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Effect of soaking and heat processing on the levels of antinutrients and digestible proteins in seeds of *Vigna aconitifolia* and *Vigna sinensis*

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The effect of soaking, cooking and autoclaving on the levels of total free phenolics, tannins, phytic acid and *in vitro* protein digestibility (IVPD) were studied in *Vigna aconitifolia* and *Vigna sinensis*. Though soaking significantly reduced the content of tannins alone in *V. aconitifolia*, both total free phenolics and tannins were markedly reduced in *V. sinensis*. Greater loss of total free phenolics as well as tannins occurred under autoclaving compared to soaking and cooking in both the legumes investigated. In *V. aconitifolia*, soaking in distilled water for 6 h and cooking for 30 min reduced the phytic acid content by up to 43%. Maximum reduction in the level of phytic acid (36%) was observed under distilled water soaking compared to cooking and autoclaving in *V. sinensis*. Limited loss in content of phytic acid was noticed under autoclaving compared to soaking and cooking in both the pulses studied. *In vitro* protein digestibility (IVPD) of *V. aconitifolia* and *V. sinensis* was enhanced to 12.5 and 14.8%, respectively, under autoclaving. Of all the processing methods, autoclaving seemed to be the most efficient for reduction in content of the antinutrients, except phytic acid, and improving IVPD in both the pulses.

INTRODUCTION

To alleviate the widely prevailing protein–calorie-malnutrition (PCM) in developing countries, food technologists and nutritionists have been searching for alternative sources of food. *Vigna aconitifolia* is cultivated as a dry land crop and also grows wild throughout India (Babu *et al.*, 1985). The fried seeds are eaten by tribal people such as Kurumbas and Malayalis of Kolli and the Chervarayan hills and low income families in rural areas (Siddhuraju *et al.*, 1994). *Vigna sinensis* is also now cultivated in the upper Gangetic plains of North India. The seeds are eaten by civilized people as curry after soaking in water overnight and mixed with chillies and spices (Jain, 1981). It is noteworthy that the seeds of both *V. aconitifolia* (Siddhuraju *et al.*, 1994) and *V. sinensis* (Rajaram and Janardhanan, 1990) are rich sources of protein, some of the essential amino acids and unsaturated fatty acids.

Utilisation of legumes in human nutrition is constrained due to their inherent antinutritional factors, which have a negative influence on protein digestibility in monogastrics including humans (Liener, 1989). Therefore, much research has been devoted to the inactivation of antinutritional factors by ordinary processing methods such as soaking, cooking and autoclaving. These are simple and inexpensive processing methods which can be carried out to increase the nutritive value of legumes. Keeping this perspective in mind, the present study has been planned to understand to what extent, and by which processing method, the antinutritional compounds in *V. aconitifolia* and *V. sinensis* can be eliminated/reduced. Furthermore, the effect of each treatment on *in vitro* protein digestibility has also been assessed.

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MATERIALS AND METHODS

Seed samples

Mature seeds of *Vigna aconitifolia* (Jacq.) Marechal and *V. sinensis* (L.) Savi. ex. Hassk were collected from Vanavasi, Salem District in Tamil Nadu and Allahabad City in Uttar Pradesh, India, respectively. Soon after collection the seeds were dried for 2 days in open

sunlight and stored in plastic containers at room temperature (25°C).

Soaking

Whole seeds of *V. aconitifolia* and *V. sinensis* were soaked in distilled water and 0.02% (w/v) sodium bicarbonate ($NaHCO_3$) solution (pH 8.6) for 2, 4 and 6 h in the bean water ratio of 1:10 (w/v). The soaked solution was drained off, then the seeds were dried at 55°C.

Cooking

Another set of seeds of *V. aconitifolia* and *V. sinensis* were cooked in distilled water (100°C) in the ratio of 1:10 (w/v) for 10, 20 and 30 min and 20, 40 and 60 min, respectively. The cooked seeds were rinsed with distilled water and dried at 55°C.

Autoclaving

Separate batches of seeds of *V. aconitifolia* and *V. sinensis* were autoclaved at 15lb pressure (121°C) in distilled water (1:10 w/v) for 7, 15 and 20 min and 10, 20 and 30 min, respectively. The seeds were rinsed with distilled water and dried at 55°C.

All the processed as well as raw seeds were powdered in a Wiley Mill to 60 mesh size.

