Published in final edited form as:
Food Control. 2007 May ; 18(5): 566–575.

Antimicrobial and antioxidant effects of sodium acetate, sodium lactate, and sodium citrate in refrigerated sliced salmon

Khalid Ibrahim Sallam*
Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Mansoura University, Mansoura, Egypt

Abstract

This study was carried out to evaluate the microbiological quality and lipid oxidation of fresh salmon slices treated by dipping in 2.5% (w/v) aqueous solution of sodium acetate (NaA), sodium lactate (NaL), or sodium citrate (NaC) and stored at 1 °C. The results revealed that these salts were efficient ($P < 0.05$) against the proliferation of various categories of spoilage microorganisms; including aerobic and psychrotrophic populations, *Pseudomonas* spp., H_2S-producing bacteria, lactic acid bacteria, and *Enterobacteriaceae*. The general order of antibacterial activity of the different organic salts used was; sodium acetate > sodium lactate > sodium citrate. Lipid oxidation, as expressed by peroxide value (PV) and thiobarbituric acid (TBA) value, was significantly ($P < 0.05$) delayed in NaA- and NaC-treated samples. The antioxidant activity followed the order: NaC > NaA > NaL. The shelf life of the treated products was extended by 4–7 days more than that of the control. Therefore, sodium acetate, sodium lactate, and sodium citrate can be utilized as safe organic preservatives for fish under refrigerated storage.

Keywords

Sliced salmon; Microbial quality; Lipid oxidation; Sodium acetate; Lactate; Citrate

1. Introduction

Fish and shellfish are excellent protein sources for human consumption; in addition they have a high content of hydrosoluble and liposoluble vitamins, minerals and polyunsaturated fatty acids (PUFAs) of the n-3 family. Interestingly, omega-3 fatty acids, found mainly in fat-rich fish such as salmon, mackerel, herring, and sardines confer health benefits in humans not found in any other foods. Omega-3 fatty acids from fish can lower blood triglycerides, reduce abnormal heart rhythms, reduce blood pressure by small but significant amounts, and improve blood clotting regulation (Nettleton, 1995). The American Heart Association (AHA) advocates consumption of fatty fish, at least two times a week, as a safe and effective way to obtain the heart health benefits of omega-3 fatty acids.

Fish, however, is more perishable than chicken or red meat as it contains relatively large quantities of free amino acids and volatile nitrogen bases compared with other meats (Ashie, Smith, & Simpson, 1996). During storage, the quality of fish degrades due to a complex process in which physical, chemical and microbiological forms of deterioration are implicated (González-Fandos, Villarino-Rodríguez, García-Linares, García-Arias, & García-Fernández, 2005). Enzymatic and chemical reactions are usually responsible for the initial loss of freshness

E-mail address: sallam.kh.i@aist.go.jp
*Present address: Proteolysis and Protein Turnover Research Group, Institute of Genome-Based Biofactory, National Institute of Advanced Industrial Sciences and Technology (AIST), 2-17-2-1 Tsukisamu-Higashi, Toyohira-ku, Sapporo 062-8517, Japan. Tel.: +81 11 857 8935; fax: +81 11 857 8980.

whereas microbial activity is responsible for the obvious spoilage and thereby establishes product shelf life (Gram & Huss, 1996). The large amount of polyunsaturated fatty acid found in fish lipids makes them highly susceptible to oxidation. Oxidative rancidity is an important organoleptic characteristic for rejection or approval of fish after prolonged storage (Amanatidou et al., 2000).

The use of good manufacturing practices and hazard analysis of critical control point (HACCP) is crucial in the production, storage, distribution and retailing of refrigerated foods, and because of consumer demand for fresh refrigerated foods with extended shelf life, considerable research has been directed toward using various preservation strategies to preserve or prolong the shelf life, while ensuring the safety, of fresh foods including fishery products.

Sodium salts of the low molecular weight organic acids; such as acetic, lactic, and citric have been used to control microbial growth, improve sensory attributes and extend the shelf life of various food systems including meat (Maca, Miller, & Acuff, 1997;Sallam & Samejima, 2004), poultry (Williams & Phillips, 1998), and fish (Boskou & Debevere, 2000;Zhuang, Huang, & Beuchat, 1996). In addition to their suppressing effect on the growth of food spoilage bacteria, organic salts of sodium acetate, lactate, and citrate were shown to possess antibacterial activities against various food-borne pathogens including *Staphylococcus aureus* and *Yersinia enterocolitica* (Lee, Cesario, Owens, Shanbrom, & Thrupp, 2002), *Listeria monocytogenes* (Qvist, Sehested, & Zeuthen, 1994), *Escherichia coli* (Lee et al., 2002;McWilliam Leitch & Stewart, 2002), as well as *Clostridium botulinum* (Anders, Cerveny, & Milkowski, 1989). Furthermore, these salts are widely available, economical, and generally ‘‘recognized-as-safe’’ (McWilliam Leitch & Stewart, 2002).

Salmon is a high quality product with considerable nutritional and economic importance. Much of the fresh salmon are sold to the consumer as gutted, whole salmon, but significant amounts also are sold as fillets or slices. The main objective of this study was to investigate the antimicrobial and antioxidant effects of sodium acetate (NaA), sodium lactate (NaL), and sodium citrate (NaC) in fresh salmon slices during tray-packaged storage at 1 °C.

2. Materials and methods

2.1. Preparation and treatment of fish samples

Fresh ice-chilled Pacific salmon (*Onchorhynchus nerka*) were purchased, within 24 h post harvesting, from a local seafood market in Sapporo, Hokkaido, Japan. Salmon samples were headed, gutted, filleted, and cut into skin-on slices (110-g average weight/slice) using the market facilities. Salmon slices were then transported to the laboratory in polystyrene boxes with an appropriate quantity of flaked ice. Within 1 h of arrival, fish slices were divided into four batches (12 kg each); three batches were treated by dipping for 10 min in pre-chilled (4 °C) aqueous solution (2.5%; w/v) of NaA, NaL, or NaC (Wako Pure Chemical Industries Ltd., Osaka, Japan), while the fourth batch was dipped in pre-chilled distilled water as a control sample. Fish to dipping solution ratio was 1:2.5. After dipping, fish slices were allowed to drain for 5 min on a sterile stainless wire mesh screen at the ambient temperature (18 °C). Five slices from each dipping treatment were placed in a Styrofoam tray and packaged by over-wrapping with polyvinylidene film. This packaging permits an aerobic condition during storage. Packaged slices were subsequently labeled and stored at 1 °C. The sliced salmon were sampled for examination at storage days 0, 3, 6, 9, 12, and 15. On each sampling occasion, three fish slices from every batch were evaluated for the microbiological quality and lipid oxidation.

2.2. Compositional analysis

Before packaging and storage, composite flesh sample (comprising; dorsal, abdominal, and caudal regions) of the sliced salmon was analyzed for its moisture, protein, fat, and ash content according to the methods of AOAC International (1999). Analyses were conducted in triplicate, and all reagents were of analytical grade.

