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Nutritional Composition and Microbiological Assessment of Three Fish Species Traded in Ile-Ife and Ejigbo, Osun State.

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Abstract - Three different species of fish were obtained from Ile Ife and Ejigbo market to determine their nutritional composition and their level of fungal contamination. These were determined by using the AOAC (2005) method and basic mycological procedures. The species of fish used for the study were namely crayfish, croaker and spiny-eel while the fungal species identified were Aspergillus, Rhizopus, penicillium, mucor and Absidia. Sample from Ife market have higher number of the fungal species than Ogiyan market (Ejigbo) with Absidia spp scarcely found in both sample. Altogether Crayfish sample from the two markets have the highest load of the fungal contamination (50%), followed by Croaker (27.8%) while the least was in Spiny-eel (22.2%). Though all the fish samples were of high protein content with appreciable amount of carbohydrate, energy value, calcium and moisture content. The high level of fungal contaminant discovered revealed that the sellers/processors should be encouraged to practice a good hygiene particularly during handling, processing and storage. Also more awareness on the health implication of fungal agents and mycotoxins- associated with contaminated fish product should be created among fish handlers and consumers.

Keywords: Fish, Fungal contamination, Markets, Nutritional composition, Proximate.

1. Introduction

Consumption of smoked and smoke-dried fish without further cooking is common in Nigeria. It has been reported that smoke-dried fish are often contaminated with microorganisms such as bacteria, yeasts and moulds from the processing units, handling, up to market centres. According to research, it was noticed that good storage practices are not observed by most wholesalers of smoked and smoke-dried fishes such as improper ventilation and easy access of pest into the storage environment. Most common post processing microbial contaminations comes from poor handling practices, while some could be by air, the fish sources, or from other degrading substances (Job *et al.*, 2016).

Some microorganism such as mycotoxins are not destroyed during food processing or cooking and several cases of human gastroenteritis, severe diarrhoea and food poisoning outbreaks have been recorded after smoked fish consumption infected with mycotoxins (Ayisi *et al.* 2017). Microbial action plays a significant part in the spoilage of fish and other sea foods. Fungi spoilage of fish is observed and notice by softening of the muscle tissue, production of slime and sometimes offensive odours (Sengor *et al.*, 2004).In humid tropical conditions, very dry smoked fish with low moisture contents are prone to insect infestation, while others having medium to high moisture contents are predisposed to both bacterial and fungal contaminations (Banwart, 2004).

Fish is known to be one of the cheapest sources of animal protein and other essential nutrient required in human diets (Fawole *et al.*, 2007). Fishes are highly nutritious and an excellent means of obtaining dietary essentials, like protein, minerals and vitamins. Fish fat contains a high proportion of polyunsaturated fatty acids, which may help to reduces the incidence of atherosclerosis and heart related diseases. Fish reduces vulnerability to hunger by providing a complementary food sources as part of diversified livelihood strategies (Abisoye *et al*, 2011).Eating of fish help in reducing the risk of heart diseases and lower the risk of developing dementia and Alzheimer's diseases (Jolaoso *et al.*, 2016).

The study of mineral elements present in living organisms is of biological importance; since many of such elements take part in some metabolic processes and are known to be dispensable to all living things (Abolude

and Abdullahi, 2005). The body usually contains small amount of these minerals, some of which are essential nutrients, been components of many enzymes system and metabolic mechanisms, and as such contribute to the growth of the fish. (Mumba and Jose, 2005).

Therefore the aim of this work is to determine the proximate, mineral element and microbiological analysis (fungi load) of three fish species, crayfish (*procambarus clarkii*), croaker fish (*pseudotolithus elongates*) and spiny-eel fish (*aethiomastecembelus cryptacanthus)* sold to people in Oja Tuntun market (Ile-Ife) and Ogiyan market (Ejigbo), Osun State, Nigeria.

