Effect of Various Processing Methods on Antinutrients and *in Vitro* Digestibility of Protein and Starch of Two Chinese Indigenous Legume Seeds

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The effect of various processing methods on antinutrients and *in vitro* protein and starch digestibility of *Phaseolus angularis* and *Phaseolus calcaratus* seeds was studied. The processes, which included soaking (12 and 18 h), cooking, autoclaving, and germination (24 and 48 h), significantly reduced ($p < 0.05$) the levels of antinutrients including phytates, tannins, trypsin inhibitors, and amylase inhibitors and significantly improved ($p < 0.05$) the digestibility of protein and starch as well. Since a significant negative correlation (ranging from −0.80 to −1.00) was found between these antinutrients and the digestibility of both protein and starch, the reduction in the levels of these antinutrients might partly account for the improved digestibility. Cooking as well as autoclaving brought about a more significant ($p < 0.05$) improvement in the digestibility of both protein and starch as compared with soaking and germination. In this study, all processing methods were more effective in improving the starch digestibility than the protein digestibility.

Keywords: *Phaseolus angularis; Phaseolus calcaratus; processing; antinutrients; protein; starch; digestibility*

INTRODUCTION

The seeds of *Phaseolus angularis* and *Phaseolus calcaratus* indigenous to China are found to be rich in proteins (25.2 and 26.5% dry weight, respectively) and starches (40.1 and 46.7% dry weight, respectively) (Chau et al., 1997). These two seeds are traditionally used as soup ingredients for therapeutic purposes such as driving away dropsy, relieving diarrhea, and tonic to the viscera (Li, 1973).

The digestibility of legume protein is limited by the protein structure (Deshpande and Damodaran, 1989a) and the presence of antinutritional factors such as trypsin inhibitors, phytates, and tannins (Nielsen, 1991). Moreover, the digestibility of legume starch is also affected by the cell-wall structural features (Tovar et al., 1991; Kataria et al., 1992) and antinutrients such as amylase inhibitors (Lajolo et al., 1991), phytates, and tannins (Yadav and Khetarpaul, 1994).

Various processing and cooking methods could improve the protein and starch digestibility of legume seeds by decreasing the levels of some antinutrients (Kataria and Chauhan, 1988; Kaur and Kapoor, 1990; Barampama and Simard, 1994; Bishnoi and Khetarpaul, 1994). Moreover, an increase in digestibility after thermal treatments may be attributed to some other factors such as disruption of protein structures and cell-wall encapsulated starch, starch gelatinization, and physical disintegration of the legume seeds (Tovar et al., 1991). The process of germination also results in the reduction of some antinutrients and improves the digestibility of legume proteins (Khalil and Mansour, 1995).

To improve the utilization of the protein and starch fractions in *P. angularis* and *P. calcaratus* seeds, the present study was undertaken to compare the effect of various processing methods on the digestibility of the protein and starch in these two seeds.

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MATERIALS AND METHODS

P. angularis and *P. calcaratus* seeds were imported from mainland China. The seeds were manually screened for damage and defects.

Preparation of Raw Legume Seed Flours. Cleaned legume seeds were ground in a Cyclotec mill (Tecator, Hoganas, Sweden) to pass through a 0.5 mm screen. Flours obtained were stored in an airtight container until use.

Preparation of Processed Legume Seed Flours. *Soaking.* Legume seed samples were soaked in distilled water for 12 and 18 h at 30 °C, and the seed-to-water ratio used was 1:5 (w/v). The soaked seeds were rinsed with distilled water and drained.

Cooking. The seed samples (without prior soaking) were directly cooked in boiling distilled water in a beaker using a seed-to-water ratio of 1:5 (w/v). Samples were cooked until soft as felt between the fingers (cooking time was ∼30 min) and drained.

Autoclaving. The seed samples (without prior soaking) were autoclaved at 1.05 kg/cm^2 pressure at 121 °C for 20 min. The ratio of seed to distilled water was 1:3 (w/v). The excessive water after autoclaving was drained off.

Germination. The legume seeds were soaked in distilled water for 12 h and then germinated in sterile Petri dishes lined with wet filter papers for 24 and 48 h at 30 °C in the dark.

All of the above processed legume seed samples were freeze-dried and ground into flour in a Cyclotec mill (Tecator) using a 0.5 mm sieve size. The flours were used for subsequent analyses.

