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The impact of different levels of nisin as a biopreservative agent on the chemical, sensory and microbiological quality of vacuum-packed sea bass (*Dicentrarchus labrax*) fillets stored at 4 ± 2 °C

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SUMMARY: Nisin is produced by *Lactococcus lactis* subsp. *lactis* and is also known as an antimicrobial agent especially effective against gram-positive bacteria. It has long been used as a preservative in foods and beverages and is generally regarded as safe (GRAS). In the present work, the effects of different concentrations of nisin (0.2, 0.4 and 0.8%) on the sensory, chemical and microbiological quality and shelf-life of vacuum-packed sea bass (*Dicentrarchus labrax*) fillets were investigated during chilled (4 ± 2 °C) storage. The sensory points for raw and cooked fillets increased with time during the storage period ($p < 0.05$). The control group, with scores of 9.08, was rejected by panelists on day 12; whereas nisin-treated groups were rejected on day 14 with scores ranging from 9.00-9.17 score. As a result of chemical analyses, lower values ($p < 0.05$) were obtained from the nisin groups with low oxidative rancidity. Moreover, nisin inhibited microbial growth, which shows antimicrobial activity. Consequently, it was concluded that the application of nisin (especially 0.8%) preserved the organoleptic quality and extended the shelf-life of sea bass fillets.

***KEYWORDS*:** *Antimicrobial activity; Dicentrarchus labrax; Nisin; Quality changes; Shelf life*

RESUMEN: ***Impacto de diferentes niveles de nisina como agente bioconservador en la calidad química, sensorial y microbiológica de filetes de lubina (*Dicentrarchus labrax*) envasados al vacío y almacenados a 4 ± 2 °C.*** La nisina es producida por *Lactococcus lactis* subsp. lactis y conocida como agente antimicrobiano, especialmente contra las bacterias grampositivas. Se ha utilizado como conservante en alimentos y bebidas durante mucho tiempo y generalmente se considera seguro (GRAS). En el presente trabajo, se investigaron los efectos de diferentes concentraciones de nisina (0,2, 0,4 y 0,8%) sobre la calidad sensorial, química y microbiológica y la vida útil de los filetes de lubina (*Dicentrarchus labrax*) envasados al vacío durante el enfriamiento y almacenamiento (4 ± 2 °C). La puntuación sensorial de los filetes crudos y cocidos aumentó con el tiempo durante el período de almacenamiento ($p < 0,05$). El grupo de control con puntuación de 9,08 fue rechazado por los panelistas el día 12, mientras que los grupos de tratamiento con nisina fueron rechazados el día 14 con un rango de puntuación de 9,00-9,17. Como resultado de los análisis químicos, se obtuvieron valores más bajos ($p < 0,05$) de los grupos de nisina con baja rancidez oxidativa. Además, la nisina inhibió el crecimiento microbiano que muestra actividad antimicrobiana. En consecuencia, se evaluó que la aplicación de nisina (especialmente 0,8%) conservó la calidad organoléptica y prolongó la vida útil de la lubina.

PALABRAS CLAVE: *Actividad antimicrobiana; Cambios de calidad; Dicentrarchus labrax; Nisina; Vida útil*

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1. INTRODUCTION

Food preservation has been seen as a serious problem throughout human history not only of fish meat but also of food products in general. This has led to the development of many traditional conservation methods in different regions of the world in order to keep the foods at their consumable levels for a long time. Today, due to the development of communication technologies and urbanization, the food industry is growing day by day. Despite this growth in the food industry, microbiological spoilage and food poisoning due to microorganisms is still an issue. According to recent information from Centers for Disease Control and Prevention in the United States, 76 million food-borne diseases occur in the United States every year and this leads to the death of approximately 5000 people (Mead *et al.,* 1999). This has led the food industry, the government and the public to examine the efficacy of current food preservation techniques (Anonymous, 2000). The negative effects of synthetic preservatives on food and human health have raised the demand for more minimally-processed or natural foods due to the development of antibiotic-resistant strains and negative perceptions on the part of consumers against synthetic preservatives. Thus, there has been a considerable amount of interest in natural antimicrobial agents.

Natural or synthetic preservatives such as antioxidants and antimicrobials are widely used to prevent or control the growth of microorganisms and undesirable compounds. One of the most prominent methods in recent years is to preserve the freshness of foods by using bacterial products. Food safety has become an increasingly crucial problem worldwide, so it is of great interest to apply antimicrobial peptides from lactic acid bacteria (LAB) which target food pathogens without causing toxicity and other adverse effects. Bacteriocins are widely used in commercial applications in nearly 50 countries and generally accepted as safe (GRAS) as approved by the Food and Agriculture Organization/World Health Organization and the European Union. LAB is well known for its bacteriocin production capacity.

Nisin, one of the emerging interesting bacteriocins, is a peptide with antimicrobial activity produced by *Lactococcus lactis* subsp. *lactis*, and generally considered safe and used to control pathogens in foods (Juneja *et al.,* 2012; Meral *et al.,* 2019; Ceylan *et al.,* 2018; Ucar *et al.,* 2020). Nisin is defined as an antimicrobial agent discovered before penicillin which showed an antimicrobial activity against a wide variety of gram-positive bacteria (vegetative cells and spores). Nisin has been included in the European Food Additives list as a biopreservative component with the code E234 (EFSA, 2006).

