COMPARATIVE EFFECTS OF LACTIC ACID, NISIN, COATING COMBINED AND ALONE APPLICATIONS ON SOME POSTMORTEM QUALITY CRITERIA OF REFRIGERATED *SARDINA PILCHARDUS*

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ABSTRACT

Fish meat is highly prone to microbiological contamination from hunting and slaughtering stages to the transportation and kitchen, especially by Pseudomonas *spp. In this study, effects of nisin (0.04 g/L/kg at pH 5.2), lactic acid (5% at pH 2.9) and their combinations were studied on coated (where vegetable oil, beeswax and distilled water mixture with pH 7.2 was used as the main coating material) and noncoated fish specimens on inhibition of* Pseudomonas *and mesophilic aerobic bacteria (MAB) at refrigeration temperatures (+4C). Lactic acid, nisin and coating reached 5.95 log units as cfu/mL on total MAB on the seventh day of shelf life at +4C, whereas control has reached 6.62 log on the fifth day. Lactic acid, nisin and coating, and lactic acid and coating applications were determined as the most preservative applications with lowest acidity losses and highest bacterial inhibition, including* Pseudomonas *spp.* ($P < 0.01$).

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INTRODUCTION

Fresh fish fillets are highly prone to microbial spoilage, in comparison to live crustacean and molluscan shellfish, because they do not have cutin and shell to protect them against superficial contamination through the skin. Therefore, the contamination of fish specimens and microbial quality decrease begins in preharvest period and continues during harvest, distribution and storage, especially starting from the skin. In the case of concerned fish spoilage, *Pseudomonas* and also *Shewanella putrefaciens* are the main specific spoilage bacteria of iced fresh fish and fish specimens under refrigerated conditions (Smulders and Woolthuis 1985; Gram and Huss 1996; Taoukis *et al.* 1999). *Pseudomonas* is also reported as the dominant spoilage microflora in the *Sardina pichardus* eggs, Nile perch, gutted and ungutted sea bass, and rainbow trout intestine (Gram *et al.* 1990; Spanggaard *et al.* 2000; Miguez and Combarro 2003; Papadopoulos *et al.* 2003). Not only is the contamination risk in gastrointestinal source caused by *Pseudomonas*, but also the contamination of surroundings, recesses, fishery equipment and devices during the evisceration of fish fillets with *Pseudomonas*, appears as one of the serious problems in the fish industry. This genus dominates the microflora of cold-smoked salmon plants, smokehouses, herring and caviar plants (Bagge-Ravn *et al.* 2003). Although fish is highly prone to microbial contamination due to its lower collagen amount and higher water content, very few studies were carried out about fish decontamination in comparison with meat. Organic acids seem to be an alternative decontaminant in fish preservation, like the treatments of pork and beef carcasses with 2% lactic acid (Smulders and Woolthuis 1985; Eppling *et al.* 1993; Hardin *et al.* 1995; Smulders and Greer 1998). Unfortunately, nisin, a bacteriocin produced by *Lactococcus lactis*, inhibiting many Gram-positive foodborne pathogens like *Listeria* and *Clostridium* (Ogden and Tubb 1985), could not be used for fish because the initial spoilage flora primarily consists of Gram-negative (especially *Pseudomonas*) bacteria. However, several studies have indicated the effect of nisin on Gram-negative bacteria in the case of its combined use with other compounds such as organic acid, ethylenediaminetetraacetic acid (EDTA) and ethyl alcohol by increasing the rate of nisin penetration into Gram-negative cell walls (Stevens *et al.* 1991; Sheffet *et al.* 1995; Taoukis *et al.* 1999). Nisin and high hydrostatic pressure alone or combined applications with lysozyme, lactoferrin and lactoferricin in foods including egg and poultry, appeared as another meat protection alternative (Ponce *et al.* 1998; Garriga *et al.* 2002; Yuste *et al.* 2002). Coating, like these procedures mentioned earlier, became another practice. Because fish spoilage takes its origin from Gram-negative bacteria, coating seems very effective in fish quality protection by the creation of an anaerobic condition which is inhibitory on Gram-negative *Pseudomonas* microflora.

