Changes in the Dietary Fiber (Resistant Starch and Nonstarch Polysaccharides) Content of Cooked Flours Prepared from Three Chinese Indigenous Legume Seeds

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The effects of cooking on the dietary fiber (DF) content, which included resistant starch (RS) and nonstarch polysaccharides (NSP), of flours from *Phaseolus angularis*, *Phaseolus calcaratus*, and *Dolichos lablab* seeds, indigenous to China, were evaluated. The cooked legume flours were prepared by milling boiled and freeze-dried legume whole seeds. Total DF contents of all cooked flours were higher than those of the raw ones. The results showed an increase of NSP and RS to various extents with increasing cooking time of the flours. In the NSP of the legume flours, both the soluble and insoluble fractions increased during cooking, and a redistribution of the soluble and insoluble NSP components was observed. Generally, cooking increased the solubilization of the NSP in the legume seed flours, which might be important for their use as soup ingredients for therapeutic purposes. The increase of RS in the legume flours could be mainly due to the presence of cell-enclosed starch and retrograded starch formed during cooking. Such increase in the RS content of the cooked legume flours might have beneficial physiological effects for humans.

Keywords: *Phaseolus angularis; Phaseolus calcaratus; Dolichos lablab; cooking; dietary fiber; nonstarch polysaccharides; resistant starch*

INTRODUCTION

Most experts now define dietary fiber (DF) as endogenous plant cell wall (PCW) material that is not digested by the secretions of the human gastrointestinal tract (Trowell, 1974; Eastwood, 1992). Nonstarch polysaccharides (NSP) are the principal component of PCW, and PCW are the major source of fiber in the diet (Selvendran and Robertson, 1990). Hence, the estimation of NSP provides a good estimate of fiber from plant foods (Englyst et al., 1994). Because of its physiological implications such as reducing postprandial glucose and insulin responses as well as lipid profiles, resistant starch (RS) has also been considered as a component of DF (Annison and Topping, 1994).

The seeds of *Phaseolus angularis* (adzuki bean), *Phaseolus calcaratus* (rice bean), and *Dolichos lablab* (hyacinth bean), indigenous to China, are traditionally used as a soup ingredients for therapeutic purposes, such as driving away dropsy, relieving diarrhea, and soothing the viscera (Li, 1973). Moreover, these three legume seeds may also have potential dietary significance because of their high protein (24.9−25.2% dry weight) and starch (45.9−51.4% dry weight) contents (Chau et al., 1997). Domestically, the usual way for cooking these beans is to boil the beans until they are softened when felt between the fingers but not pasty in texture. Thus, the usual cooking times for these two *Phaseolus* beans and *Dolichos* bean are around 1 and 2 h, respectively. Cooking of plant tissues alters the physical and chemical properties of PCW which, in turn, affect their performance as DF (McDougall et al., 1996). Thermal processing has been shown to affect the DF content in a variety of other legume seeds (Vidal-Valverde and Frias, 1991; Gooneratne et al., 1994; Nyman et al., 1994; Prasad et al., 1995; Periago et al., 1996). In general, the changes in the DF composition during cooking may be partly attributed to the redistribution of the insoluble and soluble components of NSP and partly due to the formation of RS (Thed and Phillips, 1995; Periago et al., 1996). The latter factor is very significant in legumes since legume starches have relatively high amylose content, which may produce appreciable amounts of amylose RS (Tovar et al., 1990).

The beneficial effects associated with the consumption of legumes are related to the slow rate of digestion of starch and the high content of NSP and RS in legumes (Jenkins et al., 1981; Hughes, 1991; Eastwood and Morris, 1992; Truswell, 1992; Schneeman, 1994). The purpose of this work was to study the effects of cooking on RS formation and fiber solubilization in these three legume seeds important as dietary therapeutic agents.

