



INFLUENCE OF COLLECTION TIME ON NUTRIENTS AND MICROBIAL LOADS OF SOME FROZEN MARINE FISH SPECIES SOLD IN BICHI LOCAL GOVERNMENT AREA OF KANO STATE, NIGERIA

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ABSTRACT

This study was carried to evaluate the influence of collection time on nutritional compositions and microbial loads of some frozen marine fish species; *Clupea harengus*, *Scomber scombrus* and *Trachurus trachurus* sold in Bichi local government area of Kano State, Nigeria. A total of 27 samples comprising of nine of each species were collected from the sampling station. The first collection was done upon the arrival of the stocks, the second collection was done four days after while the third collection was done when their stocks were about to finish. The samples were analyzed for proximate compositions, minerals, fatty acids, and microbial load. Results indicated that the highest crude protein content, 22.66% was recorded in *S. scombrus* from second collection. All the proximate composition parameters were affected by the species and collection time. *S. scombrus* had the highest calcium content, 1.40 mg/kg from second collection. All the mineral parameters were affected by the species except potassium, while collection time only affected potassium and iron. The highest free fatty acids content, 2.14% was observed in *T. trachurus* from third collection. *S. scombrus* recorded the highest *Salmonella spp.* count of 3.67×10^6 cfu/g from third collection, *Salmonella sp* and total coliform counts were affected by the species while collection time affected all the microbial parameters except mold. These values were within the range for safe and nutritionally balanced fish products. The study recommended *S. scombrus* as the best for human consumption due to its higher protein, least lipid and superior mineral composition.

Keywords: Minerals, Microbial Loads, Frozen, Marine Fish, Nutritional

INTRODUCTION

The widely acceptability of fish by a very diverse group of people irrespective of religious beliefs, socioeconomic backgrounds and age group makes fish an important source of animal protein in the world. These attributes plus its affordability and availability makes fish a valuable and affordable source of animal protein, particularly in developing nations (Dauda *et al.*, 2020). Fish as a rich source of protein, it has different forms of simple proteins with different essential amino acids, fats and traces of vitamin B Complex and other non-protein nitrogenous forms (Brasky *et al.*, 2010). Fish consumption is therefore, linked to the reduction of risks of various types of diseases and improvement of neurological development and other health related issues (Hellberg *et al.*, 2012). Furthermore, fish comprises of proteins and fats (macronutrient) and vitamins and minerals (micronutrient) that are required for good nutrition, thereby effectively contributing to food and nutrition security as an accompaniment to rice-based diets in Asia and maize and cassava-based diets in Africa (Glover-Amengor *et al.*, 2012). Understanding the proximate composition of fish is critical for consumers as well as managers to ensure that they meet the requirements of food regulations and commercial specifications. Also, proximate composition is a good indicator of physiology which is needed for routine analysis of fishes (Tsegay *et al.*, 2016). Generally, fish is 70 to 80% water, 20 to 30% protein, and 2 to 12% fat. Minerals in fish, such as iron, calcium, zinc, iodine from marine fish, phosphorus, and selenium, are especially concentrated in the bones. Fish is thus an excellent source of minerals, especially when eaten whole, including the bones (Glover-Amengor *et al.*, 2012). On the other hand, in different environmental

situations, the composition of the fish may vary due to variances in water quality, feeding conditions, sex, maturation status, and catch condition (Palani *et al.*, 2014).

Fish as a protein source, is highly perishable and contains a high level of free amino acids, which are metabolized by microbes to produce ammonia, biogenic amines like putrescine, histamine, cadaverine, organic acids, ketones, and sulphur compounds (Ezemba *et al.*, 2017; Ekanem and Udoma, 2021). It requires good preservation methods to ensure it retains its quality as the major goal of food technologists and fish merchants is to preserve and market frozen fish products in a high-quality and safe manner (Speranza *et al.*, 2021).

Due to the diverse nature of the marine environment, there's a significant chance of infection of fish caught there by microbes and this necessitates that immediately fish is caught, it must be stored in a good manner (such as freezer) before entering the supply chain in order to extend its shelf life and makes it accessible over long distances and period of time. For this reason, freezing is a helpful method for preserving fish and other seafood products without significantly sacrificing their quality (Baoguo *et al.*, 2020). Freezing preserves food for a longer duration of time by inhibiting the growth and proliferation of bacteria that cause food spoilage and foodborne illness, as well as inhibiting the food's own enzyme activity, which would otherwise cause the food to rot. The majority of germs do not multiply at freezing temperatures. Furthermore, germs require water to develop, and freezing converts the available water into solid ice crystals. Freezing is a common practice in the meat, fish, and other animal protein-based industries because it preserves quality for an extended period of time and provides several

benefits such as minimal changes in product dimensions and minimal deterioration in colour, flavour, and texture (USDA Food Safety Information, 2013).

It is generally recognized that fish can harbour human harmful bacteria, particularly the coliform group (Adedeji *et al.*, 2012; Ekanem and Udoma, 2021). Microbiological quality is important to public health since it directly affects fish deterioration and is a cause of food poisoning. Bacteriological risks that cause illnesses and poor health are directly tied to food safety concerns with animal proteins obtained from marketed food, such as fish, fisheries products, meat, and meat products. This raises a burning question for all consumers dealing with a high-risk item in terms of pathogenic bacteria contamination, which poses a food safety risk (Ekanem and Udoma, 2021). The majority of frozen fish marketed in Bichi Metropolitan Area, Kano State, are exposed to dust and contamination, which fosters the growth of germs due to their ubiquity, hence increasing pathogen transmission and prevalence. Furthermore, poor personal hygiene, such as failing to wash one's hands after handling contaminated objects, promotes pathogen contamination of frozen fish. There is uncertainty whether fish stocks were able to maintain their quality between when they arrived in the cold room and when they are exhausted, therefore no information on quality change over the time the fish is sold. It is therefore pertinent to evaluate the nutritional and mineral composition as well as the microbiological load of three (3) imported frozen fish most commonly sold in Bichi Metropolitan Area, Kano State at different collection times.

