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PRESERVATION EFFECT OF ELECTROLYZED WATER AND ESSENTIAL OIL COMPOUNDS IN REFRIGERATED TUNA PATTIES

BY

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ABSTRACT

Tuna (*Thunnus obesus*) meat was treated with anodic electrolyzed NaCl solution EW(+) and addition of 0.5% [0.25% carvacrol (C) + 0.25% thymol (T)] as well as its effect on the preservation of pattie during storage at 5°C was evaluated. Addition of 0.5% (C+T) and combined treatment of EW(+) and 0.5% (C+T) kept volatile basic nitrogen of patties at lower level and also significantly repressed an increase in peroxides and thiobarbituric acid-reactive substances during storage. Combined treatment of EW(+) and 0.5% (C+T) gave the strongest overall inhibition of microbial growth in patties during storage. There were significant differences between control pattie and other treated patties in taste, flavour and overall acceptability.

Key words: Electrolyzed water, antimicrobial; antioxidant, carvacrol, thymol, tuna, fish pattie, shelf life

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INTRODUCTION

Seafood products have attracted considerable attention as a source of high amounts of important nutritional components to the human diet (Ackman, 1989). Burgers and meatballs are very common and well-liked products in almost every country today. Chicken and fish burger patties or balls also became widespread around the world, especially in Turkey. Various methods are used to manufacture ready-made products from mince, such as burger patties (Kose *et al.*, 2006). On the other hand, Akkus *et al.* (2004) showed that the shelf life of the fish balls prepared from boiled and raw anchovy was 9 days at 4±1°C. Metin *et al.* (2002) found that trout burgers wrapped in gas barrier film had a shelf life of 21 days under cold storage.

Electrolyzed oxidizing water has generated much interest as a disinfectant in the food industry (Venkitanarayanan *et al.*, 1999; Park *et al.*, 2002). The electrolyzed oxidizing water is prepared through the electrolysis of a NaCl solution in the anode side of an instrument in which the anode and cathode are separated by an ion-permeable membrane, and so also named as EW(+). EW(+) is characterized by a low pH ranging from 2.3 to 2.7 and high oxidation-reduction potential (ORP) ranging from +1050 to +1100 mV, and has been reported to have strong bactericidal effects against many Gram-positive and Gram-negative pathogenic bacteria, such as *Escherichia coli* O157:H7 (Kim *et al.*, 2000; Venkitanarayanan *et al.*, 1999), *Listeria monocytogenes* (Kim *et al.*, 2003; Venkitanarayanan *et al.*, 1999), *Bacillus cereus* (Buck *et al.*, 2002), and *Salmonella* species (Fabrizio *et al.*, 2002; Venkitanarayanan *et al.*, 1999). In addition, it could disinfect hepatitis B virus and human immunodeficiency virus (Morita *et al.*, 2000) and reduce germinations of many fungal species (Buck *et al.*, 2002).

Application of essential oil is a very attractive method for controlling postharvest diseases. Production of essential oils by plants is believed to be predominantly a defence mechanism against pathogens and pests and indeed, essential oils have been shown to possess antimicrobial and antifungicidal properties (Ahmet *et al.*, 2005). Essential oils and their components are gaining increasing interest because of their relatively safe status, their wide acceptance by consumers, and their exploitation for potential multi-purpose functional use (Ormancey *et al.*, 2001). Essential oil compounds, such as carvacrol and thymol, will prevent the microbial and chemical deterioration when added to food (Burt, 2004; Ultee *et al.*, 1999). The antimicrobial and antioxidant effects of these phenolic compounds can be enhanced by combining with other natural preservatives (Yamazaki *et al.*, 2004). Carvacrol (2-methyl-5-(1-methylethyl)-phenol) is a major component of the essential oil of oregano (Rodrigues *et al.*, 2004) and used as a flavouring agent in baked goods, sweets and beverages. Carvacrol has inhibitory and biocidal effects on a range of bacteria including *Escherichia coli*, *Bacillus cereus*, *Listeria monocytogenes*, *Salmonella enterica*, *Campylobacter jejuni* and *Lactobacillus sakei* (Ultee *et al.*, 1999; Gill & Holley, 2006). Carvacrol-containing essential oils are also biostatic or biocidal against many bacteria, yeasts and fungi, and consequently have attracted considerable research attention as potential food presservatives (Burt, 2004). Thymol (5-methyl-2-(1-methylethyl)-phenol), is the major compound found in thyme (Pina-Vaz *et al.*, 2004), had been commercially available as part of a mouthwash for more than hundred years. Thymol is active against *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Campylobacter jejuni*, *Salmonella enterica* (Chiasson *et al.*, 2004), and also against insects.

