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EFFoST 2004 Conference

Food Innovations for an Expanding Europe

27-29 October 2004 ~ Warsaw, Poland

Claire S Norris

FOR AND ON BEHALF OF



ELSEVIER

[O3.4]

Correlation between sensory and instrumental characteristics of *Canestrato Pugliese* slices packed in biodegradable films

S. Di Marzo, R. Di Monaco, S. Cavella, R. Romano, I. Borriello, P. Masi
University of Naples - Federico II, Italy

This work is a part of a research whose aim is to study the application of innovative biodegradable films (Novamont, Italy) for food packaging. In this study, the possibility to predict acceptability from sensory and chemico-physical parameters of *Canestrato Pugliese* cheese, was evaluated.

The cheese was sliced and packed by using three biodegradable films having different gas and water permeabilities and a multilayer film of polyethylene and EVOH having barrier properties, used as control. The samples were stored at 8°C for 28 days and, once a week, relative moisture content, water activity, Young modulus, pH, color, acidity and mould presence were evaluated. The evolution of volatile organic compounds (VOC) during the time was also assessed by means of static headspace analysis (HSA). VOC were separated and identified by means of GC-MS. QDA and acceptability tests were also performed. The relationships between acceptability, sensory and chemico-physical data were studied by PLS analysis.

PLS results showed significant acceptability prediction from sensory and chemico-physical data (predicted variability = 71,2%). Acceptability was related positively to butter flavour and cohesiveness. Concerning instrumental parameters, relative moisture, diacetyl and pyruvic acid contents were positively related to acceptability, whereas the opposite situation was observed for ethyl-acetate, hexane and heptane contents.
Concerning the relationships between sensory and instrumental parameters, several volatile organic compounds predicted well sensory characteristics. In particular, ethyl-acetate content was related positively to the pungent and rind odor. Acetaldehyde content in the headspace predicted well the spicy attribute. These results are in accordance with the scientific literature.
The samples plot showed also that samples after fourteen days of storage received the highest acceptability score. Finally, whereas the two biodegradable films with intermediate water permeability preserved product properties for 21 days after packaging, only the barrier film maintained acceptable the cheese during all the investigated time.

Keywords: QDA, Static headspace analysis, Acceptability, PLS analysis

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[O3.5]

Effects of lactic acid, nisin and coating on some quality criteria of refrigerated *Sardina Pilchardus*

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Fish meat is highly prone to microbiological contamination from hunting and slaughtering stages to the kitchen. The microbial contamination is mostly attributed to Gram negative microorganisms, predominantly *Pseudomonas* spp. Transportation processes also exposure fishes to cross contamination by Mesophile Aerobic Bacteria (MAB). In this study, effects of nisin, lactic acid and their combinations were studied on coated and noncoated fishes on inhibition of *Pseudomonas* and MAB at refrigeration temperatures (+4 °C). The results have indicated that lactic acid, nisin and coating's combined application had more inhibition on the growth of MAB whereas lactic acid and coating's combined application has more pronounced inhibition on *Pseudomonas* spp. The lowest acidity changes in these two groups were also supporting these inhibitions ($p<0.01$). Use of 5% lactic acid had no adverse effect on organoleptic quality of fish meat according to the taste panel results ($p<0.01$).

Keywords: *Sardina pilchardus*, nisin, lactic acid, coating, *Pseudomonas*, Mesophile Aerobic Bacteria

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[O3.6]

Antimicrobial packaging materials: effectiveness evaluation of different natural substances by a biotoxicity test

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Department of Food Science and Microbiology (diSTAM), University of Milan, Italy

One of the most promising innovations in food packaging is the design of active packaging materials with antimicrobial properties, for extended shelf lives and for safer products. At present there is strong interest and demand in molecules and substances derived from vegetable matrixes and characterized by effective antimicrobial activity.

The aim of this work was to verify the possible application of a modified test of biotoxicity in determining the antimicrobial properties of substances with known and unknown effects. Several different vegetable substances were tested: extracts of grapefruit and kiwi seeds, extracts of tobacco, garlic, bergamot clove, nutmeg, rosemary, propolis and angostura, solutions of vanillin and mustard.

