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Biopreservation of the fresh Egyptian nile perch fillets using combination of bacteriocins, sodium acetate and EDTA.

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Lactococcus lactis subsp. *Lactis* (NRRL 1821) and *Lactobacillus sakei* (NRRL1917) were screened for bacteriocins production in skim milk (SM). *Lactococcus lactis* subsp.*lactis* showed the highest production and the maximum activity was noticed after 48 h of fermentation. The highest antimicrobial activity was achieved at pH 2.0 in the presence of EDTA (250 ppm). Fresh Egyptian nile perch fillets were treated by dipping in solutions of: fermented SM, (T1); FSM+ EDTA, (T2); sodium acetate, (T3); FSM+ sodium acetate, (T4) and FSM+ sodium acetate+ EDTA, (T5) and stored for 5 weeks in ice. Microbiological, chemical and sensory characteristics of the treated fillets were evaluated. All treatments succeeded in reduction initial psychrotrophic count, increasing the lag phase period and extending the microbial shelf life. T4 and T5 reduced the initial load by 2.4 log (each), elongated the lag phase by 1 and 2 weeks and extended shelf life by 2.3 and 3.0 weeks, respectively. At the end of storage period, T4 and T5 maintained the pH to 6.5 and 6.0 compared with 7.7 of control. The TBA values were 1.56 (T4) and 1.22 (T5) compared with 8.69 mg malondialdehyed Kg^{-1} fillets for control. Also, TVB-N values were 32.2 (T4) and 29.74 (T5) compared with 53.51 mg N/100 g fillets for control at the end of storage period. Similarly, T4 and T5 maintained better ($P<0.05$) sensory characteristics than control.

Keywords: Bacteriocins, bio preservation, fish fillets, nile perch, *Lactococcus lactis* subsp. *Lactis*, shelf life.

INTRODUCTION

Many food products are perishable by nature and require protection from spoilage during their preparation, storage and distribution to give them the desired shelf life (Rasooli, 2007). Food preservation is a continuous fight against microorganisms spoiling the food or making it unsafe. Traditional food preservation systems such as heating, refrigeration and addition of antimicrobial compounds can be used to reduce the risk of outbreaks of food poisoning as well as to elongate the shelf life. However, these techniques frequently have associated adverse changes in organoleptic characteristics, loss of nutrients and health concern. Therefore, the replacement of traditional food preservation techniques by new ones is seeked (Rasooli, 2007).

Recently, a novel scientific approach that has been widely employed in the food industry and gaining more attention is the bio-preservation technology. By definition this concept refers to the use of non-pathogenic antagonistic microorganisms as protective cultures and/or their antibacterial metabolites (bacteriocins) to inhibit or destroy undesirable microorganisms in foods,

improve microbiological safety and extend the shelf life**.** Lactic acid bacteria (LAB) have a great potential for use in bio-preservation because of their generally regarded as safe "GRAS" status. LAB are widely used in food industry as starter cultures, co-cultures, or bio-protective cultures in wide range of fermented foods such as dairy and meat products since ancient times without any safety risk (Singh, 2018). Several metabolic products produced by these bacteria have antimicrobial effects including organic acids, fatty acids, hydrogen peroxide and diacetyl (Holzapfel et al., 1995) as well as their ability to produce specific proteinaceous substances, bacteriocins, that inhibit the growth of pathogens such as the genera of Listeria, Clostridium, Bacillus and Enterococcus. Bacteriocins are heterogeneous group of bacterial antagonists that vary considerably in molecular weight, biochemical properties, range of sensitive hosts and mode of action. Attractive characteristics of the LAB bacteriocins which make them suitable candidates as food biopreservatives are as follows: protein nature where they are easily inactivated by proteolytic enzymes in gastrointestinal tract, non-toxic-non immunogenic, thermoresistant, broad bactericidal activity affecting most Gram positive bacteria and some damaged Gram negative bacteria and genetic manipulation is easy since the genetic determinates are generally located in plasmid (Nath et al., 2014). Various protocols for the application of LAB and /or bacteriocins in food are available: the direct inoculation with starter or protective culture where bacteriocins are directly produced insitu, the use of already fermented food with bacteriocin- producing strains as an ingredient in the food processing and/ or the direct addition of purified or semipurified bacteriocins (Raichurkar and Athawale, 2015). Nisin is the classic example of bacteriocins and is a permitted food additive in more than 50 countries including the US and Europe under the trade name Nisaplin (Delves- Broughton et al., 1996). Recently, studies aimed at broadening the bactericidal activity of LAB bacteriocins. Such studies are focused on the synergistic effects of bacteriocins, most notably nisin, with other anti-bacterial factors such as the sodium acetate, hydrolytic enzymes, various chelating agents (i.e. EDTA) and other bacteriocins (Helander et al., 1997).

Fresh fish fillets are extremely perishable and undergo enzymatic and microbiological spoilage faster than other fresh food commodities due to its biological composition. Psychrotrophic bacteria are the major group of microorganisms responsible for the spoilage of refrigerated seafoods (Zhuang et al., 1996). Reliable methods of preservation are needed to extend shelf life and to avoid health hazards. Such methods include cold storage in ice, modified ice storage, low-dose gamma radiation, cook-chill processes and organic acids and their salts (Al-Dagal and Bazaraa, 1999). Factors such as: holding temperature, oxygen, endogengous enzymes, moisture, light and most importantly microorganisms contaminating surface through the preparation step influence the shelf life and the freshness of such products (Zhou et al., 2010).

One of the most investigated new preservation technologies for fresh meat (Al-Sheddy et al., 1999; Amin, 2012) and seafoods (Kim et al., 1995; Al-Dagal and Bazaraa, 1999; Nath et al., 2014) is biopreservation. Such alternative method attempts to be mild, energy saving, environmentally friendly and guarantee natural appearance while eliminating pathogens and spoilage microorganisms. Due to the high popularity of the fresh nile perch fillets (*Lates niloticus*) in Egypt and due to the limitations in cold storage and transportation, such product faces short microbial shelf life as well as high risk of becoming a source of food borne diseases. Therefore, this study was mainly initiated to screen of two LAB for bacteriocins production and to evaluate the possibility of extending the shelf life of fresh fillets treated with LAB fermented skim milk (FSM) alone or in combination with EDTA, sodium acetate (SA) and stored in ice at 0°C.

MATERIALS AND METHODS

Microorganisms and growth conditions

LAB strains: *Lactococcus lactis* subsp. *Lactis* (1821) and *Lactobacillus sakei* (1917) were obtained from Northern Regional Research Laboratories, NRRL, (Peoria, IL, USA) and maintained on De Man Regosa Sharpe, MRS, medium (Oxoid, England). Organisms were allowed to grow in MRS for 48 h at 37°C under anaerobic conditions utilizing Oxoid anaerobic Jar and then refrigerated (4°C). Strains were monthly transferred. For fish samples treatment, cultures from refrigerated stock were daily propagated (37°C) for 3 consecutive days in bottles containing 100 ml sterile (121°C/15 min) skim milk (SM). The FSM was then diluted with sterile distilled water (3:1, v/v) and used in fish treatments.

Table 1. Code numbers and sources of the indicator bacteria

Strain	Code	Source
Bacillus cereus	33018	ATCC, American Type Culture Collection, Rockvill, Maryland, USA
Escherichia coli	35218	
Pseudomonas aeruginosa	9027	
Salmonella typhimurium	14028	
Staphylococcus aureus	25923	
Bacillus subtilis	765	NRRL, Northern Regional Research Laboratories, Peoria, IL, USA.
B. macerans	1650	
Micrococcus luteus	2618	
Bacillus amyloliqufaciense	111	FS, Food Science Department, Faculty of Agriculture, Cairo University, Giza, Egypt
Enterococcus faecalis	90	
Escherichia coli	15	
Klebsiella pneumoniae	49	
Serratia marcescens	37	
Staphylococcus aureus	112	

Indicator bacteria (Table 1) were maintained on Brain Heart Infusion, BHI, medium (Oxoid, England) at 37°C and transferred monthly.

