Nutritional Assessment and Microbial Safety of Croaker (*Micropogonias Undulatus*) Fish from Three Frozen Food Centers in Afikpo, Ebonyi State Nigeria

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Abstract— *Microbial activity on food leads to its spoilage. This is usually by enzymatic processes that bring about lost of nutrients experienced on decaying food. Food decay, affect virtually all classes of nutrients in food especially, the organic aspect with proteins and lipids being the most. This study aimed at determinig the nutritional assessment and microbial safety of croaker fish from different frozen food centers. The nutritional assessment was determined through proximate analysis by standard method of AOAC and microbial safety by aerobic count, proteolytic and lipolytic count method using approprioate media. The results for proximate analysis showed moisture (62.38, 62.58 & 64.03), protein (23. 09, 23.40 & 22.98), lipid (10.21, 10.31 & 10.25), ash (1.11, 1.12 & 1.13) and carbohydrate (2.51, 2.65 & 1.61) for center A, B & C respectively. The mean values for aerobic plate count, proteolytic, and lipolytic counts were 7.30+0.18, 4.00+0.03, and 2.00+0.06, respectively, for center A, 6.31±0.29, 3.85±0.07, 2.61±0.01 for center B, and 7.70±0.82, 4.20±0.29, 2.82±0.13 for center C all in ×10³ CFU/ml. The presence of salmonella sp and straphylococcus were confirmed. These finding however, suggest contamination with aerobes with both proteolytic and lipolytic activities and particularly with pathogenic salmonella and straphylocaccus which could endanger the health of people upon their consumption.*

Keywords— frozen food, lipolytic, microbes, proteolytic, safety, spoilage.

I. INTRODUCTION

Fish has been considered an indisputable source of animal protein available in the tropics and is widely accepted as a good quality source of protein and other nutrients for the maintenance of good health (Andrew, 2001). The l developing countries capture 50% of the world's harvest, with a large proportion of the catch consumed internally. In many Asian countries, over 50% of animal protein intake comes from fish. Methods of preservation and storage are the major factors affecting the rate of loss of quality and shelf life of fish. However, its effects bordered on the nutrient quality and safeness upon consumption (Ewo 2019).

World consumption of fish per capita increased in developing regions from 5.2kg in 1961 to 18.8kg in 2013, while in the least developed countries with deficits increased from 3.5kg to 7.6kg in the same period. Hence, fish account for about 17% of the intake of animal protein by the world population (FAO, 2016). Fish is known for its high nutritional value, and the chemical composition of fish regarding other food is unique (Simopolous, 2002). Fish tissue is the main source of long-chain polyunsaturated fatty acids, especially omega-3 and omega-6. These fatty acids have particular importance in fish since their

consumption contributes to the reduction of the appearance of cardiovascular diseases (Turkmen, Aro, Nurimi, and Kailio, 2005) and the improvement of learning ability. Fish also contains a high-quality protein with all essential amino acids, being a source of dietary minerals such as calcium, iodine, or selenium and providing an important amount of polyunsaturated fatty acid (Araujo, Soares, and Gois, 2010). Pelagic species, usually smaller ones such as sardines, are generally rich in omega-3 fatty acids, mainly eicosapentaenoic acid and docosahexaenoic acid (Pestana, 2007).

Fish is a very perishable food, being highly susceptible to oxidation and microbiological deterioration. Therefore, efficient storage strategies need to be employed in order to increase its shelf-life and guarantee its safety and quality from catch to consumption. This shelf life of fish is dependent on several factors, such as storage time, temperature, fish species, the stress suffered during the catch, and the amount of ice (Mahmud, Abraha, Samuel, Mohammedidris, and Abraham, 2018). Therefore, these preservation methods need to be optimized to increase fish shelf life to guarantee its quality and safety, with consequent satisfaction of consumer requirements, reduction of economic losses from fishing industries, and food waste. This optimization can involve the effect of freeze/chill temperature and time, thawing, fish preparation, and bleeding condition (Rong, Ruchuan, Huihui, and Qi, 2020; Nguyen and Phan, 2018)

Fish contaminants are of great concern for export earnings because of their high nutritive value, such as high protein content with little or no carbohydrate and fat value. However, fish may be contaminated at various stages of transport, handling, and processing. This contamination may be related to the raw materials, personnel, and processing tools, such as forklifts, through leakage, insects, and pest harbourage. Additionally, seafood can become contaminated during storage and processing. Contamination may be caused by foodborne pathogens that are naturally present in aquatic environments, such as vibrio spp, or derived from sewage-contaminated water, such as salmonella app (Gnanambal and Patterson, 2005). Consumption of these contaminated fish may cause infection or intoxication to the consumer.

