Nor. Afr. J. Food Nutr. Res. | 2021; 5(12): | 105-111
https://doi.org/10.51745/najfnr.5.12.105-111
https://www.najfnr.com

ORIGINAL ARTICLE

Effect of traditional processing methods on protein digestibility and chemical constituents in seeds of *Bauhinia petersiana*

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Abstract

Background: Antinutritional factors present in food may reduce the bioavailability of nutrients and cause harmful effects to human health. **Aims**: The aim of this study was to determine the effect of traditional processing methods on protein digestibility, nutrient and antinutrient constituents of seeds of *Bauhinia petersiana*. **Subjects and Methods:** The seeds were processed by soaking in water, boiling or roasting before analyzing protein digestibility, nutrient and antinutrient compositions. **Results:** Soaking resulted in no significant changes in the content of moisture, protein, fiber, phytates and trypsin inhibitor activity and significant reductions in fat, ash and tannins. Roasting resulted in no significant change in the content of moisture, ash, protein, and fiber and significant reductions in fat, phytates and trypsin inhibitor activity. Boiling resulted in a significant increase in the content of both protein and fiber and reduction in fat, ash, tannins, phytates and trypsin inhibitor activity. Mineral content of zinc, magnesium and calcium was not changed by soaking, roasting or boiling of the seeds. The calculated phytate: zinc molar ratios for both the raw and processed seeds were greater than 10, the limit for optimal absorption of zinc in the small intestine whereas phytate: iron molar ratios were less than 14, the limit for optimum absorption of iron in the intestines. In vitro digestibility of proteins in the seeds was increased when the seeds were soaked, roasted or boiled. **Conclusions**: Boiling the seeds of *B. petersiana* before consumption would effectively remove undesirable antinutrients while maintaining the nutrient content of the seeds and improving digestibility of proteins.

Keywords: Legume, nutrient, antinutrient, digestibility, phytate.

Received: July 21, 2021 / **Accepted**: October 24, 2021 / **Published**: November 08, 2021

1 Introduction

Because of their nutritional quality legumes are among the most important food sources in the world [1]. Legumes have high energy values and are rich sources of fiber, vitamins, minerals and proteins [2, 3]. With high dietary fiber content, legumes have demonstrated many health benefits such as prevention of cancer, protection against cardiovascular diseases and lowering of glycemic index for diabetics [2, 3]. People with a high intake of fiber have been reported to have lower serum cholesterol levels and lower blood pressure than those with low intake [5, 6].

Legumes add variety to the human diet and help the alleviation of hunger and malnutrition particularly in children and pregnant women, as the most vulnerable [7]. In developing countries such as India where the majority of the people are vegetarian, legumes would form an economical source of supplementary proteins [8]. The search of alternative sources of nutrition and proteins, in developing countries is important and efforts are currently underway to exploit wild legumes in tropical countries as a source of food and for processing to make food products of added value [9].

In order to promote the utilization of legumes as human food, processing methods including roasting, boiling and hydration may be used [10]. Processing of legumes destroys the heat labile antinutrients and improves both organoleptic acceptability and nutritional quality of the legumes [11]. However, some processing methods may cause considerable losses in soluble solids including vitamins and minerals [12].

Undesirable antinutrients in legumes include phytic acid, trypsin inhibitors, tannins, phytohemagglutinins, saponins, and α-galactosides [13, 14]. The ability of trypsin and chymotrypsin to digest proteins may be limited in the presence of trypsin inhibitors thus limiting the nutritional quality of the proteins and the intake of amino acids needed to synthesize new proteins [15]. The activity and amount of trypsin inhibitors in the food is inversely related to the availability of protein. Improving protein digestibility through processing helps the body to use the protein more efficiently.

Phytate, found in many plant tissues especially seeds and grains binds strongly to metals such as magnesium, calcium, iron, copper and zinc, that are necessary for good health [16]. Phytate has a particularly strong affinity for zinc, a mineral that supports brain health, protein synthesis, reproductive health, nerve function, and wound healing. It is believed that people living in developing countries are shorter than those in developed countries because of zinc deficiency caused by eating too many legumes [17]. Thus phytate is largely blamed for complexing dietary essential minerals especially zinc and copper, in cereals and legumes and rendering them poorly available in monogastric animals.

