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Chemical composition of three underutilized legume seeds grown in China

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Proteins of *Phaseolus angularis, Phaseolus calcaratus* and *Dolichos lablab* seeds (24.9 to 26.5% dry weight) were mainly contributed by their cotyledons which had a proportion of 82.9 to 90.8% by weight of the whole seeds. The levels of all the essential amino acids of the legume seeds (415 to 443 mg g^{-1} protein) were above the FAO/WHO requirement with the essential amino acid scores of the methionine and cystine being 1.02 to 1.19. Appreciable amounts of oligosaccharides of the raffinose family were found mainly in the cotyledons (3.52 to 4.72% dry weight). The total dietary fibre of the legume seeds (13.5 to 19.3% dry weight) consisted predominantly of insoluble dietary fibre (IDF). The cotyledons contained 45.9 to 51.4% dry weight of starch but had low contents of cellulose and lignin. Hulls were highly lignified and consisted predominantly of IDF, with values ranging from 70.7 to 74.0% dry weight. The principal sugar residue analysis revealed that pectins were the major polysaccharide present in the SDF of both the cotyledons and the hulls. While xylans and cellulose were the major polysaccharides present in the IDF of the hulls, xyloglucans and arabinose-rich pectic substances were the principal non-starch polysaccharides in the IDF of the cotyledons.

INTRODUCTION

In developing and underdeveloped countries, there is an urgent need of nutritious foods to meet the nutritional requirement of the ever-increasing populations (Prakash and Misra, 1988). Since legume seeds are important sources of protein, complex carbohydrate and dietary fibre in the diet (Morrow, 1991), there has been a worldwide interest in searching for potential utilization of unconventional legumes (Pandey and Srivastava, 1990). Unlike soybean, peanut, and other well-known legumes, the seeds of *P. angularis, P. calcaratus* and *D. lablab* (white bean) indigenous to China are relatively underutilized. These three native legume seeds are traditionally used as a soup ingredient for therapeutic purposes such as curing dropsy, relieving diarrhea and as a tonic to the viscera (Li, 1973). Proximate composition of several varieties of *P. angularis* and *P. calcaratus* (Baldi and Salamini, 1973; Kanamori *et al.*, 1982; Lee and Karunanithy, 1990) and some cultivars of *D. lablab* (Deka and Sarkar, 1990) are available. However, other nutrient information such as amino acid profiles, dietary fibre content and carbohydrate composition are still lacking. The aim of the present study was to determine the chemical composition of these three indigenous legume seeds in order to provide more comprehensive nutrient information.

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MATERIALS AND METHODS

Materials

P. angularis, P. calcaratus and *D. lablab* seed samples were grown in the southern part of mainland China. Fully grown seeds were chosen for composition analysis. Cleaned seeds of the three legume species were individually divided into two lots. One lot was ground in a cyclotec mill (Tecator, Hoganas, Sweden) to pass through a 0.5 mm screen. The second lot was manually dehulled after soaking in distilled water at room temperature (20°C). Samples of *P. angularis* and *P. calcaratus* seeds were soaked for 10 h while *D. lablab* seed samples were soaked for 3 only. The hulls and cotyledons were freeze-dried and ground to 0.5mm in size.

Proximate analysis

The moisture content was determined with a moisture analyzer (Mettler LJ 16, Greifensee, Switzerland) at

120°C and was expressed as percentage by weight. The crude protein content was calculated by multiplying the nitrogen content which was determined by a CHNS/O Analyzer (Perkin Elmer 2400, Connecticut, USA) with a factor of 6.25. The ash contents were estimated according to the Official Methods of Analysis (4.1.10) (AOAC, 1995). Crude lipid was determined by using hexane with a Soxhlet apparatus (Tecator, Hoganas, Sweden).

Amino acid analysis

Two milligrams of samples were hydrolyzed with 0.6 ml of 6N hydrochloric acid in airtight vials at 110°C for 24 h. Norleucine was used as an internal standard. The acid hydrolysate was evaporated to dryness using a Speedvac (Savant, New York, USA). The dried residue was dissolved in a citrate buffer (Beckman A303084, California, USA), filtered and analyzed with an Amino Acid Analyzer (Beckman 6300, California, USA). Sulfur-containing amino acids were determined after a prehydrolysis oxidation with performic acid (Gehrke *et al.*, 1985). The different amino acids recovered were presented as mg g^{-1} protein.

