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Amino Acid Profiling of Tilapia Samples in Ebonyi State, Nigeria.

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ABSTRACT

Protein-calorie malnutrition, (PCM) is the most lethal form of malnutrition/hunger and results from the inadequate uptake of quality proteins and calories in diet. This study was carried out to profile the amino acid composition of tilapia samples obtained from three different locations (two domesticated and one wild source) to establish nutritional information about the species. Chromatographic techniques were used to profile the amino acid composition of each sample. With exception of glutamate and asparagine, the results of the amino acid profiling revealed the presence of 18 amino acids (leucine, lysine, isoleucine, phenylalanine, tryptophan, valine, methionine, proline, arginine, tyrosine, histidine, cysteine, alanine, glutamic acid, glycine, threonine, serine and aspartic acid) in all the samples, which included all the essential amino acids.The results also showed amino acid concentrations ranging from 14.54 g/1100g - 0.47g/100g) with glutamic acid being the most abundant in concentration having concentrations of 9.99g/ 100g - 14.54g/100g protein, while tryptophan was least in concentration with range from 0.47g/ 100g - 0.68g/ 100g protein. Tilapia could therefore, be said to be a quality protein source and should be consumed by both adults and children as they contain all essential amino as well as functional amino acids.

Keywords: Amino acids, tilapia, Protein-calorie malnutrition, glutamic acid and tryptophan

INTRODUCTION

Proteins are the most abundant macromolecules present in all biological systems, constituting structural elements, enzymes, hormones, antibodies, receptors, signaling molecules, and have specific biological functions. Protein is essential for proper biological functioning as we know it, including provision of essential amino acids and development and maintenance of muscles and tissues [1].

Protein can be considered as being of quality when it contains all the essential amino acids in adequate quantities. The inadequate consumption of quality proteins and calories leads to protein energy malnutrition (PEM) or protein-calorie malnutrition, (PCM) which is the most lethal form of malnutrition/hunger. Kwashiorkor and marasmus, the extreme conditions of PCM are mostly observed in children and are caused by chronic deficiency of protein and energy, respectively. PCM also do occur in adults who are under chronic nutritional deficiency. About 870 million people worldwide suffer from chronic protein malnutrition and 80% of children suffering from PCM are from developing countries [2].

Amino acids are important biomolecules that both serve as building blocks of proteins and are intermediates in various metabolic pathways. They serve as precursors for synthesis ofawiderangeofbiologicallyimportantsubs tancesincluding nucleotides, peptide hormones, and neurotransmitters. If amino acids are deficient, then protein synthesis does not occur, as a result protein deficiency disease may occur. Specific amino acids are known to acutely and chronically regulate insulin secretion from pancreatic β-cells in vivo and in vitro [3]. Generally amino acids play key role in regulating multiple processes related to gene expression, including modulation of the function of the proteins that mediate messenger RNA (mRNA) translation [4]. Hence it is necessary to take balanced diet containing all essential amino acids.

Amino acids are traditionally classified as nutritionally essential amino acids (EAA), "nonessential amino acids" (NEAA) or conditionally essential (CEAA) [5]. Arginine, cystine, histidine, leucine, lysine, methionine, threonine, tryptophan, tyrosine, and valine are known as the nutritional essential amino acids (EAAs) and this is because the body cannot synthesize these amino acids in sufficient (desired) quantities. Glutamine, glutamic acid, glycine, proline, and taurine are known as conditional Essential amino acids (CEAA) their essentiality is based on the individual, their state of health and nutritional requirements. Aspartic acid, serine, and alanine are the NEAA for human nutrition [6]. However, a new classification system has been proposed based on functionality. Functional amino acids (FAAs) are those which participate and regulate key metabolic pathways to improve health, survival, growth, development, lactation, and reproduction of the organisms [7]. The FAAs also hold great promise in prevention and treatment of metabolic diseases (e.g., obesity, diabetes, and cardiovascular disorders), intrauterine growth restriction, infertility, intestinal and neurological dysfunction, and infectious disease. Arginine, cystine, leucine, methionine, tryptophan, tyrosine, aspartate, glutamicacid, glycine, proline, and taurine have been classified as FAA in human nutrition [8] Fishes are valuable sources of high grade protein and other organic products. They are most important source of animal protein and have been widely accepted as a good source of protein and other elements for the maintenance of healthy body [9]. Aquatic animal foods have higher protein contents, a low caloric density, and have a high content of omega 3 long chain polyunsaturated fatty acids (n-3 LC PUFA) compared to land living animals [10]. In comparison to the other sources of dietary animal proteins, consumers have wide choice for fish as far as affordability is concerned as there are many varieties and species of fishes available, especially in the tropical countries [11]. Fish, can play a vital role as an important and cheaper source of quality animal protein especially in developing countries where beef are expensive. Fish, has a high nutritional value regarding beneficial amounts of protein, lipids as well as essential micronutrients.

