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Effect of commercial Nisin levels on hygienic quality aspects of the frozen cultured Nile tilapia (*Oreochromis niloticus*) fillets

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ABSTRACT

This study was planned to investigate the effect of commercial nisin on the quality and safety indices of the frozen cultured Nile tilapia fillets. Fresh tilapia fillets were treated with different concentrations of nisin (0.1, 0.15, and 0.20%) for 30min, compared to the control (untreated), packed and stored at -18°C for three months. Results showed that the highest inhibition zone of nisin was found in the case of *Bacillus cereus* compared to other Gram-positive bacteria and *Escherichia coli* compared to other Gram-negative bacterial strains. In addition, investigated safety and quality indices showed that farmed raw tilapia fillets were lower than the permissible limits (MPLs). During frozen storage periods, fish fillets treated with nisin especially 0.2% could be controlled with respect to protein and fat decomposition since the values of pH, TVB-N, TMA, and TBA were more acceptable than those of the control sample. In addition, the nisin level could reduce the microbiological aspects (total viable count, psychrophilic, coliform group, yeasts, and molds) compared to the control. In conclusion, commercial nisin, especially at 0.20% could control the change rate of hygienic quality indices of farmed tilapia fillets throughout frozen storage periods. Thus, commercial nisin as a bio-preservative is recommended to improve the safety and quality of fish products.

INTRODUCTION

Rapidly than other fresh food, the fresh fish fillets are enzymatically and microbially spoiled due to their biological composition. Psychrophilic bacteria represent a basic group of microorganisms causing spoilage in seafood storage at low temperatures **(Bazaraa *et al.*, 2019)**. Traditional preservation methods, although used to reduce food poisoning and extend shelf life, are associated with negative changes in sensory properties, in addition to the loss of nutrients and health concern. Therefore, it is required to replace traditional technologies with new ones **(Rasooli, 2007)**. Biopreservation method is a modern method, based mainly on the use of anti-pathogenic microorganisms as protective cultures and/or their antibacterial metabolites (bacteriocins) as antimicrobial (bactericidal or bacteriostatic) in food. This method is applied to improve the microbial

quality and prolong their storage period. Lactic acid bacteria (LAB) can safely be used as probiotic bacteria in a wide range of processed foods such as starter cultures, co-cultures and bio-protective cultures **(Singh, 2018)**. Many bacteriocins have been isolated for use as natural food biopreservative **(Sarika *et al.*, 2018, 2019)**. Metabolites (bacteriocins) of LAB are the heterogeneous group of bacterial antagonists that vary extremely in molecular weight, biochemical properties, rang of sensitive hosts and mode of action **(Nath *et al.*, 2014)**. Its use might be metabolites of purified or semi purified bacteriocins-producing strains as an ingredient in food processing and/ or as a direct additive **(Raichurkar & Athawale, 2015)**. For a classic example of a bacteriocin, nisin is an allowed food additive in more than 50 countries including the US and Europe. It is commercially known as nisaplin; numerous studies have focused on the synergistic effects of bacteriocins, especially nisin, with other anti-bacterial factors **(Bazaraa *et al.*, 2019)**. Nisin is a broad spectrum bacteriocin with bactericidal activity, even in very low concentrations, toward a wide range of bacteria **(Amin, 2012)**, decreasing the risk for their transmission through the food chain. Nisin is highly resistant to heat. It dissolves in dilute acids and is stable to boiling. It is not toxic, even at levels much higher than those used in foods **(Françoisea, 2010)**. Therefore, this study was planned to investigate the effect of different concentrations of commercial nisin (0.1, 0.15 and 0.20%) on quality and safety indices of the cultured Nile tilapia fillets stored at -18°C for three months.

MATERIALS AND METHODS

Materials:

Bacterial Cultures

The bacterial strains used in this study were obtained from the Microbiological Resources Center (Cairo MIRCEN), Faculty of Agriculture, Ain Shams University, Egypt.

