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Food Chemistry 99 (2006) 656–662
Food Chemistry
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A new technology for fish preservation by combined treatment with electrolyzed NaCl solutions and essential oil compounds

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Received 16 May 2005; received in revised form 15 August 2005; accepted 15 August 2005

Abstract

This study was undertaken to establish a new technology, using pre-treatment with electrolyzed NaCl solutions and essential oil compounds, to extend the shelf-life of carp fillets. Samples of skinless carp fillets were treated with 100-fold (by weight) of electrolyzed NaCl solutions [cathodic solution, EW(−) and/or anodic solution, EW(+)] and 1% oil (0.5% carvacrol + 0.5% thymol) [1%(C + T)]. Then chemical [pH, volatile basic nitrogen, peroxide value, and thiobarbituric acid], microbiological (total viable count) and sensory analyses were used to evaluate the preservative effect of this new technology during storage at 5 and 25 °C. Our results from the chemical assays indicated that EW(−), followed by EW(+) and subsequently 1%(C + T) [EW(−)/EW(+)/1%(C + T)], significantly suppressed the lipid oxidation compared with other treatments. Data from sensory evaluation and microbiological assay showed that treatment with EW(−)/EW(+)/1%(C + T) extended the shelf-life of carp fillets to 16 and 1.3 days compared with 4 and 0.3 days for the control samples during storage at 5 and 25 °C, respectively.

Keywords: Antioxidant; Antimicrobial; Carp fillets; Carvacrol (C); Electrolyzed NaCl solutions (EW); Shelf-life; Thymol (T)

1. Introduction

Securing food supply and food safety for consumers is the first priority of food scientists. Fish is highly susceptible to spoilage, which can be caused by both chemical reactions and microbial growth (Gram & Dalgaard, 2002). About 25% of gross primary agricultural and fishery products is estimated to be lost every year, mostly due to chemical deterioration and microbial spoilage (Baird-Parker, 2000).

Growing population pressure and a diminishing resource base threaten food security in the coming century. It is no easy solution to increase food production but we can improve the quality and extend the shelf life of food. The development of lipid oxidation or microbial growth in fish during storage can be controlled by synthetic or natural preservatives but consumers are always concerned about the use of artificial preservatives in food, which may have potentially undesirable effects on human health (Tassou, Drosino, & Nychas, 1995).

Spices and their essential oils are the most efficient natural antioxidants and antimicrobial agents have long been used to preserve food (Burt, 2004; Delgado, Fernandez, Palop, & Periago, 2004; Harpaz, Glatman, Drabkin, & Gelman, 2003; Tsimidou, Papavergou, & Boskou, 1995). The efficacy of these compounds can be enhanced, by combining their use with other preservatives (Bagamboula, Uyttendaele, & Debevere, 2004).

Electrolyzed NaCl solution [Electrolyzed water (EW)] constitutes a new technology that has recently been studied as an antimicrobial and antioxidant agent (Miyashita,

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doi:10.1016/j.foodchem.2005.08.037

Yasuda, Ota, & Suzuki, 1999; Ozer & Demirci, 2004; Suzuki et al., 2002). The antimicrobial and antioxidant effects of pre-treating carp fillets with EW solutions, followed by essential oil compounds, have not, however, been studied.

In the present study, therefore, the preservative effects of pre-treatment with EW solutions and 1%(C + T), applied either alone or in combination, have been investigated to evaluate their efficacy as a new, natural and safe preservative technique for carp fillets during storage at 5 and 25 °C.

2. Materials and methods

2.1. Carp

Healthy common carp (*Cyprínus carpio*), with an average weight of 2.0 kg, were obtained from Miyazaki koiya Aquaculture (Hakodate, Japan). Gutting and skinless filleting were done as soon as the fish arrived in the laboratory.

2.2. Chemicals

Carvacrol and thymol (purity 99.9%) were obtained from KANTO Chemical Co., Inc. (Tokyo, Japan).

