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The Effect of Natural Edible Coatings on Chemical, Microbial, and Sensory Quality of Tilapia during Frozen Storage

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HIGHLIGHTS:

- Green tea and olive leaves extracts-based edible coatings effectively reduced chemical spoilage and microbial load in Nile tilapia during ice and frozen storage.
- The application of the edible coatings negatively influenced sensory attributes of Nile tilapia, resulting in lower Total Quality Index scores.
- This research presented a crucial challenge in the context of market acceptance of fish treated with these coatings.

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Acronyms and abbreviations
APC=Aerobic Plate Count
CFU=Colony Forming Unit
FC=Faecal Coliform
MPN=Most Probable Number
TC=Total Coliform
TQI=Total Quality Index
TVB-N=Total Volatile Basic-Nitrogen

ABSTRACT

Background: Preserving the quality and safety of fish and its products is a critical concern for food industry. In the present study, the effect of green tea extract and olive leaves extract-based preservative coatings on chemical, microbial, and sensory quality of wild type Nile tilapia (*Oreochromis niloticus*) was determined during pre-freezing ice and subsequent frozen storage.

Methods: Forty-eight ponds raised tilapia, freshly caught in April, 2021, which were divided into three groups: group CT with no coating, group GTE with green tea extract-based coating, and group OLE with olive leaves extract-based coating. Fish samples in all groups were kept in ice for five days (1±2 °C) and then subjected to freezing (-18±2 °C) for 105 days. Chemical (moisture, ash, protein, fat, Total Volatile Basic-Nitrogen (TVB-N), pH) and microbial (Aerobic Plate Count (APC), Total Coliform (TC), Faecal Coliform (FC), *Escherichia coli*) parameters as well as sensory attributes (appearance, odour, acceptability, flesh color, Total Quality Index (TQI)) of tilapia in all groups were analysed after five days of ice storage and 15, 45, 75, and 105 days of frozen storage.

Results: APC of tilapia treated with preservative coatings remained below the permissible limit ($<5\times10^5$ Colony Forming Unit (CFU)/g) throughout the storage period. After five days, content of TVB-N in untreated samples of tilapia was 32.94±2.30 mg/100 g compared to 5.05±2.19 and 7.24±2.89 mg/100 g in tilapia treated with green tea and olive leaves extract-based coating, respectively. Fat content of untreated tilapia was significantly ($p<0.05$) reduced to 0.05±0.01% at the end of the study period from the the initial content of 0.24±0.04%. However, the tilapia treated with preservative coatings achieved significantly ($p<0.05$) lower scores of TQI compared with the untreated one owing to the coloration of fish with extract solution.

Conclusion: Green tea extract and olive leaves extract-based coatings can, therefore, be used to improve fish quality and safety during storage. However, there is a need to change consumer's perception about the sensory attributes of fish.

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Introduction

Fish and fish products, being among the most internationally-traded food commodities, must comply with the established food safety and quality standards. Due to highly perishable nature of seafood, meeting consumers' expectations is a major challenge faced by the fish processing industry. Fish spoilage starts immediately after harvest and continues during storage depending upon handling and storage condition. Activities of endogenous enzymes (proteases and lipases), microbes, and lipid oxidation are the main factors responsible for deterioration of fish meat (Kumolu-Johnson and Ndimele, 2011). Fish quality was estimated using sensory evaluation as well as microbial and chemical methods under laboratory conditions. Volatile amines, collectively measured as Total Volatile Basic-Nitrogen (TVB-N) are the protein degradation products commonly used as the criteria for assessing fish quality (European Commission, 2008).

Perishable nature of fish and shellfish, in conjunction with the high demand for it necessitates continuous attention to its long-term preservation techniques. Different physical and chemical preservation methods are used worldwide on commercial scales to increase fish shelf life and maintain its quality through the cold supply chain. However, preservation methods currently used are either energy intensive (Suemitsu and Cristianini, 2019) or use synthetic chemicals which pose significant health hazards for consumers (Baran et al., 2021; Olatunde and Benjakul, 2018). The demand for fish preservation with natural products is also increasing due to increase in consumer's awareness about food safety and quality (Arshad and Batool, 2017). Use of exogenous edible coatings to reduce enzymatic and microbial spoilage is currently an active area of research in food quality preservation. Plant extracts, essential oils, and biopolymers are being investigated for their ability to preserve seafood (Olatunde and Benjakul, 2018). There is limited information on the efficacy of olive leaves extract and green tea extract-based coatings to maintain the quality of seafood and its products (EL-Hanafy et al., 2011; Testa et al., 2019). To the best of our knowledge, a comparison of their effect on fish quality has not been studied yet.

In the present study, we evaluated the effect of green tea extract and olive leaves extract-based preservative coatings on chemical, microbial, and sensory quality of tilapia during frozen storage (-18±2 °C). Prior to freezing, tilapia was stored on ice for five days to mimic fish storage during post-harvest transport as well as at retailers' shops.

Materials and methods

Experimental design

This study addressed the effect of edible preservative coatings on chemical, microbial, and sensory quality of wild-type Nile tilapia (*Oreochromis niloticus*) subjected to icing and subsequent freezing. The experimental work was conducted at Fisheries Research and Training Institute, Lahore, Pakistan, from April–July, 2021. A two-way factorial design was used for the experiment with three levels for preservative coating (no coating, green tea extract-based coating, and olive leaves extract-based coating), and six levels for fish storage (five days of ice storage and 15, 45, 75, and 105 days of frozen storage). Dependent variables were fish chemical (moisture, ash, protein, fat, TVB-N, pH) and microbial (Aerobic Plate Count (APC)), *Escherichia coli*, Total Coliform (TC), and Fecal Coliform (FC)) parameters and their sensory qualities (appearance, odour, acceptability, flesh color, Total Quality Index (TQI)).

Chemicals and reagents

Double distilled water was used for preparation of extracts and reagent solutions. Reagent grade chemicals including sulfuric acid (98%), hydrochloric acid (37%), trichloroacetic acid, sodium hydroxide, methyl red, bromocresol green, petroleum ether, copper sulphate, and potassium sulphate were purchased from Sigma-Aldrich, Germany. Butterfield's buffer solution, plate count agar, lauryl tryptose broth, brilliant green lactose broth, and *E. coli* broth were purchased from Biolife Italiana, Italy.

