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Production And Evaluation of Fish Protein Pack from Three Commercially Important Fish Species in Akwa Ibom State, Nigeria – Cat Fish (*Clarias gariepinus)*, African Bony-Tongue Fish (*Heterotis niloticus)* And Elephant Trunk Fish (*Mormyrus rume)*

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Abstract

Oven dried samples of three commercially important fish species in Akwa Ibom State, Nigeria – Clarias gariepinus, Heterotis niloticus and Mormyrus rume was milled and sieved. Proximate analysis, mineral content, free fatty acid content and microbiological qualities were determined using standard analytical procedures. The fish protein pack was formulated in the ratio of 60:20:20 for sample A, 50:40:10 for sample B and 40:30:30 for sample C respectively. Results showed moisture, protein, ash, fibre, fat, carbohydrate and energy to be 6.81%, 52.92%, 2.97%, 1.08%, 20.15%, 16.07% and 457.25kcal/100g respectively for (sample A), 5.12%, 60.17%, 2.70%, 1.01%, 21.73%, 9.27% and 473.36kcal/100g respectively for (sample B) and 5.06%, 68.13%, 2.95%, 0.95%, 22.14%, 0.76%, 474.85 kcal/100g respectively for (sample C) formulated fish protein pack. An appreciable amount of potassium, calcium, magnesium, phosphorus, iron, and zinc were also found in the developed fish protein packs. Among all the trace elements, potassium was highest in the samples. The formulated fish protein packs had potassium content of 496.80 mg/100g in sample A, 498.46 mg/100g in sample B and 499.67 mg/100g in sample C. Results of the free fatty acids showed sample C (19.61%) having the highest percentage of oleic acid followed by sample B (3.99%) and sample A (1.52%) respectively. The Total Heterotrophic Bacterial Count for the three samples varied between 8.2 x 10^5 CFU/g in sample A, 1.02 x 10^6 CFU/g in sample B and 6.0 x 10^5 CFU/g in sample C respectively. Sample B (.1 x 10^5 CFU/g) had the highest number of Total coliform count (TCC) followed by sample A (5.1 x 10^5 CFU/g) and sample C (4.6 x 10^5 CFU/g).

INTRODUCTION

Fish is one of the most important food stuff due to its high protein content and unsaturated fatty acids (Okey and Kekong, 2018). The consumption of fish as well as fish products has significantly increased during the last two decades (FAO, 2016). The popularity of fish is mainly due to the overall high quality and the positive effects on human health (Tilami *et al*., 2018). The main health benefits of fish are attributed to their high content of n-3 long-chain polyunsaturated fatty acids (FAs) (n-3 LC-PUFA) (Kris-Etherton *et al*., 2002; Lund, 2013; Khalili Tilami and Sampels, 2018).

In a world of ever-growing population, fish among foods is one of the cheapest sources for provision of high quality animal protein with values ranging from 15 to 20% (Mohanty *et al*., 2011). Fish is highly nutritious, palatable with tender flesh hence easily digestible (Effiong and Fakunle, 2011). They are also an excellent source of lipid containing omega-3 fatty acid, more specifically eicosapentaenoic acid and docosahexaenoic acid which are known to have commendatory impact on cardiovascular as well as nervous system of children during pre-natal development (Tilami *et al*., 2018). In addition to its valuable lipid and protein composition, it is also a significant source of vitamins (vitamin A and vitamin D) and minerals (Ca, P, Fe, Zn, K, Na etc) (Mansi *et al.*, 2021). Fish is recognized as the most nutritious animal protein source, but because of its high deteriorative nature, a huge number of fish are being wasted and susceptible to nutritional losses, resulting in a significant hurdle for expanding fish production (Iftekhar *et al.*, 2022).

Undernourishment counts as an invisible impediment to the success of developing countries. In a report, FAO estimated about 768 million people were undernourished worldwide (FAO, 2021). Protein-energy malnutrition (PEM) and micronutrient deficiencies are two of the major public health problems and these problems can be compensated by increasing the availability and intake of protein from different sources (Iftekhar *et al.*, 2022). Meat and meat products cover the major portion of protein sources globally but the production of meat needs vast resources (Poore and Nemecek, 2018; Mekonnen and Hoekstra, 2010) and the consumption of red meat leads to several noncommunicable diseases (NCD) (Larsson and Orsini, 2013). Fish is a good source of animal protein and it provide about 30 to 80% of the protein intake (Monterio *et al*., 2017). Fish nutritive quality is important attribute which can be affected by many factors such as food availability in ecosystem, feed composition, water quality, species and method of processing and preservation. The main issue with fish and fishery products is their shelf life. Usually, last for less than a month in modified atmosphere packaging at freezing temperature (Masniyom, 2011). Therefore, several studies suggested that using fish protein pack has a vast opportunity, as it can be blended into our regular food as a supplement. Furthermore, children as their nature, are normally not concerned about a healthy diet and rarely prefer fish in their dishes and are impatient with bakery products. If those bakery products were fortified with fish protein pack, the nutritional content of protein would increase and the daily requirement could be fulfilled (Abraha *et al*., 2018). Also, whenever there is a food scarcity problem due to natural disasters or else, an emergency food product with an extended shelf life and balanced nutrients are necessary (Purnamayati *et al*., 2019). This ready-to-consume food supply must contain all the nutrients in a compact and stable form. Fish protein pack can be a significant source of safe, nutritionally complete stable food supplements to

compensate for this problem and use as a food additive to enrich the nutritional value of various foods (Shaviklo, 2015).