2. Materials and Methods

2.1 Sample Collection, preparation, and Analysis

The commercially valued indigenous fish species were selected for the study. The traditional smoked fish samples used for these studies were randomly purchased from two local government Area of Osun State; OjaTuntun Ile-Ife and Ogiyan market in Ejigbo and was aseptically handled throughout the research process. The samples were aseptically packed into sterile zip locked polythene bags immediately after purchase, placed in labelled dry separate cooler boxes and transported to BOWEN University for mineral and proximate analysis and also Microbial Analysis was carried out Microbiology & Biochemistry Laboratory unit Federal Polytechnic Ede Osun State.

2.2 Nutritional and Proximate Analysis of the Sample

Proximate analysis of the fish samples was carried out in triplicates using AOAC 2000 method. While the mineral content was determined using Atomic Absorption Spectrophotometer (AAS PG990 MODEL).

2.3 Culture Media Preparation

Potato dextrose agar (PDA) was used for the fungi isolation. It was prepared according to the manufacturer's instruction i.e. 37 g of PDA to be dispensed in 1 litre of distilled water, therefore 18g will be dissolved in 500ml of distilled water in separate conical flask. The flask was corked with cotton wool, wrapped with Aluminium foil and then masked with paper tape and was then placed in an autoclave for sterilization at 121 °C for 15 minutes after which it was allowed to cool. The media was shaken vigorously before use **(**Barnet and Hunter 2010; Cheesberough, 2000).

2.4 Sample Preparation

A whole fish sample was used, the samples from each market were weighed and macerated with mortar and pestle and thoroughly mixed. For each fish sample, five test tubes were used for serial dilution. Ten gram of the sample was weighed aseptically and macerated with mortar and pestle and mixed thoroughly in 90 ml distilled water in a beaker. 10ml was taken from the stock solution into a conical flask. The test tubes were labelled and arranged inside a test tube rack and was filled with 9ml of distilled water each. From the 10ml solution, 1ml of the solution was withdrawn using needle and syringe into the labelled test-tubes for the first dilution. 1ml was transferred from the first dilution i.e 10^{-1} to the second labelled test-tube to make 10^{-2}. The procedure was repeated for each samples.

One millilitre (1 ml) of each dilution was dispensed in sterile Petri dishes using pour plate method and were plated in duplicate on the sterile petri-dishes. Approximately 16ml of cooled PDA fortified with streptomycin to inhibit bacteria growth was then poured into sterile petri-dishes. A slight agitation was done to allow for the mixing of the inoculums with the culture medium (PDA), thereafter incubated at room temperature (28^{o}C) and examined daily for3 to 5 days and all colonies were counted and the data was reported as Colony Forming Units CFU g^{-1}.Fungi count were done after 72 hours. The microbial isolates were observed for their cultural and morphological characteristics.

3. Statistical Analysis

Data and information collected from the study were used to analyze using SPSS version 160. All data were expressed as mean± standard deviation and probability tested at 95% level of significant ($p<0.05$).

4. Result

Table 1: Result of Mineral Element Analysis of three fish sample selected from (Ejigbo)

Fishes	Phosphorus (mg/100g)	Potassium (mg/100g)	Sodium (mg/100g)	Calcium (mg/100g)	Copper (µg/100g)	Zinc (µg/100g)
Crayfish	0.031± 0.084^{a}	0.062 ± 0.027^{a}	0.130 ± 0.110^{b}	3.403 ± 0.402^{b}	0.095 ± 0.051^{b}	0.08 ± 0.001^{a}
Spiny-eel fish	0.606 ± 0.416^{b}	0.057 ± 0.006^{a}	0.062 ± 0.024^{a}	3.591 ± 0.53^{bc}	0.057 ± 0.006^{a}	0.091 ± 0.022^{ab}
Croaker fish	0.049 ± 0.002^{ab}	0.56 ± 0.451^{b}	0.545 ± 0.471^{c}	1.531 ± 0.514^{a}	0.525 ± 0.484^{c}	0.552 ± 0.410^{b}

Values are expressed as mean ± S.D (n=3). Means with different superscripts differ significantly ($p<0.05$)