There are some studies showing the synergistic effects when nisin was applied to food without the interaction of nisin with some antioxidant extracts or sensory changes (Sallam, 2007; Behnama *et al.,* 2015; Ghomi *et al.,* 2011). Sea bass (*Dicentrarchus labrax*) is heavily consumed among commercial marine fish species due to its flavor and taste. To our knowledge, there is little information on the efficacy of nisin on the quality and safety of sea bass. Thus, in the current study, the effects of different concentrations of nisin on the sensory, chemical and microbiological quality and shelf-life of vacuum-packed sea bass fillets were investigated during refrigerated storage (4 ± 2 °C) conditions.

2. MATERIALS AND METHODS

2.1. Preparation of nisin

Nisin (in powder form) obtained from *Lactococcus lactis* (2.5% balance between sodium chloride and denatured milk solids, EC No 215-807-5, Sigma N5764) was purchased from a local company in Turkey (Tutar Lab. Chemicals, Adana, Turkey). A nisin solution was prepared according to Ceylan (2014) with minor modifications. Stock solutions (0.2%, 0.4% and 0.8% w/v) of nisin were prepared by dissolving in pure, sterile water. The chemical properties of the nisin used in this study are presented in Table 1.

2.2. Sample preparation

Sea bass (*Dicentrarchus labrax*) was provided from a local fish farm in Mersin, Turkey and they were killed by immersing them in ice-cold water (hypothermia). The samples were moved to the laboratory packed in ice within 1 hour from harvesting and death. The average weight of the sea bass was 312.06 ± 26.85g; while the length was 29.77 ± 1.02 cm. The samples were divided into four lots and then immediately gutted and filleted with the skin on. One lot was stacked onto plates (6 fillets per plate) and the rest were treated with nisin solutions.

Table 1. Physical and chemical properties of nisin

CAS-Number	1414-45-5
Formula	$C_{143}H_{230}N_{42}O_{37}S_7$ (polypeptid)
Molekular weight	3.354,07 g/mol
Solubility	Soluble in water, insoluble in non-polar solvents
Functional use	Antimicrobial preservative
E number	E-234
GRAS	Yes
ADI (acceptable daily intake)	0-2 mg/kg bw
Boiling temperature	2967ºC
Flash point	110 °C (230°F)
Storage temperature	2-8 °C
pH	for 0,2% nisin 4.40 for 0,4% nisin 4.20 for 0,8% nisin 4.12
Color	Light brown micronized white powder
Color parameters of nisin	*L** :73.26±0.02 *a**:1.36±0,01 *b**:8,38±0,03
Total phenolic content of nisin	23.56 (mg GAE/g)

GAE: equivalence of gallic acid

The fish fillets were placed in the formulated nisin solutions at various concentrations for 10 minutes. After that, all the groups were wrapped in pouches of 90 µm thick polyamide film (Polinas, Manisa, Turkey) by using a vacuum packing machine (Reepack RV50; Reepack, Seriate, Italy). Water and oxygen permeability were 8.5 g/m^2 per 24 h and 160 cm^3·m^2 per 24 h, respectively. Then, all of the samples were stored in a chilled room (4 ± 2 °C). 3 plates (total of 12 fillets) were randomly chosen from each group for each analysis daily. Analyses were carried out on days 0, 3, 6, 8, 10, 12, 14, 16 and 18.

2.3. Proximate analysis

The protein contents in the samples were adjusted using the Kjeldahl procedure (AOAC, 1984) and a Buchi Digestion System, Model K-424 (BÜCHI Labortechnic, Flawil, Switzerland) and a Kjeltec Distillation Unit B-324 (BÜCHI Labortechnic). A Kjeldahl conversion factor of N x 6.25 was used to calculate the percent protein. In addition, lipid content was determined according to the method described by Bligh and Dyer (1959). Furthermore, ash and moisture analyses were carried out according to the methods of AOAC 920.153 (2002) and 950.46 (2002), respectively**.**

2.4. Sensory analysis

For sensory analysis, the Quality Index Method (QIM) scheme improved by Bonilla *et al.,* (2007) was used for raw fish with minor modifications. The scheme comprised of quality parameters (e.g., skin brightness, skin mucus, flesh texture, flesh-blood, odor, color, brightness, gaping). The corresponding procedure is mentioned in our previous study (Özogul *et al.,* 2016).

The measurement of the freshness of cooked fish (odor, taste, and texture) was assessed according to Torry Scheme (Howgate, 1982). A hedonic scale from 10 to ≤ 3 was used to evaluate the fish. A score of 10 represents very fresh fish, while ≤ 3 represents putrid or spoiled fish. Three random fillets from each group were cooked on top of a glass plate in a microwave (Model: Siemens HF24G241, Munich, Germany) at 600 W for 2 min.

2.5. Chemical analyses

Total volatile basic nitrogen (TVB-N) content in muscle was determined according to the method of Antonocopoulos (1973); the thiobarbituric acid reactive substances (TBARS) analysis was performed according to the method of Tarladgis *et al.,* (1960); the free fatty acid (FFA) analysis was carried out according to AOCS method Ca 5a-40 (1997) and peroxide value (PV) was determined according to AOCS method Ja 8-87 (1994). All details pertaining to the analytical methods are given in our previous study (Özogul *et al.,* 2016).