Testing of the Inhibitory Activity of nisin

The antibacterial activity of nisin was tested as described in the study of **Hsu *et al.* (2008)** using different concentrations (50, 100 and 200 µg ml^{-1}) dissolved in dimethyl sulfoxide (DMCO) and added to specific medium, then used in pour plate method to demonstrate the role of bacterium in lowering *Bacillus cereus, Lactobacillus rhamnosus, Staphylococcus aureus, Salmonella typhi, Pseudomonas aeruginosa* and *Escherichia coli.*

Cultured Nile tilapia

Fresh Nile tilapia (*Oreochromis niloticus* L.) samples were obtained from EL-Ayat fish farm, EL-Giza, Egypt during November 2021. The average weight was 450±90 g. Using an icebox, Fish specimens were transferred to the Laboratory of Fish Processing, Food Science and Technology Department, Faculty of Agriculture-Cairo, Al-Azhar University within 3 hours. Fish samples were immediately washed with tap water, manually eviscerated, beheaded, filleted, re-washed, drained and packed. The prepared fish fillets

were performed following the method of **Montalvo Rodrı´guez (2014)**; they were randomly divided into four groups, including the control (C, dipped in deionized water only) and 3 treatments; T1, T2 and T3 dipped in deionized water containing different concentrations of commercial nisin (0.10, 0.15 and 0.20 % w/v, respectively) for 30min at room temperature. Treated samples were removed with sterile tongs and allowed to drain for 10min. Each treatment was packed in polyethylene bags, put in foam dishes, and all treatments were stored at -18°C for 90 days. Samples were monthly withdrawn.

Analytical Methods

Proximate Composition of Fish Fillets

The chemical composition of samples; moisture, crude protein (N×6.25), fat and ash content were determined as described by **AOAC (2012)**.

Heavy Metals Concentrations in Fish Fillets

The concentrations of Pb, Cd, Hg and As were determined in raw fish fillets samples according to **APHA (1992)**. Digested samples, blanks and standard solutions were analyzed by the atomic absorption spectrophotometer, Shimadzu AA-6800, and the results were expressed as ppm.

Biochemical Quality Criteria

The pH value, total volatile basic nitrogen (TVB-N), and thiobarbituric acid reactive substances (TBARS) values were determined **(Pearson, 1991)**. Moreover, trimethylamine nitrogen (TMA) was assessed according to **AOAC (2012)**.

Microbiological Analysis

A sample of 10 g of fish flesh was placed in 90ml of sterile saline (0.85% NaCl) and well shacked for 2min. Tenfold serial dilution was poured in a petri dish, and the specific media were poured. For total bacterial count (TBC), the psychrophilic bacterial was assayed in samples incubated on a nutrient agar. Coliform bacteria were counted on MacConkey agar, yeasts and molds were counted using potato dextrose agar (PDA), and then all plates were incubated at 30°C for 3 days, except for psychrophillic bacterial incubation, which was achieved at 7ºC for 5 days, and 20–25°C for 5 days for yeasts and molds. All microbial counts were expressed as log cfu/g as described in the work of **Difco-Manual (1984)**.

Statistical Analysis

The data obtained (n=3) were statistically analyzed using SPSS (Ver. 16) and expressed as mean ± SD, followed by different superscripts (within rows) as significantly different ($P < 0.05$).