Preparation of preservative coating solutions

Green tea extract and olive leaves extract were used to prepare preservative coatings following Cai et al. (2015) and Khemakhem et al. (2019) study, respectively. Commercially available green tea of a well-known brand was purchased from local market. Green tea leaves were ground in a food blender to form fine powder. Mixture of green tea powder and distilled water (10% w/v) was boiled for 30 min with continuous agitation. Subsequently, the mixture was centrifuged at 3,500 rpm (EBA 3S, Hettich, Germany) for 15 min, and supernatant was filtered through Whatman No. 1 filter paper (Cai et al., 2015). Olive leaves were collected from plant nurseries in Lahore, Punjab, Pakistan. Leaves were air dried for one week and then ground to form fine powder. The extract was prepared by stirring olive leaves powder with distilled water (2.5% w/v) for 60 min at room temperature. The mixture was, then, filtered through Whatman No. 1 filter paper (Khemakhem et al., 2019). Concentration of filtered extracts was determined by weighing the residue left after evaporating measured aliquots of the filtrates and was found to be 1.45% for green tea extract and 0.5% for olive leaves extract. Green tea extract was diluted to prepare 0.3% coating solution, while olive leaves extract was used

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directly as coating material.

Fish sampling and processing

Forty-eight live samples of wild Nile Tilapia (*Oreochromis niloticus*) were procured from production ponds of Fisheries Research and Training Institute, Lahore, and were immediately transferred to a chemistry laboratory where they were slaughtered on ice, gutted and washed with distilled water.

Fish treatment with preservative coating and storage

Three fish samples were used to determine sensory, chemical, and microbial quality of tilapia immediately after slaughtering. The rest of 45 tilapia samples were divided into three experimental groups with 15 samples in each group. Tilapia samples in group CT were dipped in distilled water for 60 min. Samples in group GTE and group OLE were dipped in coating solution containing green tea extract (0.3%) and olive leaves extract (0.5%) for 60 min. After dip treatment, samples were dried on stainless steel mesh screen for 1 min, packed in sterile plastic bags, and stored in ice (1±2 °C) for five days. Melted ice was regularly replaced to completely cover the samples. Subsequently, samples of each group were subjected to frozen storage at -18±2 °C

Quality assessment of fish

Tilapia samples in triplicate were used for quality assessment. Sensory, chemical, and microbial quality of tilapia samples in each group was determined at the end of ice storage and after 15, 45, 75, and 105 days of frozen storage.

Assessment of chemical quality

Samples were collected from dorsal muscles of tilapia samples to determine their chemical quality. Muscles were ground in a food homogenizer for 1 min to form homogenous mince. Muscles' proximate composition (ash, fat, protein, and moisture) was evaluated using the standard procedures of Association of Official Agricultural Chemists (AOAC International and Latimer, 2012). Weighed portions of muscles were heated at 105 and 550 ºC to the constant weight to determine their moisture and ash content, respectively. Muscles' protein content was measured by determining the nitrogen content using manual Kjeldahl digestion and distillation method (protein (%)=nitrogen content (%)×6.25).

Soxhlet extraction apparatus (Cole-Palmer, USA) using petroleum ether as extraction solvent was used to determine fat content. Trichloroacetic acid extraction with subsequent steam distillation was adopted for analysis of TVB-N, following the method of Sewwandi et al. (2016) with slight modification. To specify pH, 10 g muscle sample was homogenized with distilled water (100 ml) for 30 min. The mixture was filtered, and its pH was measured using calibrated pH meter (HI 8,424, Hanna, China).

Assessment of microbial quality

Homogeneous samples were collected from ventral muscles of tilapia for microbial analysis. Microbial parameters (APC, TC, FC, and *E. coli*) of fish were determined according to AOAC International and Latimer (2012) and FDA (2021). Fifty g fish sample was homogenized with 450 ml sterile Butterfield's buffer solution and was used to prepare serial dilutions. For APC, dilutions were mixed with molten plate count agar in petri plates and incubated at 35 °C for 48 h. Colonies on plates with 25-250 colonies were counted and reported as Colony Forming Unit (CFU)/g. Dilutions were mixed lauryl tryptose broth in test tubes and incubated at 35 °C for 48 h. A loopful of suspension from gas positive tubes was transferred to brilliant green lactose broth test tubes and *E. coli* broth tubes each; they were then incubated for 48 h at 35 °C. The Most Probable Number (MPN) of TC and FC was calculated on the basis of gas positive tubes regarding brilliant green lactose broth and *E. coli* broth, respectively. A loopful of suspension from cultures with positive reaction in *E. coli* medium was used to streak Levine's eosin-methylene blue agar plates. *E. coli* count was calculated by re-inoculation of suspicious colonies from Levine's eosin-methylene blue agar plates into tryptone water and was reported as MPN/g (AOAC International and Latimer, 2012; FDA, 2021).

Sensory assessment

Sensory assessment of tilapia quality was conducted using the quality index method, as described by Yu et al. (2017). The sensory evaluation panel consisted of three trained laboratory staff members, each with expertise in fish quality assessment. The evaluation focused on several key sensory attributes, including fish appearance, odor, overall acceptability, and flesh color. Each sensory attribute was individually assessed on a scale ranging from 1 to 5, with a score of 5 indicating the highest quality. TQI was calculated based on the combined scores for all attributes. The maximum achievable score was 20, signifying the highest quality fish. Conversely, a score of 12 was considered a borderline threshold for acceptable fish quality. The sensory evaluation process included detailed instructions and guidelines for authors to ensure consistency and reliability in scoring. The sensory assessment was conducted in a controlled environment with standardized lighting, temperature, and ventilation conditions to minimize external influences on the evaluation. Fish samples were served in a randomized design identified by random codes to avoid carry-over

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effects and potential bias.

Statistical analysis

Factorial analysis of variance was used to find significant interaction among the two factors (preservative coating and storage duration) and their effect on chemical and sensory quality of tilapia muscles. Subsequently, one-way analysis of variance using post-hoc analysis with Tukey Test was used to find significant differences in fish quality parameters with changing levels of each factor. Prior to analysis of variance, data was checked for normality and homogeneity of variance. A significance level of 0.05 was used for statistical analysis unless otherwise specified. Bivariate correlation analysis (Pearson's correlation) was used to find significant correlations among fish chemical, sensory, and microbial quality parameters. The effect size was calculated by taking square of the Pearson's correlation coefficient (Field, 2013). All the statistical analysis was carried out using SPSS version 22.

Results

Factorial analysis of variance

The results of factorial analysis to determine the individual and combined effect of edible coating and storage duration on chemical and sensory quality of tilapia are presented in Table 1. Storage duration significantly affected all the chemical quality parameters as indicated by significant *F*-ratio (Table 1). In the case of preservative coating, *F*-ratio was significant for TVB-N, moisture, ash, fat, and pH but not for protein content. A significant interactive effect of preservative coatings and storage was found on muscles' TVB-N, ash, fat, and pH (Table 1, Figure 1: a-d).

