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CEOS Human Nutrition and Food Science

Research Article

Received Date: December 26, 2024
Accepted Date: January 26, 2025
Published Date: January 29, 2025

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Citation

Ayegbusi, Temitope Bodunde, BROWN, David Eugene and IKE, Joseph E (2025) Chemical Composition and Amino Acid Profile of Fish Flour Obtained from Catfish (Ictalurus punctatus), Mackerel (Tranchurus symmetricus) and Croaker (Micropogonias undulatus)- CEOS Hum Nutr Food Sci 3(1): 102

Chemical Composition and Amino Acid Profile of Fish Flour Obtained from Catfish (Ictalurus punctatus), Mackerel (Tranchurus symmetricus) and Croaker (Micropogonias undulatus)

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Abstract

Background: Fish flour is a product derived from dried fish which are edible as a result of its nutrient composition

Objective: The study evaluated the nutritive value of fish flour obtained from catfish, mackerel and croaker fish species.

Materials and Methods: The fish species were purchased from market in Umuahia North LGA of Abia State. The three fish species were processed to fish flours using a standard method.

Results: The proximate composition showed that the moisture content (3.29%) of sample B (mackerel fish flour) was significantly ($P<0.05$) higher than the other samples. However, the crude protein and ash contents of sample C (Croaker fish flour) with values of 81.99 and 7.24%, respectively, were significantly ($P<0.05$) higher than the other fish flours. The dietary fiber of sample A (catfish flour) was significantly ($P<0.05$) higher in its solubility compared to others. The amino acid profiles of sample C (Croaker fish flour) were significantly ($P<0.05$) higher in seven of the essential amino acids which included leucine, lysine, phenylalanine, valine, methionine, histidine and threonine with values of 7.12, 7.48, 4.01, 4.42, 2.25, 2.63, 4.17g/100g, Sample B was higher in isoleucine (3.61g) and tryptophan (1.01g) contents. The non-essential amino acid contents of sample C were significantly ($P<0.05$) higher than that of samples A and B, respectively.

Conclusion: The protein and essential amino acid compositions of the three fish flour were higher and met the recommended daily intake but croaker fish flour had more nutritional value in terms of protein, essential and non-essential amino acids than others.

Keywords: Chemical Composition; Amino-Acid Profile; Fish Flour; Catfish; Mackerel and Croaker

Introduction

Fish is a scaly skinned vertebrate that swims in water and breathes using gills, they have a skeleton made from bone, just like amphibians, reptiles, birds, and mammals and there are more than 33,000 species of fish that can be found in the water depending on the composition of the water [1]. Fish is a food that contains high quality protein and essential amino acids in adequate quantities, it is also an excellent source of lipid that contains omega-3 fatty acids, especially, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), vitamins such as D (cholecalciferol) and B2 (riboflavin) [2]. Fish is also a great source of minerals such as calcium, phosphorus, iron, zinc, iodine, magnesium, and potassium [3].

Fish flour is a product derived from dried fish of which can be edible as a result of its nutrient composition, the nutritional enhancement of the food products has attracted attention due to increased interest for healthier foods in recent years [4]. Fish flour is a dried powder, prepared from dressed fish which is highly nutritious and contains high quality proteins with all essential amino acid, fatty acids and minerals, it can be likened to fish mince which have almost the same pattern of production and they are both for the basis of human consumption [5].

Furthermore, fish flour can be used as part of complementary food for children older than six months of age since it is a good source of essential amino acids, minerals, vitamins and omega 3 fatty acids which will play a vital role in their physical and cognitive development, the fish flour can also be used as a food mixture for any age bracket as it is a good source of nutrient [6]. Fish flour is a fish product like other fish products derived via fish processing, such as fish meal, fish canning, fish silage (Tawari, 2006). Fish and fish by-products are highly perishable, due to their biological composition, which may affect their nutrient composition; however, it is known that biological composition and chemical stability depend on the fish species (Lougovois and Kyrana, 2005). It is also necessary to have data on the nutritional composition of fish powder in order to make the best use of them as food and to develop the technology for processing fish powder and other fish items (Lougovois and Kyrana, 2005).

