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Evaluation of Amino Acid Profile of *Annona muricata* (Soursop) Seed Meal Utilization As A Potential Growth Factor and Fish Health Promoter in the Aquaculture Business in Owerri, Southeast, Nigeria

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ABSTRACT

Aquaculture has witnessed remarkable growth in recent decades, driven by increasing demand for fish as a source of protein and the depletion of wild fish stocks. Fish feeds constitutes 40-60% of total cost of aquaculture production, which has made the production of fish highly expensive. Fish meal and soy bean meal have been the primary protein sources in aquaculture feeds due to their excellent nutritional profiles, including well- balanced amino acid composition and high digestibility. However, the competitive nature and rising costs of theses major protein sources have prompted researchers to explore alternative plant-based protein sources. Plant-based proteins offer several advantages such as lower cost, greater availability and potential sustainability for fish feed production. Therefore, attempts are made to cut down the cost of production without actually compromising in the nutritional quality and bioavailability of feed ingredients for fish culture. The first approach towards the research was by gathering the seeds together, and subjecting them into fermentation process technique that lasted for 5 days. This was done to detoxify the *A. muricata* seeds of some anti- nutritional factors to enhance the nutrient value of the plant seed. The fermented seeds were brought out and air-dried at room temperature of 20-25°C for 4 days. The air-dried seeds were grounded into powdery form and subjected to Amino Acid Analysis, using the High Performance Liquid Chromatography (HPLC) equipped with a binary pump and UV/Vis detectors. This was followed with a fluorescence detector to separate them into columns for individual amino acid separation and identification. The results revealed a total a total of 18 Amino Acids identified, with Total Amino Acid content of 106.13g/100g Crude Protein, 45.929/100g Total Essential Amino Acids (TEAA) and 60.21 Non-Essential Amino-Acid (TNAA). Among the Essential Amino Acids identified, arginine exhibited the mean value of 8.12±0.19g/100g Crude Protein, followed by Leucine (6.55±0.16)g/100g and Phenylalanine (6.45±0.19)g/100 Protein respectively. However, Lysine content of 4.61±0.08g/100g. Protein was lower than the recommended level of 5.50g/100g Protein. The Predicted Protein Efficiency Ratio (P-PER) of 1.45 suggests a good protein quality and digestibility. The Total Sulfur Amino Acid content of 4.50g/100g Crude Protein was within the recommended range. Compared to the conventional plant- protein sources,sour sop. Seeds exhibited a higher Total Essential Amino Acid content than soy bean meal (39.60g/100g), but lower than fish meal (57.60g/100g) Crude Protein. The Amino Acid profile of *A. muricata* (Sour Sop) seeds indicates their potential as a valuable protein sources for fish feed formulation, particularly when combine with other protein sources to complement the Amino Acid composition.

INTRODUCTION

Fish feed accounts for 40-60% of the overall expenses in aquaculture, contributing to higher fish production costs. This situation has led to increased research on alternative feed components to substitute traditional sources of protein, such as fish meal and soybean meal. The growth and survival rates of aquatic species depend significantly on the formulation of nutritionally balanced diets (Tom & Van-Nostrand, 1989). Fish meal and soybean meal are recognized as the most effective ingredients in the production of fish feed due to their alignment with the protein needs of fish regarding energy and amino acid profiles. Abowei & Ekubo (2006) further noted that the rising demand for these conventional feed ingredients has sparked an interest in exploring other affordable, unconventional diets, like A. muricata, to bolster fish production. Nevertheless, efforts to reduce feed production costs have sometimes resulted in the partial or complete replacement of soybean meal with alternative ingredients without properly assessing their amino acid compositions. Numerous researchers have proposed strategies for incorporating plant protein sources into fish feed formulations as a means to lower costs and boost fish production (Fasuyi & Aletor, 2005). The use of plant protein sources in animal husbandry has gained significant scientific and commercial acceptance as a method to enhance the growth and health conditions of animals (Djakalia *et al.,* 2011; Ugwu *et al.,* 2011). In aquaculture, herbal plants such as A. muricata may offer active compounds that could help improve both the growth and immune systems of fish (Citarasu, 2024; Magsood *et al.*, 2011).