MATERIALS AND METHODS

Study area

Bichi town is the Headquarter of Bichi Local Government Area in Kano State, Nigeria. It is ranked to be second in population in Kano State with total of about 277,099 people (NPC, 2006). It has a total area of 612 km² and it lies between Coordinates: 12°14'03"N 8°14'28"E (Abdullahi *et al.*, 2020).

Sampling Size and Procedure

Three most commonly frozen marine in the study areas were used. A total of 27 samples comprising nine Atlantic herring (*Clupea harengus*), nine Atlantic mackerel (*Scomber scombrus*) and nine Atlantic horse mackerel (*Trachurus trachurus*) were purchased from the major frozen fish cold-room in Bichi Local Government Area. The first sample collection was done upon the arrival of the stocks/products, the second collection was done four days after the first collection and the third collection was done when their stocks were about to finish (7 days from arrival). The fish samples were collected into a sterile tinfoil paper individually and immediately transported in an ice cooler/cold flask to retain the freshness, to Umaru Musa Yar'adua University, Katsina (UMYU) at the Biochemistry laboratory and Microbiology laboratory for analysis of proximate compositions and microbial load respectively.

Nutritional Composition Analyses

Proximate analysis, the moisture content, crude protein, crude lipid and ash content of fish sample were analyzed using the method of analyses by Association of Official Analytical Chemists (AOAC, 2016).

Free Fatty Acids Analysis

A modified method of AOAC Official Method 940.28 Fatty Acids (Free) in Crude and Refined Oils Titration Method (AOAC, 2012)

Mineral Analysis

Mineral contents were determined using atomic absorption spectrometer (AAS) and flame photometer analysis (Ismaniza *et al.*, 2012).

Microbiological Analysis

Microbial analysis was determined using standard microbiological procedures (Alkuraieef *et al.*, 2021; Arendrup *et al.*, 2021; Cheesbrough, 2005).

Data Analysis

Data was analyzed using descriptive statistics (mean \pm standard deviation). The data was subjected to normality and homogeneity of variance tests, after which a two-way analysis of variance (ANOVA) was used to compare the fish species for each parameter, also to compare the collection times for each parameter at 95% level of probability ($P < 0.05$). Whenever a significant difference was observed Turkey's HSD test was used to separate the different means using IBM SPSS Version 23.

RESULTS AND DISCUSSION

The results of the proximate composition of the three frozen marine fish species collected at different times are shown in Table 1, highest moisture ($77.69 \pm 0.09\%$) was observed in *C. harengus* from third collection. The moisture was significantly higher in *C. harengus* ($P < 0.05$) compared to other species. The difference in moisture due to collection time was also significant with the highest at 3rd collection time. The total ash of ($2.91 \pm 0.07\%$) recorded in *T. trachurus* from third collection was the highest. The ash was significantly higher ($P < 0.05$) in *T. trachurus* than other species. It was significantly different among ($P < 0.05$) the collection times with the highest at 3rd collection time. The highest crude protein, $22.66 \pm 0.14\%$ was recorded in *S. scombrus* from second collection. Crude protein was significantly higher in *S. scombrus* compared to other species ($P < 0.05$). There was a significant difference ($P < 0.05$) in crude protein due to collection time, with at the second collection time. The highest crude lipid, $5.94 \pm 0.04\%$ was recorded in *C. harengus* from second collection, it was significantly higher in *C. harengus* than other species. It was significantly ($P < 0.05$) higher at the third collection time. The highest free fatty acids, 2.14 ± 0.03 was observed in *T. trachurus* from first collection, while the least, 0.72 ± 0.03 was recorded in *C. harengus* from third collection. Free fatty acids was higher significantly in *T. trachurus* compared to other species. The difference in free fatty acids due to collection was significant ($P < 0.05$).

The results of the mineral composition of fish at different times is shown in Table 2, highest Na (1.98 ± 0.67 mg/kg) was observed in *C. harengus* from second collection, it was significantly higher in *C. harengus* ($P < 0.05$) compared to other species but the difference in Na due to collection time was not significant ($P > 0.05$). The Ca of 1.40 ± 0.61 mg/kg recorded in *S. scombrus* from second collection was the highest, it was significantly higher ($P < 0.05$) in *S. scombrus* than the other species but it was not different significantly ($P > 0.05$) among the collection times. The highest K, 0.76 ± 0.14 mg/kg was recorded in *C. harengus* from second collection, it was not significant different ($P > 0.05$) among the species but there was a significant difference ($P < 0.05$) due to collection. The highest Fe, 1.14 ± 0.05 mg/kg was recorded in *S. scombrus* from first collection, Fe was significantly higher in *S. scombrus* than other species ($P < 0.05$). It was significantly different due to collection. The highest Mn, 0.36 ± 0.05 mg/kg was recorded in *T. trachurus*

from first collection, Mn was significantly higher in *T. trachurus* than other species ($P < 0.05$) but it was not significantly difference ($P > 0.05$) due to collection time.

The results of microbial population of the fish species collected from Bichi station is shown in Table 3, highest *Salmonella* ($3.67 \pm 1.53 \times 10^6$ cfu/g) was observed in *S. scombrus* from third collection, it was significantly different ($P < 0.05$) from other species. The difference in *Salmonella* due to collection time was also significant ($P < 0.05$), with the

highest at 3rd collection. The *E. coli* was not significantly different among the species ($P > 0.05$) but it was different significantly ($P < 0.05$) due to the collection time. The highest total coliform count, $5.00 \pm 1.00 \times 10^6$ cfu/g was recorded in *C. harengus* from first collection, it was significantly higher in *C. harengus* than other species ($P < 0.05$) and there was a significant difference ($P < 0.05$) due to collection. The mold was not different significantly among the species ($P > 0.05$) and among the collection time.

