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RESEARCH ARTICLE

EFFECTS OF PROCESSING METHODS ON ANTINUTRIENT COMPOSITION OF SEEDS FROM A WILD LEGUME *Bauhinia petersiana*

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ABSTRACT

The effects of processing on the levels of antinutrients in *Bauhinia petersiana* seeds were investigated. On dry weight basis, raw seeds had the highest levels of tannins (0.6 ± 0.1 g catechin equivalents (CE)/100 g), alkaloids (7.1 + 0.1 g/100 g), saponins (9.8 ± 0.5 g diosgenin equivalents (DE)/100 g), cyanogen glycosides (6.3 ± 0.3 µg /100 g), phytates (73 ± 8 mg/100 g), oxalates (216 ± 21 mg/100 g), and trypsin inhibitors (174 ± 60 trypsin inhibitor units (TIU) /100 g). Upon cooking, levels of most of the antinutrients decreased. The difference in phytic acid content of boiled (57 ± 9 mg/100 g) and roasted seeds (55 ± 2 mg/100 g) was not significant (P> 0.05). There was no significant difference (P> 0.05) in the levels of oxalate in the raw and roasted samples. No hemagglutination activity was observed in the raw and cooked seeds. Malonaldehyde was detected only in the cooked seeds ranging from 24 ± 4 mg/100 g in the boiled seeds to 29 ± 5 mg/100 g in roasted seeds. The oligosaccharides raffinose, starchyose and verbascose were present at low concentrations in both the raw and cooked seeds. The information obtained could be of essence in promoting consumption of this wild legume for the benefit of rural communities.

Key words: Antinutrient, *Bauhinia petersiana*, Processing, Phytochemicals.

INTRODUCTION

Neglected and underutilised plant species (NUS) include hundreds of locally domesticated and wild species, that are rich in nutrients and require minimum management (Padulosi *et al*., 2006). Commercialization of NUS can provide income opportunities and many NUS species are harbouring important traditional pharmacology information. *Bauhinia petersiana* is an underutilised legume occurring in Zimbabwe, South Africa, Democratic Republic of Congo, Tanzania, Zambia, Malawi, Mozambique, Namibia, Botswana and Angola (Drummond and Palgrave, 1973; Coates- Palgrave, 2002). Commonly known as large white bauhinia (E), coffee bauhinia (E), wild coffee (E) and traditionally known as mun'ando or mpondo in Zimbabwe, *B. petersiana* grows in a variety of woodland habitats and is widely distributed in most parts of Zimbabwe (Drummond and Palgrave, 1973). The seeds of *B. petersiana* are normally roasted followed by grinding to get a fine powder that can be used as a coffee substitute. The legume seeds have been used by early hunters and explorers and the seeds became known as the Zambezi coffee (Drummond and Palgrave, 1973; Coates- Palgrave, 2002). *B. petersiana* seeds may be consumed when green, but they are preferably eaten after ripening, and roasted. Worldwide, legumes are rich sources of relatively cheap and widely available proteins for human consumption (Doss *et al.*, 2011, Okoronkwo *et al*., 2010, Nwaogu and Udebuani, 2010). The lesser known legumes together with other conventional legumes can be used for combating protein malnutrition prevalent in developing countries (Ifeoma *et al*., 2008, Shim *et al*., 2003, Aberoumand and Deokule, 2009). This can be achieved by the consumption of the legumes whole and in various processed forms.

Legume seeds may contain antinutrients such as phytates, tannins, protease inhibitors, cyanogen glycosides, saponins, lectins and oxalates which prevent the use of the legumes as food or forage (Shim *et al.*, 2003, Aberoumand and Deokule, 2010, Nwaogu and Udebuani, 2010). The type and amount of antinutrient factors vary from one plant species to another, and with the local or regional variety of the species (Szakiel *et al*., 2011; Ullah *et al*., 2012). Most, but not all, antinutrients are destroyed or reduced during cooking (Nwaogu and Udebuani, 2010). Guided by the practices of African gatherer-hunters, it seems likely that African ancestors mainly dealt with antinutritional factors by roasting or cooking the seeds. Sometimes the seeds were soaked as well, prior to roasting and grinding (Kalidas and Mohan, 2012, Nwaogu and Udebuani, 2010, Afify *et al*., 2011, Fagbemi *et al*., 2005). The seeds of *B. petersiana* have received some attention

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because of their socio-economic importance to the communities that live in their growing areas. Seeds of *B. petersiana* have served as source of protein for Zimbabweans during times of drought since time immemorial. The powder of roasted seeds can be consumed as porridge and whole seeds are boiled and consumed as a bean relish. The seed oil may be extracted for local use (Ketshajwang *et al.,* 1998). In common with other legumes, the seeds of *B. petersiana* may contain antinutrients that include tannins, cyanogens glycosides, phytates, oxalates and trypsin inhibitors which may limit its use as food material. Our objective was to investigate the effect of boiling and roasting on the content of antinutrients from *B. petersiana* seeds.