Annona muricata, commonly referred to as Soursop, is a tropical fruit tree that is cultivated extensively across various regions of the globe. This fruit is indigenous to the Caribbean, Central, and Southern America. Belonging to the Annonaceae family, which is known as the Custard Apple family, A. muricata has approximately 130 genera and around 2,300 species. It thrives in many tropical and subtropical areas worldwide, including countries like India, Malaysia, and Nigeria (Adewole *et al.,* 2006). A. muricata is an upright, evergreen tree that can reach heights of 5-8 meters, featuring large, glossy, dark green leaves, and is found across most tropical nations (Suleman et al., 2012). The seeds of Soursop contain various phytochemicals recognized for their medicinal properties. Both qualitative and quantitative analyses reveal the presence of compounds such as Alkaloids, Tannins, Saponins, Flavonoids, and Glycosidic Glycogen. Alkaloids are predominantly found in plants, especially in many flowering varieties (Girdhar *et al.*, 2015). These compounds exhibit a range of pharmacological effects, including anti-inflammatory, anti-hypertensive, antioxidant, anti-cancer, antimicrobial, and the ability to reduce cholesterol and glucose levels (Choudbrry *et al.*, 2014), as well as anti-malarial properties

One possible use of soursop seeds in the formulation of fish feed may include employing processing methods to enhance their nutritional properties. Techniques such as thermal processing, including extrusion or roasting, have been found to boost digestibility and reduce anti-nutrients in plant-based protein sources (Krogdahl *et al*., 2010; Newkirk, 2010). Nevertheless, the impact of these processing techniques on the amino acid profile and bioavailability of soursop seeds needs a comprehensive investigation. Another method that has attracted interest is fermentation, known for its ability to improve the nutritional quality of plant-based proteins (Kuan et al., 2019). This process can enhance protein digestibility, diminish anti-nutritional factors, and even elevate the content of specific amino acids through the actions of microorganisms (Kiers et al., 2000). Exploring fermentation techniques for soursop seeds could be an avenue for increasing their viability in fish feed formulation.

Furthermore, investigating enzymatic treatments such as protease or phytase enzymes may improve the digestibility and bioavailability of amino acids from soursop seeds (Francis et al., 2001). These enzymes assist in breaking down complex protein structures and lowering the levels of anti-nutritional factors present, including tannins, alkaloids, and flavonoids. Protein is a crucial element of cell protoplasm and is an essential component of tissues in animals, humans included, where it is needed for cell repair, growth, and development (Akram et al., 2011). The basic units that make up all proteins are amino acids, which are found in both animal and plant sources and act as the fundamental building blocks of protein. Amino acids are vital for protein synthesis and carry out various functions within the body. They also serve as the foundational elements of proteins that create metabolic intermediates and support overall health and vitality (Usydus et al., 2009). Consequently, it is important to include them in the diet, as a deficiency can lead to serious declines in protein biosynthesis and reduce the total protein levels in the organism. Soursop (Annona muricata), a fruit that originates from North and South America, belongs to the genus Annona within the family Annonaceae, which consists of about 100 species of shrubs (Carl Von Linne, 1775).

