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Effect of Slightly Acidic Electrolyzed Water Immersion at Different Frequencies on Quality of Raw Chicken Legs

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Slightly acidic electrolyzed water (SAEW) is used as a disinfectant for raw chicken meat. Because its volume for a single immersion exceeds 10 times the weight of meat, a large amount of wastewater is generated. Importantly, a higher frequency of immersion is believed to reduce microbial contamination. The objective of this study was to investigate the effect of SAEW immersion at different frequencies on the disinfection and quality of raw chicken legs, thereby possibly limiting the usage of SAEW. Immersion for 1, 3, and 5 times, with a 7:1 SAEW:meat ratio, and duration of 15 min was tested. Meat quality was evaluated based on total aerobic bacteria, *Enterobactericeae*, total volatile basic nitrogen, thiobarbituric acid reactive substances, and color. A higher immersion frequency lowered the numbers of total aerobic bacteria and Enterobacteriaceae. Moreover, two immersions with a SAEW:meat ratio of 4:1 and a total immersion time of 6 min reduced the bacterial load as effectively as a single 15-min immersion with a SAEW:meat ratio of 7:1. Higher frequencies of SAEW immersion also resulted in lower total volatile basic nitrogen and lipid oxidation after 0 or 3 days of storage. They did, however, magnify the change in color, resulting in brighter meat. Overall, SAEW treatments with two to five immersions can improve the quality of raw chicken legs and reduce wastewater generation.

Key words: Keywords:chicken, frequencies, immersion, microorganism, quality, SAEW

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Introduction

Chicken was the most commonly consumed meat protein in 2020 worldwide, because poultry meat has lower prices, maintains a consistent quality, is suitable for different diets, and has a higher protein/fat ratio, its consumption has grown in virtually all countries. The Food and Agriculture Organization forecasts that poultry will represent 41% of meat sources by 2030[1]. Such high consumption of chicken meat represents a food safety risk. Several pathogens, including *Salmonella enterica*, *Clostridium perfringens*, *Campylobacter jejuni,* and *Staphylococcus aureus*, are associated with foodborne diseases in chickens. Therefore, various techniques have been used to reduce bacterial contamination and extend the shelf life of chicken meat[2,3]. However, these techniques have become a concern for consumers[1], as they employ numerous chemicals and present limited effectiveness[3–5].

An alternative method to reduce bacterial contamination in chicken meat is electrolyzed water[4–8]. Electrolyzed water contains mostly hypochlorous acid (HClO), which has a bactericidal effect[9]. Rahman *et al.*[5] showed that slightly acidic electrolyzed water (SAEW) exerted an antimicrobial effect similar to that of acidic electrolyzed water (AEW). SAEW has important advantages, such as low activated chlorine concentration, good stability, and lower corrosion[5,10]. SAEW can be obtained from electrolysis of NaCl, HCl or HCl with NaCl, thereby resulting in 10–30 mg/L total available chlorine (TAC)[5,11]. Wang *et al.*[11] showed that SAEW with 30 mg/L TAC had the same effect as AEW with 60 mg/L TAC, indicating that SAEW might improve microbial safety of chickens without being so harsh a treatment.

Previous studies have assessed electrolyzed water with single immersions of 3, 10, or 30 min at a ratio of more than 10 times the weight of chicken. Such usage generates abundant wastewater. To achieve the United Nations Sustainable Development Goals, it is necessary to reduce the use of disinfection water. We hypothesized that a higher number of washing sessions (frequency) could lower microbial contamination and, at the same time,

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lower the amount of disinfection water required. Therefore, the objective of this research was to study the effect of SAEW immersion at different frequencies on the quality of raw chicken legs and the consequent possibility of reducing SAEW usage.

Materials and Methods

Sample preparation

Twelve non-disinfected birds were shipped in a cool box from the Sanwa Oyadori factory to the laboratory within 3 h of slaughtering. As the chickens were meant for slaughter in any case, the experiments conducted in this study did not require ethical approval. In the laboratory, the animals were quartered to obtain chicken legs, which were stored at -20 °C and then thawed at 4 °C overnight prior to experimentation.

Preparation of treatment solution

SAEW with a pH of 5.8–6.2, oxidation-reduction potential (ORP) of 890–910 mV, and TAC of 30–33 ppm was used. SAEW was produced by electrolysis of a dilute 7% HCl solution in tap water (Purester, Morinaga Milk Industry Co., Ltd).

Disinfection procedure

Each filleted chicken leg (120–150 g) was fully immersed 1 (T1), 3 (T3) or 5 (T5) times for 15 min in SAEW at a total SAEW:meat ratio of 7:1 (w/w) (Table 1). Such ratio was previously shown to reduce total bacteria by more than 10:1. At each frequency, the chicken was lifted from the liquid for less than 2 min and immersed in new SAEW. As a negative control (T0), chicken legs were stored for 15 min. To evaluate SAEW, ORP was measured before and after treatment. Microbiological analyses were carried out for each immersion, and all treatments were performed on a cleaning bench at room temperature. After treatment, the samples were drained at 4 °C for 15 min, packed with sterilized packaging on a cleaning bench, and stored for 3 days at 4 °C in a refrigerator. The expiration time of stored fresh chicken is usually 1–2 days. Microbiological, chemical, and physical properties were analyzed after storage for 0 and 3 days.