Antimicrobial activity

The production of bacteriocins by the test LAB strains in SM was evaluated utilizing well diffusion assay (Zhang et al., 2010). A volume of 0.1ml of each indicator microorganisms (24 h old) containing 1.0×10^7cfu ml^{-1} was spread plated over the surface of plate count agar, PCA, medium (Oxoid, England). After the absorption of the inoculum by medium, wells (5 mm in diameter) were made using the top of sterile Pasteur pipette. Wells were filled with 50 µl of the FSM with the test LAB strains. Plates were left for 45 min at 4°C for proper diffusion of the FSM in the medium. Plates were incubated at 37°C for 48 h and the inhibition of the indicator microorganisms was measured as inhibition zone in mm.

The effect of pH of the FSM on the antimicrobial activities

Active 24 h old *Lactococcus lactis* subsp. *Lactis* (NRRL 1821) and *Lactobacillus sakei* (NRRL 1917) were allowed to grow separately in SM (100 ml) for 48 h at 37°C. The pH of the FSM was adjusted to pH levels of 2, 4, 6, and 8 using diluted HCl (1M) and NaOH (1M), then the antimicrobial activity was determined as described earlier.

Effect of fermentation time on bacteriocin production

Active 24 h old *Lactococcus lactis* subsp. *Lactis* (NRRL 1821) was allowed to grow in 100 ml SM for 24, 48, and 72 h. After fermentation, the pH was adjusted to 2 using HCl (1M) and the antimicrobial activity against *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* (ATCC 33018), *Escherichia coli* (ATCC 35218) and *Pseudomonas aeruginosa* (ATCC 9027) were tested as described earlier.

The effect of EDTA on the antimicrobial activities

Test organism was allowed to grow anaerobically at 37°C for 48 h in SM. Different concentration of EDTA (0, 50, 100, 150, 200, and 250 ppm) were added to the FSM and pH was adjusted to 2. The antimicrobial activities were then determined for each treatment as earlier described.

Fish treatments

Fresh nile perch (*Lates niloticus*) with an average weight of 1 kg/fish was purchased from Al-Moneeb fish market, Giza. Fish was directly decapitated, filleted by hand in the market and

then were directly transferred to laboratory in ice box. Upon arrival to laboratory, fish fillets were cut into pieces of 3×3×1 cm. The fish pieces were randomly divided into two lots (replicates), each of which was assigned five treatments in addition to two controls; untreated control (C1), and dipped in sterile water (C2). The treatments were: T1, dipping in diluted FSM (3:1, v/v in water); T2, FSM+ EDTA (200 ppm); T3, SA solution (10%, w/w); T4, FSM+SA (10%, w/w) and T5, FSM+SA (10%, w/w)+ EDTA (200 ppm). For each treatment, samples were dipped for 2 min. into 500 ml of treatment solution and gently swirled with sterile glass rod. Fish samples were removed with sterile tongs and allowed to drain for 2.5 min. on each side, on a pre-sterilized metal met. After draining the excess solution, treated fish samples were placed into sterile stomacher bags (Seward Medical, London) and stored in ice box containing crushed ice. The ice box was then kept at 4°C throughout the storage period of 5 weeks. Melted ice was drained daily out of the ice box and was replaced with new ice when needed. Samples were withdrawn weekly and assessed for pH, total psychrotrophs, thiobarbituric acid (TBA), total volatile base nitrogen (TVB-N) and sensory attributes.

Psychrotrophic bacterial count

Each fish sample, from each treatment, was weighed (Ca. 14 g) in sterile stomacher bag and sterile saline (0.85 % Nacl, w/w) was added to make a final dilution of 1:10. Samples were homogenized for 1 min. using stomacher lab-blender 400 (Seward Medical, London). From this mixture, serial dilutions using the same diluent were made. Form the appropriate dilutions, 0.1ml was spread plated on PCA and the inverted plates were incubated at 7°C for 10 days (Cousin et al., 1992). Colonies were then counted and expressed as $\log_{10}$ psychrotrophic colony forming units ($\log_{10}$ cfu g^{-1}). Generation time (GT) during logarithmic growth was calculated (Mossel et al., 1995).

pH measurement

Ten grams of each sample were homogenized with 20 ml distilled water for 1 min. and pH was measured using an Orion pH meter (Model 301, USA).

Total volatile base nitrogen (TVB-N)

A 100 g fish sample was homogenized (1 min) with 200 ml of 7.5 % (w/w) aqueous trichloroacetic acid, TCA, (Sigma, USA) solution in a laboratory homogenizer. The homogenate was filtered through Whatman No. 1 filter paper and TVB-N was then measured by steam distillation (Malle and Tao, 1987).

Oxidative rancidity

Oxidative rancidity in fish tissues was measured using the thiobarbituric acid,TBA, (Sigma, USA) test in which malondialdehyed, the principal compound in oxidized lipids, reacts with TBA to give red color. Twenty grams of fish was homogenized with 100 ml TCA (7.5%, w/w) for 2 minutes and filtered using Whatman filter paper No. 1. Five ml of TBA reagent (0.02 M 2-thiobarbituric acid in distilled water) were added to 5 ml of the filterate in a screw cap test tube. Tubes were placed in a boiling water bath for 40 min., then cooled in tap water and absorbance at 538 nm was measured. A standard curve using malondialdehyed was constructed (Vyncke, 1970).

Sensory evaluation

Treated fish samples were assessed for the changes in color, odor, texture and general appearance on a seven point hedonic scale, on which a score of 7 represented attributes most liked; 3 represented attributes at an unacceptable margin; and 1 represented attributes most disliked (Al-Dagal and Bazaraa, 1999). Samples were given three- digit codes and assessed by 9 member untrained panel (employees of The Food Science Department, Faculty of Agriculture, Cairo University). On analysis day, treated samples were compared with a fresh control (from frozen stock).

Statistical analysis

Data were analyzed using analysis of variance, ANOVA, (Rao and Blane, 1985). Data were presented as means of 3 experiments ± SD unless otherwise stated. All microbial data were transformed to logarithms before analysis.

RESULTS

The effect of pH of the FSM on the antimicrobial activities

The antimicrobial activities of bacteriocins produced by *Lactococcus lactis* subsp. *Lactis* (NRRL 1821) and *Lactobacillus sakei* (NRRL 1917), after growth for 48 h in SM at different adjusted pH levels (2, 4, and 6) against selected indicator microorganisms, are presented in Table 2. Generally, *Lactococcus lactis* subsp.

Table 2. Antimicrobial activities of bacteriocins of selected LAB* at different pH levels (2, 4 and 6).

