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Effect of domestic processing on the levels of certain antinutrients in *Prosopis chilensis* (Molina) Stunz. seeds

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The effects of soaking, cooking and autoclaving on the levels of certain antinutrients present in the seeds of *Prosopis chilensis* were studied. Both distilled water and sodium bicarbonate solution (salt water) soaking significantly reduced the content of total free phenolics compared to cooking and autoclaving. The reduction in content of phytic acid was found to be greater with distilled water soaking (14%) than with salt water soaking (8%). Cooking and autoclaving significantly reduced (32–35%) the phytic acid content. An insignificant loss of raffinose was noticed when the seeds were subjected to soaking, regardless of the duration of soaking. Autoclaving (45 min) markedly reduced the raffinose and stachyose content (63–71%). *In vitro* protein digestibility (IVPD) of *Prosopis chilensis* seeds was enhanced by 16·6% under autoclaving. Of all the treatments studied, autoclaving seemed to be the best method for eliminating the contents of phytic acid, raffinose and stachyose. It also improved the IVPD. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Legume seeds are a major source of protein for human beings. Food legumes have, on average, twice as much protein as cereals, and the nutritive value of the proteins of leguminous seeds is generally of high quality. *Prosopis* species are important vegetation elements in arid and semi-arid countries, where many of them offer food for man. The species grows well in poor soil conditions (Lamarque *et al.*, 1994). It is noteworthy that the crude protein, methionine and cystine contents of *Prosopis* are on the high side of the range values for legumes (de Lumen *et al.*, 1986). The seeds of *Prosopis chilensis* are cooked and eaten by tribes people of Madhya Pradesh (Rajaram & Janardhanan, 1991).

Feeding protein-rich legume seeds to monogastric animals is hampered by the presence of antinutritional factors. Polyphenols are known to decrease protein digestibility either by binding with digestive enzymes such as trypsin and chymotrypsin or by binding directly to the dietary protein (Jambunathan & Singh, 1981). Phytic acid lowers the bioavailability of minerals and inhibits proteases and amylases (Sharma & Sehgal, 1992). Oligosaccharides are involved in the production of flatulence, characterised by the production of CO_2, H_2 and small amounts of methane (CH_4), and lead to abdominal rumbling, cramps, diarrhoea and nausea (Steggerda, 1968).

Not much is known about the effect of various domestic processes in reducing/eliminating the antinutritional factors in legume seeds. Technological treatment is one possible way but this is usually a very energy-demanding and expensive process. Therefore, simpler, cost-effective processes need to be devised and effected to eliminate such factors. To date, there have been no studies regarding the effect of domestic processing on the levels of antinutritional factors in *P. chilensis*. Hence the present work has been aimed at understanding to what extent the antinutrients, total free phenolics, phytic acid and oligosaccharides can be eliminated after subjecting the seeds to various simple processes such as soaking, cooking and autoclaving.

MATERIALS AND METHODS

Collection of seed samples

Seeds of *Prosopis chilensis* (Molina) Stunz. were collected from Manickampalayam village, Erode of Periyar District in Tamil Nadu, India. Soon after collection the seeds were dried for 2 days in open sunlight. After removal of immature seeds and unwanted materials, the seeds were stored in plastic containers at room temperature (25°C).

Processing methods

Soaking
Separate batches of whole seeds were soaked in distilled water and 0·02% (w/v) sodium bicarbonate ($NaHCO_3$) solution (pH 8·6) for 4, 8 and 12 h in a bean:water ratio of 1:10 (w/v). The water was drained off, then the seeds were dried at 55°C and powdered in a Wiley Mill to 60 mesh size.

Cooking
The whole seeds were cooked in distilled water (100°C) in the ratio of 1:10 (w/v) for 40, 80 and 120 min on a hot plate. The seeds were rinsed, dried at 55°C and powdered in a Wiley Mill to 60 mesh size.