Tilapias originate from Africa and the Middle East [12]. They are hardy, prolific and fast growing tropical fishes. They are low on the food chain, adaptable and herbivorous, feeding mainly on plankton, algae, aquatic macrophytes and other vegetable matter [13]. Their mild-tasting flesh can be easily adapted to all kinds of uses. Fishmeal has also been known to contain large quantities of energy per unit weight and is an excellent source of proteins, lipids (oils), minerals, vitamins and a little carbohydrate [14]. Hence the need to generate and document nutritional information on tilapia species available locally to boost knowledge and foster improvements. The present study was undertaken to generate information on amino acid composition of tilapia with the objective of enhancing the scope for their utility in clinical nutrition for dietarycounseling.

MATERIALS AND METHODS.

The amino acid profiles of the tilapia samples were determined using the methods described by Benitez. The samples were weight, defatted, hydrolyzed and evaporated in a rotary evaporator (A-784E855, USA) and loaded into an Applied Biosystem PTH Amino Acid Analyzer (Model 120A). The entire defatting procedure can be carried out in approximately 10 minutes; the Bligh and Dyer method was used as it is efficient, reproducible, and free from deleterious manipulations. The wet tissue forms a miscible system with the water in the tissue. Dilution with chloroform and water separates the homogenate into two layers, the chloroform layer containing all the lipids and the methanolic layer containing all the non-lipids. A purified lipid extract is obtained merely by isolating the chloroform layer. A known weight (2.0g) of ground tissue was weighed into separating funnel. This was followed by addition of 15ml methanol, 30ml distilled water and 15ml chloroform. The separating funnel was wailed vigorously for 2 minutes and the liquid layer was decanted into 250ml conical flask after 10 minutes. The fat free tissue was put into a clean petri dish and dried overnight at room temperature.

For hydrolysis, a known weight of the defatted sample was weighed into glass ampoule. 7ml of 6NHCL was added and oxygen was expelled by passing nitrogen into the ampoule, this is to avoid possible oxidation of some amino acids during hydrolysis (methionine and cysteine can be oxidized as such). The glass ampoule was then sealed with Bunsen burner flame and put in an oven preset at $105^{0}C \pm 5^{0}C$ for 22 hours. The ampoule was allowed to cool before broken open at the tip and the content was filtered to remove the humins. It should be noted that tryptophan is destroyed by 6N HCL during hydrolysis hence for tryptophan determination; the sample was hydrolyzed with 4.2M Sodium hydroxide [15].

The filtrate was then evaporated to dryness using rotary evaporator. The residue was dissolved with 5ml to acetate buffer (pH 2.0) and stored in plastic specimen bottles, which was kept in the freezer for preservation. 60microlitre was dispensed into the cartridge of the analyzer. The analyzer is designed to separate and analyze free acidic, neutral and basic amino acids of the hydrolysate.