Indicator microorganisms	*Lactococcus lactis* subsp. *lactis* NRRL 1821			*Lactobacillus sakei* NRRL 1917		
	2	4	6	2	4	6
Staphylococcus aureus ATCC 25923	**$11.0^{a}_{c}\pm0.9$	`$6.9^{b}_{a}\pm0.8$	$3.9^{d}_{a}\pm0.8$	$5.8^{c}_{fg}\pm0.7$	$5.9^{c}_{a}\pm0.8$	$3.6^{d}_{ab}\pm0.5$
Staphylococcus aureus FS 112	$10.3^{a}_{cd}\pm0.7$	$5.1^{b}_{b}\pm0.6$	$2.7^{d}_{bcd}\pm0.5$	$4.4^{c}_{h}\pm0.5$	$3.1^{d}_{f}\pm0.8$	$3.2^{d}_{abc}\pm0.7$
Bacillus subtilis NRRL 765	$9.6^{a}_{de}\pm0.7$	$4.6^{c}_{bcd}\pm0.5$	$3.2^{d}_{ab}\pm0.7$	$5.7^{b}_{fg}\pm0.5$	$3.8^{d}_{ef}\pm0.8$	$3.6^{d}_{ab}\pm0.8$
Bacillus cereus ATCC 33018	$8.7^{a}_{e}\pm0.5$	$3.1^{ef}_{e}\pm0.8$	$2.6^{f}_{bcd}\pm0.5$	$6.0^{b}_{efg}\pm0.9$	$4.1^{cd}_{def}\pm0.8$	$3.0^{ef}_{abc}\pm0.7$
Bacillus amyloliqufaciense FS 111	$8.6^{a}_{e}\pm0.5$	$4.8^{b}_{bc}\pm0.7$	$2.6^{c}_{bcd}\pm0.8$	$4.9^{b}_{gh}\pm0.8$	$3.1^{c}_{f}\pm0.8$	$2.6^{c}_{bc}\pm0.5$
Bacillus macerans NRRL 1650	$8.8^{b}_{e}\pm0.4$	$3.1^{d}_{e}\pm0.6$	$2.1^{e}_{cd}\pm0.8$	$9.9^{a}_{a}\pm0.9$	$5.4^{c}_{ab}\pm0.5$	$3.1^{d}_{abc}\pm0.7$
Micrococcus luteus NRRL 2618	$12.7^{a}_{b}\pm0.7$	$4.6^{c}_{bcd}\pm0.5$	$3.0^{d}_{abcd}\pm0.7$	$7.1^{b}_{de}\pm0.8$	$5.0^{c}_{abcd}\pm0.9$	$3.0^{d}_{abc}\pm0.5$
Enterococcus faecalis FS 90	$13.0^{a}_{ab}\pm0.7$	$4.7^{c}_{bc}\pm0.6$	$2.0^{e}_{d}\pm0.7$	$6.2^{b}_{ef}\pm0.7$	$4.0^{d}_{def}\pm0.7$	$2.2^{e}_{c}\pm0.8$
Escherichia coli ATTC 35218	$13.7^{a}_{ab}\pm0.9$	$3.8^{d}_{cde}\pm0.4$	$2.7^{e}_{bcd}\pm0.5$	$8.6^{b}_{bc}\pm0.5$	$5.3^{c}_{abc}\pm0.7$	$3.2^{de}_{abc}\pm0.7$
Escherichia coli FS 15	$10.0^{a}_{cd}\pm0.9$	$4.4^{c}_{bcd}\pm0.5$	$2.7^{d}_{bcd}\pm0.5$	$7.9^{b}_{cd}\pm0.8$	$4.6^{c}_{bcde}\pm0.7$	$2.1^{d}_{c}\pm0.8$
Salmonella typhimurium ATCC 14028	$14.0^{a}_{a}\pm0.9$	$4.4^{c}_{bcd}\pm0.5$	$2.4^{e}_{bcd}\pm0.7$	$7.6^{b}_{cd}\pm0.5$	$4.9^{c}_{abcde}\pm0.9$	$3.9^{d}_{a}\pm0.7$
Pseudomonas aeruginosa ATCC 9027	$8.7^{b}_{e}\pm0.5$	$3.6^{c}_{de}\pm0.5$	$3.0^{d}_{abcd}\pm0.5$	$9.7^{a}_{ab}\pm0.9$	$3.8^{c}_{ef}\pm0.7$	$2.3^{e}_{c}\pm0.9$
Serratia marcescens FS 37	$10.9^{a}_{c}\pm0.9$	$5.1^{c}_{b}\pm0.8$	$2.6^{e}_{bcd}\pm0.5$	$7.4^{b}_{cd}\pm0.5$	$4.2^{d}_{cdef}\pm0.8$	$2.4^{e}_{bc}\pm0.7$
Klebsiella pneumoniae FS 49	$8.4^{b}_{e}\pm0.7$	$5.1^{c}_{b}\pm0.8$	$3.1^{d}_{abc}\pm0.7$	$9.6^{a}_{ab}\pm0.7$	$3.2^{d}_{f}\pm0.4$	$2.4^{e}_{bc}\pm0.5$

* LAB were allowed to grow at 37°C for 48 h in SM at initial pH of 6.
** Means activity (mm) followed by different superscripts (within rows) and different subscripts (within columns) are significantly different ($P<0.05$).

Lactis was the most active ($p<0.05$) strain against the tested indicator microorganisms. Data (Table 2) revealed that at all tested pH levels (2, 4 and 6), the highest obtained antimicrobial activites were for *Lactococcus lactis* subsp. *lactis* and were highest against 85.7 % of the tested indicator organisms, followed by *Lactobacillus sakei* (14.3 %). It is of interest to report that the antimicrobial activites were against Gram positive as well as Gram negative organisms. Nomoto (2005) reported the antimicrobial activites of LAB against both Gram positive and Gram negative organisms. *Lactococcus lactis* subsp. *lactis* is widely used for production of nisin, which is active bacteriocin against Gram positive bacteria while it is generally inactive against Gram negative bacteria due to the resistance conferred by the outer membrane (Sarika et al., 2011). The activities obtained against the Gram negative organisms could be due to the production of organic acids, hydrogen peroxide, diacetyle and possible other bacteriocins (Raichurkar and Athawale, 2015). It was also noted that the highest antimicrobial activities of *Lactobacillus sakei* were towards the Gram negative bacteria *Escherichia coli* 35218, *Escherichia coli* 15, *Salmonella typhimurium* 14028, *Pseudomonas aeruginosa* 9027, *Klebsiella pneumoniae* 49 and *Serratia marcescens* 37. Bacteriocins produced by the two test organisms were noted to have maximum antimicrobial activity at pH 2.0. Such activities significantly ($P<0.05$) decreased by increasing pH value and the activities were completely lost at pH 8.0 (data not shown). Such findings are supported by those of Messens and DeVuyst (2002) and Joshi et al., (2006) who indicated pH values of ≤ 5 to maximize antimicrobial activities of tested LAB. Similarly, Khan et al., (2015) indicated the highest activities of bacteriocins at the acidic pH range of 2 to 3 followed by sharp loss at higher pH levels. Adesina and Enerijiofi (2016) reported the acidic pH range of 2 to 5 to get the maximum bacteriocins activities. The obtained results therefore, indicate the possibility of using such product to extend the shelf life of acidic or acidified foods. *Lactococcus lactis* subsp.*lactis* was therefore chosen for further experiments.