Nisin, as a bacteriocin that inhibits only Gram-positive bacteria, could not find application platform in marine technology, because the dominant fish flora is Gram-negative as previously mentioned. The enhancement of nisin's inhibitory effect with organic acid's destructive effect on Gram-negative cell wall and coating on Gram-negative spoilage flora, was searched in this study, while trying to highlight the application possibilities of nisin as a microbial decontaminant in fish hygiene, as combined with lactic acid and coating.

MATERIALS AND METHODS

Sample Preparation

Sardina pilchardus was studied due to its liability with its high water content, low collagen amount and high lipid amount to microbial spoilage. Two-year-old samples randomly obtained from the Black Sea, were purchased from a local fish market (Aydinoglu Market, Samsun, Turkey). They were transferred immediately to the vendor by cold chain (at +4C) and brought in at the eighth hour to the laboratory after hunting without subjection to improper handling conditions. Whole samples without removal of internal organs, were separated into eight groups as each contains 1 kg of fish (as 0.5-kg sample and 0.5-kg replicate). Samples of the first group were dipped into a nisin solution. The nisin solution was prepared by adding 0.04-g nisin into 1-L tap water at pH 5.2 and this group was coded as N (Table 1). The fish specimens of the

TABLE 1.
THE NISIN, COATING AND LACTIC ACID ALONE/COMBINED APPLICATIONS WITH THEIR CODES

Code	Treatment
LA + N	Dipping into 0.04 g/L/kg (pH 5.2)/10 min then dipping into 5% lactic acid (pH 2.9)/2 min
LA	5% lactic acid (pH 2.9)/2 min
NCo	0.04 g/L/kg (pH 5.2)/10 min then coated (46.6% vegetable oil, 1.8% beeswax and 51.6% distilled water) (pH 7.04)/41C.
Co	Coating, with the same coating material, described above for NCo
LA + Co	Dipping into lactic acid 5% (pH 2.9)/2 min then coating
LA + N + Co	Dipping into lactic acid 5% (pH 2.9)/2 min then dipping into nisin solution (0.04 g/L/kg) (pH 5.2)/10 min and finally coating
C	The group brought in the eight hour to the laboratory by cool chain (+4C) after hunting like all the others but taken directly to the analysis without subjection to any kind of application
N	Dipping into 0.04 g/L/kg (pH 5.2)/10 min

second group were dipped into the nisin solution, which was prepared the same way and then transferred into the 5% lactic acid solution at pH 2.9, coded as LA + N. The fish specimens in the third group were dipped into the lactic acid solution (LA) while the fish in the fourth group were subjected to nisin solution and then coated (NCo). Coating material was prepared by adding 46.6% vegetable oil and 1.8% beeswax into 51.6% distilled water and mixing homogeneously under heat, until all ingredients were melted as described by Daniels (1973). The pH level of the coating material was adjusted to 5.2, following the preparation. The fifth group was only coated (Co). The sixth group was dipped into the lactic acid solution and then coated (LA + Co). The seventh group was dipped into the lactic acid solution, then transferred into the nisin solution and finally coated (LA + N + Co), while the last group served as control (C). All samples were kept in duplicate sterile plastic bags at +4C. Five fish from each group were randomly selected and taken to all analysis in triplicates. All experiments for determining the microbial, chemical and sensory criteria were carried out in triplicates.

Microbial Enumeration

Meat cuts, from 0.5 cm behind the gills, as 0.5 g from the fish surface and 0.5 g near the bone were collected from eight fishes for each sample group. Samples were ground and mixed homogeneously by using sterilized lancet and forceps, also treated with alcohol and flame, prior to use. Serial dilutions in peptone water (0.1%) were prepared from the homogenized samples. Plate count agar (Oxoid, Unipath, UK) for mesophilic aerobic bacteria (MAB) and MacConkey agar (Oxoid) for *Pseudomonas* counts were used in enumeration. Spread plating for *Pseudomonas* and pour plating for MAB at 37C for 24–48 h as in triplicates, were carried out for each dilution. After colony formation on MacConkey, long-wave ultraviolet was applied to the concerned colonies and bluish yellow fluorescent given colonies were counted during *Pseudomonas* enumeration (Collins *et al.* 1991). Microbial enumeration and pH determinations were initially carried out at the fourth hour, and then continued within 24-h intervals for a period of 7 days.