RESULTS

The results showed that fresh tilapia samples obtained from three different sources contained 18 of the 20 standard amino acids, in which all 10 of the essential amino acids (Leucine, Lysine, Isoleucine, Phenylalanine, Tryptophan, Valine, Methionine, Arginine, Histidine and Threonine) were present in significant quantities ($p>0.05$). The amino acids detected according to their relative concentration includes, Glutamic acid> Aspartic acid> Lysine> Leucine> Glycine> Alanine> Arginine> Isoleucine> Valine>Proline> Serine> Phenylalanine> Threonine> Tyrosine> Methionine> Histidine> Cysteine> Tryptophan as shown in figure 1.

The result, also shows that across the tilapia samples obtained from different locations (pond1 river and pond2), the amino acid with the mean highest concentration was glutamic acid (9.99 g/100g protein, 9.84 g/100g protein and 14.54 g/100g protein respectively) followed by aspartic acid (6.82 g/100g protein, 6.7 g/100g protein and 8.68 g/100g protein respectively), lysine (6.05 g/100g protein, 5.67 g/100g protein and 5.94 g/100g protein respectively) and leucine (5.31 g/100g protein, 5.08 g/100g protein and 6.01 g/100g protein respectively) while amino acids with the least concentration across the fish samples were tryptophan (0.63 g/100g protein, 0.58 g/100g protein and 0.47 g/100g protein respectively) and cysteine (0.73 g/100g protein, 0.66 g/100g protein and 0.48 g/100g protein respectively) as shown in Figure 1. The result of the statistical analysis showed that the difference in the amino acid concentrations across the tilapia samples from different were highly significant ($p<0.05$).

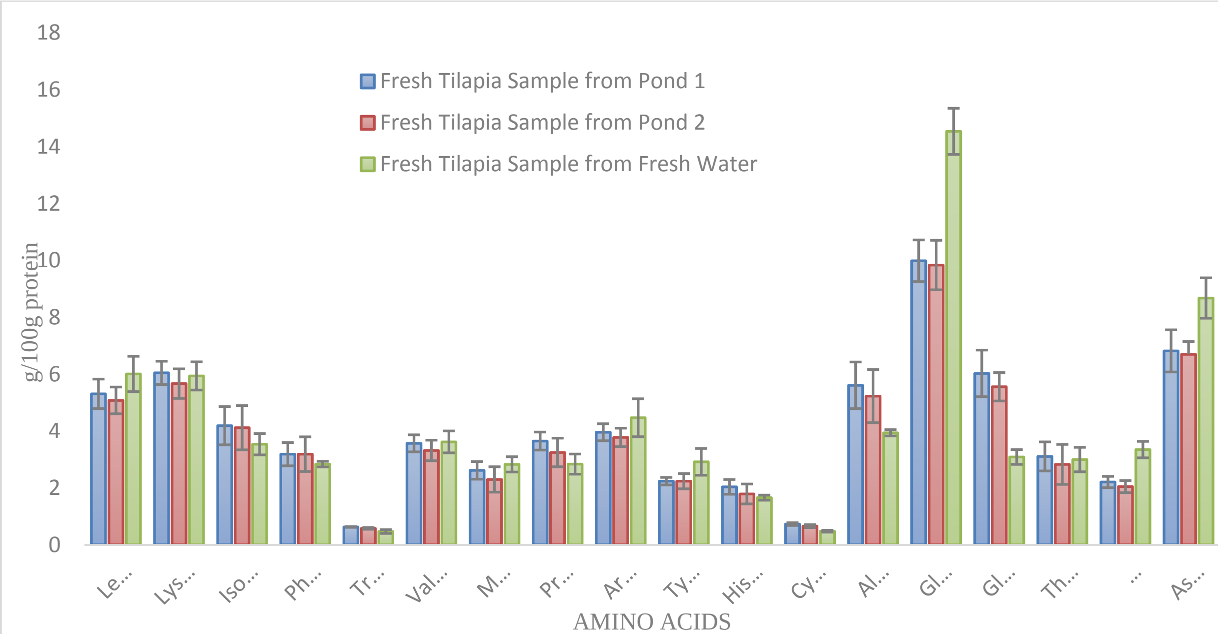

Fig1: Comparative analysis of amino acid profile across the three locations.

