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Effect of Combined Treatment with Electrolyzed NaCl Solutions and Essential Oil Compounds on the Quality of Salmon Fillets During Cold Storage

Mohamed Abou-Taleb

Laboratory of Fish Processing and Technology,
National Institute of Oceanography and Fisheries, 101 El-Kasr El-Eini, Cairo, Egypt

Abstract: Electrolyzed products of sodium chloride solution and essential oil compounds were used as pre-treatment to extend the shelf-life of salmon fillets. Salmon fillets were treated with electrolyzed NaCl solutions [cathodic solution, EW(-) and/or anodic solution, EW(+)][EW(-/+)], 1% oil (0.5%menthol+ 0.5%borneol) [1%(M+B)] and together [EW(-/+)/1%(M+B)]. Chemical [volatile basic nitrogen, trimethylamine nitrogen, peroxide value and thiobarbituric acid], microbiological [total viable count, psychrotrophic count, *Pseudomonas* count and *Enterobacteriaceae* count] and sensory analyses were used to evaluate the effect of this treatments during storage at 5°C. Significant reduction in VB-N and TMA-N of salmon fillets ($p \leq 0.05$) was recorded immediately after treatment with EW(-/+)/1%(M+B). Values of VB-N remained below the acceptable limit until the end of storage period (day 15). Treatment with EW(-/+)/1%(M+B) significantly suppressed the lipid oxidation compared with other treatments. According to the microbiological assay, the shelf-life of salmon fillets treated with EW(-/+)/1%(M+B) was extended to 13 days during storage at 5°C compared with 5 days for the control samples.

Key words: Antimicrobial · Antioxidant · Menthol (M) · Borneol (B) · Shelf-life · Salmon fillets

INTRODUCTION

Fish and shellfish are important protein sources for human consumption; in addition they have 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 [1]. During storage, the quality of fish degrades due to a complex process in which physical, chemical and microbiological forms of deterioration are implicated [2]. Enzymatic and chemical reactions are usually responsible for the initial loss of freshness whereas microbial activity is responsible for the obvious spoilage and thereby establishes product shelf life [3]. 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 [4]. Fatty fishes are a good source of omega-3 polyunsaturated fatty acids (PUFA) such as eicosapentaenoic acid (EPA; 20-5; n- 3) and docosahexaenoic acid (DHA; 22-6; n-3). The International Society for the Study of Fatty Acids and Lipids (ISSFAL) has made recommendations of aminimumintake of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) of 0.5 g/day for prevention of cardiovascular diseases [5]. An increased level of these PUFAs in the food is believed to reduce the risk and development of cardiovascular diseases [6, 7] and rheumatoid arthritis [8].

Fresh seafood has a short shelf-life, which causes substantial practical problems for its distribution. Improvements in the shelf-life of a product can have an important economic impact by reducing losses attributed to spoilage and by allowing the products to reach distant and new markets [9]. Essential oils have natural antimicrobial properties with the potential to extend the shelf-life of food when used alone or in combination with other preservation techniques [10]. Essential oils have

Corresponding Author: Dr. Mohamed Abou-Taleb, Laboratory of Fish Processing and Technology, National Institute of Oceanography and Fisheries, 101 El-Kasr El-Eini, Cairo, Egypt

been defined as the products obtained from raw material by water or steam distillation or, in the case of citrus fruits by mechanical processes [11]. In practice, four methods can be used: steam distillation applied on fresh or dried material, hydrodistillation with addition of water to the plant material, squeezing the citrus pericarp and pyrogenation of the crust or the wood of some plant materials [12]. Constituents of essential oils such as benzaldehyde, carvacrol, carvone, 1,8-cineole, cinnamaldehyde, citral, cuminaldehyde, cymene, estragole, eugenol, geranyl acetate, geraniol, isoeugenol, limonene, menthol, perillaldehyde, a-pinene, salicylaldehyde, terpineol, thymol and vanillin have been evaluated for their effects on the growth of food spoilage and foodborne pathogenic microorganisms, including Gram-positive, Gram-negative bacteria and fungi [13-17].