MATERIALS AND METHODS

Collection and preparation of plant material

Fresh and dried *B. petersiana* pods were collected from their natural habitat in Mutoko rural area of Zimbabwe. The plant and bean pods were identified with the help of plant taxonomist from the Botanical gardens of Zimbabwe. The seeds of *B. petersiana* were removed from their pods and allowed to dry in the shade for two weeks after which 300 g of the seeds were divided into three portions of 100 g each. The first portion was boiled in distilled water for 6 hours until soft, rinsed with distilled water and dried at 55 ^{o}C in a hot air oven. The second portion was roasted in an oven set at 180 ^{o}C for 30 minutes. The third portion was dried in the oven at 55 ^{o}C for 6 hours. The seeds were ground into powder which passed through a 60 mesh sieve before analysis.

Assaying for antinutritional compounds

Determination of tannins, alkaloids, saponins and cyanogen glycosides

Tannins were extracted using methanol and determined by spectrophotometric method (Price *et al*., 1978). Finely ground plant material (1 g) was defatted using diethyl ether and transferred to a 100 ml glass beaker. To the defatted material, 10 ml methanol was added and the beaker placed in an ultrasonic ice-water bath for 30 minutes at room temperature. The contents of the beaker were transferred to centrifuge tubes and centrifuged for 10 minutes at 3000 g. Tannins were determined by the modified Vanillin- HCl method using catechin (5 mg/ml, Sigma –Aldrich Chemie, Steinheim, Germany) as the standard stock solution. A 5 g sample was used for extraction and quantification of total alkaloids using a gravimetric method (Harborne, 1973). The mass of alkaloid was expressed as g/ 100 g dry weight of sample. Saponin content was determined using a spectrophotometric method (Hiai *et al*., 1976). Powdered samples (0.2 g) were extracted with 10 ml of 80% (v/v) aqueous methanol for 24 hours. The mixture was centrifuged at 3000 g for 15 minutes and the supernatant was diluted appropriately for saponin analysis. The total saponin content of the extract was determined by the vanillin -sulphuric acid method. The results were expressed as diosgenin equivalent from a standard of diosgenin (Sigma – Aldrich Chemie, Steinheim, Germany) prepared in 80% (v/v) aqueous methanol. Cyanogen glycosides were determined by a method described by Makkah, (2003). Powdered samples (4 g) were added to 125 ml water followed by 2.5 ml chloroform in a Kjeldahl flask and then distilled. HCN released was absorbed in 2 % (w/v) potassium hydroxide (total volume after extraction was 20 ml). An aliquot (5 ml) of the solution was mixed with 5 ml of alkaline picrate and heated in a boiling water bath for 5 minutes. After cooling the absorbance was read at 520 nm using potassium cyanide (240 mg KCN/ L, Sigma –Aldrich Chemie, Steinheim, Germany) as a standard.

Determination of oxalate, phytate and trypsin inhibitors

Oxalates were extracted and determined by titration (Amoo and Agunbiade, 2010). Powdered samples (2 g) were digested with 50 ml of 0.75 M H_2SO_4 for 2 hours, stirred and filtered using Whatman No. 1 filter paper. An aliquot of 125 ml of the filtrate was heated until near boiling point (80 -90 ^{o}C) and titrated against standardised 0.5 M $KMnO_4$ solution to a faint pink colour. Phytic acid content was determined by a colorimetric method (Vaintraub and Laptewa 1988). Powdered samples (1 g) were extracted with 10 ml of 0.5 M HCl for 1 hour at room temperature, diluted, centrifuged and analysed for phytic acid by addition of 1 ml of Wade reagent (0.3 g of ferric chloride ($FeCl_3.6H_2O$) and 3 g of sulphosalycylic acid dissolved in 1L of distilled water) to 3 ml of sample extract. Absorbance was read at 500 nm on a Jenway 6405 Uv/vis spectrophotometer (Jenway Ltd., Essex, UK). Trypsin inhibitor activity was determined by the method of Kakade *et al*., (1974) as modified by Fagbemi *et al*., (2005). The degree of inhibition by the extract was measured at 385 nm.