Table 1: Proximate and Free Fatty Acids Compositions of the Three Frozen Marine Fish Species Collected at Different Times from Bichi Station

Parameters (%)	Bichi Station Sample Collection				Two-way ANOVA		
	Collection	<i>C. harengus</i>	<i>S. scombrus</i>	<i>T. trachurus</i>	Species (P value)	Collection (P value)	Species * Collection (P value)
Moisture	1 st	75.56 \pm 0.04 ^{B,a}	71.10 \pm 0.01 ^b	71.18 \pm 0.02 ^c	0.000	0.000	0.000
	2 nd	73.51 \pm 0.04 ^C	70.83 \pm 0.11	68.94 \pm 0.04			
	3 rd	77.69 \pm 0.09 ^A	73.35 \pm 0.21	69.29 \pm 0.04			
Crude Protein	1 st	14.85 \pm 0.02 ^{C,c}	20.19 \pm 0.05 ^a	18.29 \pm 0.06 ^b	0.000	0.000	0.000
	2 nd	15.71 \pm 0.04 ^A	22.66 \pm 0.14	21.92 \pm 0.05			
	3 rd	15.27 \pm 0.05 ^B	19.14 \pm 0.10	19.87 \pm 0.11			
Crude Lipid	1 st	4.84 \pm 0.04 ^{A,a}	4.44 \pm 0.11 ^b	5.22 \pm 0.02 ^a	0.000	0.000	0.000
	2 nd	5.94 \pm 0.04 ^B	3.92 \pm 0.09	4.38 \pm 0.03			
	3 rd	3.32 \pm 0.07 ^C	3.86 \pm 0.12	4.48 \pm 0.03			
Total ash	1 st	2.25 \pm 0.02 ^{B,b}	2.32 \pm 0.04 ^b	2.81 \pm 0.04 ^a	0.000	0.000	0.000
	2 nd	2.25 \pm 0.03 ^C	1.90 \pm 0.08	2.45 \pm 0.03			
	3 rd	2.48 \pm 0.19 ^A	2.71 \pm 0.13	2.91 \pm 0.07			
Carbohydrate	1 st	2.51 \pm 0.11 ^{A,b}	1.94 \pm 0.12 ^c	2.50 \pm 0.03 ^a	0.000	0.002	0.000
	2 nd	2.52 \pm 0.13 ^B	0.69 \pm 0.14	2.31 \pm 0.02			
	3 rd	1.49 \pm 0.43 ^B	1.09 \pm 0.48	3.45 \pm 0.23			
Free fatty acid	1 st	0.90 \pm 0.07 ^{B,c}	1.17 \pm 0.12 ^b	2.14 \pm 0.03 ^a	0.000	0.000	0.000
	2 nd	1.15 \pm 0.03 ^A	1.43 \pm 0.09	2.04 \pm 0.02			
	3 rd	0.72 \pm .030 ^C	1.19 \pm 0.03	1.92 \pm 0.04			

*Values are presented as the means \pm standard deviation

*Different small letters as superscripts across the rows indicate significant differences ($P < 0.05$) among the fish species.

*Different capital letters as superscripts within the column for each parameters indicate significant differences ($P < 0.05$) among the stations

Key: 1st – First Collection; 2nd – Second Collection; 3rd – Third Collection

Table 2: Minerals Compositions of Three Frozen Marine Fish Species Collected at Different Time From Bichi Station

Parameters (mg/kg)	Bichi Station Sample Collection				Two-way ANOVA		
	Collection	<i>C. harengus</i>	<i>S. scombrus</i>	<i>T. trachurus</i>	Species (P value)	Collection (P value)	Species * Collection (P value)
Sodium (Na)	1 st	1.45 \pm 0.51 ^{A,a}	0.36 \pm 0.02 ^b	0.46 \pm 0.03 ^b	0.000	0.406	0.882
	2 nd	1.98 \pm 0.67 ^A	0.45 \pm 0.09	0.59 \pm 0.09			
	3 rd	1.65 \pm 0.89 ^A	0.35 \pm 0.03	0.46 \pm 0.03			
Calcium (Ca)	1 st	0.11 \pm 0.01 ^{A,b}	1.38 \pm 0.59 ^a	0.13 \pm 0.03 ^a	0.000	0.529	0.639
	2 nd	0.12 \pm 0.01 ^A	1.40 \pm 0.61	0.14 \pm 0.04			
	3 rd	0.12 \pm 0.02 ^A	0.96 \pm 0.39	0.13 \pm 0.03			
Potassium (K)	1 st	0.60 \pm 0.23 ^{B,a}	0.47 \pm 0.02 ^a	0.48 \pm 0.11 ^a	0.248	0.013	0.985
	2 nd	0.76 \pm 0.14 ^A	0.63 \pm 0.17	0.72 \pm 0.06			
	3 rd	0.55 \pm 0.25 ^B	0.46 \pm 0.04	0.48 \pm 0.11			
Iron (Fe)	1 st	0.65 \pm 0.04 ^{A,b}	1.14 \pm 0.05 ^a	0.81 \pm 0.03 ^b	0.000	0.000	0.000
	2 nd	0.56 \pm 0.06 ^B	1.07 \pm 0.04	0.34 \pm 0.03			
	3 rd	0.82 \pm 0.06 ^C	0.25 \pm 0.04	0.74 \pm 0.03			
Manganese (Mn)	1 st	0.28 \pm 0.02 ^{A,b}	0.16 \pm 0.03 ^b	0.36 \pm 0.05 ^a	0.000	0.256	0.000
	2 nd	0.25 \pm 0.03 ^A	0.23 \pm 0.03	0.35 \pm 0.03			
	3 rd	0.16 \pm 0.04 ^A	0.26 \pm 0.04	0.33 \pm 0.03			

*Values are presented as the means \pm standard deviation

*Different small letters as superscripts across the rows indicate significant differences ($P < 0.05$) among the fish species.