RESULTS AND DISCUSSION

1. Antimicrobial Activity of Commercial Nisin

The antimicrobial activity (inhibition zone, cm) of commercial Nisin against some bacterial indicators is presented in Table (1). Six bacterial strains; three Gram positive bacterial strains (*Bacillus cereus, Lactobacillus rhamnosus* and *Staphylococcus aureus*) as well as Gram negative (*Salmonella typhi, Pseudomonas aeruginosa* and *Escherichia coli*) were examined against different concentrations (50, 100 and 200 µg ml^{-1}). The results showed that the inhibition zones of Gram positive bacteria strains were ranged from 1.69-1.97, 2.83-2.95 and 3.56-3.72 cm at concentrations of 50, 100 and 200 µg ml^{-1}, respectively. The corresponding ranges of Gram negative bacteria were 1.37-1.43, 1.98-2.16 and 3.12-3.40 cm, respectively. Also, it was observed that inhibition zone increased with increasing nisin concentration. Our results agree with those mentioned by **Gyawali *et al.*, (2014)** who reported that nisin acts generally on Gram positive bacteria and some damaged Gram negative bacteria. Gram negative bacteria resist the action of nisin due to the presence of lipopolysaccharide layer which acts as a barrier preventing the entrance of nisin to its site of action. The highest inhibition zone was found in case of *Bacillus cereus* than other Gram positive bacteria and the same trend was noted for *Escherichia coli* than other Gram negative bacterial strains. These results are confirmed by **Nomoto, (2005)**, who reported the antibacterial activities against both Gram positive and negative organisms.

Table (1). Antimicrobial activity (Inhibition zone, cm) of commercial Nisin against some bacterial indicators.

Indicator	Gram reaction	Commercial Nisin concentration (µg ml^{-1})		
		50	100	200
Bacillus cereus	**(+)**	1.69	2.95	3.72
Lactobacillus rhamnosus	**(+)**	1.88	2.83	3.56
Staphylococcus aureus	**(+)**	1.97	2.91	3.67
Salmonella typhi	**(–)**	1.43	2.11	3.27
Pseudomonas aeruginosa	**(–)**	1.37	1.98	3.12
Escherichia coli	**(–)**	1.42	2.16	3.40

(+): Gram positive, (-): Gram negative.

2. Proximate analysis of cultured tilapia fillets

The proximate composition of cultured raw Nile tilapia fillets is shown in Table (1). Tilapia fillets contained (ww) moisture 78.91%, crude protein 16.80%, fat 2.48% and ash content 1.36%. Also, the values of pH, TVN, TMA and TBARS were 6.84, 4.65 mg/100g, 0.34 mg/100g and 0.23 mg MDA/kg sample, respectively.

Table (2). Chemical composition and quality indices of cultured raw Nile tilapia fillets.

Constituent	%		Physico-chemical criteria		
	*WW	*DW	Criterion	Value	*MPLs
Moisture	78.91± 2.11	-	pH	6.84± .05	6.5
Crude protein	16.80± 1.64	79.66	TVB-N (mg/100g sample)	4.65± .07	30
Fat	2.48± .43	11.76	TMA-N (mg/100g sample)	0.34± .03	10
Ash	1.36± .12	6.45	TBARS (mg MDA/kg sample)	0.23± .01	4.5

* MPL: Maximum Permissible Levels; according to EOS [23]. * WW: wet wieght , DW: dry weight.

Our results are in agreement exception fat 5–20% with those reported by **Mohanty, (2015)**; the proximate composition of fish were moisture 65 – 80%, protein 15 – 20%, and ash content 0.5 – 2%. Also, **El-Sherif *et al.*, (2016)** exception crude protein, they found that the moisture, protein and ash content of Nile tilapia were 78.3, 18.15 and 1.35% and **Ibrahim, (2018)** showed that the chemical composition of cultured Nile tilapia ranged 79.38-79.41% moisture, 17.89-18.05% crude protein, 1.12-1.23% fat and 1.14-1.21% ash content. However, the results of this work varied with those reported by **Olopade *et al.*, (2016)**; they found that proximate composition (wet weight) of Nile Tilapia (*Oreochromis niloticus*) were moisture 81.39%, protein 13.66%, fat 0.54%, and ash content 1.36%. This variation in chemical composition of fish is due to fish *spp.*, specimens within the same species, feeding, sex, spawning period, season and location of catch etc.

