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Nutritional Profile and Determination of the Biological Value of the Raw and Cooked Soursop Seeds

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Abstract

Background of study: *Annona muricata* (Soursop) seeds have been reported to contain nutrients and other bioactive components and hence utilised as a nutritional medicinal supplement.

Aim: This study was aimed at evaluating the nutritional profile and the biological value of the raw and cooked soursop seeds.

Method: The proximate, vitamin (A, C, E), mineral (Ca, Mg, K, Na, Fe, Cu, Zn), and anti-nutrient (phytae, oxalate, tannin, hydrogen cyanide) composition of the raw and cooked soursop seeds were determined using standard analytical methods.

A total of sixteen rats were grouped into four groups with four rats in each. Rats in group 1 were fed on reference protein diet, rats in group 2 were given basal diet, and rats in group 3 were fed on the raw soursop seeds while rats in group 4 were fed on the cooked soursop seeds. The experiment spanned 14 days and the weights of the rats and the amount consumed were monitored daily. Nutritional indices (net body weight, protein efficiency ratio (PER), net protein utilization (NPU) and the biological value (BV) were determined.

Results: Proximate analysis revealed the protein content of the cooked soursop (9.29%) was significantly ($p<0.05$) higher than that of the raw seed (6.57%); carbohydrate content of cooked seed (47.62%) was significantly ($p<0.05$) higher than that of the raw (43.82%) while the ash and fiber content of the raw seed was significantly ($p<0.05$) higher than that of the cooked with a slight variation in the fat content of the raw and cooked seeds. There was a significantly ($p<0.05$) higher contents of Ca, Mg, K and Na in the raw seed as compared to the cooked sample while there was a significantly ($p<0.05$) higher content of Fe, Cu and Zn in the cooked seed as compared to the raw. The levels of all the anti-nutrients were found to be significantly ($p<0.05$) higher in the raw seed as compared to the cooked seed while there was a significantly ($p<0.05$) higher contents of vitamin A, C, and E in the raw seeds as compared to the cooked. There was a significant ($p<0.05$) increase in the body weight and PER of the animals fed on the cooked seeds as compared to those fed on raw. The TD, NPU and BV values obtained for the cooked soursop seed was higher than that of the raw.

Conclusion: The cooked soursop seed has a higher nutritional advantage over the raw seed and this suggests its potential as a nutritional protein supplement.

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Keywords

Soursop; Nutrition; Protein efficiency ratio (PER); Net protein utilization (NPU) and Biological value (BV).

Introduction

Various seeds of plants and seed products have been incorporated into the diet of people due to the nutritional components of these seeds and their products [1]. Nutrition involves the relationship of food and nutrients to health. Nutrients refer to chemical substances that provide nourishment essential for the maintenance of life and for growth [2,3]. Plants have been utilised as food by man because they are a major source of nutrients with some plants possessing other inherent medicinal activities in addition to their nutritive value.

Soursop is the fruit of *Annona muricata*, an evergreen tree with a juicy flesh. The seeds are spherical with a shiny blackish brown colour [4]. Extracts from soursop fruits and seeds have been reported to possess various pharmacotherapeutic activities such as antioxidant, anti-inflammatory and analgesic activities [5,6].

Proximate analysis of food is used to describe the basic nutrient composition of foods in terms of protein, moisture, fat, fiber, ash (minerals) and carbohydrate [7]. Conventionally, various food processing methods have been employed in transforming raw ingredients or food into other forms for consumption by humans [8]. One of the food processing methods is cooking which is the art of preparing food for consumption and even involves addition of water to immerse the substance being cooked [8]. Cooking food substances in water (boiling) has been found to lead to decreased fatty acid, crude fats and the energy contents of the food samples but increases the protein content of samples [9]. Biological value is an index for measuring the proportion of protein assimilated by an organism from a food that is retained by the organism [10]. Some of the plants consumed as food have also been found to contain other anti-nutrients which hinder the absorption of some nutrients and hence the needs to investigate the processing method that will help to reduce the amounts of these anti-nutrients in these plant substances. The high cost of living and the recent hike in the price of protein form animal origin necessitated the need for cheaper and affordable plant based protein diet which might also be a viable nutritional supplement to help alleviate malnutrition and improve the health of humans on the long run. . Moreover, no information exists on the biological value of soursop seeds in the literature. This study will provide more insight into the potential use of soursop seeds as an alternative protein supplement. This study was aimed at determining the nutritional profile and the biological value of raw and cooked soursop seeds.