2.3. 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.

2.4. Treatment of carp fillets

Six samples of skinless carp fillets were used to assess the antioxidant and antimicrobial effects of EW solutions and/or 1% (0.5%C + 0.5%T) compounds. Five grammes of skinless carp fillets were prepared from different carp fish (10 fish). The samples were treated by dipping them for 15 min in: 1, a 100-fold volume of sterile 0.2% agar solution (0.2% agar was used to dissolve essential oil compounds in water); 2, EW(+); 3, EW(−), followed by EW(+) [EW(−)/EW (+)]; 4, 1% (carvacrol + thymol) [1%(C + T)]; 5, EW(+), followed by 1%(C + T) [EW(+)/1%(C + T)]; or 6, EW(−)/EW(+), followed by 1%(C + T) [EW(−)/EW(+)/1% C + T]. Treatments were carried out in a sterile 3-l flask with gentle shaking (100 rpm) by 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 and then separately packaged (in sterilised 7.6×17.8 cm bags, Sainte-Julie, Canada J3 E219) under aseptic conditions and stored at either 5 or 25 °C. Samples for chemical, sensory and microbiological analyses were taken on days 0, 1, 2, 5, 10, 15 and 20 after treatment.

2.5. pH value measurement

The pH of the carp fillets was measured on homogenized fillet samples diluted in distilled water (1:10) with a pH meter (D-14, Horiba, Tokyo, Japan).

2.6. Determination of volatile basic nitrogen (VB-N)

VB-N was determined according to the method of (Conway, 1950) which measures the content of ammonia, TMA, DMA and other basic nitrogenous compounds associated with fish fillet spoilage.

2.7. 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 shaking, the samples were titrated with 0.01 M sodium thiosulfate. The peroxide values were expressed in units of meq/kg of sample (Egan, Kirk, & Sawyer, 1981).

2.8. Thiobarbituric acid (TBA) test

TBA values were determined spectrophotometrically according to the procedure described by Siu and Draper (1978). Carp fillet samples (10 g) 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 colour 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.

2.9. Organoleptic evaluation of carp fillets

The organoleptic properties of carp fillets during storage at 5 and 25 °C were assessed by a group of 10 trained

panellists from the staff of Marine Food Science, Graduate School of Fisheries Science Hokkaido University. Samples (approximately 3 cm × 2 cm) were fried for 1–2 min before evaluation. The panellists were asked to evaluate several parameters (colour, flavour, odour, taste and texture) for the food samples on a scale from 10 to 0 indicating decreasing freshness (Gelman, Pasteur, & Rave, 1990). A general 'freshness score' was calculated as the average of all grades. A freshness score of more than six was taken to indicate acceptability of the fillet samples. The data from the ten independent panellists were pooled and the mean values and standard deviations were determined. The differences between the control and the treated samples were determined by Student's *t*-test. Differences between samples were considered to be significant when $p \leqslant 0.05$.

2.10. Microbial analyses

The total aerobic bacteria count was determined by analysing 5 g samples of carp fillets. Samples were homogenised for 1 min at room temperature in 45 ml of sterilised 0.9% NaCl saline using a stomacher 80 Lab-blender (Seward, London, UK). Serial decimal dilutions were prepared in 0.9% NaCl saline solution, and duplicate samples (0.1 ml) of each dilution were spread on the plates. The total aerobic count was determined on Plate Count Agar (Difco, Spark, MD) after incubation at 20 °C for 48 h. The experiment was carried out three times in duplicate.

2.11. 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 Student's *t*-test and were considered to be significant when $p \leqslant 0.05$ (Steel & Torrie, 1980).