More than 2 billion people are affected by micronutrient deficiency which is a condition often referred to as "hidden hunger" [7]. Micronutrient deficiency is particularly prevalent in poor rural and urban areas where limited economic resources prevent diversity of diets and the most common micronutrient deficiencies are connected to low dietary intakes of vitamin A, iron, zinc and iodine rich foods [8].

Micronutrient deficiency has been considered as a major risk factor for child survival in Nigeria, increasing the risk of death from common diseases such as acute gastroenteritis, pneumonia, and measles [9]. The prevalence of micronutrient deficiencies in Nigerian children under 5 years of age was reported by the Nigerian Food Consumption Survey as 23.3%, 34.0%, 13.0%, and 20.0% for Vitamin A deficiency, iron deficiency anemia, Iodine deficiency disorder, and zinc deficiency disorders, respectively [10].

According to [11], it is estimated that 150 million children under 5 years are underweight and more than 20 million suffer from severe malnutrition. About 47 million children under 5 years are stunted in the sub-Saharan Africa, whereas in the Eastern and Southern Africa 24 million are stunted. Stunting is an indicator of past growth failure, which is a sign of poor nutritional history. It is associated with a number of long-term factors including chronic insufficient protein and energy intake, frequent infection, sustained inappropriate feeding practices and poverty. Wasting indicates current or acute malnutrition resulting from failure to gain weight or actual weight loss. In Sudan, 31.0% of children under 5 years are moderately or severely underweight, 32.5% suffer from moderate or severe chronic malnutrition and 14.8% suffer from global acute malnutrition.

However, risk of malnutrition can be reduced by educating care-givers and mothers on how to take good care of their infants and children, making them realize the relationship between health and food. Fish flour is a nutrient dense fish by-product which when added to infant and children's diet, will improve its nutritional components as well as reduce the risk of protein energy undernutrition and micronutrient deficiencies also known as 'hidden hunger' among under 5 children in the societies. The study will improve the knowledge of people and be of great importance as it will increase the awareness about the use of fish flour as part of recipe in diet formulation and preparation for young children.

Significantly, the study would be useful because it will provide insight about the nutritional benefit of fish flour in diet as it improves its nutritional value, the balanced amino acids composition of fish flour complements and provide synergistic effect with other animal and vegetable protein in diet to promote fast growth and reduced feeding cost in the societies. The result of the study will also be useful to food and nutrition professionals, Pediatricians to incorporate the fish flour as part of ingredients in the production pediatric therapeutic diet such as fortified pap, high energy mix (Hemix) for stabilization and rehabilitation of malnourished infant and children in the society. Therefore, it is essential to investigate the nutritional composition of flour made from three different fish species from different water bodies.

Materials and Methods

Experimental Design

The work adopts experimental design method to evaluate the proximate, fibre and amino acid profile of fish flour made from catfish, mackerel and croaker fishes.

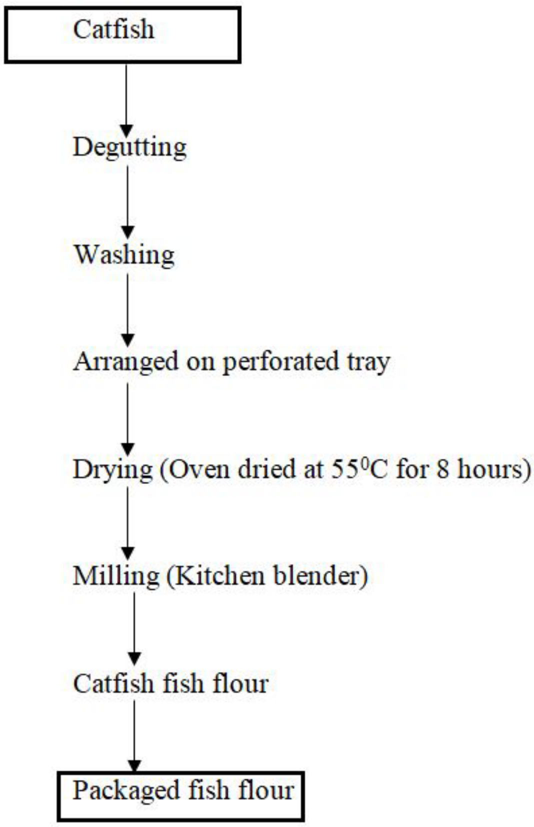

Figure 1: Flow chart for the production of catfish flour. Source: [12]

