



**PROXIMATE ANALYSIS, MINERAL AND FATTY ACID COMPOSITION OF *Annona muricata* SEED**

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**Abstract**

This study investigated the proximate, mineral and fatty acid composition of *Annona muricata* seed cultivated in Ikom, Cross River State, Nigeria. The *Annona muricata* seeds were removed from the fruit pulp, dried at room temperature and blended using a mill and further blended into powder using electric blender. Proximate composition was determined using standard methods. Atomic Absorption Spectroscopy and Gas chromatography mass spectroscopy were used to determine mineral and fatty acid contents respectively. Proximate analysis revealed high contents of fat, fiber and protein. Mineral analysis of the seed showed that Potassium (128.9 mg/100g) and Calcium (94.66 mg/100g) respectively were the most abundant elements in the seed. Iron, Sodium and Zinc were also detected. The fatty acid profile indicated 15 fatty acids (both saturated and unsaturated) with DHA (121%), DPA (61.4%) and Stearic acid (19.96%) as the major fatty acids in the *Annona muricata* seed. The nutrient composition of *Annona muricata* seed revealed its potential use in feed formulation and the seed oil could serve as a raw material for industrial applications.

**Key words:** *Annona muricata*, DHA, DPA, Proximate Ccomposition, Fatty acid.

**1.0 Introduction**

*Annona muricata*, commonly known as soursop) belongs to the family Annonaceae (Moghadamtousi *et al.*, 2015). *Annona muricata* is gaining tremendous fame owing to its rich nutritional and appropriate maturity indicators (Chang *et al.*, 2015). It is also called sour sop because the plant produces edible fruits which has characteristic sour taste. The *A. muricata* is known for its edible fruits which grows to about 5 to 6m in height. The fruit has a

fleshy endocarp size ranging from 10 to 30cm long and up to 4.5 to 6.8kg, whitish and acidic taste especially when ripe and characterized with the presence of abundant seeds. *A. muricata* seed has been used against various cancer cells (Chang, 2015). It has the longest fruits in the genus. It is astringent, cholagogic and promotes digestion (Pamphona- Roger, 2005). The plant is indigenous to the warmest tropical and subtropical parts of the world; including India, Malaysia and Nigeria (Adewole and

Caxton-Martins, 2006). *A. muricata* is an evergreen tree giving 5-8m height with large, glossy dark green leaves and large green color heart shape fruit in 15-20cm diameter, with unique pleasant, sub-acid and aromatic flavor. Each fruit may contain 55 - 170 black seeds when fresh and they turn light brown when dry (Wahab *et al.*, 2018).

*A. muricata* can be slender and upright or low spreading and bushy and becomes straggly as it matures. The hermaphrodite flowers, which have a unique fragrance, are generally produced singularly or in small clusters on ancient wood. The flowering phase begins at age 3 to 4, though this may vary depending on the environment. The fruit is heart-shaped to oval, depending on its pollination. The fruiting occurs most of the year, but it may become seasonal depending on the altitude. The fruit will become distorted with an irregular shape and undersized when it has poor pollination and unfertilized ovules fail to develop. This skin has many recurved soft spines 0.5 to 1.3cm apart (Leonti *et al.*, 2018).

The fruit's flesh comprises an edible white pulp, fiber and a core of indigestible black seeds. The pulp is also used for flavor sweets, sorbets, and ice cream, as well as fruit nectar smoothies, and fruit- juice beverages. *Annona muricata* fruit contain considerable amount of seeds and many parts of *A. muricata* are widely used for their medicinal benefits to man, yet its seeds are often discarded without consideration for domestic or industrial applications. This study was therefore conducted to determine the proximate, mineral and fatty acid composition of the seed of *A. muricata seed*.

## 2.0 Materials and methods

### 2.1 Plant materials

The *A. muricata* fruits were bought directly from farmers farmers in Okundi, Ikom Local Government of Cross River State of Nigeria, West Africa with coordinates: 6° 21' 0" N, 8° 48' 0" E. The fruit was identified by Dr. Effa A. Effa, a botanist of Department of

Plant and Ecological studies , University of Calabar on 11/12/2023. The Voucher number (BOT/Herb/UCC/048) was deposited in the herbarium for future reference. They were well packaged and transported to the Department of Biochemistry, University of Calabar, Calabar, Cross River State, Nigeria for further studies.