The aim of the present study was to evaluate the effect of treatment of tuna (*Thunnus obesus*) meat with anodic electrolyzed water EW(+) before mincing process in the pattie manufacture, and addition of essential oil (carvacrol + thymol) on the preservation of pattie mixture during refrigerated storage.

MATERIAL AND METHODS

Tuna

Frozen blocks of Big-eye tuna (*Thunnus obesus*) edible in raw were purchased from a local market, Hakodate, Japan, and stored at –65°C until use.

Chemicals and food additives

Carvacrol and thymol were obtained from Kanto Chemical (Tokyo, Japan). The other ordinary chemicals are analytical grade supplied from Wako Pure Chemical Industries (Osaka, Japan). Starch, spices and vegetable oil were bought from the local market, Hakodate, Japan.

Preparation of anodic electrolyzed water

Electrolyzed NaCl solution was prepared using a two-compartment batch-scale electrolysis apparatus (JED-020, Aoi Engineering, Kannami, Japan). After electrolysis of 0.1% NaCl solution for 10 min, the electrolyzed solution, EW(+), with a pH of 2.2, 41 ppm of available chlorine and (Oxidation Reduction Potential) ORP +1140 mV was obtained in the anodic compartment. Solution was prepared immediately before use.

Preparation of fish patties

Fish patties were prepared according to Chandrasekhar and Mohite (1978). Tuna blocks were washed, cut to small pieces, rewashed to remove any blood traces, and then drained for a few minutes. Tuna pieces were minced under hygienic conditions and then food additives were added and mixed thoroughly with the minced tuna meat. Once, the homogeneous mixture become smooth, 50 g of mixture was placed between two sheets of transparent polyethylene films and pressed gently to give the burger texture. To evaluate the effect of anodic electrolyzed water, the small pieces of tuna fish were immersed in EW(+) for 15 min before minced. To evaluate the effect of 0.5% essential oils, 0.25% carvacrol (C) and 0.25% thymol (T) were mixed with minced fish meat and other additives. Each three pieces of fish patties were packed and stored at 5°C for 20 days. Samples were analyzed from each treatment at 5 day-intervals. Following recipe was used in the preparation of fish patties; tuna minced meat 75%, vegetable oil 9%, starch 8%, sodium chloride 2.3%, sodium bicarbonate 0.4%, polyphosphate 0.3%, onion 2.5%, garlic 0.5% and spice mixture 2% (black pepper 42%, cumin 23%, coriander 5%, cubeb 7.5%, cardamom 10%, red pepper 3.5%, ginger 5% and clove 4%).

pH determination

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

Determination of volatile basic nitrogen (VB-N)

VB-N was determined according to the micro-diffusion method of (Botta *et al.*, 1984) which measures the content of ammonia, trimethylamine (TMA), dimethylamine (DMA) and other basic nitrogenous compounds associated with fish pattie spoilage.

Peroxide value determination

The peroxide value was expressed in units of meq/kg of sample was measured according to (Egan *et al.*, 1981).