*Different capital letters as superscripts within the column for each parameters indicate significant differences ($P < 0.05$) among collection time

Key: 1st – First Collection; 2nd – Second Collection; 3rd – Third Collection

Table 3: Microbial Population of Three Frozen Marine Fish Species Collected at Different Time From Bichi Station

Parameters (x10 ⁶ cfu/g)	Bichi Station Sample Collection				Two-way ANOVA		
	Collection	<i>C. harengus</i>	<i>S. scombrus</i>	<i>T. trachurus</i>	Species (P value)	Collection (P value)	Species * Collection (P value)
<i>Salmonella spp</i>	1 st	0.67 ± 0.58 ^{AB,b}	1.33 ± 0.58 ^a	1.33 ± 0.58 ^{ab}	0.010	0.044	0.037
	2 nd	0.33 ± 0.58 ^B	1.00 ± 1.00	1.33 ± 0.58			
	3 rd	1.00 ± 0.00 ^A	3.67 ± 1.53	1.00 ± 1.00			
<i>E. coli</i>	1 st	2.33 ± 1.53 ^{A,a}	3.67 ± 1.53 ^a	1.67 ± 1.53 ^a	0.387	0.039	0.083
	2 nd	2.00 ± 1.00 ^B	0.33 ± 0.58	1.67 ± 0.53			
	3 rd	1.33 ± 0.58 ^B	2.00 ± 1.00	0.67 ± 0.58			
Total coliform	1 st	5.00 ± 1.00 ^{A,a}	4.00 ± 1.73 ^b	1.00 ± 1.00 ^b	0.002	0.022	0.138
	2 nd	3.00 ± 1.00 ^{AB}	2.00 ± 1.73	1.00 ± 1.73			
	3 rd	3.00 ± 1.00 ^B	0.33 ± 0.58	1.33 ± 1.15			
Mold	1 st	6.33 ± 2.08 ^{A,a}	3.66 ± 2.08 ^a	4.67 ± 3.79 ^a	0.298	0.577	0.308
	2 nd	4.00 ± 1.00 ^A	4.67 ± 0.58	3.00 ± 1.00			
	3 rd	4.33 ± 1.53 ^A	6.33 ± 2.31	3.00 ± 2.00			

*Values are presented as the means ± standard deviation

*Different small letters as superscripts across the rows indicate significant differences (P < 0.05) among the fish species.

*Different capital letters as superscripts within the column for each parameters indicate significant differences (P < 0.05) among the stations

Key: 1st – First Collection; 2nd – Second Collection; 3rd – Third Collection

Discussion

Fish has been a food source for human worldwide from time immemorial, especially as an inexpensive source of animal protein. Therefore, the proximate compositions of the fish species help to provide important nutritional profile (Nurnadia *et al.*, 2011). Several researchers have reported proximate composition of different fish species and documented differences due to species (Abubakar, 2016; Babalola *et al.*, 2011; Bello *et al.*, 2019; Elaigwu, 2019; Mohammad *et al.*, 2019; Palani *et al.*, 2014). In this study all the proximate composition parameters were affected by both the species and collection time. For instance, highest amount of moisture was reported in *C. harengus* which was significantly higher than that of *S. scombrus* and *T. trachurus*. This is similar to study of Bello *et al.*, (2019) who studied on *C. harengus*, *T. trachurus*, *S. scombrus* and *M. undulates*. *T. trachurus* had reported higher, lipid, total ash and estimated carbohydrate. This observation may be due to difference in species of fish experimented. This is in line with observation of Elaigwu, (2019) who reported differences in proximate composition of fresh water fish species (*Schilbe mystus*, *Bagrus bayad*, *Oreochromis niloticus*, *Clarias anguillaris* and *Petrocephalus bane bane*). The result also is line with the study of Babalola *et al.*, (2011) who reported differences in proximate composition of marine and fresh water fish species (*S. scombrus*, *T. trachurus*, *S. aurita*, *M. furnieri* and *C. gariepinus*). In contradictory to this study Omolara and Omotayo, (2009) reported differences in proximate compositions of marine water fish species with *T. trachurus* having higher moisture values over *S. aurita*. They also reported *S. scombrus* to have higher protein content over *T. trachurus* and *C. harengus* (Omolara and Omotayo, 2009). The differences due to collection times in the station might be due to handling and the differences in storing between the source and the cold room. Aubourg *et al.* (2012) earlier noted that the duration of freezing storage and different methods of handling might affect proximate composition values. The biochemical composition of fishes could greatly affect the postharvest processes and storage. Understanding this may assist in determining the suitability of different species to specific processing and storage methods (Malik *et al.*, 2021). Protein is the major nutrients in fish, but composition varies depending on age, sex, environment and seasonal migration of which their percentage level helps to define the nutritional

status of a particular organism (Aberoumand and Pourshafi, 2010; Ahmed *et al.*, 2022). Generally, the proximate compositions of these fish species studied are the recommended and safety level for consumption.