pH and Total Lactic Acidity (%) Determinations

The pH of the samples was measured by a digital pH meter after homogenization (pH-meter Lab 850; Schott Instruments GmbH (Hattenbergstrabe 10-55122, Mainz, Germany; pH electrode pH 14; buffers 4.01 ± 6.87)). The meat cuts as 0.5 g from the fish surface and 0.5 g near the bone were taken from eight fishes for each sample group. Samples were grinded using sterilized lancet and tweezers, and 1-g ground sample of each application was

transferred to the shaker after pouring them into 9-mL distilled water. They were taken to pH determination followed by 15-min shaking period. The lactic acid levels were detected by high-performance liquid chromatography (HPLC). LKB-Bromma model 2150 HPLC equipment with a differential refractometer (KNAUER; Shimadzu Scientific Instruments, Kyoto, Japan) and Shimadzu C-R-4-Chromatopack monitor (Shimadzu Scientific Instruments, Kyoto, Japan) with a Phenomenex Rezex Cal monosaccharide column (300 × 7.8 μm) was used. The attenuation parameter was set to 2, speed = 2, flow rate was 0.45, at 510 nm and the oven temperature was kept as 55C throughout the experiment. The peak areas are calculated according to a reference lactic acid peak; recorded at the same conditions with the applications, as the area of reference is accepted as 100%. The percent of the area of the treated group was calculated in proportion, by division of the peak area, which belongs to the concerned application, to the peak area of the reference, which was referred as 100%.

Sensory Evaluation

Fish meats that belong to the application groups and control were fried in pans separately after cleaning, prior to the taste panelist evaluation in the third day of shelf life at (+4C). Each 250 mL of sunflower oil for 16 fishes in each application batch was used for frying. The 15 panelists were previously trained, from the point of fish sensory, especially in differentiating the cooked fish out of the standards, from the cooked fish having high sensory quality criteria. Discrimination of cooked fish groups was made by a "10-point scale" from the point of taste, texture and odor described by Huss (1995), with aroma, flavor and appearance. Four and lower than 4-point evaluations in each quality criteria indicated "rejection." The fish meats evaluated as higher than 4, in each quality criteria, were accepted. The fish batches accepted by 9 of 15 taste panelists, were selected and transferred into the descriptive test. Fifteen different panelists for this test were trained about postmortem fish quality criteria, from the point of the same sensory criteria, as 2 h each day all through 1-week sensory session. Fishes from each group were fried in pans separately and again, by using the 10-point scale (Meilgaard *et al.* 1991; Huss 1995), each quality criterion of the concerned groups was compared. Attributes included color (1 = brown/green/blue and 10 = blackish white), appearance (1 = discolored, 10 = translucent), aroma (1 = putrid, 10 = fresh), taste (1 = rotten, spoilt and 10 = meaty, no sweetness), odor/flavor (1 = H2S/sulphide and 10 = characteristic, seaweedy) and texture (1 = slippery, sloppy, greasy, 10 = firm, elastic). The numeric values given by taste panelists were taken to multivariate statistical analyses (MANOVA). All sensory assessments were performed under natural light. Natural spring water was provided between samples to cleanse the palate. All findings with MANOVA results were tabulated in Table 2.

TABLE 2.
VARIATION AMONG THE EFFECT OF NISIN, COATING AND LACTIC ACID TREATED APPLICATION GROUPS ON QUALITY CRITERIA WITH MEAN VALUES ($P < 0.01$)