Akwa Ibom State, Nigeria is blessed with network of streams, rivers and seasonal flood plains and tidal creeks, which play a major role in her development (Udo, 2012). These inland waters provide easy means of transportation, occupational activities, and means of water disposal and source of food. Inland waters are important harbours for fishes of high economic values and some intrusive marine species that use them as spawning and nursery grounds (Udo, 2012). These inland fisheries if sustainably managed will continue to provide good quality protein food for the teaming population of our people while at the same time still serving as a source of livelihood to several others. A lot has been done on rivers, estuaries, and streams in Akwa Ibom State (Usanga, 2015).

Demands for fish protein ingredients including dried fish protein to develop functional food or ready-to-eat products are gradually growing in the world (Thorkelsson *et al*., 2009). The quality and characteristics of fish protein ingredients are highly dependent on the source of the raw materials and the processing methods (Arason *et al.*, 2009, Shaviklo *et al*., 2010).

The fish protein concentrate (FPC) is a dried and stable fish product, intended for human consumption, in which the protein is more concentrated than in the original fish flesh (Shaviklo *et al*., 2010). It is accepted as human food and not animal food while fish meal is not accepted as human food because of its comparatively poor flavour stability in general requiring antioxidants for flavour maintenance, its odour and also the fact that many countries will not permit the sale of foods made from unwholesome raw materials example, fish guts. In addition to animal protein, FPC also bears other important micronutrients, such as various vitamins, minerals, and trace elements, which are beneficial for child growth, keeping human well-being, and speeding up the recovery from malnutrition and various diseases.

High nutritive value, low calorific content, can be prepared from whole edible-grade fish which be used as dietary supplements, high potential to reduce the malnutrition condition in an economically viable way on a worldwide scale, long shelf-life, good storage stability, and no requirement for refrigeration during transportation and storage (Pires *et al*., 2012). The FPC also bears a low level of anti-nutritional components and hence they can be directly used in food products preparation. The low or negligible oil content in FPC offers promising consumer acceptance due to the disappearance of the fishy taste in its edible portion (Lee *et al.*, 2016).

MATERIALS AND METHOD

Materials Procurement

Freshly caught Catfish (*Clarias gariepinus),* African bony-tongue fish (*Heterotis niloticus*), and Elephant Trunk fish *(Mormyrus rume)* were purchased from local retailers in Esuk, Nwaniba beach Market, Uruan Local Government Area, Akwa Ibom State, Nigeria and transported to the Food Processing Laboratory, Department of Food Science and Technology, University of Uyo, Nigeria, for processing into edible fish protein pack. To prevent microbial activity during transportation, the containers were kept chilled (8^{o}C) under hygienic conditions.

Preparation of Fish protein pack

Fish protein pack was prepared using the method described by Iftekhar *et al.* (2022) with some modifications. After washing with tap water, the fish samples were weighed and beheaded. The scales (African bony-tonue and elephant trunk fishes) were removed, as well as other extraneous materials. Belly flaps, blood, and viscera were also removed. The fishes were thoroughly cleaned to eliminate any lingering blood and intestinal waste and weighed. They were then filleted separately by taking out the spine using a sharp stainless-steel knife. The fillets were chopped into small pieces (size reduction) and washed in cold water (8°C) to eliminate any blood, dirt, and other impurities **(**Iftekhar *et al.*, 2022).

The fillets were thereafter immersed in a 5% NaCl solution (1:4 w/v) for 10 minutes to reduce the initial moisture content through osmotic dehydration (Owusu-Kwarteng et al., 2017) and blanched for 10 minutes at 80^{o}C in the same solution to reduce enzymatic activity and the initial microbial load (Fellows, 2017). The fillets were then filtered to eliminate excess water and spread on a stainless-steel tray, covered with clean aluminum foil (1-2mm thick) and placed in a heated oven-drier (universal oven) at 65^{0}C for 8 hours with continuous inspection until constant moisture content was obtained. A crisp fish flake was formed after drying which was crushed in a grinder and sieved through a strainer with 1mm opening to obtain a fine fish protein pack. The fish protein pack was thereafter packed in an airtight container and stored at ambient temperature for further analysis (Figure 1).