Assessment of chemical quality

Chemical quality parameters of tilapia in three experimental groups are presented in Table 2. TVB-N in fresh tilapia samples was 7.80±0.00 mg/100 g which significantly increased in all three experimental groups during frozen storage. Significant *F*-ratio for interactive effect of two factors indicates that TVB-N produced in tilapia during storage depended on the use of edible coating. Figure 1 (a) clearly shows that TVB-N was high in tilapia which was not treated with any preservative coating after five days of ice storage and 15, 75, and 105 days of frozen storage, compared to the level found in the fish treated with green tea extract or olive leaves extract-based coatings. Tilapia samples in untreated group, however, showed a significant reduction in TVB-N content after 45 days (49.61±7.58 to 29.89±3.05 mg/100 g). The observed decrease in TVB-N levels in untreated samples can be attributed to the dynamics of microbial populations during the storage period. After 15 days of storage, a notable decline in microbial load was observed in the untreated fish (Figure 2). At the end of frozen storage, muscles' TVB-N in group CT, GTE, and OLE was 67.92±4.46, 49.73±7.51, and 49.97±13.77 mg/100 g, respectively. TVB-N of tilapia treated with preservative coatings was significantly (p<0.05) lower than the untreated fish during icing and frozen storage (Table 1). A similar trend in muscles' TVB-N content was observed after 15 and 75 days of frozen storage.

Initial pH of fresh tilapia flesh was determined to be 6.71±0.01, which exhibited a decrease to 6.52±0.09 in group CT and an increase to 6.93±0.1 and 7.11±0.06 in group GTE and group OLE, respectively, following five days of ice storage. At the end of 105 days of frozen storage, pH was found to increase in all treatment groups and ranged from 7.01±0.01 to 7.09±0.32. Figure 1 (a, b) illustrates changes in pH levels of tilapia muscles across all experimental groups throughout the study period.

Moisture content of fresh fish muscles was found to be 81.95±0.13%. At the end of 105 days of study period, moisture content of tilapia samples treated with green tea extract was significantly higher (84.26±0.90%) compared to the samples treated with olive leaves extract (82.73±0.83%) and untreated ones (81.28±1.00%). Ash content of fresh tilapia muscles was found to be 1.11±0.01% and ranged from 0.94±0.08% to 1.35±0.08% during 105 days of frozen storage. In group three, ash content was significantly high after 15 days of frozen storage. Figure 1 (c) shows that ash content in untreated fish samples from group CT was reduced after 45 days of frozen storage. The fish treated with edible coating (group GTE and group OLE), however, showed highest ash content after 45 days of storage.

Initial protein content of tilapia muscles was 11.72±0.12%. Protein content ranged from 11.45±0.54 to 12.11±0.59% after 15 days and from 11.59±0.64 to 12.51±0.20% after 105 days of frozen storage in three experimental groups. Fat content of fresh tilapia samples was 0.24±0.04%. Figure 1 (d) demonstrates that the highest fat content in all treated and untreated tilapia was found after 45 days of storage; however, the difference in its content among three groups was not significant. Fat content significantly decreased to 0.05%. in group CT after 105 days of frozen storage. Non-significant increase in fat content of tilapia from group CT after 75 days and the one from group OLE after 15-45 days of frozen storage can be attributed to natural variation in composition of fish muscles.

Assessment of microbial quality

Microbial quality of fish in different groups is shown in

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Figure 2. Initial APC of tilapia (5.40×10^2 CFU/g) increased to 6.75×10^6, 9.28×10^2, and 2.175×10^3 CFU/g in Group CT, GTE, and OLE, respectively. At the end of 105 days of frozen storage, the highest APC (8.37×10^6 CFU/g) was found in fish from group CT. Initial TC, FC, and *E. coli* in fresh tilapia (15.0, 7.4, and 3.6 MPN/g) increased to 7.8×10^2, 5.7×10^2, and 5.7×10^2, respectively in untreated tilapia after five days of ice storage.

Acceptable limits for APC, TC, FC, and *E. coli* which are regarded as markers of fish hygienic conditions are $<5\times10^{-5}$ CFU/g, <100 MPN/g, <10 MPN/g, and 500 MPN/g (ICMSF, 1986). In general, microbial load of fish at the time of harvest varies from 10^2 to 10^5 CFU/g. In this study, the initial APC (5.40×10^2) of untreated tilapia increased to 6.75×10^6 CFU/g after five days of ice storage; a value which is 12.5 times higher than the permissible limit of 5×10^5 CFU/g (Figure 2). At the end of 105 days of frozen storage, APC of the untreated samples became 8.37×10^6 CFU/g, about 16 times higher than the permissible limits. In the case of tilapia treated with preservative coatings, APC remained well below the acceptable limits after five days of ice storage and during entire frozen storage period (Figure 2). TC, FC, and *E. coli* were found to be higher than the permissible limit in untreated tilapia after five days of ice storage. Among the fish treated with preservative coatings, tilapia treated with olive leaves extract showed higher FC than permissible level (29 MPN/g) after 15 days of frozen storage. For all other fish treated with preservative coatings, these microbial parameters were found to be less than the recommended range (Figure 2).

Sensory assessment

Storage time, use of edible coating, and their interactive effect, significantly affected all the sensory attributes of tilapia (Table 1, Figure 3: a-e). Sensory scores of tilapia recorded during the study are presented in Table 2. At the start of the experiment, fresh tilapia samples achieved 4.80 score for appearance, odour, acceptability, and flesh color, and 19.20 for TQI. For fish odour, no significant difference was observed among the three groups at the end of the 105 days of experimental period. Significantly lower scores were recorded for appearance, flesh color, and overall acceptability of the fish treated with preservative coatings (groups GTE and OLE) compared to the untreated ones at the end of experimental period. The lowest TQI, 15.1±0.1, was recorded for the fish treated with green tea extract-based coating. In contrast, tilapia treated with olive leaves extract-based coating obtained a TQI score of 16.3±0.4, while the untreated tilapia registered the highest score of 18.67±0.42.

Correlation analysis

The results of Pearson's correlation are presented in Table 3. A significant positive correlation was found between duration of storage and muscles' TVB-N as well as pH (r=0.837, r=0.366, respectively, p<0.01). pH was positively correlated with fat (r=0.006, p<0.01) and moisture content (r=0.503, p<0.01). Moisture content and pH were negatively correlated with all the sensory attributes of tilapia (p<0.01). TVB-N was negatively correlated with flesh color, overall acceptability (p<0.01), and TQI (p<0.5). Moreover, a significant positive correlation was recorded regarding all the sensory attributes including appearance, odor, flesh color, overall acceptability, and TQI (p<0.01). Among the microbial parameters, there was a significant positive correlation between APC, TC, FC, and *E. coli* (Table 3). TC and *E. coli* contributed 21.1 and 30.60% variability in APC, respectively. *E. coli* was the major bacterial species regarding coliform due to the high variability it shared in TC and FC (84.8 and 99.2%, respectively).