Criteria	Applications							
	LA + Co	LA + N + Co	LA + N	N + Co	LA	Co	N	C
Pseudomonas	3.55^{A*}	3.69^{B}	3.90^{D}	3.82^{C}	4.09^{E}	3.81^{C}	5.13^{F}	5.56^{G}
MAB	4.55^{B}	4.21^{A}	4.74^{C}	5.35^{F}	4.90^{D}	5.29^{E}	5.41^{G}	5^{H}
pH	5.49^{A**}	5.65^{B}	5.85^{C}	6.11^{E}	5.92^{D}	6.10^{E}	6.23^{F}	6.31^{G}
TLA (%)	$52A^{B}$	57^{A***}	41^{BC}	34^{D}	55^{AB}	39^{BC}	31^{D}	26^{E}
Tenderness	6.70^{BC}	8.09^{AB}	7.21^{B}	3.19^{DE}	4.22^{DE}	4.88^{CD}	2.20^{DE}	1.2^{E}
Aroma	5.83^{C}	9.17^{A}	8.24^{AB}	4.96^{D}	5.38^{CD}	3.19^{D}	2.82^{DE}	1.40^{E}
Texture	5.71^{C}	8.55^{AB}	7.69^{B}	5.10^{CD}	3.35^{D}	4.62^{CD}	3.11^{DE}	2.22^{DE}
Taste	6.36^{BC}	9.50^{A}	8.86^{AB}	5.26^{CD}	2.66^{DE}	4.98^{CD}	2.48^{DE}	1.44^{E}
Color	7.71^{B}	7.62^{B}	6.42^{CB}	4.11^{D}	4.51^{D}	6.95^{BC}	4.92^{D}	4.16^{D}
Odor	5.47^{CD}	7.44^{B}	6.54^{CB}	4.81^{D}	5.41^{CD}	4.69^{CD}	1.82^{E}	1.82^{E}
Appearance	5.87^{CD}	8.59^{AB}	7.21^{B}	4.24^{D}	4.14^{D}	4.36^{CD}	1.97^{E}	1.94^{E}

* The highest inhibition, lowest count ($^{a-E}$ indicates an increase in microbial count).
** The lowest pH level ($^{a-E}$ indicates an increase at pH level).
*** The highest total lactic acid level (TLA %) ($^{a-E}$ indicates a decrease in total lactic acid level [%]/total lactic acid [%] level).
C, control; N, nisin; LA, lactic acid dip; LA + Co, lactic acid dip and then coating application; N + Co, nisin and then coating application; Co, coating; LA + N, lactic acid dip and then nisin application; LA + N + Co, lactic acid dip then nisin and finally coating.

Statistical Analysis

MANOVA was applied to determine whether a variance is abundant among the sole and combined effects of nisin, lactic acid and coating. The degrees of differences among applications were determined by Duncan's multiple range test.

RESULTS AND DISCUSSION

According to the experimental results, lactic acid, nisin and coating's combined application (LA + N + Co) had significant inhibition on MAB growth in refrigerated (+4C) storage period ($P < 0.01$). MAB count for LA + N + Co was kept around 5.95 log cfu/g at the end of the seventh day, whereas the control had reached 6.62 log cfu/g at the end of the fifth day, which is in the limits of consumption risk. A reduction of 1.54 log MAB in LA + N + Co application with respect to control by the seventh day of shelf life obtained in our study, is also consistent with studies revealing 1 log reduction on MAB counts of the fishes treated with ice (Laksmanan *et al.*

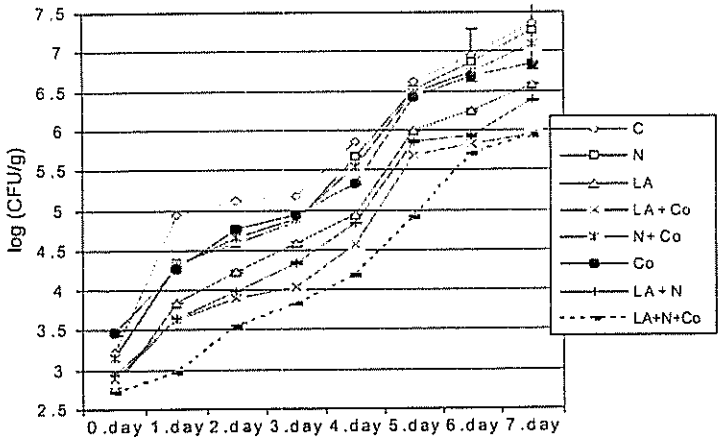


FIG. 1. MESOPHILIC AEROBIC BACTERIAL COUNTS OF CONTROL AND APPLICATION GROUPS WITH MEAN VALUES

C, control; N, nisin; LA, lactic acid dip; LA + Co, lactic acid dip and then coating application; N + Co, nisin and then coating application; Co, coating; LA + N, lactic acid dip and then nisin application; LA + N + Co, lactic acid dip then nisin and finally coating.