MATERIALS AND METHODS

Sample Preparation. *P. angularis*, *P. calcaratus*, and *D. lablab* seeds were grown in the southern part of mainland China. The cotyledons of the three legume seeds contribute 82.9−90.8% dry weight of the whole seed (Chau et al., 1997). Two hundred grams of each of the three cleaned whole legume seeds was boiled in 1 L of tap water in a 2-L beaker covered with aluminum foil for different periods of time. *P. angularis* and *P. calcaratus* seeds were cooked for 30 and 60 min. *D. lablab* seeds were cooked for 60, 90, and 120 min. The softened seeds, along with the cooking water (bean−liquor mixture), were freeze-dried and finely ground to pass a 0.5 mm screen in a Cyclotec mill (Tecator, Hoganas, Sweden). The resulting

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Table 1. Changes in the Resistant Starch and Nonstarch Polysaccharide Content (Grams per 100 g of Dry Matter) of Raw and Cooked *P. angularis* Seed Flour

	DF[a]	RS	NSP[b]	Fuc	Rha	Ara	Xyl	Man	Gal	Glc	UAc
raw											
insoluble	8.58	0.53	8.05	0.10	0.03	1.75	1.34	0.06	0.32	4.05[c]	0.40
soluble	0.62	ND[d]	0.62	0.01	tr[e]	0.19	0.03	0.12	0.10	0.08	0.09
total[f]	9.20	0.53	8.67	0.11	0.03	1.94	1.37	0.18	0.42	4.13	0.49
cooked (30 min)											
insoluble	10.4	1.36	9.03	0.11	0.04	2.13	1.38	0.08	0.38	4.48[c]	0.43
soluble	0.64	ND	0.64	0.01	tr	0.07	0.04	0.10	0.10	0.09	0.23
total[f]	11.0	1.36	9.67	0.12	0.04	2.20	1.42	0.18	0.48	4.57	0.66
cooked (60 min)											
insoluble	12.3	2.90	9.43	0.11	0.04	2.06	1.22	0.07	0.37	5.07[c]	0.49
soluble	1.31	ND	1.31	0.02	tr	0.33	0.08	0.11	0.21	0.24	0.32
total[f]	13.6	2.90	10.7	0.13	0.04	2.39	1.30	0.18	0.58	5.31	0.81

[a] Dietary fiber (DF) is the sum of resistant starch (RS) and nonstarch polysaccharides (NSP). [b] NSP includes Fuc, Rha, Ara, Xyl, Man, Gal, Glc, and UAc. [c] Glucose values corrected for resistant starch. [d] ND, not determined [e] tr, trace amount (<0.01). [f] Values are the sum of insoluble and soluble fractions.

Table 2. Changes in the Resistant Starch and Nonstarch Polysaccharide Content (Grams per 100 g of Dry Matter) of Raw and Cooked *P. calcaratus* Seed Flour

	DF[a]	RS	NSP[b]	Fuc	Rha	Ara	Xyl	Man	Gal	Glc	UAc
raw											
insoluble	12.3	1.89	10.4	0.13	0.02	1.99	1.84	0.10	0.29	5.47[c]	0.52
soluble	0.60	ND[d]	0.60	0.01	tr[e]	0.17	0.05	0.11	0.08	0.08	0.10
total[f]	12.9	1.89	11.0	0.14	0.02	2.16	1.89	0.21	0.37	5.55	0.62
cooked (30 min)											
insoluble	12.3	1.81	10.5	0.13	0.03	1.97	1.56	0.09	0.27	5.66[c]	0.75
soluble	0.82	ND	0.82	0.02	tr	0.11	0.05	0.08	0.11	0.10	0.35
total[f]	13.1	1.81	11.3	0.15	0.03	2.08	1.61	0.17	0.38	5.76	1.10
cooked (60 min)											
insoluble	14.2	4.03	10.2	0.12	0.03	1.90	1.50	0.09	0.25	5.49[c]	0.77
soluble	1.10	ND	1.10	0.02	tr	0.24	0.08	0.09	0.16	0.13	0.38
total[f]	15.3	4.03	11.3	0.14	0.03	2.14	1.58	0.18	0.41	5.62	1.15

[a] Dietary fiber (DF) is the sum of resistant starch (RS) and nonstarch polysaccharides (NSP). [b] NSP includes Fuc, Rha, Ara, Xyl, Man, Gal, Glc, and UAc. [c] Glucose values corrected for resistant starch. [d] ND, not determined [e] tr, trace amount (<0.01). [f] Values are the sum of insoluble and soluble fractions.

cooked flour samples were kept in a desiccator until used. Raw flour samples, which were used as controls, were dried and milled according to the same procedures as for the cooked samples.