The test of biotoxicity relies on the reduction of respiratory activity of yeasts cells (*Saccharomyces cerevisiae*), assumed as an index of potential antimicrobial properties. The yeasts are put in contact with different amounts of the tested substances and the uptake of oxygen by the cells is monitored by means of an optical method, based on the lifetime determination of a fluorescent emission induced in an optically excited Ruthenium complex. The fluorescence emission is inversely proportional to the dissolved oxygen concentration and this technique allows an accurate oxygen determination, without any oxygen consumption and in a closed, uncontaminated system.

The results show that the test used is able to discriminate between different substances and among different amounts. Some preliminary results in immobilising some of the selected substances in a plastic sheet and in measuring the antimicrobial activity of the sheets, are also presented.

Keywords: active packaging, antimicrobial materials, oxygen uptake, optical method

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[O3.7]

Optimised modified atmosphere packaging of pre-rigor filleted farmed cod

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Farming of cod (Gadus morhua) is increasing and diminishing availability of wild caught cod will increase the importance of the farmed alternative. However, farmed cod has differences compared with wild cod at the established packaging parameters requirements for post-rigor processed wild cod may not be the optimised packaging solution for pre-rigor filleted farmed cod.

This paper reports results from studies of the combined effect of modified atmosphere and superchilling

THURSDAY 28 OCTOBER 2004

SESSION: Innovative Preservation
Chair: Andrzej Lenart

09.00 **PLENARY LECTURE: Irradiation for better foods [O2.1]**
Jozsef Farkas, *Szent István University of Budapest, Hungary*

09.30 **PLENARY LECTURE: Gentle preservation methods for better foods [O2.2]**
Dietrich Knorr, *Berlin University of Technology, Germany*

10.00 **Refreshment Break**
Sala Balowa Ballroom Foyer

10.30 **The role of protective media in the viability retention of probiotic bacteria *Lactobacillus rhamnosus* GG during spray drying and storage [O2.3]**
Edwin Ananta, *Berlin University of Technology, Germany*

10.50 **Application of new preservation technologies on food products [O2.4]**
Lekje Berg, *TNO Nutrition and Food Research, The Netherlands*

11.10 **An innovative thermal process for microbial decontamination of food powders [O2.5]**
Frederic Fine, *Laboratoire de Génie des Procédés Alimentaires et Biotechnologiques, ENSBANA, France*

11.30 **Irradiation preservation of traditional Indian fermented foods [O2.6]**
Sheetal Khadye, *Institute of Chemical Technology, India*

11.50 **Stabilization of fruit juices by means of pulsed high pressure [O2.7]**
Paola Maresca, *University of Salerno, Italy*

12.10 **Inactivation of *S. cerevisiae* in fruit juices using pulsed electric fields [O2.8]**
Gianpiero Pataro, *University of Salerno, Italy*

12.30 **Lunch**
Sala Balowa Ballroom Foyer

SESSION: Innovative Packaging
Chair: Jozsef Farkas

14.00 PLENARY LECTURE: **Modified atmosphere packaging for better foods [O3.1]**
Jaroslaw Dobias, *Institute of Chemical Technology, Czech Republic*

14.30 PLENARY LECTURE: **Intelligent packaging for better foods [O3.2]**
Arielle de Jong, *TNO Nutrition & Food Research, The Netherlands*

15.00 **Refreshment Break**
Sala Balowa Ballroom Foyer

15.30 **The effects of lysozyme and EDTA impregnated pullulan film coatings on microbial load and color of cold stored high moisture dried tomatoes [O3.3]**
Taner Baysal, *Ege University, Turkey*

15.50 **Correlation between sensory and instrumental characteristics of *Canestrato Pugliese* slices packed in biodegradable films [O3.4]**
Sabrina Di Marzo, *University of Naples - Federico II, Italy*

16.10 **Effects of lactic acid, nisin and coating on some quality criteria of refrigerated *Sardina Pilchardus* [O3.5]**
Ugur Gogus, *Middle East Technical University, Turkey*

16.30 **Antimicrobial packaging materials: effectiveness evaluation of different natural substances by a biotoxicity test [O3.6]**
Luciano Piergiovanni, *University of Milan, Italy*

16.50 **Optimised modified atmosphere packaging of pre-rigor filleted farmed cod [O3.7]**
Morten Sivertsvik, *Norconserv, Norway*

17.10 **Bacterial growth and off-odour in chicken breast fillets as affected by packaging material and storage conditions [O3.8]**
Marit Kvalvåg Pettersen, *Agricultural University of Norway, Norway and MATFORSK – Norwegian Food Research Institute, Norway*