Carbohydrate analysis

Oligosaccharides and soluble sugars were extracted with 80% ethanol (Vidal-Valverde *et al.*, 1993) and analyzed by HPLC using a differential refractometer (Waters 410, USA) and a reversed-phase C-18 5 μm column (BIO-RAD HL 90-5, USA) in a compression unit (Waters 510, USA) at room temperature, with a flow rate of 0.8 ml min^{-1} and water as eluent. The column was calibrated using sucrose, raffinose and stachyose.

The dietary fibre fractions were determined with the Official Methods of Analysis (32.1.17) (AOAC, 1995). In 40 ml of Mes-Tris buffer, 1 g of finely ground samples were sequentially digested by heat-stable α-amylase (50 μl), protease (100 μl), and amyloglucosidase (300 μl) to remove starch and protein. Insoluble dietary fibre (IDF) was filtered from the enzyme digestate. The residue was washed twice each with 15 ml portions of 78% ethanol, 95% ethanol, and acetone. The soluble dietary fibre (SDF) in the filtrate was precipitated with 4 volumes of 95% ethanol and then filtered. After oven-drying (95°C), the dietary fibre residues were corrected for protein, ash and blank. Procedures for the determination of starch and Klason lignin were those described by Theander and Westerlund (1986).

Using the method of Englyst and Cummings, (1988), polysaccharides in IDF and SDF were hydrolyzed with 12 M H_2SO_4 at 35°C for 60 min. The acid was then diluted to 2 M in concentration and further hydrolyzed in a boiling waterbath for 60 min. Allose was used as an internal standard. Neutral sugars in the acid hydrolysate were quantified as alditol acetates using a gas chromatograph (Hewlett-Packard 6890, USA) fitted with a flame ionization detector. Helium was used as the carrier gas. The GC conditions were as follows: capillary column used was a SGE BP225 (12 m × 0.22 mm I. D.); oven temperature from 180°C (initial) to 220°C (final) at a rate of 2°C min^{-1}; final temperature hold time was 10 min; injector and detector temperature were both at 270°C; gas flow rates at 2.5 ml min^{-1} (carrier), 22 ml min^{-1} (make up), 25 ml min^{-1} (hydrogen), 250 ml min^{-1} (air); and split ratio at 20:1. For non-cellulosic glucose determination, IDF and SDF samples were only hydrolyzed with 2 M H_2SO_4 in a boiling waterbath for 60 min. Cellulose content was calculated from the difference between glucose yields from the 12 M (followed by 2 M) H_2SO_4 and 2 M H_2SO_4 hydrolysis.

Uronic acids in the acid hydrolysate were measured colorimetrically by the Official Methods of Analysis (45.4.11) (AOAC, 1995), in which the blank was prepared with 2 M H_2SO_4 and galacturonic acid was used as the standard.

All measurements were made in duplicate except that the moisture, llpid and ash contents were determined in triplicate.

RESULTS AND DISCUSSION

Table 1 shows the proximate composition of the *P. angularis, P. calcaratus* and *D. lablab* seed samples. The whole seeds of these samples possessed similar protein contents ranging from 24.9 to 25.2%. Since the cotyledons were the major seed fraction (ranging from 82.9 to 90.8% dry weight of the seeds), the cotyledons of *P. angularis, P. calcaratus* and *D. lablab* seed samples accounted for 96, 95 and 92.5% of total seed protein, respectively. The total dietary fibre (sum of IDF and SDF) content of the whole seed of *P. angularis, P. calcaratus* and *D. lablab* were 13.5, 19.3 and 18.3% dry weight, respectively. Table 1 shows that the IDF was the dominant fibre fraction in either the hulls, cotyledons or the whole seeds. The IDF constituted 70.7 to 74.0% dry weight of the hulls. High IDF content has been reported by Gooneratne *et al.* (1994) on mung bean and black gram, and by Bartolome *et al.* (1995) on lentils and cocoa beans. In spite of being a minor seed fraction, the hulls from *P. angularis, P. calcaratus* and *D. lablab* samples (Table 1) did contribute 49.5, 55.6 and 67.3% of the total dietary fibre in the whole seed, respectively. Table 1 shows that the moisture and ash contents of *P. angularis, P. calcaratus* and *D. lablab* seed samples were similar to each other. The lipid contents of these three seed samples were too low to be a potential source of edible oil.

