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PROXIMATE, MINERAL AND ANTI-NUTRITIONAL ASSESSMENT OF *Annona muricata* (SOURSOP) SEEDS

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ABSTRACT
Annona muricata is one plant whose seed can be explored as food and source of nutrient to both animal and man. The nutritional, minerals and antinutritional value of soursop dehulled seed, coat and unhulled seed were determined using AOAC (2006) method for food analysis. The results of the proximate analysis showed that the dehulled seed had the highest percentage moisture content, ash content and crude protein with 28.01±0.08 %, 2.18±0.04 % and 21.88±0.43 % respectively while the seed coat had the lowest with corresponding values of 20.63±0.93 %, 0.49±0.02 % and 5.69±0.31 % respectively. The fat content and calorific value (20.40±1.02 % and 402.20±0.01 kcal/100g respectively) of the dehulled seed were the highest while the unhulled seed (13.18±1.01% and 352.22±0.01 kcal/100g respectively)were the lowest. Value obtained for crude fibre was highest in the seed coat (6.82±1.01 %) and lowest in the dehulled seed (2.14±0.01%). Carbohydrate content was highest in the unhulled seed (50.53±0.53 %) and lowest in the dehulled seed (32.77±0.77 %). Mineral contents in the three samples were in the order: K > Ca >Mg > Na > Cu. Cyanide content was highest in the dehulled (390.20±1.70 mg/100g) and lowest in the unhulled seed (358.40±1.70 mg/100g). Oxalate and Tannins content in the seed coat (1.58±0.03 and 12.61±3.70 mg/100g respectively) were the highest while the unhulled seed (0.34±0.20 and 5.91±0.24 mg/100g respectively)had the lowest. Results obtained revealed that soursop dehulled seed, seed coat and unhulled seed have some nutritional components required for proper functioning of the body.

INTRODUCTI`ON

Seed is the small hard part of a plant from which a new plant grows. Over time, seeds have been used by human for different purposes (Okafor *et al*., 2015). The most important of those uses is as food; some seeds are eaten directly while others are used for produing flour, starch, oil and some other edible products (Okafor e*t al*., 2015; Suleiman *et al*., 2018; Batista *et al*., 2018; da Cunha *et al*., 2020). The evaluation of food nutritional composition such as protein, carbohydrate, fat and oil, vitamins, minerals and some other nourishing substances present in seeds is of great importance and the science that focus on these aforementioned nourishing substances that seed contains in relation to their health effect is refers to as nutritional science.

Annona muricata (soursop) fruit plant belongs to the family Annonaceae, which have approximately 119 species. It is a medicinal plant, known locally as shawa-shawa in Nigeria. The fruit is oval in shape; its size range from 20 to 40 cm long and the width could be up to 20 cm with a weight of up to 2 kg (Fasakin, *et al*., 2008). The fruit consists of fibrous membrane and white pulp with large seeds well dispersed within it, making it difficult to consume (Badrie and Schauss, 2009). The leaves, fruit and seeds of soursop are known to be used traditionally for the treatment of stomach upset, diarrheal, diabetes, fever and as sedatives in parts of North and South America (Sawant and Dongre, 2014). Soursop fruit is highly perishable and has a short shelf life. Soursop fruit are usually harvested in an unripe state and ripened post harvest (Badrie and Schauss, 2009).

Studies revealed that there are different varieties of wild fruits and lesser known vegetables that are in abundance in Nigeria which could be of health benefit (Thompson, 2003). The availability of fruits is short-lived due to seasonality and the perishable nature; soursop fruit is one of such seasonal and perishable fruits. The fruit is known to be consumed but its seed is believed to be under utilized and information on its nutritional, mineral

and anti-nutritional composition of the soursop dehulled seed, coat and unhulled seed is scanty and these knowledge is very important because it can lead to maximum exploration of the economic potentials of the soursop seed for animal, man and industrial purposes.

MATERIALS AND METHODS

Sample Collection

Soursop fruits used in this research were obtained from Bokkos Local Government Area of Plateau State, Nigeria. The seeds were separated from the pulp, washed thoroughly and dried at room temperature. The seeds were divided into three portions; one was gently crushed to separate the seed from the coat (dehulled), seed and its coat were crushed (unhulled) and only seed coat was crushed.

