

Effect of Transportation on Chemical And Microbial Analysis of Frozen Marine Fish (*Sardinella Species*)

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Abstract

Microbiological contamination, particularly from unspecified spoilage bacteria such as *Salmonella* sp., *Vibrio* spp., *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*, has raised concerns. This study aims to evaluate the effect of transportation on chemical and microbiological status of frozen imported fish purchased in Wukari markets, Taraba State. Proximate composition analysis and microbial assays were conducted. The highest protein content was recorded in frozen marine fish samples from Marmara market, followed by New market and Old market respectively. The bacterial count in New market was 1.94×10^4 , Old market it was 0.97×10^4 , and Marmara market was 1.1×10^3 . The fungal count in New market was 5.0×10^3 , Old market it was 4.0103 , and Marmara market was 4.1×10^3 . *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *E. coli* were isolated from both New market and Old market, with a percentage occurrence of 37.50%; however, *E. coli* and *S. aureus* were present in Marmara market with a percentage occurrence of 25%. Nonetheless, the results obtained from the sampled fish species fell within a safe range for human consumption.

Keywords: Marine Fish, Microbiological Analysis, Proximate composition, Transportation.

Introduction

Frozen marine fish displays third order biotic activity. It belongs to the class of foods in which the respiration *process is suspended, but in which biochemical, microbial and other decomposition processes which must be considered still proceed [15]. Fish and bacteria exist in a state of equilibrium, and it is only after death that spoilage bacteria can invade the tissue and spoil the fish. [13] Reported that secrete digestive juices and enzymes which breakdown the tissue and cause spoilage of fish [15]. Spoilage organisms such as *Salmonella typhi*, *Psuedomonas aeruginosa* and *Escherichia coli* causes' loss of flavour and odour. Fish carry a flora of psychrotrophic bacteria, most of which survive freezing; and are ready to grow on thawing [27]. Fish may harbor a number of biohazards as well as chemical contaminations such as biogenic amines, biotoxins, pathogenic bacteria and viruses if not properly handled [16].

Fish is one of the most important components of feed for animals and human beings, because of their excellent nutritional profile and easily digestible characteristics [28]. It provides high-quality rich protein, lipids, vitamins, minerals, essential amino acids, fatty acids and various extractable compounds required for the growth, development and maintenance of a healthy human body and prevents several nutritional deficiency diseases [23].

Fish is more perishable than other proteinaceous animal food and its freshness is the most important criteria for judging the quality [3]. Proper post-harvest handling of catch is the most crucial step in the production of a high quality finished fishery product to meet the consumer demand [9]. Contamination of fish with pathogenic bacteria reflects use of un-cleaned utensils, contaminated water and ice, inadequate amount of ice and unhygienic handling practices [26].

Microbiological quality study of frozen marine fish has great importance to public health as it is directly related to spoilage of fish and food poisoning. Microbial hazards causing infections and poor health which are closely related to food security concerning with animal proteins derived from marketed food such as fish, fishery products, meat and meat products. This poses a burning question for all consumers with high-risk related to pathogenic bacterial contaminations concerning food security challenges. Food borne disease results from the ingestion of bacteria and toxins produced by microorganisms present in the spoilt food.

The microbiological contamination concern has been on high loads of unspecified spoilage bacteria like *Salmonella spp.*, *Vibrio spp.*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* [14]. Initial micro-flora on fish is directly related to the surrounding aquatic environment while the bacterial flora in the gastrointestinal tract corresponds to the condition of the fish [19]. It has been well known that fish can harbor human pathogenic bacteria particularly the coliform group [3].

Microbiological quality is of importance to public health as it directly relates to spoilage of fish and becomes the cause of food poisoning. Microbial hazards causing infections and poor health are closely related to food safety concerns with animal proteins derived from marketed food -fish, fishery products,

meat and meat products. This creates a burning question for all consumers with a high risk commodity with regard to pathogenic bacteria contaminations alarming to food safety challenge [22].

