IOSR Journal Of Pharmacy And Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 14, Issue 3 Ser. III (May – June 2019), PP 46-51
www.Iosrjournals.Org

Effects of Smoking on Amino Acid Content in Domesticated and Wild Tilapia

[1*]Oko, Augustine Okpani, [1]Eluu Stanley, [2]Agwu Samuel C, [3]Ehihia Loveth, [4]Ngele Kalukalu,[1]Okoye, Emeka Desmond, [5]Oluwole, Akinjide Omoniyi and[6]Okorie, Joseph Michael

[1]Department of Biotechnology, Ebonyi State University, Abakaliki, Nigeria
[2]Nigerian Institute for Oceanography and Marine Research, Lagos, Nigeria
[3]Department of Biochemistry, Ebonyi State University, Abakaliki, Nigeria
4. Biology/Microbiology/Biotechnology, Federal University, Ndufu-Alike, Ikwo, Abakaliki
5. Department of Materials Science and Engineering African University of Science and Technology Abuja
6. Department of Environmental Biosafety and General Release, National Biosafety Management Agency, Abuja
Corresponding Author: Oko, Augustine Okpani

Abstract: *Amino acids are the building blocks of protein and there are about 300 known amino acids if not more, that has been described and categorized, but only about 20 amino acids are consistently involved in biological build up.Traditional technique of preserving fish (by smoking) was investigated to understand its effect on the nutritional quality of tilapia and present human dietary needs. This study is aimed at evaluating the amino acid content of tilapia before and after drying (smoking).For this study tilapias were obtained from three major sources, two domesticated sources (ponds) and a wild source (river). The method used was that described by Benitez.Results from this study showed that the amino acid with highest concentration was glutamic acid with concentrations ranging from 9.99 g/100g protein - 14.54 g/100g protein, while the least abundant one was tryptophan ranging from 0.47 g/100g protein - 0.63 g/100g protein. Sample trends could also be observed in the amino acids profiles with smoked samples showing significant increase in concentration as compared to their fresh counterparts aside from a few deviations.*

Key words: *Amino acids, smoking, tilapia, domesticated and wild*

Date of Submission: 27-06-2019 Date of acceptance: 13-07-2019

I. Introduction

Fish drying is one of the most popular methods of preserving fish in tropical countries like Nigeria, where they make use of the high ambient temperature and low humidity. The main advantage of drying is its ability to increase the shelf life of the fish by reducing the moisture content of the flesh to a limit that ensures the stability of the product in storage. Traditionally, dried fish represents a low cost source of high quality protein [1].

Among the good quality animal protein sources, fish is the most perishable. An estimated 50 % of the fish produced in the remote coastal settlements and hinterland perish before reaching the consumers, as a result of poor handling, preservation and processing practices adopted by the artisanal fishers, commercial fish farmers and fisheries entrepreneurs [2]. However, some fish mongers especially those within the rural communities, still practice a simple and inexpensive fish drying process to produce a dried fish. Though commercially accepted, fish processed through this means are exposed for longer heat period for effective drying which leads to severe nutritive losses [3] due to complex chemical reactions, such as protein-protein interactions, protein-fat interaction as a result of high processing temperatures [4].

They are favoured because the smoke-drying process imparts characteristic aroma, taste, colour and texture (crispiness) on processed fish. Dried fish are less expensive than fish prepared through any other means. The product is widely consumed as there is no religious or cultural objection to its consumption [5].

Amino acids are basic unit of protein. Amino acids contain an amino group and a carboxylic group. Amino acids play major role in regulating multiple processes related to gene expression, including modulation of the function of the proteins that mediate messenger RNA (mRNA) translation [6]. Amino acids are categorized as acidic, basic and neutral amino acids. Some amino acids are not synthesized in the body and it is necessary to take them in diet. Such types of amino acids are called essential amino acids. Some amino acids are synthesized in the body and there is no needs to take them in diet, such type of amino acids are called non-

essential amino acids. Some amino acids are synthesized in the body but their production is insufficient such type of amino acids are called semi-essential amino acids [7].