Table 1: The results of factorial analysis regarding the effect of edible coating and storage duration on the quality of tilapia

Parameter	Source	Sum of squares (SS)	df	*F*-ratio	*p*-value
TVB-N	Coating	2,234.445	2	21.833	.000
	Storage	15,976.815	5	62.445	.000
	Coating*storage	1,855.405	10	3.626	.002
Moisture	Coating	46.81	2	25.08	.000
	Storage	16.04	5	3.44	.012
	Coating*storage	15.25	10	1.64	.136
Ash	Coating	.031	2	4.28	.021
	Storage	.153	5	8.44	.000
	Coating*storage	.230	10	6.33	.000
Protein	Coating	.059	2	.088	.916
	Storage	3.796	5	2.270	.068
	Coating*storage	3.004	10	.898	.544
Fat	Coating	.445	2	10.388	.000
	Storage	1.442	5	13.469	.000
	Coating*storage	.744	10	3.476	.003
pH	Coating	1.342	2	24.615	.000
	Storage	1.134	5	8.321	.000

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	Coating*storage	.872	10	3.201	.005
Appearance	Coating	3.151	2	92.478	.000
	Storage	3.776	5	44.322	.000
	Coating*storage	2.600	10	15.261	.000
Odor	Coating	.788	2	133.000	.000
	Storage	.937	5	63.250	.000
	Coating*storage	.341	10	11.500	.000
Flesh color	Coating	2.064	2	30.283	.000
	Storage	5.684	5	33.361	.000
	Coating*storage	3.999	10	11.735	.000
Overall acceptability	Coating	2.001	2	38.600	.000
	Storage	6.368	5	49.126	.000
	Coating*storage	1.439	10	5.549	.000
Total quality index	Coating	30.179	30.179	231.489	.000
	Storage	50.433	50.433	154.736	.000
	Coating*storage	20.443	20.443	31.361	.000

Table 2: Chemical quality and sensory attributes of tilapia during storage (Mean±SD)

Parameter	Group	Fresh samples	Ice storage duration (5 days)	Frozen storage duration 15 days	45 days	75 Days	105 days
TVB-N (mg/100 g)	CT	7.80±0.00 ^{d}	32.94±2.30 ^{c,A}	49.61±7.58 ^{b,A}	29.89±3.05 ^{c,B}	50.42±2.08 ^{b,A}	67.92±4.46 ^{a,A}
	GTE	7.80±0.00 ^{d}	5.05±2.19 ^{d,B}	21.51±2.08 ^{c,B}	32.75±2.73 ^{b,A,B}	44.33±2.82 ^{a,A,B}	49.73±7.51 ^{a,A,}
	OLE	7.80±0.00 ^{c}	7.24±2.89 ^{c,B}	29.37±7.00 ^{b,B}	38.29±0.27 ^{a,b,A}	44.09±2.56 ^{a,b,B}	49.97±13.77 ^{a,A}
Moisture (%)	CT	81.95±0.13 ^{a}	80.43±1.16 ^{a,b,B}	79.18±0.74 ^{b,B}	80.55±1.58 ^{a,b,A}	79.44±0.74 ^{a,b,B}	81.28±1.00 ^{a,b,B}
	GTE	81.95±0.13 ^{a}	82.84±0.86 ^{a,A}	82.05±1.32 ^{a,A}	81.99±1.09 ^{a,A}	82.83±2.07 ^{a,A}	84.26±0.90 ^{a,A}
	OLE	81.95±0.13 ^{a}	82.05±0.34 ^{a,A,B}	81.64±0.23 ^{a,A}	81.88 ±0.57 ^{a,A}	82.57±0.91 ^{a,A,B}	82.73±0.83 ^{a,A,B}
Ash (%)	CT	1.11±0.01 ^{a}	1.15±0.06 ^{a,A}	1.03±0.05 ^{a,B}	1.05±0.06 ^{a,A}	1.10±0.03 ^{a,A}	1.03±0.05 ^{a,A}
	GTE	1.11±0.01 ^{a}	1.10±0.06 ^{a,A}	1.24±0.03 ^{a,A}	1.10±0.03 ^{a,A}	1.18±0.09 ^{a,A}	1.08±0.14 ^{a,A}
	OLE	1.11±0.01 ^{b}	0.94±0.06 ^{c,B}	1.35±0.08 ^{a,A}	1.07 ±0.03 ^{b,c,A}	1.09±0.03 ^{b,A}	1.03±0.06 ^{b,c,A}
Protein (%)	CT	11.72±0.12 ^{a}	11.57±0.31 ^{a,A}	11.45±0.54 ^{a,A}	11.35±0.60 ^{a,A}	11.35±0.97 ^{a,A}	11.72±0.49 ^{a,A}
	GTE	11.72±0.12 ^{a}	11.32±0.20 ^{a,A}	12.11±0.59 ^{a,A}	11.44±0.13 ^{a,A}	11.34±1.58 ^{a,A}	11.59±0.64 ^{a,A}
	OLE	11.72±0.12 ^{a,b}	11.31±0.28 ^{b,c,A}	11.76±0.25 ^{a,b,A}	11.58±0.41 ^{a,b,c,A}	10.75±0.61 ^{c,A}	12.51±0.20 ^{a,A}
Fat (%)	CT	0.24±0.04 ^{a,A}	0.22±0.00 ^{a,A}	0.19±0.00 ^{a}	0.71±0.05 ^{a,A}	0.07±0.01 ^{c,B}	0.05±0.01 ^{c,B}
	GTE	0.24±0.04 ^{a,B}	0.11±0.01 ^{a,B}	0.14±0.1 ^{a,A}	0.41±0.02 ^{a,A}	0.08±0.03 ^{a,B}	0.17±0.09 ^{a,A}
	OLE	0.24±0.04 ^{a,b,B}	0.12±0.01 ^{b,B}	0.63±0.45 ^{a,b,A}	0.72±0.14 ^{a,A}	0.58±0.04 ^{a,b,A}	0.14±0.04 ^{b,A,B}
pH	CT	6.71±0.01 ^{b,B}	6.52±0.09 ^{c,B}	6.59±0.06 ^{b,c,B}	6.93±0.03 ^{a,A}	6.47±0.10 ^{c,B}	7.01±0.01 ^{a,A}
	GTE	6.71±0.01 ^{a,A}	6.93±0.1 ^{a,A}	6.86±0.16 ^{a,A,B}	7.04±0.09 ^{a,A}	7.03±0.20 ^{a,A}	7.09±0.32 ^{a,A}
	OLE	6.71±0.01 ^{b,A}	7.11±0.06 ^{a,b,A}	7.00±0.07 ^{a,b,A}	7.48±0.45 ^{a,A}	7.21±0.19 ^{a,b,A}	7.01±0.17 ^{a,b,A}
Appearance	CT	4.80±0.0 ^{a}	4.73±0.23 ^{a,b,A}	4.8±0.0 ^{a,A}	4.27±0.23 ^{c,A}	4.40±0.0 $^{b,\ c,A}$	4.67±0.12 ^{a,b,A}
	GTE	4.80±0.0 ^{a}	4.27±0.12 ^{b,B}	4.33±0.12 ^{b,B}	3.53±0.12 ^{c,B}	3.60±0.20 ^{c,B}	3.6±0.2 ^{c,b,C}
	OLE	4.80±0.0 ^{a}	3.80±0.0 ^{c,C}	4.27±0.23 ^{b,B}	4.33±0.12 ^{b,A}	4.20±0.0 ^{b,A}	4.2±0.0 ^{b,B}
Odor	CT	4.80±0.0 ^{b}	5.0±0.0 ^{a,A}	4.8±0.0 ^{c,A}	4.60±0.0 ^{c,A}	4.80±0.0 ^{b,A}	4.80±0.0 ^{a,A}
	GTE	4.80±0.0 ^{a}	4.80±0.0 ^{b,B}	4.40±0.0 ^{b,C}	4.40±0.0 ^{b,A}	4.47±0.12 ^{a,b,B}	4.80±0.2 ^{b,A}
	OLE	4.80±0.0 ^{a}	4.80±0.0 ^{a,B}	4.60±0.0 ^{a,B}	4.40±0.0 ^{a,A}	4.60±0.0 ^{a,B}	4.80±0.0 ^{a,A}
Acceptability	CT	4.80±0.0 ^{a}	4.6±0.0 ^{a,b,A}	4.5±0.12 ^{a,b,A}	4.27±0.30 ^{a,b,A}	4.27±0.30 ^{a,b,A}	4.2±0.2 ^{b,A}
	GTE	4.80±0.0 ^{a}	4.20±0.0 ^{b,B}	4.33±0.12 ^{b,A}	3.73±0.12 ^{c,B}	3.40±0.20 ^{c,B}	3.50±0.2 ^{c,B}
	OLE	4.80±0.0 ^{a}	3.80±0.0 ^{c,C}	4.33±0.12 ^{b,A}	4.13±0.12 ^{b,c,A,B}	3.80±0.20 ^{c,A,B}	3.8±0.2 ^{c,A,B}
Flesh color	CT	4.80±0.0 ^{a,b}	5±0.0 ^{a,A}	4.0±0.0 ^{a,b,B}	4.67±0.12 ^{a,b,A}	4.33±0.30 ^{b,A}	4.80±0.20 ^{a,b,A}
	GTE	4.80±0.0 ^{a}	4.53±0.12 ^{a,B}	4.53±0.12 ^{a,A}	3.80±0.0 ^{b,C}	3.60±0.40 ^{b,A}	3.6±0.4 ^{b,B}
	OLE	4.80±0.0 ^{a}	4.40±0.0 ^{b,B}	4.53±0.12 ^{a,b,A}	4.40±0.0 ^{b,B}	3.67±0.23 ^{c,A}	3.7±0.2 ^{c,B}
TQI	CT	19.20±0.0 ^{a}	19.33 ±0.23 ^{a,A}	18.0±0.0 ^{b,c,A}	17.8±0.53 ^{c,A}	17.80±0.20 ^{c,A}	18.67±0.42 ^{a,b,A}
	GTE	19.20±0.0 ^{a}	17.80±0.20 ^{b,B}	17.60±0.20 ^{b,A}	15.47±0.23 ^{c,B}	15.07±0.12 ^{c,B}	15.1±0.1 ^{c,C}
	OLE	19.20±0.0 ^{a}	16.80±0.0 ^{c,d,C}	17.73±0.31 $^{b,\mathbf{A}}$	17.27±0.12 ^{b,c,A}	16.27±0.42 ^{d,B}	16.3±0.4 ^{d,B}