A pH meter (315i/SET, Weilheim, Germany)

was used to determine the pH of the fish fillets. The samples (n=3) were homogenised with a homogenizer (Ika-Werke Ultra-turrax, model T25, Staufen, Germany) at a ratio of 1:10 (w/v) in distilled water at its highest setting (12000 rpm) for 3 min.

2.6. Microbiological analysis

To estimate the mesophilic aerobic bacteria and psychrophilic viable counts, triplicate samples (one per storage plate) with duplicate measurements from each group (n=6) were taken from each of the different treatments. Violet Red Bile Agar (VRBA, Oxoid, CM0107, Hampshire, England) was carried out and prepared according to the instructions of the manufacturer for total *Enterobacteriaceae*. The detailed measurement was described in our previous study (Özogul *et al.,* 2016).

2.7. Statistical analysis

All of the experiments in the study were performed in triplicate. Therefore, the results were presented as the mean and their standard deviation of the measurements. Significant differences in the results were determined by applying a one-way analysis of variance (ANOVA) using the SPSS version 22 software (SPSS, Chicago, Illinois, USA) and the Duncan's Multiple Range Test comparisons at p-value of < 0.05.

3. RESULTS AND DISCUSSION

3.1. Proximate analysis

Proximate analyses (crude protein, lipid, moisture, and ash) of the sea bass fillets were determined as 19.52, 3.90, 74.44, and 1.18%, respectively. Yazgan *et al.,* (2017) found similar values for crude protein (19.36%), lipid (5.14%), moisture (73.85%) and ash contents (%1.36) in sea bass. The average crude protein content (N × 6.25) values ranged from 17.9 to 21.5% for sea bass as reported by Ballestrazzi and Lanari (1996), Kyrana and Lougovois (2002), Alasalvar *et al.,* (2002), which coincide with the results of the current study. It was reported that the differences were caused by environmental conditions such as region, season and cultural factors (Alasalvar *et al.,* 2002).

3.2. Sensory analyses

Sensory changes (smell, taste, and texture) during the storage of vacuum packaged raw sea bass fillets are depicted in Figure 1. The sensory points for raw fillets increased during the storage period and there were statistical differences among the control group and the treatment groups. Treated groups were found to have a longer shelf-life than those of the control group in terms of sensory parameters. Firstly, the control group with 9.08 points (d12) was rejected by panelists. 0.2% nisin group (9.17), 0.4% (9.00) and 0.8% nisin groups (9.00) were rejected later (d14). The application of nisin reduced fish odor and maintained the appearance of the fish. The use of nisin groups prolonged the shelf-life by 2 days when compared to the control group. Some researchers reported that nisin has a synergistic effect when used with different preservation techniques (Lu *et al.,* 2010). Ceylan (2014) reported that the sensory values for raw sea bass treated with nisin in combination with the irradiation method were higher than those of the group with only nisin and the irradiated group. The combination group of nisin and irradiation extended the shelf-life of the fish by 6 days, showing beneficial effects in terms of sensory quality, similar to our study.

Changes in the sensory quality of cooked sea bass are shown in Figure 1. The acceptability scores for the odor, taste, and texture of the fish decreased with storage time. Sea bass with nisin received better scores from the panelists compared to the control group during storage. Increasing nisin concentration had a positive effect on the sensory quality of the fish fillets especially for protecting the color of fillets and removing odor. The odor, taste, and texture scores for the control were 4.57, 4.71 and 4.86, respectively, at the time of rejection (d 12). The nisin-treated groups were rejected on the 14th day of storage. The applications of nisin to sea bass fillets produced a notable development ($p < 0.05$) in the odor, texture, and taste of the fish. However, among the treatment groups, the best values for odor (4.67), taste (4.75) and texture (4.83) were observed in the 0.8% nisin-treated group at the end of the storage time. Ceylan (2014) reported that during the storage period, the groups treated with nisin received higher scores for odor, texture, color, and taste in cooked sea bass fillets compared to the control group.

3.3. Chemical analyses

3.3.1. Changes in peroxide values

There are high levels of unsaturated fatty acids in seafood and these oils are very susceptible to