Analyses. The *in vitro* protein digestibility (IVPD) was determined by the multienzyme method of Hsu et al. (1977). The enzymes used were purchased from Sigma Chemical Co (St. Louis, MO). Five milliliters of an enzyme mixture (with each milliliter containing 1.6 mg of trypsin, 3.1 mg of chymotrypsin, and 1.3 mg of peptidase) was added to 50 mL of legume flour suspension (each milliliter containing 6.25 mg of protein) incubated at 37 °C. The pH change of the mixture after 10 min was used to calculate the percent of IVPD (Y) using the

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Table 1. Effect of Processing on the Levels of Phytates, Tannins, Trypsin Inhibitor Activity, and Amylase Inhibitor Activity of *P. angularis* and *P. calcaratus* Seeds[a]

treatment	phytates (mg/100 g)	tannins (mg/100 g)	trypsin inhibitor activity (TIU/g)	amylase inhibitor activity (AIU/g)
P. angularis				
raw (control)	149[b]	1500[b]	2230[b]	113[b]
soaked 12 h	124[c] (16.8)[b]	921[c] (38.6)	2080[c] (6.73)	100[c] (11.5)
soaked 18 h	109[cd] (26.9)	867[c] (42.2)	2010[c] (9.87)	85.3[d] (24.5)
cooked	96.1[d] (35.5)	433[d] (71.1)	1260[d] (43.5)	95.4[cd] (15.6)
autoclaved	67.9[e] (54.4)	468[d] (68.8)	1250[d] (44.0)	90.0[d] (20.4)
germinated 24 h	117[c] (21.5)	740[c] (50.7)	1560[e] (30.0)	87.6[d] (22.5)
germinated 48 h	85.5[f] (42.6)	536[d] (64.3)	782[f] (64.9)	56.3[e] (50.2)
P. calcaratus				
raw (control)	165[g]	1370[g]	2180[g]	104[g]
soaked 12 h	111[h] (32.7)[b]	950[h] (30.7)	2060[h] (5.51)	92.6[g] (11.0)
soaked 18 h	91.5[h] (44.6)	886[h] (35.3)	1760[i] (19.3)	66.8[h] (35.8)
cooked	96.3[h] (41.6)	500[i] (63.5)	1490[j] (31.7)	80.0[i] (23.1)
autoclaved	69.3[i] (58.0)	550[ij] (59.9)	1440[jk] (34.0)	82.9[i] (20.3)
germinated 24 h	92.6[h] (43.9)	638[j] (53.4)	1360[k] (37.6)	77.5[i] (25.5)
germinated 48 h	73.6[i] (55.4)	497[i] (63.7)	1260[k] (42.2)	44.1[j] (57.6)

[a] Values in the same column with different superscripts are significantly different (Tukey, $p < 0.05$; b−f for *P. angularis* seed, g−k for *P. calcaratus* seed). [b] Figures in parentheses indicate the percent decrease over the control values of the corresponding seed.

Table 2. Effect of Processing on the *in Vitro* Digestibility of Protein (IVPD) and Starch (IVSD) of *P. angularis* and *P. calcaratus* Seeds[a]

	IVPD (%)		IVSD (mg of maltose/g)	
treatment	*P. angularis*	*P. calcaratus*	*P. angularis*	*P. calcaratus*
raw (control)	69.3[b]	71.8[b]	37.9[b]	39.4[b]
soaked 12 h	72.6[c] (4.76)[b]	75.8[c] (5.57)	51.7[c] (36.4)	54.4[c] (38.1)
soaked 18 h	74.1[d] (6.93)	78.1[d] (8.77)	75.1[d] (98.2)	73.6[d] (86.8)
cooked	85.0[e] (22.7)	84.5[e] (17.7)	267[e] (605)	240[e] (509)
autoclaved	84.5[e] (21.9)	85.0[e] (18.4)	300[f] (692)	260[f] (560)
germinated 24 h	74.6[d] (7.65)	77.4[d] (7.80)	86.0[g] (127)	87.3[g] (122)
germinated 48 h	77.1[f] (11.3)	80.0[f] (11.4)	124[h] (227)	123[h] (212)

[a] Values in the same column with different superscripts are significantly different (Tukey, $p < 0.05$). [b] Figures in parentheses indicate the percent increase over the control values.

equation $Y = 210.464 - 18.10X$, where X is the pH change after 10 min.