Note: Means which share a similar small alphabet in the same row are not statistically significant (p>0.05). Means which share a similar capital alphabet within column for each parameter are not statistically significant (p>0.05).
TVB-N=Total Volatile Basic-Nitrogen; TQI=Total Quality Index
CT: No Coating; GTE: Green Tea Extract-Based Coating; OLE: Olive Leaves Extract Based Coating

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Table 3: Correlation matrix for chemical and microbial quality parameters of tilapia

		Storage	Moisture	Ash	Protein	TVB-N	Fat	pH	APC	TC	FC	*Escherichia coli*
Coating	*r*	0.000	0.473 **	0.081	0.052	-0.244	0.257	0.551 **	-0.290 *	-0.228	-0.227	-0.213
	Sig.	1.000	<0.001	0.561	0.707	0.075	0.060	<0.001	0.033	0.097	0.098	0.122
Storage	*r*		0.138	-0.125	0.002	0.837 **	-0.008	0.366 **	0.100	-0.181	-0.178	-0.172
	Sig.		0.319	0.369	0.988	<0.001	0.953	0.007	0.473	0.190	0.198	0.212
Moisture	*r*			-0.029	-0.061	-0.172	-0.064	0.503 **	-0.224	-0.258	-0.270 *	-0.262
	Sig.			0.836	0.664	0.214	0.644	<0.001	0.104	0.060	0.048	0.056
Ash	*r*				0.176	-0.079	0.119	-0.132	-0.024	0.111	0.062	0.054
	Sig.				0.202	0.573	0.390	0.340	0.860	0.422	0.656	0.699
Protein	*r*					0.062	-0.126	-0.173	-0.067	-0.062	-0.018	-0.046
	Sig.					0.656	0.364	0.210	0.632	0.656	0.899	0.742
TVB-N	*r*						-0.047	0.063	0.246	-0.006	-0.002	-0.001
	Sig.						0.734	0.653	0.073	0.968	0.990	0.995
Fat	*r*							0.371 **	-0.156	-0.028	-0.043	-0.027
	Sig.							0.006	0.260	0.841	0.758	0.848
pH	*r*								-0.092	-0.247	-0.248	-0.241
	Sig.								0.509	0.072	0.071	0.079
APC	*r*									0.459 **	0.558 **	0.553 **
	Sig.									<0.001	<0.001	<0.001
TC	*r*										0.920 **	0.921 **
	Sig.										<0.001	<0.001
FC	*r*											0.996 **
	Sig.											<0.001

**: Correlation is significant at the 0.01 level (2-tailed)
*: Correlation is significant at the 0.05 level (2-tailed)
r: Pearson's correlation coefficient
TVB-N=Total Volatile Basic-Nitrogen; APC=Aerobic Plate Count; TC=Total Coliform; FC=Faecal Coliform