Methodology

Plant collection and identification

A. muricata fruits were purchased from Oje market in Ibadan, Oyo State of Nigeria. The identification and authentication of the fruits was done taxonomically at the Department of Botany, University of Lagos and Lagos, Nigeria and was assigned a voucher specimen number 7551.

Processing of seeds

The soursops were washed, peeled and the pulps squeezed to remove the seeds which were then dried in the oven at 45°C for 3 hours to enable effective dehulling of the seeds to obtain the mesocarp. The dehulled seeds and the husks were then dry-milled and the powder kept in polythene bag at room temperature for further analysis. The cooked soursop seed was obtained by boiling the raw seeds in water for 30 minutes; the boiling water was discarded and the boiled seeds allowed cooling. The boiled seeds were dried in the sun for two days, after which the seed coats were cracked with the aid of small stones, thus exposing the white cotyledons. These were then boiled in water for a further one hour. The boiling water was again discarded and the white cotyledons allowed to cool. The soft seeds were then broken up into small pieces and ground into a paste. The cooked seeds were milled separately into a powdery form and then stored in a bucket kept in a freezer at 20°C until required.

Proximate composition analysis

Proximate analysis of the soursop seed powder (raw and cooked) was carried out to determine crude protein, crude fibre, total ash, total carbohydrate, crude lipid and moisture content and this was done in accordance to the methods described by AOAC (2000)[15].

Minerals composition determination

Atomic absorption spectrophotometer (Pye Unicam Sp 9 AAS) was used for the determination of calcium, magnesium, iron, copper and zinc while flame photometer was used for the determination of sodium and potassium from a solution obtained by first dry-ashing the sample at 525°C and dissolving the ash in de-ionized water with few drops of concentrated hydrochloric acid.

Vitamin composition analysis

The vitamin A, C and E content of both the raw and cooked soursop seed powder was determined by chemical methods and high precision analytical method (HPLC) in accordance to the procedures described by the association of official analytical chemist (AOAC, 2000).

Anti nutrients determination

This was done following the procedures as earlier determined. The phytate content was determined by the method of Young and Greaves [11,12]. Oxalate quantification was performed with an oxalate diagnostic kit (Trinity Biotech, St. Louis, MO) according to the manufacturer's instructions. Tannin was determined using the method of Makkar while cyanide content of the seed was determined by silver nitrate titration procedure as described by De Bruijn [13,14].

Experimental animals

A total of sixteen male littermates that were three weeks old weaning rats and weighing between 40-50 g adult, were used for the study. All the animals were housed in well ventilated, standard clean cages made of plastic and wire gauze. Wood shavings were used as beddings to keep each compartment dry. Here, normal standard ambient conditions of temperature between 28-31°C, relative humidity between 50%-60% and a photoperiodicity of 12 h natural light and 12 h dark were maintained. The animals were allowed to acclimatize for two weeks for proper adaptation to their new environment and were weighed weekly. They had access to pelletized feed and tap water ad libitum. All the experimental procedures were carried out in accordance to the guidelines of the Institutional Animal Ethics Committee (IAEC). All the ethical and humanity considerations as well as euthanasia of the animals were considered and performed.

Diet composition

The raw and cooked soursop seed samples was allocated to the rats on raw diet and cooked diet respectively with 20g of the allocated feeds. The basal and the reference protein (casein) diets were compounded with a mixture of vitamins, starch, mineral as shown in Table 1.

Experimental design

At the end of the acclimatization period, the sixteen 16 male wistar rats were divided into four groups as follows:-

Group 1: Rats fed on casein diet.

Group 2: Rats fed on nitrogen-free diet (Basal diet).

Group 3: Rats fed on raw soursop seed powder.

Group 4: Rats fed on cooked soursop seed powder.

The rats were fed on the respective diets for a period of fourteen days. Daily records of food consumption were kept for those 14 days. The weights of the rats were recorded every other day throughout the experiment. Collection of faeces was made daily for the last 14 days of the experiment. The faeces of individual rats were pooled, dried at 75°C for 3 days and ground to powder, for faecal nitrogen determination. At the end of the experiment, animals were fasted for 12 hours and sacrificed under light ether anaesthesia by cervical dislocation.