### 2.2 Seed preparation

The fresh fruits were rinsed thoroughly under running tap water, then slice into four parts to expose the seeds. The seeds were removed, washed and allowed to air dry for two (2) weeks. The dried seeds were first, grounded into coarse particle and further blended into powder using electric blender. (Binatone, BLG-675-SS Model, New Delhi, India)

### 2.3 Determination of Proximate Composition

#### 2.3.1 Estimation of crude fat content

Crude fat determined using an automated FOSS Soxtec system 8000 as described in AOAC (2005). Crude fat was extracted from 5g of the moisture free sample which was accurately weigh into a thimble and extracted for about three (3) hours with petroleum ether (40<sup>0</sup> – 60<sup>0</sup>C) using a soxhlet apparatus. and the solvent evaporated leaving behind the fat. The difference between the initial and final weight of the extraction cup was recorded as the crude fat content.

The percentage lipid or fat is calculated thus:

$$\% \text{ lipid or fat} = \frac{\text{Weight of lipid}}{\text{Weight of sample}} \times \frac{100}{1}$$

#### 2.3.2 Estimation of crude protein content

Kjedahl method was applied into determine the crude protein content using kjedahl apparatus (Kjeltec<sup>TM</sup> model 8400, Slangerupgade, Denmark). This was an

indirect method of quantifying protein in the sample (AOAC, 2005).

### 2.3.3 Estimation of moisture content

The moisture content of the sample was determined using the approved method 925.09 of the Association of Official Analytical Chemists (AOAC, 2005). Three grams of the fresh sample was dispersed into a pre-weighed aluminium can and placed in an air conventional oven (Memmert UN 55, GmbH) for 16hrs at 105°C until constant weight was attained.

### 2.3.4 Estimation of crude fibre content

The content of crude fibre was determined using standard method (AOAC, 2005). Exactly 5.0g (w) was weighed and subjected to extraction with petroleum ether using Soxhlet apparatus for 3 hours. The fat free extract obtained was transferred to a 1-litre flask. 200mls of dilute sulphuric acid in a

beaker was boiled and transferred to the flask containing the fat sample and immediately connected to the flask with a water-cooled reflux condenser and heated. The content of the flask boiled within a minute and the flask was rotated frequently. After boiling for 30 minutes the flask was removed and filtered through a fine linen cloth (about 18 threads to the centimeter) held in the funnel and washed with boiling water until the washing was no longer acidic to litmus. The crucible and its content was dried at 105±2°C in an air oven to a constant weight, cooled and weighed (W1). Also the content of the Gooch crucible was incinerated in an electric muffle furnace at 600±20°C until all the carbonaceous matter was burnt. The Gooch crucible containing the ash in a desiccators was left to cool and weighed (N<sub>2</sub>)

### Calculation

$$\text{Crude fibre(\%)} = \frac{(\text{Dry weight of digested sample} - \text{Weight of ash})}{\text{Weight of Sample}} \times 100$$

### 2.3.5 Estimation of carbohydrate content

The quantity of carbohydrate in the plant sample was calculated using the mathematical formula (AOAC, 2005) below:

$$\text{Carbohydrate} = [100 - (\% \text{ moisture} + \% \text{ fats} + \% \text{ proteins} + \% \text{ fibre} + \% \text{ ash})].$$

### 2.4 Determination of sodium and potassium

Sodium and potassium content was determined by flame photometry (A. O. A. C., 2005). About 1.0g of the sample was digested with 20ml of acid mixture (6.50ml of concentrated HNO<sub>3</sub>; 80ml perchlorate (PCA); 20ml Conc. H<sub>2</sub>SO<sub>4</sub>) and aliquots of the diluted clear digest taken for flame photometry using Gallen-kamp flask analyzer.

### 2.5 Determination of calcium, zinc and iron

Calcium, Zinc and Iron was determined using Atomic Absorption Spectrophotometer. About 1.0g of the sample was digested with 20ml of acid mixture (650ml conc. HNO<sub>3</sub>; 80ml PCA; zinc conc. H<sub>2</sub>SO<sub>4</sub>) and aliquots of the diluted clear digest taken for atomic absorption spectrophotometry using filters that match the different elements.