Determination of total free phenolic levels

The content of total free phenolics in the raw and processed samples was estimated by the method of Sadasivam and Manickam (1992) using a Spectronic 20D spectrophotometer at 650 nm. 1 g of air-dried raw and all processed seed flours were extracted with 50 ml of 1% (v/v) HCl in methanol. The samples were shaken on a reciprocating shaker for 24 h at room temperature. The contents were centrifuged at 10 000 *g* for 5 min. From the supernatants, total free phenolics and tannins were estimated. 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).

Determination of tannin content

The tannin content of both raw and treated seeds was determined by the vanillin–HCl method of Burns (1971). The vanillin reagent will react with any phenol that has an unsubstituted resorcinol or phloroglucinol nucleus and forms a coloured substitute product which is measured at 500 nm.

Determination of phytic acid content

The Wheeler and Ferrel (1971) method was used for the determination of phytic acid content. Phytic acid was extracted from 3 g seed flour with 50 ml of 3% (w/v) trichloroacetic acid (TCA) by shaking at room temperature followed by high speed centrifugation. The phytic acid in the supernatant was precipitated as ferric phytate by adding excess ferric chloride and centrifuged. The ferric phytate was converted to ferric hydroxide with a few ml of water and 3 ml of 1.5 N NaOH. The volume was brought to approximately 30 ml with distilled water and it was heated in a boiling water bath. The contents were filtered. Then the precipitate was dissolved with 40 ml of hot 3.2 N nitric acid and made up to a known volume. 5 ml of this aliquot was taken in a volumetric flask and diluted to approximately 70 ml. To this, 20 ml of 1.5 M potassium thiocyanide was added and diluted to 100 ml. Then the absorbance was immediately measured at 480 nm in a Spectronic 20 D spectrophotometer (using $Fe(NO_3)_3$ as standard). Then the iron content present in the sample was estimated. The phytate phosphorus was calculated from the iron results assuming a 4:6 iron: phosphorus molecular ratio. The phytic acid was estimated by multiplying the amount of phytate phosphorus by the factor 3.55 based on the empirical formula $C_6P_6O_{24}H_{18}$.

In vitro protein digestibility (IVPD)

The in vitro protein digestibility of raw and processed seed samples was performed according to the modified multienzyme technique of Hsu *et al.* (1977). Ten ml of distilled water was added to the powdered sample (the amount of sample was adjusted so as to contain 6.25 mg protein ml^{-1}) and hydrated for 1 h at 5°C. The three enzyme solutions (trypsin, chymotrypsin and peptidase procured from Sigma chemical Co., St Louis, USA) and sample were adjusted to pH 8.0 at 37°C. One ml of the three enzyme mixed solution was added to the sample and stirred well. After 10 min, 1 ml of bacterial protease solution (procured from Sigma chemical Co., St Louis, USA) was added and transferred to water bath maintained at 55°C immediately. After 9 min the solution was again transferred to water bath maintained at 37°C. Then the pH of the hydrolysate was measured. *In vitro* protein digestibility was calculated using the following formula

$$\% \text{ digestibility} = 234.84 - 22.56x$$

where x was the pH after 20 min of incubation. The principle involved in this method is that four proteolytic enzymes are used to digest the protein and the pH change was due to the release of amino acids at a fixed time interval.

Statistical analysis

The data were statistically analysed using Duncan's Multiple Range Test (DMRT) by the method of Alder and Roessler (1977).

RESULTS AND DISCUSSION

It is well evident that the nutritional importance of a given food/feedstuff in a diet depends not only on the nutrient composition of the raw feedstuff, but also on the amount that is utilised as well as the presence of antinutritional factors. Hence elimination or inactivation of antinutritional compounds is absolutely necessary to improve the nutritional quality of legumes and effectively utilize their full potential as human food.

The tannin causes a decrease in the digestibility of protein and carbohydrates as a result of the formation of insoluble enzyme-resistant complexes (Reddy *et al.*, 1985). Deshpande *et al.* (1986) reported that polyphenols could react with proteins and enzymes so they could also act as trypsin inhibitors and amylase inhibitors. Raw seeds of *V. aconitifolia* contain fairly high levels of total free phenolics (Table 1) compared to an earlier report in different species of the genus, *Vigna* (Rajaram and Janardhanan, 1990; Siddhuraju *et al.*, 1992, 1994), whereas the seeds of *V. sinensis* exhibit comparable levels of total free phenolics to that of different species of *Vigna* (Rajaram and Janardhanan, 1990; Siddhuraju *et al.*, 1992). The levels of tannin in both the species of the present study (Table 1) seem to be higher compared with those of some of the commonly cultivated pulse crops (Giami, 1993).