Microbiological properties

Filleted chicken legs were swabbed three times across an area of 5 × 5 cm with a sterilized flocked swab. The flocked swabs were then immersed in 10 mL of buffered peptone water and vortexed. Aliquots (1 mL) were serially diluted in 9 mL sterile buffered peptone water. Next, 1 mL of solution was spread on Aerobic Plate Count (APC) 3M Petrifilm and then incubated at 35 °C for 48 ± 2 h. Another 1 mL of solution was spread on Enterobacteriaceae Count Plate 3M Petrifilm and incubated at 35 °C for 24 ± 2 h[12].

Chemical properties

The chemical properties of the meat were analyzed after storage for 0 and 3 days. Total volatile basic nitrogen (TVB-N) was measured using the Conway method. The chopped samples (5 g) were homogenized in 45 mL of trichloroacetic acid (TCA) for 30 s, incubated for 30 min, and filtered through Whatman paper no. 2. Next, 1 mL of the filtered liquid was placed in the outer space of a Conway dish, 1 mL of 0.01 N boric acid solution containing an indicator (methyl red and bromocresol green) was placed in the inner part of the Conway dish, and 1 mL of 50% (w/v) K_2CO_3 was added to the other side of the outer space. The dish was sealed and slightly shaken so that the sample solution and K_2CO_3 could be mixed, and then incubated at 37 °C for 120 min. The solution in the inner space of the Conway dish was titrated with 0.01 N H_2SO_4. The moisture content of the sample was measured using an infrared moisture analyzer at 105 °C. TVB-N values were calculated using the following equation[13]:

Where Vs = titration volume of H_2SO_4 (mL), Vb = titration volume of the blank (mL), N = normality of H_2SO_4 (N), v = valency of H_2SO_4; Ms = weight of sample (g), MC = moisture content, $VTCA$ = volume of TCA.

Lipid oxidation values were determined using the thiobarbituric acid reactive substances (TBARS) method. The chopped sample (5 g) was homogenized in 10 mL of 10% (w/v) TCA for 20 s, centrifuged at 4032 × g for 30 min at room temperature, and filtered through Whatman paper no. 2. The supernatant (2 mL) was mixed with 2 mL of 0.15% (w/v) 2-thiobarbituric acid solution. The mixture was vortexed and incubated at 70 °C for 2.5 h. After cooling to room temperature (~1 h), the absorbance of the resulting supernatant was measured with a spectrophotometer at 531 nm, which corresponded to maximum absorbance, and 600 nm to correct for non-specific turbidity[7,14,15].

Physical properties

Water-holding capacity and color were measured after 0 and 3 days of storage, whereas muscle structure was assessed after treatment. Water-holding capacity was determined as the percentage of meat mass after centrifugation at 2800 × g for 10 min,

Table 1. **Experimental set-up**

Treatment	T0 (Control)		T1 (1 time)		T3 (3 times)		T5 (5 times)	
Frequency (times)	SAEW: Meat (w/w)	Time (min)	SAEW: Meat (w/w)	Time (min)	SAEW: Meat (w/w)	Time (min)	SAEW: Meat (w/w)	Time (min)
1	0: 1	15	7: 1	15	3: 1	5	2: 1	3
2					2: 1	5	2: 1	3
3					2: 1	5	1: 1	3
4							1: 1	3
5							1: 1	3
Total	0: 1	15	7: 1	15	7: 1	15	7: 1	15

divided by the initial meat mass[6,16]. The meat was weighed using an analytical balance (resolution = 0.0001 g). Meat color was quantified based on RGB values extracted from images using Phyton 3.9.7. Images were captured using an EPSON GT-X980 scanner with 24-bit color and resolution of 300 dpi. The images were cropped to incorporate the middle of the upper and lower legs of the chicken sample and a diameter of 7 cm. The change in color during storage was expressed as a difference using the following equation[17]:

$$Differences = \sqrt{(r_1 - r_2)^2 + (g_1 - g_2)^2 + (b_1 - b_2)^2}$$

Muscle structure was analyzed by scanning electron microscopy prior to storage. Before analysis, chicken meat was fixed with 2.5% glutaraldehyde in a phosphate-buffered solution (pH 7.2–7.4) and rinsed 2 times with distilled water for 10 min. After fixation, the meat samples were serially dehydrated by immersion in ethanol (25, 50, 70, 80, 90, and 99%) for 30 min, snap-frozen in liquid nitrogen, and dried using a freeze-dryer for 2 days[18–20]. The samples were examined and photographed under a scanning electron microscope (TM4000; Hitachi) at 5 kV voltage and 100× magnification. Porosity was measured by thresholding in ImageJ[21,22].