Autoclaving
The seeds were autoclaved at 15 lb pressure (121°C) in distilled water (1:10, w/v) for 15, 30 and 45 min. The seeds were rinsed with distilled water, dried at 55°C and powdered in a Wiley Mill to 60 mesh size.

Analysis of antinutritional factors

The content of total free phenolics in the raw and treated samples was estimated by the method of Bray & Thorne (1954) using a Spectronic 20D spectrophotometer at 650 nm. The principle involved in this method is that phenols react with phosphomolybdic acid in Folin–Ciocalteau reagent in alkaline medium and produce a blue-coloured complex (molybdenum blue).

Extraction and estimation of phytic acid

The Wheeler and Ferrel (1971) method was used to quantify the content of phytic acid in raw as well as in processed seed flours. The phytate phosphorus was calculated from the results for iron, assuming a 4:6 iron:phosphorus molecular ratio. The phytic acid was calculated using the factor 3·55, based on the empirical formula $C_6P_6O_{24}H_{18}$.

Extraction and estimation of oligosaccharides

The oligosaccharides were first extracted twice with 80% ethanol and concentrated under vacuum at 40°C. The sugars were separated by descending paper chromatography using Whatman No. 1 chromatographic paper and propanol–ethanol–water in the ratio 7:1:2 (v/v) (Tharanathan *et al.*, 1975). A standard sugar mixture containing raffinose, stachyose and verbascose (Sigma Chemical Co., St Louis, USA) was run simultaneously.

After completion of the chromatographic runs, *p*-anisidine hydrochloride reagent was sprayed onto the papers to reveal the sugar spots (Mukherjee & Srivastava, 1952). For quantification, the paper at areas (2 cm×2 cm) corresponding to each oligosaccharide spot was cut from unsprayed paper and eluted with 3 ml of distilled water. The individual sugars were then estimated by the phenol–sulphuric acid method of Dubois *et al.* (1956).

All the results are expressed on a dry weight basis.

Determination of *in vitro* protein digestibility (IVPD)

The IVPD of raw and treated seeds was estimated using the multienzyme method of Hsu *et al.* (1977).

Statistical analysis

The data were statistically analysed using Duncan's multiple range test by the method of Alder & Roessler (1977).

RESULTS AND DISCUSSION

The contents of total free phenolics, phytic acid and oligosaccharides in raw seeds of *P. chilensis* are given in Table 1. The total free phenolics content in *P. chilensis* appears to be higher than that of cowpea (Giami, 1993) and different varieties of peas (Bishnoi *et al.*, 1994) and lower than that of different varieties of rice bean (Kaur

Table 1. Effect of soaking on the levels of total free phenolics, phytic acid and oligosaccharides in *Prosopis chilensis*

Treatment	Total free phenolics (g per 100 g of seed flour)	Phytic acid (mg per 100 g of seed flour)	Oligosaccharides (g per 100 g of seed flour)		
			Raffinose	Stachyose	Verbascose
Raw seeds	0·84[a]	513[a]	1·45[a]	1·10[a]	Trace
Seeds soaked in distilled water					
4 h	0·70[b](17)	484[b](6)	1·45[ab](1)	1·05[b](5)	Trace
8 h	0·53[c](37)	451[c](12)	1·36[ab](6)	1·02[b](7)	—
12 h	0·35[d](58)	442[c](14)	1·35[b](7)	0·99[b](10)	—
Seeds soaked in sodium bicarbonate solution					
4 h	0·68[b](19)	499[a](3)	1·40[ab](3)	1·01[b](8)	Trace
8 h	0·49[c](42)	481[b](6)	1·32[b](9)	0·89[c](19)	—
12 h	0·30[d](64)	472[b](8)	1·29[b](11)	0·87[c](21)	—

All values are the average of three determinations.
Mean values in the column sharing a common letter are not statistically different ($P < 0·05$).
Values in parentheses indicate per cent loss from raw seeds.

