

Steaming as a Pre-Treatment Strategy to Improve the Microbiological Safety, Physicochemical Quality, and Sensory Attributes of Refrigerated Nile Tilapia (*Oreochromis Niloticus*) During Cold Storage

Haram Hassan Abbas Bakhiet^{1*} and Sara Abker²

¹Department of Fisheries and Wildlife Science College of Animal Production Science and Technology, Sudan University of Science and Technology P.O.BOX 204, Khartoum North, Sudan

²Department of fisheries, college of natural resources, university of Bahri, Sudan

***Corresponding author:** Haram Hassan Abbas Bakhiet, Department of Fisheries and Wildlife Science College of Animal Production Science and Technology, Sudan University of Science and Technology P.O.BOX 204, Khartoum North, Sudan

ARTICLE INFO

Received: 📅 August 27, 2026

Published: 📅 September 09, 2026

Citation: Haram Hassan Abbas Bakhiet and Sara Abker. Steaming as a Pre-Treatment Strategy to Improve the Microbiological Safety, Physicochemical Quality, and Sensory Attributes of Refrigerated Nile Tilapia (*Oreochromis Niloticus*) During Cold Storage. Biomed J Sci & Tech Res 66(4)-2026. BJSTR. MS.ID.010381.

ABSTRACT

Fish is highly susceptible to microbial spoilage during refrigerated storage, making the development of simple and effective preservation strategies essential for maintaining its safety and quality. This study evaluated the effect of steaming as a pre-treatment on the microbiological quality, proximate composition, and sensory attributes of refrigerated Nile tilapia (*Oreochromis niloticus*) during 10 days of cold storage. Fifty fish were randomly assigned to two groups: raw refrigerated fish (control) and steamed refrigerated fish. The treatment group was steamed at 100°C for 15 min before storage at $4 \pm 1^\circ\text{C}$. Microbiological, proximate composition, and sensory analyses were performed at 2-day intervals throughout storage. Steaming significantly reduced the total viable count ($P < 0.01$), with bacterial counts increasing from 5.3×10^2 to 3.4×10^3 CFU/g in steamed fish compared with 5.0×10^4 to 8.9×10^4 CFU/g in raw fish after 10 days of refrigerated storage. *Escherichia coli* and *Salmonella* spp. were not detected in either treatment throughout storage, whereas low total coliform counts were detected only in raw fish on days 8 and 10. Steamed fish exhibited lower moisture content (70.1–72.3%) and higher protein content (25.7–26.4%) than raw fish (78.9–79.9% and 21.2–22.6%, respectively), while fat (1.1–1.5%) and ash (1.8–2.1%) contents remained relatively stable during storage. Although sensory scores gradually declined in both treatments, steamed fish maintained acceptable texture, odour, and colour throughout the storage period. These findings demonstrate that steaming prior to refrigeration effectively improves the microbiological safety of Nile tilapia while preserving its nutritional quality and acceptable sensory characteristics during chilled storage. Steaming therefore represents a simple, practical, and cost-effective preservation strategy for extending the refrigerated shelf-life and enhancing the quality of freshwater fish.

Keywords: *Oreochromis Niloticus*; Steaming; Refrigerated Storage; Microbiological Quality; Physicochemical Properties; Sensory Evaluation; Fish Preservation; Food Safety; Shelf-Life

Abbreviation's: ANOVA: Analysis of Variance; LSD: Least Significant Difference; SD: Standard Deviation; MPN: Most Probable Number; TVC: Total Viable Count

Introduction

Fish and aquatic foods are important sources of high-quality protein, long-chain omega-3 fatty acids, vitamins, and essential minerals, contributing substantially to food security, human nutrition, and sustainable livelihoods. Global fisheries and aquaculture production

continues to increase, highlighting the growing importance of aquatic foods in meeting the demand for nutritious and affordable animal protein [1]. Among freshwater species, Nile tilapia (*Oreochromis niloticus*) is widely farmed and consumed because of its rapid growth, high nutritional value, affordability, and broad consumer acceptance

[1,2]. Despite these advantages, fish is highly perishable owing to its high moisture content, neutral pH, endogenous enzymatic activity, and favourable conditions for microbial growth. Spoilage is driven by microbial proliferation, enzymatic autolysis, and lipid oxidation, resulting in quality deterioration, off-odours, and a limited shelf-life during refrigerated storage [3-6]. Although refrigeration effectively slows microbial and biochemical deterioration, it cannot completely prevent spoilage or eliminate foodborne pathogens, creating a need for complementary preservation strategies [6,7].