Statistical analysis

All experiments were performed in triplicate. The obtained data were analyzed using descriptive analysis. Data are represented as means and error bars denote standard deviations. One-way analysis of variance was performed in IBM SPSS version 26 to determine significant differences between treatments. Means with significant differences were separated using Tukey's honest significant difference test[23].

Results

The ORP values of SAEW for each treatment are presented in Table 2. The initial ORP of SAEW was 900.28 ± 8.24 mV, and ranged between 504.87 ± 58.80 and 574.27 ± 16.15 mV for the different treatments.

The effect of immersion in SAEW at different frequencies on the microbial load in chicken leg fillets was evaluated (Fig. 1). Initial total aerobic bacteria amounted to 3.54 ± 1.17 colony-forming units (CFU)/cm^2 (T0), 4.03 ± 0.97 CFU/cm^2 (T1), 3.65 ± 0.60 CFU/cm^2 (T3), and 3.53 ± 0.68 log CFU/cm^2 (T5). After treatment, these values dropped to 3.31 ± 1.10, 3.26 ± 0.98, 2.38 ± 0.41, and 2.17 ± 0.53 log CFU/cm^2, respectively (Fig. 2). Five immersions resulted in the largest drop in APC (by 1.36 log CFU/cm^2) and Enterobacteriaceae (by 1.69 log CFU/cm^2) compared to control and single-immersion conditions ($P < 0.05$).

Table 3 reports the growth rate of microorganisms 3 days after SAEW treatment and the porosity of tested meat. Aerobic bacteria and Enterobacteriaceae grew fastest on three immersions, reaching 0.62 ± 0.37 log CFU/cm^2/day and 0.33 log CFU/cm^2/day, respectively, although the values were not significantly different from those of the control. Porosity was 16.51 ± 4.93% (T0), 21.63 ± 7.17% (T1), 25.89% ± 4.9% (T3), and 23.82 ± 4.48% (T5). Porosity was measured based on scanning electron micrographs (Fig. 3); however, values did not differ significantly from those of the control.

The water-holding capacity of chicken samples on day 0 of storage was 93.72 ± 3.77% (T0), 86.09 ± 1.45% (T1), 84.74 ± 2.84% (T3), and 84.64 ± 1.32% (T5). After 3 days of storage, the water-holding capacity rose to 91.89 ± 1.79%, 85.86 ± 2.93%, 87.58 ± 0.78%, and 85.47 ± 3.22%, respectively. As shown in Figure 4, SAEW treatment resulted in a lower water-holding capacity than the control ($P < 0.05$).

Figure 5 summarizes the TVB-N values for all treatments after 0 and 3 days of storage. TVB-N values decreased as the immersion frequency increased., although not by a significant margin compared to the control ($P > 0.05$). TBARS values, which offered an indication of lipid oxidation, decreased with an increase in immersion frequency (Fig. 6), even though they were not signifi-

Table 2. **Oxidation-reduction potential of SAEW before and after treatment**

Immersion frequencies (times)	Immersion Time (min)	Ratio SAEW:Chicken (w/w)	Treatment
Before			900.28 ± 8.24
1 (T1)	15	7:01	516.15 ± 26.47^{ab}
3 (T3)	5	3:01	509.67 ± 62.36^{a}
	5	2:01	504.87 ± 58.80^{a}
	5	2:01	511.77 ± 32.05^{ab}
5 (T5)	3	2:01	574.27 ± 16.15^{c}
	3	2:01	556.20 ± 7.57^{bc}
	3	1:01	542.20 ± 17.86^{abc}
	3	1:01	535.95 ± 14.16^{abc}
	3	1:01	524.27 ± 29.25^{ab}

ORP, oxidation-reduction potential; SAEW, slightly acidic electrolyzed water.
ORP values correspond to the mean ± standard deviation; those with different superscripts (a,b, and c) are significantly different ($P < 0.05$).