17.30 **END OF DAY TWO**

19.00 **Conference Dinner**
Coach departs hotel for ***Dwór Polski Pecice,*** an 18th Century Manor House, for an evening of traditional Polish Food and entertainment. This is an optional event, tickets have been booked in advance, check with the conference secretariat for late on-site availability

EFFoST 2004
Food Innovations for an Expanding Europe

27 - 29 OCTOBER 2004

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(CURRENT AT TIME OF PRINT)

Please note: Due to data protection requirements we do not supply full details of attendees

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COMPARATIVE EFFECTS OF LACTIC ACID, NISIN, COATING COMBINED AND ALONE APPLICATIONS ON SOME POST MORTEM 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, esp. 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, bees wax and distilled water mixture with pH: 7.2 was used as the main coating material) and non coated fish specimens on inhibition of *Pseudomonas* and mesophilic aerobic bacteria at refrigeration temperatures (+4 °C). Lactic acid, nisin and coating reached 5.95 log units as cfu/mL on total mesophilic aerobic bacteria in seventh day of shelf life at +4 °C, whereas control has reached 6.62 log at 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.($p<0.01$).

Keywords: *Sardina pilchardus*, nisin, lactic acid, coating, *Pseudomonas*, Mesophile Aerobic Bacteria

INTRODUCTION

Fresh fish fillets are highly prone to microbial spoilage, in comparison with live crustacean and molluscan shellfish, since 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 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; Spanggard *et al.* 2000; Miguez and Combarro, 2003; Papadopoulos *et al.* 2003). Not only 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 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 an alternative decontaminant in fish preservation, like the treatments of pork and beef carcasses with 2% lactic acid (Smulders and Woolthius, 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 since 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, 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, Yuste *et al.* 2002; Garriga, 2002). Coating, like these procedures mentioned above, became another practice. Since 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 which inhibits only Gram positive bacteria, could not find application platform in marine technology, since the dominant fish flora is Gram negative as previously mentioned above. 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 Black Sea, were purchased from a local fish market (Aydınoglu Market/Samsun/Turkey). They were transferred immediately to the vendor by cold chain (at +4 °C) and brought in 8th hour to the laboratory after hunting without subjection to improper handling conditions. Whole samples without removal of internal organs, were separated into 8 groups as each contains 1 kg fish (as 0.5 kg sample and 0.5 kg replicate) . Samples of the first group were dipped into nisin solution. The nisin solution was prepared by adding 0.04 g nisin into 1L tap water at pH 5.2 and this group was coded as (N). The fish specimens of the second group were dipped into the nisin solution, which was prepared the same 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 % bees wax into 51. 6 % distilled water and mixing homogeneously under heat, until all ingredients were melted as described by Daniels (1973). The pH level of coating material was adjusted to 5.2, following the preparation. Fifth group were only coated (Co). The sixth group was dipped into the lactic acid solution and then coated (LA+Co). The seventh group were dipped into the lactic acid solution, then transferred into the nisin

solution and finally coated (LA+N+Co), while the last group were served as control (C). All samples were kept in duplicate sterile plastic bags at (÷4 °C). 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 8 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 (PCA) (Oxoid) for mesophilic aerobic bacteria (MAB) and Mac Conkey Agar (Oxoid) for *Pseudomonas* counts were used in enumeration. Spread plating for *Pseudomonas* and pour plating for mesophilic aerobic bacteria at 37 °C for 24-48 hours as in triplicates, were carried out for each dilution. After colony formation on Mac Concey, long wave UV was applied to the concerned colonies and bluish yellow fluorescent given colonies were counted during *Pseudomonas* enumeration(Collins and Lyne, 1991). Microbial enumeration and pH determinations were initially carried out at fourth hour, and then continued within 24 hours' 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 (Hattenbergstraβe 10-55122, Mainz-Germany; pH electrode 14 pH; buffers 4.01±6.87). The meat cuts as 0.5 gram from the fish surface

and 0.5 gram near the bone were taken from 8 fishes for each sample group. Samples were grinded, using sterilized lancet and tweezers and 1 gram 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 minutes 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) and Shimadzu C-R-4-Chromatopack monitor with a Phenomenex Rezex Cal monosaccharide column (300 X 7.8 micrometer) was used. The attenuation parameter was set to 2, speed=2, flow rate was 0.45, at 510 nanometer and the oven temperature was kept as 55° C 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 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 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 (+4 °C). Each 250 mL of sunflower oil for 16 fishes in each application batch was used for frying process. The 15 panelists were previously trained, from the point of fish sensory, especially in differentiating the cooked fish out of standards, from the cooked fish having got high sensory quality criteria. Discrimination of cooked fish groups, were made by '10 point scale' from the point of taste, texture and odour described by Huss

(1995), with aroma, flavor and appearance. 4 and lower than 4 point evaluations, in each quality criteria, indicated to '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. Different 15 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' (Huss, 1995 ; Meilgaard *et al.* 1991), each quality criteria of the concerned groups were 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), odour/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 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.