Minerals play important role in maintaining body functions because they maintain acid base balance, and help blood formation (hemoglobin formation). They also control the water balance in the body, help bones formation and teeth structure, and catalyze many metabolic reactions (Njinkoue *et al.*, 2016). All aquatic animals require minerals for their vital physiological and biochemical functions and to maintain their normal life processes (Lall and Kaushik, 2021). Several researchers have reported mineral compositions of different fish species and documented differences due to species (Achionye-Nzeh *et al.*, 2011; Guizani and Moujahed, 2015; Haizhou *et al.*, 2022; Okpanachi *et al.*, 2018; Stanton *et al.*, 2020; Tenyang *et al.*, 2014). In this study all the mineral compositions parameters were affected by the species except potassium while collection time affected only potassium and iron. For instance, highest amount of Sodium (Na) was reported in *C. harengus* which was significantly higher than that of *S. scombrus* and *T. trachurus*. Furthermore, the highest amount of Calcium (Ca) was reported in *S. scombrus* which was significantly higher than that of *T. trachurus* and *C. harengus* and the highest amount of Manganese (Mn) was reported in *T. trachurus* which was significantly higher than that of *S. scombrus* and *C. harengus*. This is similar to the observation of Stanton *et al.* (2020) who studied *C. harengus*, *C. aper* and *M. poutassou* and that of Achionye-Nzeh *et al.* (2011) who studied *S. scombrus*, *T. trachurus*, *K. Pelamis* and *G. lineates*. *C. harengus* and *T. trachurus* had the highest Sodium (Na) and Manganese (Mn) contents and this observation may be due to differences in species of fish studied. The mineral compositions in this study varied significantly among the species this may be due to the fact that variations in concentrations of these mineral elements varies from one species of fish to another and also due to the chemical forms of the elements and their concentrations in the local environment (Babalola *et al.*, 2011). Expectedly, all fishes absorb minerals not only from their diets but also from the surrounding aquatic environment via their gills and skin (Nurnadia *et al.*, 2013).

Free fatty acids play important roles as a source of energy for most body tissues and their functions in energy transport

within the body are well established (Binienda *et al.*, 2013). Free fatty acids also play important role in oxidative stress following cell membrane depolarization (Binienda *et al.*, 2013; Kimura *et al.*, 2019). Many researchers have reported free fatty acids composition of different fish species and documented differences due to species (Abubakar, 2016; Fawole *et al.*, 2018; Koppers *et al.*, 2010; Mervat, 2008). In this study free fatty acids composition were affected by the species and the collection time. For instance, highest amount of free fatty acids was reported in *T. trachurus* which was different significantly higher than that of *S. scombrus* and *C. harengus*. This is similar to observation of Abubakar, (2016) who studied *C. harengus*, *T. trachurus* and *S. pilchardus* where *T. trachurus* reported higher free fatty acids compositions. This observation may be due to difference in species of fish studied. This is in line with observation of Fawole *et al.* (2018) who also reported differences in free fatty acids composition of frozen marine fish species (*Sardinella* spp. and *M. poutassou*). Mervat, (2008) also reported difference in free fatty acids composition of frozen marine fish species (*C. harengus*, *T. trachurus* and *S. scombrus*). The differences observed in the free fatty acids composition values among the collection times may be due to the duration of freezing storage and methods of handling (Alsuhailani *et al.*, 2020; Aubourg *et al.*, 2012; Fawole *et al.*, 2018). However, the free fatty acid in the samples studied were within the FAO recommended level of 2.5% for consumption (FAO, 2014), and is in line with International Fish Oils Standard (IFOS) who reported a range of 1% to 7% as standard (IFOS, 2011). The free fatty acids compositions of these fish species studied are within the acceptable and safety limits, therefore, they are safe for consumption.

The basic nutrient of protein in fish that is so important in man's diet also attracted microorganisms for their growth and multiplication (Silvia *et al.*, 2020). The association of these microorganisms in fishes depends on the environment and their proliferation due to inadequate storage facilities (Olagbemide and Akharaiyi, 2021). Many researchers have reported microbial load of different fish species and documented differences due to species (Adegunwa *et al.*, 2013; Adeparusi *et al.*, 2019; Ali *et al.*, 2020; Bello *et al.*, 2019; Daniel *et al.*, 2013; Taiwo *et al.*, 2017). In this study *Salmonella* sp and total coliform counts were affected by both the species and collection time, while *E. coli* was only influenced by the collection time. For instance, highest amount of microbial load was reported in *C. harengus* which was significantly higher than that of *T. trachurus* and *S. scombrus*. This is similar to observation of Daniel *et al.* (2013) who studied bacteria load on *T. trachurus*, *S. scombrus*, *C. gariepinus*, *E. fimbriata* and *P. croaker*. *T. trachurus* had the highest *Salmonella* spp count, *S. scombrus* had the highest *E. coli* count and *C. gariepinus* had the highest total coliform and mold count, this observation may be due to differences in species of fish experimented and difference in methods of handling. This is in line with observation of Adeparusi *et al.* (2019) who also reported difference in bacteria loads of marine water fish species (*C. harengus*, *S. scombrus* and *M. poutassou*). Taiwo *et al.* (2017) also reported difference in bacteria loads of marine water fish species *T. trachurus*, *S. scombrus*, *L. crocea*, *G. chalcogrammus* and *O. niloticus*. The variations in the bacterial loads for all the three fish species studied from is an indication that handling and preservation methods employed from the source and the station might be different. This is supported by Olutimehin *et al.* (2019) who stated that handling and preservation methods have effects on the level of microbial loads in fish. The microbial load recorded in this

study did not exceed the recommended limit of $\times 10^6$ to 10^7 cfu/g of bacteria load (Gilbert *et al.*, 2000; NAFDAC, 2015; Rubaijaniza *et al.*, 2021). This could be due to the fact that the fishes received good preservation method and handling by the fish vendors at the cold room in Bichi town (Olutimehin *et al.*, 2019; Rubaijaniza *et al.*, 2021).

Generally, it has been observed that appropriate methods of handling of fish during preservation reduces microbial loads in the final products (Huong, 2014). The significant variation noted between individual fish species in this study could be due to the fact that the fish were caught at random and they have differences in method of preservation, freezing/storage, handling, variation in sex, age, and environment (Burke, 2011). However, the microbial loads found these fish species are within the safety and recommended levels which cause no harm to human when consumed.