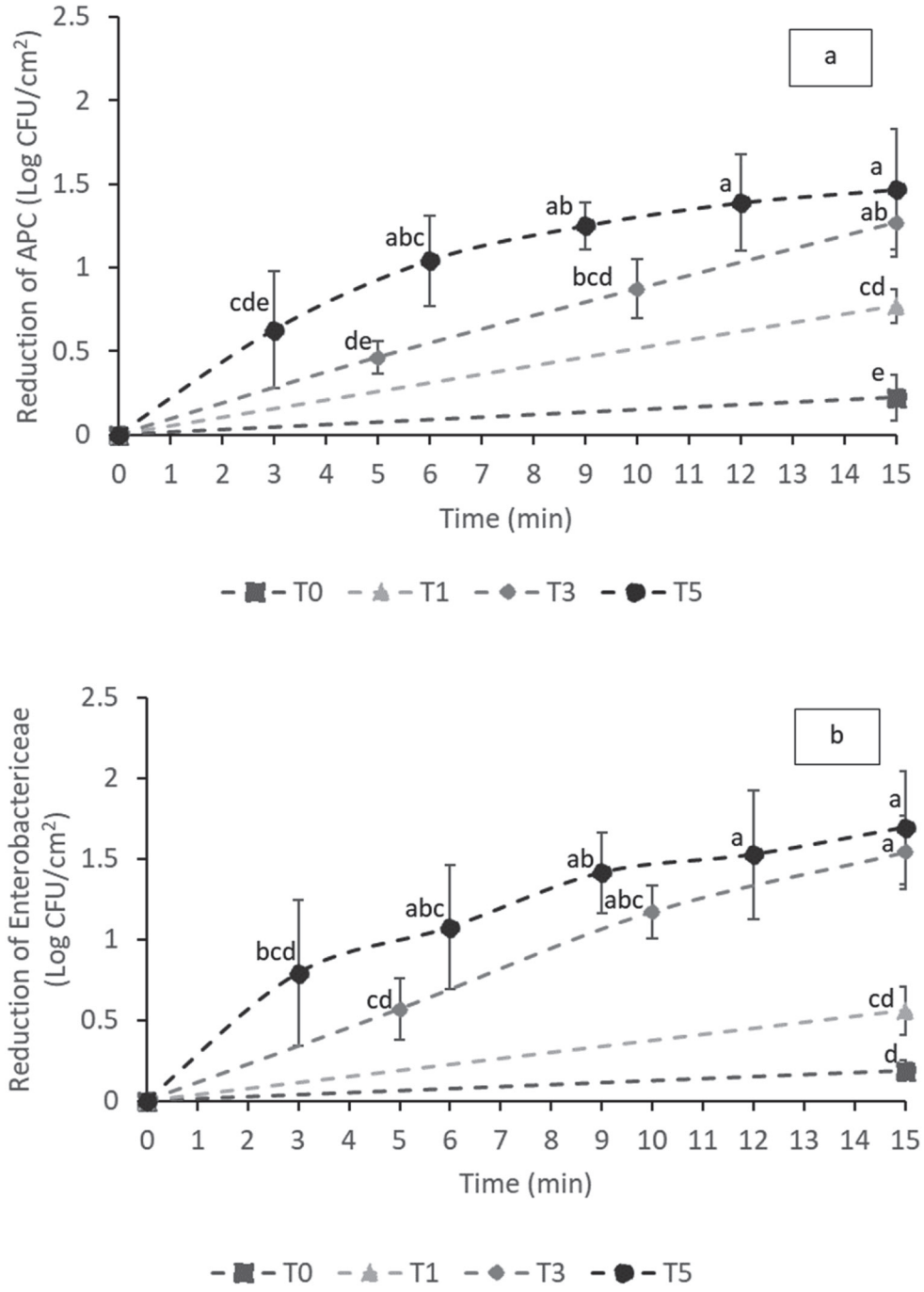

Fig. 1. **Reduction of microorganisms in raw chicken leg meat following SAEW treatment.** (a) Aerobic bacteria. (b) Enterobacteriaceae. T0 (control), T1 (single immersion), T3 (three immersions), T5 (five immersions). The data represent the mean ± SEM and circles with different letters are significantly different.

cantly different between treatments ($P > 0.05$).

Finally, an increase in immersion frequency promoted a change in color of raw chicken legs during storage (Table 4). Notably, the change in other parameters during storage was greater after three immersions than after five immersions.

Discussion

The ORP decreased after the first 5 min of immersion in SAEW to an extent similar to that observed after one, three or five immersions for 15 min each (Table 2). Xuan *et al*.[24]