DF Preparation. The DF fractions were determined according to the *Official Methods of Analysis* (991.43) (AOAC, 1995). In brief, 1 g of raw and cooked legume flour suspended in 40 mL of Mes-Tris buffer was sequentially digested by heat stable α-amylase (50 μL), protease (100 μL), and amyloglucosidase (300 μL) to remove starch and protein. Insoluble DF fraction was recovered from the enzyme digestate after filtration. The soluble DF in the filtrate was precipitated with 4 volumes of 95% ethanol and then filtered. Both DF fractions collected were washed two times each with 15-mL portions of 78% ethanol, 95% ethanol, and acetone. All fiber fractions were oven-dried (45 °C) and were corrected for residual protein, ash, and blank.

Measurement of NSP. The DF fractions of the legume flour samples obtained by using the AOAC method were further characterized for their sugar profiles according to the Englyst procedure (Englyst et al., 1994). For the NSP neutral sugars, the legume DF fractions were dispersed with 12 M H_2SO_4 at 35 °C for 60 min. The acidic mixture was then diluted with H_2O to 2 M H_2SO_4 and further hydrolyzed in a water bath at 100 °C for 60 min. Allose was added as an internal standard after the acid hydrolysis. The released monosaccharides were quantified as alditol acetates using a gas chromatograph (GC) (Hewlett-Packard 6890, Avondale, PA) fitted with a flame ionization detector. Helium was used as carrier gas. The conditions were as follows: capillary column used was a SGE BP225 (12 m × 0.22 mm i.d.); oven temperature was ranged from 180 (initial) to 220 °C (final) at a rate of 2 °C/min; final temperature hold time was 10 min; injector and detector temperatures were both 270 °C; gas flow rates were 2.5 mL/min (carrier), 22 mL/min (make up), 25 mL/min (hydrogen), and 250 mL/min (air); and split ratio was 20:1. The values of glucose determined were corrected for the RS values (see below).

Uronic acid residues in the acid hydrolysate were determined according to the colorimetric method developed by Scott (1979), with dimethylphenol solution as the reagent and D-galacturonic acid monohydrate as reference.

Measurement of RS. The procedures of solubilization, enzymatic hydrolysis, and quantification of RS were mainly those according to Goñi et al. (1996). All insoluble DF samples prepared from legume flours were treated with 4 M KOH for 30 min at room temperature, and the mixture was then neutralized with 4 N HCl. The solubilized RS was quantified as the amount of glucose released by incubation with amyloglucosidase (in 0.4 M acetate buffer at pH 4.75 for 45 min at 60 °C), which was measured enzymatically (hexokinase−glucose-6-PDH) (Sigma diagnostic kit 16-10). The RS values (in terms of glucose content) were subtracted from the values of glucose in the insoluble NSP as determined by GC in the Englyst method.

Statistical Analysis. All chemical analyses were carried out in duplicate. Data collected from this study were analyzed by one-way analysis of variance and the Tukey test (Ott, 1988).

