



AIR SANITATION IN CHICKEN PROCESSING USING SAEW: MIST VS FORCED-AIR

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Abstract

In the chilling step of chicken processing, air systems are used for cooling or draining. Sanitation also usually occurs during this step. However, an air sanitation system using SAEW has not yet been developed. This research describes evaluation of the air sanitation system using SAEW by comparing mist (SAEW-Mist) and forced-air (SAEW-Gas) against controls in terms of raw chicken quality during storage and the potential formation of trihalomethane. The air sanitation system using SAEW with both treatments reduced total microorganisms and Enterobacteriaceae, although they were not significantly different from the control ($P \geq 0.05$). However, SAEW treatments effectively slowed microbial growth over time, with SAEW-Mist showing better stability. NaOCl treatment caused higher microbial growth rates, greater porosity, and significant reductions in water holding capacity (WHC), likely due to protein denaturation. SAEW-Mist preserved WHC better and showed lower total volatile basic nitrogen (TVB-N) and lipid oxidation than SAEW-Gas and NaOCl. TVB-N and TBARS values in SAEW-Mist remained below the threshold for spoilage. Chloroform, a trihalomethane compound, was detected in NaOCl and SAEW-Gas samples but not in SAEW-Mist or control. SAEW-Mist also caused fewer physical and chemical changes during storage, produced no liquid waste, and did not generate trihalomethane. The mist-based system offers advantages such as reduced water usage, prevention of cross-contamination, and improved hygiene conditions. Therefore, SAEW-Mist can be proposed as part of an integrated and environmentally friendly sanitation approach in poultry processing facilities.

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Introduction

Chicken food safety is necessary due to high consumption. According to FAO [1] chicken has consistently been the most produced type of meat in the past four years. To ensure chicken food safety and extend shelf life, sanitation techniques, including chemical and system application, have become the focus of many studies [2–4]. The poultry industry mainly uses chemical disinfectants for microbial control. Sodium hypochlorite (NaOCl) is widely used as a sanitizing agent at a concentration of 200 ppm. It is preferred for its strong effectiveness and affordability [5,6]. Even so, the presence of organic matter with NaOCl higher ppm levels will enhance trihalomethane (THM) formation, which is a critical disadvantage. Chloroform, a marker of THM, has been found in broilers after NaOCl treatment, posing a carcinogenic risk [7]. Slightly Acidic Electrolyzed Water (SAEW) is considered in chicken processing due to its advantages over other sanitizing agents. It offers greater

stability and high bactericidal efficacy with lower chlorine concentration. Moreover, it will affect the lower possibility of trihalomethane formation, and is less corrosive [4,5,7]. Rahman et al. [8] showed that chicken samples treated with SAEW had a lower total aerobic bacterial count (1.49 log CFU/g) than the control. Our previous research also showed a reduction of aerobic plate count by 1.36 log CFU/cm² after immersion in SAEW for five times [9].

Sanitation in chicken processing usually occurs in the chilling step. Generally, the step is performed using an immersion system or an air system. The immersion system is the most frequently chosen due to its efficiency [10]. However, our previous research showed that the samples treated using the immersion system had a higher microbial growth than the control [9]. Liu et al. [11] wrote that SAEW with immersion may damage egg cuticles. In contrast, SAEW with a sprayed mist did not affect the egg cuticle. Moreover, cooling carcasses with mist has some advantages

compared to immersion. It offers reduced water consumption and waste, as well as no cross-contamination [12]. Therefore, an air system should be further explored as a sanitation method in chicken processing.

In an air sanitation system, the liquid sanitation agent should be converted into gas phase. The transformation can occur when atomizing or forced-air vaporizing is used. The atomizing method utilizes spraying or ultrasonic misting. Ultrasonic misting of hypochlorite solution has effectively sanitized bacteria and viruses on solid surfaces. The mist contains fine HOCl droplets in aqueous form with higher densities than in gas form [13]. During mist production, the loss of free chlorine was around 11.7–13.2 % [14]. Dry mist with HClO at a concentration of 300 ppm reduced *Escherichia coli* and *Salmonella enterica* by more than 5 log after 60 s [15]. However, electrolyzed water with ACC more than 10 ppm could inactivate bacteria within 0.5 min [13]. Liu et al. [11] reported that using 50 ppm significantly reduced total culturable bacteria on eggshell compared to tap water. SAEW with 30–50 ppm was proven to eliminate *Staphylococcus aureus* after 3 hours of atomizing [16].