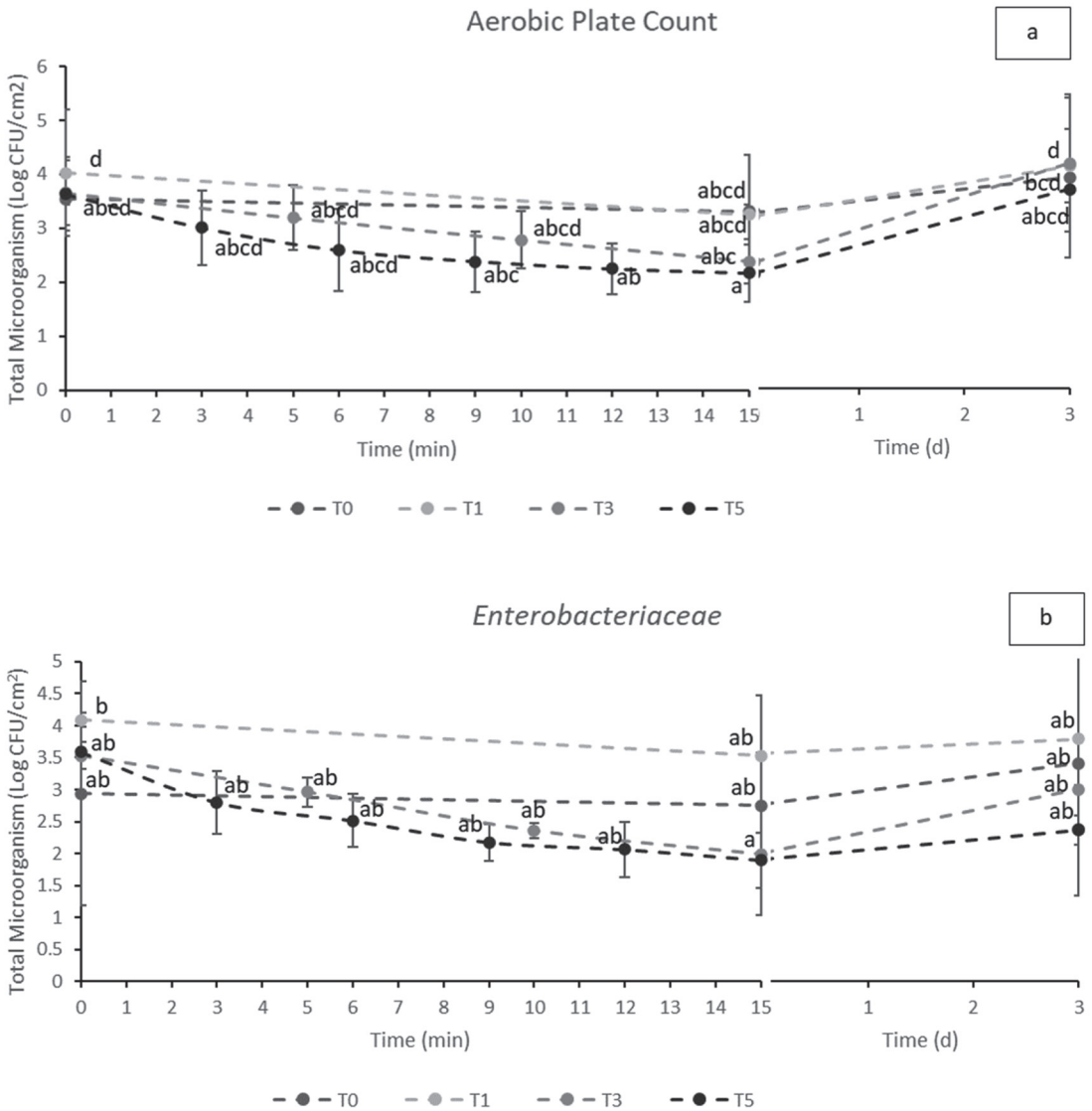

Fig. 2. **Total microbial load in chicken leg meat during SAEW treatment and after storage.** (a) Aerobic bacteria. (b) Enterobacteriaceae. T0 (control), T1 (single immersion), T3 (three immersions), T5 (five immersions). The data represent the mean ± SEM and circles with different letters are significantly different.

showed that the ORP for pork meat decreased within 1 min of immersion in SAEW. Large amounts of organic material in meat can easily react with chlorine, thereby altering the physicochemical properties of SAEW[25].

Total aerobic bacteria and Enterobacteriaceae in chicken legs dropped significantly following immersion in SAEW (Fig. 1). Vetchapitak *et al.*[26] reported lower APC (1.23–1.51 log CFU/cm^2) when using NaOCl with 200 ppm TAC for 15 s. Here, APC declined by 1.36 log CFU/cm^2 after five immersions, indicating the potential of SAEW as a disinfectant for raw chicken legs. Rahman *et al.*[5] demonstrated a reduction of 1.49 log CFU/g for total aerobic bacteria in chicken breasts using SAEW with 10 ppm TAC during a single immersion.

Three and five immersions achieved similar results ($P > 0.05$) at the endpoint of 15 min (Fig. 1). The higher was the frequency of immersion, the lower were any residual aerobic bacteria and Enterobacteriaceae at an initial stage. Specifically, a second immersion time in SAEW (6 and 10 min for T5 or T3) with a total SAEW:meat ratio of 4:1 and 5:1 produced better results than a single immersion for 15 min with a SAEW:meat ratio of 7:1 (Fig. 1). Given that ORP decreased rapidly even during the first 3 min of treatment in T5 (Fig. 1), the previously reported 15-min treatment may be unnecessarily long to disinfect raw chicken[24]. Our results demonstrated that an increased frequency of immer-

Table 3. **Effect of SAEW immersion at different frequencies on the growth rate of microorganisms in raw chicken legs after 3 days of storage**

Treatment	Aerobic Bacteria (log CFU/cm^2.d)	Enterobacteriaceae (log CFU/cm^2.d)	Porosity (%)
T0 (Control)	0.22 ± 0.22^{a}	0.21 ± 0.11^{b}	16.51 ± 4.93^{a}
T1 (1 time)	0.31 ± 0.11^{ab}	0.08 ± 0.01^{a}	21.63 ± 7.17^{a}
T3 (3 times)	0.62 ± 0.37^{b}	0.33 ± 0.02^{c}	25.89 ± 4.90^{a}
T5 (5 times)	0.53 ± 0.23^{ab}	0.15 ± 0.10^{ab}	23.82 ± 4.48^{a}

CFU, colony-forming units; SAEW, slightly acidic electrolyzed water.
Values correspond to the mean ± standard deviation; those with different superscripts (a,b, and c) are significantly different ($P < 0.05$).

