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Shelf Life of Semifried Tuna Slices Coated with Essential Oil Compounds after Treatment with Anodic Electrolyzed NaCl Solution

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ABSTRACT

The main objective of this study was to evaluate the effect of two commonly used essential oils after treatment with anodic electrolyzed NaCl solution in order to extend the shelf life of coated semifried tuna slices. Samples of tuna slices were treated with 100-fold electrolyzed water and coated with an edible solution containing 1% essential oil (0.5% eugenol [E] plus 0.5% linalool [L]). The coated slices were fried at 170°C for 1 min and then stored at 5 and 25°C. Total volatile basic nitrogen of slices treated with electrolyzed water and 1% (E+L) decreased from 15.5 for the nontreated control slices to 9.7 immediately after treatment and remained at a low level (<30) until the end of the storage period (day 20). Treatment with 1% (E+L) significantly suppressed lipid oxidation in coated semifried tuna. Sensory evaluation and microbiological assays showed that treatment with electrolyzed water and 1% (E+L) extended the shelf life of coated semifried slices to 15 and 2 days compared with 5 and 1 days for control slices during storage at 5 and 25°C, respectively.

Electrolyzed saline is the recommended ''gold standard'' for assessing bactericidal activity of electrolyzed products *(37).* Electrolyzed oxidizing water is a relatively new concept developed in Japan, and has been utilized in agriculture, livestock management, medical sterilization, food sanitation, and areas that rely on antimicrobial methodologies *(17, 18, 34, 35).* Acidic electrolyzed oxidizing water has a pH of approximately 2.6, an oxidation-reduction potential of +1,150 mV, and a chlorine concentration ranging between 40 and 90 ppm, and can inactivate most pathogenic bacteria *(18, 34).* Electrolyzed NaCl solution constitutes a new technology that has recently been studied as an antimicrobial and antioxidant agent *(22, 32).*

Lipid oxidation is a major quality problem that can lead to the development of off-flavors and off-odors in edible oils and fat-containing foods through oxidative rancidity *(23).* Spices and their essential oils, the most efficient natural antioxidants and antimicrobial agents, have long been used to preserve food *(6, 15).* The efficacy of these compounds can be enhanced, by combining their use with other preservatives *(3).* Eugenol (4-allyl-2-methoxyphenol), a major component of clove and cinnamon oil *(10),* has been granted the status of generally recognized as safe by the U.S. Food and Drug Administration. It is commonly used as a flavoring agent in cosmetics and food products, particularly in dental material. Eugenol is active against many pathogenic bacteria (e.g., *Escherichia coli, Listeria monocytogenes, Campylobacter jejuni, Salmonella enterica, Staphylococcus aureus, Lactobacillus sakei, Helicobacter pylori*) *(1, 9, 12),* fungi, and viruses. Linalool (3,7-dimethyl-1,6-octadien-3-ol), the chief constituent of linaloe oil, has also been used as a flavoring agent. Essential oil containing linalool exhibits a less broad antimicrobial spectrum and is active against *Staphylococcus epidermidis* and *Rhodococcus equi (25).*

The objective of this work was to examine the preservative effects of pretreatment with anodic electrolyzed NaCl solution and essential oils, applied either alone or in combination, to extend the shelf life of coated semifried tuna slices during storage at 5 and 25°C.

MATERIALS AND METHODS

Tuna. The species used in this study was bigeye tuna (*Thunnus obesus*). Frozen blocks of edible raw tuna were purchased from a local market in Hakodate, Japan, and stored at −65°C until use.

Coating agents. Coating agents including wheat flour, sodium chloride, and cumin were bought at a local supermarket. Chitosan (from crab shells, 75 to 85% deacetylated; a liquid low-molecular-weight grade product as designated by the Food Additives Regulation of Japan) was obtained from Kimica (Tokyo, Japan). Eugenol and linalool were obtained from Kanto Chemical (Tokyo, Japan).

Preparation of electrolyzed NaCl solution. Electrolyzed anodic NaCl solution was prepared by using a two-compartment batch-scale electrolysis apparatus (JED-020, Aoi Engineering, Kannami, Japan). After electrolysis of 0.1% NaCl in deionized water for 10 min, electrolyzed water (EW(+)) with pH 2.2, available chlorine of 41 ppm, and oxidation-reduction potential of +1,140 mV was obtained in the anodic compartment. The solution was prepared immediately before use.