2.3. Microbiological analyses

Twenty-five grams of fish samples were aseptically removed from the trays and homogenized for 1 min in a stomacher (Stomacher 400 Lab Blender; Seward Medical, London, UK) containing 225 ml pre-chilled sterile peptone-physiological saline solution (0.1% peptone + 0.85% NaCl) (Katayama Chemical, Osaka, Japan). After resuscitation for 30 min at room temperature, further decimal serial dilutions were prepared from this homogenate in the same chilled sterile diluent. The appropriate dilutions were subsequently used for enumeration and differentiation of microorganisms and particular microbial genera in the samples, at each of the pre-determined time intervals, during refrigerated storage.

2.3.1. Aerobic plate count—Aerobic plate counts (APC) were determined by inoculating 0.1 ml of the sample homogenate, at selected dilutions, onto duplicate sterile plates of pre-poured and dried Standard Method Agar (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) using the surface spread technique, then the plates were incubated for 48 h at 35 °C (APHA, 1992).

2.3.2. Psychrotrophic count—Psychrotrophic counts (PTCs) were determined in a similar method to that for APC except that plates were incubated at 7 °C for 10 days (Cousin, Jay, & Vasavada, 1992).

2.3.3. Pseudomonas count—Pseudomonas were enumerated on Pseudomonas Agar Base (CM 559; Oxoid) supplemented with cetrimide, fucidin, and cephaloridine (CFC) supplements (SR 103; Oxoid, Basingstoke, Hampshire, UK) providing a selective isolation medium for *Pseudomonas* spp. Colonies were counted after 2-days incubation at 25 °C.

2.3.4. Hydrogen sulfide-producing bacteria—H_2S-producing organisms were enumerated on iron agar Lyngby (CM 964; Oxoid, Basingstoke, Hampshire, UK) by pour plating method. After solidification, the plates were covered with a thin layer of the same growth medium and incubated at 25 °C for 3 days (Gram, Trolle, & Huss, 1987). On this medium, these bacteria are recovered as black colonies due to precipitation of ferrous sulfide. Sixty selected black colonies were isolated, pure cultured, and then characterized by morphology, Gram reaction, motility test, catalase and oxidase production, DNase test, TMAO reduction, glucose fermentation, arginine dihydrolase, lysine and ornithine decarboxylase, growth in the presence of 6.5% NaCl, growth at 4 and 37 °C, citrate utilization, as well as utilization of sucrose and D-gluconate as the sole carbon and energy source for growth.

2.3.5. Lactic acid bacterial count—For determination of lactic acid bacteria (LAB), diluted samples were plated on deMan, Rogosa, and Sharpe (MRS) agar (Merck, Darmstadt, Germany) and incubated at 30 °C for 2–3 days in anaerobic jars with disposable Anaerocult C bags (Merck, Darmstadt, Germany) for the generation of an anaerobic medium.

2.3.6. Enterobacteriaceae count—Enterobacteriaceae counts (EBC) were enumerated by the pour plating method on Violet Red Bile Glucose Agar (VRBGA; Difco, Detroit, Michigan, USA). The plates were overlaid with a virgin layer of the same growth medium before incubation at 37 °C for 24 h (ICMSF, 1978).

2.4. Assessment of lipid oxidation

The assessment of lipid oxidation was conducted via two different indices, the peroxide value (PV) measurement and the 2-thiobarbituric acid (TBA) assay.

2.4.1. Measurement of peroxide value—Peroxide value (PV) was determined according to the AOAC International (1999). The sample (5 g) was weighed in a 250-ml glass stoppered Erlenmeyer flask and heated in a water bath at 60 °C for 3 min to melt the fat, then thoroughly agitated for 3 min with 30 ml acetic acid–chloroform solution (3:2 v/v) to dissolve the fat. The sample was filtered under vacuum through Whatman filter paper to remove meat particles. Saturated potassium iodide solution (0.5 ml) was added to the filtrate, which was transferred into the burette of an automatic titrator (DL 25 Titrator, Mettler-Toledo AG, Greifensee, Switzerland) equipped with stirrer and pH electrode. The titration was allowed to run against standard solution of sodium thiosulfate (25 g/l). PV was calculated and expressed as milliequivalent peroxide per kg of sample:

$$\mathrm{PV(meq/kg)} = \frac{S \times N}{W} \times 1000,$$

where S is the volume of titration (ml), N the normality of sodium thiosulfate solution (N = 0.01), and W the sample weight (kg).

2.4.2. Measurement of TBA value—The 2-thiobarbituric acid (TBA) assay was carried out according to the procedure of Schmedes and Holmer (1989). Fish sample (10 g) was mixed with 25 ml of 20% trichloroacetic acid (w/v) and homogenized in a blender for 30 s. After filtration, 2 ml of the filtrate were added to 2 ml of 0.02 M aqueous TBA in a test tube. The test tubes were incubated at room temperature in the dark for 20 h; then the absorbance was measured at 532 nm by using UV–vis spectrophotometer (model UV-1200, Shimadzu, Japan). TBA value was expressed as mg malonaldehyde (MA) per kg of fish sample.

2.5. Statistical analysis

On each sampling occasion, three independent samples from each processing condition were subjected to the microbiological quality and lipid oxidation tests. All measurements were carried out in triplicates, and all microbial counts were converted into base-10 logarithms of colony forming units per g of sliced salmon samples ($\log_{10}$ CFU/g). Data were subjected to analysis of variance (ANOVA) using the General Linear Models procedure of the Statistical Analysis System software of SAS Institute (SAS Institute, Inc., 1990). Differences among the mean values of the various treatments and storage periods were determined by the least significant difference (LSD) test, and the significance was defined at $P < 0.05$. The differences which are equal to or more than the identified LSD values are considered statistically significant.

3. Results and discussion

3.1. Proximate composition

The mean (±SE) compositional contents of moisture, protein, lipid, and ash (g/100 g fish muscle) in the raw salmon analyzed were 68.23 ± 0.73, 19.21 ± 0.36, 11.56 ± 0.17, and 1.12 ± 0.03, respectively. Dipping of the sliced salmon into the different treatment solutions did not cause any significant changes in these contents (data not shown). The proximate composition of the raw salmon reported in different studies (González-Fandos et al., 2005;USDA, 1987) showed some degree of differences, especially for the lipid content. Such variations in the chemical composition of fish is greatly related to the nutrition, catching season (spawning cycles), sexual variation, fish size, living area, as well as the other environmental conditions

(Pacheco-Aguilar, Lugo-Sanchez, & Robles-Burgueno, 2000). In addition, the farming of fish generally increases their lipid content (González-Fandos et al., 2005). The compositional variation, due to the reasons mentioned above, may possibly lead to changes in the sensory attributes; including taste, odor, texture, color, and surface appearance, which control the acceptability of fish as food (Flick & Martin, 1992;González-Fandos et al., 2005), as it may affect the microbial growth (González-Fandos et al., 2005).