The amino acid profiles of the seed proteins of *P. angularis, P. calcaratus* and *D. lablab* samples (Table 2) revealed that the levels of all their essential amino acids (EAA) were comparable to those of the FAO/WHO requirement (1990). Although sulphur-containing amino acids (methionine and cystine) of some *Phaseolus* species or *Dolichos* beans had been reported to be the

Table 1. Proximate composition (g 100 g^{-1} dry weight) of *P. angularis*, *P. calcaratus* and *D. lablab* seeds

Sample	Proportion of seed (%)	Moisture (%)	Crude Lipid[a]	Crude Protein[b]	IDF[bc]	SDF[bc]	Ash[a]	Carbohydrate[d]
P. angularis								
Whole seed	—	12.3 ± 0.06	0.72 ± 0.04	25.2 ± 0.10	12.8 ± 0.10	0.74 ± 0.08	3.20 ± 0.06	57.1
Hull	9.33	5.10 ± 0.57	0.83 ± 0.01	12.2 ± 0.13	70.7 ± 0.03	3.88 ± 0.19	2.48 ± 0.00	11.3
Cotyledon	90.8	4.08 ± 0.16	0.68 ± 0.01	30.2 ± 0.09	7.00 ± 0.26	0.84 ± 0.06	3.65 ± 0.00	57.8
P. calcaraus								
Whole seed	—	11.7 ± 0.09	0.67 ± 0.04	26.5 ± 0.15	18.4 ± 0.33	0.85 ± 0.10	3.94 ± 0.03	49.6
Hull	13.4	7.40 ± 0.53	1.29 ± 0.01	10.8 ± 0.05	74.0 ± 0.35	4.75 ± 0.06	2.63 ± 0.00	5.48
Cotyledon	86.6	4.17 ± 0.15	0.51 ± 0.01	31.9 ± 0.14	9.02 ± 0.13	0.91 ± 0.12	4.34 ± 0.00	53.5
D. lablab								
Whole seed	—	11.4 ± 0.17	2.59 ± 0.21	24.9 ± 0.15	16.4 ± 0.08	1.92 ± 0.18	3.76 ± 0.03	49.2
Hull	17.1	4.55 ± 0.07	0.74 ± 0.01	11.7 ± 0.09	71.4 ± 0.00	6.04 ± 0.15	3.97 ± 0.01	6.59
Cotyledon	82.9	4.32 ± 0.08	2.26 ± 0.01	30.0 ± 0.12	8.45 ± 0.47	1.39 ± 0.13	4.31 ± 0.01	53.6

[a]Means ± S.D. of triplicates.
[b]Means ± S.D. of duplicates.
[c]IDF, insoluble dietary fibre; SDF, soluble dietary fibre.
[d]Calculated by difference (= 100 - crude lipid - crude protein - IDF - SDF - ash).

limiting amino acids (Baldi and Salarnini, 1973; Kanamori *et al.*, 1982; Laurena *et al.*, 1991), the seed proteins from all three legume seeds contained levels of methionine and cystine (EAA scores ranged from 1.02 to 1.19) that were above the FAO/WHO requirement. Oshodi *et al.* (1995) reported that environmental factors under which food legumes were grown could influence their amino acid composition. With respect to the total EAA in the FAO/WHO requirement, all three legume seeds seemed to be able to contribute adequate levels of total EAA.