Sample Preparation

The dehulled, unhulled and seed coats of *Annona muricata* were sorted out carefully and ground separately into fine powder using 750 watts Marlex Excella grinder. These were sieved using 2 mm sieve and further passed through a 45 µm sieveand stored separately in polyethylene bags which are air tight in preparation for analysis.

Proximate Analysis

The proximate compositions of dehulled, unhulled and seed coat of *Annona muricata* were determined using standard analytical methods for food analysis (AOAC, 2006).

Determination of Moisture Content

The crucibles were washed, dried and weighed as W_1 with an analytical weighing balance. To each of the already weighed crucibles, 2 g of the dehulled, unhulled and seed coat of *Annona muricata* were separately added and re-weighed as W_2. The crucible and its content were then placed in an oven at 105 °C until a constant reading was obtained; it was cooled in a desiccator and weighed as W_3.

% Moisture Content = $(W_2-W_3)/(W_2-W1)$ x 100 ...(1)

Where: W_1 = mass of empty crucible; W_2 = mass of crucible plus sample; W_3 = mass of dried sample and crucible.

Determination of Ash Content

A cool dried crucible was weighed as W_1 and 5 g of the sample was added and re-weighed as W_2. This was transferred using a thong into a muffle furnace at 550 °C until it fully ashes (the colour changes to grey) and was weighed as W_3.

% Ash Content = $(W_3-W_1)/(W_2-W_1)$ x 100 ...(2)

Where: W_1 = mass of the empty crucible; W_2 = mass of the crucible and sample;

W_3 = mass of ashed sample and crucible.

Determination of Crude Fat

Fat content was determined using the soxhlet extraction method. 5.00 g of the sample was weighed and wrapped with Whatman No. 1 filter paper, which was folded at the two ends. The filter paper that contains the sample was placed into extraction thimble and returned back to the soxhlet apparatus which was fitted with a weighed flat bottom flask filled with petroleum ether to three quarter of its volume and allowed to boil at a boiling point of about 40 °C to 60 °C. Extraction was done over a period of 6 hours after which extraction was completed. Petroleum ether was evaporated on water bath while the remaining portion in the flask was removed together with water by drying for 30 minutes in oven at 80 °C. This was allowed to cool in a desiccator and weighed. The percentage crude fat content was calculated thus:

$$\% \text{ Crude fat} = \frac{Weight\ of\ extracted\ fat}{Weight\ of\ sample} \times 100 \quad (3)$$

Determination of Crude Protein

Protein content was quantified based on the nitrogen contents of the sample using micro kjeldahl apparatus for distillation. Using an analytical weighing balance, 0.25 g of the dried sample was weighed into a clean 100 cm^3 kjeldahl flask, 5.00 cm^3 concentrated sulphuric acid (H_2SO_4) and a pinch of copper catalyst were added and digested on a kjeldahl digestion block in a fume cupboard at 420 °C for about 3 hours. Complete digestion was indicated by a colour change from brown to colourless (a very clear digest). The flask was then removed from heating and left to cool. The digest was filtered into 50 cm^3 volumetric flask using No. 1 filter paper and made up to the mark using distil water.

The kjeldahl distillation apparatus was set up, 10 cm^3 of the digest was pipetted into the distiller, 10 cm^3 of 40 % NaOH solution was carefully transferred into the distiller and it was properly closed. The solution was allowed to steam and ammonia was liberated. A receiving flask containing 10.00 cm^3 of boric acid with 2-3 mixed indicators was connected to the distillation

chamber under the tip of the condenser. The ammonia liberated was collected by the receiving flask having the solution of boric acid until it reaches 50 cm^3. A colour change from pink to purple was observed, which developed for 5 minutes. The distillate was titrated with 0.01 $moldm^{-3}$ HCl solution with the observation of colour change from purple to pink and the volume of acid used was taken and recorded. The titre values obtained were used to calculate the total nitrogen content, which was converted to percentage protein using the formula:

Total nitrogen (N) = $[(a-b)\times 0.01 \times 0.014 \times D\times 100]/(W\times V)$ ...(4)

% Crude protein = $N \times 6.25$ ...(5)

Where: a = titre value of the digested sample; b = titre value of the blank; V = volume of sample used; W = mass of dried sample; D = dilution factor.