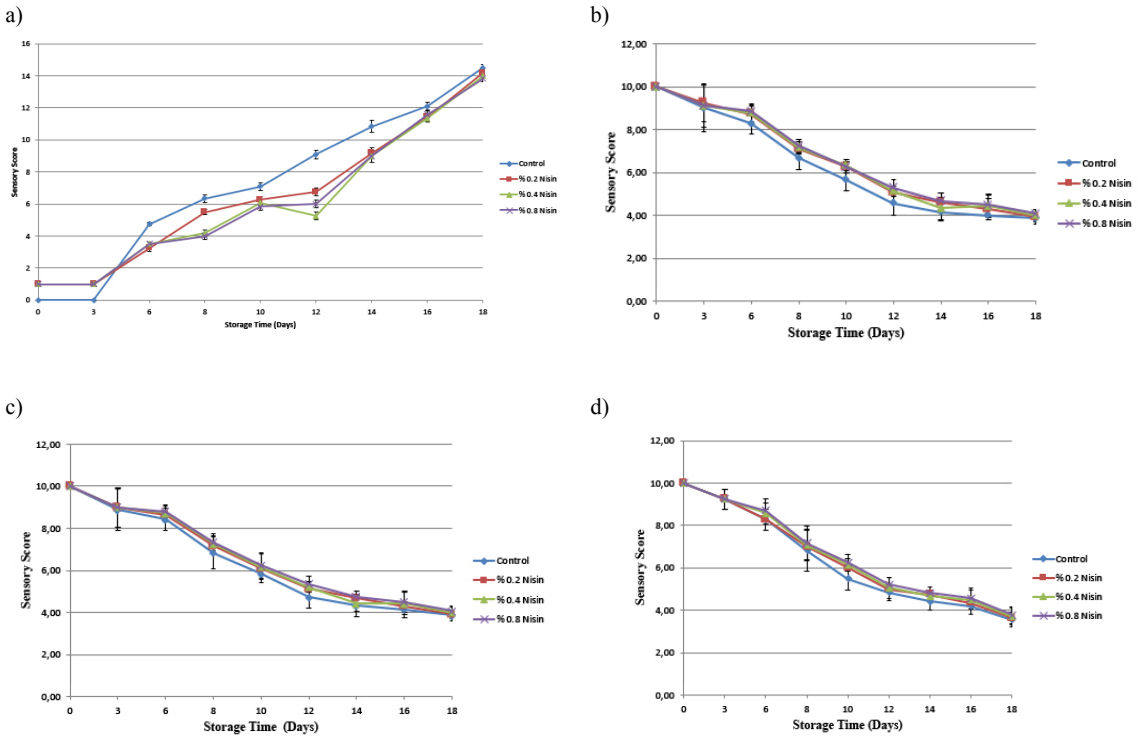

FIGURE 1. The sensory evaluation of sea bass treated with nisin. a) Sensory scores for raw sea bass; b) odor scores for cooked sea bass; c) taste scores for cooked sea bass; and d) texture scores for cooked sea bass.

oxidation. As mentioned above, PV is an indicator of the beginning of lipid oxidation and is the most common index of lipid hydroperoxides. The changes in PV of the control and nisin-treated groups are presented in Table 2. The initial PV was in the range of 2.18 and 2.65 meq O_2/kg. The PV of all treatment and the control groups fluctuated during the storage period and the PV of the nisin-treated groups was lower than the control group during storage. There were statistically significant differences for all groups ($p < 0.05$). A PV value below 5 meq O_2/kg demonstrates that the lipids are fresh due to the hydroperoxides having degraded into ketones. A PV value between 5 and 10 meq O_2/kg showed that the lipids are rancid (Gracey *et al.,* 1999). The upper limit for PV is 20 meq O_2/kg oil (Connell, 1995). The PV did not reach the upper limit in any of the groups during storage, which means the values were below the maximum recommended value for human consumption. Among the nisin groups, the lowest PV was observed in the 0.8% nisin group (2.73 meq O_2/kg) at end of the trial followed by the 0.4% nisin group (3.00 meq O_2/kg) and the 0.2% nisin group (3.84 meq O_2/kg) groups. The nisin application delayed lipid oxidation, depending on its concentration. The decrease in PV in the treatment groups was thought to be due to the influence of vacuum packaging and nisin on the antioxidant effect of lipolytic bacteria (Nykanen *et al.,* 2000) and also its antimicrobial effect on some bacteria such as *L. monocytogenes* and Pseudomonas sp. Similar results were obtained by Ghomi *et al.,* (2011) for grass carp slices and by Behnama *et al.,* (2015) for vacuum-packed rainbow trout (*O. mykiss*) treated with sodium and nisin.

3.3.2. Changes in thiobarbituric acid (TBARs)

Peroxides are formed in the primary stage of the oxidation of lipids found in seafood. In the advanced stage of oxidation, TBARs appear as a freshness parameter that measures the degree of secondary lipid oxidation. The TBAR concentration of freshly-caught fish changes from 3 to 5 mg of malondialdehyde (MA) equivalents per kilogram of flesh (Kuley *et al.,* 2012). Table 2 shows the TBAR changes in the sea bass fillets treated with or without nisin stored at 4 ± 2 °C. While the initial TBARs of the asea bass fillets were found in the range of

Table 2. The changes in PV, TBARs, FFA, TVB-N, and pH contents of sea bass fillets during storage