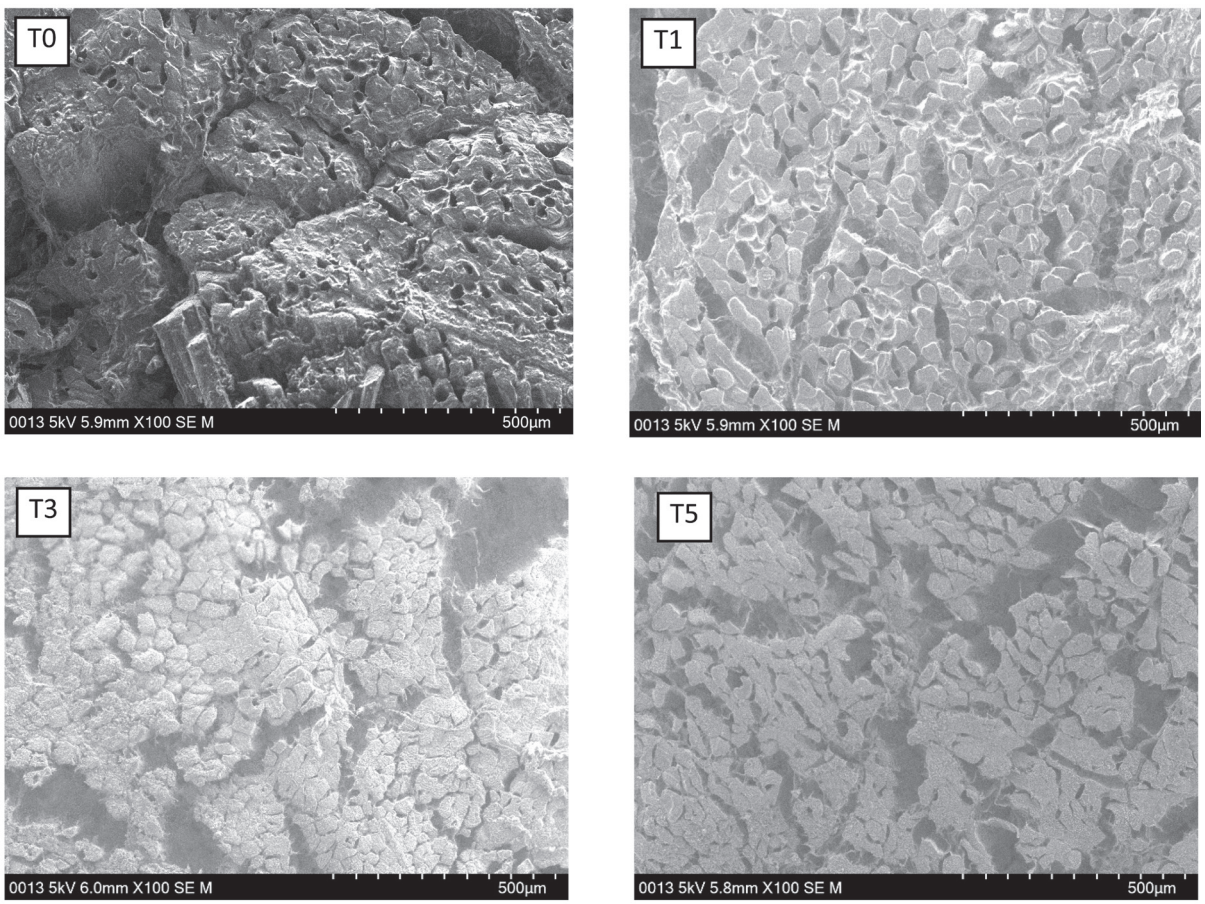

Fig. 3. **Muscle structure in raw chicken leg meat after SAEW treatment, without any storage.** T0 (control), T1 (single immersion), T3 (three immersions), T5 (five immersions).

sion could limit the time and utilization of SAEW (Fig. 1). Generally, the ratio of disinfectant water to chicken is approximately 10:1[6,7,27,28]. In 2021, 2,216,307 tons of young chickens were processed for food in Japan, which meant the generation of an estimated 22,163,070 tons of disinfectant wastewater[29]. As indicated by our data obtained with five immersions (Table 1), increasing the frequency of immersion in SAEW could lower to 4:1 the ratio of disinfectant water to chicken, thereby limiting the annual consumption of disinfectant water to 8,865,228 tons. Implementation of such an approach could reduce water usage by 13 million tons per year and effectively contribute to one of the key Sustainable Development Goals.