From the same Table (2), pH value of investigated Nile tilapia were more than 6.0 to 6.5 for fresh fish muscle as reported by **Ježek and Buchtová, (2011).** While the values of TVN, TBA in this study were lower than those findings by **El-Sherif *et al.*, (2011)**; they showed that the TVB-N and TBA of tilapia fish were 14.31 mg/100gm and 0.55 mg/kg, respectively, while **Ibrahim, (2018)** reported that farmed Nile tilapia recorded pH 6.20-6.74, TVN 10.64-12.04mg/100g, whereas the result of TBA agrees with his result (0.21-0.23 mg/kg sample). In addition, TVN content in this work is lower than the recommended limits (30, 35 and 40 mg N/100g based on fish family) as reported by **EOS, (2005).**

3. Heavy metals concentrations of tilapia fillets

Table (3) shows the concentrations of some heavy metals in farmed tilapia fillets. The levels of Pb, Cd, Hg and As were not detectable, 0.08, 0.03, and not detectable ppm, respectively. Cd level (0.08 ppm) was the most abundant metal in the studied tilapia fillets. Concerning microbial contaminants, it was found that fish fillets contaminated with TPC 3.62, Psychrophilic 2,17, coliform group 3,22, yeats 1.34 and molds 1.29 cfu g^{-1}.

Table (3). Heavy metals pollutants and microbiological contaminants of cultured raw Nile tilapia fillets.

Heavy metals pollutants			Microbiological contaminants		
Metal	**Concentration (ppm)**	****MPLs**	**Aspect**	**Value**	****MPLs**
Pb	***ND**	**0.30**	**TPC**	**3.62± 0.10**	**106**
Cd	**0.08**	**0.05**	**Psychrophilic**	**2.17± 0.17**	**10^7 - 10^8 cfu g^{-1}**
Hg	**0.03**	**0.50**	**Coliform group**	**3.22± 0.09**	**-**
As	**ND**	**-**	**Yeasts**	**1.34± 0.02**	**-**
			Molds	**1.29± 0.01**	**-**

*ND: not detected. **MPL: Maximum Permissible Levels; according to EOS [25]. (-): Not available data.

Biodegradation does not occur in heavy metals, therefore, their bioaccumulation has been reported in fish, mussels, oyster, sediments and other components of the world's aquatic ecosystems **(Kumar and Singh, 2010)**. In this work, concentrations of heavy metals in fish fillets are lower than the maximum permissible limits of Pb (0.30 ppm), and Hg (0.50 ppm) as reported by **EOS (2010)** except Cd where record value 0.08 ppm. Our results are varied with some previous studies such as **Elnimr (2011)**; the concentrations of Pb, Cd, and Hg in tilapia fish were 0.83, 0.12 and 0.004 ppm, respectively. **Younes *et al.* (2012)** found that the concentrations of Cd and Pb in muscles of tilapia obtained from Burullus Lake were 0.31-1.25and 5.75-27.35 ppm, respectively. **Abd-Allah, (2013)** found that the tilapia muscles obtained from the Nile River contained (ppm, ww) 2.68 Pb, 0.04 Cd and 0.91 Hg. **Saeed, (2013)** showed that the concentrations of heavy metals (ppm, dw) in tilapia muscles obtained from Edku lake were 0.28 Cd, and 0.92 pb. **Ibrahim *et al.*, (2013)** showed that the concentrations of heavy metals (ppm) in raw *Tilapia zillii* muscles obtained from Lake Qarun were 3.768 Pb and 0.173 Cd. **Basiouny, (2018)** showed the annual means of heavy metals (ppm) in muscles of Nile tilapia collected from Lake Burullus were 0.02 Cd and 0.17 Pb. Nowadays, **Ibrahim *et al.*, (2020)** found that the range of Cd and Pb concentrations in different fish muscles were <0.012-0.02 and <0.023-0.52 ppm of the Nile River, <0.012-0.27 and <0.023-0.50 ppm of Wadi El-Rayan Lake, <0.012 and <0.02-0.05 ppm of Edku lagoon and <0.012 and 0.03-0.06 ppm of in Burullus lagoon, respectively. This variation in heavy metals accumulated in fish muscles is due to fish *spp.*, specimens within the same species, feeding, sex, spawning period, season, location of catch and tested part too.