CONCLUSION

S. scombrus had the highest crude protein and the least lipid, it also had highest calcium, potassium and iron. *T. trachurus* appeared better in terms of *Salmonella* sp load, the total coliform count was similar between the *S. scombrus* and *T. trachurus*. While the proximate and mineral contents tended to reduce with collection time, the microbial load tended towards increase with the collection times. However, the proximate composition of all the examined fishes irrespective of the collection times showed that they contain all the required nutrients within the recommended limit and thus suitable for human consumption, though significant difference was observed in some of the parameters. The current investigation also revealed the rate of contamination by three pathogens in marine fish sold in Bichi for the first time. These results demonstrated that the pathogenic bacteria load of the studied marine fish was fairly high though not beyond the safe limit for consumption. Overall, *S. scombrus* tended to be the best in the study area followed by *T. trachurus*.

REFERENCES

- Abdullahi, B. N., Yeboah, F. K., Ahmed, N., and Muhammad, I. U. (2020). Profitability of Small-Scale Maize Production in Nigeria: A Case Study of Bichi Local Government, Kano State-Nigeria. *European Journal of Agriculture and Food Sciences*, 2(5), 11-20.
- Aberoumand, A., and Pourshafi, K. (2010). Chemical and proximate composition properties of different fish species obtained from Iran. *World Journal of Fish Marine Sciences*, 2(1), 237 – 239.
- Abubakar, R. (2016). Physical, Chemical and Quality Indices of Three Imported Frozen Marine Fish Species Sold in Zaria, Nigeria. *MSc. Dissertation. Department of Biological Sciences, Faculty of Science, Ahmadu Bello University, Zaria, Nigeria*, 1-51.
- Achionye-Nzeh, C. G., Adedoyin, O. M., Oyebanji, S., and Mohammed, M. O. (2011). Mineral composition of some marine and freshwater fishes. *Agriculture and Biology Journal of North America*, 2(7), 1113-1116.
- Adegunwa, M. O., Adebawale, A. A., Olisa, Z. G., and Bakare, H. A. (2013). Chemical and microbiological qualities of smoked herring (*sardinella eba*, valenciennes 1847) in Odeda, Ogun state, Nigeria. *International Journal of Microbiology*, 1(5), 85-87.

- Adeparusi, E. O., Adejuyigbe, A., Ode, A. D., and Oke, I. O. (2019). Microbiological Assessment Of Three Frozen Marine Commercial Fishes. *Journak of Applied Tropical Agriculture*, 24(1), 241-246.
- Adedeji, O. B., Okeretugba, P. O., Innocent-Adide, H. C., and Okonkwo, I. O. (2012). Benefits, Public Health Hazards and Risks Associated with Consumption of Sea Food. *New York Science Journal*, 9(4), 33-61.
- Ahmed, I., Jan, K., Fatma, S., and Dawood, M. A. O. (2022). Muscle proximate composition of various food fish species and their nutritional significance: A review. *Journal of Animal Physiology and Animal Nutrition*, 106, 690–719.
- Ali, I. H. A. A., Salman, A. M., El Ayis, A. A., and Hamad, E. M. (2020). Enumeration of E. coli, Coliform and Aerobic Bacteria in Marine Fish in Port Sudan, Red Sea State, Sudan. *Asian Journal of Research in Animal and Veterinary Sciences*, 6(3), 9-14.
- Alkuraieef, A. N., Alsuhaibani, A. M., Alshawi, A. H., Alfaris, N. A., and Aljabryn, D. H. (2021). Chemical and microbiological quality of imported chilled, frozen, and locally cultured fish in Saudi Arabian markets. *Food Science and Technology*.
- Alsuhaibani, A. M., Amal, N. A., Amal, H. A., and Amani, H. A. (2020). Effect of Frozen Storage on Nutritional, Microbial and Sensorial Quality of Fish Balls and Fish Fingers Produced From Indian Mackerel. *Current Research in Nutrition and Food Science*, 8(3), 852-861.
- AOAC. (2012). Official Methods of Analysis of Association of Official Analytical Chemists AOAC International. *Association of Official Analytical Chemists, 19th Edition*. Washington, DC.
- AOAC. (2016). Official Methods of Analysis of Association of Official Analytical Chemists AOAC International. *Association of Official Analytical Chemists, 20th Edition*, Arlington, Virginia, USA.
- Arendrup, M. C., Karin, M. J., Nicolien, H., and Paul, E. V. (2021). ISO standard 20776-1 or serial 2-fold dilution for antifungal susceptibility plate preparation: that is the question! *Journal of Antimicrobial Chemotherapy*, 76, 1793–1799.
- Aubourg, S. b., Lugasi, A., Losada, V., Hóvári, J., Lebovics, V., and Jakóczy, I. (2012). Effect of pre-soaking whole pelagic fish in a plant extract on sensory and biochemical changes during subsequent frozen storage. 1-22.
- Babalola, A. F., Adeyemi, R. S., Adeseye, O. O., Motolani, M. S., Olaitan, O. O., and Gbola, R. A. (2011). Proximate and mineral composition in the flesh of five commercial fish species in Nigeria. *Internet Journal of Food Safety*, 13, 208-213.
- Baoguo, L., Hoa, T. T., Heng, Z., Quanyou, G., and Song, L. (2020). Freezing methods affect the characteristics of large yellow croaker (*Pseudosciaena crocea*): use of cryogenic freezing for long-term storage. *Food Science and Technology*, 40(2), 429-435.
- Bello, M. M., Oyebola, O. O., and Lawal, T. H. (2019). Proximate composition and microbial analysis of common marine fishes consumed in Maiduguri Metropolis, Borno State, Nigeria. *International Journal of Agric. and Agricultural Tech.*, 11(1), 113-126.
- Binienda, Z. K., Sarkar, S., Silva-Ramirez, S., and Gonzalez, C. (2013). Role of free fatty acids in physiological conditions and mitochondrial dysfunction. *Journal of Food and Nutrition Sciences*, 4(1), 6-15.
- Burke, A. B. (2011). The Proximate, Fatty Acid and Mineral Composition of the Muscles of Cultured Yellowtail (*Seriola lalandi*) at Different Anatomical Locations. 1-35.
- Brasky, T. M., Lampe, J. W., Potter, J.D., Patterson, R.E. and White E. (2010). Specialty supplement and breast cancer risk in the vitamin lifestyle (VITAL) cohort. *Cancer Epidemiology preview* 19, 1696-1708.
- Cheesbrough, M. (2005). *District laboratory practice in tropical countries, part 2*. Cambridge university press.
- Daniel, E. O., Ugwueze, A. U., and Igbegu, H. E. (2013). Microbiological quality and some heavy metals analysis of smoked fish sold in Benin City, Edo State, Nigeria. *World Journal of Fish Marine Science*, 5(3), 239-243.
- Dauda, A. B., Omosalewa, O. O., and Garuba, A. M. (2020). Preference and perception of fish processors on the influence of wood types on the quality of smoked-dried fish in Katsina State. *Proceedings of the 35th Annual National Conferneve of Fisheries Society of Nigeria (FISON)*, 357-361.
- Ekanem, J. O., & Udoma, I. G. (2021). Bacteriological assessment of some frozen fishes sold at Ator market in Ikot Ekpene metropolis. *Global journal of pure and applied sciences*, 27, 355-359. copyright© bachudo science co. Ltd printed in Nigeria ISSN 1118-0579.
- Ezemba, C., Eze, S. & Archibong, E. (2017). Bacteriological Analysis of Iced Fish Retailed at Eke-Awka Market, Anambra State. *International Journal of Research in Pharmacy and Biosciences*, 4, (8), 1-9.
- Elaigwu, A. M. (2019). The proximate composition, amino acid profile and chemical indices in five freshwater fishes from Tiga Dam Reservoir, Nigeria. *Croatian Journal of Fisheries*, 77(2), 87-92.
- FAO. (2014). Food and Agriculture Organization. Quality of fish and fish products. Fisheries and Agriculture for a World Without Hunger. *Food and Agriculture Organization (FAO), Rome, Italy*.
- Fawole, O. O., Oyelese, O. A., and Etim, E. U. (2018). Organoleptic and Chemical Assessment of Two Frozen Marine Fishes Obtained From Markets in Four Agricultural Zones of Oyo State, Nigeria. *Ife Journal of Science*, 20(2), 337-343.
- Gilbert, R. J., Louvois, D. J., Donovan, T., Little, C., Nye, K., Ribeiro, C. D., J Richards, J., Roberts, D., and Bolton, F. J. (2000). Guidelines for the microbiological quality of some ready-to-eat foods sampled at the point of sale. *Communicable Disease and Public Health*, 3(3), 163-167.