Sample Collection

The fish samples used for this work include catfish, mackerel and croaker fishes, this three types of fish was used because they are reared and exposed to different living condition and water bodies which have effect on their accessibility and nutritional value. Catfish are fish species from a confined small water bodies while mackerel and croaker are from big water bodies and more expensive. The croaker and mackerel fish species were purchased from Shoprite super-market at Amuzukwu off Uwalaka road of Umuahia, Umuahia North

Local Government Area and the catfish species were also purchased from Michael Okpara University of Agriculture (MOUAU Fish Farm), Umudike of Ikwuano Local Government Area, Abia state, Nigeria.

Sample Preparation

Preparation of Catfish Flour

The catfish was killed, degutted, washed and were arranged on a perforated tray and dried using oven drier at 550C for 8 hours or until well dried with a moisture content of about 6.9%. The dried fish was milled with the use of kitchen blender [12].

Preparation of Mackerel Flour

The mackerel was thawed, degutted, washed and were arranged on a perforated tray and dried using oven drier at 550C for 8 hours or until well dried with a moisture content of about 6.9%. The dried fish was milled with the use of kitchen blender [12].

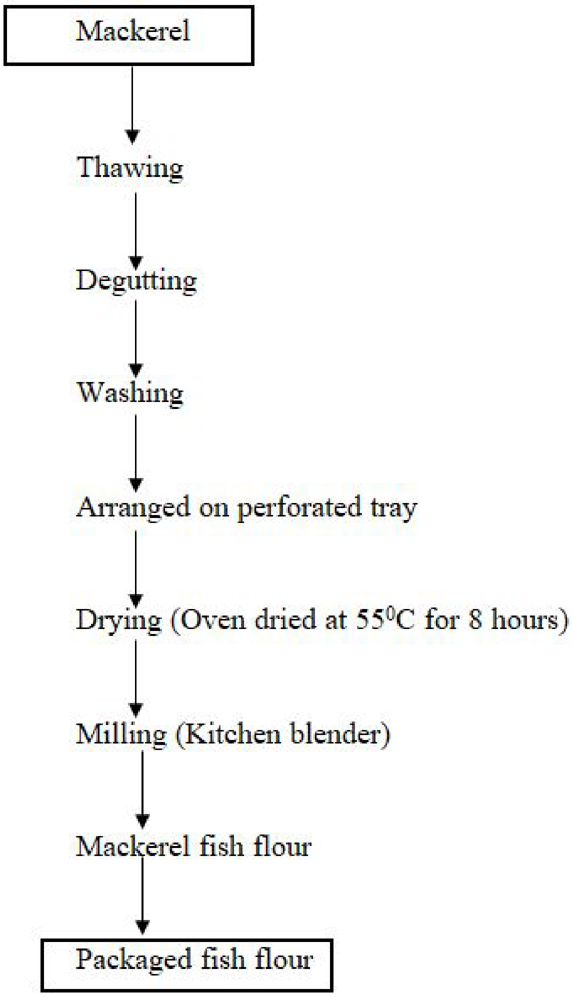

Figure 2: Flow chart for the production of mackerel flour. Source: [12]

Preparation of Croaker Flour

The croaker fish was thawed, descaled, degutted, washed and were arranged on a perforated tray and dried using oven drier at 550C for 8 hours or until well dried with a moisture content of about 6.9%. The dried fish was milled with the use of kitchen blender [12].

