IKR Publishers

IKR Journal of Agriculture and Biosciences (IKRJAB)
Journal homepage: https://ikrpublishers.com/ikrjab/
Volume-1, Issue-2 (July-August) 2025
OPEN ACCESS
ISSN: 3107-5509 (Online)

Effect of Transportation on Chemical and Microbial Analysis of Frozen Marine Fish (Sardinella species)

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DOI:*10.5281/zenodo.17214759*

ARTICLE INFO

Article history:
Received : 17-09-2025
Accepted : 22-09-2025
Available online : 27-09-2025

Citation: Mohammed AM, Modu AB, Yunisa M and Amos E (2025). Effect of Transportation on Chemical and Microbial Analysis of Frozen Marine Fish (Sardinella species). *IKR Journal of Agriculture and Biosciences (IKRJAB)*, 1(2), 66-71.

ABSTRACT **Original research paper**

Concerns have been raised over microbial contamination in frozen fish, particularly from bacteria such as Salmonella sp., Vibrio spp., Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli. This study assessed the impact of transportation on the chemical composition and microbial quality of frozen imported Sardinella spp. sold in markets within Wukari, Taraba State. Proximate composition and microbial analyses were conducted. Results showed that fish from Marmara market had the highest protein level (72.50±5.20), followed by samples from New market (68.22±2.11) and Old market (65.46±4.76). Bacterial counts were highest in New market (1.94×10^4), while Old market and Marmara market had 0.97×10^3 and 1.1×10^3 respectively. Fungal load was 5.0×10^3 in New market, 4.0×10^3 in Old market, and 4.1×10^3 in Marmara. Pathogens including S. aureus, P. aeruginosa, and E. coli were isolated in both Old and New markets (37.5% occurrence each), whereas Marmara market samples contained E. coli and S. aureus (25%). Overall, the values obtained fell within safe consumption limits.

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

Abbreviations: FAO: Food and Agriculture Organization; CFU: Colony Forming Units; ANOVA: Analysis of Variance; KOH: Potassium Hydroxide.

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Introduction

Frozen marine fish exhibit third-order biotic activity, meaning that although respiration ceases after harvest, several biochemical, microbial, and degradative processes continue to occur [1]. During life, fish maintain a balance with bacterial populations, but after death, spoilage microorganisms gain access to tissues and initiate deterioration. According to Eyo [2], digestive enzymes secreted by fish accelerate tissue breakdown, further encouraging microbial action [1]. Spoilage organisms such as Salmonella typhi, Pseudomonas aeruginosa, and Escherichia coli are associated with undesirable changes in flavor and odor. Psychrotrophic bacteria are also of concern, as many survive freezing and resume growth upon thawing [3].

Additionally, fish may contain biological hazards and chemical contaminants, including pathogenic bacteria, viruses, biotoxins, and biogenic amines, especially under poor handling conditions [4].

Fish remain one of the most important sources of food for both humans and animals due to their high nutritional value and ease of digestion [5]. They provide quality protein, lipids, vitamins, minerals, essential amino acids, and fatty acids, all of which support growth, development, and maintenance of health while preventing deficiency diseases [6]. Compared to other protein foods, fish are highly perishable, and freshness is considered the main indicator of quality [7]. Effective post-harvest handling is therefore critical to produce high-quality products that meet consumer

expectations [8]. Contamination often occurs when fish are exposed to unclean utensils, contaminated water, inadequate ice, or unhygienic handling practices [9].

Assessing the microbiological quality of frozen fish is essential for public health, since spoilage organisms and pathogenic bacteria are linked to food poisoning and food security issues. Consumption of animal protein sources such as fish, meat, and fishery products may pose risks if microbial hazards are present, leading to illness from either viable bacteria or toxins produced in spoiled food. Common contaminants include Salmonella spp., Vibrio spp., Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli [10]. The microbial flora of fish depends on both the aquatic environment and the gastrointestinal condition of the species [11]. Notably, fish are capable of harboring human pathogens, especially coliforms [7].

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 [12].

The proximate composition of fish is a key indicator of nutritional quality. Fish generally contain significant amounts of moisture, protein, fat, and ash, with smaller proportions of nitrogen-free extract, vitamins, and minerals [13]. Variations in biochemical composition often reflect feeding habits and diet [14]. Moisture levels are inversely related to lipid and protein content, thereby influencing the energy density of the fish [15]. Proteins serve not only as a source of energy but also play essential roles in hormone and enzyme production [16]. Lipids supply energy and essential fatty acids, while minerals are critical for bone development, blood composition, and osmoregulatory functions [17].