Effect of fermentation time on the antimicrobial activities of *Lactococcus lactis* subsp*. Lactis*

The highest production of bacteriocin was observed after 48 h of incubation at 37 °C. Increasing fermentation period over 48 h significantly ($P<0.05$) resulted in reduction in bacteriocin production (figure 1). Such reduction in bacteriocin biosynthesis could be due to the effect of proteases released during prolonged incubation (Piard et al.,1990). Similar results were obtained by Campos et al., (2006) and Danial et al., (2016) where fermentation period of 24 to 48 h maximized bacteriocin production**.**

The effect of EDTA on the antimicrobial activities of *Lactococcus lactis* subsp. *lactis*

Nisin acts generally on Gram positive bacteria. In contrast, Gram negative bacteria resists this bacteriocin due to that the lipopolysaccharide layer acts as a barrier, preventing the diffusion of nisin to its site of action (Gyawali and Ibrahim, 2014). However, upon the destabilization of this layer, this group of bacteria become susceptible to such antimicrobial agents. This destabilization could be achieved using EDTA as chelating agent which binds to magnesium ions of the lipopolysaccharide layer causing membrane disruption, rendering Gram negative bacteria sensetive to nisin **(**Prudêncio et al., 2016). The maximum permitlable concentration of EDTA in fresh fish is 50 – 250 ppm (Food and Agriculture Organization, 2015). Therefore, the effect of EDTA was tested in concentration of 0 to 250 ppm. Data (Table 3) indicate that the addition of EDTA to the FSM generally improved the antimicrobial activities against the tested G+ or G- organisms. The antimicrobial activities increased by increasing EDTA concentration where a range of 76.9 to 100% of the maximum activities obtained at 250 ppm were achieved at 150 ppm and the range of 85.3 to 100 % were obtained at 200 ppm. The improvement (%) range in antimicrobial activity (200 ppm) was from 78 to 116.3 % for the G- bacteria (in exception of *Klebsilla pneumoniae,* where the improvement was 18.9 % only) when compared with controls. Such results were expected since EDTA facilitated the entrance of bacteriocins inside the cell (Prudêncio et al., 2016). Khan et al., (2015) succeeded to inhibit *Escherichia coli* and *Salmonella typhimurium* when a compination of nisin (125-150 $\mu g\ ml^{-1}$) and Na- EDTA (20-30 mM) at pH of 5 to 6 was utilized. Prudêncio et al. (2016) referred the influence of temperature and pH on the susceptibility of *Salmonella typhimurium* to the combination of nisin and EDTA.

Table 3. Effect of different concentrations of EDTA (ppm) on the antimicrobial activities of bacteriocins of *Lactococcus lactis* subsp. *Lactis* NRRL1821 produced during SM fermentation (48 h at 37˚C).

Indicator microorganisms	Inhibition zone (mm)					
	0	50	100	150	200	250 ppm
Staphylococcus aureus ATCC 25923	**$11.0^{d}_{bcd}\pm0.7$	$12.1^{c}_{bcd}\pm0.5$	$12.1^{c}_{d}\pm0.5$	$16.0^{b}_{cd}\pm0.8$	$17.7^{a}_{cd}\pm0.5$	$18.3^{a}_{cd}\pm0.7$
Staphylococcus aureus FS 112	$10.3^{e}_{d}\pm0.8$	$12.8^{d}_{bc}\pm0.9$	$14.5^{c}_{c}\pm0.7$	$18.0^{b}_{bc}\pm0.8$	$20.1^{a}_{b}\pm0.7$	$20.2^{a}_{b}\pm0.6$
Bacillus subtilis NRRL 765	$9.6^{e}_{de}\pm0.8$	$11.4^{d}_{d}\pm0.7$	$12.5^{c}_{d}\pm0.8$	$13.4^{b}_{e}\pm0.8$	$15.2^{a}_{d}\pm0.8$	$15.7^{a}_{d}\pm0.7$
Bacillus cereus ATCC 33018	$8.7^{e}_{e}\pm0.7$	$9.6^{d}_{e}\pm0.6$	$11.1^{c}_{e}\pm0.7$	$12.3^{b}_{f}\pm0.5$	$12.7^{a}_{e}\pm0.7$	$12.6^{a}_{e}\pm0.8$
Bacillus amyloliqufaciense FS 111	$8.6^{e}_{e}\pm0.7$	$10.2^{d}_{e}\pm0.8$	$11.5^{c}_{e}\pm0.5$	$13.7^{b}_{e}\pm0.5$	$14.5^{a}_{a}\pm0.5$	$14.3^{a}_{de}\pm0.5$
Bacillus macerans NRRL 1650	$8.8^{c}_{e}\pm0.5$	$8.9^{c}_{ef}\pm0.5$	$10.5^{b}_{ef}\pm0.5$	$12.0^{a}_{f}\pm0.5$	$12.3^{a}_{e}\pm0.8$	$12.4^{a}_{e}\pm0.8$
Micrococcus luteus NRRL 2618	$12.7^{f}_{b}\pm0.5$	$14.7^{e}_{ab}\pm0.6$	$17.6^{d}_{b}\pm0.7$	$19.3^{c}_{b}\pm0.5$	$20.8^{b}_{b}\pm0.5$	$23.0^{a}_{ab}\pm0.5$
Enterococcus faecalis FS 90	$13.0^{c}_{ab}\pm0.5$	$13.3^{c}_{b}\pm0.5$	$17.6^{b}_{b}\pm0.8$	$20.1^{a}_{ab}\pm0.8$	$21.0^{a}_{b}\pm0.7$	$21.3^{a}_{b}\pm0.8$
Escherichia coli ATTC 35218	$13.7^{f}_{a}\pm0.5$	$16.8^{e}_{a}\pm0.5$	$18.9^{d}_{a}\pm0.5$	$22.5^{c}_{a}\pm0.4$	$25.0^{b}_{a}\pm0.9$	$28.2^{a}_{a}\pm0.7$
Escherichia coli FS 15	$10.0^{f}_{d}\pm0.9$	$11.2^{e}_{d}\pm0.8$	$13.7^{d}_{cd}\pm0.7$	$15.3^{c}_{e}\pm0.7$	$17.8^{b}_{cd}\pm0.7$	$19.9^{a}_{c}\pm0.8$
Salmonella typhimurium ATCC 14028	$14.0^{e}_{a}\pm0.5$	$14.6^{e}_{ab}\pm0.6$	$16.8^{d}_{bc}\pm0.7$	$20.4^{c}_{ab}\pm0.5$	$22.6^{b}_{ab}\pm0.5$	$26.5^{a}_{a}\pm0.9$
Pseudomonas aeruginosa ATCC 9027	$8.7^{e}_{e}\pm0.7$	$13.0^{d}_{b}\pm0.7$	$16.5^{c}_{bc}\pm0.5$	$17.3^{c}_{c}\pm0.7$	$18.4^{b}_{c}\pm0.5$	$18.8^{a}_{c}\pm0.7$
Serratia marcescens FS 37	$10.9^{f}_{cde}\pm0.9$	$13.2^{e}_{b}\pm0.8$	$14.5^{d}_{c}\pm0.7$	$15.7^{c}_{cd}\pm0.5$	$17.4^{b}_{cd}\pm0.7$	$19.4^{a}_{c}\pm0.7$
Klebsiella pneumoniae FS 49	$8.4^{c}_{f}\pm0.5$	$8.2^{c}_{f}\pm0.5$	$9.0^{b}_{f}\pm0.7$	$10.0^{a}_{g}\pm0.7$	$10.3^{a}_{f}\pm0.7$	$10.0^{a}_{f}\pm0.5$

**Means followed by different superscripts (within rows) and different subscripts (within columns) are significantly different ($P<0.05$).