3.2. Microbiological evaluation

Spoilage of fresh and lightly preserved fish is caused by the growth and activity of specific spoilage organisms (SSOs) which produce metabolites causing off-flavors or off-odors and consequently cause consumer food rejection (Gram & Dalgaard, 2002;Gram & Huss, 1996). However the SSOs are not the same in every case and the microbial flora isolated from seafoods differs considerably from one study to another, depending on the species of fish, their environment, the mode of capture, the type of fish product (whole, whole gutted, fillets, slices) as well as the climatic and storage conditions (Gram & Dalgaard, 2002). Nonetheless, pseudomonas, H_2S-producing bacteria, and lactic acid bacteria (LAB), are generally predominant in spoiled fish flora, while different Gram-negative bacteria, including *Enterobacteriaceae*, are frequently present. SSO level has found better correlation with the shelf life of fresh fish than the total bacterial level (Gram & Huss, 1996).

3.2.1. Aerobic plate count—The initial quality of fish used in this study was good, as indicated by a low initial number of bacteria (< 4 $\log_{10}$ CFU/g) before the fish slices were subjected to the different treatments. The initial APC ($\log_{10}$ CFU/g) in sliced salmon were ranged from 3.32 in NaA-treated samples to 3.85 in control (Fig. 1). This indicated that dipping of the sliced salmon in the different treatment solutions did not result in drastic reduction (only 0.38–0.53 $\log_{10}$ CFU/g) of the initial APC. By the day 6 of storage, however, APCs in sliced salmons for all of the different treatments were still below 6 $\log_{10}$ CFU/g, while that of control attained a count of 6.53, which is in close proximity to the maximal recommended limit of 7 $\log_{10}$ CFU/g for APC in raw fish (ICMSF, 1986), indicating a microbiological shelf life of about 7–8 days for the non-treated control samples. Likewise, a shelf life of less than 10 days has been estimated for sea salmon (*Pseudopercis semifasciata*) stored in ice at 0 °C (Hozbor, Saiz, Yeannes, & Fritz, 2006). A maximum shelf life of about 20 days has been estimated for iced whole salmon (*Salmo salar*) stored at 2 °C (Emborg, Laursen, Rathjen, & Dalgaard, 2002). Nonetheless, a commercial shelf life is usually limited to only 1 week when salmon is stored between 2 and 8 °C (Amanatidou et al., 2000). The shelf lives of the whole salmon fillets were always longer than those found for the sliced salmon (Truelstrup Hansen, Røntved, & Huss, 1998). The comparatively lower shelf life of sliced salmon versus whole fish or whole fillets can be attributed to the postharvest handling condition which involved higher bacterial contamination in sliced fish either from handling, processing tables and knives or from the fish viscera during preparation. Indeed, the initial microbial contamination, storage and packaging condition (aerobic storage, vacuum-packed, or packed under modified atmosphere) as well as the storage temperature can play the major role in determining the shelf lives of fishery products. At the point of sensory rejection, the common number of total spoilage bacteria in aerobically stored fish products is typically 7–9 $\log_{10}$ CFU/g (Gram & Huss, 1996;Ólafsdóttir et al., 1997). Nevertheless, standards, guidelines, and specifications often use much lower total microbial counts as indices of acceptability (Ólafsdóttir et al., 1997).

Dipping of sliced salmon in 2.5% aqueous solution of NaA, NaL, or NaC significantly delayed the microbial growth and extended the shelf life of the product up to 15, 12, and 12 days, respectively; and by the end of storage period (day 15), the salmon slices treated with NaA, NaL, or NaC revealed significant ($P < 0.05$) lower APCs (6.89, 7.63, and 7.78 $\log_{10}$ CFU/g, respectively) in comparison with the control (9.41 $\log_{10}$ CFU/g). No significant differences,

however, were found in APCs among the different treated samples although NaA-treated salmon showed 0.74- and 0.89-log lower than that of NaL- and NaC-treated samples, respectively. As may be expected, the increase in storage time produced significant proliferations in APC, whatever the treatment conditions (Fig. 1).

Sodium acetate treatment has been reported to produce significant reduction in the microbial population and extend the shelf life of various refrigerated fish such as channel catfish (*Ictalurus punctatus*) fillets (Zhuang et al., 1996) and cod (*Gadus morhua*) fillets (Boskou & Debevere, 2000). The antimicrobial effect of NaL on fish showed some variations. Williams, Rodrick, and West (1995) confirmed a significant decrease in APCs, along with shelf life extension of fresh catfish (*Ictalurus nebulosus*) fillets-treated sodium lactate during 8 days storage at 1 °C. On the contrary, Nykänen, Lapveteläinen, Kallio, and Salminen (1998) claimed that dipping of whole gutted rainbow trout (*Oncorhynchus mykiss*) into 2% sodium lactate solution for 2 min did not show any antimicrobial effect during a storage period of 10 days at 0 °C. A little antimicrobial effect was also confirmed in both shrimp (*Penaeus* spp.) and catfish fillets after dipping for 30 min into 2% NaL when compared with the control samples (Zhuang et al., 1996). The discrepancy of NaL effects on the microbial growth in fish products may be dependent on a variety of factors including the concentration of NaL used, the dipping time, the species of fish, the type of fish product, the degree of microbial contamination, as well as the storage condition.

3.2.2. Psychrotrophic bacteria—The Gram-negative psychrotrophic bacteria are the major group of microorganisms responsible for spoilage of aerobically stored fresh fish at chilled temperatures (Gram & Huss, 1996;Gram et al., 1987). In this study, the initial PTCs (day 0) of sliced salmon were ranged from 3.59 $\log_{10}$ CFU/g, in NaA-dipped samples, to 4.24 $\log_{10}$ CFU/g in control (Fig. 2). Additionally, the growth pattern of PTC showed same behavior as that of APC, with control also being the highest at day 15 (10.38 $\log_{10}$ CFU/g), followed by samples treated with NaC (8.46 $\log_{10}$ CFU/g), and NaL (8.17 $\log_{10}$ CFU/g), while lower count (7.12 $\log_{10}$ CFU/g) was detected in samples treated with NaA. The psychrotrophic population, however, were relatively higher than APCs, and by day 15 of storage, the difference in count was ranged from 0.23-log in NaA-dipped slices to 0.97-log in control samples, which was attributed to the refrigerated storage (1 °C) condition of the sliced salmon. This result is in accordance with that of Hozbor et al. (2006), who revealed similar growth pattern for psychrotrophic population in sea salmon during refrigerated storage.

Length of refrigerated storage (1 °C) had a significant ($P < 0.05$) effect on PTC, which tended to increase as the storage time increased regardless of treatment process. The results did not reveal any significant ($P > 0.05$) differences in the initial PTC among the different treatments or between the treated and control samples; however by the end of storage (day 15), significant ($P < 0.05$) differences (1.92–3.26-logs) were detected between all of the treated salmon and the control, and although the PTC in NaA-treated samples was 1.05-logs and 1.34-logs lower than those of NaL- and NaC-treated samples, no significant differences were detected among these different treatments. Conversely, Williams et al. (1995) pointed out that treatment of fresh catfish fillets with sodium lactate (1% or 2% by tumbling), resulted in an initial significant reduction in psychrotrophic counts, when compared to control, although they did not detect any difference in such counts between the treated and control samples by the end of storage (8 days at 1 °C).