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Treatment of tuna slices. Tuna blocks were washed in tap water, cut into slices (approximately 4 by 4 by 2 cm), rewashed, and drained. Slices were randomly divided into four batches. One batch designated as the control was dipped into the edible coating solution (wheat flour, 2.5% [wt/vol]; sodium chloride, 2.0% [wt/vol]; cumin, 2.0% [wt/vol]; and chitosan, 1.0% [wt/vol]). The second batch of slices was dipped in EW(+) for 15 min before being dipped into the edible coating solution (EW(+)). The third batch was dipped in an edible coating solution containing 1% essential oil (eugenol plus linalool) (1% (E+L)) for 15 min. The fourth batch was dipped for 15 min in EW(+) and then dipped for 15 min in an edible coating solution containing 1% (E+L) (EW(+)/1% (E+L)). After dipping, all tuna slices were drained for 3 to 4 min at room temperature, semifried in sunflower oil at 170°C for 1 min using an electrical fryer, and then drained to remove excess oil. Different coated tuna slices were packaged in polyethylene bags and stored at 5 and 25°C.

pH value measurement. The pH of homogenized tuna slices diluted in distilled water (1:10) was measured with a pH meter (D-14, Horiba, Tokyo, Japan).

Determination of VB-N. Volatile basic nitrogen (VB-N) was determined according to the microdiffusion method *(5)* that measures the content of ammonia, trimethylamine, dimethylamine, and other basic nitrogenous compounds associated with fish spoilage.

PV measurement. Samples (0.5 g each) were added to 25 ml of a 3:2 glacial acetic acid–loroform solution in a conical flask, followed by the addition of 1 ml of saturated potassium iodide. After maintaining this solution in the dark for about 10 min, 30 ml of distilled water and 1 ml of freshly prepared 1% starch were added. After shaking, the samples were titrated with 0.01 M sodium thiosulfate. Peroxide value (PV) was expressed as milliequivalents per kilogram of sample *(8).*

TBA test. Thiobarbituric acid (TBA)–reactive substances were determined colorimetrically according to the procedure described by Siu and Draper *(30).* Tuna samples (10 g) were homogenized in 25 ml of distilled water for 2 min and then mixed with 25 ml of 10% trichloroacetic acid. The mixture was filtered, and then 1 ml of 0.06 M TBA was added to 4-ml aliquots of the filtrate, and then boiled for 10 min for color development. Absorbance at 532 nm was measured with a spectrophotometer (U-2000, Hitachi, Tokyo, Japan). The TBA values were expressed as milligrams of malondialdehyde per kilogram of sample.

Microbial analyses. Five-gram samples of tuna were added to 45 ml of sterilized 0.9% NaCl saline and homogenized for 1 min using a stomacher 80 Lab-blender (Seward, London, UK). Serial dilutions were prepared with 0.9% NaCl saline solution, and duplicate samples (0.1 ml) of each dilution were spread plated on Plate Count Agar (Difco, Becton Dickinson, Sparks, Md.). The total aerobic count was determined after incubation at 20°C for 48 h. The experiment was carried out three times in duplicate.

Sensory evaluation. The sensory properties of tuna slices were assessed by a group of 10 trained laboratory staff panelists during storage at 5 and 25°C. The semifried samples were heated in a microwave oven for 1 to 2 min before evaluation. Panelists were asked to evaluate the samples based on several parameters (color, flavor, odor, taste, and texture) using a 10 to 0 scale indicating decreasing freshness *(11).* A general freshness score was calculated as the average of all parameters. A freshness score of >5 indicated an acceptable sample. Data from the 10 independent panelists were pooled and statistically analyzed.

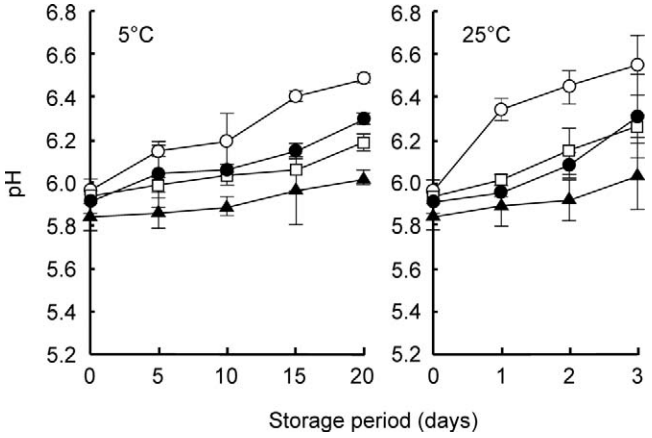

FIGURE 1. *Changes in the pH of coated tuna slices during storage at 5 and 25°C. Symbols: ○, control; ●, anodic solution (EW(+)); □, 1% (eugenol plus linalool) (1% (E+L)); ▲, EW(+)/1% (E+L). Results are shown as the means of three replicates ± SD.*