DISCUSSION

The result of the amino acid composition of fresh tilapia showed that the 3 samples, although collected from different locations, had the same trend of amino acid composition although their relative concentrations varied. From the data analyzed, all the ten (10) essential amino acids were present in all the samples. This suggests that tilapia is a good source for obtaining quality protein. The amino acid profile of the samples is also similar with the report of [16] which suggested that whole tilapia can yield 30 % to 37 % protein.

The composition of the amino acid shows that glutamic acid was the most abundant in all the samples with highest concentrations of 14.54 g/100g protein. Glutamic acid which was found in great abundance from our study plays important roles in amino acid metabolism because of its role in transamination reactions and is necessary for the synthesis of key molecules, such as glutathione which are required for removal of highly toxic peroxides and the polyglutamate folate cofactors. This was same findings by [17] while using Penaeuskerathurus. The high glutamic acid contents reported maybe as a result that Glutamic acid is a nonessential amino acid and hence is produced in large quanties. Other studies have reported high concentrations in other protein sources like in the carps (*C.catla, L.rohita,* and *C. mrigala)* and catfishes (*C. batrachus* and *H. fossilis)* [18]

Arginine's concentration in the samples was <5g/ 100g of protein but this may not be a problem as it is a non-essential amino acid. Given the proper internal environment, the body can manufacture it. Arginine maintains the body's nitrogen equilibrium, it is also involved in waste detoxification, moderate glucose tolerance, and promotes wound healing and bone repairs and from this study the samples were proven to contain sufficient arginine with highest concentrations of 4.47 g/ 100g protein in the fresh water sample. The results from the domesticated fish samples were not so different which could be as a result of their feeding.

Leucine is the only dietary amino acid that can stimulate muscle protein synthesis [19] and has important therapeutic role in stress conditions like burn, trauma, and sepsis [20]. As seen in the results the samples had a high concentration of leucine and the result was same with results carried out on*S. waitei* and *R.kanagurta,* carps (*L. rohita* and *C. mrigal*), which confirmed the high concentration of leucine in marine fishes [2]. This can be seen as the fresh water fish sample had the highest concentrations of Leucine suggesting that wild fish species contain more leucine.

Glycine is probably the most functionally important amino acid and was found in significant (p>0.05) concentrations of 6.03g – 3.09g/ 100g of protein plays important role in metabolic regulation, preventing tissue injury, enhancing anti-antioxidant activity, promoting protein synthesis, wound healing, improving immunity and treatment of metabolic disorders in obesity, diabetes, cardiovascular disease, cancer, and various inflammatory diseases [7]. In a similar study, the domesticated catfish *H. fossilis* was found to contain the highest amount of glycine with concentrations of 15.4 ± 3.6g /100g of protein [11]. This difference in amino acid concentration may be as result in feeding conditions and their environment as could be seen in the large difference between the pond samples and their fresh water counterparts.

Lysine is an Essential amino acid and also a functional amino acid which is extensively required for optimal growth and its deficiency leads to immunodeficiency [14]. Lysine was third most significant amino acid detected in this study with concentrations as high as 6.05g /100g of protein which was slightly lower than results obtained from similar studies done on *Channamicropeltes* (10.9 ± 1.05%) [9]. The difference in concentration could be as a result in size as the *Channamicropeltes* species grows 1.5m and weighs up to 20kg.

The other essential amino acids isoleucine, methionine, phenylalanine, threonine and valine were generally of low concentration as they all had concentrations <5g/100g of protein while the limiting amino acid was tryptophan which had concentrations <1g/100g of protein.

CONCLUSION

Tilapia is a quality protein source and should be consumed by both adults and children as they contain all essential amino acids and functional amino acids. Tilapia can also be used as a great meal in order to avoid protein calorie nutrition.

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