Thiobarbituric acid (TBA) test

Thiobarbituric acid-reactive substances (TBARS) were determined colourimetrically according to the procedure described by Siu and Draper (1978). The absorbance at 532 nm was measured with a spectrophotometer (U-2000, Hitachi, Tokyo, Japan). The TBA value was expressed in units of mg malondialdehyde (MDA)/kg sample.

Microbial analyses

Five gms of fish pattie samples 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 the saline solution, and duplicate samples (0.1 ml) of each dilution were spread on Plate Count Agar (Difco, Spark, MD, USA). The total aerobic count (TBC) was determined after incubation at 20°C for 48 h. The experiment was carried out three times in duplicate.

Sensory evaluation of fish patties

The Sensory properties of fish patties during storage at 5°C were assessed by a group of 10 trained panellists from the Laboratory staff. Patties were cooked in an electrical fryer pan using sunflower oil at 150°C for 5 min before evaluation. The panellists were asked to evaluate for (colour, tenderness, juiciness, taste, flavour, and overall acceptability) of the pattie samples according to 9-point headonic scale indicating decreasing freshness (Larmond, 1974). 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 patties samples. The data from the ten independent panellists were pooled and statistically analysed.

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 t-test and were considered to be significant when $p \leq 0.05$ (Steel & Torrie, 1980).

RESULTS AND DISCUSSION

Changes in VB-N and pH of tuna patties during storage at 5°C

The changes in total VB-N of fish patties stored at 5°C are shown in Fig. 1. The initial VB-N of control fish patties was 7.31±0.93 mg/100g. In fish patties that tuna meat had been dipped for 15 min in EW(+) before mincing and the 0.5% (C+T) was added to pattie mixture, the VB-N value was 5.04±0.14 mg/100g. During storage at 5°C, differences in VB-N could be observed between the treated and control pattie samples. The change in VB-N value was at a higher rate in control pattie. In this study, the control patties reached the acceptable limit of VB-N (30 mg/100g) after 14 days, whereas the patties made of EW(+)-dipped tuna meat reached this limit in 18 days. Patties added with 0.5% (C+T) and treated with EW(+) and 0.5% (C+T) did not reach this limit until the end of storage period (20 days). Harpaz *et al.* (2003) reported that a level of 30 mg/100g VB-N is considered to be the upper limit, above which fishery products are considered unfit for human consumption.

Changes in pH of tuna patties during storage at 5°C are shown in Fig. 2. The initial pH values of control patties and patties prepared with EW(+) and 0.5% (C+T) were 6.50 and 6.42, respectively. During storage at 5°C, the pH values of control and all treated patties samples slightly increased until 15 days of storage then slightly decreased. The maximum pH value was 6.72 in control patties and 6.67 in patties prepared with EW(+) and 0.5% (C+T) by the day 15 of storage ($p \leq 0.05$).

Changes in peroxide and TBA values of tuna patties during storage at 5°C

Changes in the peroxide value of tuna fish patties during storage at 5°C are shown in Fig. 3. Immediately after processing patties (day 0), there were no significant differences between control and treated patties with EW(+) and/or 0.5% (C+T). Data obtained during storage at 5°C indicated that, from day 5 onwards, there were significant differences between them until the end of storage. Control patties reached an unacceptable freshness score (6.0) by day 10. By contrast, patties prepared with EW(+) and 0.5% (C+T) did not reach an unacceptable score, even by the end of storage (day 20), and kept higher freshness score than the other pattie samples. Mahmoud *et al.* (2006) had observed antioxidant efficacy of essential oils and their combination with EW(+) treatment on carp flesh.

