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Effect of soaking and hydrothermal processing methods on the levels of antinutrients and *in vitro* protein digestibility of *Bauhinia purpurea* L. seeds

K. Vijayakumari [a], M. Pugalenthi [b,*], V. Vadivel [b]

[a] *Department of Botany, Vellalar College for Women, Erode 638 009, Tamil Nadu, India*
[b] *Department of Biotechnology, Karpagam Arts and Science College, Coimbatore 641 021, Tamil Nadu, India*

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Abstract

The effects of various domestic processing methods such as soaking, cooking and autoclaving on the levels of certain antinutritional factors and *in vitro* protein digestibility of seeds of *Bauhinia purpurea* L., an underutilised legume collected from South India, were investigated. The raw seeds were found to contain antinutritional factors like total free phenolics (2.75 g/100 g), tannins (2.35 g/100 g), phytic acid (692 mg/100 g) and flatulence factors, raffinose (0.54 g/100 g), stachyose (1.17 g/100 g) and verbascose (0.95 g/100 g). Soaking the seeds in distilled water caused maximum reduction in the phytic acid content (37%), whereas soaking in $NaHCO_3$ solution reduced significant levels of phenolics and tannins (72% and 78%, respectively). A reduction in the levels of oligosaccharides (raffinose by 63%, stachyose by 42% and verbascose by 79%) was observed during cooking. Of the attempted treatments, autoclaving appeared to be most effective in reducing levels of all the investigated antinutrients, except phytic acid, and also improved the *in vitro* protein digestibility of *B. purpurea* seeds.

Keywords: *Bauhinia purpurea*; Antinutritional factors; Processing methods; *In vitro* protein digestibility

1. Introduction

One of the serious problems facing most of the developing countries is the scarcity of food for the teeming human population and feed for the dwindling livestock industries (Carlini & Udedibie, 1997). Although conventional legumes have been playing a key role as a food and feedstuff in most of these countries, their production is not enough to meet the requirements of the increasing population and animal feed industries (Siddhuraju & Becker, 2003). Hence, there is a need for exploitation of hitherto neglected underutilised legumes (Agbede & Aletor, 2005; Janardhanan, Vadivel, & Pugalenthi, 2003). In this context, *Bauhinia purpurea* L., a promising tropical underutilised legume having good nutritional properties, merited the attention.

B. purpurea is distributed throughout India, especially in the forests of the Deccan plateau, the Western Himalayas and the Khasi hills (Janardhanan et al., 2003). Cooked young pods and seeds were eaten by certain ethnic groups, particularly the Garo, Naga, Khatkharis and Gonds living in northeastern and central India (Jain, 1981; Vijayakumari, Siddhuraju, & Janardhanan, 1997a). It exhibits many favourable agrobotanical traits such as fast vegetative growth, early flowering, a high fertility index with high pod weight (8.3 g) and number of seeds per pod (8–9) (Rajaram & Janardhanan, 1991). The plant parts are used in indigenous medicine for curing body pain, fever, cancerous growths in the stomach and indigestion (Janardhanan et al., 2003).

* Corresponding author. Tel.: +91 422 2611146/2611082; fax: +91 422 2611043.
E-mail address: pugalmuthiah@rediffmail.com (M. Pugalenthi).

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The seeds of this legume are a rich source of crude protein (25.6–27.2%), crude lipid (12.3–14.3%), fibre (4.6–5.8%), carbohydrates (51%), minerals and essential amino acids (Rajaram & Janardhanan, 1991; Vijayakumari et al., 1997a). Unsaturated fatty acids, comprise 66% of the total fatty acid content which is also a desirable nutritional property.