The phytate molecule is negatively charged at physiological pH and is reported to bind nutritionally important essential divalent cations, such as iron, zinc, magnesium and calcium. This forms insoluble complexes, thereby making minerals unavailable for absorption (Rimbach *et al.*, 1994). The seeds of both *V. aconitifolia* and *V. sinensis* are known to contain lower levels of phytic acid than various varieties of green gram and black gram (Kaur and Kapoor, 1992) and chickpea and pigeonpea (Chitra *et al.*, 1995).

Soaking

The effect of soaking on the levels of total free phenolics, tannins and phytic acid of *V. aconitifolia* and *V. sinensis* are given in Table 1. Insignificant loss of total free phenolics in *V. aconitifolia* has been noticed while soaking in distilled water (13%). More or less the same percentage loss of total free phenolics has been observed earlier during soaking in black gram (Kataria *et al.*, 1988) and green gram (Kataria *et al.*, 1989). Tannin content of *V. aconitifolia* gets reduced significantly by either distilled water or $NaHCO_3$ solution soaking. In contrast, soaking for 4 h enhanced the tannin level significantly. This accords with an earlier study in *Dolichos lablab* var. *vulgaris* (Vijayakumari *et al.*, 1995). The increase in content observed may be due to the degradation of high molecular weight insoluble polymer into small molecular weight soluble polymers that give a colour reaction with the reagent (Satwadhar *et al.*, 1981). Soaking significantly (more than 50%) reduces the content of total free phenolics and tannins in *V. sinensis*. This accords with an earlier investigation in different varieties of *Pisum sativum* (Bishnoi *et al.*, 1994) and *Vicia faba* (Sharma and Sehgal, 1992).

In general, the contents of total free phenolics and tannin in both the presently investigated pulses are reduced to the maximum extent when soaked in salt water ($NaHCO_3$ solution) compared to distilled water. This accords with an earlier report in *Dolichos lablab* var. *vulgaris* (Vijayakumari *et al.*, 1995). The marked loss of polyphenolics when subjected to soaking in $NaHCO_3$ may be attributed to its effect in creating an ionic environment. The changed ionic environment in turn might change the seed coat permeability. This enables greater and rapid solid losses (Mulimani and Supriya, 1994).

During soaking, more than 40% reduction in content of phytic acid has been noticed in *V. aconitifolia* which

Table 1. Effect of soaking on the levels of certain antinutrients in two species of *Vigna* seeds*

Treatment	*Vigna aconitifolia*			*Vigna sinensis*		
	Total free phenolics (g per 100 g of seed flour	Tannins (g per 100 g of seed flour)	Phytic acid (g per 100 g of seed flour)	Total free phenolics (g per 100 g of seed flour)	Tannins (g per 100 g of seed flour)	Phytic acid (g per 100 g of seed flour)
Raw seeds	1.36^{a}	0.14^{a}	0.48^{a}	0.72^{a}	0.19^{a}	0.32^{a}
Seeds soaked in distilled water						
2 h	$1.23^{a}(-10)$	$0.12^{b}(-18)$	$0.41^{b}(-15)$	$0.41^{b}(-43)$	$0.16^{a}(-16)$	$0.29^{b}(-9)$
4 h	$1.2^{a}(-11)$	$0.15^{c}(+7)$	$0.33^{c}(-31)$	$0.37^{b}(-49)$	$0.11^{b}(-42)$	$0.25^{c}(-22)$
6 h	$1.18^{a}(-13)$	$0.11^{b}(-20)$	$0.27^{d}(-44)$	$0.36^{b}(-50)$	$0.09^{b}(-53)$	$0.20^{d}(-38)$
Seeds soaked in sodium bicarbonate solution						
2 h	$1.29^{ab}(-5)$	$0.10^{b}(-29)$	$0.42^{b}(-13)$	$0.34^{b}(-54)$	$0.16^{a}(-16)$	$0.27^{b}(-16)$
4 h	$1.19^{ab}(-13)$	$0.20^{c}(+43)$	$0.35^{c}(-27)$	$0.31^{b}(-57)$	$0.11^{b}(-42)$	$0.25^{c}(-22)$
6 h	$1.08^{b}(-21)$	$0.06^{d}(-57)$	$0.28^{d}(-42)$	$0.26^{b}(-64)$	$0.05^{c}(-74)$	$0.22^{d}(-32)$