Statistical analysis:

Multivariate statistical analysis 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 experimental results; lactic acid, nisin and coating's combined application (LA+N+Co) had significant inhibition on MAB growth in refrigerated (+4 °C) 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 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 7th 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.* 2002) and 2 log reduction on MAB counts of the fishes treated with a bacteriocin (Microgard) 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 to the 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 to 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 it's alone application. The most interesting point among MAB counts, belong to the various lactic acid, nisin and coating alone/combined applied groups, is the greater inhibition

which 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, 2005).

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 ($p<0.01$). 1.84 log reduction made by LA+Co application as in respect to control by 7^{th} day of preservation under (+4 °C) 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 along application was not effective in inhibiting Gram negative *Pseudomonas*, expectedly. 0.24 and 0.75 log reductions belong to LA+N combined application, as 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 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 6.80 pH in 7^{th} day of shelf life, than LA and N alone applications with 6.92 and 7.23 pH 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 above.

According to the Multivariate Statistical Analyses (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 belong 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 (Fig 1, 2, 3 and Table 2). LA+Co was the third most accepted application after LA+N+Co and LA+N in tenderness, and most accepted with LA+N+Co in colour. 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 got most of the

higher sensory quality criteria, but they were the applications which were exposured to less microbial contamination and activity all through the shelf life (Fig. 1, 2, 3, 4 and Table 2). Lactic acid, degrade proteins and fats, make the sulphur compounds such as cysteine and methionine free, might have been beneficial for especially aroma, odour and taste of the sardines, which were treated with this organic acid alone/combined applications(Table 2). Briefly, since lactic acid alone/combined applications improve the texture, aroma, odour, taste and appearance, whereas they maintain microbial quality better, they might be advised as the applications, having got 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 4 °C. This result also indicates to 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 were also reflected to 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 indicates to 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 above indicates, is the lower *Pseudomonas* and Mesophilic

aerobic bacteria counts of LA+N in comparison with N and LA alone applications and Control (Fig. 1 and 2, Table 2). As in all periods of shelf life at (+4 °C), bacterial counts were always lower in LA+N fishes than the fishes treated with only nisin or lactic acid 5% and control. pH and HPLC results also supported these findings, indicating lower acidity losses in LA+N in comparison with nisin or lactic acid 5% alone applications and control. The lower acidity losses with the lower *Pseudomonas* and MAB counts obtained with LA+N, especially as 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 with 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 (+4 °C) 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, 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 (+4 °C) refrigeration conditions while maintaining cooked fish sensory quality, especially as in texture, aroma, odour and taste.

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TABLE LEGENDS

Table 1. The nisin, coating and lactic acid alone/combined applications with their codes.

Table 2. Variation among the effect of nisin, coating and lactic acid treated application groups on quality criteria with mean values.

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% bees wax and 51.6% distilled water)(pH:7.04)/41° C.
(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 solutior (0.04 g/L/Kg) (pH: 5.2)/10 min. and finally Coating
(C)	The group brought in 8th h to the laboratory by cool chain (+4 °C) after hunting all the others but taken directly to the analysis without subjection to any kind application.
(N)	Dipping into 0.04 g/L/Kg (pH: 5.2)/10 min

Table 1. The nisin, coating and lactic acid alone/combined applications with their codes.

	Applications							
Criteria ▼	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}
Colour	7.71^{B}	7.62^{B}	6.42^{CB}	4.11^{D}	4.51^{D}	6.95^{BC}	4.92^{D}	4.16^{D}
Odour	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}

Table 2. Variation among the effect of nisin, coating and lactic acid treated application groups quality criteria with mean values ($p<0.01$).

* : The highest inhibition, lowest count (a ⟶ g indicates increase in microbial count).

** : The lowest pH level (a ⟶ g indicates increase at pH level).

*** : The highest total lactic acid level (%) (a ⟶ e indicates decrease in total lactic acid level (%

": Total lactic acid (%) level.