The progressive deterioration of fish results from the accumulation of volatile and carbonyl compounds generated through biochemical and microbial processes. These changes form the basis of the present study, which seeks to assess both the nutritional and microbiological quality of frozen fish

Materials and Methods

Study Areas

The study was carried out in Wukari Local Government Area, Taraba State. Fish samples were obtained from Old market, New market, and Marmara market in Wukari town. Wukari LGA lies at latitude 7°51′N and longitude 9°47′E, covering about 4,308 km². It shares boundaries with Benue and Nasarawa States to the south and west, and with Donga, Gassol, and Ibi LGAs within Taraba State.[18; 19].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.

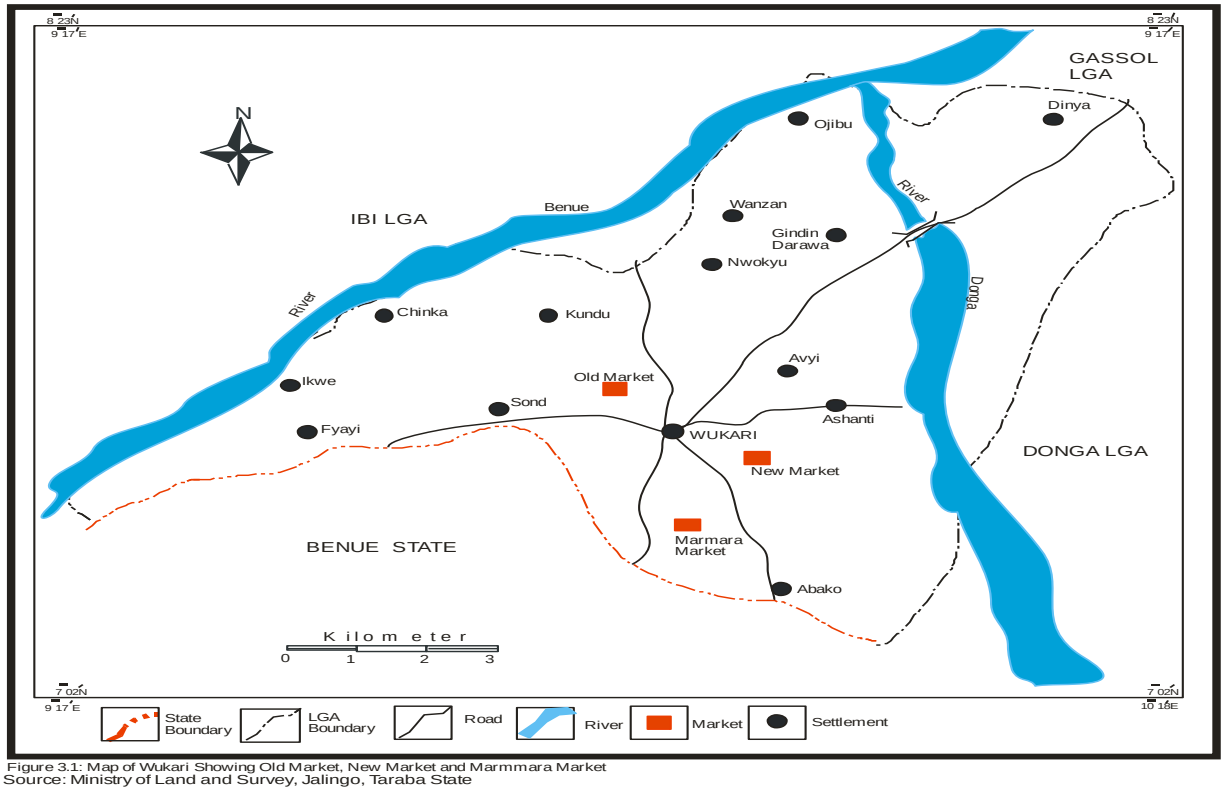

Figure 3.1: Map of Wukari Showing Old Market, New Market and Marmmara Market
Source: Ministry of Land and Survey, Jalingo, Taraba State

Figure 1: Map of Wukari town showing: Old market, New market and Mammara market

Sampling Method

Eighteen frozen imported *Sardinella spp.* were purchased from fishmongers across the three selected markets. The samples were transported under refrigeration to the Biochemical Laboratory, Department of Animal Science, Ahmadu Bello University, Zaria, for proximate and microbial analyses. Identification of fish species was done using FAO species identification sheets [20].