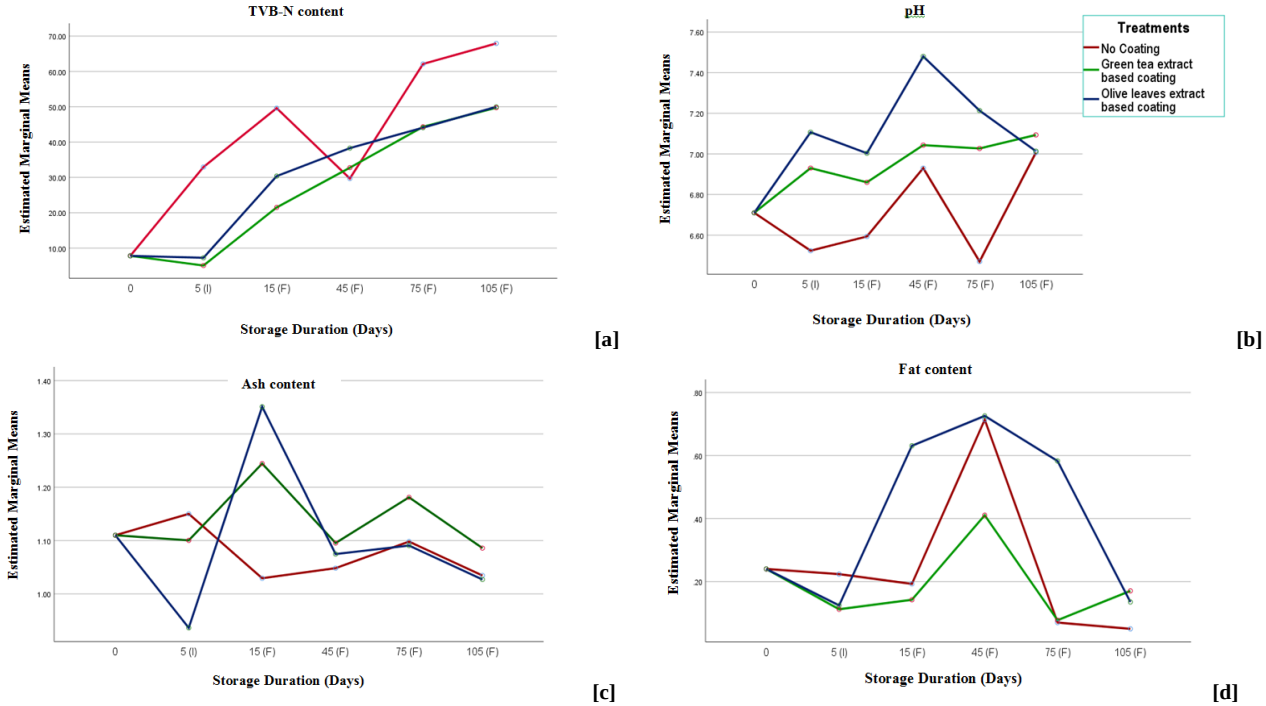

Figure 1: The interactive effect of storage duration and use of preservative coating on chemical quality of tilapia muscles **(**I: ice storage, F: frozen storage): a: Total Volatile Basic-Nitrogen (TVB-N); b: pH; c: ash content; d: fat content

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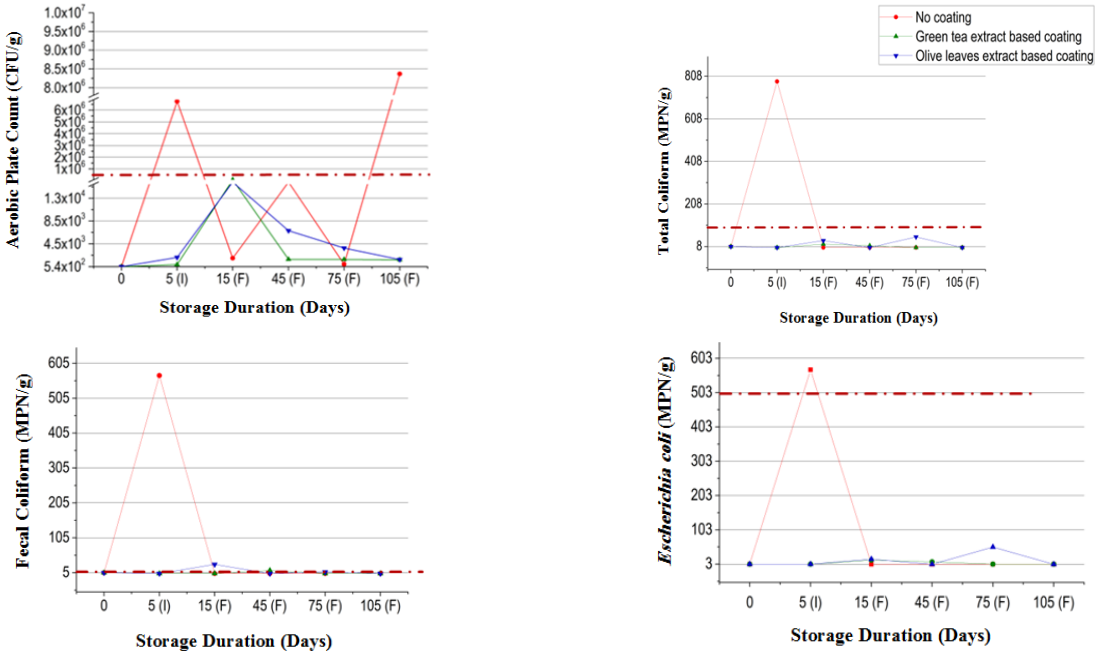

Figure 2: The microbial quality of tilapia during storage (I: ice storage, F: frozen storage)
CFU=Colony Forming Unite; MPN=Most Probable Number