### 2.6 Determination of Fatty Acid Composition.

Fatty Acid Composition of the *A. muricata* seed was determined using GC-MS

### 2.7 Statistical analysis

Statistical analysis was done using SPSS version 25, Microsoft Excel. Values were expressed as Mean ± Standard deviation

### 3.0 Results

#### Proximate, mineral and vitamin content of the *A. muricata* seed

Results of the Proximate, mineral and vitamins composition of *A. muricata* seed are shown in Table 1, 2 and 3 respectively. In decreasing order of percentage, the nutritional composition was as follows: Carbohydrate > Fat > Crude protein > Moisture > Ash content > Crude fibre. The

seeds also contain Potassium> Calcium>Sodium> Iron > Zinc in decreasing order of quantity. The *A. muricata* seed contains Vitamin A (8.415ug), Vitamin B<sub>1</sub>(0.53mg/100mg), Vitamin B<sub>2</sub>(1.85mg/100mg), Vitamin B<sub>3</sub>(0.455mg/100g), Vitamin C(2.365mg/100g) and Vitamin E(4.27mg/100mg) respectively.

Table 1: Proximate composition of the seed of *Annona muricata*

<u>Proximate composition</u>	<u>% Contents</u>
Moisture	11.41 ± 0.03
Ash	3.265 ± 0.02
Fat	26.06 ± 0.62
Crude fiber	2.935 ± 0.02
Crude protein	17.71 ± 0.12
Carbohydrate	38.625 ± 0.45
Dry matter	88.595 ± 0.03

Values are expressed as Mean ± SD of triplicate samples (n= 3)

Mathematical Formula for calculating Carbohydrate(%)

Carbohydrate (%) = [ 100 - % moisture + % fat + % protein + % fibre + % ash]

TABLE 2: Mineral contents of the seed of *Annona muricata*

<u>Minerals</u>	<u>Conc(mg/100g)</u>
Fe	1.24 ± 0.03
K	128.9± 0.12
Zn	0.625 ± 0.02
Ca	94.66 ± 0.45
Na	54.41± 0.42

Values are expressed as Mean ± SD of triplicate samples (n= 3)

TABLE 3: Vitamin contents of *A. muricata* seed

<b>Vitamins</b>	<b>Concentration</b>
A	8.415ug
B <sub>1</sub>	0.53mg/100mg
B <sub>2</sub>	1.85mg/100g
B <sub>3</sub>	0.455mg/100g
C	2.365mg/100g
E	4.27mg/100g

Values are expressed as Mean  $\pm$  SD of triplicate samples (n= 3)

#### **Fatty acids composition of *Annona muricata* seed**

Result of the GC – MS analysis of the fatty acid composition is shown in Table 4. The result shows the presence of 15 compounds. Docosa-pentaenoic acid, DPA(121%) and Docosa – hexaenoic acid ,DHA (61%) were the major fatty acid presence in the seeds.

Stearic acid (19%), Palmitic acid (13%) and Myristic acid (12%), were the major SFAs present. While Arachidonic acid (10%) and  $\alpha$ -Linolenic (6%) were among the minor PUFAs. Linoleic acid (1%) and Oleic acid (3%) were the minor fatty acids presence in the seeds.