3. Results and discussion

3.1. Changes in the pH of carp fillets during storage at 5 and 25 °C

Changes in the pH of carp fillets during storage at 5 and 25 °C are shown in Fig. 1 and Table 1, respectively. The initial pH values of the control and sample treated with EW(−)/EW(+)/1%(C + T) were 6.20 ± 0.08, and 6.14 ± 0.04, respectively. During storage at 5 °C, the pH values of the control and sample treated with EW(−)/EW(+)/1%(C + T) increased slightly. At 25 °C, the pH of the control and sample treated with EW(−)/EW(+)/1%(C + T) increased rapidly to reach values of 6.91 ± 0.27 and 6.49, respectively, on the second day of storage. These results are in agreement with previous study (Gelman et al., 1990). The lower pH of the samples treated with EW(−)/EW(+)/1%(C + T) during storage in comparison with the other samples may be due to their strong inhibitory effects on microbial growth, which delay the formation of basic nitrogen compounds.

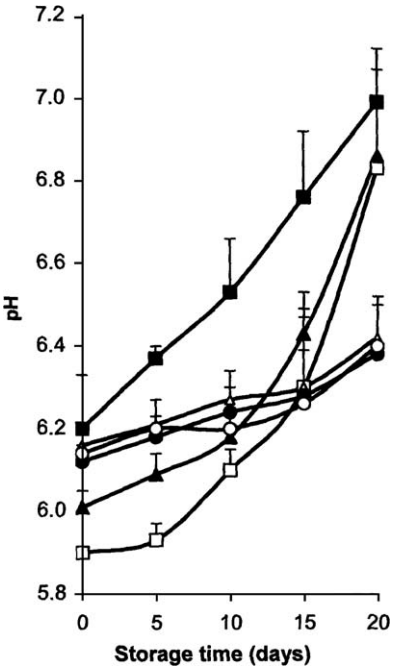

Fig. 1. Changes in the pH of carp fillets during storage at 5 °C. (–■–) control; (–□–) anodic solution [EW(+)]; (–▲–) cathodic solution EW(−), followed by anodic solution EW(+) [EW(−)/EW(+)]; (–△–) 1% (carvacrol + thymol) [1%(C + T)]; (–●–) EW(+)/1%(C + T); (–○–) EW(−)/EW(+)/1%(C + T). Results are the means of three replicates ± SD.

3.2. Changes in the VB-N of carp fillets during storage at 5 and 25 °C

Changes in the total volatile basic nitrogen (VB-N) of the carp fillets during storage at 5 and 25 °C are shown in Fig. 2 & Table 1, respectively. The VB-N of carp fillets ($p \leqslant 0.05$) decreased significantly from 11.4 ± 0.18 for the control sample to 6.8 ± 0.70 immediately after treatment (day 0) with EW (−)/EW(+)/1%(C + T). During storage at 5 °C, the VB-N of the control increased to reach the acceptable limit of fresh fish (30 mg 100 g^{-1}) on day 8. By contrast, the VB-N of samples treated with EW(−)/EW(+)/1%(C + T) remained below the limit until the end of the storage period (day 20). During storage at 25 °C, the VB-N increased rapidly and reached the acceptable limit at approximately 0.5 days for the control and 1.7 days for samples treated with EW(−)/EW(+)/1%(C + T). Extension of the acceptable VB-N limit by treating samples with EW(−)/EW(+)/1%(C + T), may be due to its strong inhibitory effects on microbial growth, which could delay the decomposition of carp fillets as compared with other treatments.

3.3. Changes in PV and TBA of carp fillets during storage at 5 and 25 °C

Lipid peroxidation, corresponding to the oxidative deterioration of polyunsaturated fatty acids in fish muscle,

Table 1
Changes in the pH, the VB-N (mg/100 g), the peroxide value (PV) (meq/kg), thiobarbituric acid (TBA) (mg MDA/kg),the organoleptic properties (overall) and the total microbial count (log, cfu/g) of carp fillets during storage at 25 °C