*All values are averages of three determinations.
Mean values in the column sharing different letters are statistically different ($p < 0.05$).
Values in parentheses indicate per cent loss/gain from raw seeds.

is in line with an earlier report of Khokhar and Chauhan (1986) on the same legume. The percentage loss of phytic acid in both the pulses investigated seems to be higher in distilled water than in salt water ($NaHCO_3$ solution). This confirms the results of Khan *et al.* (1988) in white variety of *Cicer arietinum*. Vijayakumari *et al.* (1995) investigating *Dolichos lablab* var. *vulgaris*, have reported that the loss of phytic acid is less in the presence of $NaHC0_3$. In contrast, Deshpande and Cheryan (1983) have observed that a greater reduction in phytic acid content was attained by soaking beans in 2% (w/v) $NaHCO_3$ than distilled water soaking for 12 h, but the difference could be attributed to the difference in the concentration of $NaHCO_3$. The loss of phytic acid during soaking in legumes may be attributed to the activity of phytase and diffusion.

Cooking

The effects of cooking on the levels of total free phenolics, tannin and phytic acid of *V. aconitifolia* and *V. sinensis* are shown in Table 2. The total free phenolics content of *V. aconitifolia* gets reduced to 35% when cooked for 30 min. This is in fair agreement with that of the previous findings of Vijayakumari *et al.* (1995) in *Dolichos lablab* var. *vulgaris* and Vijayakumari *et al.* (1997) in *Prosopis chilensis*. In *V. sinensis*, significant reduction in content of total free phenolics occurs when subjected to cooking for 60 min. This accords with earlier reports on mungbean (Barroga *et al.*, 1985) and pea (Bishnoi *et al.*, 1994). The tannin content of *V. sinensis* has also been significantly reduced under the same processing method (79%). More or less equal levels of loss have been observed in two different varieties of *Vicia faba* (Sharma and Sehgal, 1992). Since the polyphenolics are water soluble in nature their loss may be due to leaching out into the cooking medium or heat-degradation of tannin molecules (Uzogara *et al.*, 1990; Bakr and Gawish, 1991).

Seeds of *V. aconitifolia* and *V. sinensis* of the present study exhibit 43% and 29% of phytic acid loss, respectively, under cooking. The loss of phytic acid in *V. sinensis* is more or less comparable to that of previous investigations (Uzogara *et al.*, 1990; Sharma and Sehgal, 1992). The apparent decrease in content of phytic acid 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.

Autoclaving

Significant reduction in content of total free phenolics has been observed under autoclaving in *V. aconitifolia* (92%) and *V. sinensis* (82%) (Table 2). This accords with the previous report of Neerjarani and Hira (1993) in *Vicia faba*. Tannin content of *V. sinensis* also gets reduced significantly when subjected to autoclaving for 30 min. The loss of phenolics under autoclaving may also be due to the interaction of polyphenolics with other components of seeds such as protein to form insoluble tannin protein complexes (Babar *et al.*, 1988).

Autoclaving seems to exert less effect on destruction of phytic acid compared with cooking in both the species of *Vigna* presently investigated. Ologhobo and Fetuga (1984) and Uzogara *et al.* (1990) have also observed that autoclaving causes less loss of phytic acid compared to cooking. The results of the present study reveal that a long time is required for destroying phytates. So possibly a greater time interval would cause a pronounced effect on the destruction of phytate. This observation is in agreement with an earlier investigation (Sharma and Sehgal, 1992).