Proximate composition is traditionally used as an indicator of nutritional quality of food materials. The fishes have moisture, protein, fat and ash in abundance; nitrogen free extract in minute quantity and other constituents like vitamins and minerals in reasonable quantity [20]. In fishes, variation of biochemical composition in fish body relates closely with feed intake [25]. The higher the percentage of moisture in the composition is a good indicator of the relative energy, protein and lipid content; the lower the percentage of moisture, the greater the lipids and protein content and the higher the energy density of the fish [2]. Proteins are not only necessary for hormonal and enzyme development but also an important source of energy [18]. Fats provide much energy and essential fatty acids while minerals are the major component of bones, blood, and play an important role in osmoregulation (Gatlin, 2010).

The deteriorative changes occurring in fish results in the gradual accumulation of volatile and carbonyl compounds in the flesh due to the effect of varieties of biochemical and microbial mechanisms and this calls for this study.

Materials and Methods

Study Areas

The study was carried out in 2020 at Wukari Market, Wukari Local government area, Taraba State. The Fish samples were collected from Old market, New market and Mammara market of Wukari town, Taraba State. Wukari LGA lies on latitude 7°51'N and longitude 9°47'E which covers 4,308 km². (Fig. 1) Shows The Local Government shares borders with Benue and Nasarawa states from the South and West, respectively. It is also closely bounded by LGAs like Donga (East), Gassol (North) and Ibi (North-west) in Taraba State [4] , [7]. (Fig. 2) Zaria is a city in northern Nigeria, located at coordinates 11.1176N E. it is the second largest city in Kaduna State and is known for its historical significance as a major trading hub in the region. With a population of over one million people, Zaria is a bustling city with a mix of traditional and modern influences. The city is also home to Ahmadu Bello University, one of the largest and most prestigious universities in Nigeria.

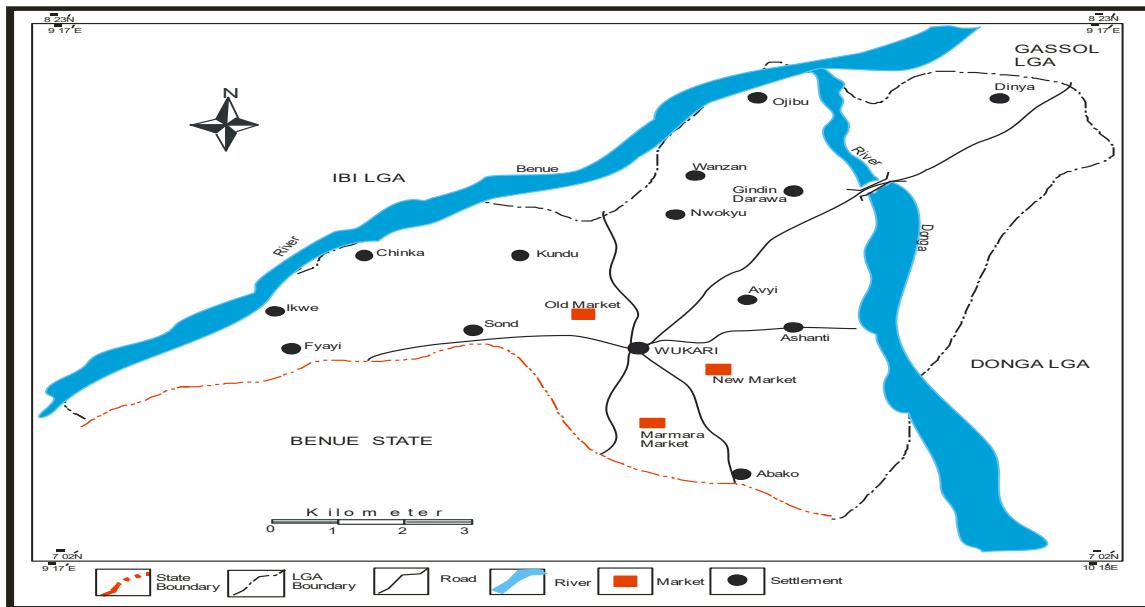


Figure 1: Map of Wukari town showing: Old market, New market and Mammara market (Study areas)

Sampling Method

Eighteen Frozen marine fish were bought from fish mongers at Wukari market for identification, chemical composition and microbial analyses and samples were preserved in a refrigerator after which it was transported in a cooler with ice packs to Biochemical Laboratory, Department of Animal Science, Ahmadu Bello University Zaria, Nigeria for analyses.