Parameters	Treatments					
	Control	EW(+)	EW(−)/EW(+)	1%(C + T)	EW(+)/1%(C + T)	EW(−)/EW(+)/1%(C + T)
pH						
0 day	6.20 ± 0.07^{a}	5.90 ± 0.10^{a}	6.01 ± 0.04^{a}	6.16 ± 0.02^{a}	6.12 ± 0.01^{a}	6.14 ± 0.04^{a}
1 day	6.64 ± 0.41^{c}	6.38 ± 0.18^{b}	6.40 ± 0.05^{b}	6.21 ± 0.04^{a}	6.19 ± 0.08^{a}	6.18 ± 0.04^{a}
2 day	6.91 ± 0.27^{c}	6.75 ± 0.14^{b}	6.78 ± 0.08^{b}	6.63 ± 0.07^{a}	6.53 ± 0.01^{a}	6.49 ± 0.22^{a}
VB-N						
0 day	11.4 ± 0.18^{c}	8.4 ± 0.32^{b}	7.0 ± 0.27^{a}	6.9 ± 0.21^{a}	6.8 ± 0.45^{a}	6.8 ± 0.70^{a}
1 day	41.5 ± 1.42^{e}	26.9 ± 0.85^{d}	24.9 ± 0.72^{c}	21.2 ± 0.66^{b}	20.7 ± 0.46^{b}	18.8 ± 1.13^{a}
2 day	69.3 ± 1.01^{d}	64.5 ± 1.50^{c}	60.1 ± 1.10^{c}	42.9 ± 1.30^{b}	40.6 ± 1.09^{b}	38.9 ± 0.80^{a}
PV						
0 day	1.0 ± 0.09^{a}	1.0 ± 0.20^{a}	1.0 ± 0.10^{a}	1.0 ± 0.10^{a}	1.0 ± 0.02^{a}	1.0 ± 0.26^{a}
1 day	4.6 ± 0.21^{c}	4.8 ± 0.20^{c}	4.0 ± 0.20^{c}	2.4 ± 0.04^{b}	2.8 ± 0.06^{b}	1.9 ± 0.15^{a}
2 day	8.5 ± 0.35^{c}	8.9 ± 0.12^{c}	$7.3 + 0.30^{b}$	6.63 ± 0.07^{b}	4.2 ± 0.13^{a}	2.9 ± 0.17^{a}
TBA						
0 day	0.15 ± 0.02^{a}	0.15 ± 0.03^{a}	0.14 ± 0.03^{a}	0.14 ± 0.02^{a}	0.14 ± 0.01^{a}	0.14 ± 0.02^{a}
1 day	0.34 ± 0.02^{d}	0.38 ± 0.01^{d}	0.26 ± 0.02^{c}	0.16 ± 0.01^{b}	0.21 ± 0.01^{c}	0.14 ± 0.01^{a}
2 day	0.80 ± 0.07^{e}	0.92 ± 0.10^{f}	0.63 ± 0.04^{d}	0.25 ± 0.04^{b}	0.30 ± 0.01^{c}	0.17 ± 0.11^{a}
Overall						
0 day	8.7 ± 0.18^{a}	8.8 ± 0.11^{a}	8.5 ± 0.11^{a}	8.4 ± 0.15^{a}	8.5 ± 0.17^{a}	8.4 ± 0.14^{a}
1 day	2.2 ± 0.48^{d}	3.3 ± 0.11^{c}	3.5 ± 0.27^{c}	5.2 ± 0.60^{b}	6.3 ± 0.18^{a}	$6.6 + 0.39^{a}$
2 day	1.2 ± 0.16^{d}	1.6 ± 0.28^{d}	1.9 ± 0.20^{c}	2.9 ± 0.15^{b}	3.8 ± 0.37^{a}	3.9 ± 0.38^{a}
Total count						
0 day	3.6 ± 0.07^{b}	2.2 ± 0.20^{a}	2.3 ± 0.10^{a}	2.6 ± 0.11^{a}	2.2 ± 0.07^{a}	2.1 ± 0.14^{a}
1 day	8.7 ± 0.16^{d}	7.6 ± 0.15^{c}	7.5 ± 0.40^{c}	6.4 ± 0.47^{b}	5.6 ± 0.52^{a}	5.4 ± 0.32^{a}
2 day	9.9 ± 0.24^{c}	9.8 ± 0.20^{c}	9.9 ± 0.15^{c}	8.3 ± 0.30^{b}	7.5 ± 0.23^{a}	7.2 ± 0.30^{a}