Although legumes constitute one of the most abundant and least expensive sources of protein in human/animal diets, their utilisation is limited largely due to the presence of antinutritional/antiphysiological compounds (Liener, 1994a). The raw seeds of *B. purpurea* exhibit low biological value, however, heat treatments could significantly improve the true protein digestibility and net protein utilisation of this wild legume (Vijayakumari et al., 1997a). The seeds of *B. purpurea* are also reported to contain many antinutritional compounds such as free phenolics, tannins, phytic acid, L-dopa, protease inhibitors, lectins, hydrogen cyanide, saponins and oligosaccharides (Rajaram & Janardhanan, 1991; Vijayakumari et al., 1997a; Young, Watson, & Williams, 1985).

Information on the nutritional quality and antinutritional factors of the presently investigated *B. purpurea* seeds is available (Rajaram & Janardhanan, 1991; Vijayakumari et al., 1997a). However, information regarding the effect of processing methods on the antinutritional factors and improvement of the *in vitro* protein digestibility of *B. purpurea* seeds appears to be lacking. Hence, the present study was carried out to analyse the effects of certain simple, cost-effective processing methods, particularly those used by Indian village people, on the levels of antinutritional compounds and their impact on *in vitro* protein digestibility of *B. purpurea* seeds.

2. Materials and methods

2.1. Collection of the seed sample

The seeds of *B. purpurea* L. were collected from Bharathiar University Campus, Coimbatore Tamil Nadu, India. Soon after collection, after removing immature and damaged seeds, the mature seeds were dried in direct sunlight for 2 days and stored in plastic containers at room temperature (25 °C) until further use.

2.2. Chemicals

The chemicals used were purchased from Merck (Darmstadt, Germany) or Sigma Chemical Company, (St. Louis, MO), throughout the study, unless otherwise specified.

2.3. Processing methods

2.3.1. Soaking

Whole seeds of *B. purpurea* were soaked in distilled water or 0.02% (w/v) sodium bicarbonate ($NaHCO_3$) solution (pH 8.6) for 2, 4 and 6 h in a bean:water ratio of 1:10 (w/v). After soaking, the water was drained off and the seeds were dried at 55 °C for 6 h in a hot air oven.

2.3.2. Cooking

Another set of seeds was cooked in distilled water (100 °C) in a bean:water ratio of 1:10 (w/v) for 20, 40 and 60 min. The cooked seeds were rinsed with distilled water and dried at 55 °C for 6 h in a hot air oven.

2.3.3. Autoclaving

Separate batches of seeds of *B. purpurea* were autoclaved at 15 psi (121 °C) in distilled water in the bean water ratio of 1:10 (w/v) for 10, 20 and 30 min. After treatment, the seeds were rinsed with distilled water and dried at 55 °C for 6 h in a hot air oven.

2.3.4. Preparation of the seed flour

All the processed and raw seeds were powdered in a Willey Mill (Scientific Equipment Works, New Delhi, India) to 60-mesh size with suitable precaution, to avoid contamination of samples. The powdered samples were stored in plastic containers at room temperature (25 °C) until further use.

2.4. Analysis of antinutritional factors

2.4.1. Determination of total free phenolics

Total free phenolics in the raw and processed seed samples were extracted and estimated by following the method of Sadasivam and Manickam (1992). One gram of air-dried seed flour was 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. They were centrifuged at $10{,}000 \times g$ for 5 min and the supernatant was analysed total free phenolics and tannins. The principle involved in this method is that the phenols react with phosphomolybdic acid in Folin–Ciocalteau reagent in alkaline medium and produce a blue-coloured complex (molybdenum blue). The absorbance was measured at 650 nm in a Spectronic 20D spectrophotometer (Thermo Electron Corporation, Waltham, MA). The amount of phenolics present in the sample was determined from a standard curve prepared with catechol. Average values of triplicate estimations were expressed as g 100 g^{-1} of seed flour on a dry weight basis.