Tilapia is a popular freshwater fish due to their nutritional benefits and wide availability. With the increase in the amount of tilapia (Oreochromisniloticus) demand and consumption, tilapias are among the most widely produced fish species around the world, with an annual production of 1,265,780 metric tons in 2000 (FAO, 2003). Tilapia meat has good organoleptic characteristics, with a fillet yield of up to 33% [8]. The consumption of fish and fish products is recommended as a means of preventing cardiovascular and other diseases and greatly increased over recent decades in many European countries [9]. Besides, this fishes are good source which possess immense antimicrobial peptide in defending against dreadful human pathogens [10]

An example, a sardine protein diet showed to lower insulin resistance, leptin and TNFa, improved hyperglycemia and decreased adipose tissue oxidative stress in rats with induced metabolic syndrome [11]. The authors suggested dietary sardine protein as a possible prophylaxis against insulin resistance. Furthermore, proteins from various fish as bonito, salmon, mackerel, herring and cod have shown anti inflammatory properties, while salmon and cod protein in addition improved insulin sensitivity in rats [12]; [13]; [14]. [15] found cod protein to better promote growth and regeneration of skeletal muscle after trauma compared to peanut protein and casein and suggested this also to be partly because of the improved resolution of inflammation by cod protein. In the more recent research, a decreased risk of metabolic syndrome in adults has been attributed to the consumption of lean fish [16]. [17] indicated that already a low dietary intake of cod protein (25%) compared to a casein only diet, improved lipid metabolism and glucose regulation in obese rats.

Albinism is an inherited disorder that occurs due to deficiency of the enzyme tyrosinase. Tyrosinase is involved in synthesis of melanin. Due to deficiency of melanin, patient becomes white (becoming an albino). Normally phenylalanine is converted to tyrosine. Also if there is deficiency of phenylalanine hydroxylase, phenylalanine is not converted to tyrosine. As a result alternative catabolites are produced. These catabolites are phenyl acetate and phenyl lactate. Phenyl acetate is conjugated with glutamine and excreted as phenyl acetyl glutamine in urine producing mouse odour in urine. Accumulation of phenyl alanine leads to defective serotonin formation, impaired melanin formation, children that are affected with this disease have fair hair and fair skin and are mentally retarded, and other features include seizure, psychosis and eczema.

Majority of fish consumers do so, because of its availability, flavor and palatability; while, few do so, because of its nutritional value. Therefore, it can be suggested that taste, size, freshness and other related external appearances should not be the only factors to be considered in making choice for marketing and consumption of tilapia [18]. Therefore, the aim of this study is to identify contents of amino acids contained in tilapia in Ebonyi State.

II. Materials and methods

Sample preparation

The tilapia samples were divided into 2 groups, one group was smoked using local smoking technique of "hot smoke drying". The samples were laid out on a platform of mesh wired supported by a semi-circular frame work of perforated metal drum. The base of the drum had an opening where wood can be combusted to generate heat. An average temperature of 160 °F (71 °C) was recorded during smoking with mercury in glass thermometer and the entire smoking process took 12minutes. Care was taken while using this traditional technique to avoid over drying as intense heating was reported to cause complex chemical reactions, such as protein-protein interactions, protein-fat interaction [19]. The samples were sliced into tiny pieces using clean surgical blades and then macerated into fine texture using homogenizer. These were used for determination of amino acid contents.

Determination of Amino acid content

The amino acid profile 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 $105^0C\pm 5^0C$ 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 [20].

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.

III. Results

The results showed that the 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 were present (Leucine, Lysine, Isoleucine, Phenylalanine, Tryptophan, Valine, Methionine, Arginine, Histidine and Threonine). Analysis on the dried tilapia samples showed the presence of 18amino acids of the 20 known standard amino acids, with Glutamic acid having the greatest concentration and tryptophan having the least concentration as Figure1, 2, 3 and 4.

The results indicated that the most abundant amino acids across the dried tilapia samples collected from different locations (vis-à-vis pond1, river and Pond2) were glutamic acid (11.96 g/100g protein, 11.51 g/100g protein and 11.66 g/100g protein respectively) followed by aspartic acid (10.42 g/100g protein, 9.6 g/100g protein and 10.3 g/100g protein respectively), glycine (7.7 g/100g protein, 7.65 g/100g protein and 7.6 g/100g protein respectively), alanine (7.28 g/100g protein, 6.98 g/100g protein and 6.98 g/100g protein respectively) and leucine (7.0 g/100g protein, 6.77 g/100g protein and 7.24 g/100g protein respectively) while amino acids with the lowest concentration across the fish samples were tryptophan (0.73 g/100g protein, 0.68 g/100g protein and 0.68 g/100g protein respectively) and cysteine (1.33 g/100g protein,1.33 g/100g protein and 1.33 g/100g protein respectively). The result of the statistical analysis showed that the difference in the amino acid concentrations across the 3 tilapia sources were highly significant ($p<0.05$).