*EW(+), anodic solution; EW(−), cathodic solution; C, carvacrol; T, thymol. Values are the means of three replicates ± SD. Within a row, values annotated with different letters differ significantly ($p < 0.05$).

leads to the production of off-flavours and off-odours, thereby shortening the shelf-life of food (Ramanathan & Das, 1992). The peroxide value (PV) and TBA value are both well-established methods for determining oxidation products in fats and oils (Kulas & Ackman, 2001). Changes in the PV of carp fillets during storage at 5 and 25 °C are shown in Fig. 3 and Table 1, respectively. Immediately after treatment (day 0), there were no significant differences ($p > 0.05$) between the control and the sample treated with EW(−)/EW(+)/1%(C + T). During storage at 5 °C, the PV values of the control and sample treated with EW(−)/EW(+)/1%(C + T) increased from 1.0 (day 0) to 13.7 ± 0.24 and 4.20 ± 0.35 meq/kg, respectively, by the end of storage. During storage at 25 °C, the PV of the control and sample treated with EW(−)/EW(+)/1%(C + T) increased rapidly to reach values of 8.5 ± 0.35 and 2.9 ± 0.17 meq/kg, respectively, by the end of storage. These results are in agreement with those of Undeland, Hall, and Lingnert (1999), who found that the PV significantly increased after only 2 days in ice storage.

Changes in the TBA of carp fillets during storage at 5 and 25 °C are shown in Fig. 4 and Table 1, respectively. Immediately after treatment (day 0), there were no significant differences ($p > 0.05$) in the TBA values between the control and the sample treated with EW(−)/EW(+)/1%(C + T). However, the TBA value was significantly affected ($p < 0.05$) by the different treatments during storage. During storage at 5 °C, the TBA values of the control and sample treated with EW(−)/EW(+)/1%(C + T) increased from 0.14 (day 0) to 1.35 ± 0.04, and 0.26 ± 0.03 mg MDA/kg, respectively, by the end of storage (day 20). During storage at 25 °C, the TBA values of the control and sample treated with EW(−)/EW(+)/1%(C + T) increased to reach values of 0.80 ± 0.07 and 0.17 ± 0.11 mg MDA/kg, respectively, by the end of 1 storage (day 2). These results are in agreement with those of (Mansour & Khalil, 2000) who reported that the TBA values of carp fillets treated with a commercial antioxidant increased with the length of storage at 5 °C. From the results of our PV 4 and TBA measurements, we can conclude that the oxidation products were initially low and increased rapidly in the control samples during storage; however, a much slower rate of increase in oxidation products was observed in carp fillet samples treated with 1%(C + T) and EW(−)/EW(+)/1%(C + T). These results are also in agreement with those obtained by Hettiarachchy, Glenn, Gnanasambandam, and Johnson (1996) who found that treatments based on natural antioxidants control lipid oxidation in ground fish and meat products. The antioxidant effect of treatment with EW(−)/EW(+)/1%(C + T) due to: 1, carvacrol and thymol stems from the presence of a phenolic OH group, which has the ability to scavenge free radicals (Farag,