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Tannins are water soluble polyphenolic compounds found primarily in the seed coat [18]. Tannins can bind to proteins and inhibit the proteolytic enzymes, chymotrypsin and trypsin, making the enzymes unavailable for digestion. The effects by tannins depend on their chemical structure and dosage. Tannins have been reported to have antiviral, antibacterial and antiparasitic effects [19].

Bauhinia petersiana, an underutilized wild legume found in Zimbabwe, has pods that mature during December to May and split explosively when ripened and dry [20]. Roast grounded seeds of *B. petersiana* can be used as a substitute for coffee [20]. Pounded meal from the seeds is used to make porridge. Seeds that are boiled in oil and water can be eaten as a bean relish. Oil extracted from the seeds can be used for various purposes [21]. The aim of this study was to determine the effect of domestic cooking methods on the reduction of antinutrients in seeds of *B. petersiana* as this would assist in identifying processing methods that are effective, simple and cost-effective, so as to fully exploit the nutritional value of the legume.

2 Materials and Methods

2.1 Collection of plant material

Fresh and dried pods of *B. petersiana* were collected from Dandara village (17°41′S, 32°23′E) in Mutoko, Zimbabwe [22]. The plants and bean pods were identified with the help of a plant taxonomist from the botanical gardens of Zimbabwe. The pods of *B. petersiana* were allowed to dry in the sun and upon drying the pods would burst open to release the seeds.

2.2 Sample processing Methods

Four batches of seeds (100 g) were processed as follows, the first batch contained the raw seeds which were dried to constant weight in a Memmert oven at 55°C [22]. Seeds (100 g) in the second batch were soaked in distilled water for 24 hours at room temperature. Two changes of the soaking water were made during the 24 hours and the weight for volume ratio of seeds to water was 1:5. The seeds were soaked until the maximum weight of the seeds was reached. After soaking the seeds were dried in the oven to a constant weight, at 55°C. A third batch of *B. petersiana* seeds (100 g) was boiled in distilled water until the seeds were soft and then dried to a constant weight in an oven at 55°C. A forth batch of the seeds (100 g) was roasted in a stainless steel pan at 180°C for 30 minutes, with constant stirring to reduce charring. All the samples of seeds were ground to a fine powder which was passed through a 60-mesh sieve before analysis.

2.3 Proximate and mineral analysis

The protein content of the powdered seeds of *B. petersiana* was determined by the Kjeldahl method [23]. Fat content was determined by the Soxhlet method [24]. The method described by Costa *et al.* [3] was used to determine the content of crude fiber of the powdered seeds.

The moisture content was determined by weighing 2 g of powdered seeds of *B. petersiana* into a previously heated, cooled and weighed porcelain crucible. The samples were heated to a constant weight in a pre-heated oven at 100°C [25]. The samples were cooled in a desiccator and weighed and the moisture content was calculated.

To determine the ash content, the samples, dried at 100°C were charred at 200°C for 2 hours to prevent foaming [25]. The charred mass was then ashed at 600°C in a muffle furnace (Phoenix, USA) for 28 hours.

To determine the mineral ions, Fe, Ca, Zn and Mg, the ash samples were transferred into beakers and the crucibles were washed with distilled water pouring the washings into the beakers containing each sample, 5 mL of concentrated HCl were added to the beaker to dissolve the ash and the mixture was boiled for 5 minutes on a hot plate in a fume hood. HCl was added when necessary in order to maintain a volume of 5 mL. The volume was adjusted to 40 mL with distilled water and the mixture was boiled for 10 minutes. The mixture was cooled and filtered through glass wool into 100 mL volumetric flasks and the beakers were rinsed into the volumetric flasks. The solution in each flask was cooled and made up to 100 ml with distilled water and used for the determination of the individual elements using a Perkin Elmer 500 atomic absorption spectrophotometer [26].

Phosphorus was determined using the vanadate colorimetric method [27]. Ash solutions (4 mL) were pipetted into 100 ml volumetric flasks. Vanadate molybdate (25 mL) reagent was added to each volumetric flask, diluted to the mark with distilled water and mixed. The solutions were allowed to stand for about 30 minutes and the absorbance of each solution was determined at 420 nm (Jenway 6405 spectrophotometer) using the 0 mL solution as the blank. Standard phosphate solutions of 300 ppm KH_2PO_4 were used for calibration.