Predigestion and determination of total nitrogen content

Pre digestion was done by placing the dead rat in a beaker containing 20 ml of sulphuric acid and the resulting solution was made up to 50 ml.

Table 1: Composition of diet.

Ingredient	Basal diet (g/kg)	Reference protein (g/kg)	Raw seed powder (g/kg)	Cooked soursop seed powder(g/kg)
Corn starch	80	70	-	-
Cotton seed oil	10	10	-	-
Milled *A.muricata* seeds powder	-	-	20	20
Casein	-	10	-	-
Mineral mix	4	4	-	-
Vitamin mix	1	1	-	-
Cellulose powder	5	5	-	-

Note: Vitamin mix (per g of diet); Vit A, Vit D- 200 mg, Vit E -280 mg; Vit K- 2 mg; Thiamin-30 mg; riboflavin- 30 mg; pyridoxine- 80 g; panthothenate- 100mg; nicotinic acid- 100 mg; vitamin B12- 50 mg; choline- 1000 mg; pteroyglutamic acid- 1.0 mg; biotin- 0.2 mg; inositol,- 220 mg; and p-aminobenzoic acid-75 mg.

Mineral mix: sodium, iodine (50 ppm), ferrocyanide.

For the determination of digestibility; the total nitrogen content in the carcass of the rat and the total faeces was determined using the Kjeldahl method [15]. Samples were weighed into a Kjeldahl flask. 10 ml of concentrated sulphuric acid was added followed by one Kjeltec tablet (Kjeltec-Auto 1030 Analyzer, USA). The mixture was digested on heating racket to obtain a clear solution. The digestate was cooled, and made up to 50 ml with distilled water and transferred onto kjeldahl distillation set up followed by 50 ml of 40% sodium hydroxide solution, the ammonia formed in the mixture was subsequently -distilled into 25 ml, 2% boric acid solution containing 0.5 ml of the mixture of 100 ml of bromocresol green solution (prepared by dissolving 100 mg of bromocresol green in 100 ml of methanol) and 70 ml of methyl red solution (prepared by dissolving 100 mg of methyl red in 100 ml methanol) indicators. The distillate collected was then titrated with 0.05M HCl. Blank determination was carried out by excluding the sample from the above procedure

$$N = \frac{1.401 \times M \times (ml\ titrant - ml\ blank)}{sample\ weight}$$

where,

N=nitrogen content (%)

$$M = Molarity\ of\ acid\ used = 0.05\left(\frac{mol}{dm}\right)$$

The balance sheet method of Mitchell (1923) was used to determine the true digestibility (TD).

The nitrogen retained in the experimental animal was calculated as the algebraic difference between the food and the sum of both the faecal nitrogen for the collection period.

NR= N1-(FN)

NR= Nitrogen retained; N1= Nitrogen in food; FN = Faecal Nitrogen

Protein efficiency ratio (PER), net protein utilisation (NPU) and biological value (BV) determination

For the determination of protein efficiency ratio (PER), net protein utilisation (NPU) and the biological value (BV), the food intake by the rats in the last 14 days period was measured. The determined crude protein content of the diet was used to calculate the amount of protein consumed during the test. The PER was calculated using the formula below [16] :-

$$PER = \frac{Weight\ gain\ or\ loss}{Protein\ consumed}$$

The NPU values were calculated using the methodology of Mitchell 1923-4a and the BV was calculated by dividing NPU by TD;

NPU=Body nitrogen (N) of Test Group – Body N of non- protein diet group + Food consumed by non-

$$NPU = \frac{protein\ diet}{N\ Consumed\ by\ test\ Group}$$

The true digestibility (TD) of the dietary nitrogen was determined by the original balance-sheet method and is defined as the percentage of the food nitrogen consumed that is absorbed. It is calculated as:-

True digestibility (%) = Nitrogen intake – (faecal nitrogen – metabolic nitrogen) / nitrogen intake

Metabolic nitrogen is the total faecal nitrogen excreted by the animals on the basal protein-free diet during the experimental period.

The biological value is then determined using the formula below:-

BV = NPU X 100

T.D

Statistical analysis

Data reported were averages of three determinations. Analysis of variance (ANOVA) was performed on each of the variables and the least significant difference (LSD) test at a significant level ($p<0.05$) was performed using SPSS 16 software to compare the differences between treatment means. Results were expressed as the means ± standard deviation of three separate determinations.