2.4.2. Determination of tannin content

The tannin content of both raw and processed seed samples was determined by the method of Burns (1971). From suitable aliquots of the seed extract, tannin content was quantified by the vanillin–HCl method using phloroglucinol as a standard. The vanillin reagent reacts with any phenol that has an unsubstituted resorcinol or phloroglucinol nucleus and forms a coloured substitute product, which is measured at 500 nm in a Spectronic 20 D spectro-

photometer (Thermo Electron Corporation, Waltham, MA).

2.4.3. Determination of phytic acid content

The phytic acid content in both raw and treated seed samples was determined by the method of Wheeler and Ferrel (1971). The phytate phosphorus was calculated from the iron results, assuming a 4:6 iron:phosphorus molecular ratio. The phytic acid content was calculated by multiplying the amount of phytate phosphorous by the factor 3.55 based on the empirical formula $C_6P_6O_2H_{18}$ and expressed as mg 100 g^{-1} seed flour on a dry weight basis.

2.4.4. Determination of oligosaccharides

The oligosaccharides were extracted from both the raw and processed seed samples according to the method of Vijayakumari, Siddhuraju, and Janardhanan (1997b). Seed flour (5 g) was extracted with 25 ml of 80% ethanol at room temperature (25 °C) by continuous shaking. The extraction was repeated thrice and the extracts were pooled and concentrated using a rotary evaporator under vacuum. The residue was made up to 5 ml with deionized water and the sugars were separated by using a descending paper chromatography technique with Whatman No. 1 chromatographic paper and the solvent mixture propanol:ethanol:water in a ration of 7:1:2. A standard sugar mixture containing raffinose, stachyose and verbascose (Sigma–Aldrich) was run simultaneously. After development, *p*-anisidine hydrochloride reagent was sprayed on the paper to reveal the sugar spots. For quantification, the paper (2×2 cm area) corresponding to each oligosaccharide spot was cut from unsprayed papers and eluted with 3 ml of deionised water. The eluted individual oligosaccharides were estimated by the phenol–sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). Values were the average of three determinations and expressed as g 100 g^{-1} on a dry weight basis.

2.5. In vitro protein digestibility (IVPD)

The IVPD of both the raw and processed seed samples was measured according to the multienzyme technique (Hsu, Vavak, Satterlee, & Miller, 1977), and was calculated by using the following equation:

$$y = 210.464 - 18.103x$$

where y is the percentage of protein digestibility and x is the pH of the protein suspension after 10 min digestion with a four enzyme solution.

2.6. Statistical analysis

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

3. Results and discussion

3.1. Antinutritional factors and IVPD of raw seeds

The results of analysis of antinutritional factors in the raw seeds of *B. purpurea* were given in Table 1. The raw seeds were found to contain fairly high levels of total free phenolics (2.25 g/100 g) and low levels of tannins (2.35 g/100 g) when compared to the earlier report in the same species (Rajaram & Janardhanan, 1991; Vijayakumari et al., 1997a) and low levels of tannins (2.35 g/100 g); (Rajaram & Janardhanan, 1991). The total free phenolics content of *B. purpurea* in the present study was found to be lower than in non-conventional legumes like *Mucuna pruriens* var. *pruriens* (7.7 g/100 g), *M. pruriens* var. *utilis* (6.1 g/100 g) and *Sesbania cannabina* (3 g/100 g) (Adebowale, Adeyemi, & Oshodi, 2005; Siddhuraju & Becker, 2001a; Siddhuraju, Osoniyi, Makker, & Becker, 2002; Vadivel & Janardhanan, 2005) and the tannin content was also lower than the values reported in *M. pruriens* (7.8 g/100 g) (Agbede & Aletor, 2005). However, the level of both phenolics and tannins of *B. purpurea* was higher than some cultivated legumes (Bishnoi, Khetarpaul, & Yadav, 1994; Giami, 1993). The higher content of total free phenolics and tannins is known to inhibit the activity of the digestive enzymes and, thus, interfere with the digestion and absorption of dietary proteins, carbohydrates, minerals and other nutrients, such as vitamin B_{12}. They may also cause damage to the mucosa of the digestive tract (Liener, 1994a) and hence, they are undesirable for human consumption from a nutritional point of view.