In forced-air vaporizing, gaseous HOCl is released along with the evaporation of water by flowing air through porous water-holding materials. A hypochlorite solution with a pH of 8.5 at 100 ppm resulted in an HOCl concentration of approximately 12 to 17 ppm after 2 hours with a flow rate of 2 m³/min in a 75 m³ room. An air sanitation system that uses chlorinated water in its gaseous state has demonstrated the capability to reduce *E. coli* cells on 0.9 % NaCl agar by more than 2.8 log CFU within 2 hours when positioned 3 meters from the vaporizer. Moreover, *V. parahaemolyticus* showed a reduction of 3.8 log CFU after being exposed to 20–50 ppb of gaseous HOCl at one meter from the vaporizer. Nevertheless, the research also indicated that the bactericidal effectiveness of HOCl diminishes in the presence of organic substances that consume chlorine [13]. Additionally, the research about an air sanitation system using SAEW has not been developed for chicken sanitizing, and its effect on the quality of raw chicken has not been investigated. Therefore, the purpose of this research is to evaluate an effect of different air sanitation systems using SAEW by comparing mist (SAEW-Mist) and forced-air (SAEW-Gas) against controls in terms of raw chicken quality during storage and the potential formation of trihalomethane.

Objects and methods

Preparation of SAEW solution

SAEW solution was produced by diluted electrolysis of saturated NaCl solution with tap water using a SAEW generator with three room-type electrolyte cells which was developed by Morinaga Milk Industry Co., Ltd. (Figure 1). Its characteristics include a pH range of 5.5–6.5, as measured by pH-meter D-51 (HORIBA, Ltd., Japan), and a total available chlorine content of 80 ppm, which was measured using a handy water meter AQUAB model AQ-202 (Sibata Scientific Technology Ltd, Japan).

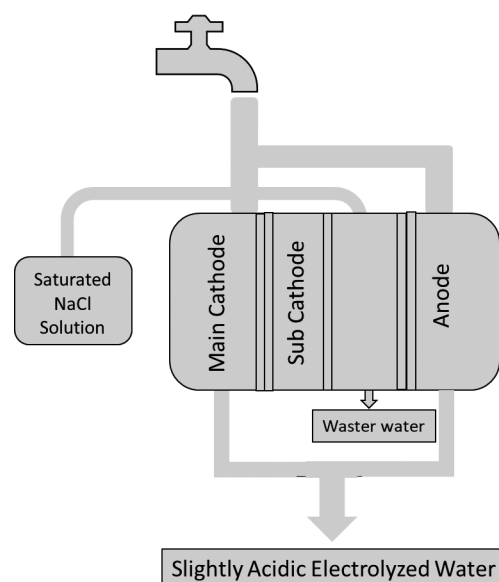


Figure 1. Three room-type electrolyte cells

Preparation of chicken samples

Chicken carcasses were transported from the Sanwa Oyadori factory to the laboratory in a cool box within 3 hours after slaughter. The chicken meat samples were cut from the thigh and breast parts closest to the gastrointestinal tract for microbiological analysis. James et al. [17] showed that the decay starts from the meat part nearest to the gastrointestinal tract. For other analyses, filleted thigh and breast parts were used. The chicken parts were stored at 4 °C before being used for the experiment.

Procedures for sanitizing

Cut chicken meat samples (120–150 g) were placed in the first rack of the chamber, positioned 30 cm above the fan in and exposed to gaseous SAEW generated either by forced-air vaporizing (SAEW-Gas) or ultrasonic misting (SAEW-Mist) for 120 min (Figure 2). The chamber was chilled by covering with a hose that was connected to a recirculating chiller, maintaining an internal temperature below 15 °C. Outside the chamber, the Rh was controlled with an automatic SALARI Pro dehumidifier Mitsubishi Electric MJ-P180RX-W (Mitsubishi Electric Corporation, Japan) to 50–60 %. For an air sanitation system using SAEW-Gas, the the speed of fan in and fan out was 5.3 m³/min and 3 m³/min, respectively, while SAEW-Mist was used to only fan out with a speed of 4.6 m³/min. Negative control samples (Control) were chicken packed in sterilized plastic and placed together in the chamber during the operation of SAEW-Gas or SAEW-Mist for 120 minutes. For positive control samples (NaOCl), the sanitation system imitated the condition of the chicken factory with some modifications, in which chicken meat samples were immersed in 200 ppm of NaOCl solution two times with a ratio of 2:1 for 15 minutes each and drained for 15 min [18]. All samples were vacuumed, packed, and stored at 4 °C for 3 and 7 days.

Microbiological properties

Microbiological properties were evaluated before and after the treatment (0 days), 3 days, and 7 days of storage.