Determination of malonaldehyde (MDA)

MDA was determined by the modified thiobarbituric acid (TBA) method (Okoronkwo *et al*., 2010). Ground sample (0.25 g) was homogenized in 5 ml of 0.1% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 3 000 g for 5 minutes. An aliquot of the supernatant (1 ml) was mixed with 4 ml of a mixture of 20% (w/v) TCA and 0.5% (w/v) TBA. The mixture was heated for 30 minutes, quickly cooled in an ice bath and centrifuged at 3000 g for 10 minutes. The absorbance was read at 532 nm. The MDA content was calculated from a standard curve constructed using the MDA derivative 1,1,3,3, tetra methoxypropane (Sigma –Aldrich Chemie, Steinheim, Germany) which hydrolyses under acid conditions to form free dialdehyde.

Determination of haemagglutination activity

The haemagglutination activity of raw and processed samples was analysed by hemagglutination assays (Swain *et al*., 1996). The assays were carried out in a round bottomed micro-titer plate using 4 % (v/v) untreated sheep erythrocytes suspended in 0.09 % (w/v) saline azide solution. One haemagglutination unit (HU) was defined as the least amount of the extract per ml of the last dilution that resulted in agglutination.

Estimation of raffinose type oligosaccharides

The oligosaccharide composition of raw and processed legume seeds was determined by the method described by Agbenorhevi *et al*., (2007). Seed flour (5 g) was extracted with 50 ml of 70% (v/v) aqueous ethanol. Separation of oligosaccharides was done by thin layer chromatography. The separated spots were compared with standard (raffinose, starchyose and verbascose, Sigma –Aldrich Chemie, Steinheim, Germany) and the sugar spots were scrapped, eluted in 2 ml of distilled water and filtered. Filtered extract

(1 ml) was treated with 1 ml concentrated HCl and kept on a boiling water bath for 6 minutes. After cooling absorbances of the solutions were read on a Jenway 6405 Uv/vis spectrophotometer (Jenway Ltd., Essex, UK) at 432 nm and the oligosaccharide contents were quantified.

Statistical analysis

The analyses were done for three replicates and each one with three samples. Data obtained were expressed as mean ± standard deviation, and were subjected to one way analysis of variance (ANOVA). Means were separated by Bonferroni's Multiple comparison test at $P< 0.05$.

RESULTS AND DISCUSSION

Effect of boiling and roasting on antinutritional compounds

Effect on tannins, alkaloids, saponins and cyanogens glycosides

As shown in Table 1, the levels of tannins in the raw sample decreased upon roasting and boiling, from 0.6 ± 0.1 g CE/100 g dry matter (DM) to 0.3 ± 0.1 g CE/100 g DM and 0.2 ± 0.1 g CE/100 g DM respectively, a result that was similar to that obtained for some wild legumes studied by Kalidass and Mohan, (2012). Roasting or boiling of *B. petersiana* seeds prior to consumption would be recommended in an attempt to improve the nutritional value of the seeds. Tannins have been reported to have the capability of decreasing the digestibility and palatability of proteins, when they interact with proteins forming insoluble complexes (Osagie, 1998). Tannins also interact with dietary iron by chelating with it and preventing iron absorption. The content of alkaloids decreased from 7.1 ± 0.1 g/ 100 g DM in the raw seeds to 4.4 g/100 g DM in roasted seeds and to 4.2 ± 0.1 g /100 g DM in boiled seeds. High levels of alkaloids were retained in the seeds of *B. petersiana* regardless of the treatment processes which maintained high antinutritional properties still being expressed. Levels of alkaloids in *B. petersiana* were greater than values reported for *Cleome rutidosperma* seeds (Nwaogu and Udebuani, 2010), *Vigna subterranean, Arachis hypogea* and *Glycine max* seeds (Mbagwu *et al*., 2011) but less than values reported for *Soja hispida* and *Triticum vulgare* (Adeniyi *et al*., 2009).