All of the different treatments (2.5% aqueous solutions of NaA, NaL, or NaC) used in this study significantly ($P < 0.05$) controlled the growth of psychrotrophic bacteria of sliced salmon. On the contrary, it has been reported that NaL (2%) treatment has a little or no effect on the psychrotrophic populations in shrimp (Zhuang et al., 1996) and rainbow trout (Nykänen et al., 1998) during refrigerated storage. Moreover, Zhuang et al. (1996) indicated that sodium acetate

(2%) treatment resulted in a significant reduction in the growth of psychrotrophic bacteria in cat-fish fillets but not in shrimp in comparison with the controls over 12 days of storage at 4 ° C.

3.2.3. Pseudomonas count—Counts of *Pseudomonas* spp. (Fig. 3) are slightly lower than the APCs, indicating the importance of these species in the spoilage of fresh salmon examined. Rasmussen, Ross, Olley, and McMeekin (2002) revealed that the shelf life of the aerobically stored Atlantic salmon (*S. salar*) fillets was governed by the growth of *Pseudomonas*, which is the specific spoilage organism identified in this species. Indeed, the microbial population of fish stored aerobically under chilling condition consists almost exclusively of *Pseudomonas* spp. and H_2S-producing bacteria (presumably, *Shewanella putrefaciens*) (Gram & Huss, 1996). These two bacterial groups have been reported to be the specific spoilage microorganisms in various fish species; including sea bass (Papadopoulos, Chouliara, Badeka, Savvaidis, & Kontominas, 2003), sea salmon (Hozbor et al., 2006), rainbow trout (*Onchorhynchus mykiss*) (Chytiri, Chouliara, Savvaidis, & Kontominas, 2004), and common octopus (*Octopus vulgaris*) (Vaz-Pires & Barbosa, 2004) harvested from different arctic, temperate, and tropical waters.

The initial *Pseudomonas* count ($\log_{10}$ CFU/g) in this study was ranged from 2.86 in NaA-treated salmon slices to 3.2 in control samples, whereas by the end of storage period, *Pseudomonas* counts in the different treated samples (6.08, 6.36, and 6.68 for salmon treated with NaA, NaL, and NaC, respectively) were in excess of 2-logs lower than that in the control (8.73).

It was clear that all of the different organic salt treatments resulted in significant reduction ($P < 0.05$) in the populations of *Pseudomonas* in sliced salmon. That is, while for the control samples it took 9 days to approach a population of 7 $\log_{10}$ CFU/g, all of the different treated samples never reached this population during the 15-days storage period (Fig. 3).

3.2.4. Hydrogen sulfide-producing bacteria—Interestingly, counts of the black colonies, which represent the H_2S-producing bacteria (Fig. 4), in the control as well as in all of the different treated samples were lower than those of the *Pseudomonas* spp. throughout the entire period of storage, and by the end of the storage (day 15), the count ($\log_{10}$ CFU/g) of H_2S-producing bacteria detected for the control non-treated salmon was 1.4-logs lower than that of the *Pseudomonas* spp. (7.33 versus 8.73) in the corresponding samples. This result is consistent with those reported for another fish species during storage at 2 °C (Chytiri et al., 2004;Papadopoulos et al., 2003). On the contrary, lower count of 4.65 $\log_{10}$ CFU/g has been reported for H_2S-producing bacteria (including *S. putrefaciens*) in ice-stored European sea bass (*Dicentrarchus labrax*) at the time of spoilage (day 15) (Kyrana & Lougovois, 2002).

The development of specific spoilage bacteria in a fish ecosystem is a result of both environmental conditions and microbial competition. In fish packed aerobically competition occurs between members of an aerobic Gram-negative flora; mainly *Pseudomonas* spp. and *S. putrefaciens*. The low count of H_2S-producing bacteria might be the result of inhibition by *Pseudomonas* spp. Actually, it was reported that *Pseudomonas* spp. can inhibit the growth of H_2S-producing bacteria (including *S. putrefaciens*) because of the ability of the former to produce siderophores, and this interaction can be the major factor governing the development of spoilage flora (Gram & Melchiorsen, 1996).

Salmon slices treated with the different organic salts contained lower count of H_2S-producing bacteria throughout the storage period when compared with the control, and by the end of the storage, a significant ($P < 0.05$) reduction was detected in H_2S-producing bacterial count in all of the different treated samples (5.23–5.98) in comparison with that of the control (7.33).

Complete inhibition of H_2S-producing bacteria has been reported in fresh cod fillets sprayed with 10% acetate buffer and stored under modified atmospheres for 12 days at 7 °C (Boskou & Debevere, 2000).

All of the 60 selected isolates of H_2S-producing microorganisms analyzed in this study were Gram-negative, motile, oxidase and catalase positive, and able to reduce TMAO. Fifty-three (88.3%) out of these 60 isolates were non-fermentative, ornithine decarboxylase positive, able to grow at 4 °C, and in the presence of 6.5% NaCl. Of the non-fermentative, 39 isolates (73.6%) were identified as *S. putrefaciens*, indicating that this species is the most common sulfide-producer organism. This finding is in accordance with those reported for salmon fillets packed under modified atmosphere and stored at 2 °C (Emborg et al., 2002) and for whole sea salmon stored in ice under aerobic condition at 0 °C (Hozbor et al., 2006).

3.2.5. Lactic acid bacteria—The count of LAB was lower than the other bacterial counts (except for the *Enterobacteriaceae*) determined in this study at the time of spoilage. The initial count ($\log_{10}$ CFU/g) of LAB was ranged from 2.33 in NaC-treated samples to 2.62 in the control (Fig. 5). A final count of 5.23, however, was reached in control samples at the end of storage period (day 15), whereas samples treated with NaA or NaL did not attained significant reduction in LAB count ($P > 0.05$) although they contained 0.85 and 0.7-logs, respectively lower than the control. On the contrary, a significant ($P < 0.05$) reduction in LAB count was realized in NaC-treated samples when compared with the control (4.08 versus 5.23). This result is in agreement with that of Lee et al. (2002), who claimed that citrate is an active agent against Gram-positive microorganisms.

The low LAB count in this study was expected since lactic acid bacteria tend to grow slowly at refrigeration temperatures and are under aerobic condition generally out-competed by pseudomonas (Huis in't Veld, 1996). In contrast, the contribution of LAB as the major spoiling microorganisms had been reported in fresh vacuum-packed Atlantic salmon portions stored at 4 °C (Rasmussen et al., 2002).

3.2.6. Enterobacteriaceae—*Enterobacteriaceae* were also found to be members of the microbial association implicated in the spoilage of fresh sliced salmon during refrigerated storage (Fig. 6). This finding is in agreement with results reported for different fish species, including fresh Atlantic salmon (Amanatidou et al., 2000), sea bass (Papadopoulos et al., 2003), rainbow trout (Chytiri et al., 2004), as well as common octopus (Vaz-Pires & Barbosa, 2004), in which *Enterobacteriaceae* were determined as a part of the microbial flora at the end of the product shelf life under refrigerated storage.