It is the most tropical member of this group and produces the largest fruits (Nagy and Shaw, 1980). The mesocarp features numerous shiny, dark brown seeds, which are ovoid and measure around 2 cm in length and 1 cm in width (NAS, 1978). The tree can produce up to 10 tons per hectare, with individual fruits weighing between 0.5 and 2.0 kg (Oyenuga, 2009). The seeds contain 12.2% to 14% crude protein, 24.4% to 27.4% fat, 3.4% to 4.2% ash, 40.4% to 45.5% carbohydrate, 6.5% to 8.5% moisture, and fiber content ranging from 4.5% to 5.6% (Neela et al., 2010). However, the precise values vary based on seed variety, growing conditions, and processing methods (Necla et al., 2010). Aquaculture, or the farming of aquatic species, has seen an increased demand for fish as a protein source alongside the depletion of wild fish stocks (FAO, 2020). Historically, fish meal has been a primary ingredient in aquaculture feed due to its superior nutritional profile, characterized by a balanced amino acid composition and high digestibility (Olsen and Hasan, 2012). However, the competitive market and rising costs of fish meal have led researchers to seek alternative plant-based protein sources (Hardy, 2010). Plant-derived proteins present numerous benefits, including lower costs, greater availability, and the potential for sustainable production (Catlin et al., 2007). Still, their incorporation into aquaculture feeds is hindered by issues such as suboptimal amino acid profiles, the presence of anti-nutritional factors, and diminished digestibility compared to animal-based proteins (Francis et al., 2001). In this regard, the exploration of underutilized locally abundant plant resources has attracted attention as a viable solution to meet the protein needs in aquaculture (Aderolu and Akinremi, 2009). One such resource is the seeds of Annona muricata (soursop), often discarded as waste after consumption (Moghadamtousi et al., 2015). These seeds are acknowledged for their potential nutrient content, which includes proteins, fats, and minerals (Jimenez et al., 2001). Analyzing the amino acid profile of A. muricata seeds is crucial for assessing their nutritional value and for investigating possible applications in the creation of functional feed. Therefore, the objective of this study is to determine the accurate amino acid composition of the seeds to better understand their potential role as a growth factor and promoter of fish health in aquaculture

Materials And Methods

The research was carried out at the FUTO SAAT farm in the fisheries department unit. The area lies within latitudes 4° 45' N, and 7° 15' N and longitudes 6° 50' E and 7° 25' E with an area of 5,100sq Km. It has an average annual temperature of above 20°C (68.0°F), with annual relative humidity of 74%, which can reach 90% in the rainy season. The dry season experiences two months of Harmattan from December to February, with the hottest months being January to March.

Collection of *A. muricata* (Sour Sop) Seeds

Soursop seeds were gathered from the bush located near the World Bank housing area in Owerri, Imo State. The seeds were then transported to the research farm of the Department of Fisheries and Aquaculture Technology at the Federal University of Technology, Owerri, where they underwent separation, rinsing to eliminate debris, and fermentation for five days before being dried at a room temperature of 20-25°C to facilitate the grinding process. Following grinding, the seeds were sent to the Department of Food Science and Technology Laboratory (FST) for amino acid analysis. A phytochemical analysis of the soursop (Annona muricata) revealed both qualitative and quantitative results from the A. muricata seed meal, indicating the presence of key phytochemicals such as alkaloids, tannins, flavonoids, and saponin, while cyanogenic glycosides were found to be absent, as determined by the Colorimetric method developed by Savithramma et al. (2011) and quantified using the method established by Vijay and Prajendra (2014) to determine their concentration levels. Alkaloids were found at 28.75%, tannins at 0.45%, flavonoids at 6.10%, and saponins at 3.5%, all of which fell within acceptable limits, with no detectable amounts of cyanogenic glycosides in the seeds. The proximate composition of the soursop (A. muricata) seed indicated the following nutritional values: moisture content of 6.5 – 8.5%, fat content of 24.4 – 27.4%, crude protein of 12.5 – 14.5%, ash content of 3.4 – 4.2%, fiber content of 4.5 - 5.6%, and NFE of 40.4 – 45.5% (A.O.A.C., 2005).

Amino Acid Analysis.

The process of acid hydrolysis and derivation of proteins aimed at determining 18 amino acids from soursop (A. Muricata) seeds began, incorporating slight modifications to the methodologies described by Eroglu et al. (2016). The ground seeds were mixed with HCl (16m, 20 mL) in hydrolysis tubes and subjected to hydrolysis for 24 hours at 110ºC in an oven under a nitrogen atmosphere. Afterward, the mixture was allowed to cool to ambient temperature. Immediately following protein hydrolysis, precolumn derivatization using phenyl isothiocyanate was performed. The dry samples were reconstituted in 20µL of ethanol: water: triethylamine (2:2:1) and subsequently evaporated under vacuum. Derivatization occurred using 20µL of a reagent mixture consisting of ethanol: water: triethylamine: phenyl isothiocyanate (7:1:1:1) at room temperature (20-25°C) for 20 minutes, after which the reagent was removed under vacuum at 45°C. The derivatized samples were then dissolved in 0.1 mL of 0.14M sodium acetate and the pH was adjusted to 6.4 using diluted acetic acid. To determine tryptophan, alkaline hydrolysis was conducted following the method outlined by Cevikkalp et al. (2016). The powdered seeds were combined with 20ml of 5N NaOH under a nitrogen atmosphere. Subsequently, the hydrolyzed mixture was placed in an oven at 120°C for 12 hours, after which the hydrolysates were cooled to room temperature and the pH adjusted to 6.3 using diluted HCl. The analysis of amino acids was performed using a prominent ultrafast liquid chromatography system (amino acid analyzer), which included a binary pump and UV/Vis detectors. A reversed-phase analytical column (shrink-pact XR-ODS (75mm X 3.0mm i.d) with a fluorescence detector facilitated the separation and detection of the amino acids. The results were expressed as mg of amino acid per 100g of dry sample (protein). Amino acid analysis was conducted in triplicates.