Changes in the TBA value of tuna patties during storage at 5°C are shown in Fig. 4. The TBA index is widely used as an indicator of degree of lipid oxidation. The TBA values in this study were found to be quite low for the all pattie samples over the storage period. However, the TBA value during storage was significantly affected by the different treatments of patties. The initial TBARS of control pattie was 0.24 mg MDA/kg. During the storage, TBARS gradually increased to reach 0.75 for the control by the end of storage. There were significant differences in the TBA values between the control pattie and the prepared patties with EW(+) and 0.5% (C+T) during all the stored period. Patties prepared with 0.5% (C+T) showed a stronger inhibitory effect of the lipid oxidation. The presence of the –OH group in carvacrol and thymol, which has a strong ability to scavenge the free radical during the propagation phase of oxidation may account for this finding (Farg *et al.,* 1989). While, pattie samples prepared with EW(+) and 0.5% (C+T) showed the strongest inhibition effect for lipid oxidation of all the pattie samples, possibly related to either combined effects or stronger individual abilities.

Changes in (TBC) total bacterial count of tuna patties during storage at 5°C

Changes in TBC of tuna patties during storage at 5°C are shown in Fig. 5. Treatment of fish meat with EW(+) and/or addition of 0.5% (C+T) to the pattie mixture resulted in significant reduction ($p \leq 0.05$) in the initial microbial counts of pattie samples as compared with the control untreated. Kim *et al.* (2000, 2003), and Ultee *et al.* (1999) reported that EW (+), carvacrol and thymol had strong bactericidal effect against most bacteria including pathogenic bacteria such as *Aeromonas*, *Bacillus cereus*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella* and *Staphylococcus*. Recently, Liao *et al.* (2007) reported that the higher ORP of electrolyzed oxidizing water could damage the outer and inner membranes of *E. coli* O157:H7, thus leading to the inactivation. The inactivation mechanism was proposed that: ORP could affect and damage the redox state of GSSG/ 2GSH first, penetrated the outer and inner membranes of *E. coli* O157:H7, and then resulted in the necrosis of the bacterium. During storage at 5°C, the TBC of control patties considerably increased and reached around 10^6 CFU/g after 5 days. By contrast, the TBC of patties added with 0.5% (C+T) reached the same value after 15 days and that of patties prepared with EW(+) and 0.5% (C+T) reached this value after 20 days at 5°C. In addition, Harpaz *et al.* (2003) observed that treatment with 0.05% oregano and/or thyme extended the shelf-life of Asian sea bass fish to 33 days at 0–2°C, as compared to 12 days for the control. Carvacrol and thymol are hydrophobic compounds that can dissolve in the hydrophobic domain of cytoplasmic membrane. They cause to increase in ATP permeability and results in lethal damage to the bacterial cell (Burt, 2004; Ultee *et al.*, 1999).

Sensory evaluation of tuna patties stored at 5°C

Table 1 summarizes the sensory properties of tuna patties during storage at 5°C. No significant differences were detected between control and the other treated patties before storage in all sensory properties and during storage in tenderness and juiciness properties. During storage at 5°C, a gradual decrease for all the sensory parameters of tuna patties was observed. On the fifth day of storage, no significant difference among control and other treated patties at 5°C. From day 10 onwards there was significant differences ($p \leq 0.05$) between control patties and other treated fish patties in taste, flavour and overall acceptability properties, and control patties reached the unacceptable score (5), while the patties samples added with 0.5% (C+T) and samples prepared with EW(+) and 0.5% (C+T) did not reach to unacceptable score until the end of storage (20 days).