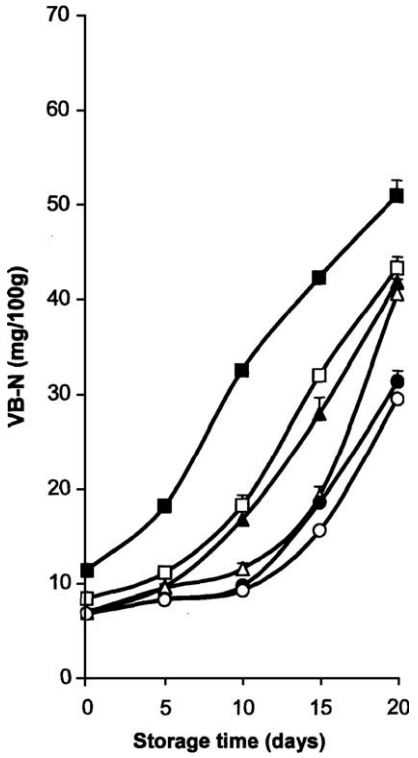

Fig. 2. Changes in the VB-N of carp fillets during storage at 5 °C. (–■–) control; (–□–) anodic solution [EW(+)]; (–▲–) cathodic solution EW(−), followed by anodic solution EW(+) [EW(−)/EW(+)]; (–△–) 1% (carvacrol + thymol) [1%(C + T)]; (–●–) EW(+)/1%(C + T); (–○–) EW(−)/EW(+)/1%(C + T). Results are the means of three replicates ± SD.

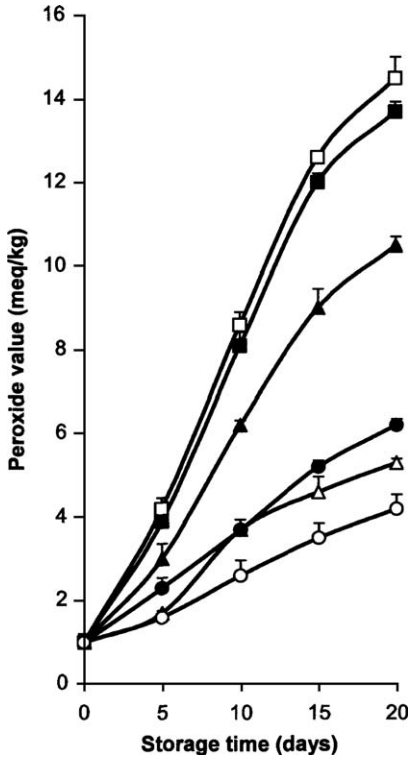

Fig. 3. Changes in the peroxide value (PV) of carp fillets during storage at 5 °C. (–■–) control; (–□–) anodic solution [EW(+)]; (–▲–) cathodic solution EW(−), followed by anodic solution EW(+) [EW(−)/EW(+)]; (–△–) 1% (carvacrol + thymol) [1%(C + T)]; (–●–) EW(+)/1%(C + T); (–○–) EW(−)/EW(+)/1%(C + T). Results are the means of three replicates ± SD.

Daw, Hewedi, & El-Baroty, 1989). 2, EW(−), likely due to its ability to break the chain reaction during the propagation phase of oxidation by scavenging or reacting with free radicals to produce nonradical compounds, through its high level of dissolved hydrogen and low level of dissolved oxygen (Miyashita et al., 1999).

3.4. Changes in organoleptic properties of carp fillets during storage at 5 and 25 °C

Sensory evaluation of fish quality is used to measure, analyse and interpret reactions to food characteristics perceived through the senses of colour, taste, flavour, odour and texture. Changes in the overall freshness and acceptability score of carp fillets during storage at 5 and 25 °C are shown in Fig. 5 and Table 1, respectively. The sensory evaluation demonstrated that, after subjecting the carp fillets to all treatments, including EW(−)/EW(+)/1%(C + T), there were no significant differences ($p > 0.05$) in any of the parameters, namely colour, taste, flavour, odour, and texture (freshness score), between the control and any of the other treatments. These results are in agreement with those of Deza, Araujo, and Garrido (2003) who reported that treatment with anodic electrolyzed water significantly reduced the amount 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 in all samples. Data obtained during storage at 5 °C indicated that, from day 10 onwards, there were significant differences ($p \leqslant 0.05$) between the control and all of the treatments; furthermore, the control had reached an unacceptable freshness score (6.0) by day 10. By contrast, samples treated with EW(−)/EW(+)/1%(C + T) had not reached an unacceptable score, even by the end of storage, and maintained the freshness score of carp fillets more than the other treatments. A possible explanation for this observation may lie in the results of the statistical analysis, which indicated that there is a significant relationship between the sensory score and other parameters (pH, VB-N, PV, TBA and total microbial count) of carp fillets during storage at 5 and 25 °C. The deterioration of carp fillets (freshness score) during storage was faster at 25 °C than at 5 °C; consequently, the freshness score of the control had decreased to 2.2 ± 0.48 by day 1. However, samples treated with EW(−)/EW(+) 1%(C + T) reached an unacceptable freshness score at approximately 1.7 days of storage.