2.4 Determination of antinutrients

2.4.1 Determination of phytate

Powdered seeds of *B. petersiana* (2 g) were extracted with 10 ml of 0.5 M HCl overnight and filtered [28]. The filtrate was collected and analyzed for total phytate by adding 1 ml of Wade's reagent (0.3 g ferric chloride and 3 g sulphosalicylic acid in 1L of distilled water) to 1ml of sample extract and the mixture was allowed to stand for 20 minutes. Absorbance was read at 500 nm (Jenway 6405 spectrophotometer) against a blank of distilled water. Standard phytate solutions of 0.5 mg/ml were used for calibration.

2.4.2 Determination of trypsin inhibitor activity (TIA)

Powdered seeds of *B. petersiana* (1.0 g) were defatted by mixing with hexane at a ratio of 1:5 (w/v) for 20 minutes [29]. The mixture was filtered through Whatman Grade 1 filter paper and the sediments were rinsed twice with hexane and dried at room temperature. The defatted samples were extracted with 50 ml of 0.01 M NaOH and the pH was adjusted to 9.5 using 0.1 M NaOH and 0.1 M HCl. The mixture was allowed to shake for one hour on a Thermolyne Rotomix M50800 (Iowa, USA) at 250 rpm. The mixture was centrifuged at 3000 rpm for 30 minutes at room temperature and the supernatant was collected. Sample

extracts (1.8 mL) were pipetted into duplicate sets of test tubes and adjusted to 2.0 mL with distilled water. Trypsin solution (2 mL) was added to each tube and the tubes were placed in a water bath at 37°C. Benzoyl-DL-arginine-p-nitroanilide (BAPA) solution (5 mL) previously warmed at 37°C was added to each tube. The reaction was terminated after 10 minutes by the addition of 30% acetic acid (1 mL). After mixing the absorbance was measured at 410 nm (Jenway 6405 spectrophotometer) against a reagent blank. The reagent blank was prepared by adding 1ml of 30% acetic acid to a test tube containing trypsin and water (2 mL each) before 5 mL of BAPA was added. Trypsin inhibitor activity (TIA) was calculated as:

$$TIA = \frac{2.632 \times A}{S} = \text{mg pure trypsin/g}$$

Where: A= change in absorbance (pure trypsin and sample extract). S= sample mass (g).

2.4.3 Determination of tannins

Powdered seeds of *B. petersiana* (2 g) were defatted using 20 ml of diethyl ether for 5 hours after which the defatted samples were extracted with 20 mL of methanol overnight at room temperature. The mixtures were filtered through Whatman Grade 1 filter paper and the filtrates were kept at 4°C for tannin determination using the vanillin-HCl method [30]. Gallic acid was used as a standard at a concentration of 5 mg/mL.

2.5 Determination of in-vitro multi-enzyme protein digestibility

Aqueous sample suspension (50 mL) containing 6.25 mg powdered *B. petersiana* seeds per ml in distilled water were adjusted to pH 8.0 using 0.1 M HCl or 0.1 M NaOH while stirring in a water bath at 37°C. A multi-enzyme system consisting of 1.6 mg trypsin, 3.1 mg chymotrypsin and 1.3 mg carboxypeptidase per mL was maintained in an ice water bath and adjusted to pH 8.0 using 0.1 M HCl or 0.1 M NaOH. A 5 mL sample of the multi-enzyme solution was added to the sample suspension with constant stirring at 37°C. After 30 minutes the pH of the suspension was recorded and the in vitro digestibility was calculated using regression equation (1) of Hsu *et al.* [31].

$$Y = 210.46 - 18.10X \quad \ldots\ldots\ldots (1)$$

Where: Y was the in vitro digestibility.

X is the pH of the sample suspension after 30 minutes of digestion with the multi-enzyme solution.

The multi-enzyme solution was freshly prepared before each series of tests and its activity was determined using casein of known in vitro digestibility.

2.6 Statistical analysis

Statistical analysis of the data was done using Graphpad Prism 5.03 software. The data obtained was expressed as means ± standard deviation and one way analysis of variance (ANOVA) was done. Comparisons between means were done using Bonferroni's multiple comparison test and significant difference between the means was accepted at $p<0.048$.