Determination of Crude Fibre

Defatted sample of 2 g was weighed into a 500 cm^3 conical flask and 200 cm^3 of 1.25 % H_2SO_4 was added using a measuring cylinder and allowed to boil for 30 mins on a heating mantle, after which it was left to cool then filtered through a Whatman No. 1 filter paper placed in a conical flask. The residue was collected and transferred into a previously washed conical flask, 200 cm^3 of 1.25 % NaOH was added and allowed to boil for another 30 mins and the solution was filtered. The residue was washed using 10 % HCl, then with ethanol. The resulting residue was transferred into dried and weighed crucible, dried at 105 ^oC for an hour in an oven and allowed to cool in a desiccator to room temperature. The dried residue was ashed in a furnace at 550 ^oC for 30 min then allowed to cool and reweighed. Percentage crude fibre was determined as:

$$\% \text{ Crude fibre} = \frac{\text{Weight of defatted sample} - \text{weight of ashed sample}}{\textit{Weight of defatted sample}} \times 100 \quad ...(6)$$

Determination of Carbohydrate

Percent carbohydrate content was estimated by difference:

Carbohydrate (%) = 100-%(crude protein+crude fat+ash+crude fibre+moisture). ...(7)

Determination of Energy Value

Energy content was quantified using equation (8):

Energy (kcal) = $[(\%CHO\times 4) + (\%CP\times 4) + (CF\times 9)]$

Where: CHO = Carbohydrate; CP = Crude protein; CF = Crude fat.

Mineral Quantification

6 g of powdered sample was weighed into triplicates dry crucibles, placed in a muffle furnace and ignited at 600 ^oC until it turned greyish white ash. The ash was cooled in a desiccator and 5 cm^3 of 1 $moldm^{-3}$ HNO_3 was added to the ashed sample and evaporated on a steam bath to dryness. The treated sample was placed in a muffle furnace and heated until greyish ash was obtained. The sample was then removed and left to cool in a desiccator then retreated with 10 cm^3 of 1 $moldm^{-3}$ HCl followed by filtration into 100 cm^3 volumetric flask. Na and K concentrations were determined using flame emission photometer while the concentrations of Ca, Mg and Cu were determined using Atomic Absorption Spectrophotometer (AAS Model SP9) (AOAC, 2006). Concentration of phosphorus was obtained using Jenway 6100 spectrophotometer at 420 nm (Ceirwyn, 1998).

Determination of Anti-nutritional Content

This was determined using standard analytical methods for food analysis (AOAC, 2005)

Determination of Tannins

0.20 g of the sample was weighed into a 50 cm^3 beaker, 20 cm^3 of 50 % methanol was measured and added then covered with Para film, and placed in a water bath at 77 ^oC for 1 hr. This was shaken vigorously to obtain uniform mixture. The extract was filtered with a double layer Whatman No. 1 filter paper into a 100 cm^3 volumetric flask and 50 % methanol was used to rinse. The volume was made up to mark with distilled water and vigorously mixed. 1 cm^3 of the extract was placed into a 50 cm^3 volumetric flask and 20 cm^3 distilled water was added followed by 2.50 cm^3 Folin Denis reagent and 10 cm^3 of 17 % Na_2CO_3 then mixed well. The mixture was made up to mark with distilled water and allowed to stand for 20 minutes for bluish green colour to develop. Absorbances of tannic acid standard solutions and the sample were obtained after colour development at a wavelength of 760 nm on UV spectrophotometer (Model 752).

Determination of Phytate

1.02 g of the sample was measured into 250 cm^3 conical flask, soaked with 50 cm^3 of 2 % concentrated HCl for 3 hours and then filtered using Whatman No. 1 filter paper. 25 cm^3 of the filtrate was measured into a conical flask and 10.00 cm^3 of distilled water was added in order to obtain normal acidity. 10 cm^3 of 0.30 % ammonium thiocyanate solution was also added and titrated

against standard iron chloride solution containing 0.00195 g iron/ cm^3 and the end point was observed to be brownish-yellow colour that occurred for 5 minutes.