The contaminant of fish is one of the leading causes of foodborne diseases or gastroenteritis, characterized by diarrhea, abdominal cramps, vomiting, nausea, and fever. According to the Centers for Disease Control and Prevention, salmonella is the leading cause of bacterial foodborne illness, causing approximately 1.4 million non-typhoidal illnesses, 15000 hospitalizations, and 400 deaths in the USA annually (Center for Disease Control and Prevention (CDC), 2011).

Water and ice quality is also an important factor for quality fish because water and ice used for fish processing may contaminate the whole processing plant. So, it is important to find out the quality of fish we consume as well as the frozen fish that are exported (Andrew and Hammack, 2001).

This study, therefore, aimed to assess the nutritional value and microbial safety of frozen croaker fish from three different frozen centers in Afikpo Ebonyi State, Nigeria, in order to assess their microbial wholesomeness. The specific objective of the study includes the determination of the nutritional value and microbial safety and comparing the various outcomes of frozen croaker fish obtained from three different frozen centers.

II. MATERIALS AND METHODS

2.1 Sample:

Frozen croaker fish (*Micropogonias undulatus*)

2.1.1 Sampling and Sample Preparation:

A freshly frozen croaker fish of about 1kg size was randomly sourced from three different frozen centers. The sample was put in sterile plastic bags and immersed immediately in an ice-containing flask. The samples were divided into two portions, one for chemical (proximate) evaluation and the other for microbiological analysis.

2.2 Nutritional Analysis:

The moisture content estimation was done following the method AOAC (AOAC, 1995). The crude protein content of the samples was determined by estimating total nitrogen by the Kjeldhal method AOAC (AOAC, 1996). The crude fat of the sample was determined by the Soxhlet extraction method AOAC (AOAC, 1996). The Ash content of the samples was determined by the method described in AOAC (AOAC, 1996). **Carbohydrate Content;** this was done by calculating the percent remaining after all the other components had been measured. Carbohydrate (%) = 100 - (%moisture + %protein + %lipid + % ash).

2.3 Microbiological Analysis:

2.3.1 Sample Preparation.

Ten grams of the sample was aseptically cut and transferred into sterile polyethylene and blended with 90ml of sterile normal saline, then 1ml of homogenate was aseptically transferred to 9ml of sterile normal saline in a test tube. Further decimal serial dilution is required before inoculation.

A. Enumeration of Aerobic Plate Count.

The plating was done by adding a loopful from each dilution on plate count algae medium using the pour plate method. The colonies formed after incubation at 35°C for two days under aerobic conditions were counted.

B. For *Staphylococcus* species.

It was cultured in mannitol salt algae, and purified colonies were confirmed using a coagulase test.

C. For *Salmonella* species.

It was cultured using a tetrathionate broth supplement with iodine.

2.3.2 Determination of Total Proteolytic Count:

It was done as recommended by APHA (APHA, 1996) as follows: 1ml of previous serial dilution was inoculated in a skim milk algae medium aseptically and incubated at 37°C for 48 hours and examined for a clear zone around the growth.

2.3.3 Determination of Total Lipolytic Count:

One ml of each dilution was mixed with tributyrin nutrient media and incubated at 37°C for 48 hours; lipolytic activity was determined by measuring the clear zone.

2.4 Statistical Analysis:

The data from different center samples were presented as mean and standard deviation. Analysis of variance (one-way ANOVA) was performed in order to compare the differences in croaker fish obtained from different frozen centers. The significance of the difference was defined at $p<0.05$.

III. RESULTS

Food safety is of principal importance to the meat industry. Chemical and microbial contamination of fish meat is a critical global problem (Farag, 2002).