In vitro protein digestibility (IVPD)

The IVPD of raw and processed seeds of *V. aconitifolia* and *V. sinensis* is shown in Table 3. The raw seeds of

Table 2. Effect of cooking and autoclaving on the levels of certain antinutrients in two species of *Vigna* seeds*

Treatment	*Vigna aconitifolia*			Treatment	*Vigna sinensis*		
	Total free phenolics (g per 100 g of seed flour	Tannins (g per 100 g of seed flour)	Phytic acid (g per 100 g of seed flour)		Total free phenolics (g per 100 g of seed flour)	Tannins (g per 100 g of seed flour)	Phytic acid (g per 100 g of seed flour)
Raw seeds	1.36[a]	0.14[a]	0.48[a]	Raw seeds	0.72[a]	0.19[a]	0.32[a]
Cooked				Cooked			
10 min	1.08[b](−21)	0.11[b](−21)	0.45[b](−6)	20 min	0.41[b](−43)	0.07[b](−66)	0.29[b](−9)
20 min	1.01[b](−26)	0.11[b](−21)	0.33[c](−31)	40 min	0.38[c](−47)	0.05[b](−74)	0.24[c](−25)
30 min	0.88[c](−35)	0.09[c](−36)	0.27[d](−44)	60 min	0.30[c](−58)	0.04[b](−79)	0.22[d](−31)
Autoclaved				Autoclaved			
7 min	0.94[b](−31)	0.09[b](−36)	0.46[b](−4)	10 min	0.52[b](−28)	0.15[b](−21)	0.30[b](−6)
15 min	0.60[c](−56)	0.09[b](−36)	0.40[c](−17)	20 min	0.36[b](−50)	0.11[c](−42)	0.29[b](−9)
20 min	0.11[d](−92)	0.05[c](−64)	0.36[d](−25)	30 min	0.13[c](−82)	0.04[d](−79)	0.28[c](−13)

*All values are averages of three determinations.
Mean values in the column sharing different letters are statistically different ($p < 0.05$).
Values in parentheses indicate per cent loss from raw seeds.

Table 3. *In vitro* protein digestibility in raw and processed seeds of two species of *Vigna*

Treatment	Protein digestibility percentage	Percentage increase of protein digestibility
Vigna aconitifolia		
Raw seeds	72.4[a]	—
Soaked in distilled water for 6 h	72.4[a]	0
Soaked in $NaHCO_3$ solution for 6 h	74.7[a]	3.2
Cooked in boiling water for 30 min	79.2[b]	9.4
Autoclaved for 20 min	81.4[b]	12.5
Vigna sinensis		
Raw seeds	61.1[a]	—
Soaked in distilled water for 6 h	61.1[a]	—
Soaked in $NaHCO_3$ solution for 6 h	63.7[a]	4.2
Cooked in boiling water for 60 min	67.9[b]	11.1
Autoclaved for 30 min	70.2[b]	14.8

Mean values in the column sharing different letters are statistically different ($p < 0.05$).

V. aconitifolia exhibit 72% IVPD, which is comparable to the literature value of 71% as reported by Satwadhar *et al.* (1981) in the same pulse. Soaking does not seem to enhance the IVPD of either of the pulses reported in the present study. Cooking gives more protein digestibility than soaking (9.35% and 11.07% improvement in *V. aconitifolia* and *V. sinensis*, respectively). A Similar percentage improvement of IVPD under cooking has been noticed previously in *Dolichos lablab* var. *vulgaris* (Vijayakumari *et al.*, 1995). In both *V. aconitifolia* and *V. sinensis*, protein digestibility of autoclaved sample is significantly higher than their raw and soaked seeds (12 and 14%, respectively). Rayas Duarte *et al.* (1988) and Wu *et al.* (1994) in *Phaseolus vulgaris* reported that processed/autoclaved beans registered IVPD value of 82.3–83.7%. Our findings are in conformity with these. Antinutritional factors in raw beans apparently inhibit enzymatic digestion of protein resulting in lower protein digestibility values than in heated beans (Nielson, 1991). Higher protein quality after heat-treatment may be due to increased accessibility of the protein to enzymatic attack (Wu *et al.*, 1994) and structural disintegration of enzyme inhibitors (Vijayakumari *et al.*, 1995).

This study has shown that autoclaving seems to be responsible for the maximum reduction of antinutrients except phytic acid and the highest value of protein digestibility in both the pulses. So, among the various treatments attempted, autoclaving can be considered as the best processing method for *V. aconitifolia* and *V. sinensis* seeds. After conducting suitable animal feeding experiments, the grains of *V. aconitifolia* and *V. sinensis* may be advocated as an alternate source of low cost protein which might aid eradication of protein–calorie malnutrition in developing countries such as India.

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