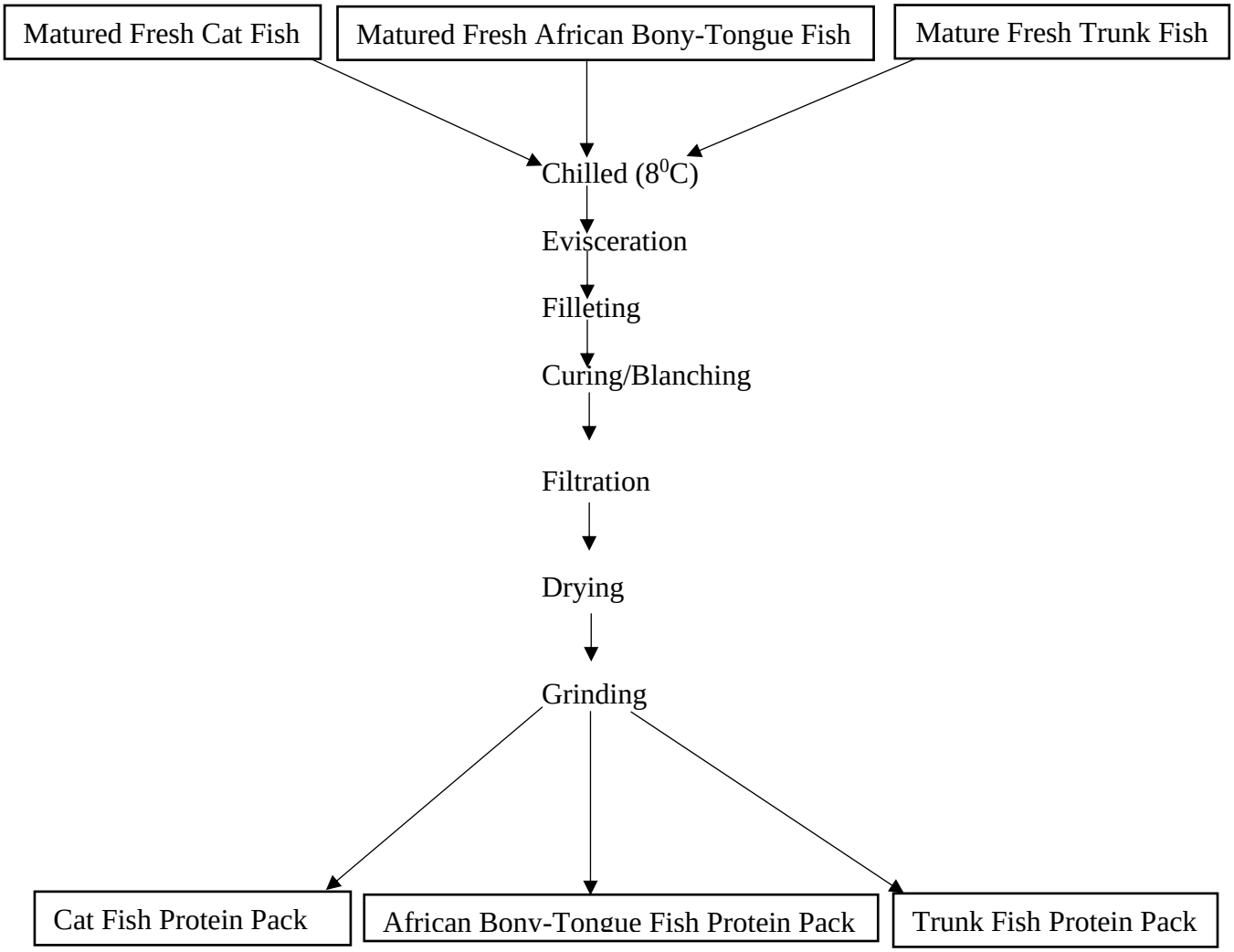

Figure 1: Flowchart for the production of fish protein pack.

Percentage yield

The Percentage yield of fish protein pack from fresh fish was determined as per the formula given by Islam *et al.* (2018).

$$\text{Percentage yield (\%)} = \frac{\text{Final product obtained}}{\text{Raw weight of sample}} \times 100$$

Table 1: Formulation of the Fish Protein Pack for Analysis

Samples (%)	*Clarias gariepinus*	*Heterotis niloticus*	*Mormyrus rume*
A	60	20	20
B	50	40	10
C	40	30	30

Where Sample:

A = *Clarias gariepinus* 60%, *Heterotis niloticus* 20% and *Mormyrus rume* 20%

B = *Clarias gariepinus* 50%, *Heterotis niloticus* 40% and *Mormyrus rume* 10%

C = *Clarias gariepinus* 40%, *Heterotis niloticus* 30% and *Mormyrus rume* 30%

Proximate Analysis

Proximate composition of the fish protein pack samples was determined using the method of AOAC (2016).

Mineral Analysis

Minerals of fish protein pack products such as potassium (K), calcium (Ca), magnesium (Mg), phosphorus (P), iron (Fe), zinc (Zn), and boron (B) were determined using established procedures (Uddin *et al*, 2016). The flame photometer was calibrated using a standard stock solution of potassium. Ca, Mg, Fe, and Zn were measured using an Atomic Absorption Spectrometer. The AAS was calibrated with a standard solution before measuring digested samples (Santos *et al.*, 2019; Jasim *et al.*, 2020). A flame photometer was used to determine K levels. The boron (B) and phosphorus (P) of the digested sample were determined using a spectrophotometer.

Fatty Acid Profile Analysis

Fatty acid profile was determined using gas chromatography/mass spectrophotometer (GC-MS) after extraction of the sample with a Soxhlet extractor, purification and cleanup using a packed column. The sample was analyzed using agilent technologies 7890A GC and 5977B MSD with Experimental conditions of GC-MS system were as follows: Hp 5-MS capillary standard non-polar column, dimension: 30M, ID: 0.25 mm, Film thickness: 0.25µm.