Statistical analysis. For each treatment, data from three independent replicate trials were pooled, and the mean values and standard deviations (SD) were determined. Differences between samples were determined by the *t* test and were considered to be significant when $P \leq 0.05$ *(31).*

RESULTS AND DISCUSSION

pH and VB-N values for coated tuna slices during storage. The pH values for coated tuna slices stored at 5 and 25°C conditions are shown in Figure 1. The initial pH of the control and slices treated with EW(+), 1% (E+L), and EW(+)/1% (E+L) were 5.96 ± 0.01, 5.91 ± 0.06, 5.94 ± 0.08, and 5.84 ± 0.06, respectively. During storage at 5°C, the pH of the control and treated slices increased slightly. At 25°C, the pH increased rapidly on the third day of storage to 6.55 ± 0.14 in the control slices and 6.31 ± 0.19, 6.27 ± 0.05, and 6.03 ± 0.16 in slices treated with EW(+), 1% (E+L), and EW(+)/1% (E+L), respectively. These results are similar to those of previous studies for refrigerated tuna steaks and whole bigeye tuna stored under controlled atmosphere *(21, 27).*

Changes in total VB-N of the coated tuna slices during storage at 5 and 25°C are shown in Figure 2. The VB-N of coated tuna slices decreased significantly ($P \leq 0.05$) from 15.5 ± 0.6 for the control slices to 9.7 ± 0.8 immediately after treatment (day 0) with EW(+)/1% (E+L). After 10 days of storage at 5°C, VB-N in the control slices increased to 26.1 ± 0.8 near the upper acceptable limit of 30 mg/100 g for fresh fish. By contrast, VB-N in slices treated with EW(+)/1% (E+L) remained below this limit (28.6 ± 0.9 mg/100 g) until the end of storage (day 20). During storage at 25°C, VB-N increased rapidly and reached the acceptable limit at approximately 2.0 days for slices treated with EW(+)/1% (E+L) and 1.0 days for the control slices.

Lipid oxidation in coated tuna slices during storage. Lipid peroxidation, corresponding to the oxidative deteri-

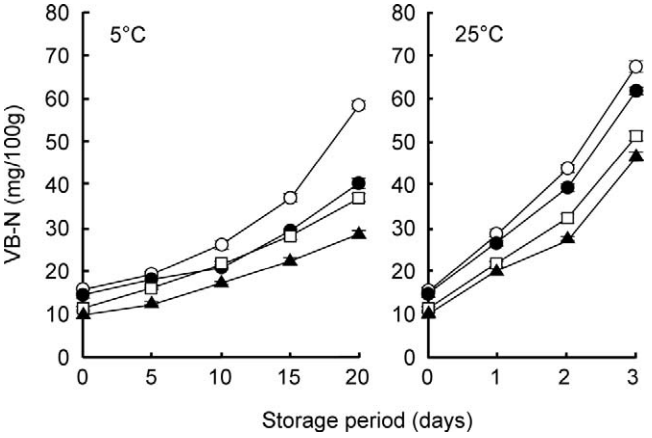

FIGURE 2. *Changes in the VB-N of coated tuna slices during storage at 5 and 25°C. Results are shown as the means of three replicates ± SD. Symbols are the same as those in Figure 1.*

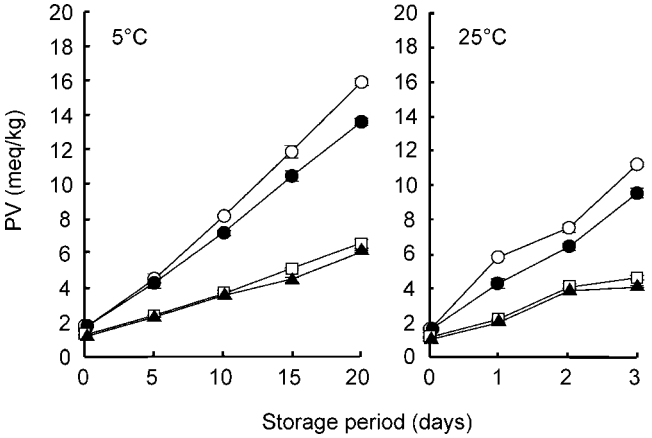

FIGURE 3. *Changes in the peroxide value (PV) of coated tuna slices during storage at 5 and 25°C. Results are shown as the means of three replicates ± SD. Symbols are the same as those in Figure 1.*