3.5. Changes in the total microbial count of carp fillets during storage at 5 and 25 °C

Microbial growth is the major cause of food spoilage. Changes in the viable total microbial count of the carp

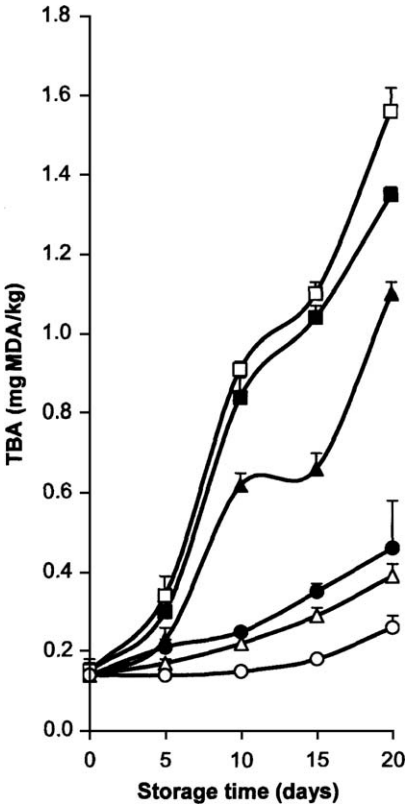

Fig. 4. Changes in the thiobarbituric acid (TBA) of carp fillets during storage at 5 °C. (–■–) control; (–□–) anodic solution [EW(+)]; (–▲–) cathodic solution EW(−), followed by anodic solution EW(+) [EW(−)/EW(+)]; (–△–) 1% (carvacrol + thymol) [1%(C + T)]; (–●–) EW(+)/1%(C + T); (–○–) EW(−)/EW(+)/1%(C + T). Results are the means of three replicates ± SD.

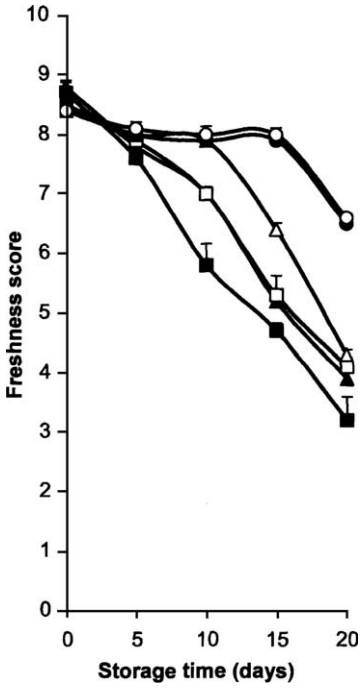

Fig. 5. Changes in the organoleptic properties of carp fillets during storage at 5 °C. (–■–) control; (–□–) anodic solution [EW(+)]; (–▲–) cathodic solution EW(−), followed by anodic solution EW(+) [EW(−)/EW(+)]; (–△–) 1% (carvacrol + thymol) [1%(C + T)]; (–●–) EW(+)/1%(C + T); (–○–) EW(−)/EW(+)/1%(C + T). Results are the means of three replicates ± SD.