Microbial analysis

Standard Microbiological techniques described by Harrigan and McCance (1990), Prescott *et al.* (2005) were employed for the microbiological analysis of the fish protein pack. Standard characterization tests (such as Gram staining, catalase, coagulase, motility, starch hydrolysis, methyl-red, Voges Proskauer, indole, citrate utilization, Oxidase test, urease, spore staining, hydrogen sulfide production and sugar fermentation) were performed. The pure cultures were identified on the basis of their cultural, morphological and physiological features with those in Bergey's Manual of Determinative Bacteriology (Cowan, 1974; Buchanan and Gibbons, 1974).

Statistical Analysis

All experiments were carried out in triplicate. Data obtained were subjected to analysis of variance (ANOVA) to compare the difference between means using SPSS 21.0 software for windows. Significant difference between means were determined by turkey test. A p–value less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Table 2: Percentage yield of fish protein pack

Fish species	Fresh fish weight (kg)	Dressed fish weight (kg)	Dried weight (kg)	Total yield percentage (%)
Cat fish	1.3	0.95	0.40	30.76
Bony-Tongue Fish	1.6	1.25	0.30	18.75
Trunk Fish	1.5	1.25	0.30	20.00

Percentage yield of fish protein pack

The processing yield of fish protein pack prepared from Catfish (*Clarias gariepinus)*, African Bony-Tongue fish (*Heterotis niloticus*), and Elephant Trunk fish *(Mormyrus rume)* as shown in Table 2 was 30.76%, 18.75 and 20.00 of processed fish, respectively. A lower yield percentage in the range of 13.00% to 14.66% was reported by Mansi *et al*. (2021) which involved preparation of edible fish protein pack from small fish species of *Amblypharyngodon mola* and *Puntius sophore*. Also, Iftekhar *et al*. (2022) reported 2.11% to 12.69% of Fish Powder produced from Tilapia (*Oreochromis mossambicus)* and Silver Carp (*Hypophthalmichthys molitrix).*

Table 3: Proximate composition and Energy Values of Fish Protein Pack produced from Cat Fish (*Clarias gariepinus)*, Bony-Tongue Fish (*Heterotis niloticus)* and Trunk Fish (*Mormyrus rume*)

Sample	Moisture (%)	Protein (%)	Ash (%)	Fibre (%)	Fat (%)	Carbohydrate (%)	Energy (kcal/100g)
A	6.81±0.01^{a}	52.92±0.03^{c}	2.97±0.02^{a}	1.08±0.02^{a}	20.15±0.04^{a}	16.07±0.03^{a}	457.25±0.33^{c}
B	5.12±0.02^{b}	60.17±0.03^{b}	2.70±0.02^{b}	1.01±0.01^{b}	21.73±0.04^{b}	9.27±0.02^{b}	473.36±0.36^{b}
C	5.06±0.05^{b}	68.13±0.03^{a}	2.95±0.06^{a}	0.95±0.02^{c}	22.14±0.04^{c}	0.76±0.11^{c}	474.85±0.26^{a}

Values are mean of the triplicate determination ± standard deviation. Means with different superscript on the same column are significantly ($p<0.05$) different.

Keys: A = 60:20:20; B = 50:40:10; C = 40:30:30.

Proximate composition and energy values of fish protein pack

Fish provides important nutrients to large number of people worldwide and therefore makes a very significant contribution to nutrition (Adewumi *et al.*, 2014). Moisture, protein, ash, fibre, lipid, and carbohydrate were evaluated in fish protein pack samples as proximate components**.** The proximate composition values for fish protein pack produced from the three different fish species: Cat Fish (*Clarias gariepinus)*, Bony-Tongue Fish (*Heterotis niloticus)* and Trunk Fish (*Mormyrus rume*) in a formulated ratio of sample A (60:20:20); sample B (50:40:10) and sample C (40:30:30) respectively is shown in Table 3. The moisture content which has effects on spoilage ranged from 5.06-6.81% with the highest value in sample A (6.81%) which was significantly higher than that of sample B (5.12%) and C (5.06%). The moisture content of the formulated Cat Fish (*Clarias gariepinus)*, Bony-Tongue Fish (*Heterotis niloticus)* and Trunk Fish (*Mormyrus rume*) protein pack was similar to the report of Shaviklo (2015). Also, the values obtained on the basis of dried matter were similar to those reported by Effiong and Fakunle and Ande *et al* in fish species from the Kainji Lake and River Lafia respectively which gave the range of 5.10 - 10.50% and 5.67 - 9.50% respectively (Effiong and Fakunle, 2012; Ande *et al*., 2012). The values obtained in this study are within the range of 5-8 % for moisture content for fish products on dry basis (FAO, 2011). The microbiological activity in a product is directly proportional to its low moisture content. According to other studies, microbial development is flattened below 8% moisture level, preserving quality and sensory features for a longer period (Laudeceased, 2014). Thus, it can be concluded that both samples might be in a safe range for storage.