Strorage days	Control	0.2%	0.4%	0.8%
PV values (meq O_2/kg)				
0	2.65 ± 0.14^{Ea}	2.18 ± 0.08^{Fb}	2.32 ± 0.11^{Gb}	2.22 ± 0.09^{Fb}
3	2.85 ± 0.05^{Ea}	2.32 ± 0.07^{Fbc}	2.42 ± 0.06^{Gb}	2.19 ± 0.10^{Fc}
6	5.20 ± 0.15^{Ca}	4.53 ± 0.23^{Cbc}	4.63 ± 0.25^{Cb}	4.25 ± 0.08^{Cc}
8	6.26 ± 0.13^{Ba}	4.08 ± 0.15^{DEbc}	4.33 ± 0.17^{Db}	4.03 ± 0.16^{Cc}
10	4.58 ± 0.17^{Dc}	5.01 ± 0.28^{Bab}	5.13 ± 0.16^{Ba}	4.70 ± 0.08^{Bbc}
12	5.38 ± 0.53^{Ca}	4.41 ± 0.20^{CDb}	3.96 ± 0.22^{Ebc}	3.70 ± 0.14^{Dc}
14	6.92 ± 0.37^{Aa}	6.53 ± 0.37^{Aa}	5.63 ± 0.22^{Ab}	5.74 ± 0.22^{Ab}
16	7.29 ± 0.22^{Aa}	4.21 ± 0.24^{CDEb}	4.25 ± 0.07^{Db}	4.13 ± 0.06^{Cb}
18	4.46 ± 0.14^{Da}	3.84 ± 0.18^{Eb}	3.00 ± 0.12^{Fc}	2.73 ± 0.13^{Ec}
TBARs content (mg malonaldehyde (MA)/kg fillet)				
0	0.27 ± 0.01^{Ea}	0.26 ± 0.01^{Eab}	0.24 ± 0.01^{Gc}	0.25 ± 0.01^{Ebc}
3	0.28 ± 0.01^{Ea}	0.27 ± 0.01^{Ea}	0.25 ± 0.01^{FGb}	0.27 ± 0.02^{Ea}
6	0.43 ± 0.04^{CDa}	0.36 ± 0.02^{Cb}	0.36 ± 0.02^{Db}	0.37 ± 0.02^{BCDb}
8	0.32 ± 0.04^{Ea}	0.27 ± 0.01^{Eb}	0.27 ± 0.00^{Fb}	0.27 ± 0.00^{Eb}
10	0.46 ± 0.03^{Ca}	0.36 ± 0.01^{Ca}	0.39 ± 0.01^{Ca}	0.35 ± 0.17^{CDa}
12	0.52 ± 0.04^{Ba}	0.42 ± 0.01^{Bb}	0.42 ± 0.01^{Bb}	0.40 ± 0.05^{BCb}
14	0.62 ± 0.06^{Aa}	0.53 ± 0.03^{Ab}	0.47 ± 0.01^{Ac}	0.44 ± 0.03^{ABc}
16	0.39 ± 0.06^{Da}	0.31 ± 0.00^{Db}	0.31 ± 0.00^{Eb}	0.31 ± 0.00^{DEb}
18	0.59 ± 0.06^{Aa}	0.52 ± 0.04^{Ab}	0.45 ± 0.04^{Ac}	0.51 ± 0.01^{Ab}
FFA content (% FFA as oleic acid)				
0	1.72 ± 0.08^{Ea}	1.54 ± 0.07^{Fb}	0.91 ± 0.03^{Ed}	1.18 ± 0.06^{Ec}
3	1.85 ± 0.04^{Ea}	1.57 ± 0.03^{Fb}	0.96 ± 0.06^{Ed}	1.22 ± 0.07^{Ec}
6	3.67 ± 0.18^{Da}	2.98 ± 0.11^{DEb}	3.01 ± 0.16^{Cb}	3.17 ± 0.15^{Cb}
8	3.95 ± 0.05^{CDa}	2.86 ± 0.16^{Eb}	2.63 ± 0.11^{Dc}	2.89 ± 0.13^{Db}
10	3.66 ± 0.12^{Da}	3.03 ± 0.14^{DEb}	3.08 ± 0.11^{Cb}	3.26 ± 0.19^{Cb}
12	5.25 ± 0.31^{Ba}	3.82 ± 0.16^{Cb}	3.62 ± 0.05^{Bbc}	3.32 ± 0.14^{Cc}
14	5.14 ± 0.28^{Ba}	4.22 ± 0.09^{Bb}	3.68 ± 0.21^{Bc}	4.04 ± 0.08^{Bb}
16	6.56 ± 0.35^{Aa}	5.50 ± 0.20^{Ab}	4.96 ± 0.25^{Ac}	4.51 ± 0.21^{Ac}
18	4.23 ± 0.34^{Cb}	3.10 ± 0.07^{Dc}	4.95 ± 0.25^{Aa}	4.25 ± 0.03^{Bb}
TVB-N content (mg/100g fillet)				
0	19.70 ± 0.47^{Ea}	19.57 ± 0.03^{Da}	18.83 ± 0.96^{Ea}	19.78 ± 0.34^{Ea}
3	20.04 ± 0.99^{Ea}	20.61 ± 0.55^{Da}	19.84 ± 0.47^{Ea}	20.82 ± 0.16^{Ea}
6	28.96 ± 0.02^{Da}	26.42 ± 0.65^{Cb}	23.87 ± 0.67^{Dc}	24.93 ± 0.23^{Dc}
8	16.99 ± 0.33^{Fa}	16.17 ± 0.36^{Eab}	15.19 ± 1.11^{Fb}	15.87 ± 0.14^{Fab}
10	31.48 ± 1.12^{Ca}	27.11 ± 0.82^{Cb}	26.76 ± 0.60^{Cb}	25.83 ± 0.02^{Db}
12	38.47 ± 0.41^{Aa}	32.36 ± 0.04^{Bb}	31.50 ± 1.37^{Bb}	28.40 ± 1.20^{Cc}
14	35.36 ± 1.95^{Ba}	35.95 ± 1.03^{Aa}	34.66 ± 1.70^{Aa}	32.98 ± 0.23^{Ba}
16	38.50 ± 0.52^{Aa}	37.03 ± 0.07^{Aab}	35.68 ± 0.53^{Abc}	34.67 ± 1.08^{Ac}
18	39.31 ± 1.33^{Aa}	36.78 ± 1.89^{Aab}	34.66 ± 0.52^{Ab}	35.29 ± 0.33^{Ab}
pH values				
0	6.60 ± 0.04^{CDa}	6.59 ± 0.00^{Ca}	6.53 ± 0.05^{Ca}	6.48 ± 0.19^{BCa}
3	6.62 ± 0.03^{CDa}	6.60 ± 0.01^{BCab}	6.58 ± 0.02^{Bb}	6.60 ± 0.02^{Aab}
6	6.78 ± 0.04^{Ba}	6.64 ± 0.00^{Bb}	6.66 ± 0.01^{Ab}	6.64 ± 0.03^{Ab}