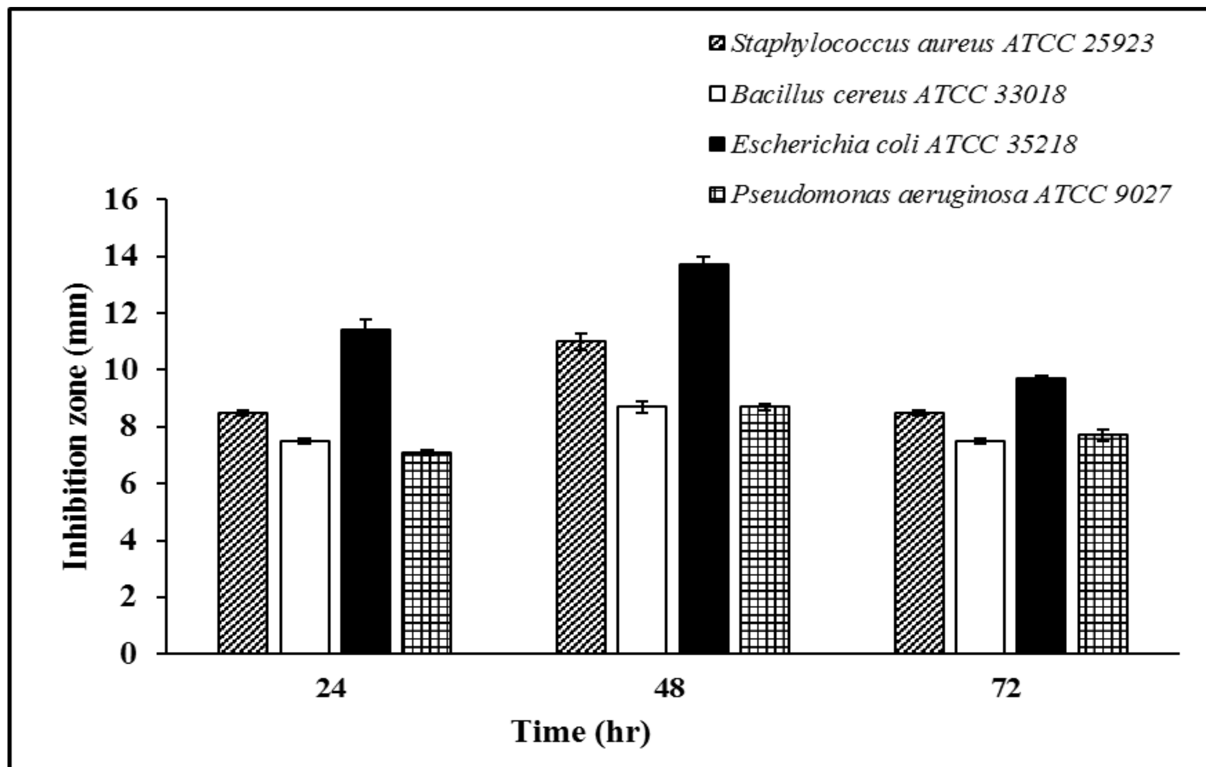

Figure 1. Effect of fermentation time on the antimicrobial activities *Lactococcus lactis* subsp. *lactis* NRRL 1821 against selected indicator microorganisms.

Psychrotrophic bacterial count

Total psychrotrophic bacterial count (Log_{10} cfu g^{-1}) in controls and in treated samples are presented in Figure 2. An initial count of 6.4 Log_{10} cfu g^{-1} for the untreated control (C1) indicated low microbial quality. This high microbial load was due to the bad hygiene followed during preparation of fillets in market. However, the fillets were still acceptable since the onset of microbial spoilage of shrimp and fish fillets is considered to be 10^7 to 10^8 cells per gram (Ayers, 1960). Dipping in water (C2) was able to reduce microbial load by only 0.3 log and this was due to the washing effect of surface microorganisms. On the other hand, dipping in FSM (T1) resulted in one Log reduction ($P<0.05$) in psychrotrophic bacterial count. Such decrease was expected and could be attributed to compounds: lactic acid and other antimicrobial substances (bacteriocins) produced during fermentation of SM using *Lactococcus lactis* subsp. *lactis*. Treatments of fillets with FSM+EDTA (200 ppm), SA (10%, w/w), FSM+SA (10%, w/w) and FSM+SA (10%, w/w) + EDTA (200 ppm) resulted in significance ($P<0.05$) reduction of total psychrotrophs and the decrements were 2.3, 2.1, 2.4, and 2.4 log, respectively. During storage at 0°C in crushed ice, C1 and C2 showed gradual increase in psychrotrophs reaching the onset of spoilage of 10^7cfu g^{-1} after 1.8 weeks. Treatment with FSM (T1) resulted in reduction of the initial count from 6.4 to 5.4 log_{10} cfu g^{-1} followed by a slight decrease in numbers reaching 4.7 log_{10} cfu g^{-1} after 2 weeks of storage after which a significant gradual increase in number was observed reaching spoilage onset after 3.3 weeks. Treatments T2 (FSM+ EDTA), T3 (SA) and T4 (FSM+SA) significantly ($P<0.05$) reduced the initial numbers by 2.3, 2.1 and 2.4 log_{10} cfu g^{-1}. Also, lag phases were extended for an additional one week followed by gradual increase in numbers reaching the spoilage onset after 3, 3 and 4.1 weeks, respectively. Such findings resulted in shelf life extension by 1.2, 1.2 and 2.3 weeks (Table 4). Treatment 5 (FSM+SA + EDTA) was the highest in extending lag phase up to 3 weeks and shelf life to 4.8 weeks. It is of interest to report that the generation time (Table 4) of the spoilage microorganisms in the controls was around 63.2 h.

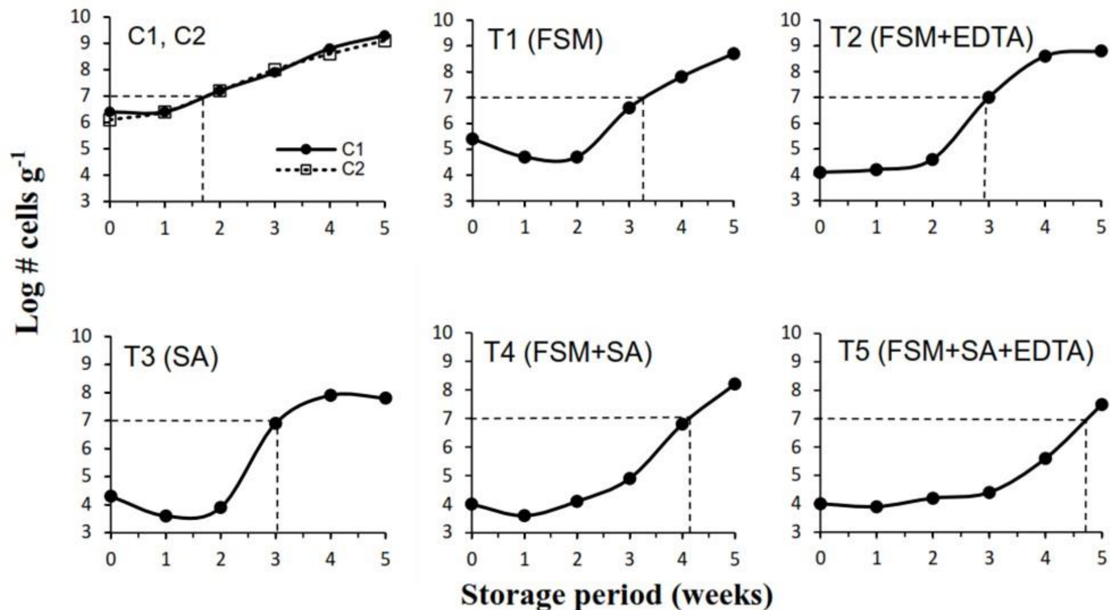

Figure 2. Total psychrotrophic count of treated nile perch fillets during 5 weeks storage in ice.

Table 4. Initial microbial load, lag phase, generation time (GT) of the spoilage microorganism and shelf life of fish fillets treated with different treatments during ice storage.

*T	Initial load (log_{10} cfu g^{-1})	Lag phase (week)	GT (h)	Shelf life (weeks)
C1	6.4	1	63.2	1.8
C2	6.1	1	63.1	1.8
T1	5.4	2	26.6	3.3
T2	4.1	2	21.1	3.0
T3	4.3	2	16.8	3.0
T4	4.0	2	26.6	4.1
T5	4.0	3	26.6	4.8

*T= Treatment: C1= untreated control, C2= dipped in water control, T1= dipped in FSM, T2= dipped in FSM+EDTA, T3= dipped in SA, T4= dipped in FSM+SA and T5= dipped in FSM+SA+EDTA.