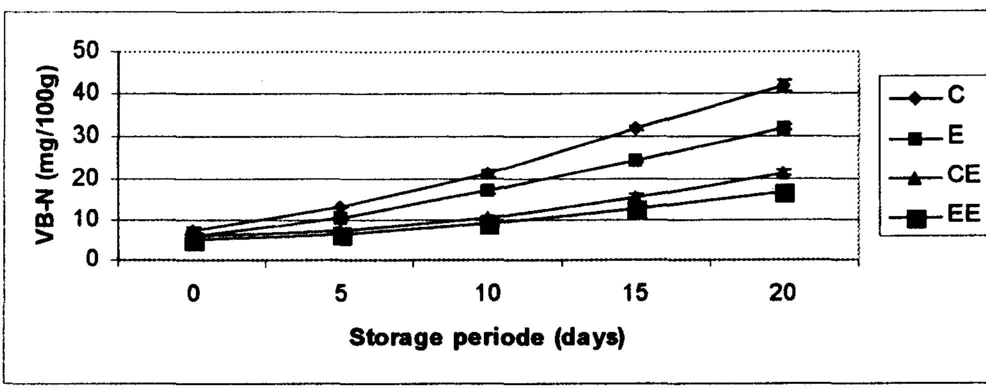

Figure (1): Changes in VB-N of tuna patties during storage at 5°C.
Symbols: C, control pattie; E, pattie treated with EW(+); CE, pattie added with 0.5% (Carvacrol+Thymol) [0.5% (C+T)]; EE, pattie prepared with EW(+) and 0.5% (C+T). Results are shown as the means of three replicates ± SD.

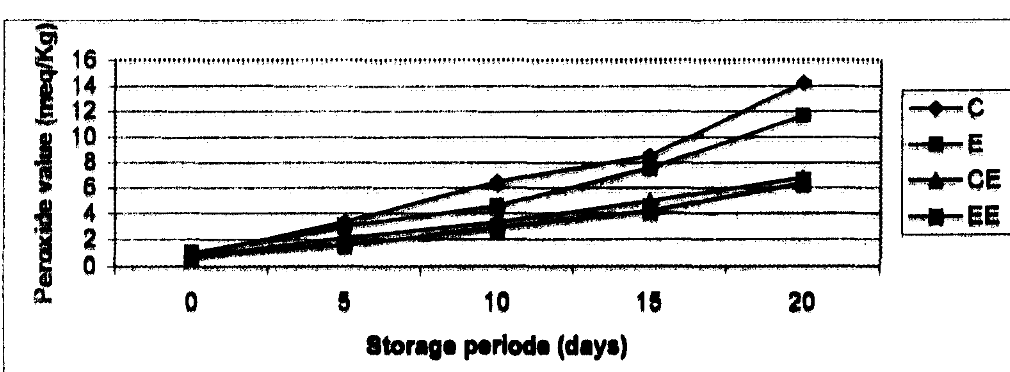

Figure (2): Changes in pH of tuna patties during storage at 5°C.
Symbols: C, control pattie; E, pattie treated with EW(+); CE, pattie added with 0.5% (Carvacrol+Thymol) [0.5% (C+T)]; EE, pattie prepared with EW(+) and 0.5% (C+T). Results are shown as the means of three replicates ± SD.

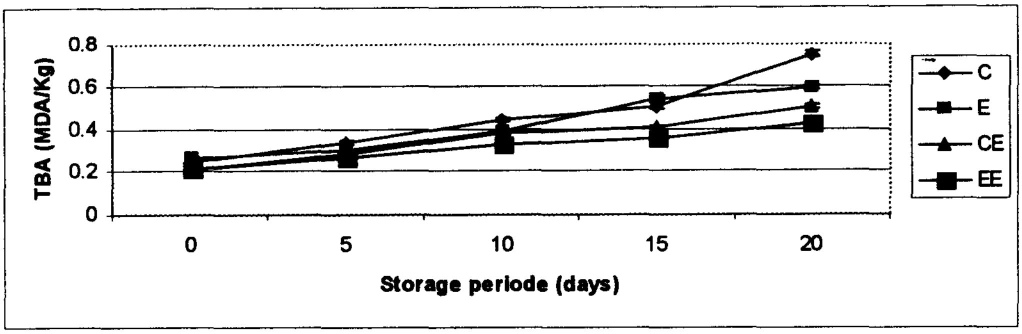

Figure 3 Changes in peroxide value of tuna patties during storage at 5°C.
Symbols: C, control pattie; E, pattie treated with EW(+); CE, pattie added with 0.5% (Carvacrol+Thymol) [0.5% (C+T)]; EE, pattie prepared with EW(+) and 0.5% (C+T). Results are shown as the means of three replicates ± SD.