The developed fish protein packs had high protein content of 52.92% in sample A, 60.17% in sample B and 68.13% in sample C. The protein content of the protein pack showed significant ($p<0.05$) increase from 52.92% in sample A to 68.13% in sample C. Jahan *et al*. (2018) reported protein content of fish protein pack prepared from *Puntius sophore* to be 54.31% which is similar to the findings of this investigation. Kasozi *et al*. (2018) in their study on nutrient composition of fish protein powder prepared from *Brycinus nurse*, a small-sized pelagic fish also obtained protein level (50.40%) similar to that observed in the present study. In the study of Shaviklo *et al.* (2012), protein content of 14.04% and 71.51% was observed in fish protein isolate produced by freeze drying alone and freeze drying with

addition of 5% sucrose and 0.2% phosphate. Sathivel *et al.* (2006) also reported higher protein content (71.01%) in freeze dried Pollock trimming soluble protein.

Several other studies (Islam *et al*., 2018; Jeyasanta *et al.,* 2013; Kasozi *et al*., 2018) have also reported protein contents of similar ranges in fish powder developed from some other fish species. The fish protein pack's high protein content may play a significant role in its applications. Since it contains this huge amount of protein in a compact form, it can open up a variety of uses both nutritionally and commercially (Iftekhar *et al.,* 2022). Also, being protein rich, the developed fish protein packs can be effective in combating protein energy malnutrition which is a major public health concern for developing countries.

The Ash content of the protein pack increased significantly ($p<0.05$) from 2.70% in sample B, 2.95% in sample C to 2.97% in sample A. The ash contents from this study varied from the result reported by Kasozi *et al*. (2014). These researchers reported a considerably higher value than those obtained in the present study. The variations may be attributed to the inherent differences in the fish species. Uzzaman *et al*. (2018) and Rathnakumar and Panchraja (2018) in their research findings reported a similar ash content in the range of 2.06% to 3.06% which might be due to removal of bones during fish powder preparation.

Nutritionally, fish fat are of prime importance as it is a rich source of omega-3 Poly unsaturated fatty acid (PUFA) (Mansi *et al*., 2021). The developed fish protein pack had high fat content of 20.15% in sample A, 21.73% in sample B and 22.14% in sample C respectively which compares with the results of Islam *et al*. (2018). A much lower fat content of 0.50 and 0.78 g/100g was reported in the studies of Jeyasanta *et al.* (2013) and Rathnakumar and Pancharaja (2018) which could be due to varietal difference or discrepancy in the processing technique adopted for preparation of fish protein pack. However, excess lipid concentration in fish protein pack can oxidize quickly, resulting in a rancid flavour that renders the protein pack unusable (Torkelsson *et al*., 2008). Another study discovered that, depending on how the fish protein pack is processed, fat content in one species can range from 3.5% to 17.80% (Savlak *et al*., 2020). This also indicates that the fat content in the current sample can be similarly reduced by treating fish fillets with sodium hydroxide (NaOH), sodium hypochlorite (NaOCl), ethyl alcohol (EtOH), hydrochloric acid (HCl), or citric acid (Iftekhar *et al.,* 2022).

The order in which the carbohydrate composition of the fish protein packs studied were significantly different ($p < 0.05$) from each other was sample A (16.07%), sample B (9.2%) and sample C (0.76%). The results were high, except that of sample C which was comparable to that obtained by Effiong and Fakunle (1.95- 11.95 %) in five fish species from the Kainji Lake (Effiong and Fakunle, 2012). Also, Ayeloja *et al* obtained the range of 2.10-12.57% for freshwater fish species from the Western part of Nigeria (Ayeloja *et al*., 2013).

The current study showed that compared to sample B and C, sample A had a higher energy content (474.85 kcal). This result is similar to the findings of fish protein pack from different species reported by Mahmud *et al*. (2019). Energy content is a direct expression of total proximate composition. The proximate composition of fish varies based on species, food, age, sex, capturing time, environment, and other factors (Boran and Karaçam, 2011).

Table 4: Mineral composition of Fish Protein Pack produced from Cat Fish (*Clarias gariepinus),* Bony-Tongue Fish (*Heterotis niloticus)* and Trunk Fish (*Mormyrus rume*)

Sample	Calcium (mg/100g)	Magnesium (mg/100g)	Iron (mg/100g)	Zinc (mg/100g)	Phosphorous (mg/100g)	Potassium (mg/100g)	Boron (mg/100g)
A	8.67 ± 0.02^{a}	4.09 ± 0.01^{a}	0.54 ± 0.02^{a}	0.06 ± 0.02^{b}	279.25 ± 0.45^{b}	496.80 ± 0.06^{c}	0.03 ± 0.00^{b}
B	8.13 ± 0.02^{b}	4.04 ± 0.05^{a}	0.44 ± 0.04^{b}	0.13 ± 0.02^{a}	281.72 ± 0.05^{a}	498.46 ± 0.01^{b}	0.06 ± 0.01^{a}
C	7.92 ± 0.03^{c}	3.86 ± 0.01^{b}	0.41 ± 0.01^{b}	0.15 ± 0.01^{a}	281.99 ± 0.01^{a}	499.67 ± 0.01^{a}	0.07 ± 0.01^{a}

Values are mean of the triplicate determination ± standard deviation. Means with different superscript on the same column are significantly ($p<0.05$) different.