The *in vitro* starch digestibility (IVSD) was assayed by employing porcine pancreatic amylase (EC 3.2.1.1, 790 units/mg of protein; catalog no. A6255, Sigma) (Singh et al., 1982). One unit of amylase liberated 1 mg of maltose from starch in 3 min at pH 6.9 at 20 °C. In brief, 50 mg of legume flour samples was incubated with 0.5 mL of pancreatic amylase solution (0.4 mg/mL) at 20 °C for 2 h. After the incubation, 2 mL of 3,5-dinitrosalicyclic acid reagent was added, and the mixture was boiled for 5 min. After cooling, the absorbance of the filtered solution was measured at 550 nm with maltose used as the standard. The values of starch digestibility were expressed as milligrams of maltose released per gram of sample in dry weight.

For the determination of antinutritional factors, tannin in the legume flour was assayed according to the modified vanillin−HCl method of Price et al. (1978), and its concentration was expressed in milligram catechin equivalents. The colorimetric procedure of Haug and Lantzsch (1983) was used to estimate the phytate content in the legume flour. Since phytates precipitated with an acidic ferric solution of known concentration, a decrease in the ferric ion level (determined colorimetrically with 2,2′-bipyridine solution) would be a measure for the phytate content. Trypsin inhibitor activity (TIA) was determined spectrophotometrically by using the enzymatic assay of Kakade et al. (1974). One trypsin unit was defined as an increase of 0.01 absorbance unit at 410 nm per 10 mL of reaction mixture, and the TIA was expressed in terms of trypsin inhibitory unit (TIU). Amylase inhibitor activity (AIA) was evaluated spectrophotometrically according to the method of Deshpande et al. (1982). To set the amylase activity reference standards, 0.25 mL of sodium phosphate buffer (pH 7.0) and 0.25 mL of amylase enzyme solution (30 μg/mL) were incubated with 0.5 mL of 1% starch solution at 37 °C for 3 min. After the incubation, 2 mL of 3,5-dinitrosalicyclic acid reagent was added and the mixture was boiled for 10 min. After cooling, the absorbance of the filtered solution was measured at 550 nm with maltose used as the standard. One unit of amylase activity was defined as 1 mg of maltose liberated in 3 min at 37 °C. To measure the AIA in the legume flours, 25 mg of the sample was dispersed in 0.25 mL of distilled water into which 0.25 mL of amylase solution and 0.5 mL of 1% starch solution were added. The mixture was incubated at 37 °C for 3 min, and the enzymatic activity assay was conducted as described above. One unit of amylase activity inhibited was expressed as one amylase inhibitory unit (AIU).

Statistical Analysis. In this study, all chemical analyses were carried out in triplicate. All data were analyzed by one-way analysis of variance and the Tukey test (Ott, 1988).

RESULTS AND DISCUSSION

Antinutrients. Table 1 shows that various processing methods such as soaking, cooking, autoclaving, and germination significantly ($p < 0.05$) reduced the levels of the phytates, tannins, TIA, and AIA of *P. angularis* and *P. calcaratus* seeds. Previous findings in other legume seeds such as faba bean, mung bean, *Vigna aconitifolia*, and *Pisum sativum* also indicated that these processing methods significantly decreased the levels of antinutrients (Khokhar and Chauhan, 1986; Kataria et al., 1989; Bishnoi and Khetarpaul, 1994; Khalil and Mansour, 1995). Among the cooking and autoclaving treatments, there was no significant difference in the levels of tannins, TIA, and AIA for both of the legume seeds, but autoclaving brought about a significantly ($p < 0.05$) lower phytate content than cooking (Table 1). In general, the decrease in the levels

of these antinutrients during heat treatment might be due to thermal degradation and denaturation of the antinutrients as well as the formation of insoluble complexes (Kataria et al., 1989). Compared to the raw seeds, significant ($p < 0.05$) reductions in the levels of all four antinutrients in both legume seeds were observed after 18 h of soaking as well as after 48 h of germination. Generally, a longer time of soaking (18 vs 12 h) and germination (48 vs 24 h) resulted in lower levels of the four antinutritional factors (Table 1). Such a decrease in the levels of antinutrients might be partially attributed to the leaching-out effect during hydration (Kataria et al., 1992; Beleia et al., 1993). Moreover, seed germination is known to reduce the levels of phytates and tannins as well as to suppress the activities of trypsin inhibitors in legume seeds (Rao and Deosthale, 1982; Khokhar and Chauhan, 1986; Kataria et al., 1989).