Electrolysed NaCl solutions (EW) have attracted much recent attention as a high-performance, new technology for potential use in the food industry. The advantage of EW is that it can be easily generated at the point of use and it does not require the transportation, storage or mixing of chemicals. It is also very cost-effective, as water and salt are the only ingredients. Therefore, it is nontoxic, environmental friendly and not harmful to humans [18]. Two solutions are produced during the electrolysis of aqueous NaCl: anodic solution [EW (+)] containing hypochlorous acid (HOCl) and O_2; and cathodic solution [EW (-)] containing NaOH, H_2, H_2O_2 and O_2 [19, 20]. EW (+) has been reported to have strong bactericidal effects in the food industry [21-23]. Electrolyzed oxidizing (EO) water is a relatively new disinfecting compound that has shown promise against cell suspensions of *Escherichia coli* (EC) O157:H7, *Salmonella enteritidis* and *Listeria monocytogenes* [24], *E. coli* O157:H7 and *L. monocytogenes* attached to cutting boards [25].

The main objective of this study is to investigate the antimicrobial and antioxidant effects of electrolyzed solutions EW(-/+) and essential oil compounds (menthol + borneol) on the quality of salmon fillets during cold storage at 5°C.

MATERIALS AND METHODS

Salmon Fish: Pacific salmon (*Onchorhynchus nerka*) were purchased from a local market, Hokkaido, Japan. Gutting and skinless filleting were done as soon as the fish arrived in the laboratory.

Chemicals: Menthol (M) and borneol (B), 98% purity, were obtained from KANTO Chemical Co., Inc. (Tokyo, Japan).

Preparation of electrolyzed NaCl solutions: Electrolyzed NaCl solutions were prepared by using a two-compartment batch-scale electrolysis apparatus (Super Oxseed Labo, Aoi Electronic Corp., Kannami, Shizuoka, Japan). Diluted NaCl solutions were prepared by dissolving 0.1% NaCl in deionised water. The voltage was automatically maintained between 11 and 12 V of direct current. After electrolysis for 10 min, the anodic solution, EW(+), with a pH of 2.22±0.03, 40.8±0.05 ppm of available chlorine and ORP + 1137 mV and cathodic solution, EW(-), with a pH of 11.6 ± 0.07 and ORP-800 mV, were prepared in the anode and cathode compartments, respectively. Both solutions were prepared immediately before use. The available chlorine concentration was measured by electrotitration, using an available-chlorine meter (type HC-30, Central Kagaku Co. Inc., Tokyo, Japan). The oxidation/ reduction potential and pH were measured by an ORP tester (ML-300; SUDO, Tokyo, Japan) and a pH meter (D-14; Horiba, Tokyo, Japan), respectively.

Treatment of salmon fillets: Four samples of skinless salmon fillets were used to assess the antioxidant and antimicrobial effects of EW solutions and/or 1% (0.5%M + 0.5%B) compounds. The samples were dipped for 15 min in: (i) a 100-fold volume of sterile 0.2% agar solution (control treatment) according to [26]; (ii) EW(-), followed by EW(+) [EW(-)/EW (+)]; (iii) 1%(menthol + borneol) [1%(M + B)]; or (vi) EW(-)/EW(+), followed by 1%(M+B) [EW(-)/EW(+)/1% M + B]. These treatments were carried out in a sterile 3 l flask with gentle shaking (100 rpm) in a multi-shaker (MMS, Tokyo Rikakikai, Co., LTD, Tokyo, Japan) at room temperature (25°C). After treatment, samples were allowed to drip for 1 min. Samples were separately packaged under aseptic conditions in polyethylene bags and stored at 5°C±1. The chemical, sensory and microbiological analyses were carried out periodically on days 0, 3, 6, 9, 12 and 15 after treatment.