Table 2: Result of Mineral Analysis of three fish sample selected from New market

Fish	Phosphorus (mg/100g)	Potassium (mg/100g)	Sodium (mg/100g)	Calcium (mg/100g)	Copper (µg/100g)	Zinc (µg/100g)
Crayfish	0.078± 0.026^{b}	0.09 ± 0.020^{a}	0.065 ± 0.006^{a}	0.362 ± 0.251^{b}	0.086±0.005^{b}	0.089 ± 0.008^{a}
Spiny-eel fish	0.283 ± 0.222^{a}	0.606± 0.495^{b}	0.078±0.026^{ab}	0.558 ± 0.484^{c}	0.600±0.472^{c}	0.099 ± 0.000^{a}
Croaker fish	0.195 ± 0.009^{a}	0.085 ± 0.015^{a}	0.063 ± 0.009^{a}	0.069 ± 0.033^{a}	0.054 ± 0.015^{a}	0.059 ±0.030^{b}

Values are expressed as mean ± S.D (n=3). Means with different superscripts differ significantly ($p<0.05$)

Table 3: Result For Proximate Analysis for three fishes selected from Ejigbo LGA

Fishes	Moisture %	Crude fat %	Crude fibre%	Ash%	Protein %	% CHO	Energy value (kcal)
Crayfish	22.64 ±0.07^{b}	0.77 ±0.11^{c}	0.22 ±0.01^{b}	1.775 ± 0.06^{a}	29.45± 0.02^{b}	65.22^{c}	385.61^{c}
Spiny-eel fish	24.62 ±0.01^{b}	0.545 ± 0.02^{b}	0.13 ± 0.00^{a}	2.125 ±0.03^{b}	31.94±0.02 ^{c}	37.62^{a}	283.15^{a}
Croaker fish	18.53 ± 0.03^{a}	0.24 ± 0.09^{a}	0.29 ± 0.01^{b}	1.77 ± 0.17^{a}	26.50± 0.01^{a}	52.58^{b}	318.84^{b}

Value are expressed as mean ± S.D (n=3). Means with different superscripts differ significantly ($p< 0.05$)

Table 4: Result For Proximate Analysis for three selected fishes from Ife LGA

Fishes	Moisture %	Crude fat %	Crude fibre%	Ash %	Protein %	% CHO	Energy value (kcal)
Crayfish	22.30 ±0.08^{c}	0.38±0.05 ^{bc}	0.80± 0.02^{b}	1.75±0.01^{b}	26.51±0.06 ^{ab}	48.27^{ab}	302.50^{a}
Spiny-eel	17.84 ± 0.63^{a}	0.36± 0.03^{b}	0.99 ± 0.01^{c}	1.92 ± 0.02^{c}	25.46± 0.05^{a}	53.43^{b}	472.88^{c}
Croaker fish	18.9 ±0.05^{b}	0.27 ±0.01^{a}	0.63 ± 0.01^{a}	1.59 ± 0.05^{a}	30.64±0.01 ^{b}	47.97^{a}	316.87^{b}

Values are expressed as mean ± S.D (n=3). Means with different superscripts differ significantly ($p<0.05$).

4.1 Microbiological Examination of the Fish Samples

Table 5: Isolated fungus from samples

Samples	Rhizopus spp	Aspergillus spp	Penicillum spp	Mucor spp	Absidia spp
Cray fish Ife	+	+	+	+	+
Croaker fish (Ife)	+	+	+	+	-
Spinyl-eel fish (Ife)	+	-	-	+	-
Crayfish (Ejigbo)	+	+	+	+	-
Croaker fish (Ejigbo)	-	-	-	+	-
Spinyl-eel fish (Ejigbo)	+	-	-	+	-

Where + means present, where – means not present.