TABLE 4: Fatty acid composition of *Annona muricata* seeds

PK#	RT (min)	Name of compound	No. Of Carbon Atom	Amount (ppm)	Peak area (%)	Molecular Structure
1	2.533	n-Dodecanonic acid (Lauric acid)	C <sub>12</sub>		-	CH <sub>3</sub> (CH <sub>2</sub> ) <sub>10</sub> COOH
2	5.260	n-Tetradecanic acid (Myristic acid)	C <sub>14</sub>	4.75041	12.497	CH <sub>3</sub> (CH <sub>2</sub> ) <sub>12</sub> COOH
3	5.996	n-Hexadecanoic acid (Pamitic acid)	C <sub>16</sub>	4.99372	13.136	CH <sub>3</sub> (CH <sub>2</sub> ) <sub>14</sub> COOH
4	7.294	n-Octadecanoic acid (Stearic acid)	C <sub>18</sub>	7.62389	19.964	CH <sub>3</sub> (CH <sub>2</sub> ) <sub>16</sub> COOH
5	9.150	Cis-9-Hexadecenoic acid (Oleic acid)	C <sub>18:1</sub>	1.43271	3.885	CH <sub>3</sub> (CH <sub>2</sub> ) <sub>7</sub> CH=CH(CH <sub>2</sub> ) <sub>7</sub> COOH
6	9.418	Cis-cis-9,12-Octadecadienoic acid (Linoleic acid)	C <sub>18:2</sub>	5.44620	1.590	CH <sub>3</sub> (CH <sub>2</sub> ) <sub>4</sub> CH=CHCH <sub>2</sub> CH=CH(CH <sub>2</sub> ) <sub>7</sub> COOH
7	10.002	Cis-cis-9,12,15-Octadecatrienoic acid ( $\alpha$ -Linolenic)	C <sub>18:3</sub>	2.37446	6.342	CH <sub>3</sub> CH <sub>2</sub> CH=CHCH <sub>2</sub> CH=CHCH <sub>2</sub> CH=CH(CH <sub>2</sub> ) <sub>7</sub> COOH
8	10.604	n-Eicosanoic acid (Arachidic acid)	C <sub>20:4</sub>	1.98312	5.327	CH <sub>3</sub> (CH <sub>2</sub> ) <sub>18</sub> COOH
9	10.765	Arachidonic acid	C <sub>20:4</sub>	3.77261	10.188	CH <sub>3</sub> (CH <sub>2</sub> ) <sub>4</sub> CH=CHCH <sub>2</sub> CH=CHCH <sub>2</sub> CH=CHCH <sub>2</sub> CH=CH(CH <sub>2</sub> ) <sub>3</sub> COOH
10	11.425	Docosanoic acid	C <sub>22</sub>	1.97515	5.304	- C <sub>22</sub> H <sub>44</sub> O <sub>2</sub>
11	12.729	Docosa-tetraenoic acid	C <sub>22:4</sub>	3.14909	8.365	- C <sub>22</sub> H <sub>44</sub> O <sub>2</sub>
12	13.729	Docosa-pentaenoic acid	C <sub>22:5</sub>	46.80918	121.695	- - <u>C<sub>22</sub>H<sub>34</sub>O<sub>2</sub></u>
13	13.804	Docosa-hexaenoic acid	C <sub>22:6</sub>	22.25785	61.496	- - <u>C<sub>22</sub>H<sub>32</sub>O<sub>2</sub></u>
14	17.874	Eicosa-penaenoic acid	C <sub>20:5</sub>		-	- <u>C<sub>20</sub>H<sub>30</sub>O<sub>2</sub></u>
15	18.063	Eicosatrienoic acid	C <sub>20:3</sub>		-	- <u>C<sub>20</sub>H<sub>34</sub>O<sub>2</sub></u>

Rt = Retention time

#### 4.0 Discussion

*Annona muricata* seeds contain a significant percentage of protein, carbohydrate and fats. In decreasing order of percentage, the nutritional composition was as follows: Carbohydrate > Fat > Crude protein > Moisture > Ash content > Crude fibre. Similar pattern of proximate composition was reported by Nwaehujor *et al.* (2020). However, Kimbonguila *et al.* (2010) reported 40% fat content for seeds obtained from Congo Brazzaville. This value is quite high compared to the results of our findings, though, they reported a low fiber content (3.2%) which is in agreement with our findings (2.93%). Moreover, Nwaehujor *et al.* (2019) reported a very high fiber content (35.2%) as against the low fiber content of our sample. Fiber is known to increase bile acid excretion that increases hepatic uptake of LDL-c, to supply the substrate for bile acid synthesis (Bassey *et al.*, 2023).

This study also shows that *A. muricata* seeds contains Iron, Fe(1.4mg/100g), Potassium, K(128.92mg/100g), Zinc, Zn (0.625mg/100g), Calcium, Ca (94.46mg/100g) and Sodium, Na(54.41mg/100g) respectively. The *A. muricata* seed also contains Vitamin A (8.415ug), Vitamin B1 (0.53mg/100mg) Vitamin B2 (1.85mg/100mg), VitaminB3(0.455mg/100g), Vitamin C(2.365mg/100g) and Vitamin E(4.27mg/100mg) respectively. But, Nwaehujor *et al.*, 2020 reported Calcium (3.5mg/g) as the most abundant element in the seed.