The phytate molecule is negatively charged at physiological pH and is reported to bind with essential, nutritionally important divalent cations such as Fe, Zn, Mg and Ca, etc., and forms insoluble complexes, thereby making minerals unavailable for absorption (Rimbach, Inglmann, & Pallauf, 1994). It also formed complexes with proteins and starch and inhibits their digestion (Oatway, Vasanthan, & Helm, 2001). The phytic acid content of *B. purpurea* (692 mg/100 g) is lower than common legumes like green gram, chickpea, pigeon pea, mung bean, urd bean, *Phaseolus angularis* and *P. calcaratus* (937–1750 mg/100 g) (Chau & Cheng, 1997; Chitra, Vimala, Singh, & Geervani, 1995; Kaur & Kapoor, 1992) and the non-conventional legume, *Mucuna* (1.2–2.5 g/100 g) (Adebowale et al., 2005; Siddhuraju & Becker, 2001a) but higher than cow pea (115–210 mg/100 g) (Giami, 2005).

Ingestion of large quantities of beans is known to cause flatulence in humans and animals. The oligosaccharides of the raffinose family (raffinose, stachyose and verbascose) from beans have been identified as one of the important contributors to flatulence in human beings. Monogastric animals cannot hydrolyse these oligosaccharides, due to the lack of α-galactosidase activity in the small intestine. The microbes in the large intestine utilise these α-galactosides and produce flatus gases, abdominal rumbling, diarrhoea and discomfort (Liener, 1994b). Among the

Table 1
Effect of soaking in distilled water and $NaHCO_3$ solution on the levels of certain antinutrients in the seeds of *Bauhinia purpurea* L

Treatment	Antinutritional factors[a]											
	Total free phenolics (g/100 g)	% loss	Tannins (g/100 g)	% loss	Phytic acid (mg/100 g)	% loss	Oligosaccharides (g/100 g)					
							Raffinose	% loss	Stachyose	% loss	Verbascose	% loss
Raw seeds	2.75 a	–	2.35 a	–	692.18 a	–	0.54 a		1.17 a		0.95 a	–
Seeds soaked in distilled water												
2 h	1.15 b	58	0.84 b	64	611.45 b	12	0.53 b	2	1.14 a	3	0.90 b	5
4 h	1.07 bc	61	0.74 c	69	507.10 c	27	0.49 ab	9	1.11 ab	5	0.84 c	12
6 h	0.95 c	65	0.68 c	71	436.07 d	37	0.46 b	15	1.05 b	10	0.77 d	19
Seeds soaked in $NaHCO_3$ solution												
2 h	1.08 b	61	0.73 b	69	653.98 b	6	0.50 ab	7	1.10 b	6	0.90 b	5
4 h	0.86 c	69	0.58 c	75	590.39 c	15	0.47 bc	13	1.07 ab	9	0.80 c	16
6 h	0.76 c	72	0.51 d	78	505.29 d	27	0.44 c	19	0.99 d	15	0.70 d	26

Mean values in same columns sharing different letters are statistically different ($p < 0.05$).
[a] All values are averages of three determinations.

Table 2
Effect of cooking and autoclaving on the levels of certain antinutrients in the seeds of *Bauhinia purpurea* L