Data Analysis

The descriptive statistics such as mean, range, standard deviation and coefficient of variation were calculated for analysis. Also, amino acid parameters such as leucine/isoleucine ratio (LEU/ILE), isoelectric point (PL) and the Predicted Protein Efficiency Ratio (P-PER) were calculated using the method of Olaofe & Akintayo (2000) and Alsmeyer *et al.,* (1974) respectively, among others.

Isoelectric Point (PL) $=\sum_{t=1}^{n=1}$ pliXi…………………. (1)

Where P^{I}_{n}= the isoelectric point of mixture of amino acids

Pli = the isoelectric point of the ith amino acids in the mixture.

Xi = the mass or mole fraction of the amino acids in the mixture.

Pred. Prot efficiency ratio (P-PER) = - 0.468+0.454 (LEU) - 0.105 (Try)……………………. (2)

Where

LEU= Leucine

Try = Tyrosine

All were achieved with the help of Microsoft, Excel 2019, Windows 10 Pro.

Results And Discussion

Table 1, Fig. 1, presents the Amino Acid composition of *Annona muricata* (sour sop) Seed meal, expressed as grams per 100 grams of Crude Protein. 18 different Amino Acids were listed along with their mean values and coefficient of variation (CV% %) obtained from the analysis. For each Amino Acid, the Mean Value represents the average concentration in soursop seeds, while the CV % indicates the extent of variation or dispersion around the mean. The Amino Acid with the highest mean concentration in soursop seeds is Glutamic acid at 12.25±0.45g/100g Crude Protein. The next highest are the Aspartic Acid (11.30±0.43g/100g) and Tyrosine (8.61±0.41g/100g), both of which are within or above their recommended levels. Other Amino Acids were within the required standards recommended by several authors.

TABLE 1: Amino Acid Composition Of *Annona Muricata* (G/100g Crude Protein)

AMINO ACID	MEAN	CV %	RECOMMENDED STANDARDS (PAAESP g/100g PROTEIN)
Lysine (Lys)[e]	4.61±0.08	1.74	5.5 (FAO/WHO, 1991)
Histidine (His)[e]	2.82±0.09	3.19	1.5-4.5 (Aremu *et al.*,2006)
Arginine (Arg)[e]	8.21±0.19	2.34	5-10 (Odue, 2010)
Aspartic (Asp)	11.30±0.43	3.80	8.5 – 14.5 (FAO/WHO,1991)
Threonine (Thr)[e]	2.28±0.31	13.59	< 3.0 (Paul *et al.*,2012)
Serine (Ser)	6.74±0.16	2.37	5-10 (Odue, 2010)
Glutamic acid (Glu)	12.25±0.45	3.67	> 10 (Paul *et al.*,2012)
Proline (Pro)	6.72±0.09	1.34	5-10 (Odue, 2010)
Glycine (Gly)	6.26±0.22	3.51	> 5.0 (FAO/WHO,1991)
Alanine (Ala)	5.91±0.11	1.86	4-6.0 (Paul *et al.*,2012)
Cystine (Cys)	2.41±0.22	9.54	1.5-4.5 (Aremu *et al.*,2006)
Valine (Val)[e]	6.48±0.24	3.70	5.0 (Paul *et al.*,2012)
Methionine (Met)[e]	2.09±0.23	10.04	1.5-4.5 (FAO/WHO,1991)
Isoleucine (Ileu) [e]	3.39±0.41	12.09	1.5-4.5 (Addy and Eteshola, 2006)
Leucine (Leu) [e]	6.55±0.16	2.44	> 5.0 (Aremu *et al.*,2006)
Tyrosine (Tyr)	8.61±0.41	4.87	> 5.0 (Paul *et al.*,2012)
Tryptophan (Typ) [e]	3.05±0.10	3.27	3.0 (FAO/WHO, 1991)
Phenylalanine (Phe) [e]	6.45±0.18	2.79	4-8.5 (Odue, 2010)