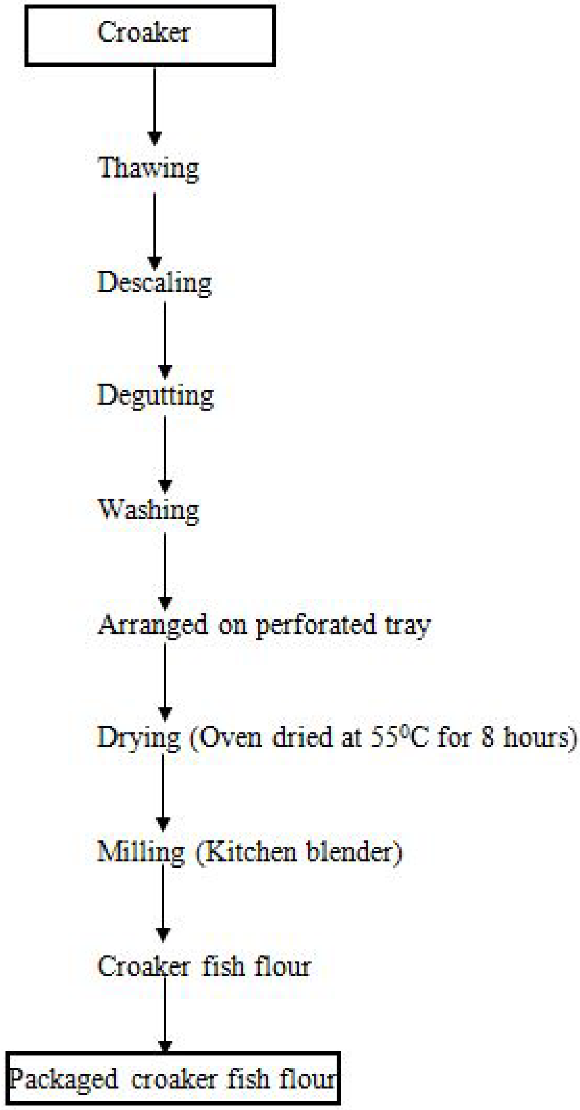

Figure 3: Flow chart for the production of croaker flour. Source: [12]

Packaging and Storage of Sample

The fish flour samples were packaged into different polythene bag, well labelled and were stored at 18^{0}C until ready for use.

Determination of Moisture Content

Moisture content was determined according to the standard methods of Association of official Analytical Chemist (AOAC), (2010). Stainless steel oven dishes were cleaned and dried in the oven at 100^{0}C for 1 hour to achieve a constant weight. They were cooled in a desiccator and then weighed. Two grams of each of the sample were placed in each dish and dried in oven at 100^{0}C until a constant weight was achieved. The dishes together with the sample were cooled in a desiccator and weighed.

$$\%\ Moisture\ content = \frac{(W2 - W3)}{(W2 - W1)} \times \frac{100}{1}$$

Where

W1= Weight of dish

W2= Weight of dish + sample before drying

W3= Weight of dish + sample after drying

Determination of Ash

Ash determination was carried out according to AOAC (2010) procedure. Two grams of sample was placed in silica dish which had been ignited, cooled and weighed. The dish and sample were ignited first gently and then at 55^{0}C in a muffle furnace for 3 hours, until a white or grey ash was obtained. The dish and content were cooled in a desiccator and weighed.

$$\%\ Ash = \frac{(W3 - W1)}{(W2 - W1)} \times \frac{100}{1}$$

Where

W1= Weight of dish

W2= Weight of dish + sample before ashing

W3= Weight of dish + sample after ashing

Determination of Fat

The fat content was determined according to AOAC (2010) Soxhlet extraction method. A 500ml capacity round bottom flask was filled with 300ml petroleum ether and fixed to the Soxhlet extractor. Two grams of sample was placed in a labeled thimble. The extractor thimble was sealed with cotton wool. Heat was applied to reflux the apparatus for six hours. The thimble was removed with care. The petroleum ether was recovered for reuse. When the flask was free of ether, it was removed and dried at 105^{0}C for 1 hour in an oven. The flask was cooled in a desiccator and weighed.

$$\%\ Fat = \frac{Weight\ of\ Fat}{Weight\ of\ Sample} \times \frac{100}{1}$$

Determination of Crude Protein

Crude protein was determined using the kjedahl method (AOAC, 2010). Two grams of sample was placed in the kjeldahl flask. Anhydrous sodium sulphate (5g of kjeldahl catalyst) was added to the flask. Concentrated tetra-oxo sulphate (VI) acid (H_2S0_4) ((25ml) was added with few boiling chips. The flask was heated in the fume chamber until the sample solution become clear. The sample solution was allowed to cool to room temperature, then transferred into a 250ml volumetric flask and made up to volume with distilled water. The distillation unit was cleaned, and the apparatus set up. Five millimeters of 2% boric acid solution with few drops of methyl red indicator was introduced into a distillate collector (100ml conical flask). The conical flask was placed under the condenser. Then 5ml of the sample digest was pipetted into the apparatus and washed down with distilled water. Five milliliters of 60% sodium hydroxide solution was added to the digest. The sample was heated until 100ml of distillate was collected in the receiving flask. The content of the receiving flask was titrated was 0.049M H_2S0_4 to pink coloured endpoint. A blank with filter paper was subjected to the same procedure.