Keys: A = 60:20:20; B = 50:40:10; C = 40:30:30

MINERAL COMPOSITION OF THE FISH PROTEIN PACK

Determination of the mineral content of foodstuff is essential due to their nutritional importance, toxicological potential, interactive effects with processing and texture of particular meals, as well as flavor (Iftekhar *et al.*, 2022). The fish protein packs developed in the present study were prepared from whole fish without discarding their bones in order to improve the mineral contents of the product (Mansi *et al.*, 2021). The results of the mineral content is presented in Table 4. Samples were observed to be abundant in mineral components like potassium, phosphorus, calcium and magnesium. The source of adequate mineral contents of these samples was mainly the bones of the fish (Iftekhar *et al.*, 2022). Among all the trace elements, potassium was highest per 100g of the sample.

The developed fish protein packs had potassium content of 496.80 mg/100g in sample A, 498.46 mg/100g in sample B and 499.67 mg/100g in sample C. Although the bulk of the mineral content was derived from fish bones that was discarded, yet the presence of the tiny bones may have enhanced the availability of potassium (Iftekhar *et al.*, 2022). Moreover, sample C showed higher contents of potassium (K) than samples B and A.

The Phosphorous content of sample C (281.99 mg/100g) and sample B (281.72 mg/100g) were significantly ($p<0.05$) similar while sample A (279.25 mg/100g) had the lowest Phosphorous Content. Similar phosphorous content of 287.54 mg/100g was reported in the studies of Rathnakumar and Pancharaja (2018). Several other studies have reported phosphorus content as high as 3510 mg/100g in the study of Kasozi *et al.* (2018). The low phosphorus concentration obtained in the present study could be attributed to the removal of the fish's head, and scales (Jahan *et al*., 2017).

Peters *et al*. (2016) reported that calcium is a macronutrient essential to health and wellbeing, which performs diverse biological functions in the human body. The concentration of calcium in the fish protein pack varied significantly ($p < .05$) with 7.92 mg/100g in sample C, 8.13 mg/100g in sample B and 8.67 mg/100g in sample A. The values obtained in the study was lower than what was reported by

Kasozi *et al*. (2018). Calcium is extremely soluble in water and a significant amount of it may have been lost during the washing, blanching, and drying process.

The concentration of magnesium in the fish protein packs ranged from 3.86 to 4.09 mg/100g. The recommended range is 4.5-452 mg/100g (FAO, 2011). This result was lower than those obtained by Adeniyi *et al*. (2012), who reported a range of mg/l00g to 20 mg/l00g and higher than the result obtained by Alfa *et al*. (2014) which were 0.06 mg/l00g to 1.19 mg/l00g.

The concentration of iron in the fish protein pack samples ranged from 0.41 mg/100g to 0.54 mg/100g, which was below the standard concentration range of 1.0 – 5.6mg/100g (FAO, 2011). The amount of zinc in the samples was very similar to the study of Kasozi *et al.* (2018) where they prepared fish powder from Tilapia. Sample A had the lowest Zinc content of 0.06 mg/100g while sample B (0.13 mg/100g) and C (0.15 mg/100g) were significantly ($p<0.05$) similar. Boron had the least content in the three fish protein pack samples. Sample A (0.03 mg/100g), while sample B (0.06 mg/100g) and C (0.07 mg/100g) were significantly ($p<0.05$) similar. The amount of trace elements in fish samples varies by species, age, sampling time and season of capture (Mendil *et al.*, 2010; Medeiros *et al*., 2012). Based on the sample mineral content in both protein packs, it can be suggested that consuming low-priced fish protein pack could improve micronutrient levels in the lower socio-economic class (Iftekhar *et al.*, 2022).

Table 5: The Total Plate Count (TPC) of the Fish Protein Pack

Samples	THBC	TCC	TFC	SSC	SC	VC	FC	CC
A	8.2×10^5 CFU/g	5.1×10^5 CFU/g	NG	NG	2.3×10^5 CFU/g	NG	3.0×10^5 CFU/g	1×10^5 CFU/g
B	1.02×10^6 CFU/g	7.1×10^5 CFU/g	NG	1.3×10^5 CFU/g	3.8×10^5 CFU/g	NG	2.0×10^5 CFU/g	NG
C	6.0×10^5 CFU/g	4.6×10^5 CFU/g	NG	5.0×10^5 CFU/g	1.7×10^5 CFU/g	NG	3.3×10^5 CFU/g	NG

NG = No growth

THBC – Total heterotrophic bacteria count; TCC -Total coliform count; TFC - Fecal coliform
SSC – Salmonella and Shigella count**;** SC – Staphylococcus count**;** VC – Vibrio count**;** FC – Fungal count
CC – Clostridium count
CFU – Colony forming unit
Keys: A = 60:20:20; B = 50:40:10; C = 40:30:30.

