



RESEARCH PAPER

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Evaluation of the microbiological and nutritional quality of two types of fish flours from *Ethmalosa fimbriata* for their use as a dietary supplement

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Abstract

Post-harvest fish conservation remains a recurring fishing industry problem in Benin. *Ethmalosa fimbriata* is a non-oily fish commonly found on the Benin artisanal fisheries. Thus, this study aims to produce fish flour from *Ethmalosa fimbriata* and evaluate their physicochemical and microbiological characteristics and their potential nutritional qualities. The results indicated that fish flours produced are high in protein content ($53.84 \pm 0.09\%$) and minerals such as phosphorus (0.48 ± 0.03 to $1.83 \pm 0.01\%$), calcium (0.26 ± 0.04 to $3.02 \pm 0.08\%$), magnesium (0.09 ± 0.01 to $0.18 \pm 0.05\%$) and iron (194.86 ± 0.06 to 246.82 ± 0.05 mg/kg). The results of the assessment of the sanitary quality of fish flour produced showed that the use of these fish whole alter the microbiological quality of flour produced and it would be better to use headless fish in feed formulation especially for children and immunodeficiency people

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Introduction

According to the World Health Organization, malnutrition is the cellular imbalance between the supply of nutrients and energy and the body's demand for them to ensure growth, maintenance, and specific functions (Tierney *et al.*, 2010). The term protein-energy malnutrition (PEM) applied to a group of related disorders that include marasmus, kwashiorkor, and intermediate states of marasmus-kwashiorkor (Tierney *et al.*, 2010). The most common form of malnutrition in Africa is protein energy deficiency affecting over 100 million people, especially 30 to 50 million children under 5 years of age (Jildeh *et al.*, 2010).

Fish and fish products have an important place in the diet of the people of West Africa (FAO, 2000). They are rich in proteins and the nutritional value of these proteins is comparable to that of the egg, milk and meat (Heen and Kreuzer, 1962).

In Benin, fishing has an important place in the national socio-economic balance as it sustains some 500,000 people and accounts for 3% of GDP (Tossou, 2010). Fishes are processed into various products to increase the bioavailability of its proteins. Among these products, fish flours appear to have a high nutritional value (FAO, 2006). However, the conservation of fish is very difficult due to the lack of adequate conservation system and post-harvest losses are estimated at about 20% (Anihouvi *et al.*, 2005). Indeed, there are many problems related to the conservation of fish, such as microbiological deterioration and fat oxidation.

The most commonly used methods of preservation, are salting, drying, fish fermentation, fish smoking, refrigeration or freezing. Despite these methods, the full-time availability of this important resource of animal protein still persists and according to FAO (2000) the lack of protein in the diet of people is one of the most common nutritional deficiencies and affected many people in tropical countries. Traditionally, fish flours were produced to be used for food fortification, especially in animal protein

deficient diets. However, these fish flours were not storable for a long duration due to problems of lipid oxidation, because the fishes used were often fatty fishes. Thus, the present work aims to produce fish flours using *Bonga* fish (*Ethmalosa fimbriata*) (figure 1), and evaluate their physicochemical, microbiological and nutritional qualities.

Materials and methods

Collection of fishes and drying

Fishes (*Ethmalosa fimbriata*) are collected from fishermen of *Agbalilamè Tokpa* in southern Benin (figure 2). Collected fishes were cleaned by rinsing with water, followed by gutting and salting brine (20g/L). These fish so treated have dried. Two types of drying was made: a solar drying on a site of fish drying located in the town of Ouidah in southern Benin, and an artificial drying by using an oven. Solar drying conditions are those found on the site of drying: after salting, fishes were simply spread out in the sun on racks raised, allowing water to evaporate from the flesh of fish. In this study, fish spread in the sun are protected by filter cloths to prevent insect infestation that could constitute a source of contamination which can affect the quality of fish during drying. Periodical reversals are performed during drying to facilitate the process by exposing more of the surface of the fish in the air. After a period of approximately one (01) months of sun exposure, natural drying was replaced by artificial drying by baking with a thermostatic oven. The drying temperature is 50 °C. Monitor the water content of the fish placed in drying is carried out periodically until relatively constant water content (about 11%). After that, drying fishes were divided into two groups A and B. A first group (A) was carefully headed to serve for the production of fish flour labeled (F1), and fishes of second group (B) were directly used for the production of fish flour (F2).