Steaming is a mild thermal treatment that can reduce microbial contamination and partially inactivate endogenous enzymes while preserving nutritional quality more effectively than many conventional cooking methods. It also helps maintain muscle structure and limits lipid oxidation, thereby reducing the initial microbial load before refrigerated storage and potentially delaying quality deterioration [4,6,8,9]. Although several preservation techniques, including refrigeration, modified-atmosphere packaging, vacuum packaging, edible coatings, natural preservatives, and thermal treatments, have been investigated, most studies have evaluated microbiological, physicochemical, or sensory quality separately. In addition, steaming has mainly been studied as a cooking method rather than as a pre-treatment before refrigerated storage. Consequently, information on the combined effects of steaming and refrigerated storage on the microbiological quality, proximate composition, and sensory characteristics of Nile tilapia remains limited, particularly under conditions relevant to developing countries [6,7]. Therefore, this study evaluated the effects of steaming as a pre-treatment before refrigerated storage on the microbiological quality, proximate composition, and sensory attributes of *Oreochromis niloticus* during 10 days of refrigerated storage.

Materials and Methods

Experimental Site

The study was conducted at the Department of Fisheries and Wildlife Science, College of Animal Production Science and Technology, Sudan University of Science and Technology, Sudan. All microbiological and proximate analyses were performed under controlled laboratory conditions using standard analytical procedures.

Fish Sampling, Experimental Design and Storage

Fifty fresh Nile tilapia (*Oreochromis niloticus*) of comparable market size and quality were obtained from the university fish farm. Fish were washed, eviscerated, and randomly assigned to two groups (n = 25 each): raw fish (control) and steamed fish (treatment). Fish assigned to the treatment group were steamed using saturated steam at 100°C for 15 min. Before steaming, fish were split longitudinally while remaining intact to ensure uniform heat penetration. The internal muscle temperature reached approximately 70°C, as monitored using a calibrated digital probe thermometer. After cooling to room temperature under hygienic laboratory conditions, all samples were

individually packed in sterile polyethylene bags and stored at $4 \pm 1^\circ\text{C}$ for 10 days. Sampling was performed on days 0, 2, 4, 6, 8, and 10. At each sampling time, independent fish were randomly removed from storage for microbiological, proximate composition, and sensory analyses. Each fish was analysed only once to ensure independent observations. Unless otherwise stated, all analyses were performed in triplicate.

Microbiological Analysis

Microbiological quality was evaluated under aseptic conditions using standard methods. Twenty-five grams of fish muscle were homogenized with 225 mL of sterile 0.1% peptone water to prepare the initial (10^{-1}) dilution, followed by serial decimal dilutions. Total viable count (TVC) was determined on Plate Count Agar (Oxoid Ltd., UK) using the pour plate method after incubation at $35 \pm 1^\circ\text{C}$ for 48 h [10]. Coliform bacteria were enumerated by the Most Probable Number (MPN) method using MacConkey broth and confirmed in Brilliant Green Bile Lactose Broth according to [11]. Faecal coliforms were detected in E.C. broth at 44.5°C , while *Escherichia coli* was confirmed on Eosin Methylene Blue agar and by conventional biochemical tests [12]. *Salmonella* spp. were detected using Selenite Cystine Broth enrichment followed by isolation on Bismuth Sulphite Agar and biochemical confirmation according to [10]. Microbial results were expressed as mean $\pm$ standard deviation.

Sensory Evaluation

Sensory quality was assessed by ten trained panelists on days 0, 2, 4, 6, 8, and 10. Samples were randomly coded and presented in random order. Colour, odour, and texture were evaluated using a 10-point hedonic scale (1 = extremely poor; 10 = excellent quality). Mean scores were used for statistical analysis.