The growth of *Enterobacteriaceae* was slower than that of the other microbial groups, starting by less than 2 $\log_{10}$ CFU/g and never exceeding 4 $\log_{10}$ CFU/g in the control samples. By the end of the storage (day 15), however, much lower ($P < 0.05$) counts of 1.2, 1.63, and 2.7 $\log_{10}$ CFU/g were achieved in salmon slices treated with NaA, NaL, and NaC, respectively when compared with control (Fig. 6). On the other hand, complete inhibition of *Enterobactericeae* has been reported in fresh cod fillets sprayed with 10% acetate buffer during storage under modified atmospheres at 7 °C (Boskou & Debevere, 2000).

Although, *Enterobacteriaceae* can grow at low temperatures, their proliferation was slow during refrigerated storage, possibly because their growth rate is lower than that of other Gram-negative psychrotrophic spoilers (Papadopoulos et al., 2003). Nevertheless, the spoilage potential of *Enterobacteriaceae* must be taken into consideration especially in the case of polluted water or delay in chilling after fish catch.

On the basis of the microbiological analyses reported in this study, the general order of antibacterial activity of the different organic salts used for treatment of fresh sliced salmon was; sodium acetate > sodium lactate > sodium citrate. The antimicrobial activity of NaA, NaL, and NaC is attributed to both the metabolic inhibition by the undissociated acetic, lactic and citric acid molecules and to the depression of pH below the growth range of many bacteria (Jay, 2000). Undissociated organic acids were thought to freely permeate the bacterial membrane, where it release toxic acid anions and protons intracellularly, and causing acidification of the cytoplasm along with dissipation of the transmembrane proton potential (Eklund, 1983).

3.3. Lipid oxidation

Fatty fish are, of course, particularly vulnerable to lipid oxidation which can create severe quality problems such as unpleasant (rancid) taste and smell, and also it may produce alterations in texture, color, and nutritional value, even on storage at subzero temperatures (Huss, 1995;Ólafsdóttir et al., 1997). The various reactions involved in the lipid oxidation are either non-enzymatic or catalyzed by microbial enzymes or by intracellular or digestive enzymes from the fish themselves. The relative significance of these reactions, therefore, mainly depends on fish species and storage temperature (Huss, 1995).

The peroxide value is the most common measure of lipid hydroperoxides; it also called primary lipid oxidation products (Ólafsdóttir et al., 1997). The initial PV (meq peroxide/kg fish sample) in the sliced salmon analyzed was ranged from 1.12 to 1.23. No difference ($P > 0.05$) has been observed throughout the first 9 days of the storage among the different samples. By the end of the storage time, however, significant differences ($P < 0.05$) were observed in the PV between the control (4.23) and each of NaC- and NaA-treated samples, which exhibited lower values of 2.43 and 3.14, respectively (Fig. 7). PV of NaC-treated samples was also significantly lower than that of NaL-treated samples. Storage time has a significant effect on the PV for each of the control and treated samples, nonetheless the PV in all samples were well below the proposed acceptable level of 10–20 meq peroxide/kg fish fat (Huss, 1995). Peroxide values reported in the current study are in accordance with those reported in farmed Coho salmon (*Oncorhynchus kisutch*) during 17-days storage in ice at 2 °C (Losada, Gómez, Maier, Vinagre, & Larraín, 2004).

TBA assay is a widely used indicator for the assessment of degree of lipid oxidation. The result of TBA assay corroborated that obtained by the PV. The initial TBA values (mg MA per kg of fish sample) were ranged from 0.34 in NaC-treated salmon to 0.39 in NaL-treated samples. TBA values of the control and treated salmon slices were significantly increased ($P < 0.05$) with the storage time; and by the end of the storage period (day 15), however, NaC-treated samples achieved significant ($P < 0.05$) lower TBA value of 0.82 in comparison with the control or NaL-treated samples, which attained a higher levels of 1.73 and 1.41, respectively (Fig. 8). Also, NaA-treated samples showed significantly ($P < 0.05$) lower TBA value when compared with the control (1.03 versus 1.73). Significant effect of storage time on TBA values had been verified in control and sodium lactate (2%) treated catfish fillets after 8-days storage at 1 °C (Williams et al., 1995).

Dipping of sliced salmon in NaL did not produce any significant reduction in the PV or TBA value ($P > 0.05$) in comparison with the control. Conversely, Williams et al. (1995) demonstrated a significantly lower TBA value for catfish fillets treated with 2% sodium lactate after 2 days and through 8 days storage at 1 °C as compared with the control. It is possible that effects of NaL on lipid oxidation in fish products may be dependent on a variety of factors including the extent of microbial growth, packaging method, and storage time.

It has been proposed that the maximum level of TBA value indicating the good quality of the fish (frozen, chilled or stored with ice) is 5 mg malonaldehyde/kg, while the fish may be consumed up to the level of 8 mg malonaldehyde/ kg (Schormüller, 1969). In the current study, TBA values for control and treated sliced salmon samples analyzed were much lower than such proposed limit throughout the 15-days storage period. On the contrary, much higher TBA values were recorded in whole (16.21 μg/g) and filleted (19.41 μg/g) rainbow trout after 18 days of storage in ice at 2 ± 0.5 °C (Chytiri et al., 2004), and also in different Mediterranean fatty fish species (7.43–20.98 mg MA/ kg) by the day 6 of ice storage in a cold room kept at 1–3 °C (Simeonidou, Govaris, & Vareltzis, 1998). However, much lower TBA ranges were measured in whole (1.52– 4.48 μg/kg) and gutted (1.35–7.31 μg/kg) sea bass stored in ice at 2 ± 2 °C for 16 days (Papadopoulos et al., 2003).

The relatively low increase in the PV and TBA values for both of the control and treated samples in this study indicated that salmon lipids are somewhat stable during the refrigerated storage when compared to other fatty fishes like sardines, mackerel and rainbow trout (Aubourg, 2001;Chytiri et al., 2004;Simeonidou et al., 1998). The results of PV and TBA values of the present work suggesting that NaC has a potent antioxidant effect more than NaA, while NaL provoked the lowest effect.

4. Conclusion

The current study concluded that dipping of salmon slices in aqueous solutions (2.5%) of the sodium salts of organic acids; namely sodium acetate, sodium lactate, and sodium citrate was efficient against the proliferation of various categories of spoilage microorganisms, as it delayed lipid oxidation and extended the shelf life of the product during refrigerated storage; therefore sodium acetate, sodium lactate, and sodium citrate can be utilized as safe organic preservatives for fish under refrigerated storage.

References

Amanatidou A, Schlüter O, Lemkau K, Gorris LGM, Smid EJ, Knorr D. Effect of combined application of high pressure treatment and modified atmospheres on the shelf life of fresh Atlantic salmon. Innovative Food Science & Emerging Technologies 2000;1:87–98.

Anders, RJ.; Cerveny, JG.; Milkowski, AL. United States Patent: 4,888,191. Oscar Mayer Foods Corporation; Madison, WI, USA: 1989. Method for delaying *Clostridium botulinum* growth in fish and poultry. Appl. No. 287252 (19881220)

Cunni, P., editor. AOAC International. Official methods of analysis of AOAC international. 16th ed. Gaithersburg, MD, USA: AOAC International; 1999.