fillets during storage at 5 and 25 °C are shown in Fig. 6 and Table 1, respectively. All treatments, including EW(−)/EW(+)/1%(C + T), gave a significant reduction ($p \leqslant 0.05$) in the total microbial count immediately after treatment (day 0) as compared with treatment with the control solution. The reduction caused by treatment with EW(−)/EW(+)/1%(C + T) was about 2.5 $\log_{10}$CFU/g as compared with control. Our results are in agreement with those of Barstad, Cutter, and Demirci (2001) who reported that the microbial count of *Listeria* in artificially inoculated chicken frankfurters and ham slices was significantly reduced by treatment with EW (−)/EW(+), thereby extending the shelf-life to 7 days at a refrigerated temperature. Subsequently, the total microbial count of the carp fillets increased during storage at 5 and 25 °C. The total microbial count of the control samples exceeded the acceptable limit (10^6 CFU/g) after approximately 4 days at 5 °C and 0.3 days at 25 °C. By contrast, the total microbial count in samples treated with EW(−)/EW(+)/1%(C + T) increased slowly as compared with the other samples and reached the acceptable limit at roughly 16 and 1.3 days at 5 and 25 °C, respectively. The possible explanation is that EW(+) is considered to protect food material by damaging the outer cell membrane of bacteria and penetrating

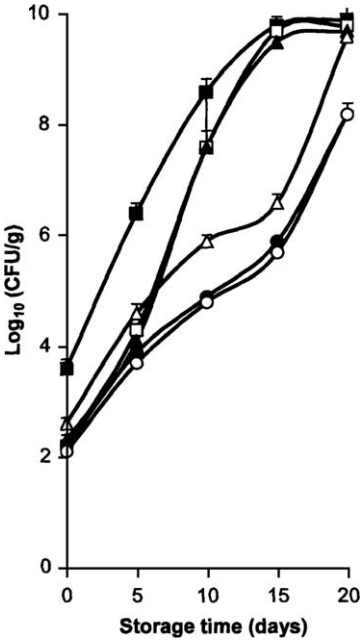

Fig. 6. Changes in the total microbial count of carp fillets during storage at 5 °C. (–■–) control; (–□–) anodic solution [EW(+)]; (–▲–) cathodic solution EW(−), followed by anodic solution EW(+) [EW(−)/EW(+)]; (–△–) 1% (carvacrol + thymol) [1%(C + T)]; (–●–) EW(+)/1%(C + T); (–○–) EW(−)/EW(+)/1%(C + T). Results are the means of three replicates ± SD.

the cytopasmic membrane, thereby causing the inactivation of cytoplasmic enzymes by hypochlorous acid (HOCl), which produces OH and Cl radicals (Kiura et al., 2002). Similarly, treatment of carp fillets with the hydrophobic compounds [1%(C + T)] are, likely to mediate their antimicrobial effects by dissolving the hydrophobic domain of the bacterial membrane, in addition, they disintegrate the outer membrane, increase the permeability of the cytoplasmic membrane to ATP, and have been shown to cause damage and lead to death in bacterial cells (Burt, 2004; Harpaz et al., 2003; Helander et al., 1998).

4. Conclusion

In this study, we have established a new preservation technique based on the pre-treatment of carp fillets, first with EW solutions and then with essential oil compounds. Our results indicate that pre-treatment with EW(−)/EW(+) strongly increases the efficacy of subsequent treatment with 1%(C + T) and can extend the shelf-life of carp fillets from 4 day for control sample, or 12 days when only 1%(C + T) is used, to 16 days when EW(−)/EW(+)/1%(C + T) is used at 5 °C. Thus, this treatment protocol might be a powerful technology, to maintain the quality and extend the shelf-life of fish fillets during refrigerated storage.

Acknowledgement

The first author expresses his thanks to the Ministry of Education, Science and Culture (MONBUKAGAKUSHO) for providing the opportunity to study in Japan.

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