Production of fish flour

After milling, fish flours were then baked into oven with a temperature of 90 °C for one (01) hours in order to further reduce the water content of the fishes. After this operation, the final water content of

fish flours is approximately 7%. Fish flours thus obtained is sieved and packed in sterile packaging. Figure 3 showed the different stages of the production.

Physicochemical and nutritional analysis

Moisture content of fish flour was determined by desiccation using the method of De Knecht and Brink (1998). A clean platinum dish was dried in an oven and cooled in a desiccator and weighed. From each sample, 5 g was weighed and spread on the dish, the dish containing the sample was weighed. It was then transferred into the air oven at 105°C to dry until a constant weight was obtained and the loss in mass was determined. Protein was analyzed by the Microkjedhal nitrogen method, using a conversion factor of 6.25 and fat content was obtained by Soxhlet extraction as described by Pearson (1976). Ash was determined according to the standard methods described by the Association of Official Analytical Chemists (AOAC, 1990). Minerals were analyzed by the method reported by Oshodi (1992). Minerals were analyzed by dry-ashing 1 g of the sample at 550°C in a furnace. The ash obtained was dissolved in 10% HCl, filtered with filter paper and made up to standard volume with deionised water. Minerals content were determined using atomic absorption spectrophotometer (Perkin Elmer, Model 403).

Microbiological analysis

To 25 g of each sample, 225 ml of peptone water was added and homogenized. From the initial concentration, appropriate decimal dilutions were prepared and aliquots were plated in duplicates on various media. Plate count agar was used for the total bacterial count. Plates were incubated at 30°C for 72 h. Desoxycholate was used for the total Coliforms count and plates were incubated at 30°C for 24 h. Desoxycholate was also used for the Faecal coliforms count. In this case, plates were incubated at 44°C and the identification was made using EMB (Eosine Methylene blue). Tryptone Sulfito Neomicin Agar was used for Anaerobic Sulfito-Reducer (ASR) count and tubes were incubated at 37°C for 24 h. After incubation, the number of colonies was tracked using

a colony counter. The number of bacteria expressed as Colony Forming Units per gram (CFU/g) was then determined by calculation, bearing in mind the factors of dilution (Singh *et al.*, 1991). The isolation of fungi from samples was performed using dilution plating method. 10 g of each sample were separately added to 90 ml of sterile water containing 0.1% peptone water. This was thoroughly mixed to obtain the 10⁻¹ dilution. Further 10-fold serial dilutions up to 10⁻⁴ were made. One millilitre of each dilution was separately placed in Petri dishes, over which 10 to 15 ml of Potato Dextrose Agar with 60 µg/ml of chloramphenicol (PDAC) was poured. The plates were incubated at 28 ± 2°C for 7 days (Rampersad *et al.*, 1999).

Statistical analyses

The data generated from these studies were analyzed using Statistical Analysis Software (SAS) and SYSTAT 5.05. The statistical analyses carried out were mean and standard deviation and analysis of variance (ANOVA) (Alder and Roessler, 1977; Ogbeibu, 2005).

Results

Physicochemical and nutritional quality

Tables 1 and 2 showed the physicochemical, nutritional and microbiological quality of different fish flours produced. The results of physicochemical characterization of two samples of fish flour (Table 1) showed that the moisture content of different samples ranged from 7.00±0.51% to 7.33±0.84%. Fish flours are rich in nutrients such as proteins (53.78±0.04-53.84±0.09%) and ash (12.26±0.18-13.24±0.09%). All samples analyzed were rich in minerals such as calcium, magnesium, potassium and iron, with a higher content of calcium (0.26±0.04 -3.02±0.08 %). Fat content was very low. However, by comparing the values obtained in the two types of fish flours, there is a significant difference in parameters such as the levels of iron, calcium, magnesium and phosphorus (p <5%). The highest levels were recorded in the flour obtained from non headless fishes.