Strorage days	Control	0.2%	0.4%	0.8%
8	6.56±0.02^{Da}	6.47±0.01^{Db}	6.49±0.01^{Db}	6.48±0.01^{BCb}
10	6.64±0.03^{Ca}	6.51±0.02^{Dc}	6.58±0.02^{Bb}	6.57±0.03^{ABb}
12	6.78±0.07^{Ba}	6.50±0.01^{Db}	6.50±0.01^{CDb}	6.38±0.01^{CDc}
14	6.92±0.07^{Aa}	6.60±0.04^{BCb}	6.48±0.02^{Dc}	6.48±0.01^{BCc}
16	6.70±0.07^{Ba}	6.42±0.01^{Eb}	6.36±0.04^{Ebc}	6.34±0.03^{Dc}
18	6.88±0.03^{Aa}	6.75±0.08^{Ab}	6.60±0.05^{Bc}	6.57±0.02^{ABc}

Values represent mean ± SD of 3 replicates in duplicate. Means sharing the same letter in the same column (a–f) and in the same row (A-F) are not significantly different ($P < 0.05$) using Duncan's multiple range test.

0.24 - 0.27 mg MA/kg, at the end of the trial it was between 0.45 mg MA/kg and 0.59 mg MA/kg. The TBAR value remained below the limit value. TBAR values were found statistically significant for both the control and nisin groups during the storage periods ($p < 0.05$). The highest TBAR value was found for the control group (0.62 mg MA/kg) on day 14, compared to those of all groups. These results demonstrated low oxidative rancidity because of the nisin and also the effects of vacuum packaging. Mendes and Golcalves (2008) reported that TBAR values in vacuum packed sea bream and sea bass were lower since the removal of oxygen from the package prevented oxidation. Ceylan (2014) found that the TBAR values of sea bass fillets at the beginning of storage were 0.81 and 1.32 mg MA/kg for the control and nisin groups, respectively, and 4.70 mg MA/kg on the first day of storage in the nisin + irradiation group.

3.3.3. Changes in free fatty acids (FFA)

Free fatty acids are known to result from the enzymatic hydrolysis of esterified lipids. The changes in FFA values of sea bass fillets treated with or without nisin and stored at 4 ± 2 °C are given in Table 2. The initial FFA value of raw fillets was determined as 1.72 (oleic acid %) for the control group and 1.54, 0.91 and 1.18 for 0.2%, 0.4% and 0.8% nisin-treated groups, respectively. Fluctuations were observed in FFA values in all groups during storage and FFA values among the groups were found to be statistically significant ($p < 0.05$). FFA values ranged from 0.91 to 6.56 (oleic acid %) for all groups during the storage period. Although the minimum FFA values for sea bass fillets treated with nisin (0.4% group) were determined to be 0.91% at the beginning of storage, the maximum FFA values for sea bass fillets treated with nisin (0.2% group) were found to be 5.50% on day 16 of storage. The highest FFA value was found as 6.56% in the control group on the 16th day of storage, while the lowest FFA value was found to be 0.91% in the 0.4% nisin-treated group at the beginning of storage. FFA and their oxidation products interact with myofibrillar proteins and promote protein aggregation. Therefore, they affect the muscle texture and functionality (Gracey *et al.,* 1999). In the current study, it was determined that nisin-treated groups had lower FFA values compared to the control group. It was determined that nisin application depends on the concentration used and vacuum packaging delayed lipid hydrolysis. Similar results for the FFA values were reported by Kyrana and Lougovois (2002) for sea bass. Durmuş *et al.,* (2019) reported that the FFA value fluctuated with the storage period of sea bass fillets (*D. labrax*) stored in cold and vacuum-packed nanoemulsions using vegetable oil.

3.3.4. Changes in total volatile basic nitrojen (TVB-N)

TVB-N is one of the most important chemical parameters used to determine fish quality during storage. Generally, volatile bases are obtained from the microbial degradation of protein and non-protein nitrogenous compounds such as trimethylamine, dimethylamine, ammonia and other volatile basic nitrogen compounds. The TVB-N contents in sea bass fillets stored at 4 ± 2 °C are shown in Table 2. While the TVB-N value was determined as 19.70 mg/100g for the control group, this parameter ranged between 18.83 and 19.78 mg/100g for the nisin-treated groups at the beginning of storage. Mendes and Gonçalves (2008) found that the initial TVB-N value in the sea bass was 21.9 mg/100g. Castro *et al.,* (2006) reported the initial TVB-N value of sea bass to be 19-22 mg/100g. The non-protein nitrogen content in fish, depending on the type of fish feeding, season of catching, fish size, various environmental factors as well as the initial microbiological quality of the fish

tissue may cause these differences in TVB-N values (Connell, 1995). In addition, the TVB-N content was reported to be related to the initial microbial activity in fish meat (Connell, 1995).