Protein and Starch Digestibilities. Table 2 shows that both the IVPD and IVSD of the two legume seeds were increased significantly ($p < 0.05$) by the processing methods such as soaking, cooking, autoclaving, and germination. These results were in agreement with previous findings that various processing methods such as soaking, heat treatment, and germination improved both the IVPD and IVSD of many legume seeds such as rice bean, faba bean, quinoa seed, and amphidiploids (black gram × mung bean) (Kaur and Kapoor, 1990; Sharma and Sehgal, 1991; Kataria et al., 1992; Ruales and Nair, 1994). Compared with the control, a longer time of soaking (18 vs 12 h) as well as germination (48 vs 24 h) resulted in a significantly ($p < 0.05$) higher values of the IVPD and IVSD for both legume seeds (Table 2). As shown in Table 2, both the cooking and autoclaving processes could bring about a more significant improvement in both the IVPD and IVSD than the soaking and germination processes. Among these two heat treatments, autoclaving would be the most energy-saving method. As compared with the control, the increase in the IVSD of both legume seeds upon soaking (36.4−98.2%), heat treatments (6−7-fold), and germination (1−2-fold) was much larger than those in the IVPD upon soaking (4.76−8.77%), heat treatments (17.7−22.7%), and germination (7.65−11.4%), respectively. The relatively greater improvements in the IVSD than in the IVPD might be partially explained by the fact that globulins, the major storage protein in legumes, are intrinsically more resistant to proteolysis.

Among the different processing methods including soaking, heat treatment, and germination, there were some very strong and negative correlations ($r = -0.80$ to -1.00, $p < 0.01$) between the levels of the antinutrients and both the IVPD and IVSD of the two *Phaseolus* seeds. Generally, many previous studies indicated that the decrease in the levels of antinutrients during soaking, heat treatment, and germination might be partly responsible for the improved IVPD and IVSD (Singh et al., 1982; Thompson and Yoon, 1984; Kataria et al., 1989; Kaur and Kapoor, 1990). The improvement in the IVPD during the above processes might also be partly attributed to the changes in the activities of endogenous hydrolytic enzymes or the alternation of the storage proteins structures (Nielsen et al., 1988; Deshpande and Damodaran, 1989b; Bishnoi and Khetarpaul, 1994). Furthermore, the enhanced IVSD during the above processes might be partly due to the swelling and rupturing of starch granules as well as the activation of amylase and phosphorylase (Kataria and Chauhan, 1988; Kaur and Kapoor, 1990).

Conclusion. In this study, all of the various processing methods investigated appeared to be effective in reducing the levels of antinutrients and improving both the IVPD and IVSD of *P. angularis* and *P. calcaratus* seeds. There was a significant ($p < 0.01$) and negative correlation between the levels of antinutrients and both the IVPD and IVSD. All of the various processing methods used were more effective in improving the IVSD to a much greater extent when compared with the IVPD. Overall, the various processing methods, especially that involving heat treatment, are important for more effective utilization of food legume proteins and carbohydrates for human nutrition. Further *in vivo* studies to compare the protein digestibilities of these two legume seeds are underway.

LITERATURE CITED

Barampama, Z.; Simard, R. E. Oligosaccharides, antinutritional factors, and protein digestibility of dry beans as affected by processing. *J. Food Sci.* **1994**, *59*, 833−838.

Beleia, A.; Thu Thao, L. T.; Ida, E. I. Lowering phytic phosphorus by hydration of soybeans. *J. Food Sci.* **1993**, *58*, 375−378.

Bishnoi, S.; Khetarpaul, N. Protein digestibility of vegetable and field peas (*Pisum sativum*): varietal differences and effect of domestic processing and cooking. *Plant Foods Hum. Nutr.* **1994**, *46*, 71−76.

Chau, C. F.; Cheung, P. C. K.; Wong, Y. S. Chemical composition of three underutilized legume seeds grown in China. *Food Chem.* **1997**, in press.

Deshpande, S. S.; Damodaran, S. Structure-digestibility relationship of legume 7S proteins. *J. Food Sci.* **1989a**, *54*, 108−113.

Deshpande, S. S.; Damodaran, S. Heat-induced conformational changes in phaseolin and its relation to proteolysis. *Biochim. Biophys. Acta* **1989b**, *998*, 179−188.

Deshpande, S. S.; Sathe, S. K.; Salunkhe, D. K.; Cornforth, D. P. Effects of dehulling on phytic acid, polyphenols, and enzyme inhibitors of dry beans (*Phaseolus vulgaris* L.). *J. Food Sci.* **1982**, *47*, 1846−1850.