4. Effect of frozen storage periods on pH value

The pH value of tilapia fillets treated with commercial nisin levels is shown in Table (4). At zero time, the pH value recorded 6.84 for all samples. A little increase in pH value was attributed with the progress frozen storage periods. The pH value increased markedly especially in control samples when compared with samples treated with nisin. The significant differences ($P<0.05$) were found between control and treatments at the end of storage. Control samples recorded a high value of pH (8.15) while it was ranged

6.84-7.11 of treatments at the 90 day. Also, samples treated with 2.0% nisin have a lower pH value than other treatments at the end of frozen storage period.

Table (4). Effect of frozen storage on pH value of tilapia fillets treated with commercial nisin levels.

Storage periods (month)	Tilapia fillets treated with commercial Nisin levels;			
	Control	0.10%	0.15%	0.20%
0	6.84± 0.05[a]	6.84± 0.04[a]	6.84± 0.00[a]	6.84± 0.03[a]
1	6.89± 0.03[a]	6.87± 0.03[a]	6.87± 0.02[a]	6.86± 0.02[a]
2	7.02± 0.11[a]	6.90± 0.05[a]	6.89± 0.04[a]	6.88± 0.06[a]
3	8.15± 0.15[a]	7.11± 0.10[b]	7.04± 0.00[b]	6.94± 0.04[b]

The values represent Mean ± SD of three experiments followed by different superscripts (within rows). a, b, c and d are significantly different ($P < 0.05$)

In this work, increase in pH value of frozen fish samples was attributed to formation of volatile basic nitrogen components as affected by biochemical changes under low temperature. This high post-mortem pH as reported by **Gram and Huss, (1996) and Francisco *et al,* (2014).** Our results agree with those findings by **Manju *et al.,* (2007),** the increase of pH may be due to the increase in volatile basic compounds, such as ammonia, by psychrotrophic bacterial activities.

5. Effect of frozen storage periods on TVB-N content

Effect of frozen storage on TVB- N content (mg/100g sample) of tilapia fillets treated with commercial nisin levels is presented in Table (5). The TVN values were 4.65, 4.62, 4.62 and 4.63 mg/100g sample of control, treatments; 0.10%, 0.15% and 0.20% nisin, respectively at zero time of storage. During frozen storage, significant differences (*$P<0.05$*) were found between all treatments. TVN content increased gradually in all samples especially control (24.20 mg/100g) at the end of storage period. The lowest content of TVN (12.17 mg/100g) was found in treatment contained 2.0% nisin than other ones. In addition, a high concentration of nisin could be controlled in TVN formation compared to control sample.

Table (5). Effect of frozen storage on TVB -N content (mg/100g sample) of tilapia fillets treated with commercial nisin levels.

Storage periods (month)	Tilapia fillets treated with commercial Nisin levels;			
	Control	0.10%	0.15%	0.20%
0	4.65± 0.07[a]	4.62± 0.08[a]	4.62± 0.03[a]	4.63± 0.05[a]
1	9.17± 0.03[a]	8.33± 0.00[b]	7.03± 0.07[c]	6.44± 0.06[d]
2	14.66± 0.04[a]	11.26± .06[b]	10.21± 0.06[c]	9.24± 0.01[d]
3	24.20± 0.02[a]	18.34± 0.11[b]	15.40± 0.00[c]	12.17± 0.08[d]

The values represent Mean ± SD of three experiments followed by different superscripts (within rows). a, b, c and d are significantly different ($P < 0.05$).

From the Table (5), the results of TVN are in agreement with those findings by **Nath *et al.*, (2014); Ibrahim, (2018) and Sarika *et al.*, (2018)**. The increment in TVN of tilapia fillets is as a result of protein and non-protein nitrogenous compounds degradation by spoilage microorganisms **(Dalgaard, 2000).** Beside, its content sharply decreased in all treatments, especially in case of samples treated with nisin concentration 2g/liter. However, other study **(Aly et al., 2006)** reported that there was no significant difference in TVB-N between the oysters packed in antimicrobial bacteriocin-coated films and those packed in plain low density polyethylene film.