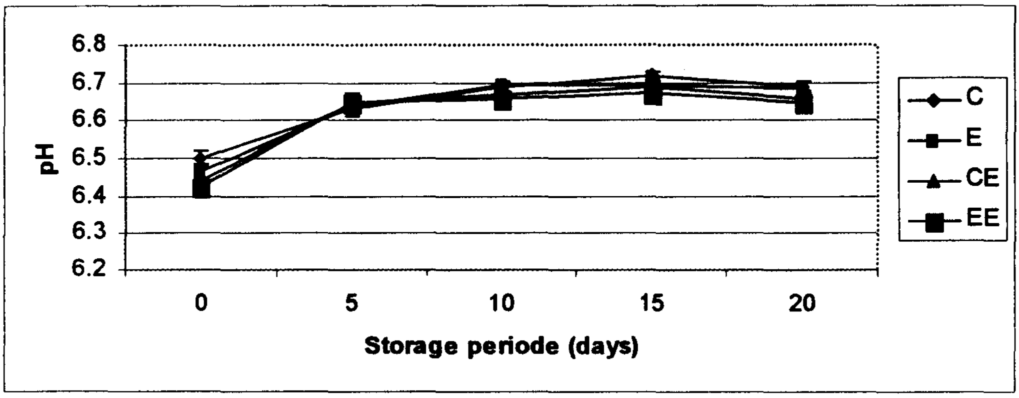

Figure (4): Changes in TBA value of tuna patties during storage at 5°C.
Symbols: C, control pattie; E, pattie treated with EW(+); CE, pattie added with 0.5% (Carvacrol+Thymol) [0.5% (C+T)]; EE, pattie prepared with EW(+) and 0.5% (C+T). Results are shown as the means of three replicates ± SD.

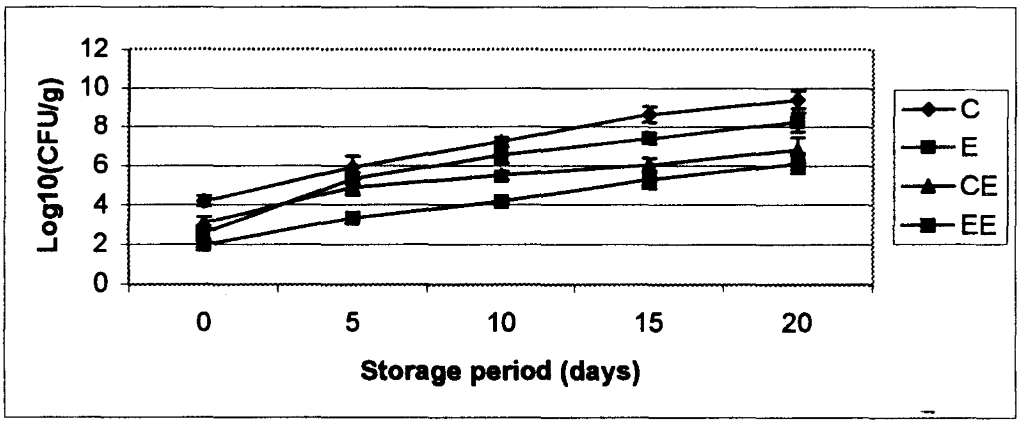

Figure (5): Changes in TBC of tuna patties during storage at 5°C.
Symbols: C, control pattie; E, pattie treated with EW(+); CE, pattie added with 0.5% (Carvacrol+Thymol) [0.5% (C+T)]; EE, pattie prepared with EW(+) and 0.5% (C+T). Results are shown as the means of three replicates ± SD.

Table (1): Sensory scores of tuna patties stored at 5°C.