Microbiological quality

The evaluation of the microbiological quality of different fish flours produced (Table 2) indicated that the microbial flora of the flours obtained with dried headed fish is very low, with a total flora count of 5.10^4 ufc /g. The enumeration of total coliforms and faecal coliforms was less than 10 cfu / g with an absence of spores of anaerobic sulphite reducers (ASR). Fungal flora was 02 ufc/g. However,

microbiological results from the flours obtained with non-headless fish, indicated the high microbial count, characterized by a total flora of 8.10^6 cfu/g. The enumeration of total coliforms and fecal coliforms was high than 10 cfu/g with the presence of spores of anaerobic sulphite reducers (ASR). Fungal flora was also high (5.10^2 ufc/ g).

Table 1. Physico-chemical and nutritional quality of fish flours produced.

Parameters	Moisture (%)	Protein (%)	Ash (%)	Iron (mg/kg)	calcium (%)	magnesium (%)	phosphorus (%)
Fish flour (F1)	$7,00 \pm 0,51^a$	$53,78 \pm 0,04^a$	$12,26 \pm 0,18^a$	$194,86 \pm 0,06^a$	$0,26 \pm 0,04^a$	$0,09 \pm 0,02^a$	$0,48 \pm 0,03^a$
Fish flour (F2)	$7,33 \pm 0,84^a$	$53,84 \pm 0,09^a$	$13,24 \pm 0,09^a$	$246,82 \pm 0,05^b$	$3,02 \pm 0,08^b$	$0,18 \pm 0,05^b$	$1,83 \pm 0,01^b$

Values are mean (n=3) \pm SE. The means followed by same letter in the same column are not significantly different according to ANOVA and Tukey's multiple comparison tests.

Table 2. Microbiological quality of fish flours produced (ufc/g).

	Total bacterial Count	Total coliforms count	Faecal coliforms count	<i>E.coli</i>	<i>Staphylococcus aureus</i>	A.S.R count	spores	Mould and yeast count
Fish flour (F1)	5.10^4	<10	<10	00	00	00	02	
Fish flour (F2)	8.10^6	1.10^3	4.10^2	6.10^1	00	08	5.10^2	
European Union - criteria (2005)	-	10	10	10	-	Absence/10 g	Absence/10 g	

A.S.R: Anaerobic Sulfito-Reducer.

Discussion

The nutritional potential of fish flour (proteins and minerals content) indicated that the use of small dried fishes as ingredient for making infant flour is an interesting alternative that should help to diversify infant complementary feeding. They are animal proteins of good nutritional quality and digestibility. Mothers who fed their child with infant flour enriched with small dried fishes should be encouraged (Dossa *et al.*, 2011). Indeed, infants and young children are often regarded as two particularly vulnerable groups in terms of food safety. Furthermore, the requirements for essential nutrients due to rapid growth and development put these groups at risk of deficiencies of essential minerals. It is therefore essential that products intended for use by infants and young children contain minerals in amounts that

satisfy their nutritional requirements without leading to adverse effects. In addition, products must not contain contaminants in amounts that could lead to negative health effects. Then, great attention should be paid to the quality of raw food ingredients used for the formulation of infant flour as done in this study. This has allowed to remarks, by observing the results of physico-chemical, microbiological and nutritional analysis, that despite the high levels of minerals in flour obtained with non-headless fish (F2), there are potential microbiological risks associated with their incorporation into food for children. The presence in high proportion of microorganisms such as *E.coli*, Anaerobic Sulfite Reducer, mould and yeast in fish flour (F2) compared to those obtained with headless fish flour (F1) confirmed that the important source of contamination is localized in the head of the fish. This

could be explained by the fact that the gills of fish are important organs in the phenomena of respiratory gas exchange are also richly vascularized and therefore constituted a center of concentration of microorganisms (Degnon *et al.*, 2013). It would be better to use the headless fish in feed formulation for children as done in this work.



Fig. 1. Bonga fish (*Ethmalosa fimbriata*).

It has been reported that proteins content for infant flours produced in must fluctuate from 8.2% to 21.3% (Trèche, 1995). Then, based on the protein and minerals contents of this fish flours, their used as supplement in infant feeding could contribute to reduce many nutritional problems occurs in developing country. Indeed, minerals are needed to form body structures and regulate chemical reactions. Like vitamins, minerals are needed in small amounts and do not provide energy. Also much like vitamins, minerals are required to regulate many body processes, such as heart beat, nerve response and reactions, blood clotting, fluid regulation and energy metabolism (release of energy from food). Minerals form part of the structure of bones, teeth, nails, muscles and red blood cells. The body does not function properly unless all are supplied in sufficient quantities.