Vanderzant, C.; Splittsloesser, DF., editors. APHA (American Public Health Association). Compendium of methods for the microbiological examination of foods. 3rd ed. Washington, DC: APHA; 1992.

Ashie INA, Smith JP, Simpson BK. Spoilage and shelf life extension of fresh fish/shellfish. Critical Reviews in Food Science and Nutrition 1996;36:87–122. [PubMed: 8747101]

Aubourg S. Damage detection in horse mackerel (*Trachurus trachurus*) during chilled storage. Journal of the American Oil Chemists' Society (JAOCS) 2001;78:857–862.

Boskou G, Debevere J. Shelf life extension of cod fillets with an acetate buffer spray prior to packaging under modified atmosphere. Food Additives and Contaminants 2000;17:17–25. [PubMed: 10793851]

Chytiri S, Chouliara I, Savvaidis IN, Kontominas MG. Microbiological, chemical, and sensory assessment of iced whole and filleted aquacultured rainbow trout. Food Microbiology 2004;21:157–165.

Cousin, MA.; Jay, JM.; Vasavada, PC. Psychrotrophic microorganisms. In: Vanderzand, C.; Splittstoesser, DF., editors. Compendium of methods for the microbiological examination of foods. 3rd ed. Washington, DC: American Public Health Association; 1992. p. 153-168.

Eklund T. The antimicrobial effect of dissociated and undissociated sorbic acid at different pH levels. Journal of Applied Microbiology 1983;54:383–389.

Emborg J, Laursen BG, Rathjen T, Dalgaard P. Microbial spoilage and formation of biogenic amines in fresh and thawed modified atmosphere-packed salmon (*Salmo salar*) at 2 °C. Journal of Applied Microbiology 2002;92:790–799. [PubMed: 11966922]

Flick, GJ.; Martin, RE., editors. Advances in seafood biochemistry, composition and quality. Lancaster, PA, USA: Technomic Publishing; 1992.

González-Fandos E, Villarino-Rodríguez A, García-Linares MC, García-Arias MT, García-Fernández MC. Microbiological safety and sensory characteristics of salmon slices processed by the sous vide method. Food Control 2005;16:77–85.

Gram L, Dalgaard P. Fish spoilage bacteria – problems and solutions. Current Opinion in Biotechnology 2002;13:262–266. [PubMed: 12180103]

Gram L, Huss HH. Microbiological spoilage of fish and fish products. International Journal of Food Microbiology 1996;33:121–137. [PubMed: 8913813]

Gram L, Melchiorsen J. Interaction between fish spoilage bacteria *Pseudomonas* spp. and *S. putrefaciens* in fish extracts and on fish tissue. Journal of Applied Bacteriology 1996;80:589–595. [PubMed: 8698659]

Gram L, Trolle G, Huss HH. Detection of specific spoilage bacteria from fish stored at low (0 °C) and high (20 °C) temperatures. International Journal of Food Microbiology 1987;4:65–72.

Hozbor MC, Saiz AI, Yeannes MI, Fritz R. Microbiological changes and its correlation with quality indices during aerobic iced storage of sea salmon (*Pseudopercis semifasciata*). LWT-Food Science and Technology 2006;39:99–104.

Huis in't Veld JHJ. Microbial and biochemical spoilage of foods: an overview. International Journal of Food Microbiology 1996;33:1–18. [PubMed: 8913806]

Huss, HH. FAO Fisheries Technical Paper No. 348. Food and Agriculture Organization (FAO) of the United Nations; Rome, Italy: 1995. Quality and quality changes in fresh fish.

ICMSF ''International Commission on Microbiological Specification for Foods''. Microorganisms in foods. 1. Their significance and methods of enumeration. 2nd ed. Toronto: University of Toronto Press; 1978.

ICMSF ''International Commission on Microbiological Specification for Foods''. Microorganisms in foods. 2. Sampling for microbiological analysis: principles and specific applications. 2nd ed. Buffalo, NY: University of Toronto Press; 1986.

Jay, JM. Modern food microbiology. 6th ed. Jay, James M., editor. Gaithersburg, MA, USA: Aspen Publishers; 2000. p. 268

Kyrana VR, Lougovois VP. Sensory, chemical and microbiological assessment of farm-raised European sea bass (*Dicentrarchus labrax*) stored in melting ice. International Journal of Food Science and Technology 2002;37:319–328.

Lee YL, Cesario T, Owens J, Shanbrom E, Thrupp LD. Antibacterial activity of citrate and acetate. Nutrition 2002;18:665–666. [PubMed: 12093451]

Losada, V.; Gómez, J.; Maier, L.; Marín, Ma E.; Vinagre, J.; Larraín; Ma, A., et al. Lipid damage assessment during Coho salmon (*Oncorhynchus kisutch*) chilled storage. 34th WEFTA meeting; 12–15 September 2004; Lübeck, Germany. 2004.

Maca JV, Miller RK, Acuff GR. Microbiological, sensory and chemical characteristics of vacuum-packaged ground beef patties treated with salts of organic acids. Journal of Food Science 1997;62:591–596.

McWilliam Leitch EC, Stewart CS. Susceptibility of *Escherichia coli* O157 and non-O157 isolates to lactate. Letters in Applied Microbiology 2002;35:176–180. [PubMed: 12180936]

Nettleton, JA. Omega-3 fatty acids and health. New York, USA: Chapman & Hall; 1995.

Nykänen A, Lapveteläinen A, Kallio H, Salminen S. Effects of whey, whey-derived lactic acid and sodium lactate on the surface microbial counts of rainbow trout packed in vacuum pouches. Lebensmittel-Wissenschaft und-Technologie/LWT-Food Science and Technology 1998;31:361–365.

Ólafsdóttir G, Martinsdóttir E, Oehlenschläger J, Dalgaard P, Jensen B, Undeland I, et al. Methods to evaluate fish freshness in research and industry. Trends in Food Science & Technology 1997;8:258–265.

Pacheco-Aguilar R, Lugo-Sanchez ME, Robles-Burgueno MR. Post-mortem biochemical and functional characteristic of Monterey sardine muscle stored at 0 °C. Journal of Food Science 2000;65:40–47.

Papadopoulos V, Chouliara I, Badeka A, Savvaidis IN, Kontominas MG. Effect of gutting on microbiological, chemical, and sensory properties of aquacultured sea bass (*Dicentrarchus labrax*) stored in ice. Food Microbiology 2003;20:411–420.

Qvist S, Sehested K, Zeuthen P. Growth suppression of *Listeria monocytogenes* in a meat product. International Journal of Food Microbiology 1994;24:283–293. [PubMed: 7703021]

Rasmussen SKJ, Ross T, Olley J, McMeekin T. A process risk model for the shelf life of Atlantic salmon fillets. International Journal of Food Microbiology 2002;73:47–60. [PubMed: 11885573]

Sallam, KhI; Samejima, K. Microbiological and chemical quality of ground beef treated with sodium lactate and sodium chloride during refrigerated storage. Lebensmittel-Wissenschaft und-Technologie/ LWT-Food Science and Technology 2004;37:865–871.