Sensory property	Pattie sample*	Storage period (days)				
		0	5	10	15	20
Colour	Control	7.8 ± 0.45^{a}	7.6 ± 0.55^{a}	7.1 ± 0.22^{a}	6.8 ± 0.45^{b}	6.5 ± 0.35^{b}
	EW(+)	7.9 ± 0.22^{a}	7.6 ± 0.24^{a}	7.5 ± 0.50^{a}	7.0 ± 0.35^{ab}	6.7 ± 0.45^{ab}
	0.5% (C+T)	7.9 ± 0.27^{a}	7.8 ± 0.45^{a}	7.8 ± 0.45^{a}	7.1 ± 0.22^{ab}	7.0 ± 0.50^{ab}
	EW(+)/ 0.5% (C+T)	8.1 ± 0.22^{a}	8.0 ± 0.35^{a}	7.8 ± 0.45^{a}	7.5 ± 0.35^{a}	7.5 ± 0.50^{a}
Tenderness	Control	7.9 ± 0.22^{a}	7.7 ± 0.45^{a}	7.2 ± 0.45^{a}	7.2 ± 0.45^{a}	6.7 ± 0.45^{a}
	EW(+)	8.2 ± 0.45^{a}	8.0 ± 0.35^{a}	7.6 ± 0.55^{a}	7.2 ± 0.45^{a}	6.8 ± 0.27^{a}
	0.5% (C+T)	8.1 ± 0.22^{a}	7.6 ± 0.55^{a}	7.4 ± 0.55^{a}	7.2 ± 0.45^{a}	7.0 ± 0.35^{a}
	EW(+)/ 0.5% (C+T)	8.2 ± 0.45^{a}	7.6 ± 0.55^{a}	7.4 ± 0.55^{a}	7.2 ± 0.45^{a}	7.1 ± 0.22^{a}
Juiciness	Control	7.8 ± 0.45^{a}	7.4 ± 0.55^{a}	7.4 ± 0.55^{a}	6.8 ± 0.45^{a}	6.0 ± 0.35^{a}
	EW(+)	7.9 ± 0.22^{a}	7.4 ± 0.55^{a}	7.4 ± 0.55^{a}	7.2 ± 0.45^{a}	6.5 ± 0.50^{a}
	0.5% (C+T)	8.0 ± 0.71^{a}	7.8 ± 0.27^{a}	7.6 ± 0.55^{a}	7.4 ± 0.55^{a}	6.8 ± 0.45^{a}
	EW(+)/ 0.5% (C+T)	8.3 ± 0.45^{a}	7.8 ± 0.45^{a}	7.6 ± 0.55^{a}	7.6 ± 0.55^{a}	7.2 ± 0.45^{a}
Taste	Control	7.9 ± 0.22^{a}	7.2 ± 0.67^{a}	5.7 ± 0.45^{b}	4.6 ± 0.55^{c}	4.1 ± 0.55^{b}
	EW(+)	8.1 ± 0.22^{a}	7.6 ± 0.22^{a}	6.8 ± 0.76^{a}	6.1 ± 0.22^{b}	4.8 ± 0.45^{b}
	0.5% (C+T)	8.2 ± 0.27^{a}	7.6 ± 0.89^{a}	7.4 ± 0.55^{a}	7.0 ± 1.00^{ab}	6.7 ± 0.84^{a}
	EW(+)/ 0.5% (C+T)	8.2 ± 0.45^{a}	7.8 ± 0.84^{a}	7.4 ± 0.55^{a}	7.4 ± 0.55^{a}	7.3 ± 0.45^{a}
Flavour	Control	8.0 ± 0.35^{a}	7.4 ± 0.55^{a}	5.4 ± 0.55^{b}	4.3 ± 0.45^{c}	4.0 ± 0.50^{b}
	EW(+)	8.0 ± 0.71^{a}	7.6 ± 0.55^{a}	6.2 ± 0.45^{b}	5.6 ± 0.55^{b}	4.5 ± 0.50^{b}
	0.5% (C+T)	8.4 ± 0.89^{a}	7.8 ± 0.84^{a}	7.4 ± 0.55^{a}	7.0 ± 0.71^{a}	7.0 ± 0.50^{a}
	EW(+)/ 0.5% (C+T)	8.6 ± 0.55^{a}	7.9 ± 0.74^{a}	7.6 ± 0.55^{a}	7.4 ± 0.55^{a}	7.4 ± 0.55^{a}
Overall acceptability	Control	8.1 ± 0.55^{a}	7.0 ± 1.00^{a}	5.5 ± 0.50^{b}	4.2 ± 0.45^{c}	3.6 ± 0.55^{c}
	EW(+)	8.2 ± 0.45^{a}	7.4 ± 0.55^{a}	6.7 ± 0.45^{a}	6.2 ± 0.45^{b}	5.1 ± 0.55^{b}
	0.5% (C+T)	8.4 ± 0.55^{a}	7.6 ± 0.55^{a}	7.4 ± 0.55^{a}	7.0 ± 1.00^{ab}	6.8 ± 0.45^{a}
	EW(+)/ 0.5% (C+T)	8.5 ± 0.50^{a}	7.6 ± 0.89^{a}	7.6 ± 0.55^{a}	7.4 ± 0.55^{a}	7.0 ± 1.00^{a}