Determination of volatile basic nitrogen (VB-N): VB-N associated with fish fillets spoilage was determined according to the method of Conway [27].

Determination of Trimethylamine-nitrogen (TMA-N): Trimethylamine-nitrogen (TMA-N) was determined as described in A.O.A.C.[28].

Peroxide value: Samples of 0.5 g were mixed with 25 ml of a solution of glacial acetic acid and chloroform (ratio 3:2) in a conical flask and then 1 ml of saturated potassium iodide was added. The mixture was kept in the dark for about 10 min and then 30 ml of distilled water and 1 ml of freshly prepared 1% starch were added. After hand shaking, the samples were titrated with 0.01 M sodium thiosulfate. The peroxide values were expressed in units of meq/kg of sample [29].

Thiobarbituric acid (TBA) test: TBA values were determined spectrophotometrically according to the procedure described by Siu and Draper [30]. Ten grams of salmon fillets were homogenized in 25 ml of distilled water for 2 min and then mixed with 25 ml of 10% trichloroacetic acid (TCA). The mixture was mixed and filtered and then 1 ml of 0.06 M thiobarbituric acid was added to 4 ml aliquots of the filtrate and heated in a boiling water bath (10 min) for color development. The absorbance at 532 nm was measured with a Hitachi U- 2000 spectrophotometer (Hitachi Ltd, Tokyo, Japan). The TBA values of antioxidant-treated fillets were compared to those of control fillets. The TBA values were expressed in units of mg malonaldehyde/kg (mg MDA/kg) sample.

Organoleptic evaluation of salmon fillets: The organoleptic properties of salmon fillets during storage at 5 ± 1 °C were assessed by a group of 10 trained panelists from the staff of the Laboratory of Marine Products and Food Science, Faculty of Fisheries Sciences, Hokkaido University. Samples were frying in an electrical fryer pan using sunflower oil at 150 °C for 5 min before evaluation. The panelists were asked to evaluate several parameters (color, odor, texture, taste, flavor and overall acceptability) for the food samples according to 9-point hedonic scale indicating decreased freshness [31]. A general freshness score was calculated as the average of all grades. A freshness score of more than five was taken to indicate acceptability of the fillets samples. The data from the ten independent panelists were pooled and statistically analyzed.

Total aerobic plate count: Five grams of salmon fillet were homogenized for 1 min at room temperature in 45 ml of sterilized 0.9% NaCl saline using a stomacher 80 Lab-blender (Seward, London, UK). Serial decimal dilutions were prepared in 0.9% NaCl saline solution. Spreading plate technique was applied to determine the total aerobic count on plate count agar (Difco, Spark, MD). The cfu were enumerated after incubation at 20 °C for 48 h. The experiment was carried out three times in duplicate.

Psychrotrophic count: Psychrotrophic count (PTCs) was determined in a similar method to that for APC except that plates were incubated at 7 °C for 10 days [32].

Pseudomonas count: *Pseudomonas* counts was 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. The cfu were counted after incubation at 25 °C for 2 days.

***Enterobacteriaceae* count:** Enterobacteriaceae counts (EBC) were enumerated by the pour plate technique on Violet Red Bile Glucose Agar (VRBGA; Difco, Detroit, Michigan, USA). The plates were overlaid with a virgin layer of the same growth medium and then incubated at 37 °C for 24 h [33].

Statistical analysis: For each treatment, data from three independent replicate trials were pooled and the mean values and standard deviations were determined. Differences between samples were determined by t-test and were considered to be significant when $p \leq 0.05$ [34].