SAS. SAS/STAT user's guide. Cary, NC, USA: Statistical Analysis System Institute; 1990.

Schmedes A, Holmer G. A new thiobarbituric acid (TBA) method for determination of free malonaldehyde (MDA) and hydroperoxides selectivity as a measure of lipid peroxidation. Journal of American Oil Chemistry Society 1989;66:813–817.

Schormüller, J. Handbuch der lebensmittelchemie (Band IV). Berlin, Heidelberg: Springer-Verlag; 1969.

Simeonidou S, Govaris A, Vareltzis K. Quality assessment of seven Mediterranean fish species during storage on ice. Food Research International 1998;30:479–484.

Truelstrup Hansen L, Røntved SD, Huss HH. Microbiological quality and shelf life of cold-smoked salmon from three different processing plants. Food Microbiology 1998;15:137–150.

USDA. Fish and shellfish. Agricultural Handbook number 8. US Government Printing Office; Washington, DC: 1987. Composition of foods, 15.

Vaz-Pires P, Barbosa A. Sensory, microbiological, physical and nutritional properties of iced whole common octopus (*Octopus vulgaris*). Lebensmittel-Wissenschaft und-Technologie/LWT-Food Science and Technology 2004;37:105–114.

Williams SK, Phillips K. Sodium lactate affects sensory and objective characteristics of tray-packed broiler chicken breast meat. Poultry Science 1998;77:765–769.

Williams SK, Rodrick GE, West RL. Sodium lactate affects shelf life and consumer acceptance of fresh catfish (*Ictalurus nebulosus, marmoratus*) under stimulated retail conditions. Journal of Food Science 1995;60:636–639.

Zhuang RY, Huang YW, Beuchat LR. Quality changes during refrigerated storage of packaged shrimp and catfish fillets treated with sodium acetate, sodium lactate or propyl gallate. Journal of Food Science 1996;61:241–244. 261–261.

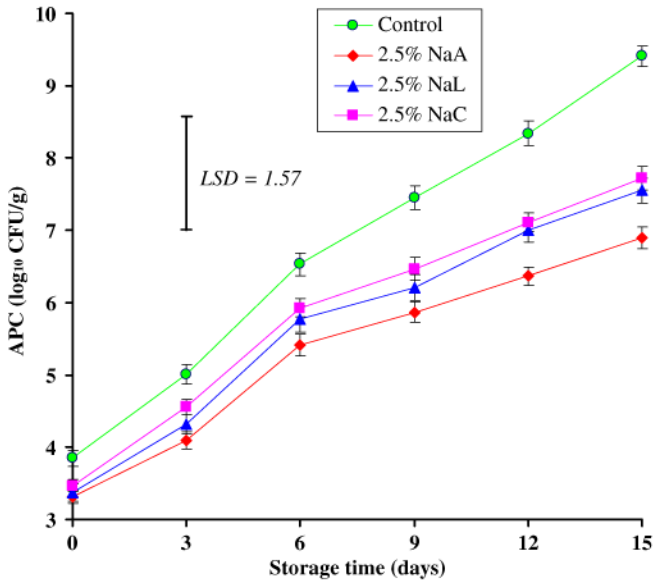

Fig 1.

Effects of sodium acetate (NaA), sodium lactate (NaL), and sodium citrate (NaC) treatments on aerobic plate counts (APC) of sliced salmon during storage at 1 °C. Values represent means ± *SE* of three replicates; *LSD* is defined at $P < 0.05$.

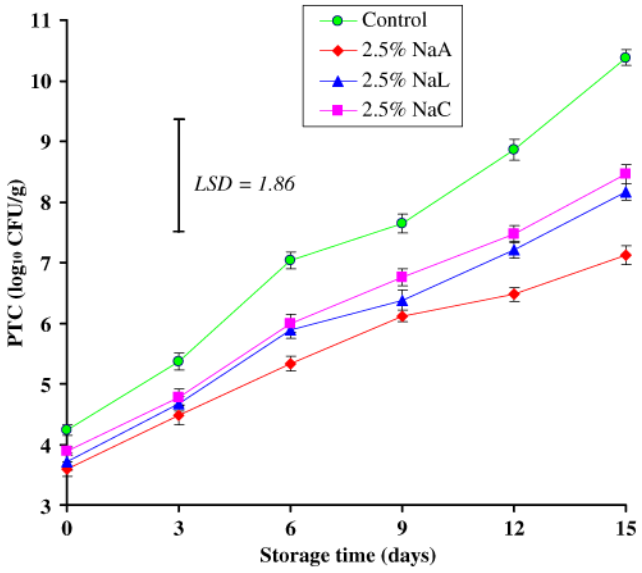

Fig 2.

Effects of sodium acetate (NaA), sodium lactate (NaL), and sodium citrate (NaC) treatments on psychrotrophic counts (PTC) of sliced salmon during storage at 1 °C. Values represent means ± *SE* of three replicates; *LSD* is defined at $P < 0.05$.

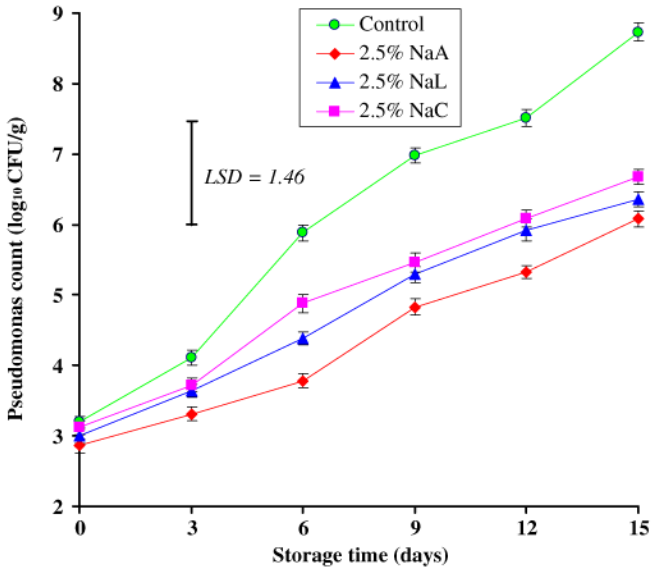

Fig 3.

Effects of sodium acetate (NaA), sodium lactate (NaL), and sodium citrate (NaC) treatments on Pseudomonas counts of sliced salmon during storage at 1 °C. Values represent means ± *SE* of three replicates; *LSD* is defined at $P < 0.05$.

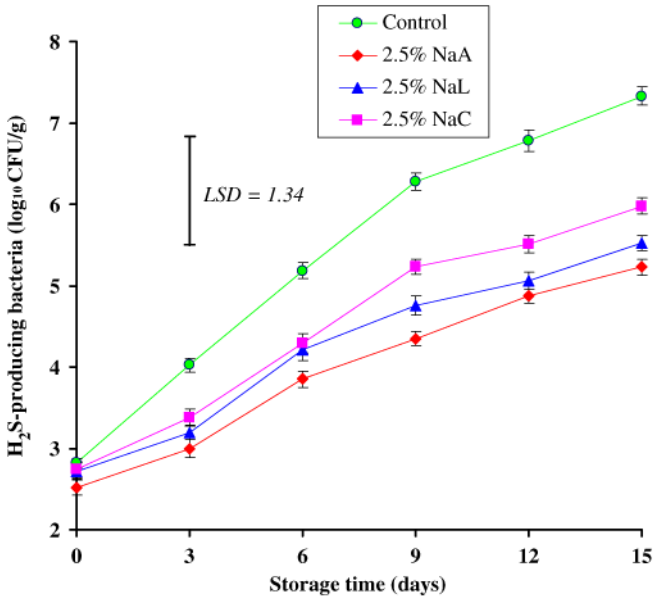

Fig 4.