% Phytic acid = y×1.19×100 ...(9)

where y = titre value×0.00195 ...(10)

Determination of Cyanide

Cyanide was determined by alkaline picrate method. 5 g of the sample was dissolved in 50 cm^3 of distilled water in a conical flask well covered. The extraction was left to stand overnight then filtered. 1 cm^3 of the filtrate was mixed with 4 cm^3 alkaline picrate in a corked test tube then incubated for 5 minutes in a water bath. After reddish brown colour development, absorbance was read at 490 nm, the absorbance of the blank containing 1 cm^3 distilled water and 4 cm^3 alkaline picrate solutions were also read. Cyanide concentration was extrapolated from the standard curve of cyanide prepared from different concentrations of KCN solution.

Cyanide (mg/g) = (Absorbance × GF×DF)/(Sample weight) ...(11)

Where GF = gradient factor and DF = dilution factor

Determination of Oxalate

Oxalate in the sample was determined by permanganate titrimetric method as described by AOAC (2005). 2 g of the sample was suspended in 190.00 cm^3 of distilled water in a 250 cm^3 volumetric flask. 10 cm^3 of 6 $moldm^{-3}$ HCl was added and the suspension digested at 100 °C for 1 hr. It was allowed to cool made up to mark then filtered. About 125 cm^3 of the filtrate was measured into a beaker followed by the addition of4 drops of methyl red indicator. This was followed by the addition of NH_4OH solution in drops until test solution colour changes from salmon pink to faint yellow. Each portion was then heated to 90 °C, allowed to cool, precipitate containing ferrous ion was removed by filtration. The filtrate was heated again to 90 °C followed by addition of 10 cm^3 of 5 % $CaCl_2$ solution while being stirred continuously. After heating, it was left to cool overnight at 5 °C, then centrifuged at 2500 rpm for 5 minutes. The supernatant was then decanted and the precipitate dissolved in 10 cm^3 of 20 % H_2SO_4solution. The total resulting filtrate from the digestion was made up to 300 cm^3. Aliquots of 125 cm^3 of the filtrate was heated to near boiling then titrated against 0.05 $moldm^{-3}$ standardized $KMnO_4$ solution until a faint pink colour which persisted for 30 seconds was obtained. Oxalate content was calculated thus:

$$\text{Oxalate content} = \frac{T \times (Vme)\,(Df)\times 10^5)}{(ME)\times Ms)} \quad ...(12)$$

Where: T = titre value of $KMnO_4$; Vme = the volume-mass equivalent (1cm^3 of 0.05$moldm^{-3}$ $KMnO_4$ solution is equivalent to 0.00225g anhydrous oxalic acid); Df = dilution factor (2.5) (Df = VT/A); VT = total volume of titrate (300 cm^3); A= Aliqout used (125 cm^3); ME= molar equivalent of KMnO4 in oxalate; Ms= Mass of sample.

RESULTS AND DISCUSSION

The proximate compositions of the dehulled, hulled and seed coat of *Annona muricata* are presented in Table 1.Results indicate that moisture content were 28.01±0.08, 20.63±0.93 and 22.02±0.98 % for dehulled, seed coat and hulled seeds respectively. The high moisture content in the dehulled seed implies that it cannot be stored for a long time because high moisture content of food makes it easily susceptible to microbial attack and possibilities of spoilage (Musah *et al*., 2020). Values obtained for moisture content were higher than the 5.56±0.02, 4.46±0.02 and 6.54±0.03 % reported for the whole seed, kernel seed and shell of *Jatropha curcas* (Abou-Arab and Abou-Salam, 2010).