3 Results

3.1 Effect of soaking, boiling and roasting on nutrient content of *B. petersiana* seeds

As shown in table1, a significant increase in protein content was observed when the seeds were boiled (33.0 ± 1.5%). There was no significant difference in protein content of the soaked (27.6 ± 0.1%) or roasted seeds (29.1 ± 0.1%) when compared with the raw seeds (30.0 ± 0.4%).

Table 1: Effect of soaking, boiling and roasting on nutrient content of seeds of *B. petersiana*

Sample	Ash (%)	Moisture (%)	Protein (%)	Fat (%)	Crude Fiber (%)
Raw	4.0 ± 0.0^a	5.63 ± 0.47^a	30.0 ± 0.4^a	22.3 ± 0.4^a	4.2 ± 0.0^a
Soaked	3.6 ± 0.1^b	7.38 ± 0.48^a	27.6 ± 0.1^a	18.8 ± 0.4^a	4.3 ± 0.2^a
Boiled	3.6 ± 0.1^b	6.18 ± 0.25^a	33.0 ± 1.5^b	16.0 ± 0.7^c	6.4 ± 0.3^b
Roasted	4.0 ± 0.1^a	5.02 ± 0.64^a	29.1 ± 0.1^a	16.3 ± 0.4^c	4.6 ± 0.2^a

Values are on dry weight basis and are means of duplicate samples ± SD. The superscripts a, b, and c denote significant difference at a level of $p<0.048$. In the same column means with different superscripts are significantly different and means with the same superscript are not significantly different.

Significant reduction in the content of fat (22.3 ± 0.4%) was observed when the seeds of *B. petersiana* were either, soaked (18.8 + 0.4%), boiled (16.0 ± 0.7%) or roasted (16.3 ± 0.4%). A significant increase in the content of crude fiber was observed when the seeds were boiled (6.4 ± 0.3%). Soaking or roasting of *B. petersiana* seeds resulted in no significant difference in the fiber content. As shown in Table 1, the ash content of the soaked (3.6 %) and boiled (3.6 %) seeds was significantly lower than the ash content of the raw seeds (4.0%).

3.2 Effect of soaking, boiling and roasting on antinutrient content and protein digestibility of *B. petersiana* seeds

As shown in Table 2, a significant reduction in phytate content, from 10.7 ± 1.5 mg/100 g in the raw seeds to 9.4 ± 1.6 mg/100 g in the boiled seeds was observed. There was no significant difference in the phytate content of the soaked and roasted seeds when compared with the raw seeds. The trypsin inhibitor activity (TIA) was significantly reduced by heating treatments such as boiling and roasting. There was no significant difference in the activity of trypsin inhibitors when the seeds of *B. petersiana* were soaked in water for 24 hours (Table 2). As shown in Table 2, boiling or soaking of the seeds of *B. petersiana* resulted in significant reduction in the content of tannins whereas roasted samples had higher levels of tannins.

A significant improvement in the in-vitro protein digestibility (IVPD) of the seeds of *B. petersiana* was observed when the seeds

were soaked, boiled or roasted. Protein digestibility increased by 12.4%, 10.5% and 6.8% when the seeds were boiled, roasted or soaked respectively.

Table 2: Effect of soaking, boiling and roasting on antinutrient content and *in vitro* protein digestibility of seeds of *B. petersiana*

Sample	Phytate (mg/100g)	TIA (mg trypsin/100g)	Tannins (mg GAE/100g)	IVPD (%)
Raw	10.7 ± 1.5^{a}	111.6 ± 0.0^{a}	14.6 ± 6.9^{a}	72.2 ± 0.5^{a}
Soaked	11.3 ± 1.3^{a}	110.3 ± 0.2^{a}	12.5 ± 2.0^{b}	79.0 ± 0.6^{b}
Boiled	9.4 ± 1.6^{b}	36.3 ± 0.1^{c}	8.1 ± 2.4^{c}	82.7 ± 0.9^{b}
Roasted	10.2 ± 1.4^{a}	80.5 ± 0.1^{b}	15.6 ± 4.3^{d}	84.6 ± 1.5^{b}

Values are on dry weight basis and are means of duplicate samples ± SD. GAE-Gallic acid equivalents, TIA-Trypsin inhibitor activity, IVPD-*in vitro* protein digestibility. The superscripts a, b, c and d denote significant difference at a level of p<0.048. In the same column means with different superscripts are significantly different and means with the same superscript are not significantly different.