& Kapoor, 1992) and faba bean (Neerjarani & Hira, 1993). The seeds of *P. chilensis* are known to contain higher levels of phytic acid than the seeds of *Prosopis glandulosa* (Harden & Zolfaghari, 1988), different varieties of rice bean, black gram and green gram (Kaur & Kapoor, 1992) and different genotypes of pigeonpea, chickpea, mungbean, urd bean and soyabean (Chitra *et al.*, 1995). Raffinose is found to be the major oligosaccharide in *P. chilensis*, as has been reported earlier for *P. glandulosa* and *P. velutina* (Becker & Grosjean, 1980). The level of raffinose in the present study is comparable to those in different cultivars of soyabean (Trugo *et al.*, 1995).

Effect of soaking

The effects of soaking on the levels of total free phenolics, phytic acid and oligosaccharides are shown in Table 1. Both distilled water and salt water soaking significantly reduce the content of total free phenolics in *P. chilensis*. In general, salt water soaking appears to be more effective than distilled water soaking in reducing the levels of polyphenols. Since the polyphenolic compounds are water-soluble in nature (Kumar *et al.*, 1979) and mostly located in the seed coat (Singh, 1988), the decrease in content of polyphenolics in pulses during soaking may be attributed to leaching out of the phenol into the soaking medium under the influence of the concentration gradient.

Soaking reduces the phytate content only by up to 14%. The same level of loss has been observed in mungbean by Kataria *et al.* (1989). A soaking period of 12 h may be inadequate for reducing the phytic acid content (Table 1). Ologhobo & Fetuga (1984) also reported that it required 3 days of soaking for a reduction in phytic acid of about 28% in cowpea. The distilled water soaking seems to be more effective compared to salt water soaking in lowering the content of phytic acid. This confirms the results of Khan *et al.* (1988) for the white variety of *Cicer arietinum*, where the loss of phytic acid was less in the presence of sodium bicarbonate. The removal of phytate in legumes on soaking has been attributed to the enzymatic (phytase) hydrolysis of phytate followed by diffusion. This is confirmed by an earlier investigation in faba bean (Eskin & Wiebe, 1983).

Soaking of *P. chilensis* in distilled water does not result in significant reduction in the content of raffinose, regardless of soaking time, which agrees with an earlier study with *Dolichos lablab* (Revilleza *et al.*, 1990). Verbascose occurs as a trace in raw seeds and is completely eliminated after 8 h of soaking. In general, the reduction of oligosaccharides seems to be significantly higher in seeds soaked in salt solution than in those soaked in distilled water. The solubility of the individual oligosaccharides and rate of diffusion are the two factors that are responsible for the sugar loss during soaking (Upadhyay & Garcia, 1988).

Cooking effect

The effects of cooking on the levels of total free phenolics, phytic acid and oligosaccharides are given in Table 2. In *P. chilensis*, a cooking time of 120 min seems to be significant in reducing the content of total free phenolics (37%). This is in agreement with an earlier study in *Dolichos lablab* var. *vulgaris* (Vijayakumari *et al.*, 1995). Since the polyphenols are water-soluble in nature, the loss may be due to leaching out into the cooking medium (Kumar *et al.*, 1979; Uzogara *et al.*, 1990).

The seeds of *P. chilensis* in the present study show a loss of phytic acid (32%) that is more or less comparable to that of previous investigations (Uzogara *et al.*, 1990; Sharma & Sehgal, 1992). The apparent decrease in phytic acid content of legume seeds during cooking may be partly due to the formation of insoluble complexes between phytate and other components, such as phytate–protein and phytate–protein–mineral complexes.