Effects of sodium acetate (NaA), sodium lactate (NaL), and sodium citrate (NaC) treatments on H_2S-producing bacteria of sliced salmon during storage at 1 °C. Values represent means ± *SE* of three replicates; *LSD* is defined at $P < 0.05$.

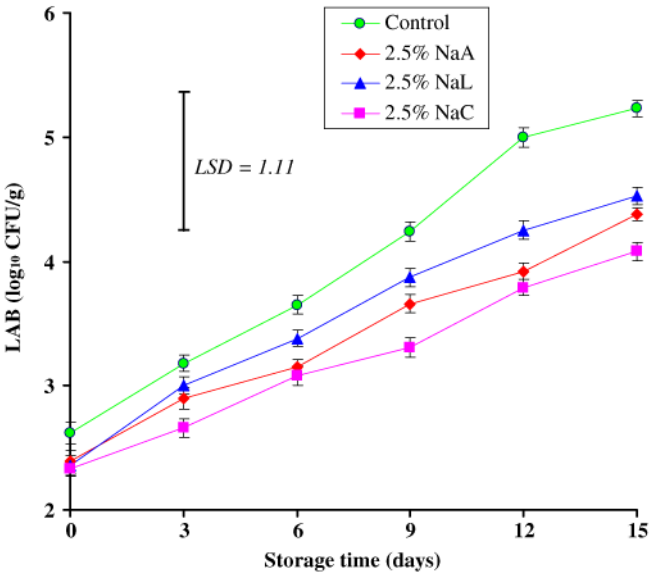

Fig 5.

Effects of sodium acetate (NaA), sodium lactate (NaL), and sodium citrate (NaC) treatments on lactic acid bacteria (LAB) of sliced salmon during storage at 1 °C. Values represent means ± *SE* of three replicates; *LSD* is defined at $P < 0.05$.

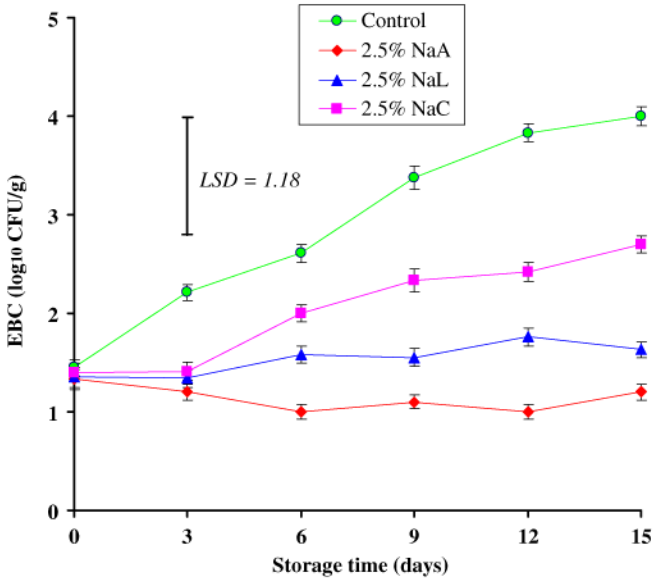

Fig 6.

Effects of sodium acetate (NaA), sodium lactate (NaL), and sodium citrate (NaC) treatments on *Enterobactericeae* counts of sliced salmon during storage at 1 °C. Values represent means ± *SE* of three replicates; *LSD* is defined at $P < 0.05$.

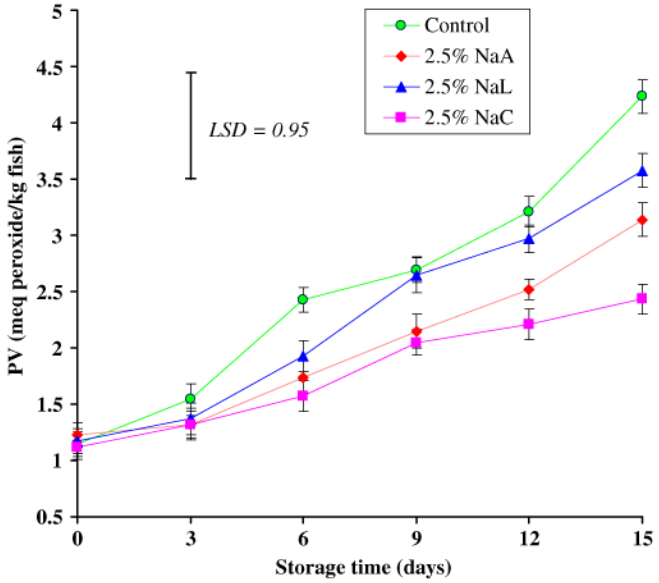

Fig 7.

Effects of sodium acetate (NaA), sodium lactate (NaL), and sodium citrate (NaC) treatments on lipid oxidation (peroxide value; PV) in sliced salmon during storage at 1 °C. Values represent means ± *SE* of three replicates; *LSD* is defined at $P < 0.05$.

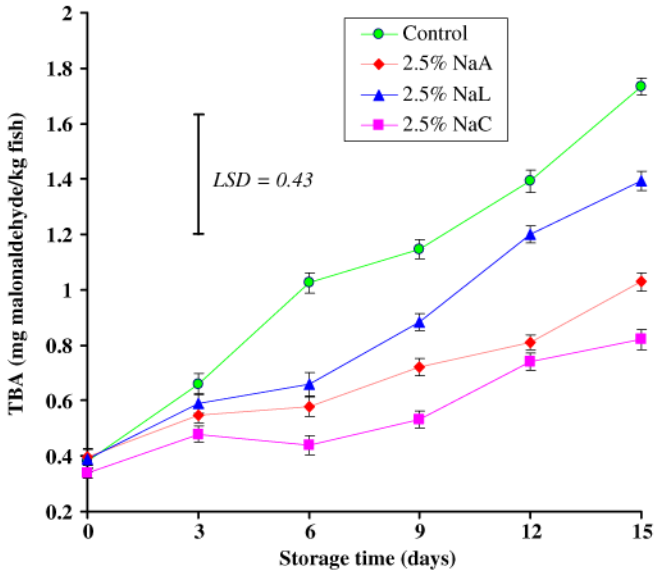

Fig 8.

Effects of sodium acetate (NaA), sodium lactate (NaL), and sodium citrate (NaC) treatments on lipid oxidation (TBA value) in sliced salmon during storage at 1 °C. Values represent means ± *SE* of three replicates; *LSD* is defined at $P < 0.05$.