Calculation:

$$\%\ Total\ Nitrogen\ = \frac{(Titre - Blank)\ \times\ Normality\ of\ acid \times N2}{Weight\ of\ sample}$$

Nitrogen Factor = 6.25

Crude protein = % total N X 6.25

Determination of Dietary Fiber

The dietary fibers of the fish flour samples were determined according to the AOAC (2010) method. Two grams of each of the fish flour were boiled under reflux for 30 minutes with 200 ml of solution containing 1.25g of tetra-oxo-sulphate (VI) acid (H_2S0_4) per 100ml of solution. The solutions were filtered through linen on a flaunted funnel and wash with water until the washing is no longer acidic. The residue was transferred to a beaker and boiled for 30 minutes with 100 ml of solution. The final residue were filtered through a thin but closer pad of washed and ignited asbestos in a gosh crucible. The residue were dried in an electric oven and weighed. The residue were incinerated, cooled and weighed. Dietary fiber content of the fish flour was calculated as follows:

$$\%\ Dietary\ fiber = \frac{W2 - W1}{W1}$$

Where:

W1 = Weight of the sample used

W2 = Weight of crucible plus sample

W3 = Weight of sample crucible

Determination of Carbohydrate Content

The carbohydrate content was calculated by Nitrogen free extractive method as described pearson (1976). It was given as a difference between 100 and a sum total of the other proximate components. Hence, the formula below was used, the carbohydrate (NFE) was given by:

% CHO = 100 - % (A+B+C+D+E)

NFE = Nitrogen Free Extract

CHO = Carbohydrate

A = Moisture content

B = Ash content

C = Fat content

D = Crude fiber content

E = Protein content

Determination of Amino Acid Profile

Reagents/Chemicals and Materials

- Deionised water
- Petroleum spirit
- Dichloro methane
- Amino acid standards
- Sodium Carbonate
- Ethylchoroformate
- Isooctane
- Soxhlet Arrangement
- Assorted Glassware
- Agilent 6890 coupled with FID/PFP

Extraction and Analysis for Amino acids

Extraction and the instrumentation analysis were carried out by the following modified method [14,15] in the simultaneous identification and determination of total content of amino acids in food by gas chromatography.

Procedure for Amino Acids Analysis

The dried and pulverized sample was made to be free of water by ensuring constant weight for a period of time in the laboratory. The sample of 0.5g was weighed into the 250ml conical flask capacity, The sample was defatted by extracting the fat content of the sample with 30ml of the petroleum spirit three soxhlet extractor that was equipped with thimble. The sample was hydrolyzed three times for complete hydrolysis to be achieved for the totality of amino acids recovery.

The pulverized and defatted sample was soaked with 30 ml of the 1ml potassium hydroxide solution and was incubated for 48 hours at 11^{0}C in hermetically closed borosilicate glass container. After the Alkaline hydrolysis, the hydrolysate was neutralized to get pH in the range of 2.5-5.0. The solution was purified by cation-exchange solid-phase extraction. The amino acids in purified solution were derived with ethylchloroformate by the established mechanism.

Statistical Analysis

Statistical analysis was done using excel and statistical package for service solution (SPSS) version 23. Triplicate results of each samples was inputted into SPSS software and Analysis of variance (ANOVA) was used to find the average figure for each sample parameters and was expressed as Mean + Standard deviation. Duncan test was used for the separation and comparison of the mean and to also test the significant differences between the samples nutritional values at (P<0.05).