The use of FSM resulted in a shorter generation time (26.6 h) calculated from the logarithmic phase. However, improvement of an additional 1.5 week in shelf life was obtained due to the extension in lag phase by 1 week. Generation time of spoilage bacteria again were reduced to 21.1, 16.8 and 26.6 h for T2, T3 and T4, respectively. However, shelf life was extended to 3, 3 and 4.1 weeks compared to 1.8 weeks for control due to the reduction of the initial loads to 4.1, 4.3 and 4.0 log_{10} cfu g^{-1}, respectively and due to the extension of the lag phase by additional one week for each of them. Therefore, treatments didn't succeed in increasing the generation time of the spoilage microorganisms, but significantly (P<0.05) improved the shelf life of fish fillets through the reduction of the initial microbial load as well as extending the lag phase periods. Kim et al., (1995) reported that the combination of 2.5% *Bifidobacterium infantis* with sodium acetate extended both lag phase and generation time of spoilage bacteria during cold storage of catfish fillets and as a result 3 days shelf life extention was achieved. Al-Dagal and Bazaraa (1999) succeeded in prolonging the microbiological shelf life of whole shrimp by 3 days using sodium acetate (10 %, w/w) or potassium sorbate (1.5 %, w/w) with bifidobacteria through increasing the generation time from 12.8 h (control) to 30.1 or 31.4 h, respectively. They also stated that sodium acetate (10%, w/w) extended the lag phase (from 3 to 6 days), lengthened the generation time and prolonged the shelf life from 10 to > 26.8 days in case of peeled shrimp. Sallam (2007) reported a shelf life of 12, 12 and 15 days at refrigerated storage (1°C) for sliced salamon treated with sodium lactate (2.5 %, w/w), sodium citrate (2.5 % w/w) and sodium acetate (2.5%, w/w), respectively. Sarika et al., (2011) studied the effect of using *Enterococcus faecalis* CD1 bacteriocin as a biopreservative of fish under different storage conditions. The use of enterocin CD1 (10%) resulted in reduction in total viable count by 2 log units (from 11.72 to 9.82 log_{10} cfu g^{-1}) at the end of storage period (28 days) at 4°C.

Changes in pH

Changes in pH values of fish fillets during ice storage are reported in Table 5. The initial pH value of the fresh nile perch fillets (C1) was 6.2. Similarly, with no significant differences (P<0.05) with C1, samples of C2, T2 (FSM+EDTA) and T3 (SA) showed initial pH values of 6.1 ,6.1 and 5.8, respectively. On the other hand, T1 (FSM), T4 (FSM+SA) and T5 (FSM+SA+EDTA) resulted in a significant decrease in pH values to reach 4.6, 4.7 and 4.8, respectively. This could be attributed to the acidity of the FSM (adjusted to pH of 2.0). During ice storage (0°C) for 5 weeks, the pH values of T2 and T3 samples showed no significant (P<0.05) changes in pH . On the other hand, other samples showed a gradual but significance (P<0.05) increase in pH values to reach 7.7, 7.8, 5.2, 6.5 and 6.0 at the end of storage for C1, C2, T1, T4 and T5, respectively. It is of interest to report that all tested treatments met the Egyptian standards that indicated that the pH value should not exceed 6.5. The increase of pH values up to 7.7 and 7.8 for control samples may be due to the increase in volatile basic compounds, such as ammonia, by psychrotrophic bacterial activities (Manju et al., 2007).

Changes in TBA

TBA values indicate about the oxidative rancidity of the samples. The Egyptian standards (2005) specified 4.5 mg malondialdihyde kg^{-1} fish fillets as the maximum permitable limit. The changes in TBA values of nile perch fillets are depicted in Table 6. The initial values for controls and treatments ranged from 0.16 to 0.20. During ice storage for 5 weeks, gradual significant (P<0.05) increase was noted in all samples. Treatments (T1, T2, T3, T4 and T5) succeeded in keeping TBA values much lower than that of the Egyptian standards and values were 2.56, 2.12, 1.74, 1.56 and 1.22, respectively. On the other hand C1, C2 samples exceeded the value of 4.5 of the Egyptian Standards and considered spoiled after 3 weeks of storage where values were 4.79 and 4.63, respectively. Rajesh et al., (2002) observed reduction in TBA values of seer fish steaks treated with sodium acetate compared with control during chill storage. Similarly, Manju et al., (2007) reported TBA values increase from 0.08 to 0.39 for fresh pearl spot fish treated with sodium acetate in vaccum pack within 17 days of ice storage. Langroudi et al., (2011) reported the effectiveness of the treatments of grass carp fillets with sodium acetate and nisin at different concentrations, during refrigeration storage (16 days) at 4°C, in lowering TBA values. They reported TBA values in the range of 0.19 to 0.46 mg malondialdehyde kg^{-1} muscle after 16 days of storage.

Table 5. Changes in pH values of nile perch fillets during storage for 5 weeks in ice at 0°C.

*T	Storage (weeks)					
	0	1	2	3	4	5
C1	**6.2^{c}_{a} ± 0.07	6.8^{b}_{a} ± 0.07	6.9^{b}_{a} ± 0.14	7.1^{b}_{a} ± 0.14	7.6^{a}_{a} ± 0.07	7.7^{a}_{a} ± 0.07
C2	6.1^{b}_{a} ± 0.14	6.5^{b}_{a} ± 0.14	6.5^{b}_{ab} ± 0.07	7.2^{a}_{a} ± 0.28	7.5^{a}_{a} ± 0.00	7.8^{a}_{a} ± 0.07
T1	4.6^{b}_{b} ± 0.14	4.7^{b}_{c} ± 0.14	4.7^{b}_{c} ± 0.07	4.7^{b}_{c} ± 0.14	4.7^{b}_{c} ± 0.07	5.2^{a}_{c} ± 0.00
T2	6.1^{a}_{a} ± 0.14	6.2^{a}_{ab} ± 0.07	5.7^{a}_{b} ± 0.14	5.9^{a}_{b} ± 0.14	5.9^{a}_{b} ± 0.14	5.9^{a}_{c} ± 0.14
T3	5.8^{a}_{a} ± 0.28	6.3^{a}_{ab} ± 0.14	6.0^{a}_{ab} ± 0.57	6.2^{a}_{b} ± 0.28	6.2^{a}_{b} ± 0.28	6.3^{a}_{b} ± 0.42
T4	4.7^{c}_{b} ± 0.14	5.7^{b}_{b} ± 0.35	5.8^{b}_{b} ± 0.07	6.1^{ab}_{b} ± 0.14	6.0^{ab}_{b} ± 0.07	6.5^{a}_{b} ± 0.14
T5	4.8^{c}_{b} ± 0.07	5.7^{b}_{b} ± 0.07	5.7^{b}_{b} ± 0.07	6.0^{a}_{b} ± 0.07	5.9^{ab}_{b} ± 0.07	6.0^{a}_{b} ± 0.00

*T= Treatment: C1= untreated control, C2= dipped in water control, T1= dipped in FSM, T2= dipped in FSM+EDTA, T3= dipped in SA, T4= dipped in FSM+SA and T5= dipped in FSM+SA+EDTA.
** Mean pH values followed by different superscripts (within rows) and different subscripts (within columns) are significantly different ($P<0.05$).

Table 6. Changes in TBA values during storage for 5 weeks in ice at 0°C.