Cooking effects a greater reduction in the level of oligosaccharides than soaking (Table 2). A significant loss of raffinose content (57%) has been observed in *P. chilensis* when cooked for 120 min, which agrees with

Table 2. Effect of cooking and autoclaving on the levels of total free phenolics, phytic acid and oligosaccharides in *Prosopis chilensis*

Treatment	Total free phenolics (g per 100 g of seed flour)	Phytic acid (mg per 100 g of seed flour)	Oligosaccharides (g per 100 g of seed flour)		
			Raffinose	Stachyose	Verbascose
Raw seeds	0·84[a]	513[a]	1·45[a]	1·10[a]	Trace
Cooked in water					
40 min	0·73[b](13)	464[b](10)	1·23[b](15)	0·92[b](16)	Trace
80 min	0·59[c](30)	393[c](23)	0·95[c](34)	0·75[c](32)	—
120 min	0·53[d](37)	349[d](32)	0·63[d](57)	0·56[d](49)	—
Autoclaved					
15 min	0·74[b](12)	456[b](11)	0·95[b](34)	0·86[b](22)	—
30 min	0·61[c](27)	384[c](25)	0·61[c](58)	0·67[c](39)	—
45 min	0·58[c](31)	334[d](35)	0·42[d](71)	0·41[d](63)	—

All values are average of three determinations.
Mean values in the column sharing a common letter are not statistically different ($P < 0·05$).
Values in parentheses indicate per cent loss.

Table 3. *In vitro* protein digestibility in raw and processed seeds of *Prosopis chilensis*

Treatment	Protein digestibility (%)	Percentage increase of protein digestibility
Raw	67·9[a]	—
Soaking in distilled water for 12 h	70·2[b]	3·33
Soaking in $NaHCO_3$ solution for 12 h	74·7[c]	9·96
Cooking in boiling water for 120 min	76·9[d]	13·3
Autoclaving for 45 min	79·2[e]	16·6

Mean values in the column sharing a common letter are not statistically different ($P < 0.05$).

earlier work by Revilleza *et al.* (1990) in *Dolichos lablab*. In marked contrast, Udayasekhara Rao & Belavady (1978) reported an increase in the level of raffinose after cooking. The seeds of *P. chilensis*, when subjected to cooking, show a significant reduction in stachyose content. This trend tallies with an earlier report of Jood *et al.* (1985) for cultivated legumes. The decrease in levels of oligosaccharides due to cooking might be attributed to heat hydrolysis of the oligosaccharides with the formation of simple disaccharides and monosaccharides or other compounds (Onigbinde & Akinyele, 1983).

Effect of autoclaving

Autoclaving seems to reduce the total free phenolics by up to 31% in *P. chilensis* (Table 2). This may be due to reduced extractability due to their changed chemical reactivity (Satwadhar *et al.*, 1981). The seeds of *P. chilensis* exhibit significant reduction in the level of phytic acid (35%). This gains support from the observations of Khokhar and Chauhan (1986) in *Vigna aconitifolia* and Neerjarani and Hira (1993) in *Vicia faba*. Autoclaving reveals losses of raffinose and stachyose to the extent of 71% and 63%, respectively (Table 2), when compared to the raw seeds. In general, maximum reduction in raffinose and stachyose contents occurs with autoclaving.

Protein digestibility

The IVPD of raw and processed seeds of *P. chilensis* is given in Table 3. Raw seeds of *P. chilensis* exhibited 67·9% IVPD. A similar percentage IVPD has recently been observed in different genotypes of pigeonpea (Chitra *et al.*, 1995). Protein digestibility of autoclaved samples is significantly higher than that of the seeds subjected to soaking and cooking treatments. Cooking causes more protein digestibility (13% improvement) than soaking. A similar observation has previously been made in field bean and horse gram, where cooking improved IVPD by 15% (Rajyalakshmi & Geervani, 1990). The improvement of IVPD of legumes on autoclaving may be attributed not only to the removal of antinutrients (Moneam, 1990) but also to the structural disintegration of the native protein, including enzyme inhibitors.

Of the various processing methods, autoclaving seems to be the best for inactivation/reduction in levels of all the antinutrients investigated, except total free phenolics. It also improves the IVPD. After conducting suitable feeding trial experiments, the seeds of *P. chilensis* may be advocated for consumption by economically weaker sections of populations throughout the developing countries, including India, to alleviate the widely prevailing protein malnutrition.