RESULTS AND DISCUSSION

Changes in the total volatile basic nitrogen (VB-N) and trimethylamine nitrogen (TMA-N) of salmon fillets during storage at 5°C: Different Chemical indicators, such as total volatile basic nitrogen (TVB-N) and trimethylamine nitrogen (TMA-N) have been used as indices to assess the spoilage of refrigerated fish [35]. Changes in the total volatile basic nitrogen (VB-N) of the salmon fillets during storage at 5 °C are shown in Table 1. The initial VB-N values of the control and samples treated with EW(-/+), 1%(M+B) and EW(-/+)/1%(M+B) were 12.85±0.43, 8.47±0.37, 8.42±0.24 and 8.28±0.23, respectively. The VB-N of salmon fillets ($p \leq 0.05$) decreased significantly from 12.85±0.43 for control sample to 8.28±0.23 immediately after treatment with EW(-/+)/1%(M+B). During storage at 5 °C, the VB-N of the control sample increased to reach the acceptable limit of fresh fish (30 mg /100 g) on day 7. By contrast, the VB-N of samples treated with EW(-/+)/1%(M+B) remained

Table 1: Changes in the VB-N, the TMA-N, the peroxide value and TBA of salmon fillets during storage at 5°C

Parameters	VB-N (mg/100g)				TMA-N (mg/100g)			
Storage Period/day	Control	EW(-/+)	1%(M+B)	EW(-/+)/ 1%(M+B)	Control	EW (-/+)	1%(M+B)	EW(-/+)/1%(M+B)
0	12.85± 0.43^{b}	8.47± 0.37^{a}	8.42± 0.24^{a}	8.28± 0.23^{a}	1.81± 0.06^{c}	1.36± 0.05^{b}	1.32± 0.05^{b}	1.17± 0.06^{a}
3	18.02± 0.34^{c}	13.06± 0.41^{b}	12.14± 0.49^{b}	10.40± 0.40^{a}	3.78± 0.15^{c}	3.07± 0.23^{b}	2.89± 0.19^{b}	2.14± 0.23^{a}
6	26.19± 0.57^{d}	19.65± 0.30^{c}	17.74± 0.74^{b}	12.71± 0.26^{a}	6.44± 0.48^{c}	4.96± 0.40^{b}	4.31± 0.34^{b}	3.21± 0.37^{a}
9	35.10± 0.56^{d}	25.46± 0.87^{c}	22.60± 0.53^{b}	17.67± 0.60^{a}	8.58± 0.86^{c}	6.87± 0.56^{b}	5.83± 0.42^{ab}	4.68± 0.59^{a}
12	41.65± 0.58^{d}	34.50± 0.66^{c}	29.00± 0.87^{b}	21.98± 0.71^{a}	10.16± 0.90^{c}	8.01± 0.65^{b}	7.21± 0.65^{ab}	5.85± 0.43^{a}
15	46.70± 0.61^{d}	41.40± 0.72^{c}	36.82± 0.40^{b}	28.20± 0.44^{a}	11.72± 0.62^{c}	9.89± 0.67^{b}	8.42± 0.79^{ab}	7.02± 0.47^{a}
	Peroxide value (meq/kg)				TBA (mg MDA/kg)			
0	1.27± 0.19^{a}	1.26± 0.18^{a}	1.23± 0.11^{a}	1.21± 0.17^{a}	0.31± 0.04^{a}	0.31± 0.07^{a}	0.30± 0.06^{a}	0.30± 0.09^{a}
3	3.32± 0.28^{c}	2.64± 0.23^{b}	2.25± 0.22^{ab}	1.87± 0.24^{a}	0.64± 0.08^{b}	0.56± 0.06^{ab}	0.47± 0.08^{ab}	0.41± 0.07^{a}
6	5.62± 0.36^{c}	4.28± 0.35^{b}	3.62± 0.43^{ab}	2.85± 0.35^{a}	1.01± 0.13^{cd}	0.86± 0.08^{bc}	0.64± 0.06^{ab}	0.52± 0.10^{a}
9	8.22± 0.58^{c}	5.84± 0.62^{b}	4.71± 0.60^{b}	3.21± 0.39^{a}	1.25± 0.12^{cd}	1.11± 0.19^{bc}	0.81± 0.15^{ab}	0.67± 0.13^{a}
12	9.51± 0.60^{d}	7.04± 0.36^{c}	5.32± 0.65^{b}	3.66± 0.40^{a}	1.33± 0.19^{b}	1.31± 0.16^{b}	0.94± 0.12^{ab}	0.75± 0.12^{a}
15	11.53± 0.73^{d}	8.20± 0.63^{c}	6.34± 0.43^{b}	4.50± 0.41^{a}	1.82± 0.17^{b}	1.54± 0.14^{b}	1.11± 0.11^{a}	0.86± 0.16^{a}