*T	Storage (weeks)					
	0	1	2	3	4	5
C1	**0.20^{f}_{a} ± 0.01	0.81^{e}_{a} ± 0.04	2.63^{d}_{b} ± 0.05	4.79^{c}_{a} ± 0.07	6.92^{b}_{a} ± 0.03	8.69^{a}_{a} ± 0.01
C2	0.20^{f}_{a} ± 0.01	0.83^{e}_{a} ± 0.05	2.68^{d}_{a} ± 0.08	4.63^{c}_{b} ± 0.04	6.80^{b}_{a} ± 0.01	8.73^{a}_{a} ± 0.02
T1	0.18^{e}_{ab} ± 0.01	0.20^{e}_{c} ± 0.00	0.61^{d}_{c} ± 0.01	1.06^{c}_{c} ± 0.01	1.69^{b}_{b} ± 0.04	2.56^{a}_{b} ± 0.06
T2	0.17^{f}_{ab} ± 0.00	0.30^{e}_{b} ± 0.00	0.55^{d}_{c} ± 0.02	0.99^{c}_{d} ± 0.06	1.38^{b}_{c} ± 0.04	2.12^{a}_{c} ± 0.04
T3	0.18^{e}_{ab} ± 0.00	0.19^{e}_{cd} ± 0.00	0.38^{d}_{d} ± 0.00	0.63^{c}_{e} ± 0.00	0.96^{b}_{d} ± 0.00	1.74^{a}_{d} ± 0.00
T4	0.17^{e}_{ab} ± 0.01	0.19^{e}_{cd} ± 0.00	0.37^{d}_{d} ± 0.01	0.63^{c}_{e} ± 0.00	0.92^{b}_{d} ± 0.01	1.56^{a}_{e} ± 0.01
T5	0.16^{e}_{b} ± 0.00	0.17^{e}_{d} ± 0.00	0.36^{d}_{d} ± 0.00	0.59^{c}_{e} ± 0.01	0.90^{b}_{d} ± 0.01	1.22^{a}_{f} ± 0.01

*T= Treatment: C1= untreated control, C2= dipped in water control, T1= dipped in FSM, T2= dipped in FSM+EDTA, T3= dipped in SA, T4= dipped in FSM+SA and T5= dipped in FSM+SA+EDTA.
** Means (mg malondialdehyde/kg nile perch fillets) followed by different superscripts (within rows) and different subscripts (within columns) are significantly different ($P<0.05$).

Changes in TVB-N

TVB-N is an important parameter for spoilage of fresh seafood (Dalgaard, 2000). TVB-N in fish is mainly composed of ammonia, primary, secondary, and tertiary amines produced as a result of protein and non-protein nitrogenous compounds degradation by spoilage microorganisms. A level of 30 mg N/100g fish muscle (Egyptian standard, 2005) and 35-40 mg N/100g of fish muscle (Lakshmanan, 2000) is usually regarded as spoiled. Table 7 indicates the changes in TVB-N of nile perch fillets during storage for 5 weeks at 0°C. The initial TVB-N values ranged from 4.13 to 6.57 mg N/100g fish muscle. A gradual significant increase ($P<0.05$) was noted during storage for controls and all tested treatments. The controls C1 and C2 were considered spoiled after 2 weeks of storage since they exceeded the limit of 30 set by the Egyptian Standards. While 3 weeks were needed to reach the spoilage onset reported by Lakshmanan (2000). After 5 weeks of storage in

Table 7. Changes in TVB-N values (mg N/100 g nile perch fillets) during storage for 5 weeks in ice at 0°C.

*T	Storage (weeks)					
	0	1	2	3	4	5
C1	**6.57^{f}_{a} ± 0.32	13.88^{e}_{a} ± 0.39	36.54^{d}_{a} ± 0.35	44.99^{c}_{a} ± 0.41	50.65^{b}_{a} ± 0.35	53.51^{a}_{a} ± 0.37
C2	6.57^{f}_{a} ± 0.32	13.98^{e}_{a} ± 0.25	34.76^{d}_{b} ± 0.34	44.10^{c}_{a} ± 0.00	48.75^{b}_{b} ± 0.64	51.50^{a}_{b} ± 0.00
T1	5.39^{f}_{b} ± 0.38	12.23^{e}_{b} ± 0.32	29.72^{d}_{c} ± 0.40	34.25^{c}_{b} ± 0.35	36.63^{b}_{c} ± 0.13	39.51^{a}_{c} ± 0.02
T2	5.09^{f}_{bc} ± 0.00	11.59^{e}_{bc} ± 0.37	26.85^{d}_{d} ± 0.35	33.65^{c}_{b} ± 0.35	35.98^{b}_{c} ± 0.35	38.78^{a}_{c} ± 0.39
T3	5.07^{f}_{bc} ± 0.04	10.34^{e}_{cd} ± 0.23	20.01^{d}_{e} ± 0.28	24.53^{c}_{c} ± 0.28	31.45^{b}_{d} ± 0.35	34.25^{a}_{d} ± 0.35
T4	4.28^{f}_{c} ± 0.35	9.81^{e}_{d} ± 0.27	17.77^{d}_{f} ± 0.33	22.41^{c}_{d} ± 0.33	28.61^{b}_{e} ± 0.35	32.20^{a}_{e} ± 0.00
T5	4.13^{f}_{c} ± 0.24	8.20^{e}_{e} ± 0.42	14.86^{d}_{g} ± 0.21	19.66^{c}_{e} ± 0.21	24.62^{b}_{f} ± 0.40	29.74^{a}_{f} ± 0.35

*T= treatment: C1= untreated control, C2= dipped in water control, T1= dipped in FSM, T2= dipped in FSM+EDTA, T3= dipped in SA, T4= dipped in FSM+SA and T5= dipped in FSM+SA+EDTA.
** Mean TVB-N values followed by different superscripts (within rows) and different subscripts (within columns) are significantly different (P<0.05)

ice, the TVB-N values for treatment T1, T2, T3, and T4 were 39.51, 38.78, 34.25 and 32.20, respectively.

Such values were within the acceptable limit of 35-40 mg N/ 100g fish muscle reported by Lakshmanan (2000). The only treatment which met the limit of the Egyptian Standards was T5, where TVB-N value was 29.74 after 5 weeks of storage followed by T4 and T3, where TVB-N values of 28.61 and 24.53 were recorded after 4 weeks and 3 weeks of storage, respectively. Manju et al., (2007) reported a significant increase from initial value of 5.6 to 19.5 mg N/100g fish muscle in controlled air pack after 10 days of storage in ice while to 21.6 mg N/100g fish muscles treated with sodium acetate in vaccum packaging and stored for 17 days. Nath et al., (2014) studied the effect of the treatment of fresh horse mackeral fillets with fresh culture of *Lactobacillus sakei* (ATCC 15521) on the shelf life and safety of fillets at 6 ± 1°C. They indicated an increasing trend in control and treatments during the 15 days of storage. The highest values was 32.91 for control while significantly lower (P<0.05) value of 24.65 and 25.21 mg N/100 g fillets were obtained for treatments with *L. sakei* under aerobic condition and vaccum, respectively. Sarika et al. (2018) treated Listeria challenged fish fillets with bacteriocin from *Lactococcus lactis* and stored at 0°C for 28 days. The TVB-N values showed significant (P<0.05) increase from 5 to 22 mg N/100g fish muscle.