Experimental Procedure

Collection of Sample Fish

The fish samples were analysed for moisture content, crude protein, crude fat, crude fibre, ash content and nitrogen free extracts using the method outline by the Association of Official Analytical Chemists(AOAC) in the year 2000.

Determination of Moisture Content

Approximately 10g of sample were oven dried at 105°C for 6 hours, it was weighed after cooling. The sample was weighed periodically (after every hour) until a consecutive uniform weight is obtained. The formula below was used to calculate the moisture content of the sample.

$$(\%) \text{ MC} = \frac{\text{Weight fresh sample} - \text{Weight dry sample}}{\text{Weight fresh sample}} \times 100$$

Where;

MC = moisture content,

W1 = weight of processed sample,

W2 = weight of dried sample.

Determination of Ash Content

Approximately 3g of sample was weighed and placed in a crucible prior to hashing in a furnace. The sample was heated at 550°C for 12 hours and allowed to cool. The weight of ash was obtained by weighing the crucible on the analytical balance. The crude ash content was obtained using the formula below;

$$\% \text{ AC} = \frac{(\text{Weight of ash})}{\text{weight of sample}} \times 100$$

Where;

AC = ash content

W1 = weight of ash

W2 = weight of processed sample

Determination of Crude Protein

The crude protein was determined by Kjeldahl method of digestion, distillation and titration. The percentage of crude protein will be determined using the following calculation for standard HCl titrant:

$$\% \text{ N} = \frac{\text{ml acid} \times \text{normality standard acid}}{\text{Wt of sample (g)}}$$

Where

A = NHCl required for sample,

B = NHCl required for blank,

N = normality of titrant,

W = weight of sample (g)

Crude protein content (%) = %Nitrogen × 6.25

Determination of Crude Lipid

Approximately 5g of sample was weighed into a bag made of muslin cloth and placed in a soxhlet extraction unit. The unit was then connected to a round bottom flask containing 2/3 full of petroleum ether (boiling point is 60 - 80°C). The petroleum ether was allowed to boil for 6 hrs. Then, the ether evaporated into a fume hood and the flask was still allowed to cool down at room temperature. The fat content was calculated using the formula below:

$$\% \text{ CLC} = \frac{\text{Wt of fat}}{\text{Wt of sample}} \times 100$$

Where

CLC = crude lipid Content;

W1 = weight of round bottom flask with sample;

W2 = weight of clean empty round bottom flask

Determination of Crude Fibre

Fibre was determined using trichloroacetic acid method as recommended by (AOAC, 2000). The percentage of crude fibre was obtained using this formula:

$$\% \text{ CF} = \frac{Wt \text{ of crucible} + \text{dried residue} - wt \text{ of crucible} + \text{ashed residue}}{Wt \text{ of sample}} \times 100$$

Where

CF = crude fibre;

W1 = initial weight;

W2 = final weight

W3 = weight of processed sample

Determination of Nitrogen Free Extracts

NFF was determined using differential method as recommended by [6]. The

Percentage carbohydrate was obtained using the formula:

$$\% \text{ CHO} = 100 - \text{Ash\%} + \text{fibre\%} + \text{protein\%} + \text{fat\%}.$$

Microbiological Analysis

Preparation of Culture Media

All media used were weighed appropriate and prepared according to the manufacturer protocol. They were autoclaved at 121°C for 15 minutes at 15 psi. The cooled were poured into Petri dish and then allowed to cool and solidify.

Culturing of Sample

Each plate was carefully labeled on top and one milli-liter (1ml) each dilution from 10¹-10¹⁰ and pipette into nutrient agar plates. Shaking of the plates were done as soon as the agar was poured, so as to have the microorganisms separated during growth. The medium was allowed to set on a flat top bench after anaerobically at 37° for 24 hours. After 24hrs the plate was observed for colour, shape, size. And it was sub-culture on a nutrient agar for pure colonize. The plate was incubated in an incubator 24hours. After 24 hours the following bio-chemical analysis was carried out on the pure culture isolated. Gram's stain, Catalase test, Coagulase test, Citrate utilization test, Indole test, Oxidase test, Voges prokauer test and Motility test (Chen *et al.*, 2014).