* Pattie sample used were "control" without any treatment, "EW(+)" prepared from EW(+)-treated flesh, "0.5% (C+T)" added with 0.25% carvacrol and 0.25% thymol, and "EW (+)/0.5% (C+T)" prepared with EW(+)-treated flesh and added woth 0.5% (C+T).
Data are expressed as mean ± SD from the ten independent panelists.
Different superscript letters mean significant differences between samples ($p \leq 0.05$).

CONCLUSION

This result shows that essential oils, carvacrol and thymol, have effective anti-microbial and antioxidant activities in tuna patties during refrigerated storage. Also, it was demonstrated that tuna treated with EW(+) before mincing process in pattie manufacture led to a reduction in the microbial count, consequently extending the shelf-life of the patties. These findings could contribute to the quality and safety of ready-made seafood products.

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التأثير الحافظ للماء المحلل كهربيا ومركبات الزيوت العطرية في باتيه سمك التونة المبرد.

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تهدف هذه الدراسة لتقييم تأثير معاملة لحم أسماك التونة بالمحلول الموجب الناتج من تحليل كلوريد الصوديوم كهربيا ثم إضافة ٠,٥% مخلوط مركبات الزيوت العطرية يحتوى على ٠,٢٥ % كارفا كرول و٠,٢٥ % ثيمول على حفظ باتيه أسماك التونة أثناء التخزين على ٥° م.

وقد أوضحت النتائج المتحصل عليها أن المعاملة بإضافة مخلوط الزيوت العطرية والمعاملة التي تشمل إضافة مخلوط الزيوت العطرية بعد معاملة لحم أسماك التونة بالمحلول الموجب الناتج من تحليل كلوريد الصوديوم كهربيا حافظت على معدل منخفض من محتوى النيتروجين القاعدي المتطاير في باتيه السمك وكذلك أثرت معنويا على خفض الزيادة في رقم البيروكسيد ورقم حامض الثيوباربيتيوريك أثناء التخزين. كما أدت هذه المعاملة لاختزال العدد الميكروبي في بداية التخزين وأعطت تثبيط قوى للنمو الميكروبي أثناء التخزين.

نتائج التقييم الحسي أظهرت فرق معنوي بين معاملة الكنترول وباقي المعاملات في الطعم والنكهة والتقبل الكلى من اليوم العاشر للتخزين فصاعدا وعندها وصلت العينة الكنترول لدرجة عدم القبول بينما عينات الباتيه المعاملة بإضافة مخلوط الزيوت العطرية بعد معاملة لحم أسماك التونة بالمحلول الموجب الناتج من تحليل كلوريد الصوديوم كهربيا لم تصل لدرجة عدم القبول حتى نهاية مدة التخزين (٢٠ يوما).