Alkaloids are not strictly regarded as antinutrients but are rather grouped with other natural food toxicants although most alkaloids are known for their pharmacological effects rather than for their toxicity (Okaka *et al*., 1992). When alkaloids occur in high levels in plants, they cause gastrointestinal upset and neurological disorders. Alkaloids levels above 20 mg/ 100 g are considered unsafe for human consumption (Chango *et al*., 1993). The levels of saponins were 9.8 ± 0.5 g DE / 100 g DM in the raw seeds, 5.1 ± 1.0 g DE/100g DM in roasted seeds and 0.8 ± 0.1 g DE /100 g DM in boiled seeds greater than that reported for *Glycine max* and *Vigna vexillata* (Shim *et al.*, 2003), *Vigna subterranea* (Mbagwu *et al*., 2011), and *Pennisetum purpureum* (*Schumach*) (Okaraonye and Ikewuchi, 2009). The saponin levels in *B. petersiana* were significantly ($P < 0.05$) reduced by boiling of the seeds. Saponins have been shown to possess both beneficial cholesterol lowering and deleterious cytotoxic and permeabilisation of the intestine properties (Price *et al*., 1989). Although some saponins have been reported to be highly toxic under experimental conditions, acute saponin poisoning is rare both in animals and man (Nwinuka *et al*., 2005). As shown in Table 1, the levels of cyanogen glycosides in *B. petersiana* seeds were low and decreased from 6.3 ± 0.3 µg HCN equivalents / 100 g DM in the raw seeds to 3.4 ± 0.2 µg HCN equivalents / 100 g DM in roasted seeds and to 1.6 + 0.1 µg HCN equivalents / 100 g DM in boiled seeds. The acute oral lethal dose of HCN for humans was reported to be 0.5 -3.5 mg /kg body weight (Speijers, 1993). The amount of cyanide detected in the raw and processed seeds was of non-lethal consequence. Cyanogenic glycosides in the gut can be hydrolysed by β-glucosidase produced by intestinal bacteria to glucose, HCN and benzaldehyde or acetone (Oke, 1979). Benzaldehyde is oxidized to benzoic acid and subsequently to salicylic acid isomers. Hydrogen cyanide absorbed from the gut can be detoxified by metabolic conversion to thiocyanate (Rosling, 1987). Acute toxicity can be observed when the rate of absorption of HCN exceeds the metabolic detoxification capacity of the body.

Table 1: Antinutritional compounds from *B. petersiana* seeds processed using different methods

Antinutrient	Raw seeds	Roasted seeds	Boiled seeds
Tannins[d] (g / 100 g)	0.6^{aA} ± 0.1	0.3^{bB} ± 0.1	0.2^{b} ± 0.1
Saponins[e] (g/ 100 g)	9.8^{a} ± 0.5	5.1^{b} ± 1.0	0.8^{c} ± 0.1
Alkaloids (g/ 100 g)	7.1^{a} ± 0.1	4.4^{b} ± 0.0	4.2^{c} ± 0.1
Cyanogen glycosides[f] (µg /100 g)	6.3^{a} ± 0.3	3.4^{b} ± 0.2	1.6^{c} ± 0.1
Phytic acid (mg/ 100 g)	73^{a} ± 8	57^{b} ± 9	55^{b} ± 2
Oxalates (mg/ 100 g)	216^{a} ± 21	170^{a} ± 16	115^{b} ± 2
Trypsin inhibitor activity (TIU/ 100 g)	174^{a} ± 60	74^{b} ± 12	36^{c} ± 1
Hemagglutination (mg/ ml)	None	None	None
Malonaldehyde (mg/ 100 g)	0.0^{a} ± 0.0	29^{b} ± 5	24^{b} ± 4
Raffinose (mg/ 100 g)	2.1^{a} ± 0.9	0.7^{b} ± 0.2	0.2^{c} ± 0.1
Stachyose (mg/ 100 g)	0.4^{a} ± 0.2	0.3^{a} ± 0.1	0.4^{a} ± 0.1
Verbascose (mg/ 100 g)	1.7^{a} ± 0.1	0.2^{b} ± 0.1	0.4^{b} ± 0.1

[A] Values are means ± SD.
[B] In the same row, means with different superscripts are significantly different ($P < 0.05$).
[d] Catechin equivalents
[e] Diosgenin equivalents
[f] HCN equivalents