Results of the Findings

Table 1: Proximate Composition of Fish Flours Obtained From Catfish, Mackerel and Croaker Fish Species

Parameter	Sample A	Sample B	Sample C
Moisture content (%)	$0.30^c \pm 0.04$	$3.29^a \pm 0.45$	$1.75^b \pm 0.12$
Crude protein (%)	$69.57^c \pm 0.32$	$75.38^b \pm 0.55$	$81.99^a \pm 0.42$
Fat (%)	$15.31^a \pm 0.50$	$11.62^b \pm 0.14$	$8.34^c \pm 0.01$
Ash (%)	$6.08^b \pm 0.05$	$5.72^c \pm 0.06$	$7.24^a \pm 0.08$
Carbohydrate (%)	$9.01^a \pm 0.00$	$4.99^b \pm 0.00$	$0.68^c \pm 0.00$
Energy Value (KJ)	$1,775.04^a \pm 0.01$	$1,717.43^b \pm 0.01$	$1,687.34^c \pm 0.01$

Values of mean ± standard deviation of duplicate sample
$^{a-c}$Mean with similar super script in each row are not significantly different (P>0.05)
KEY: Sample A- Catfish flour
Sample B- Mackerel fish flour
Sample C – Croaker fish flour

Table 2: Dietary Fibre Compositions of Fish Flours Obtained From Catfish, Mackerel and Croaker Fish Species

Parameter	Sample A	Sample B	Sample C
Insoluble fiber (g/100g)	$0.21^a \pm 0.01$	$0.18^a \pm 0.02$	$0.09^b \pm 0.01$
Soluble fiber (g/100g)	$0.39^a \pm 0.03$	$0.25^b \pm 0.00$	$0.19^b \pm 0.01$
Total dietary fiber (g/100g)	$0.60^a \pm 0.02$	$0.43^b \pm 0.03$	$0.28^c \pm 0.02$

Values of mean ± standard deviation of duplicate sample
$^{a-c}$Mean with similar super script in each row are not significantly different (P>0.05)
KEY: Sample A- Catfish flour
Sample B- Mackerel fish flour
Sample C – Croaker fish flour

Table 3: Amino Acid Profiles of Fish Flours Obtained From Catfish, Mackerel and Croaker Fish Species

Amino acid	Sample A (g/100g)	Sample B (g/100g)	Sample C (g/100g
Leucine	$6.61^{b} \pm 0.01$	$6.37^{c} \pm 0.01$	$7.12^{a} \pm 0.01$
Lysine	$6.77^{c} \pm 0.01$	$7.06^{b} \pm 0.01$	$7.48^{a} \pm 0.01$
Isoleucine	$2.95^{c} \pm 0.01$	$3.61^{a} \pm 0.01$	$3.34^{b} \pm 0.01$
Phenylalanine	$3.37^{c} \pm 0.01$	$3.73^{b} \pm 0.01$	$4.01^{a} \pm 0.01$
Tryptophan	$0.85^{c} \pm 0.01$	$1.01^{a} \pm 0.01$	$0.95^{b} \pm 0.01$
Valine	$4.00^{b} \pm 0.00$	$3.52^{c} \pm 0.01$	$4.42^{a} \pm 0.01$
Methionine	$2.21^{a} \pm 0.01$	$2.24^{a} \pm 0.01$	$2.25^{a} \pm 0.01$
Proline	$3.97^{b} \pm 0.01$	$4.07^{a} \pm 0.01$	$3.86^{c} \pm 0.01$
Arginine	$5.86^{b} \pm 0.01$	$5.34^{c} \pm 0.01$	$6.11^{a} \pm 0.01$
Tyrosine	$2.42^{c} \pm 0.01$	$2.76^{b} \pm 0.01$	$3.11^{a} \pm 0.01$
Histidine	$2.47^{b} \pm 0.01$	$2.37^{c} \pm 0.01$	$2.63^{a} \pm 0.01$
Cysteine	$0.73^{c} \pm 0.01$	$0.81^{b} \pm 0.01$	$0.85^{a} \pm 0.01$
Alanine	$4.81^{c} \pm 0.01$	$5.32^{b} \pm 0.01$	$5.77^{a} \pm 0.01$
Glutamic acid	$13.63^{b} \pm 0.01$	$13.02^{c} \pm 0.00$	$14.71^{a} \pm 0.01$
Glycine	$4.71^{c} \pm 0.01$	$5.21^{b} \pm 0.01$	$6.32^{a} \pm 0.01$
Threonine	$4.21^{a} \pm 0.01$	$3.84^{b} \pm 0.01$	$4.17^{a} \pm 0.01$
Serine	$3.36^{c} \pm 0.01$	$3.63^{b} \pm 0.01$	$4.06^{a} \pm 0.01$
Aspartic acid	$8.71^{b} \pm 0.01$	$8.57^{c} \pm 0.01$	$8.96^{a} \pm 0.01$

