling properties to the relative ratio of different constituents such as proteins, lipids and carbohydrates in different legume flours. Moreover, Fleming *et al.* (1975) suggested a direct correlation between LGC and the level of globulin in legume seed. Since the globulin content of *D. lablab* flour (4.85%, dry weight of flour) was about half of that in *P. angularis* and *P. calcaratus* flours (8.37 and 9.92%, dry weight of flour, respectively) (Chau C.F., unpublished), this could be one of the reasons why *D. lablab* flour had the lowest LGC.

Water- and oil-holding capacity

The water- and oil-holding capacities (WHCs and OHCs) of *P. angularis*, *P. calcaratus*, *D. lablab* and soybean flours are presented in Table 1. There was no significant difference between the WHCs of *P. angularis* and *P. calcaratus* flours with the WHC of *D. lablab* flour being the lowest. Table 1 shows that the WHCs of these three flours were significantly ($p < 0.05$) lower than that of soybean flour. The higher WHC of soybean flour could be due to its higher protein level (52.8%), which was about one and a half times those of the other three flours (30.4 to 32.1%). The observed WHCs of *P. angularis* and *P. calcaratus* flours were comparable to that of pigeon pea flour (1.38 g g^{-1}) (Oshodi and Ekperigia, 1989). Moreover, the WHC of *D. lablab* flour was comparable to those of the flours from faba bean, lentil and lima bean which ranged from 1.04 to 1.08 g g^{-1} (Sosulski *et al.*, 1976).

The OHCs of *P. angularis*, *P. calcaratus* and *D. lablab* flours, as shown in Table 1, were significantly ($p < 0.05$) lower than that of the reference soybean flour. Besides, *P. angularis* flour was found to have a higher OHC than *P. calcaratus* and *D. lablab* flours. Using the same procedure as Carcea Bencini (1986), the OHCs of chickpea, soybean and yam bean were found to be 1.40, 1.93 and 1.42 ml g^{-1}, respectively (Carcea Bencini, 1986; Eke and Akobundu, 1993). Basically, the mechanism of OHC is mainly due to the physical entrapment of oil by capillary attraction (Kinsella, 1976). Moreover, the hydrophobicity of proteins also plays a major role in fat absorption (Voutsinas and Nakai, 1983). Among the four flours tested (Table 1), variations in their OHCs might be partially due to the different proportions of nonpolar side chains of the amino acids on the surfaces of their protein molecules.

Emulsifying properties

The profiles of emulsifying activity (EA) against pH of *P. angularis*, *P. calcaratus* and *D. lablab* flours compared favorably with that of the reference soybean flour (Fig. 2). However, the EA of the soybean flour was significantly ($p > 0.05$) higher than those of the others over a pH range of 2 to 10. The minimum EA, ranging from 45.8 to 54.2%, was found at pH 4 with EAs increasing on either side of pH 4. The EAs of these four flours at

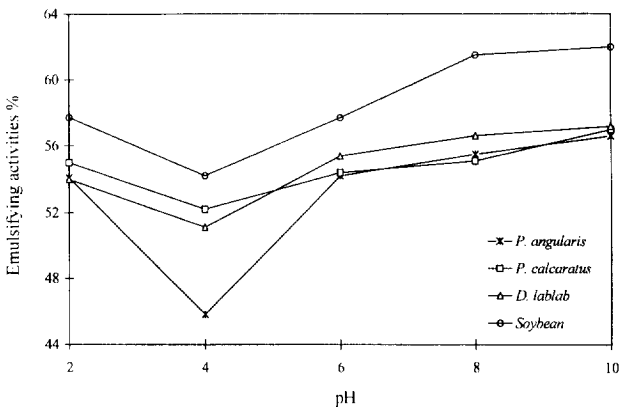

Fig. 2. Effect of pH on the emulsifying activities of *P. angularis*, *P. calcaratus*, *D. lablab* and soybean flours.

pH 2 ranged from 54.0 to 57.7%, and those at pH 10 ranged from 56.6 to 62.0%. Since the relationships between EA and pH (Fig. 2) were similar to those between nitrogen solubility and pH (Fig. 1), EA may depend on the amount of nitrogen solubilized. Therefore, the minimum EA observed at pH 4 was attributed to the proteins of the four flours reaching their isoelectric points (pI) which were also at around pH 4. The results are in agreement with the general correlation between EA and nitrogen solubility found in previous studies (Abbey and Ibeh, 1988; Shanmugasundaram and Venkataraman, 1989).

Similar to the results of EA (Fig. 2), the emulsion stabilities (ESs) of the emulsions formed by the *P. angularis*, *P. calcaratus*, *D. lablab* and soybean flours were pH-dependent with a V-shaped pattern (Fig. 3). The profiles of ES against pH of *P. angularis*, *P. calcaratus* and *D. lablab* flours were similar to each other. At pH 4 the ESs of these three flours (11.4 to 22.1%) were found to be at a minimum, which was much lower than that of the reference soybean flour (69.7%). On either side of pH 4, heating the emulsion at 80°C did not significantly affect the ES of the emulsions, since more than 80.2% of ES remained at these pH values. The relatively high ES observed at the two extreme pHs could possibly be attributed to the higher levels of solubilized proteins, which influenced ES through film encapsulation and a balance of the attractive van der Waals and repulsive electrostatic forces (Volkert and Klein, 1979).