2002), and 2 log reduction on MAB counts of the fishes treated with a bacteriocin (Orvec Microgard Ltd., Hull, UK) and nisin combination (Zuckerman and Avraham 2002). The MAB reduction obtained in our study also indicated a comparable bacteriostatic activity rate of LA + N + Co, in comparison with 1.8 log reduction on Gram-positive bacteria count of nisin alone application, applied for beef carcass decontamination (Nettles and Siragusa 1994). Lactic acid and coating (LA + Co) and lactic acid and nisin (LA + N) applications were found to be less effective when compared with LA + N + Co combination on inhibiting MAB growth ($P < 0.01$), indicating a clear synergy among lactic acid, nisin and coating applications. Coating alone was less effective on MAB growth when compared with nisin and coating (LA + Co) and lactic acid (LA) applications (Table 2 and Fig. 1), indicating the synergy between lactic acid and coating application when used in combination. Therefore, it can be clearly expressed that coating should be applied as combined with lactic acid application, rather than its alone application. The most interesting point among MAB counts, which belong to the various lactic acid, nisin and coating alone/combined applied groups, is the greater inhibition that was made by lactic acid and nisin combined (LA + N) application on MAB than lactic acid (LA) alone application. This greater inhibition of LA + N with 0.89 log reduction may be due to the lactic acid's increasing effect of nisin's penetration into also Gram-negative bacteria, by decomposing the Gram-negative cell wall prior to nisin application (Helander and Mattila-Sandholm 2000; Samelis *et al.* 2005).

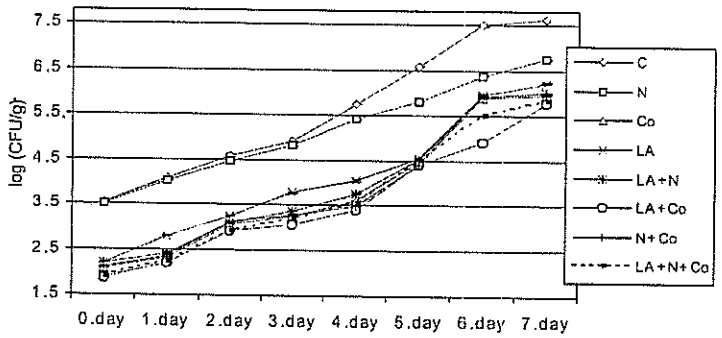


FIG. 2. *PSEUDOMONAS* SPP. COUNTS OF CONTROL AND APPLICATION GROUPS WITH MEAN VALUES

C, control; N, nisin; LA, lactic acid dip; LA + Co, lactic acid dip and then coating application; N + Co, nisin and then coating application; Co, coating; LA + N, lactic acid dip and then nisin application; LA + N + Co, lactic acid dip then nisin and finally coating.

Pseudomonas spp., which is a member of the fish flora, at refrigerated storage, were found to be significantly inhibited by LA + N + Co and LA + Co applications ($P < 0.01$). A reduction of 1.84 log made by LA + Co application with respect to control by the seventh day of preservation under (+4C) refrigerated storage is in line with a study which implied 1 log reduction obtained by the combined use of lactoferrin, lactoferricin, high hydrostatic pressure and nisin (Yuste *et al.* 2002). Coating alone was the third most effective application on growth inhibition of *Pseudomonas* spp. possibly due to the creation of anaerobic environment. More inhibition, which was made by LA + N combination, indicates a synergistic effect of lactic acid on the effectiveness of nisin, when compared to applications of lactic acid and nisin alone, which caused less inhibition on *Pseudomonas* spp. There was no additional beneficial effect of nisin application when combined with coating, whereas nisin alone application was not effective in inhibiting Gram-negative *Pseudomonas*, expectedly. Reductions of 0.24 and 0.75 log belonging to LA + N combined application, in comparison with LA and N alone applications orderly, indicates a clear synergy of lactic acid and nisin applications in inhibiting *Pseudomonas* spp. as the initial spoilage Gram-negative flora of fish, which suggests the necessity of combined use of nisin and lactic acid in fish meat decontamination ($P < 0.01$) (Table 2 and Fig. 2). Similarly, the second highest reduction on *Pseudomonas* spp., which was made by LA + N + Co, also necessitates the combined use of lactic acid, nisin and coating applications for fish meat decontamination.