The increase in TVB-N values in sea bass by the time of storage was also reported in previous studies (Ceylan, 2014; Durmus *et al.,* 2019). Unlike the results of these studies, TVB-N values showed fluctuations in the early stages of storage and then increased regularly after 8 days of storage and significant differences ($p < 0.05$) were observed between the control and treated groups throughout the storage period. The TVB-N level exceeded the upper acceptability limit (35 mg N/100g of fish flesh) set by the Commission of the European Communities (CEC, 1995) for all groups at the end of the storage period. At the end of the trial, TVB-N values were in the range of 34.66 and 39.31 mg/100 gr. The lowest TVB-N value ($p < 0.05$) was obtained from the 0.4% nisin group (34.66 mg TVB-N/100 g) followed by the 0.8% nisin group (35.29 mg TVB-N/100 g) at end of the storage period, but the highest TVB-N value was obtained from the control (39.31 mg TVB-N/100 g) followed by the 0.2% nisin group (36.78 mg TVB-N/100 g). The fish fillets treated with nisin were observed to have better quality than the control group, depending on its concentrations. Similar findings were observed for Atlantic salmon and rainbow trout fillets treated with nisin together with vacuum packaging (Han *et al.,* 2016; Behnama *et al.,* 2015). During storage, decomposition of the nitrogenous compounds caused an increase in the pH of the fish meat, which may be partly related to the production of alkali compounds.

3.3.5. Changes in pH

As one of the most important factors, pH affects microbial growth and the deterioration of foods, especially seafood. The pH value of fish meat changes due to microbial and enzymatic activities, giving information on the freshness and quality of the seafood. The typical pH of live fish muscle is ≈ 7.0 (Abbas *et al.,* 2008). However, post-mortem pH can oscilate from 6.0 to 7.1, based on the season, species, and the other factors (Simeonidou *et al.,* 1998). The pH changes in vacuum-packed sea bass fillets treated with nisin in cold storage are shown in Table 2. pH was measured at 6.48 and 6.60 at the beginning of storage and fluctuations were observed throughout the storage period. Statistical differences ($p < 0.05$) were observed between nisin-treated groups and the control group during the storage period. The pH changed from 6.34 to 6.92 (Table 2). Among the nisin groups, lower pH values were determined for the 0.8% nisin group (6.34) at end of the storage period, followed by the 0.4% and 0.2% nisin groups, but the control group (6.92) gave the highest pH value. This may be due to the inhibitory effect of nisin on microbial growth. Similar findings were observed for farmed sea bass (Durmus *et al.,* 2019). Ceylan (2014) reported that the pH values of sea bass fillets stored in the cold increased at the end of storage. Behnama et al. (2015) measured the pH values of rainbow trout fillets for the control and the groups treated with nisin at 6.37 and 6.36, on day 0 of storage, respectively.

3.4. Microbiological changes

The degradation of most seafood products is mainly caused by microorganisms. Figure 2 shows microbiological changes in vacuum-packed sea bass fillets treated with different concentrations of nisin.

3.4.1. Total mesophilic bacteria

Figure 2(a) shows total mesophilic bacteria changes in vacuum-packed sea bass fillets treated with different concentrations of nisin. The initial total mesophilic bacterial count was 2.55 log cfu/g and reached a maximum level of 7.89 log cfu/g for the control group at the end of the trial. The lowest bacterial count was obtained in fish fillets treated with nisin. When fish was unacceptable by panelists, total mesophilic counts were above 7.39 log cfu/g for the control and about 6.65 log cfu/g for the other nisin-treated groups. Meral *et al.,* (2019) reported that total mesophilic bacteria in nisin-curcumin- treated rainbow trout fillets were 6.12 log cfu/g after 12 days of storage.

Durmuş *et al.,* (2019) reported that the control group exceeded the limit on the 12th day of storage (day of sensory rejection) with 7.38 log cfu/g in sea bass. Ceylan (2014) reported that the control group exceeded the limit value (6 log cfu/g) on the 9th day and the nisin group exceeded the limit value at 11th days of storage for sea bass. Mesophilic bacteria counts were reported to be above 8 log cfu/g at the time of sea bass rejection (Ozogul *et al.,* 2016). With a combination of nisin and vacuum packaging, total aerobic mesophilic bacteria counts were found to be lower than those of the control, extending the shelf-life