Bacterial growth has been reported to increase in pork with a higher porosity following ultrasound treatment. Higher porosity

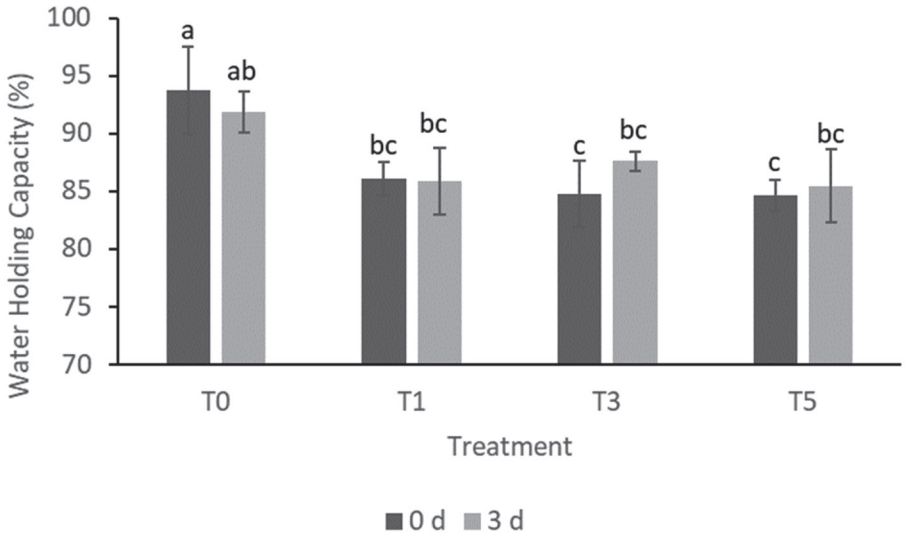

Fig. 4. **Water-holding capacity in raw chicken leg meat after SAEW treatment and successive storage for 3 days.** T0 (control), T1 (single immersion), T3 (three immersions), T5 (five immersions). The data represent the mean ± SEM and bars with different letters are significantly different

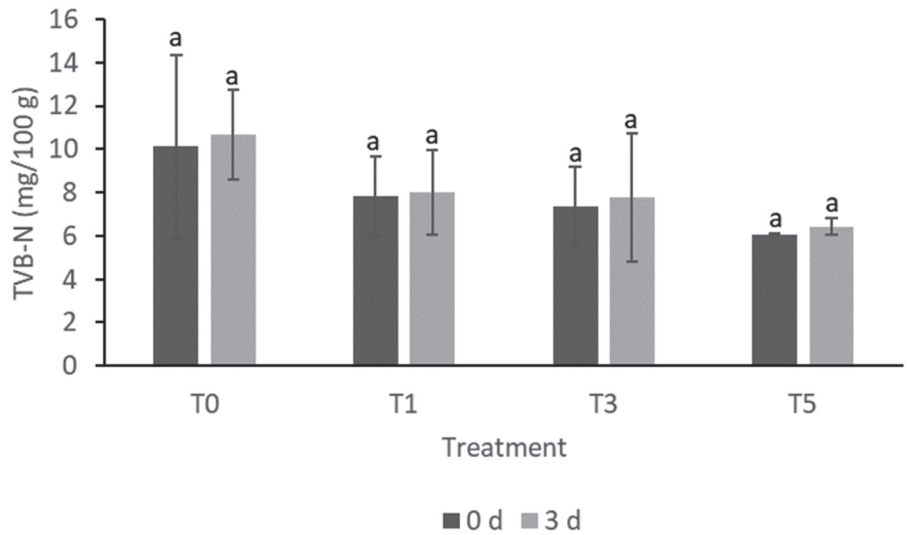

Fig. 5. **TVB-N in raw chicken leg meat after SAEW treatment and successive storage for 3 days.** T0 (control), T1 (single immersion), T3 (three immersions), T5 (five immersions).

promotes oxygen and nutrient transport to the cells, which influences the growth of bacteria[30]. In this study, the rate of bacterial growth did not differ significantly between SAEW-treated or untreated samples (Table 3), although it correlated positively with porosity. Hence, SAEW treatments, and in particular five immersions, could complement ultrasound and surpass its effectiveness.

Electrolyzed water has been reported to suppress growth of Enterobacteriaceae, such as *Salmonella* sp. and *Escherichia coli*[4–6]. As shown in Figure 3, more frequent immersions in SAEW caused larger gaps between muscle bundles, which may be related to the lower water-holding capacity of meat just after SAEW treatment[31,32]. Importantly, Figure 4 shows that the water-holding capacity of chicken legs after three and five immersions tended to increase following 3 days of storage. The loss of water at 0 days due to muscle breakdown increases protein extractability and has a "sponge effect" that augments water-holding capacity[33].