below the acceptable limit until the end of storage period (day 15). Extension of the shelf-life of the samples treated with EW(-/+)/1%(M+B) may be due to the inhibitory effects of this treatment on the microbial growth, which delay the formation of basic nitrogen compounds.

Changes in the trimethylamine nitrogen (TMA-N) of the salmon fillets during storage at 5°C are shown in Table 1. The TMA-N of salmon fillets ($p \leq 0.05$) decreased significantly from 1.81±0.06 for the control sample to 1.36±0.05, 1.32±0.05 and 1.17±0.06 immediately after treatments with EW(-/+), 1%(M+B) and EW(-/+)1%(M+B), respectively. During storage at 5 °C, the TMA-N values of the sample treated with EW(-/+)/1%(M+B) increased slightly until reached 7.02±0.47 after 15 days of storage, while, of the control sample increased rapidly to reach value of 11.72±0.62 after the same period. These results are in agreement with previous studies [35-37].

Changes in the peroxide value (PV) and thiobarbituric acid (TBA) of salmon fillets during storage at 5 °C: The various reactions involved in the lipid oxidation are catalyzed either by non-enzymatic, microbial enzymes, intracellular enzymes or digestive enzymes from the fish themselves. The relative significance of these reactions, therefore, mainly depends on fish species and storage temperature [37]. Changes in the PV of salmon fillets during storage at 5 °C are shown in Table 1. The initial PV in the salmon fillets analyzed was ranged from 1.21±0.17 in fillets treated with EW(-/+)/1%(M+B) to 1.27±0.19 in control sample. No significant difference ($P>0.05$) has been observed immediately after treatments (day 0) between the control and all treated samples. During storage at 5 °C, the PV values of the control and samples treated with EW(-/+), 1%(M+B) and EW(-/+)/1%(M+B) increased from 1.27±0.19, 1.26±0.18, 1.23±0.11 and 1.21±0.17 (day 0) to 11.53±0.73, 8.20±0.63, 6.34±0.43 and 4.50±0.41 meq/kg, respectively, by the end of storage (day 15) and significant differences ($P<0.05$) were observed between the control and each treated samples. The PV in all treated samples were below the acceptable level of 10-20 meq/kg fish [37]. These results are in agreement with those of Undeland [38], who found that the PV significantly increased in herring fillets after only 2 days in ice storage.

Changes in the TBA of salmon fillets during storage at 5°C are shown in Table 1. No significant differences ($P>0.05$) in the TBA values between the control and all treated samples were noticed immediately after treatment (day 0). The TBA values of control and samples treated with EW(-/+)/1%(M+B) were significantly ($p<0.05$) increased from 0.30 (day 0) to 1.82±0.17 and 0.86±016 mg MDA/kg, respectively, at the end of storage at 5°C (day 15). From obtained results of PV and TBA measurements, it can be concluded that the oxidation products were increased rapidly in the control samples during storage, however, slower rate of increasing the oxidation products was observed in salmon fillets treated with EW(-/+)/1%(M+B). This result could be explained by the antioxidant effect menthol and borneol, which has ability to scavenge free radicals and the effect of EW(-), which reacts with radicals compounds to produce nonradical compounds. This is to be expected from the