Table 3 shows that the starch content in the cotyledons of *P. angularis*, *P. calcaratus* and *D. lablab* seeds was ranging from 45.9 to 51.4 % dry weight while that of their hulls was 1.31 to 4.30% dry weight only. The oligosaccharide fraction found in the legume seeds was the raffinose family typical of legume seeds (Reddy *et al.*, 1984; Trugo and Almeida, 1988; Vidal-Valverde *et al.*, 1993). The amount of oligosaccharides found in the cotyledons (3.52 to 4.72% dry weight) was larger than that of the hulls (0.56 to 1.59% dry weight). The Klason lignin contents of the hulls of the three seed samples

Table 2. Amino acid profiles (mg g^{-1} protein)[a] of the whole seeds of *P. angularis*, *P. calcaratus* and *D. lablab*

Amino acids	*P. angularis*	*P. calcaratus*	*D. lablab*	FAO/WHO (1989) requirement pattern
Aspartic acid	113	124	115	
Threonine	33.5 (0.99)[b]	35.0 (1.03)	38.3 (1.13)	34
Serine	44.0	51.5	53.9	
Glutamic acid	169	172	159	
Proline	41.6	43.9	42.2	
Glycine	37.6	40.9	40.6	
Alanine	40.8	43.5	43.4	
Valine	52.3 (1.49)	54.9 (1.57)	53.5 (1.53)	35
Methionine	15.8 (1.17)[c]	15.3 (1.19)[c]	12.5 (1.02)[c]	25[c]
Cystine	13.5	14.5	13.0	
Isoleucine	42.4 (1.51)	44.7 (1.60)	41.4 (1.48)	28
Leucine	75.3 (1.14)	78.3 (1.19)	77.2 (1.17)	66
Tyrosine	31.6 (1.34)[d]	35.2 (1.47)[d]	36.6 (1.40)[d]	63[d]
Phenylalanine	53.1	57.5	51.5	
Histidine	30.2 (1.59)	29.9 (1.57)	28.2 (1.48)	19
Lysine	72.9 (1.26)	77.2 (1.33)	63.1 (1.09)	58
Arginine	62.2	65.1	55.9	
Tryptophan	ND[e]	ND	ND	11
Total EAA[f]	421	443	415	328

[a]All values are mean of duplicate determinations.
[b]Data in parentheses show the essential amino acid (EAA) score.
[c]Value includes Met and Cys.
[d]Value includes Tyr and Phe.
[e]Not determined.
[f]Total EAA (mg g^{-1} protein) excludes Trp.

Table 3. Starch and oligosaccharide content[a] (% of dry weight) of cotyledons and hulls from *P. angularis*, *P. calcaratus* and *D. lablab* seeds

	Cotyledons			Hulls		
	P. angularis	*P. calcaratus*	*D. lablab*	*P. angularis*	*P. calcaratus*	*D. lablab*
Starch	51.4 ± 0.84	45.9 ± 1.06	49.1 ± 1.08	1.31 ± 0.09	2.50 ± 0.07	4.30 ± 0.14
Sucrose	1.57 ± 0.10	1.44 ± 0.04	1.49 ± 0.06	0.78 ± 0.05	0.83 ± 0.03	0.35 ± 0.02
Raffinose	1.19 ± 0.10	1.03 ± 0.01	0.84 ± 0.03	0.60 ± 0.05	0.60 ± 0.02	0.28 ± 0.01
Stachyose	3.53 ± 0.07	3.34 ± 0.06	2.68 ± 0.13	0.74 ± 0.02	0.99 ± 0.02	0.28 ± 0.02

[a]Means ± SD of duplicate determinations.

Table 4. Monosaccharide composition[a] of the insoluble and soluble dietary fibre from cotyledons of *P. angularis*, *P. calcaratus* and *D. lablab* seeds

	Fucose	Rhamnose	Ribose	Arabinose	Xylose	Mannose	Galactose	Glucose		Uronic acids	Glucosamine
								NC[b]	C[c]		
Insoluble fibre											
P. angularis	0.09	0.04	0.03	2.66	0.34	0.06	0.36	0.56	1.23	0.13	0.39
P. calcaratus	0.11	0.03	0.03	2.95	0.36	0.09	0.31	0.94	1.53	0.19	0.54
D. lablab	0.11	0.03	0.02	2.05	0.35	0.04	0.69	2.38	0.95	0.18	0.52
Soluble fibre											
P. angularis	0.01	tr[dz]	0.01	0.18	0.03	0.10	0.07	0.09	—	0.07	0.11
P. calcaratus	0.01	tr	0.01	0.15	0.03	0.10	0.07	0.08	—	0.04	0.10
D. lablab	0.01	tr	0.01	0.24	0.05	0.11	0.15	0.14	—	0.04	0.15

[a]Expressed as g per 100 g dry weight of the cotyledons.
[b]Noncellulosic glucose.
[c]Cellulosic glucose.
[d]tr, trace amount (< 0.01).