RESULTS AND DISCUSSION

The effect of cooking on the DF content of *P. angularis*, *P. calcaratus*, and *D. lablab* seed flours is given in Tables 1−3, respectively. In general, DF contents (total NSP and RS) of all the cooked legume flours were higher than those of the raw ones (Tables 1−3). Furthermore, the total DF content (soluble and insoluble fractions) of the legume flours increased with increasing cooking time (Tables 1−3). The maximum percent increases in the total DF content of the cooked legume flours of *P. angularis* (60 min), *P. calcaratus* (60 min),

Table 3. Changes in the Resistant Starch and Nonstarch Polysaccharide Content (Grams per 100 g of Dry Matter) of Raw and Cooked *D. lablab* Seed Flour

	DF[a]	RS	NSP[b]	Fuc	Rha	Ara	Xyl	Man	Gal	Glc	UAc
raw											
insoluble	12.3	0.46	11.8	0.12	0.02	1.17	1.89	0.06	0.44	7.56[c]	0.54
soluble	1.40	ND[d]	1.40	0.03	tr[e]	0.33	0.17	0.12	0.22	0.24	0.29
total[f]	13.7	0.46	13.2	0.15	0.02	1.50	2.06	0.18	0.66	7.80	0.83
cooked (60 min)											
insoluble	14.3	1.21	13.1	0.16	0.03	1.62	1.85	0.08	0.62	8.00[c]	0.73
soluble	1.81	ND	1.81	0.03	0.01	0.35	0.24	0.14	0.31	0.23	0.50
total[f]	16.1	1.21	14.9	0.19	0.04	1.97	2.09	0.22	0.93	8.23	1.23
cooked (90 min)											
insoluble	16.5	2.47	14.0	0.17	0.04	1.79	1.88	0.09	0.71	8.63[c]	0.67
soluble	1.97	ND	1.97	0.05	0.01	0.50	0.23	0.14	0.31	0.24	0.49
total[f]	18.5	2.47	16.0	0.22	0.05	2.29	2.11	0.23	1.02	8.87	1.16
cooked (120 min)											
insoluble	16.9	2.94	14.0	0.17	0.03	1.55	2.16	0.10	0.63	8.66[c]	0.70
soluble	2.14	ND	2.14	0.05	0.01	0.55	0.24	0.14	0.33	0.30	0.52
total[f]	19.0	2.94	16.1	0.22	0.04	2.10	2.40	0.24	0.96	8.96	1.22

[a] Dietary fiber (DF) is the sum of resistant starch (RS) and nonstarch polysaccharides (NSP). [b] NSP includes Fuc, Rha, Ara, Xyl, Man, Gal, Glc, and UAc. [c] Glucose values corrected for resistant starch. [d] ND, not determined. [e] tr, trace amount (<0.01). [f] Values are the sum of insoluble and soluble fractions.

Table 4. Effects of Cooking on the Proportion[a] of Insoluble and Soluble Components of the Nonstarch Polysaccharides (NSP) of *P. angularis, P. calcaratus,* and *D. lablab* Seed Flours

	cooking time				
sample	raw	30 min	60 min	90 min	120 min
P. angularis					
insoluble NSP	92.8	93.4	87.8		
soluble NSP	7.20	6.60	12.2		
P. calcaratus					
insoluble NSP	94.5	92.8	90.3		
soluble NSP	5.50	7.20	9.70		
D. lablab					
insoluble NSP	89.4		87.9	87.7	86.7
soluble NSP	10.6		12.1	12.3	13.3

[a] Expressed as percent of total NSP, which is the sum of insoluble and soluble components of NSP.

and *D. lablab* (120 min) were 47.8, 18.6, and 38.7, respectively. Among these three seed flours (both raw and cooked), the insoluble NSP constituted about 87−95% of the total NSP content (Table 4).

For the *P. angularis* and *D. lablab* flours, the values of both their total NSP and RS contents increased with increasing cooking time, having their highest values at a cooking time of 60 and 120 min, respectively (Tables 1 and 3). The total NSP contents of the *D. lablab* flour cooked for 90 and 120 min were almost the same (Table 3). While the total NSP contents of the raw and cooked flours of *P. calcaratus* seeds were almost identical, the RS content of the cooked flour was 2 times higher than that of the raw one (Table 2). The increase in the total NSP content in the *P. angularis* and *D. lablab* flours after cooking should not be due to losses of nonfiber material such as free sugars because the cooking water was freeze-dried together with the cooked seeds.