Foaming properties

The foam capacities (FCs) of *P. angularis*, *P. calcaratus*, *D. lablab* and soybean flours (Fig. 4) were pH-dependent and showed a V-shaped pattern. At pH 4, the FCs of *P. angularis*, *P. calcaratus*, *D. lablab* and soybean flours were at a minimum (80.80, 64.4, 76.4 and 110%, respectively), which might be due to the protein behavior at their isoelectric points. The FCs of *P. angularis*, *P. calcaratus* and *D. lablab* flours at pH 2 (range from 106 to 111%) and at pH 10 (range from 114 to 122%) were lower than those of the reference soybean flour which were found to be 140 and 142%, respectively (Fig. 4). The higher FC at the two extreme pHs was attributed to the increased flexibility of the protein, which diffused more rapidly to the air–water interface to encapsulate air particles and then enhanced the foaming (Aluko and Yada, 1995). Furthermore, the higher FC of the soybean flour might be due to its higher protein level and nitrogen solubility. Several authors have suggested a direct relationship between FC and nitrogen solubility of leguminous flours (Nath and Narasinga Rao, 1981; Narayanan and Narasinga Rao, 1982).

Since the FCs given in Fig. 4 are equivalent to the foam stability (FS) at the first 30 s, the FS after standing for 5, 30 and 120 min are presented in Table 2. The FSs of all four flours decreased significantly ($p < 0.05$) as time increased. In each pH condition used, the reference soybean flour was found to have higher FS than the other three flours after standing for 5, 30 and 120 min (Table 2). It has been reported that soybean flour has higher FC and FS than other legume flours (Sosulski *et al.*, 1976). Compared with the reference soybean flour, the lower FSs of *P. angularis*, *P. calcaratus* and *D. lablab* flours might be explained by their lower nitrogen solubilities and protein contents. In Table 2, the FSs observed at pH 4 for all four flours did not change appreciably beyond 30 min as compared to those in the other pH conditions. Such stabilization of foam might be due to the more stable protein conformations at their isoelectric points (Yatsumatsu *et al.*, 1972).

In conclusion, *P. angularis*, *P. calcaratus* and *D. lablab* flours had higher gelation capacities than soybean flour, and their EAs were comparable to that of soybean flour. The lower WHCs, OHCs and FCs of *P. angularis*, *P. calcaratus* and *D. lablab* flours were probably due to their lower levels of soluble proteins compared with soybean flour. The desirable functional characteristics, including gelation capacities, EAs and ESs, of *P. angularis*, *P. calcaratus* and *D. lablab* flours revealed in this study might have useful applications in functional food ingredients.

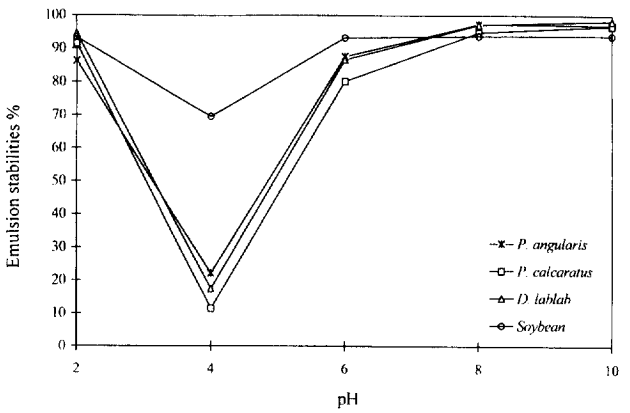

Fig. 3. Effect of pH on the emulsion stabilities of *P. angularis*, *P. calcaratus*, *D. lablab* and soybean flours.

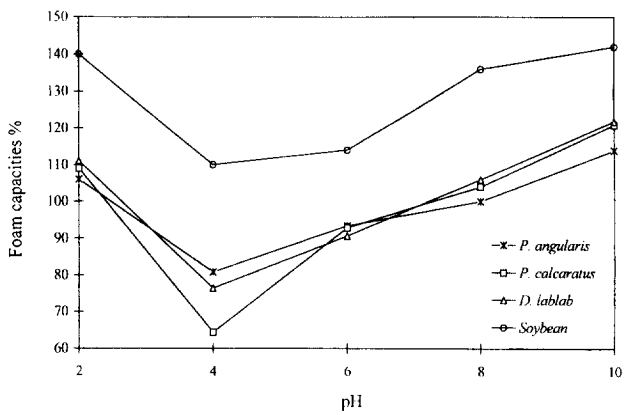

Fig. 4. Effect of pH on the foam capacities of *P. angularis*, *P. calcaratus*, *D. lablab* and soybean flours.

Table 2. Effect of pH on the foam stabilities of *P. angularis*, *P. calcaratus*, *D. lablab* and soybean flours

	pH 2			pH 4			pH 6			pH 8			pH 10		
Flours	5[a]	30	120	5	30	120	5	30	120	5	30	120	5	30	120
P. angularis	105[b]	92.0[c]	84.0[d]	80.0[e]	76.0[f]	73.0[f]	91.0[h]	86.0[i]	80.0[j]	96.0[k]	84.0[l]	78.0[m]	112[n]	102[o]	89.0[o]
P. calcaratus	107[b]	102[c]	94.0[d]	83.0[e]	80.0[ef]	79.0[f]	90.0[h]	82.0[i]	78.0[j]	102[k]	100[k]	90.0[l]	116[n]	108[o]	96.0[p]
D. lablab	108[b]	104[c]	96.0[d]	75.0[e]	70.0[f]	68.0[fg]	89.0[h]	82.0[i]	76.0[j]	102[k]	96.0[l]	90.0[m]	120[n]	114[o]	104[p]
Soybean	138[b]	136[b]	128[c]	109[e]	106[ef]	103[f]	108[h]	106[h]	101[i]	134[k]	131[k]	126[l]	140[n]	138[n]	130[o]

[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).

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