Table 5.1: Biochemical Characterization and Identification of Bacterial Isolated from Fish Protein Pack Samples

Gram Reactions	Shape	Catalase	Coagulase	Motility	Starch hydrolysis	Citrate	Urease	MR	VP	Spore formation	H_2S	Oxidase	Indole	Glucose	Maltose	Xylose	Lactose	Fructose	Sucrose	Mannitol	Galactose	Probable organisms
+	Long rod	-	-	-	+	+	_	+	-	+	+	-	-	-	A	A	-	A	-	A	A	*Lactobacillus sp*
+	Rod	+	-	+	+	+	-	-	+	+	-	-	-	AG	A	A	-	A	-	-	A	*Bacillus sublilis*
+	Cocci in pairs	+	-	-	+	+	+	+	-	-	-	+	-	-	A	A	-	A	-	A	A	*Micrococcus sp*
+	Cocci in clusters	+	-	-	-	+	-	-	+	-	-	-	-	A	A	-	-	A	A	AG	A	*Staphylococus albus*
+	Cocci in clusters	+	+	-	-	+	-	-	+	-	-	-	-	A	A	-	-	AG	-	AG	A	*Staphylococcus aureus*
-	Short rod	+	-	+	+	+	-	+	-	-	+	-	-	A	A	A	AG	AG	AG	AG	A	*Citobacter sp*
+	Thick rod	+	-	+	+	+	+	-	+	+	-	-	-	A	A	A	-	A	A	-	A	*Bacillus sp*
-	medium rod	+	-	+	+	-	+	-	+	-	+	-	-	AG	AG	A	AG	AG	AG	AG	A	*Proteus sp*

Key: + = positive; - = negative; AG = Acid and Gas production; A = Acid production.

Table 5.2: Macroscopic and Microscopic Characteristics of Fungal Isolated from Fish Protein Pack Samples

Colony colour	Types of Soma	Nature of hyphae	Vegetation structure	Asexual spore	Special reproductive structure	Conical head	Vesicle shape	Probable organism
Brownish colony becoming darker with age	Filamentous	Septate	Footcell	Globose conidia	Short conidiophores	Long columnar	Hemispherical	*Aspergillus terrus*
Blue-green colony	Filamentous	Septate	Broom-like appearance	Subglobose conidia	Highly 3-stage branched conidiophores	-	-	*Penicillium sp*
Milky colony	Pseudo-hyphae	Septate	Anamorphs	Blastoconidia	Budding cells	Radiate	Dome shape	*Candida sp*
Smooky white colony	Filamentous	Septate	Footcell	Globose conidia	Short conidiophores	Long columnar	Hemispherical	*Aspergillus fumigatus*

Table 5.3: The Occurrence and Distribution of Diverse Species of Bacteria and Fungi Isolated from Fish Protein Pack Samples.

	Samples		
Organisms	**A**	**B**	**C**
BACTERIA			
Lactobacillus sp	+	-	+
Bacillus sublilis	+	+	+
Micrococcus sp	+	+	+
Staphylococus albus	+	+	+
Staphylococcus aureus	-	+	+
Citobacter sp	-	+	-
Bacillus sp	+	+	+
Proteus sp	+	-	-
FUNGI			
Aspergillus terrus	+	+	+
Penicillium sp	-	+	+
Candida sp	+	+	+
Aspergillus fumigatus	-	+	+

KEY: +ve = Organism present; -ve = Organism absent
A = 60:20:20; B = 50:40:10; C = 40:30:30.

Total Plate Count (TPC) of The Fish Protein pack

Quantitative microbiological analysis helps to assess the quality of dried fish (Iftekhar *et al.*, 2022). As shown in Table 5, the Total Heterotrophic Bacterial Count in the three samples varied between 8.2 x 10^5 CFU/g in sample A, 1.02 x 10^6 CFU/g in sample B and 6.0 x 10^5 CFU/g in sample C respectively. Sample B (.1 x 10^5 CFU/g) had the highest number of Total coliform count (TCC) followed by sample A (5.1 x 10^5 CFU/g) while sample C (4.6 x 10^5 CFU/g) had the lowest Total coliform count (TCC). They were no growth of Total Fecal coliform (TFC) detected in the three different samples. Salmonella and Shigella count (SSC) were found to be 5.0x 10^5 CFU/g in sample C and 1.3 x 10^5 CFU/g in sample B while there was no growth of Salmonella and Shigella count (SSC) in sample A. Sample B (3.8 x 10^5 CFU/g) had the highest number of Staphylococcus count (SC) followed by sample A (2.3 x 10^5 CFU/g) and sample C having the least number of Staphylococcus count (SC). Fungal count (FC) were found to be 3.3 x 10^5 CFU/g in sample C, 3.0 x 10^5 CFU/g in sample A and 2.0 x 10^5 CFU/g in sample B. There was no growth of Clostridium count in sample B and sample C while in sample A, Clostridium count was found to be 1 x 10^5 CFU/g. In their studies, Abbey *et al.* (Abbey *et al*, 2017) found that mechanical drying minimizes microbial attack, hence the protein pack should be safe. In addition, these fish items will be subjected to a heating or cooking process to eliminate the presence of microorganisms. The safe and acceptable TPC limit for fishery products is approximately 10^6 CFU/g (Jeyasanta *et al*., 2013).