Key: The Superscript (**e**) means Essential Amino Acid, PAAESP=Provisional Amino Acid (Egg) Scoring Pattern;

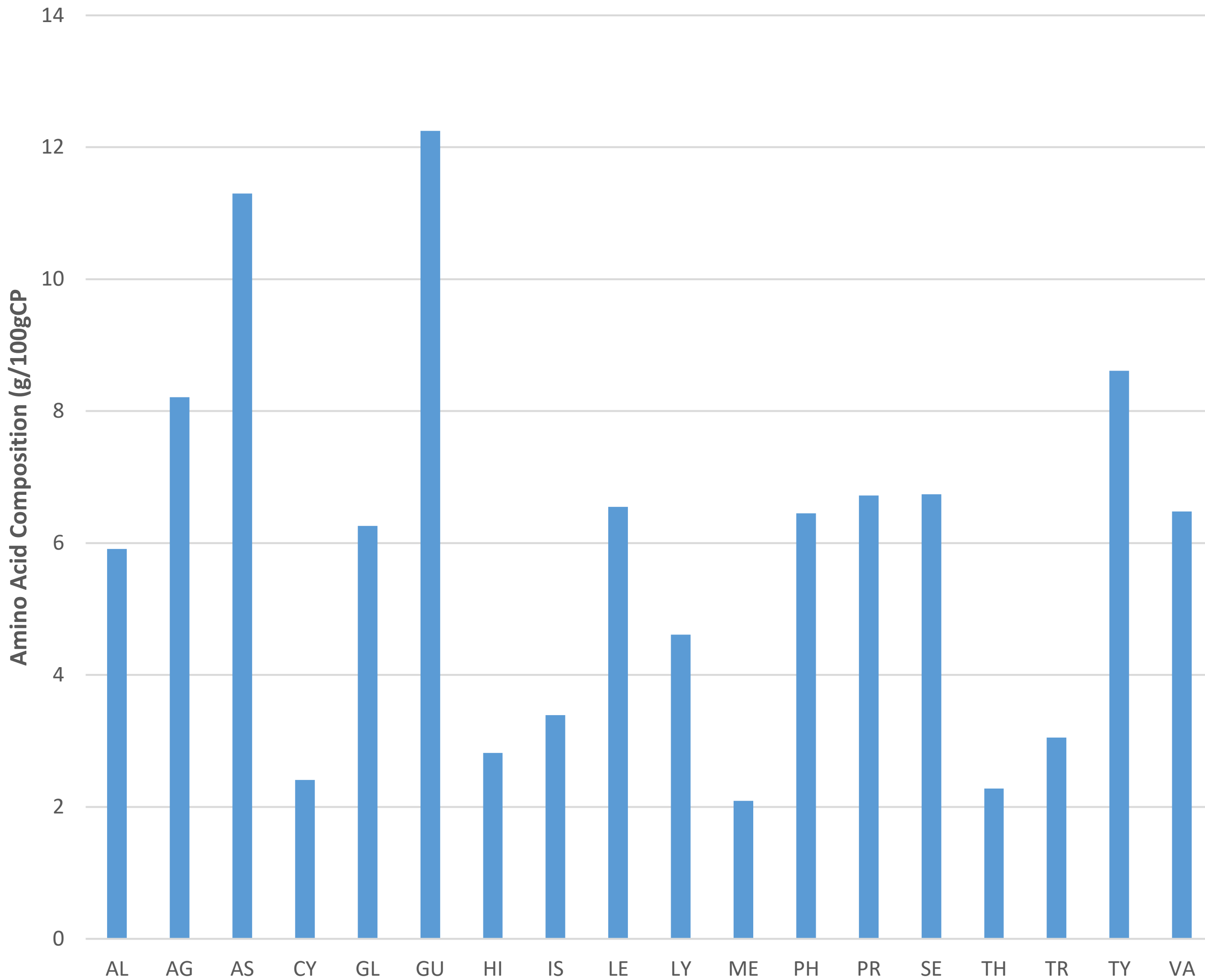

Fig 1: Showing the multiple variate bar chart for the 18 Amino acids

FOOTNOTE: AL = Alannine, AG = Arginine, AS = Aspartic, CY= Cystine, GL= Glyeine, GU= Glutamic, Hi= Histidime, IS= Isoleucine, LE= Leucine, LY=Lysine, ME= Methnonine, PH= Phytalannine, PR= Prohine, SE= Serine, TH= Threonine, TR= Trytophan, TY= Tyrosine and VA= Valine

Table 2: Concentrations Of Essential, Non-Essential, Acid, Neutral, Sulphur, Aromatic, Etc. (G/100g Protein) Of *Annona muricata* Seeds.

AMINO ACID PARAMETRS	*A. muricata* SEED
Isoelectric Point (PL)	5.39
Predicted Protein Efficiency Ratio (P-PER)	1.45
Leucine/Isoleucine Ratio (LEU/ILE)	1.96
Total Amino Acid (TAA)	106.13
Total Non-Essential Amino Acid (TNAA)	60.21
Total Essential Amino Acid (TEAA)	45.92
Essential Aliphatic Amino Acid (EAAA)	16.42
Essential Aromatic Amino Acid (EArAA)	9.50
Total Neutral Amino Acid (TNAA)	49.20
Total Acidic Amino Acid (TBAA)	23.55
Total Basic Amino Acid (TBAA)	15.64
Total Sulphur Amino Acid (TSAA)	4.5