Results

Proximate analysis of raw and cooked *A. muricata* seed powder samples

The results of the proximate composition of raw and cooked samples of *A. muricata* as shown in Table 2 reveals that the carbohydrate, crude fiber, ash content, fat, and nitrogen free extract in the raw samples were found to be statistically ($p<0.05$) higher than that of the cooked samples. However, the moisture content and the protein content of the cooked sample was significantly ($p<0.05$) higher than the raw sample.

Mineral composition

The results as shown in Table 3 revealed that the levels of calcium, magnesium, potassium and sodium in the raw soursop seed sample is significantly ($p<0.05$) higher when compared to the cooked samples. However, the iron, copper and zinc levels in the cooked samples were significantly ($p<0.05$) higher than that of the raw samples.

Vitamin composition

As shown in Figure 1, the levels of vitamin A, vitamin C, and vitamin E content in the raw soursop seeds is significantly ($p<0.05$) higher than that of the cooked.

Table 2: Proximate Composition of raw and cooked soursop seed powder.

Parameter (%)	Raw	Cooked
Nitrogen Free Extract	1.03 ± 0.03^a	1.31 ± 0.16^b
Protein	6.57 ± 0.04^b	9.29 ± 0.03^a
Moisture	21.6 ± 0.03^b	27.65 ± 0.28^a
Fat	0.018 ± 0.001^a	0.002 ± 0.001^b
Ash	15.54 ± 0.13^a	12.52 ± 0.097^b
Fiber	12.26 ± 0.0467^a	11.96 ± 0.452^b
Carbohydrate	43.82 ± 0.04^b	47.62 ± 0.17^a

Values are means of three duplicate ± Standard deviation (SD); means with same superscript in the same column are not significantly ($p<0.05$) different; means with different superscript in the same column are significantly ($p<0.05$) different.

Table 3: Mineral composition of soursop.

Mineral (mg/100 g)	Raw	Cooked
Calcium	2.48 ± 0.032^b	2.087 ± 0.032^a
Magnessium	0.88 ± 0.056^b	0.85 ± 0.04^a
Potassium	2.79 ± 0.11^a	1.18 ± 0.101^b
Sodium	0.88 ± 0.005^a	0.24 ± 0.009^b
Iron	64.40 ± 0.84^a	73.49 ± 0.104^b
Copper	0.33 ± 0.02^a	0.95 ± 0.03^b
Zinc	54.26 ± 0.042^a	58.41 ± 0.024^b

Values are means of three duplicate, ± standard deviation; means with same superscript in the same column are not significantly ($p<0.05$) different; means with different superscript in the same column are significantly ($p<0.05$) different.

Anti nutrients composition of raw and cooked soursop seeds

The result shown in Table 4 revealed that the levels of phytate, oxalate, tannin and hydrogen cyanide in the cooked samples were significantly ($p<0.05$) lower as compared to the raw samples with the percentage reduction corresponding to 18.2%, 15.7%, 60.1% and 93.3% respectively.

Result of PER, TD, NPU AND BV

As shown in Table 5, there was an increase in the body weight of the animals fed on the cooked soursop seed as compared to those fed on the raw soursop seed sample. The PER value obtained from the animals fed on cooked was higher (2.22 ± 0.53) as compared to those fed on raw (2.10 ± 0.81). The NPU value obtained from the cooked soursop seed was higher (63.20 ± 0.01) than that obtained for the raw seed sample (58.47 ± 0.004). The TD value obtained from the cooked soursop seed was higher (87.02 ± 0.01) than that obtained for the raw seed sample (85.02 ± 0.004). The BV obtained from feeding the animals on the cooked soursop seed samples was higher (72.63 ± 0.002) than that obtained for those fed on raw seed sample (68.77 ± 0.00).

Discussion

Nutrients are vital to the maintenance of growth and all metabolic processes. Different methods have been employed in the processing of food in order to increase its palatability, taste and for easier consumption and digestion. Cooking was employed in this study as a food processing method. Proximate analysis of the raw and cooked soursop seed revealed that the seed contains considerable amount of carbohydrate, moisture, ash and fiber as well as protein with the cooked seed containing a significantly ($p<0.05$) higher content of carbohydrate, protein and moisture. This implies that the cooked seed is a good source of energy and nutrients that can help the body to maintain its normal metabolic processes.