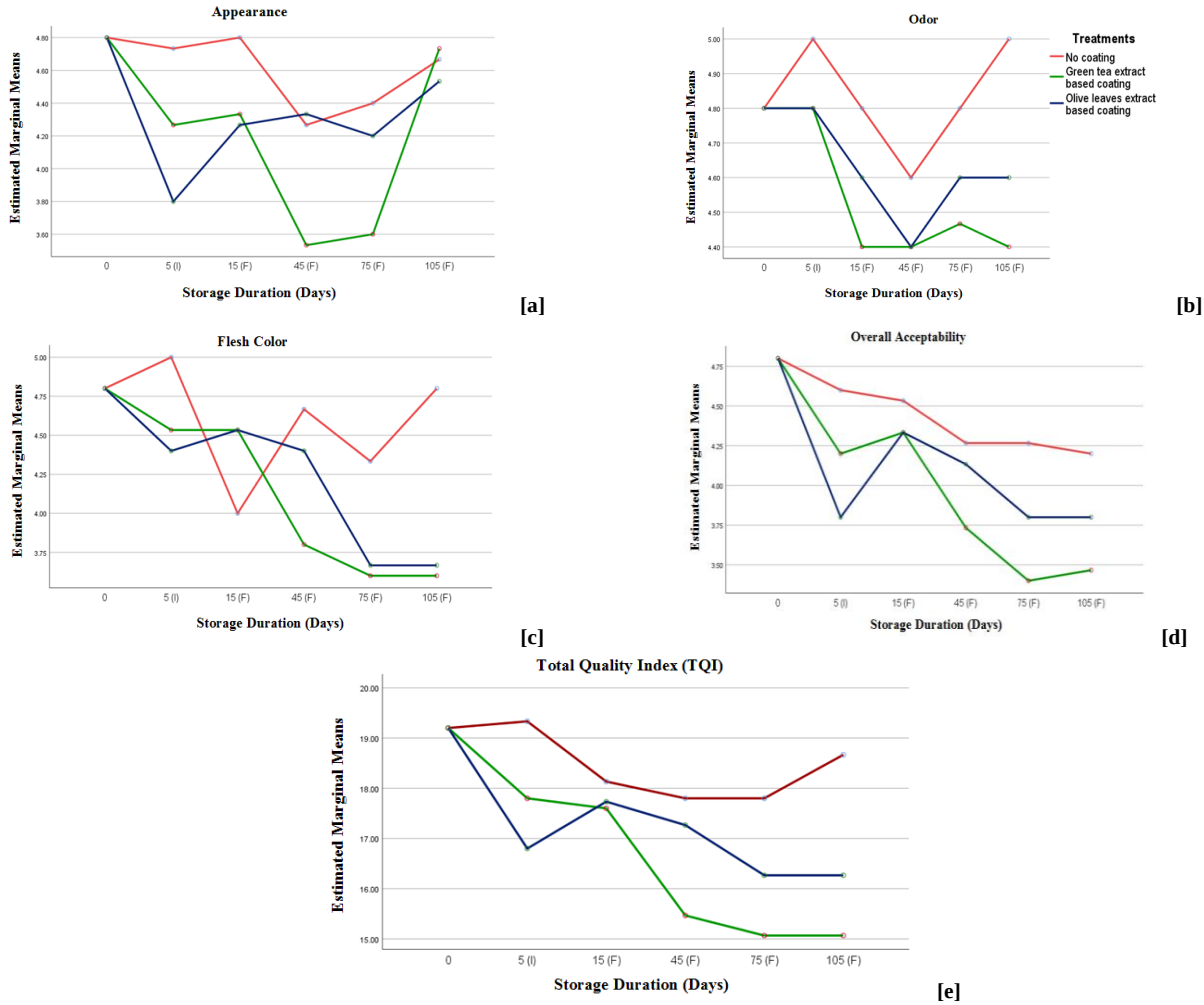

Figure 3: The interactive effect of storage duration and use of preservative coating on sensory quality of tilapia muscles **(**I: ice storage, F: frozen storage): a: appearance; b: odor; c: flesh color; d: overall acceptability; e: Total Quality Index (TQI)

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Discussion

Antimicrobial and antioxidant characteristics of preservative coatings depend on the concentration of active compounds in the coating material. Cai et al. (2015) reported that green tea extract with a concentration greater than 0.3% showed negative influence on the sensory attributes of shrimp paste. In another study conducted by Ahmed et al. (2014), it was found that olive leaves extract with 0.5% concentration significantly reduced the microbial load of raw shrimps, and the antimicrobial effect was reported to be concentration dependent. In this study, the authors carefully selected the concentrations of green tea and olive leaf extracts to optimize the balance between their antimicrobial efficacy and their impact on the sensory attributes of the fish.

The European Commission (EC) has recommended 25-35 mg/100 g fish as acceptable TVB-N limit concerning muscles of various fish species; however, the reported commission regulation did not include a threshold limit for chichlids (European Commission, 2008). According to some reports, 20 mg/100 g is considered the proposed limit for freshwater fish (Moosavi-Nasab et al., 2021). Egyptian Standards Specifications propose 30 mg/100 g fish as the permissible limit for freshwater fish (Talab et al., 2016). According to this criterion, TVB-N in the tilapia treated with preservative coatings remained below the permissible limit after five days of ice storage. The fish which did not receive any coating treatment showed higher TVB-N compared with the permissible value after five days of ice storage. TVB-N gradually increased in fish samples of all the three groups. It, however, remained below the permissible limit in the fish treated with preservative coatings after 15 days of frozen storage. With the exception of untreated tilapia samples analyzed after 45 days of storage, further increase in frozen storage duration led to higher TVB-N compared with the permissible level of 30 mg/100 g in all the three groups. Lower TVB-N content in the untreated fish after 45 days of freezing, may be attributed to the reduction in microbial activity observed within these samples by the 15th day of storage (Figure 2: a). The reduced microbial load potentially resulted in a diminished rate of protein degradation, subsequently leading to a decreased production of volatile nitrogen compounds. It's also noteworthy that the treated fish consistently exhibited lower TVB-N levels in comparison to the untreated samples. Green tea extract and olive leaves extract-based coatings were found to be equally effective in reducing TVB-N of tilapia and maintain its quality (Table 2).

The results were in line with earlier investigations which reported that natural edible coatings can reduce chemical and microbial spoilage of fish during storage. Emire and Gebremariam (2010) found that TVB-N in muscles of tilapia increased from 12.04±0.48 to 21.75±0.35 mg/100 g during 90 days of frozen storage. Castro et al. (2012) reported that TVB-N content in muscles of fresh gilthead sea bream (15.56±0.29 mg/100 g) increased to 22.0±0.24 mg/100 g after 10 days of storage in ice at 4 °C. Khidhir (2015) reported that common carp fillets treated with olive leaves extract demonstrated lower total plate count and psychrophilic count (1.065×10^5 and 1.434×10^3 CFU/g) compared to the untreated fillets (2.25×10^5 and 2.775×10^3 CFU/g) after 7 days of refrigerated storage. Testa et al. (2019) described preservative and antimicrobial characteristics of olive leaves extract to maintain the quality of anchovy fillets during 22 days marination process. The use of olive leaves extract reduced TVB-N in fish fillets at the end of marination (11.40±0.19 mg/100 g) compared to the one found in fillets from control batch (15.81±0.11 mg/100 g). Psychrophilic count was found to be <10 CFU/g in the treated fillets compared to the 4.5×10^2 CFU/g in untreated ones. According to EL-Hanafy et al. (2011), frozen green tea extract increased the shelf life of tilapia from 6 days (untreated fish) to 14 days (fish treated with 4-6% extract). TVB-N and total viable content in untreated fish samples were 37.68 mg/100 g and 7.89±0.1 CFU/g after 6 days of ice storage. For the tilapia stored on 2, 4, and 6% of frozen green tea extract, 36.97, 35.90, and 34.81 mg/100 g TVB-N content and 7.41±0.05, 7.14±0.08, and 7.11±0.09 CFU/g of total viable count were recorded after 10, 14, and 14 days of storage. The antimicrobial activity of olive leaves extract has also been reported by Ahmed et al. (2014); they found 1 log cycle CFU/g reduction in APC and coliform bacteria of the shrimps treated with 1% leaves extract compared to the untreated groups. Ali et al. (2021) reported the efficacy of green tea extract to improve shelf life of shrimps during refrigerated storage. They recorded the total coliform as $1.0\times10^2\pm5.0\times10$ MPN/g in imported unpeeled shrimps treated with 0.1% green tea extract compared to $2.0\times10^3\pm2.0\times10$ MPN/g in untreated shrimps after 6 days of storage at 4 °C.