Proximate Composition

Moisture, crude protein, crude fat, and ash contents were determined according to the Official Methods of [13]. Analyses were performed in triplicate and expressed as percentages.

Statistical Analysis

Data were analysed using two-way analysis of variance (ANOVA) with treatment and storage time as fixed factors. When significant differences were detected ($P < 0.01$), means were separated using Fisher's Least Significant Difference (LSD) test. Statistical analyses were performed using IBM SPSS Statistics version 21.0 (IBM Corp., Armonk, NY, USA), and results are presented as mean $\pm$ standard deviation (SD).

Results

Microbiological Quality

(Tables 1-3).

Table 1: Effect of steaming on the microbiological quality of Nile tilapia (*Oreochromis niloticus*) during refrigerated storage (mean $\pm$ SD).

Microbiological parameter	Treatment	Day 0	Day 2	Day 4	Day 6	Day 8	Day 10	Significance
Total viable count (CFU g ⁻¹)	Raw	$5.0 \times 10^4 \pm 0.6^a$	$7.5 \times 10^4 \pm 0.5^b$	$8.7 \times 10^4 \pm 0.7^c$	$8.7 \times 10^4 \pm 0.86^d$	$8.8 \times 10^4 \pm 0.45^e$	$8.9 \times 10^4 \pm 0.40^f$	*
	Steamed	$5.3 \times 10^2 \pm 0.56^a$	$5.1 \times 10^2 \pm 5.20^b$	$4.7 \times 10^2 \pm 6.10^c$	$4.3 \times 10^2 \pm 3.50^d$	$3.6 \times 10^3 \pm 4.20^e$	$3.4 \times 10^3 \pm 1.90^f$	*
Total coliform (MPN g ⁻¹)	Raw	ND	ND	ND	ND	0.4 ± 1.0	1.3 ± 1.5	
	Steamed	ND	ND	ND	ND	ND	ND	NS
Escherichia coli (MPN g ⁻¹)	Raw	ND	ND	ND	ND	ND	ND	NS
	Steamed	ND	ND	ND	ND	ND	ND	NS
Salmonella spp.	Raw	ND	ND	ND	ND	ND	ND	NS
	Steamed	ND	ND	ND	ND	ND	ND	NS

Note: Abbreviations: CFU = colony-forming units; MPN = most probable number; ND = not detected; SD = standard deviation. Values within the same row followed by different superscript letters (a-f) are significantly different ($P < 0.01$) during refrigerated storage.

Table 2: Effect of steaming on the proximate composition of Nile tilapia (*Oreochromis niloticus*) during refrigerated storage (mean $\pm$ SD).

Parameter	Treatment	Day 0	Day 2	Day 4	Day 6	Day 8	Day 10	Significance
Protein (%)	Raw	21.2 ± 0.15^a	21.9 ± 0.14^b	22.1 ± 0.15^c	22.3 ± 0.15^d	22.4 ± 0.15^e	22.6 ± 0.19^f	*
	Steamed	26.4 ± 0.25^a	26.3 ± 0.30^b	26.2 ± 0.30^c	26.1 ± 0.60^d	25.8 ± 1.00^e	25.7 ± 0.20^f	*
Fat (%)	Raw	1.5 ± 0.05^a	1.4 ± 0.14^b	1.3 ± 0.17^c	1.2 ± 0.20^d	1.1 ± 0.05^e	1.1 ± 0.10^e	*
	Steamed	1.3 ± 0.15^a	1.2 ± 0.20^b	1.1 ± 0.20^c	1.1 ± 0.05^c	1.1 ± 0.20^c	1.1 ± 0.05^c	*
Moisture (%)	Raw	79.9 ± 0.30^a	79.8 ± 1.05^b	79.7 ± 0.50^c	79.6 ± 0.40^d	79.5 ± 1.30^e	79.4 ± 0.30^f	*
	Steamed	70.1 ± 0.60^a	70.1 ± 0.05^a	70.5 ± 0.10^b	71.5 ± 1.50^b	71.8 ± 1.40^c	72.3 ± 1.05^d	*
Ash (%)	Raw	2.12 ± 0.17^a	2.1 ± 0.19^a	1.9 ± 0.01^b	1.8 ± 0.05^c	1.8 ± 0.10^c	1.8 ± 0.30^c	*
	Steamed	2.12 ± 0.17^a	2.10 ± 0.19^a	2.0 ± 0.01^b	2.0 ± 0.10^b	2.0 ± 0.30^b	2.0 ± 0.05^b	*

Note: *Values are presented as mean $\pm$ standard deviation (SD). Means within the same row followed by different superscript letters (a-f) are significantly different during refrigerated storage ($P < 0.01$).