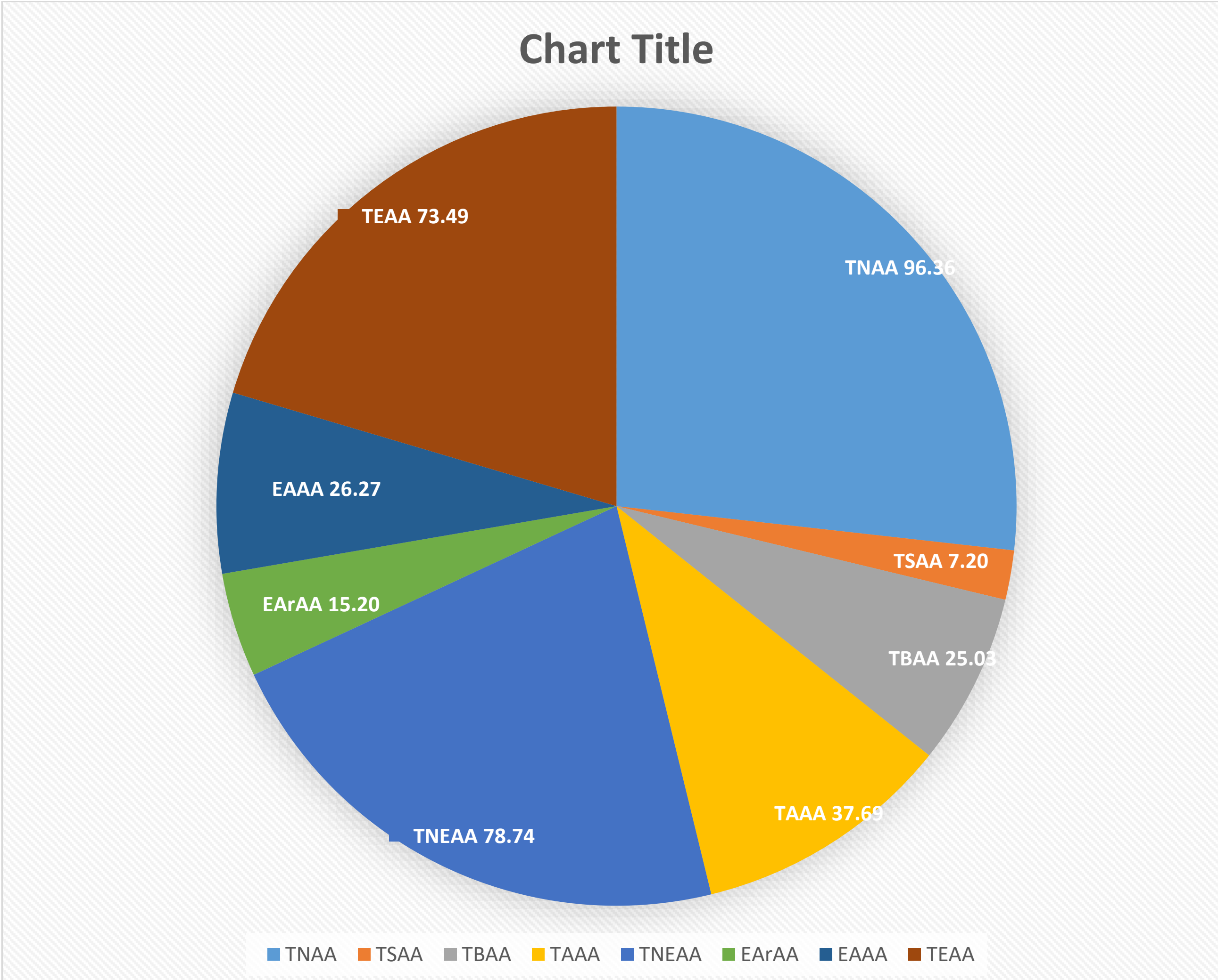

Fig 2: Pie Chart showing the concentration of the Amino Acids in the *Annona muricata* Seed. Table 2/Fig 2 shows the Concentrations of Essential, Non-essential Amino Acids, Neutral, Sulphur, Aromatic, etc. (g/100g crude protein) of *A. muricata* seed meal. The Isoelectric Point is 5.39, which represents the Theoretical Isoelectric Point of the mixture of Amino acids in soursop seeds based on their composition. Predicted Protein Efficiency Ratio (P-PER) is an estimate of the protein quality and digestibility based on the Amino Acid composition, especially the levels of Leucine and Tyrosine. A P-PER Value of 1.45 suggests moderately good protein quality. The Leucine and Isoleucine Ratio of the two Amino Acids is 1.96 for soursop seeds, Total Amino Acid (TAA) is 106.13g/100g Crude Protein, Total Non-essential Amino Acid (TNAA) is 60.21g/100g, Total Essential Amino Acid (TEAA) is 45.92g/100g Crude Protein, Essential Aliphatic Amino Acid (EAAA) is 16.42g/100g. This includes the Aliphatic Essential Amino Acids with Leucine, Isoleucine, and Valine. Also, the Essential Aromatic Amino Acid (EArAA) is 9.50g/100g and covers the Aromatic Essential Amino Acids, such as Phenylalanine and Tyrosine. There are also results from the Total Neutral Amino Acid (TNAA), 49.20g/100g Crude Protein, Total Acidic Amino Acid (TAAA), 23.55g/100g Crude Protein, and the total Basic Amino Acid (TBAA) with a value of 15.64g/100g Crude Protein. The Total Sulphur Amino Acid (TSAA) is 4.5g/100g Crude Protein, and this includes the Sulphur-containing Amino Acids of Methionine and Cystrine. Overall, the table provides the standard calculated parameters based on the Amino Acid Profile that give more insight into the protein quality and the characteristic values of soursop seed meal.