Table 6: Fatty Acid Composition of the formulated fish protein pack

Free Fatty Acid	RT	Area (%)
Sample A		
Dodecanoic acid, ethenyl ester	5.169	1.91
Fumaric acid, trans-hex-3-enyl undecyl ester	7.764	6.13
Hex-3-enyl isobutyl carbonate	7.764	6.13
Fumaric acid, cis-3-enyl nonyl ester	7.764	6.13
D-Limonene	11.458	14.78
Oleic acid	23.988	1.52
Undec-10-ynoic acid, dodecyl ester	23.988	1.52
Hexadecanoic acid, methyl ester	32.440	0.36
6-otadecenioc acid, methyl ester	33.839	0.55
trans-13-octadecenoic acid, methyl ester	33.839	0.55
11-octadecenoic acid, methyl ester	33.839	0.55
Sample B		
9,12-Octadecadienoyl chloride, (Z, Z)-	33.647	3.99
Oleic Acid	33.647	3.99
Oleic Acid	33.757	2.93
9-Octadecenoic Acid	33.757	2.93
1-(hydroxymethyl) ethyl ester	33.757	2.93
Sample C		
Fumaric acid, trans-hex-3-enyl undecyl ester	7.795	2.15
Oxalic acid, isobutyl nonyl ester	8.000	0.46
Oxalic acid, isobutyl nonyl ester	8.814	0.65
D-Limonene	11.430	2.70
Limonene	11.430	2.70
Limonene	11.430	2.70
9-Eicosenoic acid	22.146	0.39
Hexadecanoic acid, Z-11-	23.487	0.50
Oleic Acid	31.322	6.32
Cis-9, 10 - Epoxyoctadecan -1- ol	32.163	0.05
Oleic Acid	36.708	19.61

Note: RT = Retention time
Sample A = 60:20:20; B = 50:40:10; C = 40:30:30.

Fatty Acid Composition

One of the most important health benefits of eating fish is its complex fatty acid profile (Okomoda *et al*., 2020). Saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) are defined. Fish oil contains long-chain fatty like saturated, monounsaturated, polyunsaturated fatty acids of carbon chains ranging from 14 to 22 carbon atoms (Shweta and Ravi, 2022). Table 6 present the free fatty acid composition of the three fish protein pack samples. The Fish Protein Pack samples had

differences in the composition of free fatty acids with sample C (19.61%) having the highest percentage of monounsaturated fatty acid (oleic acid and 9-Eicosenoic acid) followed by sample B (3.99%) and sample A (1.52%) with the lowest percentage of oleic acid (monounsaturated fatty acid). These results were similar to Choi and Lee (2015) which stated that the unsaturated fatty acids of fish oil were oleic. The ratio of fatty acids in fish oil depends on species and type of fish, geographical location of fish (Shweta and Ravi, 2022). Each of the protein pack sample was found to be composed of different free fatty acids. However, some fatty acids were not detected in some of the fish samples studied. Sample A showed dominant composition of free fatty acids followed by sample C.

Conclusion

The results of this study showed that an efficient amount of dry fish protein pack is achievable from Cat fish (*Clarias gariepinus)*, African Bony-Tongue fish (*Heterotis niloticus)* and Trunk fish *(Mormyrus rume)* which is rich in protein, potassium, calcium, and other valuable micro nutrients. The developed edible fish protein pack from three commercially important fish species in Akwa Ibom State, Nigeria – Cat fish (*Clarias gariepinus)*, African Bony-Tongue fish (*Heterotis niloticus)* and Trunk fish *(Mormyrus rume)* with formulated sample ratio of sample A (60:20:20), sample B (50:40:10) and sample C (40:30:30) had good nutritional characteristics indicating greater potential towards food and nutrition of the consumers. The study showered that sample C (fish protein pack from 40% *Clarias gariepinus*, 30% *Heterotis niloticus* and 30% *Mormyrus rume*) had the highest values of crude protein, ash, energy, zinc, potassium and Oleic acid, sample A (fish protein pack from 60% *Clarias gariepinus*, 20% *Heterotis niloticus* and 20% *Mormyrus rume*) had the highest values of moisture content, fibre, carbohydrate, calcium, magnesium, fumaric acid, trans-hex-3-enyl undecyl ester and D-Limonene while the highest values of 9-Octadecenoic acid and 1-(hydroxymethyl) ethyl ester was observed in sample C (fish protein pack from 50% *Clarias gariepinus*, 40% *Heterotis niloticus* and 10% *Mormyrus rume*).The developed fish protein pack will not only provide an avenue to increased utilization of fish but will also enhance the shelf life of the fishes making it available throughout the year for human consumption. Due to its high protein and mineral content, it can serve as a food vehicle for fortification and production of functional foods.

Recommendations

Based on the results of the study, it is recommended that:

1. A high-quality nutritious fish protein pack from formulated Cat fish (*Clarias gariepinus)*, African Bony-Tongue fish (*Heterotis niloticus)* and Trunk fish *(Mormyrus rume)* can be prepared and adopted as a food fortifier.
2. Experiments on the stability of the fish protein pack during storage should be carried out to determine the storage life of the product.

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