Treatment	Antinutritional factors[a]											
	Total free phenolics (g/100 g)	% loss	Tannins (g/100 g)	% loss	Phytic acid (mg/100 g)	% loss	Oligosaccharides (g/100 g)					
							Raffinose	% loss	Stachyose	% loss	Verbascose	% loss
Raw seeds	2.75 a	–	2.35 a	–	692.18 a	–	0.54 a	–	1.17 a	–	0.95 a	–
Cooking in water												
20 min	1.18 b	57	0.93 b	60	644.57 b	7	0.50 b	7	0.96 b	18	0.84 b	12
40 min	1.03 bc	63	0.81 c	66	559.14 c	19	0.39 c	28	0.79 c	32	0.43 c	52
60 min	0.92 c	67	0.72 c	69	491.45 d	29	0.20 d	63	0.68 d	42	0.20 d	79
Autoclaving												
10 min	1.84 b	33	0.82 b	65	623.29 b	10	0.43 b	20	0.95 b	19	0.74 b	22
20 min	0.97 c	65	0.65 c	72	570.45 c	18	0.24 c	56	0.69 c	41	0.33 c	65
30 min	0.49 d	82	0.46 d	80	546.82 d	21	0.10 d	81	0.35 d	70	0.20 d	79

Mean values in same columns sharing different letters are statistically different ($p < 0.05$).
[a] All values are averages of three determinations.

oligosaccharides, stachyose is found to induce more flatulence than the other oligosaccharides (Mulimani & Devendra, 2000), and is present at high levels in the presently investigated *B. purpurea* seeds (1.19 g/100 g), which is followed by verbascose (0.95 g/100 g) and raffinose (0.54 g/100 g). These results are similar to those in cowpea (Onigbinde & Akinyele, 1983) but lower than *M. pruriens* var. *utilis* (Siddhuraju & Becker, 2001b). The verbascose level (0.9 g/100 g) of *B. purpurea* is very low when compared to *Cajanus cajan* and *M. pruriens* var. *utilis* (4–9.3 g/100 g) (Janardhanan, Gurumoorthi, & Pugalenthi, 2003; Mulimani & Devendra, 2000). The stachyose and verbascose levels of *B. purpurea* were similar but the raffinose content was lower when compared with levels in *M. pruriens* (1.2, 0.9 and 1.6, respectively) (Adebowale et al., 2005).

In vitro protein digestibility of raw seeds of *B. purpurea* is shown in Table 3. Raw seeds of *B. purpurea* exhibit 70% IVPD, which is comparable to that of many common legumes like pigeon pea (69%), mung bean (67%), soya bean (71%) and *Phaseolus angularis* (69%) and the wild legume *M. pruriens* var. *utilis* (68–69%) and higher than rice bean (58%), faba bean (53%) and urd bean (56%) (Chau & Cheng, 1997; Chitra et al., 1995; Saharan, Khetarpaul, & Bishnoi, 2002; Siddhuraju & Becker, 2001a). The relatively low protein digestibility of raw legume seeds may be due to the presence of antinutritional compounds and their structural characteristics. Numerous studies have indicated that globulin is the major storage protein, which is quite resistant to attack by proteolytic enzymes (Deshpande, 1992). Antinutritional factors in raw beans may also inhibit the enzymatic digestion of protein, resulting in lower protein digestibility values than in processed beans (Nielson, 1991).

3.2. Effect of soaking in distilled water

The results of soaking the seeds in distilled water on the levels of antiphysiological substances are shown in Table 1. The percentages of reduction in the content of total free phenolics and tannins of *B. purpurea* during soaking in distilled water were higher than the values reported in *M. pruriens* (Vijayakumari, Siddhuraju, & Janardhanan, 1996). Significant reductions ($p < 0.05$) were observed in the levels of phenolics and tannins during the first 2–4 h of soaking in distilled water. Thereafter, prolonging the soaking time did not cause any significant reduction. Since polyphenolic compounds are water-soluble in nature and mostly located in the seed coat, the decrease in the level of phenolics and tannins during soaking may be attributed to leaching into the soaking medium (Vijayakumari et al., 1997b).