Ash content indicates the level of the minerals stuffing in a food sample. The ash content of the dehulled seed (2.18±0.04 %) was higher than that of the seed coat (0.49±0.02 %) and unhulled seed (1.75±0.20 %). This is an indication that the dehulled seed will have more mineral stuffing

Table 1: Proximate Composition of the Dehulled, Seed Coat and Hulled of Soursop

Parameters	Dehulled Seed	Seed Coat	Unhulled Seed
Moisture %	.....	20.63±0.93	22.02±0.98
Ash %	2.18±0.04	0.49±0.02	1.75±0.20
Fat %	20.40±1.02	15.40±1.00	13.18±1.00
Crude fibre %	2.14±0.01	6.82±1.00	4.65±0.00
Crude protein %	21.88±0.43	5.69±0.31	7.87±0.13
Carbohydrate %	32.77±0.77	48.59±1.41	50.53±0.53
Calorific value (kcal/100g)	402.20±0.01	355.72±0.00	352.22±0.01

Values are means of triplicate readings ± standard deviation

when compared to the seed coat and unhulled seed. These values were lower than the 3.30 % reported in *Afzelia Africana* seed (Nzekwe *et al*., 2016). Values obtained revealed that seed coat (6.82±1.00 %) had higher crude fibre content than the dehulled seed (2.14±0.01 %) and unhulled seed (4.65±0.00 %). High dietary fiber aids digestion and absorption of nutrients from food in the body (Nzekwe *et al*., 2016). This implies that the seed coat could aid digestion and absorption of nutrients in the body when compared to the dehulled and unhulled seeds. The value of crude protein in the dehulled seed, seed coat and unhulled seed were 21.88±0.43, 5.69±0.31 and 7.87±0.13 % respectively. The value obtained for the dehulled seed indicates that it is richer in crude protein than the seed coat and unhulled seed; the value is also higher than the 17.49±0.01 % reported for dehulled *Artocarpus altilis* seed (Tukura and Obliva, 2015). Proteins are essential nitrogenous compound needed by both man and animal for growth, development and repair of damage tissues, they do so by providing crucial body nutrients, maintaining body fluid balance and contributing to body immune function and enzymes formation. The fat content of the dehulled seed, the seed coat and the unhulled seed were 20.40±1.02 %, 15.40±1.00 % and 13.18±1.00 % respectively. The dehulled seed was richer in fat implying that it is a good source of energy. Values obtained were lower than 47.18±2.71 and 27.36±3.98 % reported for the dehulled and unhulled *Jathropha curcas* seeds respectively (Abuo-Arab and Abu-Salam, 2010). One major importance of dietary fat is that it helps to absorb and retain flavour, thereby increasing the palatability of food. Any food that supplies 1-2 % of its caloric energy as fat is good for the body, as too much of fat can lead to some cardiovascular diseases (Antia *et al*., 2006). Carbohydrate content obtained for the dehulled seed was 32.77±0.77 %, coat was 48.59±1.41 % and unhulled seed was 50.53±0.53 %. The seemingly high carbohydrate content of the unhulled seed was lower than the 70.05±.14 % reported for *Vigna unguiculata spp sesquipedalis* seed (Musah *et al*., 2020). The high carbohydrate content of soursop seeds implies that they could be utilized as good source of energy.

Values of mineral composition of soursop dehulled seed, coat and unhulled seed are presented in Table 2. Results revealed that dehulled seed contained 0.505±0.02 mg/100g, the coat had 0.505±0.12 mg/100g and the unhulled seed contained 0.500±0.09 mg/100g calcium respectively. Calcium in the samples was lower than the 34.21±4.47, 51.41±3.49 and 28.01±0.52 mg/100g obtained for whole seed, kernel seed and shell of *Jathropha curcas* seeds respectively (Abuo-Arab and Abu-Salam, 2010). Calcium is known to play a vital role in blood clothing, functioning of muscles and formation of strong bone (Bell *et al*., 1996).

Magnesium contents were 0.22±0.07, 0.22±0.03 and 0.21±0.21 mg/100g for dehulled seed, seed coat and unhulled seed respectively. Magnesium, together with calcium can assist in blood clothing and regulation of blood pressure (Adeyeye and Agesin, 2007). Potassium content in the dehulled seed was 0.70±0.13 mg/100g, seed coat was 0.72±0.19 mg/100g and unhulled seed contained 0.72±0.01 mg/100g. These values are lower than 24.15±1.15 mg/100g in *Vigna unguiculata spp sesquipedalis* seed (Musah *et al*., 2020).