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REFERENCES

Alder, H. L. & Roessler, E. B. (1977). *Introduction to Probability and Statistics*, 6th edn. W.H. Freeman, San Francisco, pp. 1–426.

Becker, R. & Grosjean, O. K. (1980). A compositional study of pods of two varieties of mesquite (*Prosopis glandulosa*, *P. velutina*). *J. Agric. Food Chem.*, **28**, 22–25.

Bishnoi, S., Khetarpaul, N. & Yadav, R. K. (1994). Effect of domestic processing and cooking methods on phytic acid and polyphenol contents of pea cultivars (*Pisum sativum*). *Plant Foods Hum. Nutr.*, **45**, 381–388.

Bray, H. G. & Thorne, W. V. (1954). Analysis of phenolic compounds. *Methods Biochem. Anal.*, **1**, 27–52.

Chitra, U., Vimala, V., Singh, U. & Geervani, P. (1995). Variability in phytic acid content and protein digestibility of grain legumes. *Plant Foods Hum. Nutr.*, **47**, 163–172.

de Lumen, B. O., Becker, R. & Reyes, P. S. (1986). Legumes and a cereal with high methionine/cysteine contents. *J. Agric. Food Chem.*, **34**, 361–364.

Dubois, M., Gilles, K., Hamilton, T. K., Rebers, P. A. & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Anal. Chem.*, **28**, 350–356.

Eskin, N. A. M. & Wiebe, S. (1983). Changes in phytase activity and phytate during germination of two faba bean cultivars. *J. Food Sci.*, **48**, 270–271.

Giami, S. Y. (1993). Effect of processing on the proximate composition and functional properties of cowpea (*Vigna unguiculata*) flour. *Food Chem.*, **47**, 153–158.

Harden, M. L. & Zolfaghari, R. (1988). Nutritive composition of green and ripe pods of honey mesquite (*Prosopis glandulosa* Fabaceae). *Econ. Bot.*, **42**, 522–532.

Hsu, H. W., Vavak, D. L., Satterlee, L. D. & Miller, G. A. (1977). A multienzyme technique for estimating protein digestibility. *J. Food Sci.*, **42**, 1269–1271.

Jambunathan, R. & Singh, U. (1981). Grain quality of pigeonpea. In *Proceedings of the International Workshop on Pigeonpea*, Vol. I, ICRISAT, Hyderabad, Andhra Pradesh, India, 15–19 December 1980.

Jood, S., Mehta, U., Singh, R. & Bhat, C. M. (1985). Effect of processing on flatus-producing factors in legumes. *J. Agric. Food Chem.*, **33**, 268–271.

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

Kaur, D. & Kapoor, A. C. (1992). Nutrient composition and antinutritional factors of rice bean (*Vigna umbellata*). *Food Chem.*, **43**, 119–124.

Khan, N., Zaman, R. & Elahi, M. (1988). Effect of processing on the phytic acid content of bengal grams (*Cicer arietinum*) products. *J. Agric. Food Chem.*, **36**, 1274–1276.

Khokhar, S. & Chauhan, B. M. (1986). Antinutritional factors in moth bean (*Vigna aconitifolia*) varietal differences and effects of methods of domestic processing and cooking. *J. Food Sci.*, **51**, 591–594.

Kumar, N. R., Reddy, A. N. & Rao, K. N. (1979). Levels of phenolic substances in the leachates in *Cicer* seed. *Indian J. Exp. Biol.*, **17**, 114–116.

Lamarque, A. L., Maestri, D. M., Grosso, N. R., Zygadlo, J. A. & Guzman, C. A. (1994). Proximate composition and seed lipid components of some *Prosopis* (Leguminosae) from Argentina. *J. Sci. Food Agric.*, **66**, 323–326.

Moneam, N. M. A. (1990). Effects of presoaking on faba bean enzyme inhibitors and polyphenols after cooking. *J. Agric. Food Chem.*, **38**, 1479–1482.