Values of mean ± standard deviation of duplicate sample
$^{a\text{-}c}$ Mean with similar super script in each row are not significantly different (P>0.05)
KEY: Sample A- Catfish flour
Sample B- Mackerel fish flour
Sample C – Croaker fish flour

Discussion

Findings of the study showed that mackerel fish flour had higher moisture content (3.29%) than the other samples making it to have a reduce shelf life because moisture in food substances encourage microbial growth. The crude protein content of the fish flour ranged from 69.57 to 81.99% in the samples with croaker having the highest amount of protein. These differences in the protein content could be as a result of variation in their habitats and the feeding patterns, catfish are mostly reared in a confined pond and fed with formulated feeds but mackerel and croaker are mostly species of big river and lakes, they feed on worm and crustaceans which may be seen as proven fact why their protein contents was higher than that of catfish. Highest value of crude fat was found in catfish flour compared to mackerel and croaker which conformed to the result of [13] that reported catfish having the highest amount of crude fat contents to other fish samples. Croaker fish flour had the highest amount of ash contents while mackerel fish flour had the least ash content with value of 5.72% which were higher than the values reported for catfish (1.60%), mackerel (1.18%) and croaker (2.00%) by [13]. The difference found in the present study and that Bouriga can be linked the processing techniques the fishes was subjected.

The results of total dietary fiber which catfish flour has the highest value (0.60g/100g) is in line with the result reported by (United State Department of Agriculture (USDA), (2015) of 0.60g for catfish in their study. The result indicated that croaker fish flour was significantly ($P<0.05$) higher in seven of the essential amino acids which include: histidine, leucine, lysine, phenylalanine, methionine, valine and threonine with values of 2.63, 7.12, 7.48, 4.01, 2.25, 4.42 and 4.17g/100g, respectively. These results agreed and authenticate the proximate analysis that showed that croaker fish flour was significantly higher in protein content compared to catfish and mackerel flour, respectively.

However, the result showed that mackerel fish flour which was significantly ($P<0.05$) higher in the remaining essential amino acids which are isoleucine and tryptophan with the values of 3.61 and 1.01g/100g, respectively. All the isoleucine values (3.61, 3.34 and 2.95g) of the fish flours were higher than the FAO/WHO/UNU (2007) recommended value of 2.8g/100g to meet human requirements. But the tryptophan values of the samples were all lower than the daily recommended intake value of 1.1g/100g (FAO/WHO/UNU, 2007). The Table also showed that croaker fish flour had the significant ($P<0.05$) highest composition of non-essential amino acids compared to catfish flour and mackerel fish flour, respectively. The glutamic acid contents of the fish flours obtained from the three fish species ranged from 13.02 to 14.71g/100g, respectively.

Conclusion

In conclusion, croaker and mackerel fish flour was found significantly ($P<0.05$) higher in crude protein compared to catfish and it was authenticated by the study amino acid analysis because it was evidenced result-based that amino acids profile of the fish flour shows that croaker and mackerel fish flour was higher in seven out of the nine essential amino acids that is needed in the body through food and nutrients consumption.

Acknowledgements

My deep sense of honor and profound gratitude goes to Mrs. I.C Asomugha for her total support in putting up this project work. May God Almighty bless and reward you abundantly in all your endeavors.

My undiluted appreciation and gratitude to my academic mentor in the person of Prof. Sunday Abayomi Adebisi (1st Occupier of Dr. Mike Adenuga Junior Professorial Chair) for his financial and mentorship support towards writing this work, God almighty will increase you in all your endeavors.

My sincere gratitude also goes to my professional colleagues: Brown David, Ifesinachi Peter, Olarenwaju Isaac (RDN), Abiola Lawal (RDN), Jimoh Adekunle, Omolorun Olamide, Njoku Godwin for their tireless efforts rendered during this research work, I salute you all.

To my co-researcher, Ayanleye Stephen Foluso, I appreciate your effort towards making this research work a reality.

To the woman that supported my dream and activities in the person of Jones Patience Idara, may Almighty God grant us peace (Amen).

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