Table 6: Comparative distribution of fungal isolates from the two sources (Market)

Fungal isolates	Oja tuntun mkt,Ile-ife (%)	Ogiyan mkt, Ejigbo (%)	Total (%)
Aspergillus sp	2 (18.2)	1 (14.3)	3 (16.7)
Rhizopus sp	3 (27.3)	2 (28.6)	5 (27.8)
Penicillium sp	2 (18.2)	1 (14.3)	3 (16.7)
Mucor sp	3 (27.3)	3 (42.9)	6 (33.3)
Absidia sp	1 (9.1)	0	1 (5.6)
Total	11 (100.1)	7 (100.1)	18 (100.1)

Fig 1: Pie chart showing Fungi load contamination in the three fish species

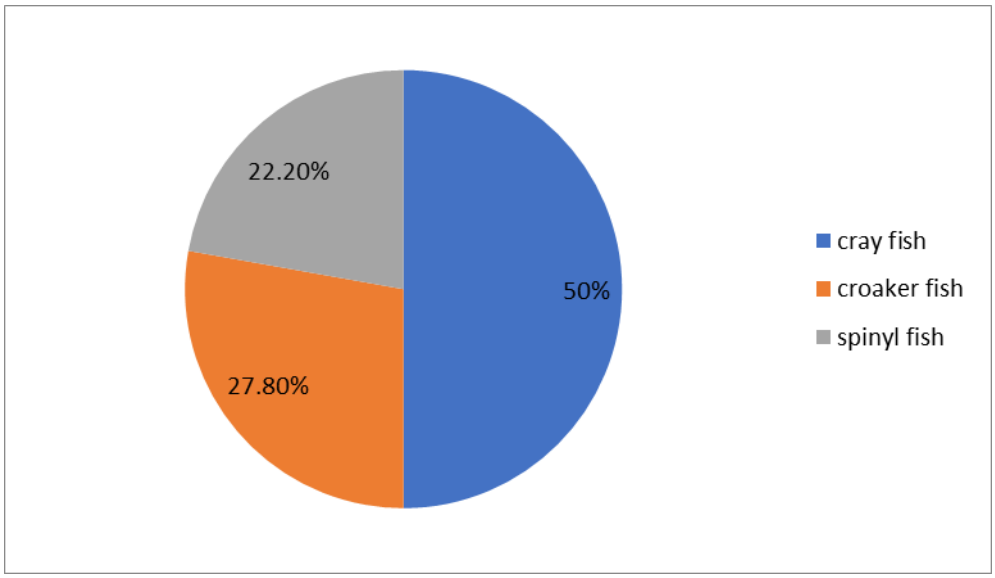

Table 7a: Fungi colony count in samples from Ile-Ife market in cfu/g

S/N	Sample	Total colony count (Cfu/g X 10^5)
1.	Crayfish	2.05 ± 0.25^a
2.	Croakerfish	1.9 ± 0.15^b
3.	Spinyl-eel fish	1.8 ± 0.25^c

Table 7b: Fungi colony count in samples from Ogiyan market in cfu/g

S/N	Sample	Total colony count (Cfu/g X 10^5)
1.	Cray fish	1.9 ± 0.25^a
2.	Croaker fish	1.75 ± 0.29^c
3.	Spinyl-eel fish	1.85 ± 0.27^b

5. Discussion

The concentration of mineral elements in three dry fish in Ogiyan market in Ejigbo and oja-tuntun market in Ile-Ife are presented in **Tables 1 and table 2**. Results show that the three fishes are rich in calcium, potassium, copper, zinc, sodium and phosphorous. Cray fish and Spiny-eel fish from Ogiyan market in Ejigbo recorded high amount of calcium 3.403 ± 0.402 and 3.591±0.53 respectively. Which implies that it will help in teeth and bone formation and in prevention against osteomalacia and osteoporosis, This result is in accordance with Njinkoue et al (2016), who got calcium of spiny-eel fish to be 0.41±0.25.