The Amino Acid Profile of *Annona muricata* seed as shown in Table 1 and Figure 1, revealed its potential as a protein source, particularly for fish feed formulation. the seeds contained appreciable levels of most of the Essential Amino Acids, which are crucial for growth, development, and maintenance of physiological functions in fish (Gatlin *et al.*,2007). Among the Essential Amino Acids of Arginine exhibited the highest mean value of 8.21±0.19g/100g Crude Protein, falling within the recommended range of 5-10g/100g Protein (Odue, 2010). Arginine plays a vital role in various metabolic processes including Protein Synthesis, ammonia detoxification, and immunefunction (Li *et al.*, 2009). The Lysine content of 4.61±0.08g/100g was lower than the FAO/WHO (1991) recommendation of 5.5g/100g protein, which could limit its use as a solo protein source. However, this deficiency can be compensated by incorporating other lysine-rich ingredients or by adding Synthesis Lysine in fish feed formulations (Ami-Sah *et al.*,2009). The seeds were found to be a good source of Leucine (6.55± 0.16g/100g), exceeding the recommended level put above 5.0g/100g protein (Aremu *et al.*, 2006). Leucine is an Essential Amino Acid with a crucial role in protein synthesis, muscle growth, and energy regulation (Ruthen *et al.*, 2005).
The leucine/Isoleucine ratio of 1.96 in soursop seeds was comparable to the ratio reported for other plant-based protein sources, such as Soybean (1.90) and Peanut (1.80), (Mosse, 1990). The Total Essential Amino Acid (TEAA) Content of of 45.92g/100g protein was higher than that reported for some conventional plant protein sources like Soybean meal (36.6g/100g) (Mosse, 1990) and Cotton Seed Meal (42.1g/100g protein) (Aremu *et al.*,, 2011). However, it was Lower than the TEAA content of Animal-based protein sources like fish meal (57.6g/100g protein) (Mohanty & Kaushik,1991). The Predicted Protein Efficiency Ratio (P-PER) of 1.45 for soursop seed meal suggests good protein quality and digestability (Alsmeyor *et al.*,1974). This value is comparable to that of other plant-based protein sources like soybean (1.48) and