3.3 Effect of soaking, boiling and roasting on mineral content of seeds of *B. petersiana*

As shown in Table 3, the iron content of seeds of *B. petersiana* was significantly reduced when the seeds were soaked in water. No significant reduction in the content of iron was observed when the seeds were either boiled or roasted. The content of zinc, magnesium and calcium was not affected by soaking, boiling or roasting of the seeds of *B. petersiana*.

Table 3: Effect of soaking, boiling and roasting on the mineral content of seeds of *B. petersiana*

Sample	Mineral concentration (mg/100g) ± the standard deviation			
	Zinc	Magnesium	Iron	Calcium
Raw	0.055 ± 0.005^{a}	5.0 ± 0.1^{a}	0.4 ± 0.1^{a}	1.07 ± 0.02^{a}
Soaked	0.063 ± 0.007^{a}	4.9 ± 0.1^{a}	0.4 ± 0.2^{a}	1.24 ± 0.02^{a}
Boiled	0.050 ± 0.003^{a}	5.2 ± 0.1^{a}	0.2 ± 0.1^{b}	1.06 ± 0.03^{a}
Roasted	0.052 ± 0.010^{a}	5.1 ± 0.0^{a}	0.4 ± 0.1^{a}	1.08 ± 0.02^{a}

Values are means of triplicate samples ± SD. The superscripts a and b denote significant difference at a level of p<0.048. In the same column means with different superscripts are significantly different and means with the same superscript are not significantly different.

As shown in Table 4, a significant increase in the content of phosphorus was observed in the soaked (11.6 ± 0.2%), roasted (11.3 ± 0.2%) and boiled (13.7 ± 0.2%) seeds. As shown in Table 4, the calculated phytate: iron (PA:Fe) molar ratios for the raw (2.3), soaked (2.4), roasted (2.2) and boiled (4.0) seeds of *B. petersiana* were less than the critical value of phytate to iron ratio, of 14. The calculated phytate: zinc molar ratios for the raw (19.3), soaked (17.8), roasted (19.4) and boiled (18.6) seeds were greater than 10, the limit for optimal absorption of Zn in the small intestines.

Table 4: Phosphorus content and phytate mineral ion molar ratios of seeds of *B. petersiana*

Sample	Phosphorus content (%)	PA:Zn	PA:Fe
Raw	9.7 ± 0.3^{a}	19.3	2.3
Soaked	11.6 ± 0.2^{b}	17.8	2.4
Boiled	13.7 ± 0.2^{c}	18.6	4.0
Roasted	11.3 ± 0.2^{b}	19.4	2.2

PA:Zn – phytate: zinc molar ratio ; PA:Fe – phytate: iron molar ratio. Values for phosphorus content are means of two replicates ± SD. The superscripts a, b, and c denote significant difference at a level of p<0.048. In the same column means with different superscripts are significantly different and means with the same superscript are not significantly different.

4 Discussion

Preparation of food before consumption is important to maintain good nutrition. The significant increases in both the protein and fiber content of the seeds of *B. petersiana* upon boiling of the seeds (Table 1) could have resulted from modifications that occur during cooking resulting in the formation of protein-fiber complexes [12]. Soaking or roasting of seeds of *B. petersiana* resulted in no significant changes in the moisture, protein and fiber content of the seeds, a result that is similar to what other workers have observed with other legumes [32, 33]. Retention of protein upon soaking and cooking of *B. petersiana* seeds is beneficial for people consuming the legume as a source of food. High fiber foods are desirable for health maintenance and retention of fiber in seeds of *B. petersiana* during cooking is desirable for health [2,4]. Soaking, boiling and roasting, resulted in a significant reduction in fat content with boiling and roasting giving the greatest losses of 6.3 % and 6 % respectively (Table 1). The loss in fat upon boiling or roasting of the seeds may be a result of denaturation of the lipid fraction during boiling and roasting at temperatures above 100°C[34]. Soaking of seeds in water would result in increased activity of lipase enzymes that break down lipids to form glycerol and fatty acids [35].