- Glover-Amengor, M., Ottah, Atikpo, M.A., Abbey, L.D., Hagan, L., Ayin, J., & Toppe, J. (2012). Proximate Composition and Consumer Acceptability of Three Underutilised Fish Species and Tuna Frames. *World Rural Observation*, 4(2), 65-70. <http://www.sciencepub.net/rural>. 11
- Guizani, E. S., and Moujahed, N. (2015). Atlantic mackerel amino acids and mineral contents from the Tunisian middle eastern coast. *International Journal of Agricultural Policy and Research*, 3(2), 77-83,.
- Haizhou, W., Bitu, F., Mehdi, A., and Ingrid, U. (2022). Five cuts from herring (*Clupea harengus*): Comparison of nutritional and chemical composition between co-product fractions and fillets. *Journal of Food Chemistry*, 10(16), 1-12.
- Hellberg, R. S., Dewitt, C. A. M., & Morrissey, M. T. (2012). RiskBenefit Analysis of Seafood Consumption: A Review. *Comprehensive Reviews in Food Science and Food Safety*, 11(5), 490-517.
- Huong, D. T. T. (2014). The effect of smoking methods on the quality of smoked Mackerel. *United Nations University Fisheries Training Programme, Iceland [final project]*.
- IFOS. (2011). International Fish Oils Standard. *Fish oil purity standards*.
- Ismaniza, I., Idaliza, M., and Saleh. (2012). Analysis of heavy metals in water and fish (*Tilapia* sp.) samples from Tasik Mutiara, Puchong. *Malaysian Journal of Analytical Sciences*, 16(3), 346-352.
- Kimura, I., Atsuhiko Ichimura, Ryuji Ohue-Kitano, and Igarashi, M. (2019). Free Fatty Acid Receptors in Health and Disease [Reviews]. *Journal of american Physiological society*, 10(2), 1-45.
- Koppers, A. J., Garg, M. L., and Aitken, R. J. (2010). Stimulation of mitochondrial reactive oxygen species production by unesterified, unsaturated fatty acids in defective human spermatozoa. *Free radical biology and medicine*, 48(1), 112-119.
- Lall, S. P., and Kaushik, S. J. (2021). Nutrition and Metabolism of Minerals in Fish. *Animals*, 11(9), 2711.
- Malik, I. A., Elgasim, A. E., Oladipupo, Q. A., Asmahan, A. A., and Isam, A. M. A. (2021). Effect of frozen storage on the biochemical composition of five commercial freshwater fish species from River Nile, Sudan. *Food Science and Nutrition*, 9, 3758–3767.
- Mervat, K. I. R. (2008). Evaluation of Nutritional and Hygienic Quality of Herring Fish (*Clupea harengus*) and its Products. *Assiut Vet. Med. J.*, 54(119), 1-14.
- Mohammad, R., Saad, A., and Yasin, M. (2019). Study of Changes in Fatty Acids Contents ($\omega 3$, $\omega 6$) and Chemical Quality Indicators in Frozen (*Scomber japonicus*) from the Marine Waters of Tartus. *Tishreen University Journal for Research and Scientific Studies - Biological Sciences Series*, 14(6), 93-111.
- Murthy, L. N., Kumar, A., Jeyakumari, A., and Nilavan, E. (2019). Training Manual on “Microbiological examination of seafood pathogens with special reference V.mimicus & V.valnificus”. *Mumbai Research Center of Central Institute of Fisheries Technology (Indian Council of Agricultural Research)*, 69.
- NAFDAC. (2015). Nigeria Agency for Drug Administration and Control. *NAFDAC, Nigeria*.
- Njinkoue, J. M., Gouado, I., Tchoumboungang, F., Nguenguim, J. Y., Ndinteh, D. T., Fomogne-Fodjo, C. Y., and Schweigert, F. J. (2016). Proximate composition, mineral content and fatty acid profile of two marine fishes from Cameroon coast: *Pseudotolithus typus* (Bleeker, 1863) and *Pseudotolithus elongatus* (Bowdich, 1825). *Nutrition and Food Science Journal*, 4, 27-31.
- NPC. (2006). National Population Commission. *National Population Commission, Census 2006*.
- Nurnadia, A., Azrina, A., Amin, I., Yunus, M. A., and Effendi, M. H. I. (2013). Mineral contents of selected marine fish and shellfish from the west coast of Peninsular Malaysia. *International Food Research Journal*, 20(1), 431–437.
- Nurnadia, A. A., Azrina, A., and Amin, I. (2011). Proximate composition and energetic value of selected marine fish and shellfish from the West coast of Peninsular Malaysia. *International Food Research Journal*, 18(1), 137-148.
- Okpanachi, M. A., Yaro, C. A., and Bello, O. Z. (2018). Assessment of the Effect of Processing Methods on the Proximate Composition of *Trachurus trachurus* (Mackerel) Sold in Anyigba Market, Kogi State. *American Journal of Food Science and Technology*, 6(1), 26-32.
- Olagbemide, P. T., and Akharaiyi, F. C. (2021). Evaluation of microorganisms associated with vended frozen fish in Ado Ekiti locality. *Food Research*, 5(4), 21-28.
- Olopade, O. A. (2015). Effect of Poor Handling on The Nutritional Composition of Some Imported Frozen Fishes in Nigeria. *Bulletin UASVM Food Science and Technology*, 72(1), 20-26.
- Olutimehin, I. O., Adejuyigbe, I. J., and Fasuhanmi, O. S. (2019). Effects of Handling and Storage Systems on the Lipid Oxidation and Fatty Acid Level of the African Snakehead Fish (*Parachanna Obscura*). *International Journal of Engineering Research & Technology (IJERT)*, 8(7), 82-90.
- Omolar, O. O., and Omotayo, O. D. (2009). Preliminary Studies on the effect of processing methods on the quality of three commonly consumed marine fishes in Nigeria. *An international journal published by the Nigerian Society for Experimental Biology*, 21(1), 1-7.
- Padmavati, G. (2017). Proximate and elemental composition of *Stolephorus commersonnii* (Lacepede, 1803) from the coastal waters of South Andaman. *Indian Journal of Geo Marine Sciences*, 46(5), 1000–1007.
- Palani, K. M., Ruba, A. A., Jeya, S. R., and Shanmugam, S. A. (2014). Proximate and major mineral composition of 23 medium sized marine fin fishes landed in the Thoothukudi Coast of India. *Journal of Nutrition and Food Science*, 4(1), 1-7.