The increase in the insoluble NSP content of *P. angularis* and *D. lablab* cooked flours was mainly due to the increase of glucose (up to 1% dry weight of the flour). Such an apparent increase in glucose had also been observed in cooked peas (Englyst et al., 1988). Since the glucose content in the insoluble NSP had been corrected for RS, this extra amount of glucose could be a result of an improved depolymerization of cellulose in the hulls of cooked legume flour compared to that of the raw ones. Cellulose in raw hull is resistant to dispersion in 72% sulfuric acid within 1 h at 35 °C, but during cooking the cellulose swells and subsequently becomes more readily dispersible with the acid. It had been found that the amount of cellulosic glucose determined in cooked legume hulls (Chau et al., 1997) was larger than that of uncooked legume hulls (Cheung, unpublished results). Hence, the apparent increase in NSP during cooking is mainly due to an underestimation of NSP (particularly cellulosic glucose) in cooked legume flours.

The soluble NSP content of all cooked legume flours showed a trend of increase with longer cooking time. A maximum increase (≈1-fold) in the solubilization of NSP in the flour samples of *P. angularis*, *P. calcaratus*, and *D. lablab* with cooking times of 60, 60, and 120 min, respectively, was observed (Tables 1−3). Such increase in the soluble NSP content could be due to solubilization of cell wall pectic substances as a result of dissolution of the middle lamellae and some breakdown of pectins through β-elimination during boiling (McDougall et al., 1996). This could be further justified from the fact that the contents of pectic sugars such as arabinose, galactose, and uronic acids of the soluble NSP of all the cooked flours were higher than those of their raw flours (Tables 1−3). The increase in the solubilization of pectic polysaccharides during the cooking of these legumes was important for their therapeutic values as soup ingredients.

Furthermore, the proportion of soluble NSP in the total NSP content of the cooked flour was increased with cooking time, suggesting a redistribution of the total NSP content from insoluble to soluble components (Table 4). Cooking time of 30 min seemed to have minimum effect on *P. angularis* seed flour in terms of the redistribution of the insoluble and soluble components of NSP (Table 4). These results were in agreement with those for other legume seeds subjected to thermal processing (Gooneratne et al., 1994; Periago et al., 1996).

RS contributed substantially (about half of the increase) to the increase of the total DF content of all the legume flours (Tables 1−3). The RS content of all cooked flours was increased with increasing cooking time except in the *P. calcaratus* flour cooked for 30 min. The RS in the legume flours may be made up of physically inaccessible starch and retrograded starch (Goñi et al., 1996). Physically inaccessible starch would

result in a lower α-amylolysis rate (Tovar et al., 1990). The relatively lower in vitro digestibility of starch in cooked leguminous seed flours as compared to raw ones has been reported (Tovar et al., 1990, 1991). This could be due to the presence of physical entrapment of starch granules by intact cotyledon cell walls in the cooked flours after milling, which contrasted with the predominance of free starch granules in the milled flour from raw seeds (Tovar et al., 1991). Cell-enclosed starch is less easily attacked by amylolytic enzymes, which results in a limited availability in vitro. As mentioned earlier, the starch (especially high-amylose content) in legume seeds is very susceptible to retrogradation during cooking (Tovar, 1992). When hot gelatinized starch is cooled, a certain portion (in particular the high-amylose forms) can retrograde to a less soluble form that is resistant to acid and amylase action (Berry, 1986). Since the formation of retrograded starch in legumes depends on the amylose/amylopectin ratio, degree of gelatinization, thermal treatments, and cooling and storage times (Tovar, 1992), it is not surprising that there were some variations in the amount of RS formed in the three cooked legume flours.

In summary, cooking of *P. angularis*, *P. calcaratus*, and *D. lablab* seed flour samples resulted in an increase in the solubilization of NSP (mainly pectic polysaccharides) and the RS content, which have therapeutic value and potential physiological benefits to humans, respectively. The influence of thermal processing other than boiling in water on the DF content of these legume flours still needs to be investigated. Until then, the use of these legume flours as a DF supplement in processed food products cannot be evaluated.

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