Haug, W.; Lantzsch, H. Sensitive method for the rapid determination of phytate in cereals and cereal products. *J. Sci Food Agric.* **1983**, *34*, 1423−1426.

Hsu, H. W.; Vavak, D. L.; Satterlee, L. D.; Miller, G. A. A multi-enzyme technique for estimating protein digestibility. *J. Food Sci.* **1977**, *42*, 1269−1273.

Kakade, M.; Rackis, J. J.; McGhee, J. E.; Puski, G. Determination of trypsin inhibitor activity of soy products: A collaborative analysis of an improved procedure. *Cereal Chem.* **1974**, *51*, 376−382.

Kataria, A.; Chauhan, B. M. Content and digestibility of carbohydrates of mung beans (*Vigna radiata* L.) as affected by domestic processing and cooking. *Plant Foods Hum. Nutr.* **1988**, *38*, 51−59.

Kataria, A.; Chauhan, B. M.; Punia, D. Antinutrients and protein digestibility (*in vitro*) of mungbean as affected by domestic processing and cooking. *Food Chem.* **1989**, *32*, 9−17.

Kataria, A.; Chauhan, B. M.; Punia, D. Digestibility of protein and starch (*in vitro*) of amphidiploids (black gram x mung bean) as affected by domestic processing and cooking. *Plant Foods Hum. Nutr.* **1992**, *42*, 117−125.

Kaur, D.; Kapoor, A. C. Starch and protein digestibility of rice bean (*Vigna umbellata*): effect of domestic processing and cooking methods. *Food Chem.* **1990**, *38*, 263−272.

Khalil, A. H.; Mansour, E. H. The effect of cooking, autoclaving and germination on the nutritional quality of faba beans. *Food Chem.* **1995**, *54*, 177−182.

Khokhar, S.; Chauhan, B. M. Antinutritional factors in moth bean (*Vigna aconitifolia*): varietal differences and effects

of methods of domestic processing and cooking. *J. Food Sci.* **1986**, *51*, 591−594.

Lajolo, F. M.; Filho, F. F.; Menezes, E. W. Amylase inhibitors in *Phaseolus vulgaris* beans. *Food Technol.* **1991**, *9*, 119−121.

Li, S. C. *Chinese Medicinal Herbs*; translated by Smith, F. P., Stuart, G. A.; Georgetown Press: San Francisco, CA, 1973; pp 155, 316.

Nielsen, S. S. Digestibility of legume proteins. *Food Technol.* **1991**, *9*, 112−114, 118.

Nielsen, S. S.; Deshpande, S. S.; Hermodson, M. A.; Scott, M. P. Comparative digestibility of legume proteins. *J. Agric. Food Chem.* **1988**, *36*, 896−902.

Ott, L. *An Introduction to Statistical Methods and Data Analysis*, 3rd ed.; PWS-KENT: Boston, MA, 1988; pp 437−466.

Price, M. L.; Scoyoc, S. V.; Butler, L. G. A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. *J. Agric. Food Chem.* **1978**, *26*, 1214−1218.

Rao, P. U.; Deosthale, Y. G. Tannin content of pulses: varietal differences and effects of germination and cooking. *J. Sci. Food Agric.* **1982**, *33*, 1013−1016.

Ruales, J.; Nair, B. M. Effect of processing on *in vitro* digestibility of protein and starch in quinoa seeds. *Int. J. Food Sci. Technol.* **1994**, *29*, 449−456.

Sharma, A.; Sehgal, S. Protein and starch digestibility of faba bean in dependence on processing and cooking methods. *Nahrung* **1991**, *35*, 891−893.

Singh, U.; Kherdekar, M. S.; Jambunathan, R. Studies on desi and kabuli chickpea (*Cicer arietinum* L.) cultivars, the levels of amylase inhibitors, levels of oligosaccharides and *in vitro* starch digestibility. *J. Food Sci.* **1982**, *47*, 510−512.

Thompson, L. U.; Yoon, J. H. Starch digestibility as affected by polyphenols and phytic acid. *J. Food Sci.* **1984**, *49*, 1228−1229.

Tovar, J.; de Francisco, A.; Bjorck, I; Asp, N. G. Relationship between microstructure and *in vitro* digestibility of starch in precooked leguminous seed flours. *Food Struct.* **1991**, *10*, 19−26.

Yadav, S.; Khetarpaul, N. Indigenous legume fermentation: effect on some antinutrients and *in vitro* digestibility of starch and protein. *Food Chem.* **1994**, *50*, 403−406.

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