Table 3 and Table 4: Presents the proximate compositions of three dry fishes (Spiny-eel, Croaker fish and crayfish) in Ejigbo and ile-ife respectively. Proximate composions carried out are protein, lipid, fat, fiber content, carbohydrate, moisture and ash content. Every consumer wants to obtain a good quality nutrient especially protein from fish. From this study, it has been observed that protein is more concentrated in spiny-eel, followed by croaker fish with the value of 31.94% and 30.64% respectively. The result is high than the one gotten by Nahid and fayza, (2009) got protein to be (13.88±1.477) and (Alainserges *et al*., 2013) 19.19±1.19. The protein content in fish range with species due to certain factors such as the season of the year, effect of spawing and migration, food availability etc. (Abdullahi, 2001).

The ash content is higher in the three fish species. the result implies that the three fish has high mineral content. The result of ash is higher than the one reported by Oguz et al., 2013 and (Alainserges *et al.,* 2013). The carbohydrate values of the two fish species were higher than the IFIC (2011) recommended values of 12-16g/day and 12g/100g reference value of FAO, (2004),WHO, (2001). This shows that the species could be dependable sources of high energy. The significant reduction of fibre observed in this study posed no threat because fish is usually consumed as adjuncts or additives to other food.

Microbiological assessment: revealed that fungal load (contamination) associated with contamination of smoke-dried fish and crayfish sold in the markets. The organism isolated were; *Aspergillus, Penicillum, Mucor, Rhizopus and Absidia* (**Table 5).**

(Table 6) Shows the Comparative distribution of fungal isolates from the two source market, Ojatuntun market in Ile ife and Ogiyan market in Ejigbo. And the total percentages of each organism isolated are: *Aspergillus* (16.7%) *Mucor* (33.3%), *Penicillum* (16.7%) and *Rhizopus* (27.8%) were the dominant fungi recorded in association with the smoke-dried fish contamination. Absidia (5.6%) occurred less frequently.

Fig 1: Shows the pie chart illustration of total fungi load in the three fishes. Crayfish sample from the two market have the highest load of fungi contamination (50%,) followed by croaker fish (27.8%), while the least was spiny eel fish (22.2%).

Table 7a and 7b Indicate average fungi colony count in Cfu/g at ($p<0.05$). Cray fish in new market in Ile Ife having the highest fungi colony count 2.05± $0.25x10^5$cfu/g, while Spiny-eel fish recorded the lowest, 1.8 ± $0.25x10^5$cfu/g. Also in Ogiyan market in Ejigbo Crayfish also recorded the highest 1.9±$0.25x10^5$cfu/g while Croaker fish had the lowest $1.75x10^5$cfu/g still indicating crayfish having the highest fungi load in the two market source.

The microbial load on the dry Cray fish and smoke dried fish(spiny-eel fish and croaker fish) in this study fall within the maximum recommended bacterial count for good quality fish product i.e. $5x10^5$ (5.7log10 cfu/g) (ICMSF, 1986). Cheesebrough (2000) reported that fish with microbial load of less than10^6cfu/g is acceptable from the microbial point of view.

The fungi isolated in this study are all opportunistic pathogens Job et al (2016) of medical and veterinary importance. The presence of toxigenic fungi, for example some species of *Aspergillus*, and *Penicillium*, in foods as contaminants increases the risk for mycotoxins production which could induce gastrointestinal and metabolic disturbances when contaminated foods like smoke-dried fish are consumed Martin A (2008). Among the moulds isolated by Job et al. (2016), only strains of *Aspergillus flavus* presented aflatoxigenic producing potentials.

Thus, the growth of fungi in smoke-dried fish can be affected by the storage method employed. It was observed, during sample collection for the present study, that smoke-dried fishes were stored either in baskets, wooden boxes or metal containers with covers Osibona et al (2018) observed in their study that storage containers play a vital role in the preservation and shelf-life of smoked dried fish, and recommended the use of air-tight storage containers for smoked fish. The high level of fungal contaminant discovered revealed that the sellers/processors

should be encouraged to practice a good hygiene particularly during handling, processing and storage. Also more awareness on the health implication of fungal agents and mycotoxins- associated with contaminated fish product should be created among fish handlers and consumers, most especially readymade food.

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