Proximate Composition Analysis

Standard methods described by [21] were used to determine:

- Moisture content by oven-drying samples at 105°C until constant weight.
- Ash content by incinerating samples at 550°C in a muffle furnace.
- Crude protein via the Kjeldahl method, where nitrogen content was multiplied by 6.25.
- Crude lipid through Soxhlet extraction with petroleum ether.
- Crude fibre using trichloroacetic acid digestion.
- Nitrogen-free extract (NFE) was obtained by difference.

Microbiological Analysis

Culture media were prepared according to manufacturer's instructions and sterilized by autoclaving. Serial dilutions (10^{-1}–10^{-10}) of homogenized samples were inoculated onto nutrient agar plates and incubated at 37°C for 24 hours. Distinct colonies were purified and subjected to biochemical tests including Gram staining, catalase, coagulase, citrate utilization, indole, oxidase, Voges-Proskauer, and motility tests to identify isolates[21; 22; 23; 24].

Statistical Analysis

Data were analyzed using Analysis of Variance (ANOVA). Means were compared using Least Significant Difference (LSD) at 5% probability, with IBM SPSS V25 software.

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 Wukarimarkets (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 obtainedfrom 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 (Cfc/g) In Frozen Marine Fish Bought of Wukari Markets.

The New market recorded bacterial count (1.94 x 10^4±0.20), Old market has bacteria count (0.97 x 10^3±0.30)and Marmara market revealed bacterial count (1.1 x 10^3±0.25). New market presented fungal count (5.0 x 10^3±0.10), Old market reported (4.0x10^3±0.10) and Marmara market has(4.1 x 10^3±0.30). *E. coli* has(7.0 x 10^3±0.20), (7.0 x 10^3±0.40) and (7.3 x 10^3±0.10) obtained from New market, old market and Marmara market respectively. Also *S. typhi*recorded (1.0 x 10^7±0.10), (1.3 x 10^7±0.20) and (1.5 x 10^7±0.10) from New market, Old market and Marmara market. The highest *E. coli* count recorded at Marmara market (7.3 x 10^3±0.10) followed by New and Old market (7.0 x 10^3±0.20 and 7.0 x 10^3±0.40) respectively. The highest *S. typhi*count was recorded at Marmara market (1.5 x 10^7±0.10) followed by Old and New market (1.3 x 10^7±0.20and 1.0 x 10^7±0.10) respectively.

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

Location	Bacterial Count	Fungal Count	*E. coli*	*S. typhi*
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 aeroginosa* 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

Location	Freq. of occurrence	Percentage (%)	Bacteria Isolated
New Market	3	37.50	*S. aureus, P. aeroginosa and E. coli*
Old Market	3	37.50	*S. aureus, P. aeroginosa and E. coli*
Marmara	2	25.00	*S. aureus and E. coli*
Total	8	100	

Discussion

From (Table. 1) 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 [25] whose reported high (80-85) moisture content. Higher protein content is generally favorable for fish, as they are proteinaceous animals. All samples belong to the high protein categories with mean value ranging from 65.46±4.73 to 72.50±5.20. 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 that reported by [26]. The high crude protein (68.22±2.11) in New market agreed with the result of [27] whose reported 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 [28]. 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 [29]. 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 [29, 28].

From (Table. 2) New market recorded highest $1.94 \times 10^4 \pm 0.20$ bacterial count while low $0.97 \times 10^3 \pm 0.30$ was observed from Old Market and Marmara market has intermediate bacterial count of $1.1 \times 10^3 \pm 0.25$. The presence or absence of bacteria can be indication of hygiene or contamination level of these fish samples. From the study of [30] who's reported that micro-organisms may contaminated 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. From (Table 3) *Staphylococcus aureus*, *Pseudomonas aeroginosa* and *E. coli* were present in both samples collected at New market and Old market with percentage occurrence of 37.50% for each, 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^{o}C the occurrence of *Staphylococcus* in the smoked dried fish samples was in accordance to [31]. 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

Seafood is an important dietary component, supplying essential proteins, fatty acids, vitamins, and minerals, yet its perishable nature makes it highly susceptible to microbial contamination. This study evaluated the nutritional and microbial quality of frozen Sardinella spp. sold in Wukari markets. Results showed high protein content with moderate fat and ash levels, confirming the species as a valuable nutritional resource. Microbial analysis revealed the presence of Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli, although counts remained within acceptable safety limits. The findings highlight the need for improved hygienic handling, stricter monitoring during transportation and marketing, and stronger quality control measures. Such interventions are essential to reduce contamination risks, safeguard consumer health, and enhance public confidence in seafood products.

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