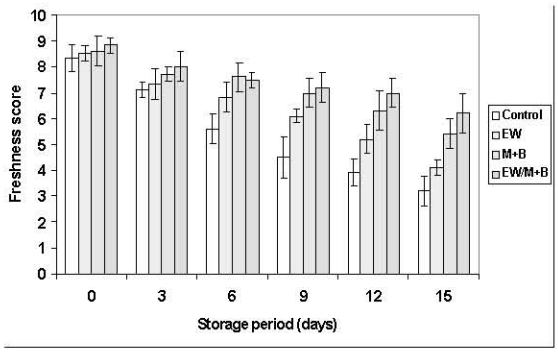

Fig. 1: Changes in organoleptic properties of salmon fillets during storage at 5°C. (EW) cathodic solution EW(-), followed by anodic solution EW(+) [EW(-/+)]; (M+B) 1% (menthol + borneol) [1%(M+B)]; (EW/M+B) [EW(- /+)/1%(M+B)]. Results are the means of three replicates±SD.

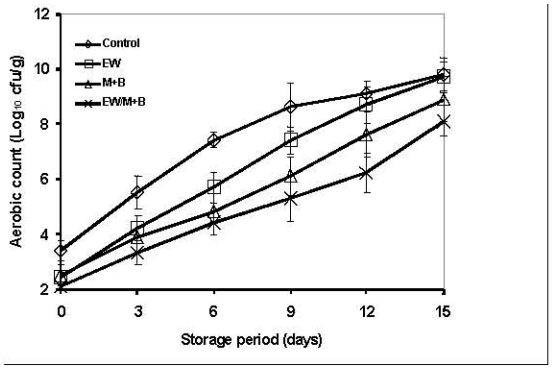

Fig. 2: Changes in the aerobic count of salmon fillets during storage at 5°C. (EW) cathodic solution EW(-), followed by anodic solution EW(+) [EW (-/+)]; (M+B) 1% (menthol+borneol) [1%(M+B)]; (EW/M+B) [EW(-/+)/1%(M+B)]. Results are the means of three replicates±SD.

high level of dissolved hydrogen in EW(-) [39, 19]. This results are in agreement with those obtained by [40, 41] who found that treatments based on natural antioxidants control lipid oxidation in ground fish and meat products.

Changes in organoleptic properties of salmon fillets during storage at 5°C: Changes in the overall freshness and acceptability scores of salmon fillets during storage at 5 °C are shown in Fig. 1. Sensory evaluation of fish quality is used to measure, analyze and interpret reaction to food characteristics perceived through the senses of color, odor, texture, taste, flavor and overall acceptability. The sensory evaluation demonstrated that, after subjecting the salmon fillets to all treatments, there is no significant difference ($P>0.05$) in any of the parameters between the control and any of the other treatments. During storage at 5 °C, a gradual decrease in all of these parameters was noticed in all samples. Data obtained indicated that, from day 6 onwards, significant differences ($P<0.05$) between the control and all of the treatments were recorded. Furthermore, the control had reached an unacceptable freshness score (5.0) by day 7. By contrast, fillets treated with 1%(M+B) and EW(-/+)/1%(M+B) had not reach an unacceptable score, even by the end of storage. Fillets treated with EW(-/+)/1%(M+B) maintained the freshness score more than the other treatments. These results are in agreement with those of [42, 26].

Changes in the total microbial count of salmon fillets during storage at 5°C: Changes in the total microbial counts of the salmon fillets during storage at 5 °C are shown in Fig. 2. Treatment with EW(-/+)/1%(M+B) resulted in a significant reduction ($P<0.05$) in the initial microbial counts comparing with the control. The reduction caused by treatment with EW(-/+)/1%(M+B) was about 1.4 $\log_{10}$ CFU/g. No significant differences in the total microbial count were recorded among control salmon fillets and the samples treated with EW(-/+) and 1%(M+B) just after treatment. The total microbial count of the control fillets exceeded the acceptable limit (10^6 CFU/g) after approximately 5 days during storage at 5°C. While, the total microbial count in fillets treated with EW(-/+)/1%(M+B) was slowly increased comparing with the other fillets and reached the acceptable limit at roughly 13 days. By the day 6 of storage at 5°C, all treatments showed significant differences ($P<0.05$) in the total microbial count as compared with control samples. These results are in agreements with those obtained by previous studies [26, 43, 44]. The antimicrobial effect of treatment with EW (-)/EW (+) is initiated by EW (-), which acts like a surface-active agent to decrease the hydrophobicity of food materials. As a result, microorganisms on the surface are readily accessible to EW (+), which produces hydroxyl (OH) and chlorine (Cl) radicals by dissociation of hypochlorous acid (HOCl) within the microenvironment. The (OH) and (Cl) radicals inactivate the cytoplasmic enzymes and damage the outer membrane of bacteria, which leads to bacterial death [26, 43, 45]. Essential oil compounds exert antimicrobial activity, first, by interfering with the phospholipids bilayer of the cell membrane, which causes an increase in permeability and a loss of cellular constituents; second,