- Raffic, A. S. S., Abdhakar, E. S., and Muthukkaruppan, R. (2020). Proximate and mineral composition of commercially important marine fin fishes from the Kasimedu fish landing centre Chennai. *Journal of Fisheries and Life Sciences*, 5(1), 20-25.
- Rubaijaniza, A., Jessica, L. N., Clovice, K., and John, D. K. (2021). Microbiological Quality of Traditionally Smoked Fish from Lake Victoria Crescent, Uganda. *Food Science and Quality Management*, 104(1), 47-53.
- Silvia, T., Ana, C. D. A., Saldanha, P., Dario, M., Picone, G., Francesca, S., Maria, T. R.-E., Massimiliano, P., Francesco, C., and Pietro, R. (2020). Quality Changes during Frozen Storage of Mechanical-Separated Flesh Obtained from an Underutilized Crustacean [open access]. *Food and Nutrition Bulletin*, 9(1485).
- Speranza, B., Racioppo, A., Bevilacqua, A., Buzzo, V., Marigliano, P., Mocerino, E., Scognamiglio, R., Corbo, M. R., Scognamiglio, G., and Sinigaglia, M. (2021). Innovative Preservation Methods Improving the Quality and Safety of Fish Products: Beneficial Effects and Limits. *Foods*, 10(11), 2854. <https://doi.org/10.3390/foods10112854>
- Stanton, C., Egerton, S., Mannion, D., Culloty, S., Whooley, J., and R.P, R. (2020). The proximate composition of three marine pelagic fish: Blue whiting (*Micromesistius poutassou*), Boarfish (*Capros aper*) and Atlantic herring (*Clupea harengus*). *Irish Journal of Agricultural and Food Research*, 59(1), 185-200.
- Taiwo, I. O., Olopade, O. A., and Bamidele, N. A. (2017). Microbial load of some imported frozen fish species in Lagos, Nigeria. *Nigerian Journal of Animal Production*, 44(3), 152-160.
- Tenyang, N., Womeni, H. M., Linder, M., Tiencheu, B., Villeneuve, P., and Tchouanguep Mbiapo, F. (2014). The chemical composition, fatty acid, amino acid profiles and mineral content of six fish species commercialized on the Wouri river coast in Cameroon. *La Rivista Italiana Delle Sostanze Grasse Grasse*, 91(1), 129-138.
- Tsegay, T., Natarajan, P. and Zelealem T., (2016). Proximate and mineral composition of some commercially Imported fish species of tekeze reservoir and lake Hashenge, Ethiopia. *International Journal Fisheries and Aquaculture Studies*; 4(1): 160-164
- USDA Food Safety Information. (2013). Freezing and Food Safety, http://www.fsis.usda.gov/wps/wcm/connect/cce745c9-0fc9-4ce6-a50c-84363e5b5a48/Freezing_and_Food_Safety.pdf?MOD=AJPERES.



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