LA + Co and LA + N + Co indicated the lowest pH values in all periods of shelf life followed by LA + N, LA, Co, N + Co and N orderly, while the

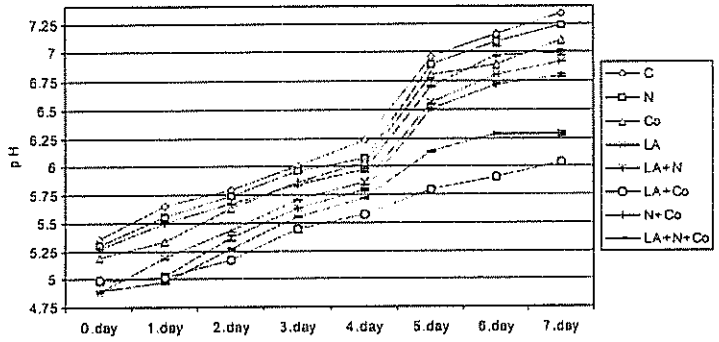


FIG. 3. pH LEVELS OF CONTROL AND APPLICATION GROUPS WITH MEAN VALUES
C, control; N, nisin; LA, lactic acid dip; LA + Co, lactic acid dip and then coating application; N + Co, nisin and then coating application; Co, coating; LA + N, lactic acid dip and then nisin application; LA + N + Co, lactic acid dip then nisin and finally coating.

highest pH levels were observed in control ($P < 0.01$), as clearly indicated in Fig. 3 and Table 2. The lower pH values of LA + Co and LA + N + Co were also confirmed by lower lactic acidity losses (Fig. 4 and Table 2). On the other hand, the lower acidity loss, which belongs to LA + N combined application with pH 6.80 on the seventh day of shelf life, than LA and N alone applications with pH of 6.92 and 7.23 levels orderly, also confirms the synergistic effect of lactic acid and nisin applications when combined, that the same synergy was also observed in inhibiting MAB and *Pseudomonas* spp., as mentioned earlier.

According to the MANOVA of the taste panelist evaluations in descriptive test, all application groups appeared as better than control ($P < 0.01$). LA + N + Co application appeared as the most preferred in aroma, taste, tenderness and appearance. These highest sensory quality criteria belonging to this application, might have been due to the lower microbial load and activity, which can also be confirmed by its higher microbial quality criteria and lower acidity losses (Figs. 1, 2, 3 and Table 2). LA + Co was the third most accepted application after LA + N + Co and LA + N in tenderness, and the most accepted with LA + N + Co in color. LA + N + Co was the most preferred one in aroma, taste, tenderness and texture among all others. LA + N combined application followed LA + N + Co, as the second most preferred in aroma, tenderness, texture and appearance. When these three mentioned applications, LA + N + Co, LA + N and LA + Co, are concerned, they are not only the applications, having the most of the higher sensory quality criteria, but they were the applications that were exposed to less microbial contamination and activity all through the shelf life (Figs. 1, 2, 3, 4 and Table 2). Lactic acid, which degrades proteins and fats, and makes the sulfur compounds such as cysteine and methionine free, might have been beneficial for especially aroma,

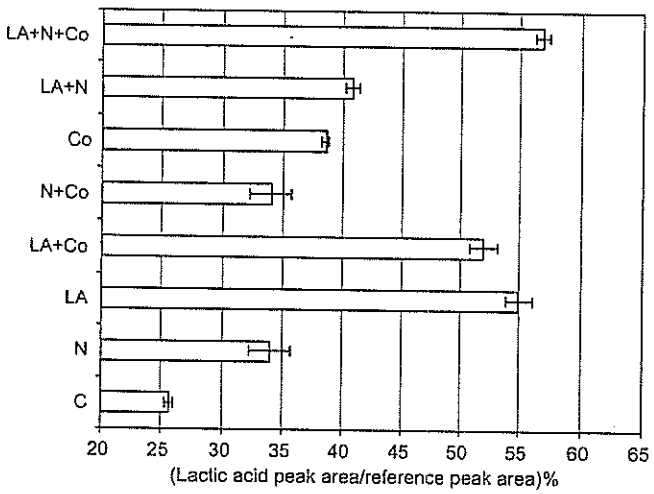


FIG. 4. LACTIC ACID PEAK AREA OF APPLICATIONS/REFERENCE PEAK AREA % RATIOS

C, control; N, nisin; LA, lactic acid dip; LA + Co, lactic acid dip and then coating application; N + Co, nisin and then coating application; Co, coating; LA + N, lactic acid dip and then nisin application; LA + N + Co, lactic acid dip then nisin and finally coating.

odor and taste of the sardines, which were treated with this organic acid alone/combined applications (Table 2). Briefly, because lactic acid alone/combined applications improve the texture, aroma, odor, taste and appearance, whereas they maintain microbial quality better, they might be advised as the applications having superiority in postmortem fish sensory quality.