The maximum reduction in the level of phytic acid content (37%) was observed when the seeds of *B. purpurea* were soaked in distilled water for 6 h, which is in agreement with the results in *M. pruriens* and *Vigna aconitifolia* (Vijayakumari et al., 1996; Vijayakumari, Siddhuraju, Pugalenthi, & Janardhanan, 1998) and higher than for cowpea (28%) (Ologhobo & Fetuga, 1984). The percentage of reduction in the phytic acid content increased by increasing the soaking time up to 6 h. The loss in the phytic acid content is mainly due to leaching and is particularly favoured when the compounds possess low molecular weight and ionic character (Siddhuraju & Becker, 2001a). The removal of phytic acid during soaking has also been attributed to the degradation of phytate molecule followed by diffusion of the phytase enzyme, which is activated in the seeds due to imbibition (Vijayakumari et al., 1997b).

The effect of soaking on the levels of oligosaccharides in *B. purpurea* is shown in Table 1. Although oligosaccharides are water-soluble, soaking the seeds in distilled water resulted in a limited reduction in the flatulence factors, regardless of the soaking time. These results are in agreement with earlier studies in *Dolichos lablab*, *M. pruriens* var. *utilis*, *Lathyrus sativus* and cowpea (Revilleza, Mendoza, & Raymundo, 1990; Siddhuraju & Becker, 2001b; Somiari & Balough, 1993; Vijayakumari, Siddhuraju, & Janardhanan, 1995). The loss of sugars during soaking was influenced by two factors, the solubility of the individual oligosaccharides and the diffusion rate (Upadhyay & Garcia, 1988). The diffusion rate in turn would depend on the thickness and permeability of the seed coat, which may limit the sugar losses in *B. purpurea* seeds because the mature seeds have a hard and thick seed coat.

Even though the soaking treatment (soaking the seeds for 6 h in distilled water) is effective in reducing significant levels of tannins and phytic acid content, it does not cause any improvement in the protein digestibility of *B. purpurea* seeds, which is in agreement with an earlier study in *Vigna aconitifolia* (Vijayakumari et al., 1998). But in some cases like *Prosopis chilensis* and *Dolichos lablab* var. *vulgaris,* an improvement in the IVPD (3%) was recorded (Vijayakumari et al., 1995; Vijayakumari et al., 1997b).

Table 3
In vitro protein digestibility of raw and processed *Bauhinia purpurea* L. seeds

Treatment	*In vitro*protein digestibility[a] (%)	% increase in protein digestibility
Raw seeds	70.16 a	–
Seeds soaked in distilled water for 6 h	70.16 a	0
Seeds soaked in $NaHCO_3$ solution for 6 h	74.66 b	6
Seeds cooked in boiling water for 60 min	79.18 c	13
Seeds autoclaved for 30 min	81.44 c	16

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

[a] All values are averages of three determinations.

3.3. Effect of soaking in $NaHCO_3$ solution

Soaking the seeds in $NaHCO_3$ solution for 6 h reduced the level of phenolics (72%) and tannins (78%) to a greater degree than soaking in distilled water (Table 1). The con-

tent of phenolics and tannins was decreased with an increase in soaking time. The decrease in the level of phenolics and tannins during soaking in $NaHCO_3$ solution may be attributed to leaching to the soaking medium under the influence of concentration gradient (Saharan et al., 2002) or due to solubilisation of those compounds in alkaline solution. Such losses may also have been a function of increased permeability of the seed coat, due to the alkaline environment (Siddhuraju & Becker, 2001a).

Soaking in $NaHCO_3$ solution was less efficient in reducing the phytic acid content (27%), than water (37%). Similar results were observed in *Cicer arietinum* and *Dolichos lablab* var. *vulgaris* (Khan, Zaman, & Elahi, 1988; Vijayakumari et al., 1995). Conversely, in a study by Deshpande and Cheryan (1983), *Phaseolus vulgaris* soaked in 2% $NaHCO_3$ solution for 12 h displayed a marked reduction in phytic acid content. That may be due to the concentration of $NaHCO_3$ used by them and/or the soaking time; inherent differences in testa and membrane characteristics among pulse species may also have contributed to the disparate results. The level of reduction in phytic acid in *B. purpurea* during soaking in $NaHCO_3$ solution is greater than the losses reported in *Mucuna pruriens* var. *utilis* and *Lathyrus sativus* (Janardhanan et al., 2003; Siddhuraju & Becker, 2001a; Srivastava & Khokhar, 1996).