The result of the comparative analysis as shown in figure 4 of amino acid composition between fresh tilapia samples and dried samples collected from 3 different locations each revealed that the following amino acids; leucine, isoleucine, phenylalanine, tryptophan, valine, methionine, proline, arginine, tyrosine, histidine, cysteine, alanine, glycine, threonine, serine and aspartic acid were lower in fresh samples compared to their respective concentrations in the dried samples. However, glutamic acid was higher in the fresh sample from pond2 compared to the dried ones from pond2 (14.54 g/100g protein and 11.66 g/100g protein respectively). Also, lysine content in the fresh sample from pond1 was higher than its dried counterpart (6.05 g/100g protein and 5.62 g/100g protein respectively). The difference in the amino acid compositions between the fresh and dried tilapia samples was very significant ($p<0.05$).

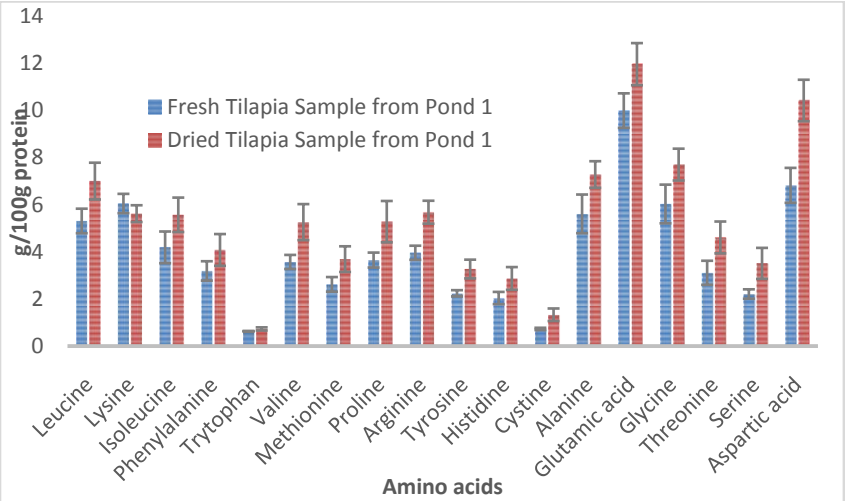

Figure 1: Amino acid profile of fresh and dried sample from pond 1.

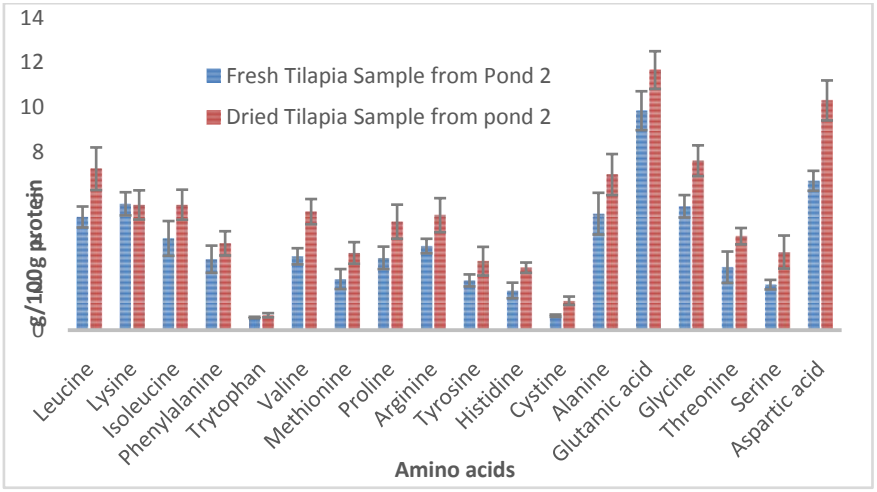

Figure 2: Amino acid of fresh and dried samples from pond 2.