Increase of the muscles' pH during frozen storage was consistent with earlier reports and can be attributed to enzymatic degradation of muscles and production of volatile basic compounds. EL-Hanafy et al. (2011) reported that the pH value of tilapia increased from 6.51±0.02 to 6.83±0.02 during 6 days of ice storage. Concerning the initial decrease in pH of the tilapia treated with green tea-based coating and the untreated fish as observed in present study, the literature provided varying reports about alterations in fish muscle pH during storage. For instance, Mezhoudi et al. (2022) documented an initial pH of 6.11 in *Mustelus mustelus* fillets, which exhibited a significant (p<0.05) decrease after 6 days of storage at 4 °C. In contrast, Volpe et al. (2015) reported an increase in pH in

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Oncorhynchus mykiss fillets stored at 4 °C for 15 days, attributing this change to the formation of biogenic amines.

The moisture content of fresh fish muscles (81.95±0.13%) was in line with Alsaggaf et al.'s paper (2017), reporting 79.6±0.3% moisture content in fresh tilapia fillets. Ruelas-Chacon et al. (2020), however, reported higher moisture content in fresh tilapia fillets ranging from 89.95±0.33 to 90.02±0.25%. According to the findings of the present research, the significant increase in moisture content of tilapia treated with green tea extract is not in line with that of Ruelas-Chacon et al.'s study (2020); they found that the moisture content of either the untreated tilapia fillets or the fillets treated with bioactive protective coatings significantly decreased with refrigerated storage during 15 days of the study period. With regards to ash content, the results of the current study were in agreement with Ruelas-Chacon et al.'s paper (2020); they reported that the treatment of tilapia fillets with preservative edible coating did not significantly affect ash content during 15 days of refrigerated storage. The significant increase in muscles' ash content observed in the tilapia treated with the olive leaves extract-based coating after 15 days of freezing was more likely attributed to inherent variability in the proximate composition of the fish, rather than the treatment with the edible coating.

In the study by Shafi et al. (2020), the protein content of silver carp stored in ice for five days prior to freezing, decreased significantly after 60 days of frozen storage. In the present project, any noticeable effect of edible coating and storage duration on fish's protein content could not be detected. Morachis-Valdez et al. (2017), however, reported that amino acids content significantly reduced in untreated common carp fillets after five months of frozen storage compared to those treated with chitosan-based edible coatings. The fat content of fresh tilapia muscles (0.24±0.04%) was low compared to 1.47±0.57% reported by Ruelas-Chacon et al. (2020). The significant decrease in fat content of untreated tilapia may be attributed to fish spoilage due to lipid oxidation (Hematyar et al., 2019). Ruelas-Chacon et al. (2020) found that the fat content of the tilapia treated with edible coating demonstrated no significant increase after 15 days of storage at 4 °C.

Reduction in sensory quality of the treated tilapia can be attributed to color of coating solutions which masked the original color and appearance of fish. Nonetheless, the sensory quality of the treated tilapia samples were not reduced to the unacceptable limit (TQI=12). Contrary Ali et al.'s study (2021) who found no negative effect of green tea extract on sensory quality of shrimps, in this study, a reduction was observed in sensory quality of the shrimps treated with *Moringa oleifera* leaves extract. Moreover, the green tea extract with the concentration >0.3% reduced the sensory quality of shrimp paste (Cai et al., 2015). A significantly lower score for fish odor was recorded for untreated tilapia after 45 days of storage, which can be attributed to unsystematic variation among the experimental conditions.

This study provides significant implications for food industry, particularly food safety, preservation, and the growing demand for sustainable and eco-friendly food production practices. The use of green tea extract and olive leaves extract-based preservative coatings demonstrated remarkable effectiveness in controlling chemical and microbial spoilage in tilapia, indicating their potential to significantly extend the shelf life of seafood. Extended shelf life not only reduces food waste, but also enhances the availability of safe and high-quality fish in the market. However, it is crucial to acknowledge that these coatings impacted the sensory attributes of the fish, emphasizing the delicate balance between safety and sensory appeal. Future research in this area should explore methods to mitigate or manage these sensory changes while preserving the safety benefits of the coatings. Additionally, educating consumers about the sensory changes associated with preservative coatings is crucial. Effective communication can help bridge the gap between improved safety and potential consumer concerns regarding the appearance of raw fish. Recognizing the broader implication of this research, it becomes evident that market acceptance and consumer perception play pivotal roles in commercialization of food edible coatings. The study's findings have the potential to influence consumer preferences and purchasing decisions concerning seafood products. As such, it is vital for the food industry to be proactive in addressing these perceptual shifts.

Conclusion

The present study has addressed the potential of green tea extract and olive leaves extract-based coatings to maintain the quality and safety of tilapia during frozen storage. Both preservative coatings proved effective in maintaining chemical and microbial quality of fish during freezing. The tilapia without any preservative coating become unacceptable for human consumption after five days of icing due to its high TVB-N content and poor microbial quality. Lower sensory scores of the tilapia treated with preservative coatings indicate that consumers' awareness needs to be raised regarding the role of plant-based coatings in control of fish spoilage and their possible effects on its sensory quality. Future research in this area may delve deeper into the ecological impact of such coatings, considering the factors such as sourcing, production, and waste management to further enhance the sustainability profile of seafood preservation methods.

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Conflict of interest

The authors declared no conflict of interest.

Author contributions

Z.S.M. conceptualized the study, wrote, reviewed, and edited the manuscript; A.M.C. provided technical support and funding acquisition and reviewed the manuscript; J.S. conducted the experimental work and wrote and edited the manuscript; K.N.W. reviewed the manuscript; and S.S. and M.M.H. conducted the microbial analysis of the samples. All authors read and approved the final manuscript.

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