TABLE 1
A TABLE SHOWING THE CHEMICAL COMPOSITION OF ESTIMATES FOR FROZEN CROAKER FISH IN THREE DIFFERENT CENTERS WITHIN AFIKPO

Parameters	Center A (%)	Center B (%)	Center C (%)
Moisture	63.38	62.52	64.03
Protein	23.09	23.4	22.98
Lipids	10.21	10.31	10.25
Ash	1.11	1.12	1.13
Carbohydrate	2.51	2.65	1.61

The moisture, protein, lipid, ash, and carbohydrates content for frozen croaker fish obtained from center A were 63.38, 23.09, 10.21, 1.11, and 2.51, respectively, 62.52, 23.40, 10.31, 1.12, and 2.65, respectively for center B, and 64.03, 22.98, 10.25, 1.13 and 1.61 respectively for center C.

TABLE 2
SUMMARY OF MICROBIAL SAFETY SHOWING AEROBIC PLATE COUNT (APC), PROTEOLYTIC COUNT, AND LIPOLYTIC COUNT OF FROZEN CROAKER FISH OBTAINED FROM THREE DIFFERENT FROZEN CENTERS WITHIN AFIKPO AND ITS ENVIRONS

Parameters	Center A (%) (×10³ CFU/ml)	Center B (%) (×10³ CFU/ml)	Center C (%) (×10³ CFU/ml)
Aerobic plate count	7.30±0.18	6.31±0.29	7.70±0.82
Proteolytic count	4.00±0.03	3.85±0.07	4.20±0.19
Lipolytic count	2.00±0.06	2.61±0.01	2.85±0.13

No significant difference ($p<0.05$) among the centers under study was revealed by the student t-test.

Microbiological quality: Aerobic plate count is a commonly recommended microbiological method for estimating the shelf-life of fish meat and others. The bacteriological content of frozen croaker fish obtained from different frozen centers is revealed in Table 2. The mean values for aerobic plate count, proteolytic, and lipolytic counts were 7.30+0.18, 4.00+0.03, and 2.00+0.06, respectively, for center A, 6.31±0.29, 3.85±0.07, 2.61±0.01 for center B, and 7.70±0.82, 4.20±0.29, 2.82±0.13 for center C. All in ×10³ CFU/ml. No significant difference ($p<0.05$) among the centers under study using the student t-test.

TABLE 3
SUMMARY OF MICROBES PRESENT IN THE ANALYZED SAMPLES FROM DIFFERENT FROZEN CENTERS.

Parameters	Present
Staphylococcus	+
Salmonella	-

Staphylococcus and *Salmonella* were present in all samples obtained from the three different frozen centers under study.

IV. DISCUSSION

Frozen fish are popularly consumed processed food in many countries of the world. They are generally produced by refrigeration methods, which play important roles in the physiochemical and sensory properties of fish products (Rasul et al., 2018). The moisture, protein, lipids, ash, and Carbohydrate values reveal the nutritional quality of every food. It also gives the impression of shelf life and safety. The moisture content of frozen foods is always high, which is consistent with the findings of frozen croaker fish obtained from three different frozen centers within Afikpo and its environs. This is a further indication that outside refrigeration temperature, spoilage, and safety of frozen fish are not guaranteed. The protein content was found to be high in the three centers, suggesting croaker fish is a good source of animal protein, which is substantial enough to supply the protein needs of the body. The lipid composition is also significant, with important essential fatty acids such as omega-3 and others needed for normal physiological conditions. It is also rich in retenol and vitamine E. These are also important for good health. The ash content also points to the mineral composition of croaker fish. As seafood, it has been revealed to be a source of selenium and iodine.

The findings on nutritional assessment are nearly similar to those obtained by (Steffens, 2006, Tawfik, 2009, Nisa and Asadullah, 2011, Nail and Raju, 2015) for some tested parameters. Similar studies with different results were reported by Topper, Albrektsen, Hope, and Aksnes (2007) and Ondo-Azi, Kumulungui, Meworo, Mbina-Kounmba and Ella-Missang (2013). However, some parameters were higher in some centers, which may be attributed to the quality of feed the fish were fed with and the processing procedure used in handling the from catch to refrigeration.

The proteolytic and lipolytic microorganisms grow well in fish meat, leading to loss of fish meat quality and reduction in its shelf-life due to protein and lipid hydrolysis, which may lead to deterioration in the color, flavor, and texture of displayed fish meat. The presence of *salmonella* and *staphylococcus* spp indicates food poisoning and potent health hazards(2017). In conclusion, the croaker fish from the three centers are contaminated but not above the recommended limits, with moderately

low lipolytic and proteolytic activities. However, worrisomely is the presence of pathogenic microbes with potent health hazards.

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