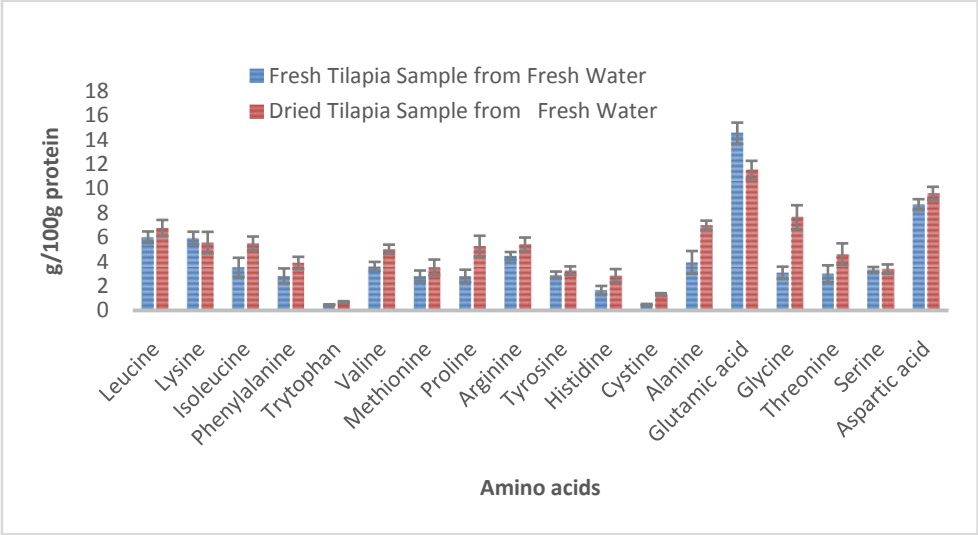

Figure 3: Amino acid profile of fresh and dried samples from fresh water sample.

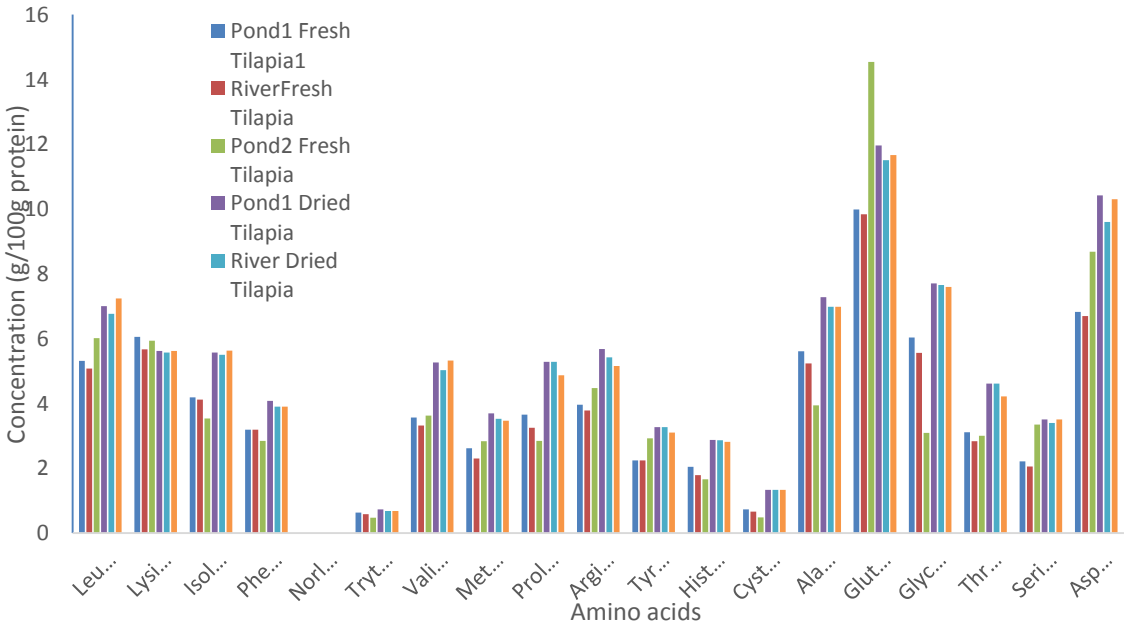

Figure 4: Comparism of amino acid profile of fresh and dried samples from three locations

IV. Discussion

The result of comparative analysis of amino acid profile between fresh and dried samples from the three sources showed that the each of the 18 amino acids detected were significantly higher ($p<0.05$) in dried samples compared to fresh samples across the three (3) sources. This was in agreement with the findings of [21] who reported that there was overall increase in amino acid compositions fish species after processing by smoking. [22] reported same findings after using Penaeuskerathurus. The values of glycine, theorine, serine, aspartic acid, alanine, tyrosine and valine were also significantly raised ($P<0.05$) after drying.