Table 3: Effect of steaming on the sensory attributes of Nile tilapia (*Oreochromis niloticus*) during refrigerated storage (mean $\pm$ SD).

Sensory attribute	Treatment	Day 0	Day 2	Day 4	Day 6	Day 8	Day 10	Significance
Texture	Raw	9.8 ± 0.4	9.4 ± 0.5	9.2 ± 0.8	8.1 ± 0.4	7.8 ± 0.4	7.7 ± 0.8	*
	Steamed	9.9 ± 1.9	9.9 ± 0.5	9.6 ± 0.8	8.8 ± 1.9	8.4 ± 0.54	8.3 ± 1.5	*
Odour	Raw	10.0 ± 0.0	9.4 ± 0.5	9.1 ± 0.8	8.6 ± 0.8	8.3 ± 0.9	8.2 ± 1.3	*
	Steamed	9.8 ± 2.07	9.7 ± 1.3	9.6 ± 0.4	9.5 ± 0.4	9.4 ± 0.0	9.1 ± 1.2	*
Colour	Raw	9.6 ± 1.9	9.6 ± 0.8	9.6 ± 0.8	9.5 ± 0.4	9.4 ± 0.4	9.0 ± 0.5	*
	Steamed	9.7 ± 1.2	9.6 ± 0.8	7.6 ± 1.5	7.6 ± 1.5	7.5 ± 0.5	7.5 ± 1.5	*

Note: *Values are presented as mean $\pm$ SD of ten trained panelists ($n = 10$). Sensory attributes were evaluated using a 10-point hedonic scale (1 = extremely poor; 10 = excellent quality). * ($P < 0.01$).

Discussion

Steaming before refrigerated storage markedly improved the microbiological quality of Nile tilapia by substantially reducing the initial bacterial load and suppressing microbial proliferation throughout storage. The lower total viable counts observed in steamed fish confirm that steam treatment effectively inactivated a considerable proportion of spoilage microorganisms before refrigeration, thereby

slowing bacterial growth during chilled storage. Similar findings have been reported for thermally pre-treated fish products, where mild heat treatment reduced spoilage microbiota and improved microbiological stability during refrigerated storage [6,14]. The absence of *Escherichia coli* and *Salmonella* spp. throughout storage further indicates satisfactory hygienic handling and effective processing conditions.

Moreover, the detection of low total coliform counts only in raw fish on days 8 and 10 highlights the additional microbiological protection provided by steaming [7,9]. Steaming also influenced the proximate composition of refrigerated tilapia. The lower moisture content and higher apparent protein concentration observed in steamed fish were mainly associated with moisture loss during thermal processing rather than an actual increase in protein content, consistent with previous reports [8]. Regarding sensory quality, steamed fish maintained acceptable sensory characteristics throughout refrigerated storage despite a gradual decline in quality over time. Overall, steaming before refrigeration improved the microbiological quality of Nile tilapia while preserving its nutritional and sensory quality during refrigerated storage.

Conclusion

Steaming before refrigerated storage significantly improved the microbiological quality of Nile tilapia by reducing bacterial growth throughout 10 days of storage. It also maintained a higher apparent protein concentration due to moisture loss during steaming, with only minor changes in fat and ash contents, although moisture content decreased following thermal processing. Despite slight reductions in sensory scores, steamed fish remained organoleptically acceptable. Therefore, steaming combined with refrigeration can be recommended as a simple, economical, and practical preservation method for improving the microbiological safety, nutritional quality, and refrigerated shelf-life of Nile tilapia.

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ISSN: 2574-1241

DOI: 10.26717/BJSTR.2026.66.010381

Haram Hassan Abbas Bakhiet . Biomed J Sci & Tech Res



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