6. Effect of frozen storage periods on TMA-N content

Table (6) shows the effect of frozen storage on TMA content (mg/100g sample) of tilapia fillets treated with commercial nisin levels. The values of TMA were 0.34 mg/100g sample of control, treatments at zero time of storage. TMA values increased sharply in all investigated fish samples throughout frozen storage period. Control sample recorded a high value of TMA (9.33 mg/100g) at the end of storage period compared to nisin treatments. Furthermore, the changes in TMA-N of samples were attributed to storage periods prolonged. A significant increase ($P<0.05$) was found between control ant treatments from the 1st month till the 3th of storage. Fish fillets samples treated with nisin especially 0.20% could be reduce TMA formation as a result of protein decomposition in fish fillets comparing with the control samples.

Table (6). Effect of frozen storage on TMA -N content (mg/100g sample) of tilapia fillets treated with commercial nisin levels.

Storage periods (month)	**Tilapia fillets treated with commercial Nisin levels;**			
	Control	**0.10%**	**0.15%**	**0.20%**
0	0.34± 0.03^{a}	0.34± 0.02^{a}	0.34± 0.03^{a}	0.34± 0.02^{a}
1	2.62± 0.00^{a}	1.33±0 .03^{b}	1.03± 0.03^{c}	0.83± 0.00^{d}
2	5.22± 0.03^{a}	3.29± 0.01^{b}	2.99± 0.03^{c}	1.24± 0.04^{d}
3	9.33± 0.02^{a}	6.85± 0.05^{b}	4.16± 0.04^{c}	2.67± 0.05^{d}

The values represent Mean ± SD of three experiments followed by different superscripts (within rows). a, b, c and d are significantly different ($P < 0.05$)

In this work, the TMA values of tilapia fillets were less than acceptable value (10 mg/ 100 g). Similar findings were reported by **Daboor and Ibrahim, (2008) and Ibrahim and El-Sherif, (2008).** In general, nisin at 1, 1.5 and 2g/liter levels reduced protein changes in fish fillets comparing with the control samples. It could be speculated that nisin was more effective as antimicrobial agent in particular at concentration 0.20% level.

7. Effect of frozen storage periods on TBARS value

Effect of frozen storage on TBARS value (mg MDA/kg sample) of tilapia fillets treated with commercial nisin levels is shown in Table (7). The TBA values were 0.23, 0.23, 0.22 and 0.21 mgMDA/kg sample of control, treatments; 0.10%, 0.15% and 0.20% nisin, respectively at zero time of storage. The values of TBA increased markedly in all treatments, especially in control (5.62 mg MDA/kg) at the end of storage period. A significant increase (*P<0.05*) was found between control ant treatments from the 1st month till the 3th of storage. TBA values of treatments are taken the following order: 0.20% < 0.15% < 0.10%.

Table (7). Effect of frozen storage on TBARS value (mg MDA/kg sample) of tilapia fillets treated with commercial nisin levels.

Storage periods (month)	Tilapia fillets treated with commercial Nisin levels;			
	Control	**0.10%**	**0.15%**	**0.20%**
0	0.23± 0.02^{a}	0.23± 0.01^{a}	0.22± 0.00^{a}	0.21± 0.01^{a}
1	2.34± 0.06^{a}	0.92± 0.02^{b}	0.83± 0.02^{c}	0.63± 0.00^{d}
2	4.49± 0.06^{a}	2.04± 0.04^{b}	1.56± 0.07^{c}	0.97± 0.00^{d}
3	5.62± 0.06^{a}	3.90± 0.05^{b}	3.50± 0.05^{c}	1.92± 0.02^{d}

The values represent Mean ± SD of three experiments followed by different superscripts (within rows). a, b, c and d are significantly different (P < 0.05).