FIGURE LEGENDS

Figure 1. Mesophilic aerobic bacterial counts of control and application groups with mean values.

Figure 2. *Pseudomonas* spp. counts of control and application groups with mean values.

Figure 3. pH levels of control and application groups with mean values.

Figure 4. Lactic acid peak area of applications/ reference peak area ratios %.

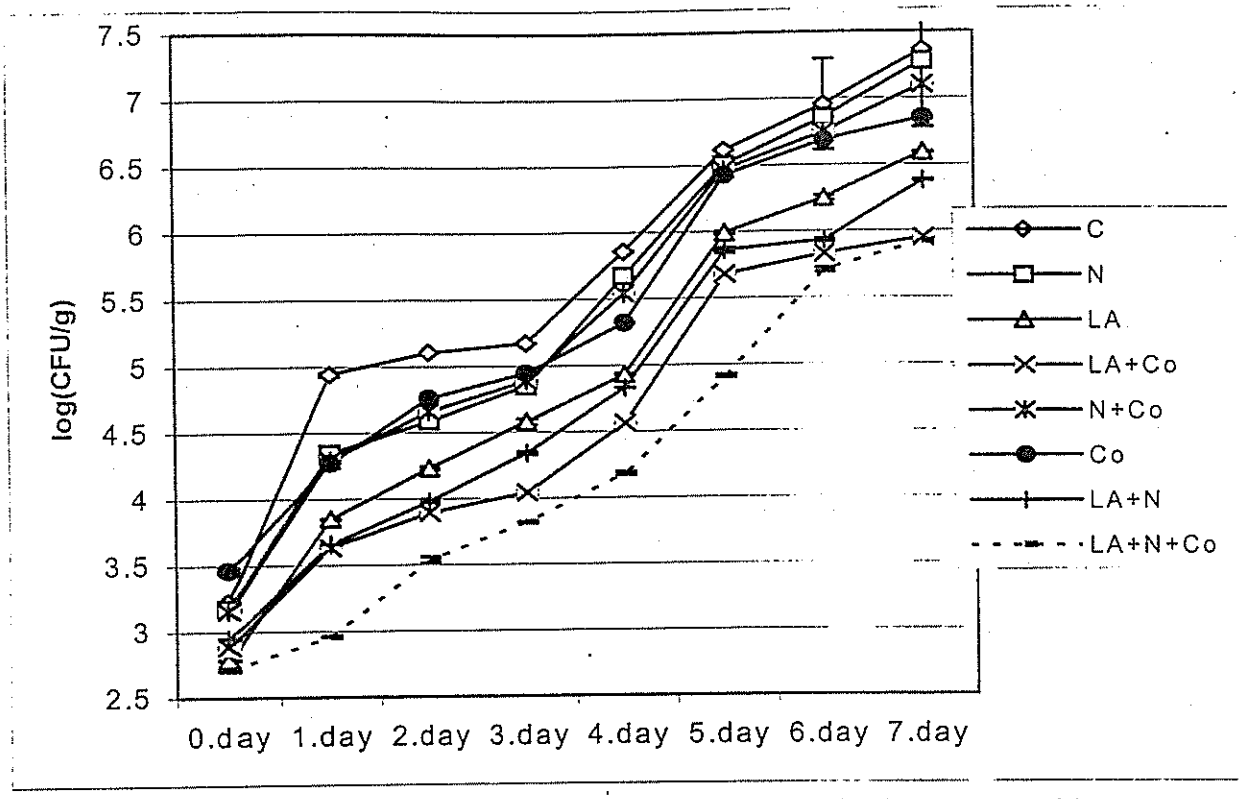


Fig. 1 Mesophilic aerobic bacterial counts of control and application groups with mean values.

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.