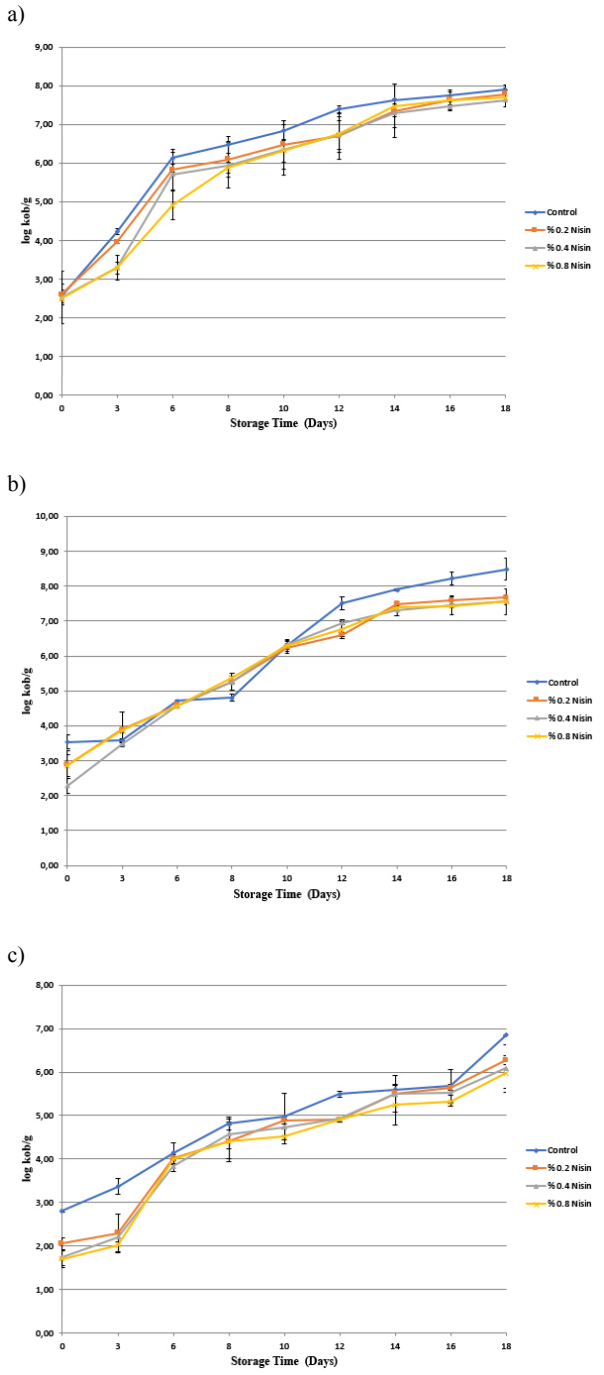

FIGURE 2. Microbiological changes in sea bass treated with nisin. (a) Total mesophilic bacteria, (b) total psychrotrophic bacteria, (c) total *Enterobacteriaceae* counts

of sea bass fillets. Thus, the total aerobic mesophilic bacteria count correlated with the sensory analysis.

3.4.2. Total psychrotrophic bacteria

Psychrotrophic bacteria in the seafood stored in cold causes the deterioration of products. Figure 2(b) shows the total psychrotrophic bacteria counts in vacuum-packed sea bass fillets treated with different concentrations of nisin. The initial total psychrotrophic bacterial count was 3.53 log cfu/g and reached the maximum level of 8.48 log cfu/g for the control group at the end of the trial. At the beginning of storage, total psychrotrophic bacteria was found to be within the range of 2.28 and 2.88 log cfu/g for nisin-treated groups and differences were observed in the total psychrotrophic bacteria during storage. In contrast to the control group, the application of nisin and vacuum packaging decreased total psychrotrophic bacteria growth. It was reported that the growth of psychrotrophic bacteria was delayed in fish treated with nisin (Behnama *et al.,* 2015). Similarly, in the current study, the treatment of fish fillets with nisin and vacuum packaging resulted in lower bacterial growth. When fish was unacceptable by panelists, total psychrophilic counts were above 7.52 log cfu/g for the control (day 12) and about 6.90 log cfu/g for all nisin-treated groups (day 14). Durmus *et al.,* (2019) reported that total psychrotrophic bacteria for the control group of vacuum-packed seabass fillets exceeded the limit value with 7.09 log cfu/g on the 16th day of storage. Psychrotrophic bacteria count was reported to be above 7.2 log cfu/g at the time of sea bass rejection (Ozogul *et al.,* 2016).

3.4.3. Total Enterobacteriaceae counts

Paleologos *et al.,* (2004) found that *Enterobacteriaceae* were part of the spoilage microflora of whole gutted and filleted sea bass stored in ice. In this study, gradual increases in total *Enterobacteriaceae* counts were observed during the storage period. Nisin applications combined with vacuum packaging generally showed the lowest bacterial load. At the beginning of storage, total *Enterobacteriaceae* counts were found to be 2.80, 2.06, 1.74 and 1.71 log cfu/g for the control, 0.2%, 0.4% and 0.8% nisin-treated groups, respectively and statistically crucial divergences were obtained between the control group and the nisin-treated groups during the storage period. The total *Enterobacteriaceae* number increased in direct proportion to the storage period and the highest increase was observed in the control group. At the rejection time of fish by panellists, total *Enterobacteriaceae* counts were 5.49 log

cfu/g (12 d), 5.49 and 5.51, 5.50 ve 5.25 log cfu/g for for the control, 0.2%, 0.4% and 0.8% nisin-treated groups (14 d), respectively. Although total *Enterobacteriaceae* counts were below 6.85 log cfu/g for all groups at the end of the trial, the maximum load of *Enterobacteriaceae* was observed in the control group throughout the storage period. Durmuş *et al.,* (2019) reported that the amount of *Enterobacteriaceae* increased with the duration of storage of sea bass (*D. labrax*) fillets and the highest value of 6.20 log cfu/g on the last day of storage (day 18). Nisin combined with vacuum packaging was effective in the deceleration of the growth rate of *Enterobacteriaceae* in refrigerated sea bass fillets.

4. CONCLUSIONS

The combined use of nisin with vacuum packaging in the cold storage of sea bass fillets led to the extention of shelf-life. Nisin inhibited microbial growth and lowered the incidence of oxidation in sea bass fillets, with antimicrobial and antioxidant activity. As a result, it was concluded that the application of nisin, especially 0.8% nisin, preserves the nutrient and organoleptic quality and extends the shelf-life of sea bass (2 days) at 4 ± 2 °C.

COMPLIANCE WITH ETHICAL STANDARDS

The authors declare there are no conflicts of interest.

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