The reduction in oligosaccharides content during the soaking of the seeds in $NaHCO_3$ solution is slight but higher than that due soaking in distilled water. The loss of raffinose (19%), stachyose (15%) and verbascose (26%) was observed when soaking the seeds for 6 h. These results are similar to those in an earlier study in *M. pruriens* var. *utilis* (Siddhuraju & Becker, 2001b). The loss of oligosaccharides during soaking in $NaHCO_3$ solution may partly be due to leaching into the medium because of change in the permeability of the seed coat caused by the ionic strength of the soaking medium.

Soaking in $NaHCO_3$ solution is effective in significantly reducing levels of total free phenolic and tannins content, compared to other processing methods and it improves the protein digestibility of *B. purpurea* seeds by 6%. The percentage of improvement in IVPD of *B. purpurea* seeds during soaking in $NaHCO_3$ solution is higher than that reported in *Vigna aconitifolia* (3.2%) and *V. sinensis* (4.2%) but lower than in *Prosopis chilensis* (9.9%) (Vijayakumari et al., 1997b; Vijayakumari et al., 1998). Conversely, Vijayakumari et al. (1995) reported nil improvement in the IVPD of *Dolichos lablab* var. *vulgaris*.

3.4. Effect of cooking

The results of the effect of cooking on the levels of investigated antinutritional factors of *B. purpurea* are shown in Table 2. Cooking the seeds in distilled water for 60 min resulted in the loss of 67% of phenolics and 69% of tannins. Cooking eliminated phenolics and tannins at significant levels ($p < 0.05$) up to 40 min and thereafter, there was no significant reduction. Since the phenolic compounds are water-soluble, the reduction in total free phenolic and tannin level during cooking in the present study might be due to either the increased leaching out of phenolic substances or degradation by heat (Siddhuraju & Becker, 2001a; Uzogara, Morton, & Daniel, 1990).

Seeds of *B. purpurea* lose of 29% of phytic acid during cooking, which is comparable to that of previous reports in other common legumes (Uzogara et al., 1990). The apparent decrease in the content of phytic acid of legume seeds during cooking may be partly due to leaching into the cooking medium or degradation by heat or formation of insoluble complexes between phytate and other components, such as protein and minerals (Siddhuraju & Becker, 2001a).

Significant reduction ($p < 0.05$) in the level of α-galactosides was observed when cooking the seeds up to 60 min. Reduction in the level of oligosaccharides during cooking was higher than for *Mucuna monosperma* (Pugalenthi, Vadivel, Siddhuraju, Gurumoorthi, & Janardhanan, 2003) and similar to other common legumes (Jood, Mehta, Singh, & Bhat, 1985) but lower than for red gram (Mulimani & Devendra, 1998). The loss in raffinose content of *B. purpurea* (63%) during cooking is similar to the loss in *Dolichos lablab* (Revilleza et al., 1990). However, the raffinose level increased during cooking in *Cajanus cajan, Cicer arietinum, Phaseolus aureus* and *P. mungo* (Udhayasekhara Rao & Belavady, 1978). Significant reduction in the level of verbascose was noticed is similar to that in *C. arietinum* (Jood et al., 1985). The reduction in the levels of oligosaccharides during cooking probably results from their molecular decomposition to from simple di- and monosaccharides or form their reaction with other metabolites (Onigbinde & Akinyele, 1983).