Particularly, histidine concentration (2.87 g/100g protein) was highest in the smoked samples of Pond 1 and perhaps offered better deal when in quest for growth and repair of tissue as well as in the production of blood cells [23]. Nevertheless, this study has confirmed the tendency of histidine to be increased when subjected to increase temperatures because of its scavenge of free radicals (as suggested by [24] produced on exposure to heating. This implies on consumption, the dried samples will provide functions such as prevention of seafood allergy and according to [25] dietary foods functioning as antioxidant prevent many human diseases including cancer, atherosclerosis, stroke, rheumatoid arthritis, neurodegeneration, and diabetes. Importance of histidine in infant and growing children have been emphasized at many world conferences and advocated by many nutritionist (FAO/ WHO/ONU 1985).

Arginine's concentration in the samples was generally low 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. It helps maintain the body's nitrogen equilibrium, involved in waste detoxification, moderates glucose tolerance, and promotes wound healing and bone repairs and from this study the samples were proven to contain sufficient arginine with highest concentrations found in the dried samples (5.68 - 5.16 g/ 100g protein) while those of the fresh samples had lower concentrations of 3.78 – 4.47g/ 100g of protein. Similar levels of arginine have been reported in the small forage fish capelin (Mallotusvillosus) ($5.70 \pm 0.02\%$).The results from similar studies on cold water fishes O. mykissobtain slightly higher concentrations of 6.5 ± 0.3 g100g−1 protein [26]. The difference in results could be as a result in species as different fish species showed difference in concentration, although the difference is not significantly great.

The reduction of available lysine from 6.05 g/g protein in fresh pond 1 to 5.62 g/g protein in its dried counterpart and 5.94 g/g protein in fresh sample of pond 2 was reduced to 5.62 g/100g of protein was a deviation of increase in amino acid concentration as seen with other amino acids upon drying. [6] also reported loss of lysine in milk fish subjected to salt and sun drying. This indicates heating has an adverse action lysineand moreover higher concentrations have always been seen in fresh fish samples as reported by [17] in a study with S. commersonii which had concentrations of 16.1 ± 0.9 g 100^{-1} g protein. Methionine values increased relatively across all samples ranging from 2.6g/ 100g of protein to 3.69g/ 100g of protein indicating the impact of heating on methionee. However, the values for lysine and methionine in the samples were able to meet the 3 g/kg per day and 1 g/kg per day recommended for adults.

Cysteine was highest in the dried samples in equal concentrations (1.33g/ 100g of protein) and is useful in dietary formulation for convalescing individual since cysteine is useful during the healing of wounds after surgical operations and in increasing the activities of white blood cells.Generally, =[9ooo0the amino acids composition of the dried samples obtained from pond1, river and pond2 locations, glutamic acid was significantly higher (11.96 g/100g protein, 11.51 g/100g protein and 11.66 g/100g protein respectively) followed by aspartic acid (10.42 g/100g protein, 9.6 g/100g protein and 10.3 g/100g protein respectively) meanwhile tryptophan and cysteine remained predominantly low in concentration across the samples. However, majority of the non-essential amino acids were higher in samples from pond 2. [12] indicated that leucine regulates the blood sugar levels therefore, the smoked form may be recommended in diabetes treatment. Although phenylalanine values were elevated in the smoked samples and may have contributed in the Maillard-type reaction, this study suggest that the impact of drying (smoking) on leucine was higher than in its fresh water counterpart.

However, the result of comparative analysis of amino acid profile between fresh and dried samples from the three sources showed that the each of the 18 amino acids detected were significantly higher ($p<0.05$) in dried samples compared to fresh samples across the three (3) sources. However, there were few deviations from this general trend. Glutamic acid was higher in the fresh sample from pond2 compared to its dried sample counterpart from pond2 (14.54 g/100g protein and 11.66 g/100g protein respectively) as seen in figure 5. Also, lysine content in the fresh sample from pond1 was higher than its dried counterpart (6.05 g/100g protein and 5.62 g/100g protein respectively). Hence, this study has shown that processing of tilapia by drying helps increase some of its amino acids although the underlying mechanism is yet to be properly understood.

Glutamate was the most dominant amino acid and like arginine, isoleucine, threonine and proline the slight increase in concentration by smoking were considered not important. Maillard reactions lead to increased values of leucine, histidine, tyrosine and lysine leading to the browning observed in the smoked and sundried samples. Nevertheless, Protein degradation and Amino Acids Score (AAS) and Protein Digestibility Corrected-

Amino Acid Score (PDCAAS) were positive indicating that the proteins from tilapia (fresh, and smoked forms) were well balanced in their essential amino acid compositions indicating that tilapia is a high quality protein source.

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