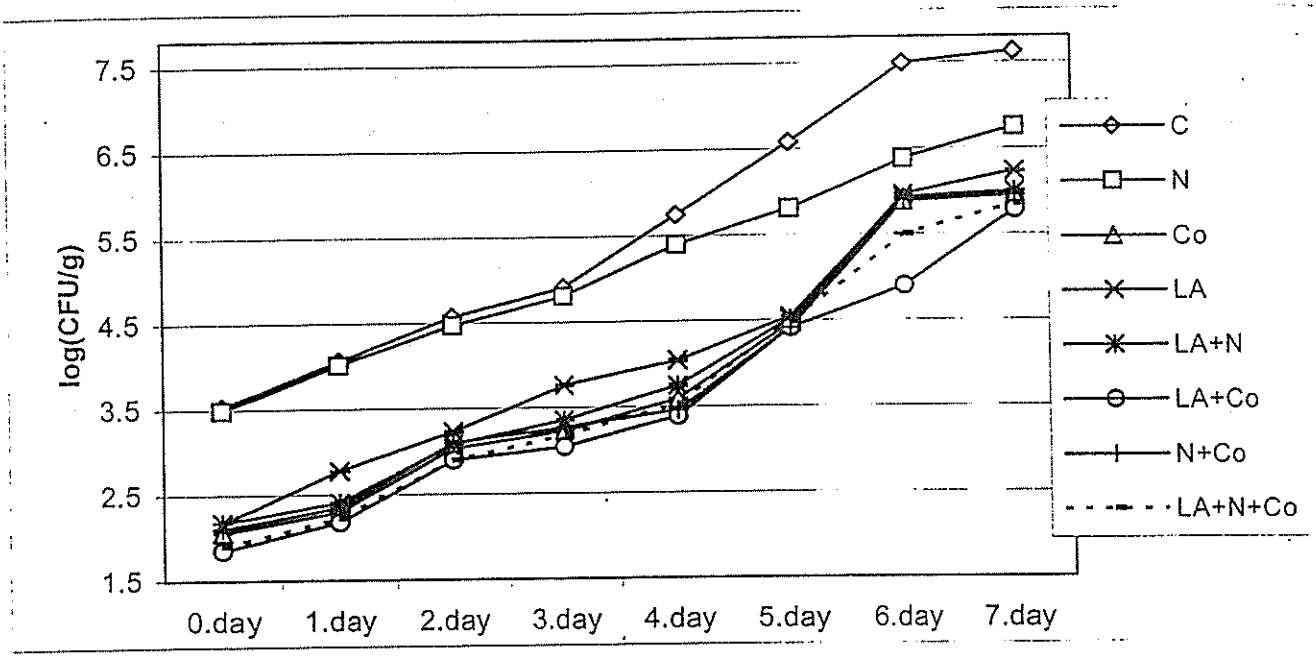


Fig. 2 *Pseudomonas* spp. counts of control and application groups with mean values.

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.

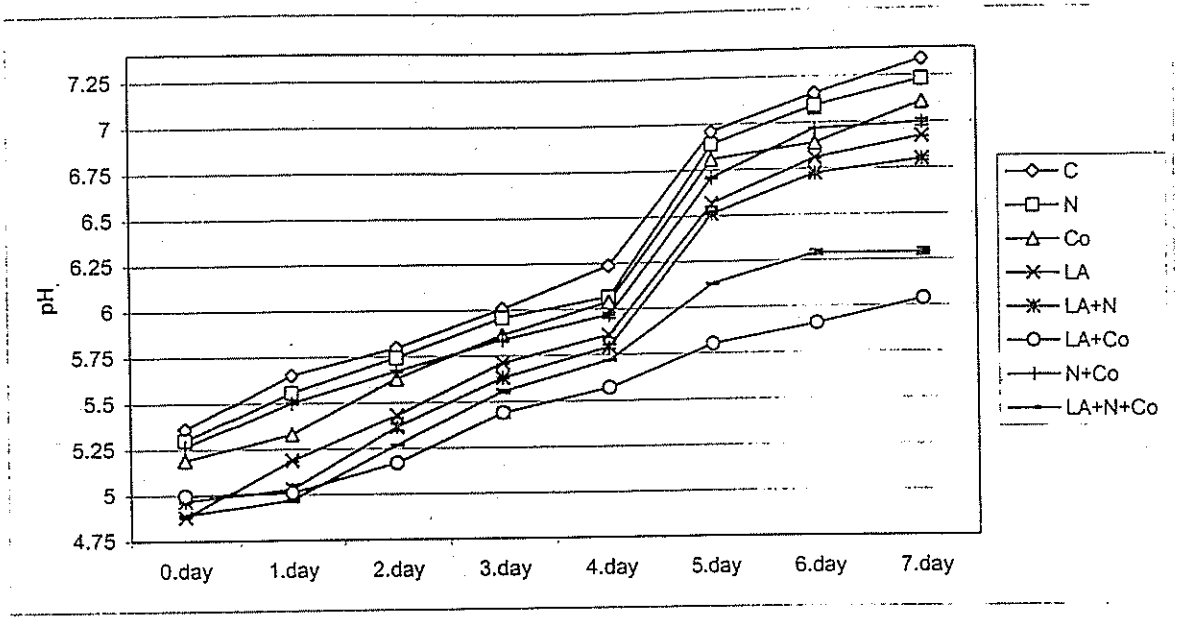


Fig. 3 pH levels of control and application groups with mean values.