Our results are in harmony with those reported by **Ibrahim and Desouky, (2009) and Langroudi *et al.*, (2011).** An increase in TBA value in samples studied could be due to lipid hydrolysis and secondary products formation under low temperature **(Amin, 2012).** Based on these results, the TBA values of tilapia fillets were less than 4.5mg MDA/kg sample in treatments compared to control (untreated) as the maximum permissible limit **(EOS, 2005).**

8. Microbiological aspects

8.1 Total Bacterial Count (TBC)

Table (8) shows the effect of frozen storage on TBC (log cfu/g) of tilapia fillets treated with commercial nisin levels. Initial TBC for control fish fillets was 3.62, 3.62, 3.49 and 3.25 log cfu/g of control, 0.10%, 0.15% and 0.20% nisin treatments, respectively. With the progress of frozen storage, TBC increased significantly (*P<0.05*) to record 9.64, 7.11, 6.42 and 5.30 log cfu/g for control, treatments; 0.10%, 0.15% and 0.20% nisin, respectively at the end of storage at -18°C. In addition, metabolites of nisin at different concentrations had more inhibitory activity, especially in treatment of 0.20% nisin under same conditions of storage.

Table (8). Effect of frozen storage on TPC (log cfu/g) of tilapia fillets treated with commercial nisin levels.

Storage periods (month)	Tilapia fillets treated with commercial Nisin levels;			
	Control	**0.10%**	**0.15%**	**0.20%**
0	3.62± 0.10[a]	3.62± 0.08[a]	3.49± 0.06[a]	3.25± 0.10[b]
1	5.19± 0.11[a]	4.57± 0.13[b]	4.11± 0.09[c]	3.94± 0.06[c]
2	7.11± 0.06[a]	5.93± 0.07[b]	5.35± 0.10[c]	4.22± 0.08[d]
3	9.64± 0.06[a]	7.11± 0.11[b]	6.42± 0.03[c]	5.30± 0.10[d]

The values represent Mean ± SD of three experiments followed by different superscripts (within rows). a, b, c and d are significantly different (P < 0.05).

8.2 Psychrophilic bacterial

Effect of frozen storage on psychrophilic bacteria (log cfu/g) of tilapia fillets treated with commercial nisin levels is shown in Table (9). Initial psychrophilic for control fish fillets was 2.17, 2.17, 2,14 and 2.13 log cfu/g of control, 0.10%, 0.15% and 0.20% nisin treatments, respectively. With the progress of frozen storage, psychrophilic counts increased significantly (*P<0.05*) to record 8.31, 6.25, 5.13 and 4.70 log cfu/g for control, treatments; 0.10%, 0.15% and 0.20% nisin, respectively at the end of storage at -18°C.

Table (9). Effect of frozen storage on Psychrophilic bacteria (log cfu/g) of tilapia fillets treated with commercial nisin levels.

Storage periods (month)	Tilapia fillets treated with commercial Nisin levels;			
	Control	**0.10%**	**0.15%**	**0.20%**
0	2.17± 0.17[a]	2.17± 0.08[a]	2.14± 0.14[a]	2.13± 0.08[a]
1	3.52± 0.10[a]	3.15± 0.15[b]	2.86± 0.04[c]	2.51± 0.09[d]
2	5.13± 0.13[a]	4.66± 0.10[b]	3.79± 0.01[c]	3.21± 0.00[d]
3	8.31± 0.31[a]	6.25± 0.05[b]	5.13± 0.15[c]	4.70± 0.06[d]

The values represent Mean ± SD of three experiments followed by different superscripts (within rows). a, b, c and d are significantly different (P < 0.05).

8.3 Coliform group

The effect of frozen storage on coliform group (log cfu/g) of tilapia fillets treated with commercial nisin levels is exhibited in Table (10). Initial counts of coliform group were 3.22, 3.46, 3.23 and 3.23 log cfu/g of control, 0.10%, 0.15% and 0.20% nisin treatments, respectively. Coliform counts increased significantly (*P<0.05*) to be 7.12, 5.90, 5.14 and 4.80 log cfu/g for control, treatments; 0.10%, 0.15% and 0.20% nisin, respectively at the end of storage at -18°C.

Table (10). Effect of frozen storage on coliform group (log cfu/g) of tilapia fillets treated with commercial nisin levels.