Peanut (1.40) (Mosse, 1990). However, it is lower than the P-PER of animal-based protein sources like fish meal (2.93) (Mohauty & Kaushok, 1991), indicating the need for supplementation with other high-quality protein sources or incorporating that feed with synthetic Amino acids to beef up the quality.

Furthermore, the Total Sulfur-Amino Acid (TSAA) content of 4.5g/100g Crude Protein in soursop seed meal was within the recommended/ range of 1.5-4.5g/100g protein (Aremu *et al.,*, 2006; FAO/WHO,1991), Suggesting adequate levels of these Amino Acids Essential for flesh production in animals (McDonald *et al.,*2011). Compared to other Non-Conventional Protein sources, the Amino Acid composition of soursop seed meal was similar to that of Artocarpus altilis (Breadfruit) Seeds, which had a TEAA content of 43.1g/100g protein and P-PER of 1.41(Adepoju *et al.,*2011). However, Soursop seeds had higher levels of essential amino acids like Leucine, Isoleucine, and Phenylalanine compared to the breadfruit seeds.

CONCLUSION

The current research examined the amino acid composition of Annona muricata (Soursop) seeds to assess their viability as a protein source for formulating fish feed. The findings indicated that Soursop seeds possess significant amounts of most essential amino acids, making them a viable alternative source of plant-based protein. The seeds displayed a favorable amino acid profile, with adequate levels of Arginine, Leucine, and Phenylalanine. The total essential amino acid content and the estimated protein efficiency ratio suggest a moderately good quality of protein. Nevertheless, the comparatively low quantities of certain essential amino acids, like Lysine and Threonine, may require supplementation with other protein sources or synthetic Lysine amino acids to fulfill the specific nutritional needs of fish. Recent studies by various researchers demonstrated that A. muricata (Soursop) inclusion levels for soybean meal yielded excellent growth results for Oreochromis niloticus without adverse effects. This positive outcome is attributed to the initial fermentation process applied to the Soursop seeds, which helped mitigate some of the anti-nutritional factors present in the seeds. (Anyanwu Personal Communication, 2023). Moreover, the constituents of A. muricata showed the presence of alkaloids, tannins, flavonoids, and saponins, which provide health benefits such as anti-cancer, antioxidant, anti-inflammatory, antimicrobial, and antihypertensive properties, thereby reinforcing its potential advantages as both a fish growth enhancer and a health contributor in aquaculture. In summary, the results of this study underline the promise of A. muricata seeds as a valuable protein resource for fish feed formulation, thereby aiding in the creation of sustainable and cost-effective aquaculture feed production.

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