The crude protein content for the cooked seed was significantly higher ($p<0.05$) than the raw seed. This finding is in agreement with Fasakin [11]. This could be attributed to the increased bioavailability of the protein due to the effect of the processing method which helped to reduce the anti-nutritional factors. Also, the moisture content reflected a significant ($p<0.05$) difference, with the cooked sample having significantly higher values than the raw sample which might be attributed to the fact that in the process of cooking with water, more moisture had been absorbed and this is indicative of a decreased shelf life. The levels of crude fibre in soursop was found to be low with that of the cooked sample significantly ($p<0.05$) lower than that of the raw samples. Fibre cleanses the digestive tract by removing potential carcinogens from the body and prevents the absorption of excess cholesterol. Fibre also adds bulk to the diet and prevents the intake of excess

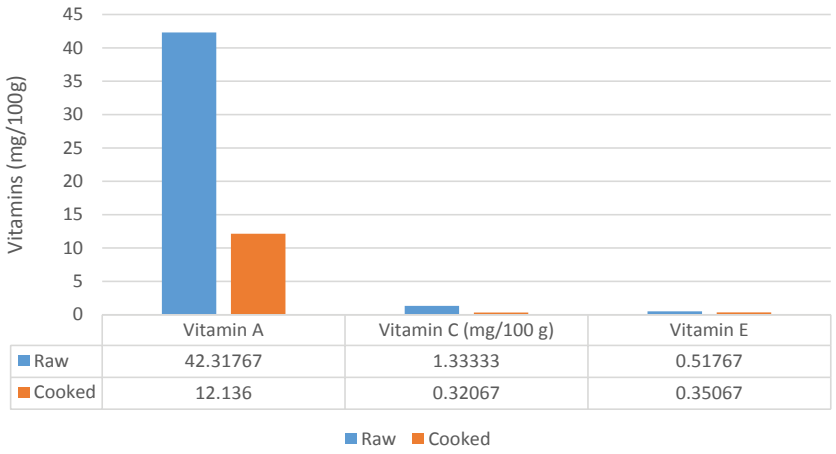

Figure 1: A graph showing the vitamin composition of the raw and cooked soursop seeds.

Table 4: Anti-nutrient composition of raw and cooked soursop seeds.

Anti-nutrient (mg/100g)	Raw	Cooked
Phytate	8.91 ± 0.093[a]	7.29 ± 0.019[b]
Oxalate	6.61 ± 0.009[b]	5.57 ± 0.027[a]
Tannin	8.43 ±0.044[a]	3.36 ± 0.007[b]
HCN	0.015 ± 0.005[b]	0.001 ± 0.001[a]

Values are means of three duplicate ± Standard deviation; means with same superscript in the same column are not significantly ($p<0.05$) different; means with different superscript in the same column are significantly ($p<0.05$) different.

Table 5: The total protein consumed, Net body weight of the rats fed on the control (casein) diet and the raw and cooked soursop seeds and the PER, TD, BV, NPU of the raw and cooked soursop seeds.

Diet	Total Protein Consumed	Net Body weight gain(g)	PER	NPU	TD	BV
Casein (Control diet)	18.91± 5.91	14.02 ± 9.66	2.26 ± 0.05	67.26 ± 1.14	80.67 ± 0.46	83.38 ± 0.07
Raw soursop seed	11.14± 0.24	6.44 ± 15.88	2.10 ± 0.81	58.47 ± 0.39	85.02 ± 0.004	68.77 ± 0.00
Cooked soursop seed	11.23± 0.02	12.83 ± 3.28	2.22 ± 0.53	63.20 ± 0.21	87.02 ± 0.01	72.63 ± 0.002

Each figure represents the mean values; PER = Protein efficiency ratio; TD= Total Digestibility; BV= Biological Value; NPU=Net Protein Utilization

starchy food and may therefore guard against metabolic conditions such as hypercholesterolemia and diabetes mellitus [17].

The level of carbohydrate in the cooked seed was significantly ($p<0.05$) higher than that in the raw seed. This is in alignment with an earlier report that suggests that cooking causes the granules to break down, softens the cellulose and makes the starch more available [11]. The percentage of ash content ranges from 12.52% to 15.55%, with the cooked seed having the least value (12.52%) while the raw seed had the highest value (15.55%). This reduction could be attributed to leaching of the minerals during cooking. Cooked soursop seeds still has higher carbohydrate content compared to other seeds such as soya beans (7.97%) and cocoa shell (46.5%); and higher ash content compared to soya beans (4.99%), guinea corn (2.36%) and cocoa shell (6.70%) and also higher fiber content than soya beans (5.52%) [18].