Sensory analysis

The nile perch fillets treated with FSM, FSM+SA, FSM+SA+EDTA as well as untreated control were evaluated for the changes in color, odor, texture and overall acceptability during storage in ice (Table 8). During the first week of storage, no significant (P<0.05) changes in color were noted for C1 (untreated control) and T1 (FSM) samples followed by a gradual significant decrease in color scores till the end of storage. Samples were completely rejected after 5 weeks of storage. Samples of T4 (FSM+SA) and T5 (FSM+SA+EDTA) showed constant color scores within the first 2 weeks of storage followed by slight but significant (P<0.05) reduction in color scores (T4) and with almost no significant differences in case of T5. By the end of the 5th week of storage both treatments significantly improved color scores compared with C1 and T1. In case of odor scores, no significant changes (P<0.05) were noted within the first week of storage for C1, T1 and T4 samples. On the other hand, T5 resulted in high odor stability with no significant changes through the first 3 weeks of storage followed by slight decrease till the end of storage. Only C1 sample was rejected (score of 2.2) at the end of week 5 of storage, while scores were 3.7, 4.6 and 5.3 for samples of T1, T4, and T5, respectively. Similarly, texture scores were constant within the first week of storage for C1

Table 8. Mean sensory characteristics of nile perch fillets treated with FSM, FSM+SA and FSM+EDTA+SA during storage in ice at 0°C.

*T	Storage (weeks)	Mean sensory scores ± SD			
		Color	Odor	Texture	Over all acceptability
C1	0	**6.3^{ab} ± 0.9	5.9^{abc} ± 1.3	6.4^{abcd} ± 0.5	6.4^{abc} ± 0.7
	1	6.0^{abc} ± 0.9	5.7^{bcd} ± 1.2	6.1^{abcde} ± 0.8	6.0^{abcde} ± 0.9
	2	5.4^{cdef} ± 0.9	5.3^{cde} ± 1.4	5.4^{efg} ± 0.7	4.3^{f} ± 0.5
	3	4.7^{gh} ± 1.1	4.1^{fg} ± 1.7	4.4^{hi} ± 1.2	3.6^{g} ± 0.5
	4	3.9^{i} ± 0.7	2.9^{hi} ± 1.1	3.0^{k} ± 0.7	2.4^{h} ± 0.7
	5	2.9^{j} ± 0.7	2.2^{i} ± 1.3	2.1^{l} ± 0.8	1.7^{i} ± 0.7
T1	0	5.9^{abcd} ± 0.5	6.2^{abc} ± 0.4	6.0^{abcde} ± 0.7	5.4^{e} ± 0.5
	1	5.7^{bcde} ± 0.6	6.0^{abc} ± 0.0	5.6^{def} ± 0.9	5.4^{e} ± 0.5
	2	5.2^{defg} ± 0.6	5.3^{cde} ± 0.5	5.0^{fgh} ± 1.1	4.6^{f} ± 0.5
	3	4.2^{hi} ± 0.5	4.2^{fg} ± 1.3	4.6^{ghi} ± 1.2	4.3^{f} ± 0.5
	4	3.5^{ij} ± 1.0	3.9^{fg} ± 1.1	3.9^{ij} ± 1.1	3.5^{g} ± 0.5
	5	2.0^{j} ± 1.2	3.7^{gh} ± 1.2	3.4^{j} ± 1.3	2.9^{gh} ± 1.0
T4	0	6.6^{a} ± 0.5	6.0^{abc} ± 0.7	6.7^{ab} ± 0.5	6.6^{ab} ± 0.5
	1	6.4^{a} ± 0.5	6.1^{abc} ± 0.3	6.7^{ab} ± 0.5	6.6^{ab} ± 0.5
	2	6.0^{abc} ± 0.5	5.6^{bcd} ± 0.7	6.6^{abc} ± 0.5	6.2^{abcd} ± 2.0
	3	5.6^{cde} ± 0.9	5.4^{bcde} ± 0.9	6.3^{abcd} ± 0.7	5.9^{bcde} ± 0.6
	4	5.0^{efg} ± 0.5	4.8^{def} ± 0.7	6.1^{abcde} ± 0.8	5.8^{cde} ± 0.7
	5	4.8^{fgh} ± 0.8	4.6^{efg} ± 0.9	5.9^{bcde} ± 1.1	5.6^{de} ± 0.5
T5	0	6.3^{ab} ± 0.5	6.8^{a} ± 0.4	6.8^{a} ± 0.4	6.7^{a} ± 0.5
	1	6.1^{abc} ± 0.3	6.3^{ab} ± 0.5	6.2^{abcde} ± 0.4	6.0^{abcde} ± 0.0
	2	5.9^{abcd} ± 0.3	6.0^{abc} ± 0.7	6.1^{abcde} ± 0.3	6.0^{abcde} ± 0.0
	3	5.7^{bcde} ± 0.5	6.0^{abc} ± 0.7	6.0^{abcde} ± 0.5	6.0^{abcde} ± 0.0
	4	5.6^{cde} ± 0.5	5.3^{cde} ± 0.5	5.9^{bcde} ± 0.6	5.9^{bcde} ± 0.3
	5	5.4^{cdef} ± 0.5	5.3^{cde} ± 0.5	5.8^{cdef} ± 0.7	5.8^{cde} ± 0.4

*T= Treatment: C1= untreated control, T1= FSM, T4= FSM+SA, T5= FSM+EDTA+SA.
** Means followed by different superscripts (within) columns are significantly different ($P<0.05$).For sensory scores, 7= most liked, 3= unacceptable margin and 1= most dislike

and T1 followed by significant ($P<0.05$) decrease during storage reaching the rejection limit of 3 in case of C1 sample after 4 weeks. T4 significantly ($P<0.05$) improved fish fillets texture, where scores persisted with no significant changes during the 5 weeks of storage (scores of 6.7 to 5.9). T5 followed T4 in improving and extending the texture acceptability and scores were constant with no significant changes during the first 3 weeks of storage (scores of 6.8 to 6.0) followed by a slight decrease thereafter reaching the score of 5.8. Such persistence in texture scores during the course of storage in case of T4 and T5 was probably due to inhibition of the endogeneous fish alkaline proteases by: the acetate moiety present in both solutions, the low initial pH of both solutions where pH of 4.7 and 4.8 were recorded compared with 6.2 for control and finally due to the presence of EDTA which acts as protease inhibitor (Liang et al., 2016; Zhang et al., 2016). Zhang et al., (2016) reported optimal pH values of 7.0 and 11.0 for acidic and alkaline endogenous proteases from lizard fish viscera, respectively. Finally, panalists gave T5 the highest scores of overall acceptability, where scores didn't significantly ($P<0.05$) changed during the first 3 weeks of storage followed by T4. Scores of 5.6 and 5.8 were obtained at the end of storage for T4 and T5, respectively. On the other hand, T1 and C1 samples were rejected and scored 2.9 and 2.4 after 5 and 4 weeks, respectively. The sensory shelf life was 3, 4, > 5 and > 5 weeks for C1, T1, T4 and T5, respectively.

CONCLUSION

Surface treatment of fresh fish fillets by dipping in solutions of FSM (containing bacteriocins of *Lactococcus lactis* subsp. *Lactis*) in combinations with SA and/ or EDTA resulted in significant reduction of psychrotrophic bacteria, oxidative rancidity and TVB-N values. Also, high sensory quality was maintained. Such application

resulted in microbial shelf life extension up to 4.8 weeks compared with 1.8 for control with keeping both chemical and sensory quality. A further study on the sensory quality of cooked treated fish fillets is needed. Therefore, such treatment is considered to be safe, easy to apply, cheap and very effective in extension the shelf life of fresh fish and seafoods.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

AUTHOR CONTRIBUTIONS

Prof. WAB designed, supervised all experimental trials and wrote the manuscript. SNA conducted research laboratory work and statistical analysis. Drs. MEA and HAG participated in editing of the manuscript and co-supervised the experimental trials. All authors read and approved the final copy of the manuscript.

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