Gram's Staining

The tests was done according to [21]. Small amount of the fish sample was placed on a slide and heat fixed by passing the slide through a bunsen burner three times, Crystal violet was added to the slide and incubated for 60seconds. The slide was rinsed with a gentle stream of water for 5seconds to remove unbound crystal violet. Iodine was added for 60seconds to help fix the crystal violet bacteria cell wall it was rinsed with acetone for 3seconds and rinsed gently with water. Safarin was added to for 5 seconds. A purple color signifies Gram (+) positive while pink color background indicates Gram (-) negative.

Catalase Test

This test was done according to [10]. The test was performed by dropping a loopful of the isolate mix with the hydrogen peroxide on the slide.

Citrate Utilization Test

This test was done according to [10]. The test was used to identify which of the isolates can utilize citrate as the sole sources of carbon for metabolism. The test is usually used as an aid in the differentiation of organisms in the Enterbacteriacca group. Inoculate Simon's citrate medium in sterile test tubes with a loopful of culture. Incubate tube at 37°C for 24hours. The change from green to blue is a positive result.

Oxidise Test

This test was done by dropping 2-5 drops of freshly prepared oxidase (p-aminodimethylamine) reagent in a filter paper, the suspected organisms is picked using a sterile wire loop and mix with the oxidize reagent.

Vogas Proskaeur Test

This test was carried out in accordance to [10] to detect the presence of acetoin and indole production in bacteria. The sample was cultured into a VP broth and incubated at 37°C for 24-48 hours, After incubation, 1-2 drops of alpha-naphthol reagent was added to the broth and 1-2 drops of KOH was also added to the broth well mixed which gives pink color.

Coagulate Test

This test was done according [10] to differentiate *Staphylococcus aureus* and other *Saphylococcus species*. Add 2-3 drops of normal saline on a grease free slide to the normal saline mix the suspected organism and add 1-2 drops of plasma and rock, the presence of agglutination means a positive result while no agglutination mean a negative results.

Indole Test

This test was done according to [24] to differentiate members of enterobacteriacea, *Escheichia Coli* with Indole positive. The test organism was inoculated in a test tube containing 3ml of sterile trytone water. Incubation was done at 37°C for 24 hours. The test for indole was done by adding 0.5ml of Kovae's reagent and shaken gently.

Motility Test

This test is to identify members of vibranaceae and most members of the enterobacteriaceae which are also motile. The mobility medium was inoculated using a needle to make 5 stabs of the test organism to the depth of 1 – 2cm of the bottom of the tube was incubated at 37°C 24hours the line of incubation was examined for cloudiness showing the organism is motile [10].

Statistical Analysis

All data was subjected to the analysis of variance (ANOVA) and a statistical significances of mean were calculated using descriptive statistics using the least significance difference (LSD) in the mean comparison of means at $p<0.05$ level of significance with aid of statistics 10.0 version.

Results

Table 1. Shows the Mean Proximate Composition of Frozen Marine Fish Bought in Wukari Markets. The proximate composition of frozen marine fishes bought in Wukari markets (New, old and marmara). The highest protein content of the frozen marine fish was recorded from the samples purchased at Marmara market (72.50 ± 5.20), followed by frozen marine fish obtained from New market and Old market with (68.22 ± 2.11 and 65.46 ± 4.73) respectively. New market has highest moisture content (12.55 ± 0.88), followed by Old market (12.49 ± 6.27) and Marmara market (10.635 ± 1.04). Old market and Marmara market recorded the lowest ash content (10.35 ± 0.295 and 10.35 ± 0.050) while New market recorded highest ash content (11.195 ± 0.035). The high crude lipid mean value (5.885 ± 0.005) was recorded at New market with it lowest (4.500 ± 0.005) at Marmara market. Marmara market has the highest crude fibre (0.035 ± 0.005), followed by Old market (0.028 ± 0.0125) and New market (0.025 ± 0.005). The highest Nitrogen free extract was recorded in Old market (48.720 ± 5.35), follow by New market and Marmara market (44.665 ± 1.945) and (44.605 ± 3.615) respectively.