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.

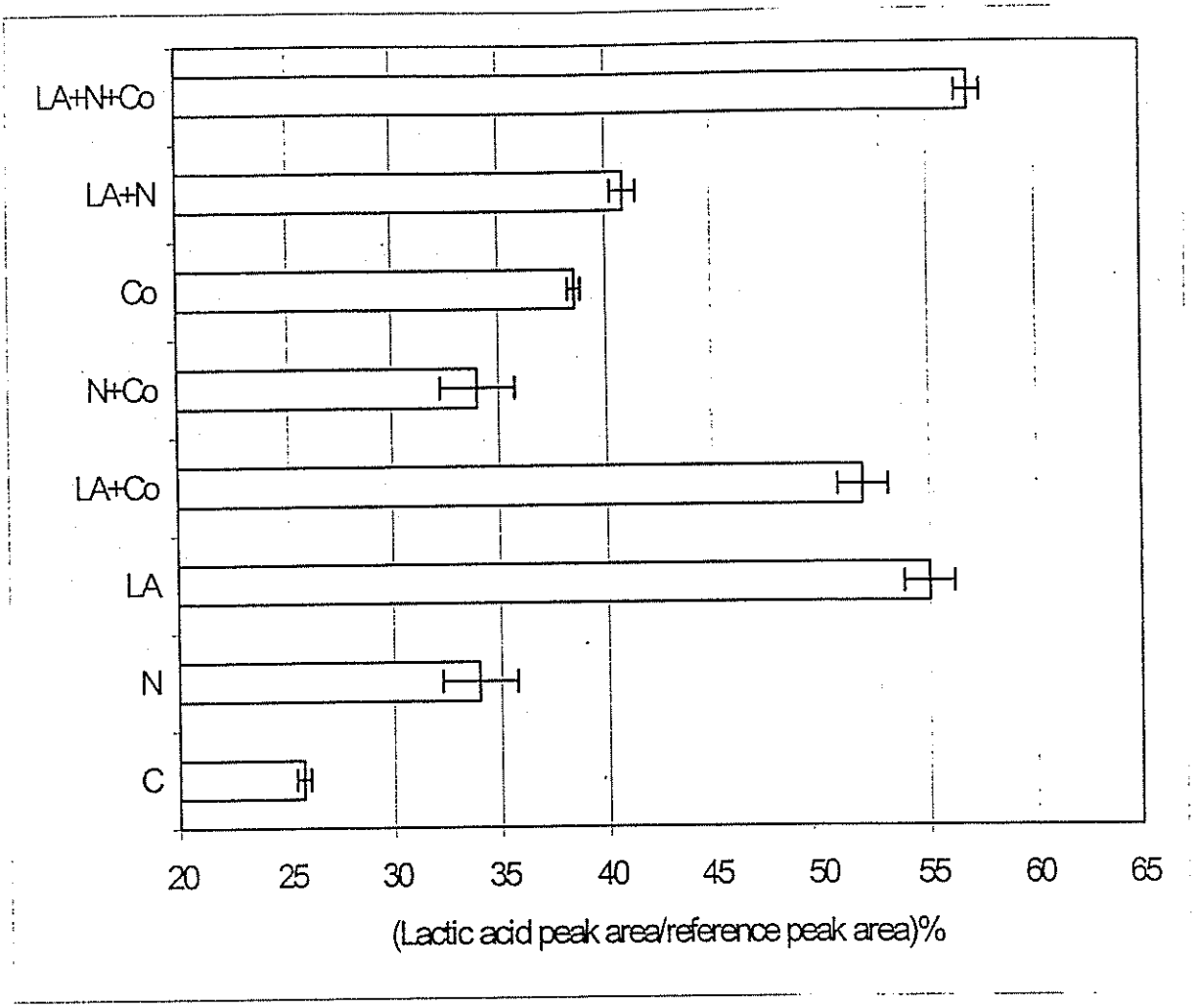


Fig. 4. (Lactic acid peak area of applications/ reference peak area) % ratios.

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.