Effects on phytates, oxalates and trypsin inhibitors

Phytic acid in the seeds decreased from 73 ± 8 mg/100 g DM in the raw seeds to 57 ± 9 mg/100g DM in roasted seeds and to 55 ± 2 mg/ 100 g dry weight in boiled seeds. Differences in the phytic acid content of the roasted and boiled seeds were not significant ($P > 0.05$). Phytic acid content of the seeds was less than that reported for Brazilian jack beans (*Canavalia braziliensis*) (Oseni *et al.* , 2011), kidney beans, peas, chickpeas and cowpeas (El-Niely, 2007), but more than that reported for mung beans (Okoronkwo *et al.*, 2010). The knowledge of the phytate level in foods is necessary as high concentration can cause adverse effects, phytate forms stable complexes with Cu^{2+}, Zn^{2+}, Co^{2+}, Mn^{2+}, Fe^{2+}, Ca^{2+} and phosphorus thereby preventing the proper utilization of these metals (Jack *et al*., 1985). Phytases destroy phytates during processes such as, roasting of nuts, pre-soaking beans, cooking and fermentation (Ross, 2007). The presence of phytic acid is however, also beneficial because it may have a positive nutritional role as an antioxidant and anti-cancer agent (Phillippy *et al*., 2004, Minihane and Rimbach, 2002). The levels of oxalate in the samples decreased from 216 ± 21 mg/ 100 g DM in the raw seeds to 170 ± 16 mg/ 100 g DM in

roasted seeds and to 115 ± 2 mg/ 100 g DM in boiled seeds. The oxalate content of raw seeds of *B. petersiana* is unlikely to pose toxicity problems to man since they are below 2-5 g/ 100 g stated by Munro and Bassir, (1969). Oxalates were significantly reduced ($P < 0.05$) by boiling of the seeds than roasting. Reduction could result from leaching that occurs during boiling. Cooking has been found to be an effective measure in reducing the oxalate levels of foods thus making the food safe for human consumption (Lewu *et al*., 2009). Oxalic acid as an antinutrient interferes with mineral availability particularly calcium. Oxalate binds with calcium and forms insoluble calcium oxalate, which cannot be absorbed in the body and is involved in the formation of kidney stones (Giami *et al*., 1999). The levels of trypsin inhibitor were 174 ± 60 TIU/ 100g DM in raw seeds, 74 ± 12 TIU/100 g DM in roasted seeds and 36 ± 1 TIU/ 100 g DM in boiled seeds. Trypsin inhibitor activity was more than that reported for *Phaseolus aureus* (mung beans) (Okoronkwo *et al*., 2010) and other wild legumes (Kalidass and Mohan, 2012). Trypsin inhibitors are heat labile and can be partially or completely denatured when exposed to elevated temperature. The treatments by roasting or boiling were effective in inactivating the trypsin inhibitors in *B. petersiana* seeds.

Effects on malonaldehyde, hemagglutinins, raffinose type oligosaccharides

No malonaldehyde was detected in *B. petersiana* seeds, but seeds processed by roasting and boiling contained malonaldehyde at 29 ± 5 mg/ 100 g DM and 24 ± 4 mg/ 100 g DM respectively. The difference in malonaldehyde content in the roasted and boiled seeds was not significant ($P > 0.05$). Malonaldehyde in both the roasted and boiled seeds could result from thermal oxidation of lipids. Higher temperatures favour thermal oxidation reactions (Ifeoma *et al*., 2008). Malonaldehyde is metabolized *in vivo* and *in vitro* by oxidation to malonic semialdehyde and by decarboxylation to acetaldehyde (NIOSH, 2005). Acetaldehyde can form DNA adducts hence *in vivo* metabolism of malonaldehyde may lead to formation of other biologically toxic compounds (Ifeoma *et al*., 2008). Although the physiological effects of malonaldehyde at the levels observed is not clear, but it would be prudent to minimize the levels of malonaldehyde in foods.

No haemagglutination activity was observed in the raw and processed seeds of *B. petersiana*, indicating that there was no lectin activity in the analysed seeds. Raw and processed seeds of *B. petersiana* had a low content of the oligosaccharides, raffinose, starchyose and verbascose. The amount of raffinose 2.1 ± 0.9 mg/ 100 g DM in the raw seeds which decreased to 0.7 ± 0.2 mg/100 g DM in roasted seeds and to 0.2 ± 0.1 mg/ 100 g DM in boiled seeds is far below the level that could cause flatulence in humans which, is an added advantage from a nutritional perspective. Legumes contain β-galactosidases, which hydrolyse raffinose type oligosaccharides to sucrose and galactose (Kalidas and Mohan, 2012).

Conclusions

The seeds of *B. petersiana* contained low levels of tannins, oxalates, phytates, cyanogen glycosides, trypsin inhibitors and raffinose type oligosaccharides, which may be further reduced by processing thus rendering the seeds safe for human use. This chemical and biochemical information could be of essence to promote consumption of this wild legume for the benefit of rural communities.

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