Table 1: Chemical Composition of Frozen Marine Fish Bought in Wukari Markets

Location	%M	%CP	% CF	%CL	%Ash	%NFE
New Market	12.55 ± 0.88^a	68.22 ± 2.11^b	0.025 ± 0.005^b	5.885 ± 0.005^a	11.195 ± 0.035^a	44.665 ± 1.945^b
Old Market	12.49 ± 6.27^a	65.46 ± 4.73^c	0.028 ± 0.0125^b	5.455 ± 0.015^b	10.35 ± 0.295^a	48.720 ± 5.35^a
Marmara	10.635 ± 1.04^b	72.50 ± 5.20^a	0.035 ± 0.005^a	4.500 ± 0.005^c	10.35 ± 0.050^a	44.605 ± 3.615^b

The mean values with different letters superscript are significantly different ($p<0.05$)

Table 2. Shows Microbial Population (Cfu/g) In Frozen Marine Fish Bought of Wukari Markets.

The New market recorded bacterial count ($1.94 \times 10^4\pm0.20$), Old market has bacteria count ($0.97 \times 10^3\pm0.30$) and Marmara market revealed bacterial count ($1.1 \times 10^3\pm0.25$). New market presented fungal count ($5.0 \times 10^3\pm0.10$), Old market reported ($4.0 \times 10^3\pm0.10$) and Marmara market has ($4.1 \times 10^3\pm0.30$). *E. coli* has ($7.0 \times 10^3\pm0.20$), ($7.0 \times 10^3\pm0.40$) and ($7.3 \times 10^3\pm0.10$) obtained from New market, old market and Marmara market respectively. Also *S. typhi* recorded ($1.0 \times 10^7\pm0.10$), ($1.3 \times 10^7\pm0.20$) and ($1.5 \times 10^7\pm0.10$) from New market, Old market and Marmara market. The highest *E. coli* count recorded at Marmara market ($7.3 \times 10^3\pm0.10$) followed by New and Old market ($7.0 \times 10^3\pm0.20$ and $7.0 \times 10^3\pm0.40$)

respectively. The highest *S. typhi* count was recorded at Marmara market ($1.5 \times 10^7 \pm 0.10$) followed by Old and New market ($1.3 \times 10^7 \pm 0.20$ and $1.0 \times 10^7 \pm 0.10$) respectively.

Table 2. Microbial population (cfu/g) in frozen marine fish bought of Wukari markets

Location	Bacterial Count	Fungal Count	<i>E. coli</i>	<i>S. typhi</i>
New Market	$1.94 \times 10^4 \pm 0.20$ a	$5.0 \times 10^3 \pm 0.10$ a	$7.0 \times 10^3 \pm 0.20$ b	$1.0 \times 10^7 \pm 0.10$ c
Old Market	$0.97 \times 10^3 \pm 0.30$ c	$4.0 \times 10^3 \pm 0.10$ b	$7.0 \times 10^3 \pm 0.40$ b	$1.3 \times 10^7 \pm 0.20$ b
Marmara	$1.1 \times 10^3 \pm 0.25$ b	$4.1 \times 10^3 \pm 0.30$ b	$7.3 \times 10^3 \pm 0.10$ a	$1.5 \times 10^7 \pm 0.10$ a

Table 3. Present the Percentage Bacteria Isolation in Frozen Marine Fish (*Sardinlalla spp.*) Bought in Wukari Markets.

The percentage bacteria isolation in frozen marine fish (*Sardinlalla spp.*) bought in Wukari markets shows that three (3) different species bacteria were isolated; *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *E. coli*. All the bacteria species isolated were also present in New market and Old market with percentage occurrence of 37.50% and 37.50%; *Escherichia coli* and *S. aureus* were present at Marmara market with percentage of occurrence of 25%.