The results of the present study revealed that the cooking treatment (cooking the seeds for 60 min) is more effective than soaking treatment but less effective than autoclaving in reducing the levels of oligosaccharides. Cooking is found to significantly ($p < 0.05$) improve the IVPD (13%) of *B. purpurea* seeds. This is similar to *Prosopis chilensis* (13.3%), higher than *Dolichos lablab* var. *vulgaris* (9%) and two different species of *Vigna* (9.4–11%) (Vijayakumari et al., 1997b; Vijayakumari et al., 1998) but lower than the values (17–22%) reported by Chau and Cheng (1997) in *Vigna* species. The improvement in the IVPD of *B. purpurea* seeds during cooking may partly due to degradation of antinutritional factors by heat followed by leaching in to the cooking medium.

3.5. Effect of autoclaving

Autoclaving the *B. purpurea* seeds for 30 min significantly ($p < 0.05$) reduced the content of total free phenolics and tannins (Table 2). These results are in agreement with the previous report in *Vicia faba, Vigna aconitifolia, V. sinensis* and *V. unguiculata* (Neerjarani & Hira, 1993; Uzogara et al., 1990; Vijayakumari et al., 1998). The loss of phenolics due to autoclaving may be due to degradation or inter-

action with other components of seeds, such as proteins, to form insoluble complexes (Uzogara et al., 1990).

The loss of phytic acid in *B. purpurea* seeds during autoclaving is similar with that of *M. pruriens* var. *utilis*, *Lathyrus sativus* and *Vigna unguiculata* (Siddhuraju & Becker, 2001a; Uzogara et al., 1990; Srivastava & Khokhar, 1996). The apparent decrease in phytic acid content of legume seeds during autoclaving may be partly due to either formation of insoluble complexes between phytate and other components, such as phytate–protein and phytate–protein–mineral complexes, or hydrolysis of inositol hexaphosphate to penta and tetraphosphates. Phytic acid is relatively heat-stable, hence, significant and prolonged inputs of energy are required for its destruction. Autoclaving for longer periods may result in additional phytate loss. Prolonged heat treatment reduced phytate content effectively in an earlier investigation (Sharma & Sehgal, 1992).

Autoclaving caused significant ($p < 0.05$) reduction on the level of oligosaccharides of *B. purpurea* seeds, raffinose by 81%, stachyose by 70% and verbascose by 79%. These results were in agreement with some earlier reports on certain common legumes (Jood et al., 1985; Vidal-Valverde, Frias, & Valverde, 1993) and some wild legumes, like velvet bean (Janardhanan et al., 2003). Such loss of heat stable oligosaccharides during hydrothermal processing is primarily due to leaching into the autoclaving water. However, the total loss cannot be explained solely on the basis of the solubility of the individual sugars in water. The heat under pressure may enhance the leaching out of oligosaccharides into the medium by increasing the permeability of the seed coat.

Of all the treatments, autoclaving seems to be most effective in reducing the maximum levels of all the investigated antinutrients, except phytic acid, and also improved the IVPD (16%) of *B. purpurea* seeds. The results of the present study were in agreement with the improved protein digestibility in autoclaved faba bean, field bean, horse gram, *Phaseolus calcaratus, P. angularis* and *M. pruriens* var. *utilis* (Chau & Cheng, 1997; Rajyalakshmi & Geervani, 1990; Siddhuraju & Becker, 2001a). The improvement in the protein digestibility after treatment may partly be due to reduction in the levels of various antinutrients during autoclaving and also due to increased accessibility of the proteins to enzymatic attack (Nielson, 1991).

4. Conclusion

Considering the various processing methods attempted in the present study to improve the nutritional quality of *B. purpurea* seeds, autoclaving appears to be most effective in reducing levels of all the investigated antinutritional compounds, except phytic acid, and improves the protein digestibility of *B. purpurea* seeds. Adoption of such viable, cost-effective processing methods may further enhance the utilisation of the seeds of *B. purpurea* as an alternative protein source for both human beings and animals. After conduction of animal feeding trials, incorporation of such processed potential underutilised legumes in the diets of humans/animals as protein supplements may reduce the over dependence on conventional pulses.

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