(8.37 to 13.4% dry weight) (data not shown) were much higher than that of the cotyledons (0.28 to 0.68% dry weight) (data not shown).

The monosaccharide composition of the IDF and SDF of the cotyledons from *P. angularis*, *P. calcaratus* and *D. lablab* seed samples is shown in Table 4. For all legume cotyledons, the major sugar constituents of the IDF were arabinose and glucose, followed by xylose and galactose. Such monosaccharide composition suggested that hemicelluloses, like xyloglucans and pectic substances rich in arabinose, were the major polysaccharides in the IDF of the cotyledons. The noncellulosic glucose released from the IDF of *P. angularis*, *P. calcaratus* and *D. lablab* cotyledons by 2 M H_2SO_4 hydrolysis constituted 0.56, 0.94 and 2.38% dry weight, respectively. This noncellulosic glucose was probably derived from hemicelluloses such as xyloglucan. The content of arabinose, galactose, and uronic acids constituted about half of the total amount of monosaccharide in the SDF, which implied that pectic polysaccharides were the major noncellulosic polysaccharides in the SDF of the cotyledons (Table 4). Both the IDF and SDF of the cotyledons possessed different levels of mannose and glucosamine, the presence of which could

Table 5. Monosaccharide composition[a] of the insoluble and soluble dietary fibre from hulls of *P. angularis*, *P. calcaratus* and *D. lablab* seeds

	Fucose	Rhamnose	Ribose	Arabinose	Xylose	Mannose	Galactose	Glucose		Uronic acids	Glucosamine
								NC[b]	C[c]		
Insoluble fibre											
P. angularis	0.51	0.06	—	3.70	13.8	0.15	1.00	1.09	37.6	0.98	2.16
P. calcaratus	0.66	0.06	—	3.73	12.6	0.27	0.85	1.75	41.8	1.63	3.94
D. lablab	0.67	0.06	—	2.57	11.2	0.33	1.21	2.37	45.0	1.31	3.72
Soluble fibre											
P. angularis	0.09	0.01	tr[d]	0.53	0.14	0.17	0.36	0.09	—	0.11	0.71
P. calcaratus	0.08	0.01	tr	0.41	0.24	0.21	0.25	0.18	—	0.08	0.70
D. lablab	0.12	0.01	tr	0.46	0.81	0.18	0.44	0.16	—	0.12	1.23

[a]Expressed as g per 100 g dry weight of the hulls.
[b]Noncellulosic glucose.
[c]Cellulosic glucose.
[d]tr, trace amount (< 0.1).

originate from intracellular glycoproteins, including lectins (Lis and Sharon, 1978; Nagahashi *et al.*, 1980). Moreover, in the presence of high levels of arabinose, the glycoproteins could be partially composed of arabinogalactan, containing glycoproteins (Aspinall, 1980).

In Table 5, the monosaccharide profile in the hulls suggested that the SDF of the hulls was mainly constituted of pectic substances that were rich in arabinose and galactose. Table 5 shows that the noncellulosic glucose in the IDF of the hulls was low (1.09 to 2.37% dry weight) and cellulose (37.6 to 45.0% dry weight) was the major component of these IDF. Moreover, the xylose content of the IDF of the hulls ranged from 11.2 to 13.8% dry weight which implied the presence of xylose - containing polysaccharides. As indicated earlier, the level of the noncellulosic glucose of the IDF of the hulls was low, and from the knowledge that xylans had also been isolated from soybean hulls (Aspinall *et al.*, 1966) and pea hulls (Ralet *et al.*, 1993), it was inferred that the bulk of the xylose was mainly from xylans rather than xyloglucan.

In conclusion, the above analytical data revealed that *P. angularis, P. calcaratus* and *D. lablab* seeds seemed to be a potential source of proteins, essential amino acids, dietary fibre and starch. The results suggest that the consumption of the whole seed is recommended because, while the cotyledon is the major source of protein and starch, the hull is a good source of dietary fibre (mainly the IDF). Further investigations on the nutritional value of these legume seeds using animal feeding experiments are under way.

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