Calcium is essential for healthy bone growth and for nerve and muscle functions; it may protect against high blood pressure. Calcium cannot be made in the body so it is essential that babies receive the calcium they need from their diet. In their first year, babies double the mass of their skeleton (Duggan *et al.*, 2008). The structure of the body depends on calcium. About 99% of the body's entire supply is deposited as

calcium salts in the bones and teeth (Thomas and Bishop, 2007). During these periods of rapid growth, bone mass will depend on appropriate amounts of calcium being available from dietary sources (Matkovic, 1991) and has implications for bone health in later life. It is also found in body fluids and tissues where it is important for cell membrane transport and stability (Thomas and Bishop, 2007), and is needed for coagulation (Lovinger, 1980); low plasma levels being associated with a reduced ability to form blood clots (Geissler and Powers, 2005). Calcium is also involved in digestion and muscle contraction. In childhood and adolescence, it is particularly important to eat and drink calcium rich foods to ensure maximum calcium storage and strong bones. Iron has several roles. It is a component of both hemoglobin in blood and myoglobin in muscle as well as being important for the body's metabolic processes (Thomas and Bishop, 2007). It is also important in brain function and development (Beard, 2008). At birth the brain is only about a quarter of its eventual size (Dobbing and Sands, 1979) but grows rapidly up to the age of two. Insufficient iron in the diet during the first two years of life has been linked to cognitive and behavioral problems later in life (Walter, 2003; Hulthen, 2003).

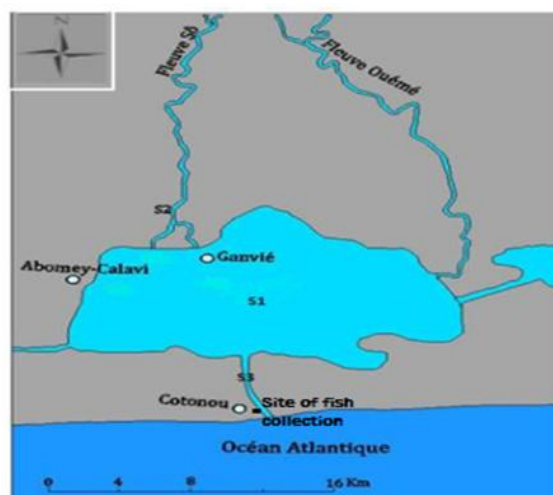


Fig. 2. A map showing the site of fish's collection.

Appropriate levels of phosphorus in an infant's diet are also important to achieve optimum bone mineralization. 85% of the body's phosphorus is found in the bones (Thomas and Bishop, 2007). The remainder is within DNA and RNA and in

phospholipid membranes. Phosphorus is associated with the release of energy and oxygen to cells associated with energy metabolism (Geissler and Powers, 2005; Lee, 2009). Based on the nutritional potential of this fish flours produced, it should be also recommended as food supplement in infant feeding.

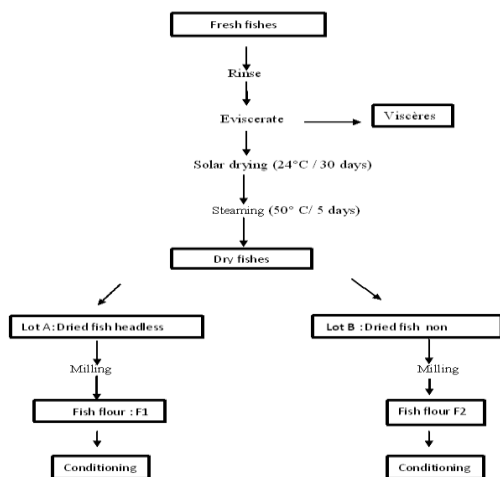


Fig. 3. Technological Diagram of fish flours production.

Conclusion

This survey underlined the nutritional potentiality of fish flours produced using *Ethmalosa fimbriata* and recommended its use as a dietary supplement. However, it would be better to use the headless fish in feed formulation for children in order to preserve children health.

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