oration of polyunsaturated fatty acids in fish muscle, leads to production of off-flavors and off-odors, thereby shortening product shelf life *(26).* The peroxide and TBA values are both well established methods for determining oxidation products in fats and oils *(20).* PV is the most common measure of lipid hydroperoxides, which are primary lipid oxidation products *(24).* Changes in the PV of coated tuna slices during storage at 5 and 25°C are shown in Figure 3. Immediately after treatment (day 0), no significant differences were seen between the control and slices treated with EW(+) and, also, between slices treated with 1% (E+L) and EW(+)/1% (E+L). At 5°C, PV increased in all slices from 1.66 ± 0.08, 1.61 ± 0.09, 1.24 ± 0.03, and 1.08 ± 0.07 (day 0), to 15.92 ± 0.24, 13.58 ± 0.21, 6.54 ± 0.28, and 6.12 ± 0.08 meq/kg in the control, EW(+), 1% (E+L), and EW(+)/1% (E+L), respectively, by the end of storage (day 20). During 3 days of storage at 25°C, PV of the control slices increased rapidly to 11.18 ± 0.17 meq/kg, compared with 4.14 ± 0.04 meq/kg, for slices treated with EW(+)/1% (E+L). These results are similar to those of Undeland et al. *(33),* Mahmoud et al. *(22),* and Sallam *(28)* for fillets of herring, carp, and sliced salmon, respectively.

The TBA assay is widely used to assess the extent of lipid oxidation. The results of TBA assay corroborated that obtained by PV. Changes in TBA values for coated tuna slices during storage at 5 and 25°C are shown in Figure 4. At day 0 immediately after treatment, no significant differences in TBA values were seen between the control slices and slices treated with EW(+), or between slices treated with 1% (E+L) and slices treated with EW(+)/1% (E+L). However, for slices treated with 1% (E+L) and EW(+)/1% (E+L), the TBA values were significantly affected ($P < 0.05$) by the different treatments during storage. A maximum level TBA value of 5 mg of malondialdehyde per kg has been proposed for good fish quality, whereas the product may be consumed up to the level of 8 mg of malondialdehyde per kg *(29).* In the current study, TBA values for slices treated with 1% (E+L), and EW(+)/1% (E+L) were well below this proposed limit throughout 20 and 3 days of storage at 5 and 25°C, respectively.

Total microbial counts for coated tuna slices during storage. Spoilage of fresh and lightly preserved fish is caused by the growth and enzymatic activity of specific spoilage organisms that produce metabolites causing off-flavors or off-odors leading to consumer rejection *(13, 14).* Changes in viable total microbial counts of the tuna slices during storage at 5 and 25°C are shown in Figure 5. Immediately after the treatments, all total microbial counts decreased with a significant reduction ($P \leq 0.05$) of 1.3 log CFU/g seen for EW(+)/1% (E+L) compared with the control. Sallam *(28)* and Barstad et al. *(4)* reported that the numbers of *Listeria* in artificially inoculated chicken frankfurters and ham slices decreased significantly after treating with EW(−)/EW(+), thereby extending the refrigerated shelf life to 7 days.

During subsequent storage, the total microbial count in samples treated with EW(+)/1% (E+L) increased slowly compared with the other samples and reached the acceptable limit after about 15 and 2 days at 5 and 25°C, respectively. One possible explanation is that EW(+) can reportedly damage the outer cell membrane of bacteria and pen-

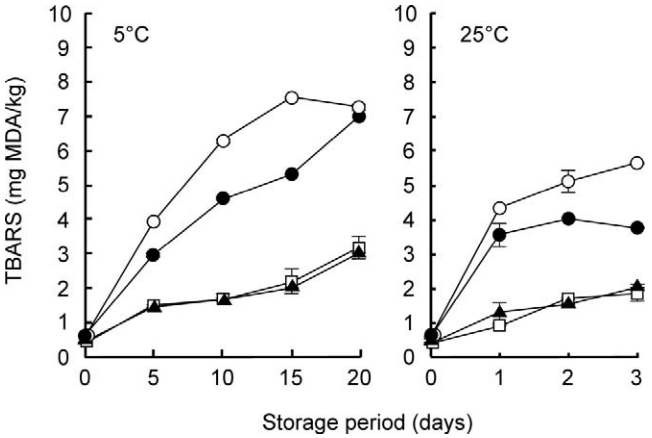

FIGURE 4. *Changes in the thiobarbituric acid-reactive substance (TBARS) content of coated tuna slices during storage at 5 and 25°C. Results are shown as the means of three replicates ± SD. Symbols are the same as those in Figure 1.*