The reduction of antinutrients that occurs during cooking of legumes is necessary to prevent poisoning and improve biological utilization [11]. Boiling of seeds of *B. petersiana* resulted in a greater reduction of trypsin inhibitor activity of 75% compared to roasting (30%) (Table 2). The loss of trypsin inhibitor activity during boiling and roasting could have resulted from the destruction of trypsin inhibitors by high temperatures as trypsin inhibitors are protein in nature hence are denatured by heating[36]. Trypsin inhibitors limit the digestion of proteins by the enzymes trypsin and chymotrypsin and their reduction would result in overall improved protein digestibility of the legume as shown in Table 2, where protein digestibility of the seeds was improved by 12.4% and 10.5% respectively upon boiling and roasting. Reduced content of tannins after soaking and boiling may be due to the solubility and consequent leaching of tannins into the soaking and boiling water [37]. The highest reduction in tannin content was observed after boiling and this could be because tannins are heat labile and degrade upon heating [38].

Significant reduction in phytate content, observed upon boiling of seeds of *B. petersiana* could have resulted from hydration of the beans resulting in an increased phytase activity that occurs as the temperature of the water is increased before the enzyme can be denatured [39]. Phytate is heat stable hence it was not affected by roasting [40]. The presence of phytate in foods has been associated with reduced mineral absorption in the intestines as phytic acid chelates or binds strongly to some metals that are necessary for good health [16]. Phytic acid impairs the absorption of iron and zinc and to a lesser extent calcium [41]. A molar ratio of phytate: zinc (PA: Zn) of 10 has been described as the limit for optimal absorption of zinc [42]. In our study, the calculated molar ratios of phytate: zinc were all greater than 10 (Table 4), meaning that the phytate present in raw, soaked, roasted and boiled seeds of *B. petersiana* could impair the absorption of zinc in the digestive tract and may contribute to zinc deficiency. A molar ratio of phytate: iron (PA:Fe) of 14 as the limit for optimal absorption of iron has been described by Lestienne *et al.* [42]. The phytate: iron molar ratios of seeds of *B. petersiana* were less than the critical value of 14; therefore it is unlikely that iron absorption would be significantly impaired by the phytate present in raw, soaked, boiled or roasted seeds of *B. petersiana*. Further tests are required in order to determine the actual bio-accessibility of the mineral ions.

Soaking, roasting and boiling of seeds of *B. petersiana* had no effect on the content of zinc, calcium and magnesium (Table 3). The results are similar to the findings by Duhan *et al.* [43], who reported a total retention of calcium in different types of beans that could result from a strong association of calcium with proteins in seed cells. A strong association of zinc with phytates and proteins in the cells of seeds has been described by Karkle and Beleia [44]. The significant increase in phosphorus during soaking, boiling and roasting of seeds of *B. petersiana* may have resulted from the activity of phytase which hydrolyses phytate and the hydrolysis products that include phosphorus would remain in the seeds. Boiling resulted in a greater increase in phosphorus content, which might have resulted from increased phytase activity at higher temperature before the temperatures were too high for the enzyme. During boiling the seeds were placed in cool water at room temperature and the temperature of the water increased gradually before boiling.

5 Conclusions

According to this study, boiling seems to be the best method to maintain the nutritional quality of seeds of *B. petersiana* while reducing the levels of the antinutrients, tannins, phytates and trypsin inhibitors. Cooking *B. petersiana* seeds also improved protein digestibility which is useful when preparing weaning foods that can prevent protein-energy malnutrition. Although the phytate levels obtainable in the seeds after processing could interfere with the absorption of zinc.

Acknowledgments: None.

Author contribution: C.C. conceived and designed the study, and undertook the literature research. All authors participated in the experiment and data acquisition. A.V. and C.C. performed the data analysis. A.V. and C.C. carried out the statistical analysis, prepared, reviewed and drafted the manuscript. All authors approved the final version before submission. All authors have read and agreed to the published version of the manuscript.

Funding: Funding for the research was provided by the Third World Academy of Sciences grant number 11-428 RG/BIO/AF/AC_1.

Conflict of interest: The authors declare no conflicts of interest.

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Cite this article as: Vengesai A., Nyanga L. K., & Chidewe C. (2021). Effect of traditional processing methods on protein digestibility and chemical constituents in seeds of *Bauhinia petersiana*. *The North African Journal of Food and Nutrition Research,* 5(12): 105-111. https://doi.org/10.51745/najfnr.5.12.105-111