Storage periods (month)	Tilapia fillets treated with commercial Nisin levels;			
	Control	0.10%	0.15%	0.20%
0	3.22± 0.09[a]	3.46± 0.04[a]	3.23± 0.07[a]	3.23± 0.23[a]
1	4.14± 0.23[a]	3.74± 0.06[b]	3.71± 0.06[b]	3.55± 0.09[c]
2	5.96± 0.04[a]	5.32± 0.03[b]	4.67± 0.01[c]	4.37± 0.08[d]
3	7.12± 0.12[a]	5.90± 0.11[b]	5.14± 0.14[b]	4.80± 0.06[c]

The values represent Mean ± SD of three experiments followed by different superscripts (within rows). a, b, c and d are significantly different ($P < 0.05$).

8.4 Yeasts and Molds

The effect of frozen storage on yeasts and molds count (log cfu/g) of tilapia fillets treated with commercial nisin levels is presented in Table (11). Initial of yeasts and molds count were 1.31, 1.30, 1.27 and 1.26 log cfu/g of control, 0.10%, 0.15% and 0.20% nisin treatments, respectively. Yeasts and molds count increased significantly (*P<0.05*) to record 4.94, 3.58, 3.35 and 2.62 log cfu/g for control, treatments; 0.10%, 0.15% and 0.20% nisin, respectively at the end of storage at -18°C.

Table (11). Effect of frozen storage on total count yeasts and molds (log cfu/g) of tilapia fillets treated with commercial nisin levels.

Storage periods (month)	Tilapia fillets treated with commercial Nisin levels;			
	Control	0.10%	0.15%	0.20%
0	1.31± 0.02[a]	1.30± 0.06[a]	1.27± 0.03[a]	1.26± 0.10[a]
1	2.25± 0.09[a]	2.13± 0.07[a]	1.91± 0.02[b]	1.74± 0.04[b]
2	3.36± 0.07[a]	2.91± 0.07[b]	2.65± 0.04[b]	2.24± 0.12[b]
3	4.94± 0.10[a]	3.58± 0.08[b]	3.35± 0.00[b]	2.62± 0.05[c]

The values represent Mean ± SD of three experiments followed by different superscripts (within rows). a, b, c and d are significantly different ($P < 0.05$).

The results of TBC and Psychrophilic (Tables 8 and 9) showed that fish fillets treated with commercial nisin are lower than the maximum permissible limits compared with control sample since the onset of microbial spoilage of seafood is considered to be 10^7-10^8 cells/gm. Similar results were noted as showed by **Allende *et al.* (2004)**. A reduction in coliform group (Table 10) of samples treated with nisin levels indicated that, the bacterium produce antibacterial peptides against *E. coli* DPC 6053 **(Hayes *et al.*, 2006)**. Our results (Table 11) are in accordance with those mentioned by **Collins and Hardt, (1980)** who reported that filtrates of *Lactobacillus acidophilus* at pH 7.7 retarded the growth of *Candida albicans*. The results presented in Table (12) showed that commercial nisin, especially at 0.20% caused a high depression of molds count. Our results agree with those reported by **Ibrahim and Desouky, (2009)** who found that molds inhibited by Lactic acid bacteria metabolites. **EL-Sherif *et al.*, (2011)** found that the TBC of tilapia fish were 2.35 $\log_{10}$ cfu g^{-1}. TBC was within the permissible limit of 6 log cfu/g **(ICMSF, 1986).** Fresh tilapia fish samples obtained from two farms A and B. the TBC of tilapia samples from two farms were 2.25×10^4 and 1.7×10^4cfu/ g respectively. While the

yeast and mold counts were 9.5×10^2 and 4.66×10^2 cfu/ g for farms A and B, respectively, **(Mohamed et al., 2019).**

CONCLUSION

Based on the results of pollutants and contaminants, farmed Nile tilapia fillets are safe for human consumption. Using commercial nisin especially at 0.20% could be controlled in the change rate of biochemical and microbiological quality indices of farmed tilapia fillets throughout frozen storage periods compared to control sample. So, this study recommends that commercial nisin as bio-preservative to improve the safety and quality of seafood.

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