Table 2: Mineral Content of Soursop Dehulled Seed, Coat and unhulled Seed in mg/100g

Parameters	Dehulled Seed	Coat	Unhulled Seed
Calcium	0.505±0.02	0.505±0.12	0.500±0.09
Magnesium	0.220±0.07	0.220±0.03	0.220±0.21
Copper	0.100±0.11	0.100±0.17	0.110±0.37
Potassium	0.700±0.13	0.720±0.19	0.720±0.01
Sodium	0.210±0.05	0.210±0.07	0.210±0.03

Values are means of triplicate readings ± standard deviation

Potassium is required for normal tissue protein and normal function of the heart and kidney muscle (Ogbuagu *et al*., 2011). Values of sodium content in dehulled seed, seed coat and unhulled seed were 0.21±0.05, 0.20±0.07 and 0.21±0.03 mg/100g respectively. Sodium is required for maintaining osmotic pressure within the cell and preventing them from collapsing (Eddy and Udoh, 2005). Copper content in dehulled seed was 0.100±0.17 mg/100g; 0.101±0.17 mg/100g in seed coat and 0.200±0.37 mg/100g was the value for unhulled seed. These values are slightly lower than 0.44±0.64 mg/100g obtained for *Boerhavia elegana choisy* (Al-Farga *et al*., 2016).

The anti-nutrient components of soursop seeds are presented in Table 3. Results revealed the presence of tannin in dehulled seed (8.41±1.20 mg/100g), seed coat (12.61±1.07 mg/100g) and unhulled seed (5.91±0.24 mg/100g). Tannin helps to improve the healing of wound due to its ability to bind to protein of exposed tissues and precipitate the protein, which forms a slight anti-septic protective effect. Tannin contains tannic acid which when ingested in large amount can cause effects such as stomach irritation, vomiting and liver damage. Regular consumption of herbs with high tannin concentration can lead to increased chances of developing nose or throat cancer (Sharma *et al*., 2019).

Phytate content in dehulled seed, seed coat and unhulled seed were 263.40±1.70, 240.79±0.97 and 259.29±1.04 mg/100g respectively. Phytic acid is known to chelate some metals like Ca, Mg, Fe, and Zn leading to the formation of insoluble complexes that are not easily broken down and may pass through the digestive tract unchanged, making these essential metals unavailable for human body (Ekholm *et al*., 2003). Values obtained for oxalate content indicate 0.66±0.03 mg/100g for dehulled seed, 1.58±0.03 mg/100g for seed coat and 0.34±0.20 mg/100g for unhulled seed. When consumed in large quantity, oxalate can cause the formation of kidney stones (Gemede, 2014). Cyanide content in the dehulled seed, seed coat and unhulled seed was higher than other anti-nutrients determined. Values obtained were 390.20±1.70, 364.60±1.07 and 358.40±1.7mg/100g for dehulled seed, seed coat and unhulled seed respectively. Cyanide in the body can prevent cells from using oxygen and eventually these cells dies. The heart, respiratory system and central nervous system are most susceptible to poisoning by cyanide (Graham and Traylor, 2021).

Table 3: Anti-nutrient composition of soursop dehulled seed, coat and unhulled seed in mg/100g

Parameters	Dehulled Seed	Coat	Un hulled Seed
Cyanides	390.20±1.70	364.60±1.07	358.40±1.70
Oxalates	0.66±0.03	1.58±0.03	0.34±0.20
Phytates	263.40±1.74	240.79±0.97	259.29±1.04
Tannins	8.41±1.20	12.61±1.07	5.91±0.24

Values are means of triplicate readings ± standard deviation

CONCLUSION

From the results obtained in this study, it is apparent that the dehulled seed of soursop(*Annona muricata*) had the highest percentage moisture, ash, crude fat, crude protein and energy values. However, the crude fibre and carbohydrate values were found to be the lowest when compared to the seed coat and unhulled seed of *Annona muricata*. Also, the seed coat had the highest crude fibre while the unhulled seed had the highest carbohydrate value. More so, the dehulled seed had the highest cyanides and phytates content while the seed coat had the highest oxalates and tannins content. Furthermore, the dehulled seed, seed coat and unhulled seed contains substantial amount of some essential minerals like calcium, potassium, sodium and copper in trace quantities. The nutritional qualities of dehulled seed, coat and inhulled seed of soursop can be exploited to provide high-quality diets.

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