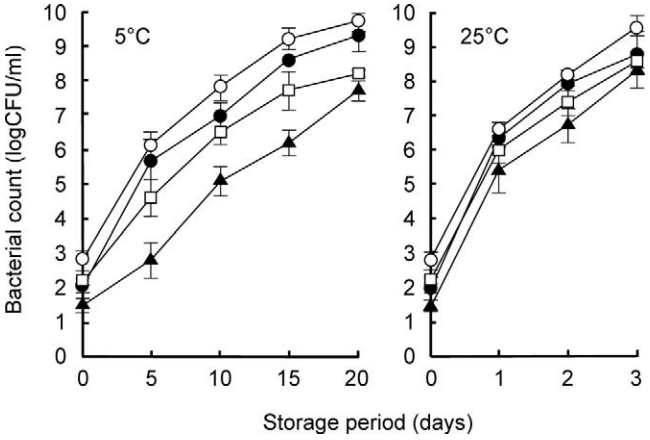

FIGURE 5. *Changes in the total microbial count of coated tuna slices during storage at 5 and 25°C. Results are shown as the means of three replicates ± SD. Symbols are the same as those in Figure 1.*

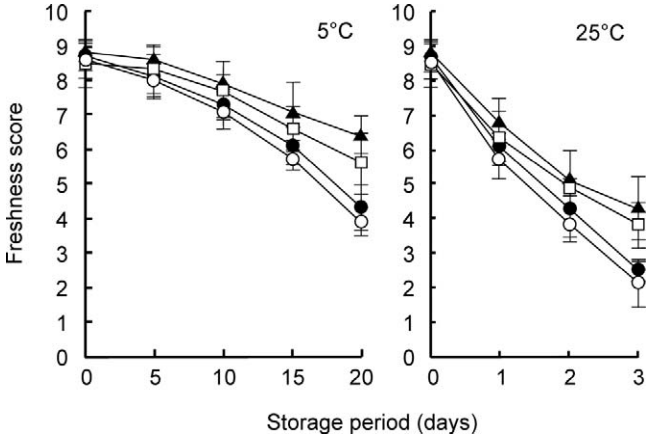

FIGURE 6. *Changes in the sensory properties of coated tuna slices during storage at 5 and 25°C. Results are shown as the means of three replicates ± SD. Symbols are the same as those in Figure 1.*

etrate the cytoplasmic membrane, thereby causing the inactivation of cytoplasmic enzymes by hypochlorous acid (HOCl), which produces OH and Cl radicals *(19).* In addition, the outer membrane disintegrates, thereby increasing the permeability of the cytoplasmic membrane to ATP, which can lead to bacterial cell death *(6, 15, 16).*

The lower pH of the coated slices treated with EW(+)/1% (E+L) during storage compared with the coated control slices (Fig. 1) is also likely responsible for greater microbial inhibition, followed by delayed formation of basic nitrogen compounds as reported by Mahmoud et al. *(22).* Additionally, the combined strong inhibitory effects of EW(+) and essential oil compounds may also be partly responsible for extending the acceptable VB-N for slices treated with EW(+)/1% (E+L) (Fig. 2).

Sensory properties of coated tuna slices during storage. Sensory evaluation of fish quality is used to assess perceived changes in color, taste, flavor, odor, and texture *(22).* Figure 6 shows the changes in freshness scores for overall acceptability of coated tuna slices during storage at 5 and 25°C.

After subjecting the tuna slices to the various treatments, no significant differences in freshness score were seen between the control and any of the treated slices until 10 days of storage at 5°C. Deza et al. *(7)* reported that treatments with anodic electrolyzed water significantly reduced the numbers of pathogenic microorganisms on tomatoes without affecting the organoleptic properties of the fruit. During storage at both 5 and 25°C, a gradual decrease in all of these parameters was noticed for all slices. Following 15 days of storage at 5°C, significant differences ($P \leq 0.05$) were seen between the control slices and those treated with EW(+)/1% (E+L); furthermore, the control slices had reached an unacceptable freshness score of 6.0 by day 10. By contrast, slices treated with EW(+)/1% (E+L) remained acceptable, even at the end of storage. Deterioration of semifried tuna slices during storage was faster at 25 than 5°C, which agrees with the findings of Aubourg et al. *(2),* Yesim et al. *(36),* Ruiz-Capillas and Moral *(27),* and Mahmoud et al. *(22)* for raw materials of turbot, European eel, bigeye tuna, and carp, respectively.

Overall, these findings indicate that dipping tuna slices in a EW(+) solution followed by an edible solution containing 1% (E+L) increases the efficacy of the latter treatment and can extend the shelf life of semifried coated tuna slices.

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