It is well established that most nutritional disorders are generated by diets lacking in minerals and vitamins (Rouphael *et al.*, 2017). Micronutrients are dietary components, often referred to as vitamins and minerals, which although only required by the body in small amounts, are vital to development, disease prevention, and wellbeing (Centers for Disease Control and Prevention, 2024) of the human body.

Micronutrients are not produced in the body and must be provided by the diet (Kraemer, 2021). Some minerals play important roles in biological functions of plants and animals. Calcium for example, performs a significant function in the uptake of dietary Vitamin B and activation of lipase. It also functions in the synthesis of neurotransmitter acetylcholine. Calcium supplements lowers blood pressure. It helps in the building and maintaining of bone mass. Iron is the main component of hemoglobin and many enzymes that play significant function in the oxygenation of red blood cells. It is needed to improve the immune system and for energy production, its deficiency results in anemia. (Olaniyi *et al.*, 2018). Zinc is a major component of many enzymes. It plays a significant function in alcohol dehydrogenase, ribonucleic polymerases, alkaline phosphatase and carbonic anhydrase. Deficiency of Zinc during pregnancy may lead to developmental disorder in offspring (Olaniyi *et al.*, 2018). Zinc deficiency can also cause coronary disease. These elements were determined in the seeds of soursop (Table 2), so they could be further applied as a source of minerals in food products.

The DHA (C22:6) and DPA (C22:5) were the major fatty acid found in *A. muricata* seeds in this work. The seeds also contain Omega 3 PUFAs such as linoleic acid (C18:2) and linolenic acid (C18:3) which are essential fatty acids because human must obtain from diets. But, Zaha *et al.*, 2019 reported that the major monounsaturated and saturated fatty acids in *A. muricata* seeds were oleic acid (39.2%) and palmitic acid (19.1–19.2%), respectively, whereas the  $\alpha$ -linolenic acid (1.2%) and linoleic acid (34.9%) were polyunsaturated fatty acid. Emerging evidence suggests that docosapentaenoic acid(DPA) the intermediate fatty acid between EPA and DHA may also play a role in imparting health benefits previously attributed solely to EPA and DHA.(Richter *et al.*, 2016). A

lower serum concentration of DPA has been associated with greater risk of myocardial infarction. (Nsor *et al*, 2024). Also, plasma Omega 3 DPA has been inversely associated with total mortality particularly from stroke-related deaths, (Mozaffarian *et al.*, 2013), non fatal myocardial infarction and incident CVD in some ethnic groups (de Oliveira Otto., 2023).

An inverse association with the inflammatory marker C- reactive protein has also been reported for plasma, serum, and RBC Omega 3 DPA concentration. (de Oliveira Otto., 2023).

Endogenous DPA stores may also serve as a source of EPA, which could have important implications for disease states. EPA and DPA are highly interconvertible and EPA concentrations have been increased by DPA supplementation in both cell-based and clinical studies (Miller *et al.*, 2013). Again, 22 – carbon fatty acids may be preferentially stored in specific tissue compartments, and in the case of Omega 3 DPA, may serve to replenish plasma EPA that has been utilized. However, less research has been conducted on DPA compared to EPA and DHA and consequently the contribution of DPA to total Omega 3 PUFA intake has not been a focus of nutrition information messaging. Linoleic and linolenic acids are not synthesized in the human body, but are considered essential to the human diet (Egydio & Santos, 2021; Santos *et al.*, 2016). Also, DHA is the most important n-3 long-chain polyunsaturated fatty acids in the tissues, especially in the brain and retina. In infants, it has been shown that DHA is essential for brain development and functions, as the major component of neural membrane phospholipids (Guo *et al*, 2018) and could prevent cardiovascular diseases and neurodegenerative disorders in adults (Guo *et al*, 2018). All the same, the *A. muricata* seeds are potential alternative sources of oil as raw materials for industrial

purposes in order to prevent to post harvest loss.

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**Contributions by authors:** All authors contributed, read and approved the manuscript

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