The most abundant mineral present in soursop is iron, followed by zinc and it contains small amounts of calcium and potassium. Iron is a constituent of some metalloenzymes, myoglobin and haemoglobin which aids the transport of oxygen and carbon dioxide during respiration and also other metabolic processes [19]. Haemoglobin (containing iron) also helps to modulate changes in blood pH thereby serving as a buffer Zinc plays an important role as a cofactor in enzyme catalysis in the body and is also needed for making protein and genetic material [19-21]. Calcium is involved in muscle contraction and blood clotting and it is a major constituent of bone and teeth [19, 22,23]. Potassium exists in the intracellular fluid and helps to preserve electrolyte balance and membrane fluidity [19].

Vitamins are assorted collection of organic molecules that are essential in the diet in minute quantities for health, growth, and survival [24]. The raw soursop seed has a significantly ($p<0.05$) higher content of vitamin A, C and E than the cooked samples; however, vitamin contents of the seed was similar to the value obtained in some commonly consumed fruits which are established dietary sources of vitamins [25]. Vitamins A, C and E all exhibit antioxidant property *in vivo* [26]. Vitamin A is a fat-soluble vitamin which helps maintain normal reproduction, vision and immune function [27]. Vitamin C is required for immune function, collagen and thyroxin synthesis and also boosts the absorption of iron [26]. Vitamin E is also involved in immune function, cell growth, reproduction and DNA repair [26,28,29]. As seen from the results, there was a significant ($p<0.05$) decrease in the phytate, oxalate, tannin and hydrogen cyanide (HCN) in the cooked samples as compared to the raw soursop seed with a higher percentage reduction observed for hydrogen cyanide and the tannin content. Phytate is an anti-nutrient that sequesters minerals like Cu, Ca, Mg, Zn and Fe in the gut, making them less available to our bodies [30]. Oxalate is an anti-nutrient that can over accumulate, causing kidney stones and other health problems; oxalate has also been reported to chelate with Ca^{2+} forming insoluble calcium oxalate [31]. Tannin is a naturally occurring complex chemical that is found in plant, toxic and usually forms insoluble complexes with proteins, thereby interfering with their bioavailability [25]. The lower content of tannin in the cooked sample can be attributed to the fact that tannins are water soluble compounds are easily eliminated by soaking in water [32]. HCN is an anti-nutrient that affects the ash, carbohydrate, and nitrogen-free extract (NFE) content of food [33]. Subjecting foods to heat treatment has been reported to reduce the cyanogen content of foods significantly and this reason can be adduced to the highest percentage reduction in HCN recorded in the cooked sample [32].

Biological value (BV) is an index for the measurement of the proportion of the absorbed protein from a food which becomes incorporated into the proteins of the organism's body [10]. The biological value of cooked soursop seed is relatively high (68.21) which imply that it contains a considerate amount of essential amino acids. Essential amino acids are amino acids that cannot be synthesised in the body and must definitely be obtained from the diet. Examples are histidine, lysine, methionine, isoleucine, leucine, valine, threonine, tryptophan, and phenylalanine [10]. The biological value is an index for measuring the quality of a protein. Plant proteins are a source of food nutrient especially for the less privileged population in developing countries including Nigeria. Proteins are one of the macromolecule and it is an alternate energy source when other energy sources are in short supply. They are building block units and food protein is needed to make vital hormones, important brain chemicals, antibodies, digestive enzymes, and necessary elements for the manufacture of DNA. Some proteins are involved in structural support, while others are involved in bodily movement, or in defense against germs [34]. The cooked sample has a higher BV than the raw sample which might be due to the effect of processing which led to a reduction in the anti-nutrients; thereby increasing the bioavailability of the protein content.

Conclusion

The results of the present study indicated that subjecting soursop seed to cooking treatment is suitable and effective in reducing various anti-nutritional compounds in the seed; leading to the increased bioavailability of the protein as evidenced by its relatively high biological value (BV) and therefore the cooked seed could be adopted as an alternative protein source which might be incorporated into confectioneries and other food items as a protein supplement. However, more studies can be done on the effect of other processing methods on the nutritional profile of the seed to determine the best method that gives the highest BV.

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