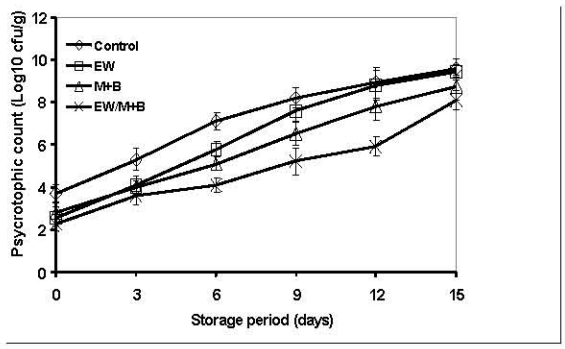

Fig. 3: Changes in the psychrotrophic count of salmon fillets during storage at 5 °C. (EW) cathodic solution EW(-), followed by anodic solution EW(+) [EW(-/+)]; (M+B) 1% (menthol + borneol) [1%(M+B)]; (EW/M+B) [EW(-/+)/1%(M+B)]. Results are the means of three replicates±SD.

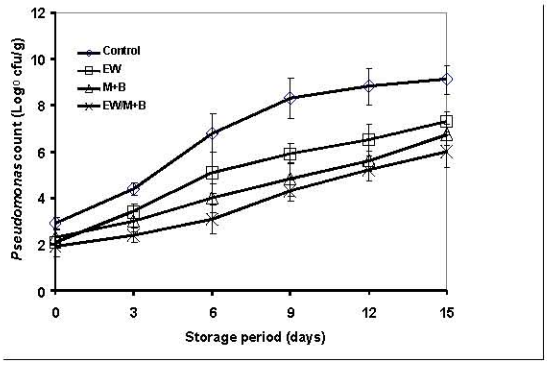

Fig. 4: Changes in the *Pseudomonas* count of salmon fillets during storage at 5 °C. (EW) cathodic solution EW(-), followed by anodic solution EW(+) [EW(-/+)]; (M+B) 1% (menthol + borneol) [1%(M+B)]; (EW/M+B) [EW(-/+)/1%(M+B)]. Results are the means of three replicates±SD.

by impairing a variety of enzyme systems, including those involved in the production of cellular energy and the synthesis of structural components [46]. The chemical composition of the oils that give rise to inhibitory effects could be due to the presence of an aromatic nucleus containing a polar functional group [47].

Changes in the psychrotrophic count of salmon fillets during storage at 5°C: The Gram-negative psychrotrophic bacteria are the major group of microorganisms responsible for spoilage of aerobically stored fresh fish at chilled temperatures [3, 48]. Changes in the psychrotrophic counts of treated salmon fillets during storage at 5°C are shown in Fig. 3. Treatment with EW(-/+), 1%(M+B) and EW(-/+)/1%(M+B) resulted in significant reduction ($P<0.05$) in the initial psychrotrophic counts compared with the control. No significant difference in the psychrotrophic count among samples treated with EW(-/+), 1%(M+B) and EW(-/+)/1%(M+B) on day 0. Subsequently, the psychrotrophic count of the salmon fillets increased during storage at 5 °C. Furthermore, there was a significant difference in the psychrotrophic counts between treatment with EW(-/+)/1%(M+B) and control during storage. Additionally, the growth pattern of psychrotrophic counts showed same behavior as that of total microbial counts but relatively higher than total microbial counts. This result is in accordance with that of [49], who revealed similar growth pattern for psychrotrophic population in sea salmon during refrigerated storage.