Mukherjee, S. & Srivastava, H. S. (1952). Improved spray reagent for detection of sugars. *Nature*, **169**, 330–331.

Neerjarani & Hira, C. K. (1993). Effect of various treatments on nutritional quality of faba beans (*Vicia faba*). *J. Food Sci. Technol.*, **30**, 413–416.

Ologhobo, A. D. & Fetuga, B. L. (1984). Distribution of phosphorus and phytate in some Nigerian varieties of legumes and some effects of processing. *J. Food Sci.*, **49**, 199–201.

Onigbinde, A. O. & Akinyele, I. O. (1983). Oligosaccharide content of 20 varieties of cowpea in Nigeria. *J. Food Sci.*, **48**, 1250–1251, 1254.

Rajaram, N. & Janardhanan, K. (1991). Studies on the under exploited tree pulses, *Acacia catechu* Willd., *Parkinsonia aculeata* L. and *Prosopis chilensis* (Molina) Stunz: chemical composition and antinutritional factors. *Food Chem.*, **42**, 265–273.

Rajyalakshmi, P. & Geervani, P. (1990). Studies on tribal foods of South India: effect of processing methods on the vitamin and *in vitro* protein digestibility (IVPD) of cereals/millets and legumes. *J. Food Sci. Technol.*, **27**, 260–263.

Revilleza, M. J. R., Mendoza, E. M. T. & Raymundo, L. C. (1990). Oligosaccharides in several Phillippine indigenous food legumes: determination, localization and removal. *Plant Foods Hum. Nutr.*, **40**, 83–93.

Satwadhar, P. N., Kadam, S. S. & Salunkhe, D. K. (1981). Effect of germination and cooking on polyphenols and *in vitro* protein digestibility of horse gram and moth bean. *Qual. Plant. Plant Foods Hum. Nutr.*, **36**, 71–76.

Sharma, A. & Sehgal, S. (1992). Effect of processing and cooking on the antinutritional factors of faba bean (*Vicia faba*). *Food Chem.*, **43**, 383–385.

Singh, U. (1988). Antinutritional factors of chickpea and pigeonpea and their removal by processing. *Plant Foods Hum. Nutr.*, **38**, 251–261.

Steggerda, F. R. (1968). Gastro-intestinal gas following food consumption. *Ann. N.Y. Acad. Sci.*, **150**, 57–66.

Tharanathan, R. N., Wankhede, D. B. & Ragavendra Rao, M. R. (1975). Carbohydrate composition of groundnuts (*Arachis hypogaea*). *J. Sci. Food Agric.*, **26**, 749–754.

Trugo, L. C., Farah, A. & Cabral, L. (1995). Oligosaccharide distribution in Brazilian soya bean cultivars. *Food Chem.*, **52**, 385–387.

Udayasekhara Rao, P. & Belavady, B. (1978). Oligosaccharides in pulses: varietal differences, effects of cooking and germination. *J. Agric. Food Chem.*, **26**, 316–319.

Upadhyay, J. K. & Garcia, V. V. (1988). Effect of soaking and cooking on reduction of oligosaccharides of cowpea (*Vigna unguiculata* (L.) Walp.). *Philipp. J. Food Sci. Technol.*, **12**, 21–28.

Uzogara, S. G., Morton, I. D. & Daniel, J. W. (1990). Changes in some antinutrients in cowpeas (*Vigna unguiculata*) processed with 'Kanwa' alkaline salt. *Plant Foods Hum. Nutr.*, **40**, 249–258.

Vijayakumari, K., Siddhuraju, P. & Janardhanan, K. (1995). Effect of various water or hydrothermal treatments on certain antinutritional compounds in the seeds of the tribal pulse, *Dolichos lablab* var. *vulgaris* L. *Plant Foods Hum. Nutr.*, **48**, 17–29.

Wheeler, E. L. & Ferrel, R. E. (1971). A method for phytic acid determination in wheat and wheat fractions. *Cereal Chem.*, **48**, 312–320.