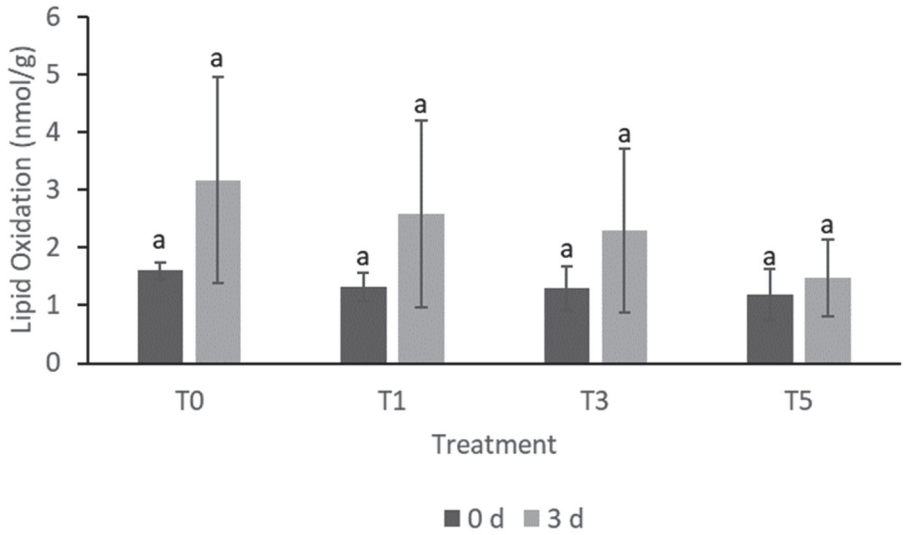

Fig. 6. **Lipid oxidation in raw chicken leg meat after SAEW treatment and successive storage for 3 days.** T0 (control), T1 (single immersion), T3 (three immersions), and T5 (five immersions).

Table 4. **Effect of SAEW immersion at different frequencies on raw chicken leg color after 3 days of storage**

Treatment	Storage time (d)	R	G	B
T0 (Control)	0	141.59 ± 0.87[a]	95.69 ± 85.07[a]	85.07 ± 1.74[a]
	3	139.80 ± 1.69[a]	85.38 ± 3.11[a]	86.68 ± 2.06[a]
	Difference		2.90 ± 1.36[a]	
T1 (1 time)	0	147.92 ± 12.31[a]	107.06 ± 12.12[a]	96.80 ± 9.89[a]
	3	144.20 ± 5.78[a]	104.30 ± 3.73[a]	93.04 ± 4.27[a]
	Difference		5.11 ± 4.57[a]	
T3 (3 times)	0	145.18 ± 5.78[a]	104.30 ± 3.74[a]	93.04 ± 4.27[a]
	3	142.28 ± 4.43[a]	99.82 ± 2.12[a]	89.59 ± 2.57[a]
	Difference		6.36 ± 2.68[a]	
T5 (5 times)	0	143.16 ± 0.99[a]	100.88 ± 3.19[a]	88.84 ± 0.60[a]
	3	137.01 ± 6.27[a]	94.94 ± 7.47[a]	84.70 ± 4.51[a]
	Difference		9.52 ± 7.77[a]	

RGB, red-green-blue; SAEW, slightly acidic electrolyzed water.
RGB values correspond to the mean ± standard deviation; those with different superscripts (a,b, and c) are significantly different ($P < 0.05$).

TBV-N is typically used as a reference for protein and amine degradation, from which to determine meat freshness[34]. More frequent immersions in SAEW decreased TVB-N values (Fig. 5). Hernández-Pimentel *et al*.[8] reported that treatment with electrolyzed water yielded a lower TVB-N value than the control. SAEW contains Cl_2 and HClO, which can absorb electrons and maintain the stability of the primary, secondary or tertiary structure of proteins[31]. Microbiological spoilage leads to higher TVB-N values[35]. TVB-N tended to increase after 3 days of storage in all SAEW treatment groups, although the highest absolute TVB-N value remained for the control, regardless of storage period. This result is consistent with other studies, which showed that TVB-N increased more slowly upon SAEW treatment[8,35].

Poultry meat is rich in polyunsaturated fatty acids, which makes it sensitive to lipid oxidation[36]. Rahman *et al*.[5] and Shimamura *et al*.[7] also reported a slower increase in lipid oxidation during storage of SAEW-treated samples. This finding appears in line with our data showing that higher immersion frequency prevented lipid oxidation (Fig. 6).

As reported previously, the change in color was amplified by SAEW treatment, followed by 5 days of storage[7]. Here, although the RGB values of chicken legs slightly decreased after

3 days of storage in all SAEW-treated samples, the differences were not significant ($P > 0.05$). The RGB values for black and white were 0 and 255, respectively. Higher RGB values indicate greater brightness in an image[37]. Shimamura *et al*.[7] reported that electrolyzed water resulted in a brighter color compared to the untreated control. In summary, our SAEW treatments (especially the second immersion in the T5 sample) improve the microbial safety, as well as chemical and physical quality of raw chicken legs, with direct repercussions on freshness.

Acknowledgments

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Author Contributions

Muliasari Kartikawati conducted the experiments, analyzed the data, and wrote the manuscript; Yutaka Kitamura designed the experiment, reviewed, and edited the manuscript; Mito Kokawa reviewed and edited the manuscript; Mareto Hamatani reviewed and acquired funding; and Takashi Soejima reviewed and acquired funding.

Conflicts of Interest

Mareto Hamatani and Takashi Soejima are employees of Morinaga Milk Industry Co., Ltd, which manufactured the purester SAEW generator used in this study. Yutaka Kitamura received a research grant from Morinaga Milk Industry Co., Ltd.

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