Changes in *Pseudomonas* count of salmon fillets during storage at 5°C: Changes in the *Pseudomonas* counts of treated salmon fillets during storage at 5 °C are shown in Fig. 4. Treatment with EW(-/+)/1%(M+B) caused a significant reduction ($P<0.05$) in the initial *Pseudomonas* count compared with the control. Rasmusssen and Ross [50] revealed that the shelf life of the aerobically stored Atlantic salmon 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 [3]. These two bacterial groups have been reported to be the specific spoilage microorganisms in various fish species; including sea salmon [49]. Results revealed that the counts of *Pseudomonas* are lower than the total microbial counts, indicating the importance of these species in the spoilage of fresh fish. The initial *Pseudomonas* count was ranged from 2.9±0.30 $\log_{10}$ CFU/g in control fillets to 1.9±0.44 $\log_{10}$ CFU/g in fillets treated with EW(-/+)/1%(M+B). Whereas, by the end of storage (day 15), *Pseudomonas* counts in the different fillets samples reached to 9.1±0.62, 7.3±0.44, 6.7±0.46 and 6.0±0.72 $\log_{10}$ CFU/g in control, EW(-/+), 1%(M+B) and EW(-/+)/1%(M+B) fillets samples, respectively.

Changes in the *Enterobacteriaceae* count of salmon fillets during storage at 5°C: Changes in the *Enterobacteriaceae* count of the salmon fillets during storage at 5°C are shown in Fig. 5. Immediately after treatment, there is no significant difference ($P>0.05$) in the *Enterobacteriaceae* counts between the control and

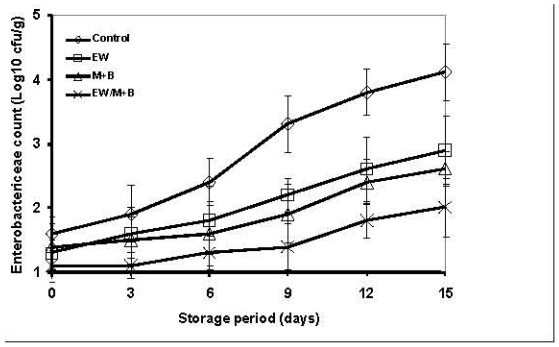

Fig. 5: Changes in the *Enterobacteriaceae* count of salmon fillets during storage at 5°C. (EW) cathodic solution EW(-), followed by anodic solution EW(+) [EW(-/+)]; (M+ B) 1% (menthol + borneol) [1%(M+B)]; (EW/M+B) [EW(-/+)/1%(M+B)]. Results are the means of three replicates ± SD.

treated fillets. Subsequently, the *Enterobacteriaceae* count of the salmon fillets was increased during storage at 5°C. Data obtained during storage at 5°C indicated that, from day 9 onwards, there were significant differences ($P<0.05$) between the control fillets and all treatments. *Enterobacteriaceae* were also found to be members of the microbial association implicated in the spoilage of fresh salmon during refrigerated storage [40, 4]. The growth of *Enterobacteriaceae* was slower than that of the other microbial groups and never exceeding 3 $\log_{10}$ CFU/g in the all treated fillets and reached to 4.1±0.44 $\log_{10}$ CFU/g in control fillets by the end of storage (day 15).

CONCLUSION

The current study concludes that using EW solutions [EW(-/+)] and essential oil compounds [1%(0.5% menthol + 0.5% borneol)] as a pre-treatment is efficient against the proliferation of various categories of spoilage microorganisms and suppression of lipid oxidation resulting in extension of the shelf-life of the salmon fillets during cold storage at 5°C.

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