When different applications are compared, lactic acid, nisin and coating's combined application appears as the most effective treatment to increase microbial quality and retard fish spoilage, especially by inhibiting MAB growth at 4C. This result also indicates the synergetic effect of "nisin, lactic acid and coating" when compared with the nisin, lactic acid and coating alone applications, with their higher MAB counts than the combined use of lactic acid, nisin and coating. The inhibition made by "lactic acid, nisin and coating" and "lactic acid and coating" combined applications on MAB counts was also reflected in the *Pseudomonas* counts. These applications were the most effective on the inhibition of *Pseudomonas*. The lower *Pseudomonas* counts observed in lactic acid, nisin and coating's combined application, in comparison with the higher *Pseudomonas* counts of lactic acid and nisin alone applications, indicate the synergetic effect of lactic acid, nisin and coating on *Pseudomonas*, a Gram-negative, which is not inhibited by nisin under normal conditions. One important point which experimental

microbial data mentioned previously indicates, is the lower *Pseudomonas* and MAB counts of LA + N in comparison with N and LA alone applications and control (Figs. 1 and 2; Table 2). As in all periods of shelf life at +4C, bacterial counts were always lower in LA + N fishes than the fishes treated with only nisin or 5% lactic acid and control. pH and HPLC results also supported these findings, indicating lower acidity losses in LA + N in comparison with nisin or 5% lactic acid alone applications and control. The lower acidity losses with the lower *Pseudomonas* and MAB counts obtained with LA + N, especially in comparison with control, clearly indicate that the effect of nisin on Gram-negative *Pseudomonas* spp. can be enhanced with organic acid, which appears perhaps as the most important finding among the experimental data. Not on MAB but on the inhibition of *Pseudomonas*, coating appeared as another effective protection alternative to retard the fish spoilage, creating an anaerobic media in fish, strongly bactericidal on *Pseudomonas* as an aerobic Gram-negative bacteria. The lower acidity losses (Fig. 4) observed in LA + N + Co and LA + N combined applications, also supported the inhibitory effects of the concerned applications on MAB and *Pseudomonas* counts. These inhibitory effects are very clear especially when compared with control.

Nisin, as a bacteriocin, which is studied in many previous studies, and observed as inhibitory on Gram-positive bacteria, could also show its bacteriostatic effect on Gram-negative bacteria such as *Pseudomonas* spp., if combined with lactic acid application. According to the experimental results, "lactic acid, nisin and coating" and also "lactic acid and coating" combined applications can be advised as protective treatments in fish preservation under +4C by inhibiting MAB and *Pseudomonas* with synergetic effect of "lactic acid, nisin and coating" without any adverse effect on sensory quality. Coating material in lipid origin can also be advised for industrial applications by protecting surface contamination and inhibiting predominant aerobic bacteria spoilage flora, mainly *Pseudomonas* spp., without adverse effect on fish sensory. This experimental data can be used in preparation of antibacterial solutions in the near future, which is consisted of nisin, lactic acid or a similar organic acid and a coating material, to prolong the shelf life by increasing the microbial quality of the fish batches, which are displayed in market benches.

CONCLUSION

Although nisin addition alone had no significant effect on *Pseudomonas* spp., the combination of nisin with lactic acid and coating can be successfully applied to retard the growth of them as one of Gram-negative spoilage

microflora, during preservation at +4C refrigeration conditions while maintaining cooked fish sensory quality, especially texture, aroma, odor and taste.

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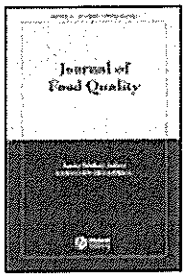


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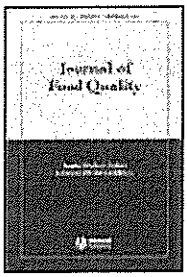
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