Table 3: Percentage Bacteria Isolation in Frozen Marine Fish (*Sardinlalla spp.*) Bought in Wukari Markets in 2020

Location	Frequency of occurrence	Percentage (%)	Bacteria Isolated
New Market	3	37.50	<i>S. aureus</i> , <i>P. aeruginosa</i> and <i>E. coli</i>
Old Market	3	37.50	<i>S. aureus</i> , <i>P. aeruginosa</i> and <i>E. coli</i>
Marmara	2	25.00	<i>S. aureus</i> and <i>E. coli</i>
Total	8	100	

Discussion

The moisture content ranges between 10.63% (minimum) to 12.55% (maximum) obtained in sample from New market and Marmara market respectively while Old Market has 14.49%. The finding disagreed with the study of [8] who reported high (80-85) moisture content. Higher protein content is generally favorable for fish, as they are proteineous animals. All samples belong to the high protein categories. The highest value was observed in Marmara market with 72.50 ± 5.20 which might be considered as having a good protein profile, potentially suitable for fish feed. There is a significant difference ($p < 0.05$) between the samples obtained from frozen marine fish bought in Wukari markets, this is in conformity with the high crude protein (64.88-66.48) reported by [11]. The high crude protein (68.22 ± 2.11) in New market agreed

with the result of [17] whose reported high 68.05 ± 1.64 crude protein from their study. Fish also require essential fatty acids for growth and development. The crude lipid content is not exceptionally high in the provided samples. New Market has the highest crude lipid content 5.885 ± 0.005 which could be of interest for its potential as a source of essential fatty acids [12]. Fish generally do not have a significant requirement for crude fiber. The crude fibre range between 0.025 ± 0.005 to 0.035 ± 0.005 which is significantly low. The low CF values in the samples are desirable, indicating a lower indigestible plant material content as similarly reported by [5]. The ash content for the samples ranged from 10.35 ± 0.050 to 11.195 ± 0.035 with the highest value observed in New market. There is a significance difference ($p<0.05$) between the tested samples. The observed range of ash value indicates that all tested samples are good sources of minerals such as calcium, potassium, zinc, iron and magnesium [5], [12].

New market recorded highest $1.94 \times 10^4\pm0.20$ bacterial count while low $0.97 \times 10^3\pm0.30$ was observed from Old Market and Marmara market has intermediate bacterial count of $1.1 \times 10^3\pm0.25$. The presence or absence of bacteria can be indication of hygiene or contamination level of these fish samples. From the study of [1] reported that micro-organisms may contaminate fish through human handling, air and soil. The presence of the micro-organisms in fish samples might be due to increase in moisture content of the product during storage and also increase in temperature which favours the growth of the organisms.

Staphylococcus aureus, *Pseudomonas aeruginosa* and *E. coli* were present in both samples collected at New market and Old market with percentage occurrence of 37.50%, however, *Escherichia coli* and *S. aureus* were present at Marmara market with percentage of occurrence of 25%. During handling of fish, the natural flora of the fish environment will be contaminated with organisms associated with man, such as members of *Enterobacteriaceae* and *Staphylococcus aureus* as which grow well at 30-70°C the occurrence of *Staphylococcus* in the smoked dried fish samples was in accordance with regular monitoring and quality control measures are crucial for safe and high-quality fish products, benefiting public health and the local economy. However, from the result obtained from this research, the sampled fish species fell within safe range for fish consumption.

Conclusion

Although Sea food is part of a healthful diet, its consumption is not out of risk worldwide continues outbreak of sea food associated infections have rendered the existing control strategies questionable. The chemical and microbiological assessment of frozen *Sardinella* species fish bought from Wukari market's highlights the need for better monitoring and regulation to ensure fish consumer safety. The mechanism of contamination that are amenable to control is thus necessary for the prevention of sea food associated infection outbreak. Coordinated effort from government sectors and private industries together with federal agencies are urgently in this contest. There is need for surveillance systems using pathogens specific techniques to avoid sea food infection outbreaks. However, the microbial assessment carried out on frozen marine fish (*Sardinellai spp*) bought in Wukari markets shows it's within the limit of the ICMSF so it can be concluded that this species were processed with properly treated pathogen free water and ice

and finally maintained at a good storage condition. The investigated frozen marine fishes were qualified enough for transport as well human consumption.

Conflict of Interest

The authors declare no conflict of interest.

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