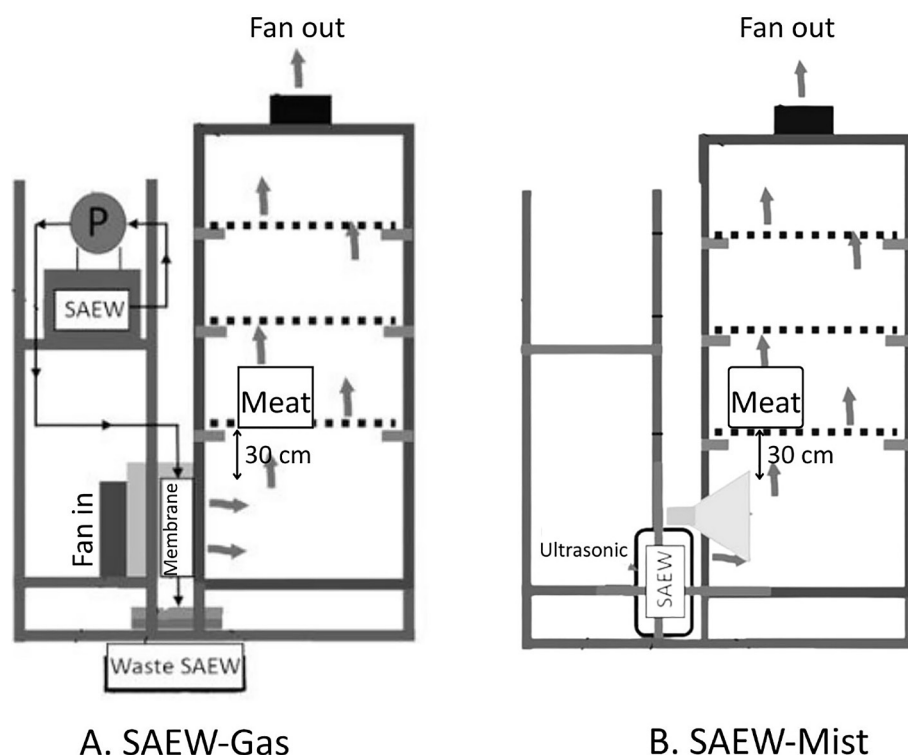


Figure 2. The air sanitation system

Chicken was swabbed in an area of 5 cm × 5 cm for 3 times with a sterilized wetted swab. Then, the swab was immersed in 10 mL of buffered peptone water (BPW), mixed with vortex TTM-1 (Sibata Scientific Technology, Ltd, Japan), and diluted serially with a ratio of 9:1 in sterile BPW. Total microorganisms and *Enterobacteriaceae* were evaluated by spreading 1 mL of aliquots of dilution to the top of 3M Petrifilm Aerobic Count Plate 6406 with the following incubation at 35 °C for 48 ± 2 hours and 3M Petrifilm Enterobacteriaceae Count Plate 6420 with the following incubation using CN-25C (Mitsubishi Electric Corporation, Japan) at 35 °C for 24 ± 2 hours [19,20].

Chemical properties

The chemical properties of the samples were evaluated using total volatile basic nitrogen (TVB-N) and lipid oxidation analyses. These chemical parameters were analyzed on 0, 3 and 7 days of storage.

TVB-N levels were determined using the Conway method. Chicken meat was cut into samples with a size of 1x1 cm and chopped using 100 mL portable mini food processor for 1 min. (WBLGG, China). A 5-g portion of chopped meat was homogenized (Physoctron homogenizer NS-52K, Microtec, Japan) with 45 mL of trichloroacetic acid (TCA) at 17000 rpm for 30 seconds. The mixture was allowed to stand for 30 minutes. It was then filtered using Whatman No. 2 filter paper. One milliliter of the resulting filtrate was placed into the outer ring of a Conway dish. Meanwhile, 1 mL of 0.01 N boric acid solution containing methyl red and bromocresol green indicators was added to the inner chamber. Additionally, 1 mL of 50 % (w/v) potassium carbonate (K₂CO₃) solution was placed on the opposite side of the outer ring. The Conway dish was

sealed and gently shaken to mix the sample and K₂CO₃. It was incubated (LTI-600SD, Tokyo Rikakikai, Japan) at 37 °C for 120 minutes. After incubation, 0.01 N sulfuric acid (H₂SO₄) was added to the inner chamber until a pink color appeared. The TVB-N was calculated as mg/100 g of sample [21].

Lipid oxidation was assessed using TBARS value. A 5-g portion of chopped meat obtained using the same method as for TVB-N was homogenized (Physoctron homogenizer NS-52K, Microtec, Japan) at 17000 rpm for 20 seconds in 10 mL of 10 % (w/v) trichloroacetic acid (TCA), then centrifuged (CN-1050, AS-ONE, Japan) at 4032 × g for 30 minutes over ice. The resulting mixture was filtered using Whatman No. 2 filter paper. From the obtained supernatant, 2 mL was collected and combined with 2 mL of 0.15 % (w/v) 2-thiobarbituric acid (TBA) solution. The mixture was vortexed and incubated at 70 °C for 2.5 hours in a water bath (Advantec Hotting Bath B-CS, Advantec Group, Japan). After cooling to room temperature for approximately one hour, the absorbance was measured using a spectrophotometer (V-630, JASCO Corporation, Japan) at 531 nm (maximum absorbance) and 600 nm (for nonspecific turbidity correction). The TBARS value was determined as equivalents of malondialdehyde (mg MDA eq/kg meat) [9,21].

Physical properties

The physical properties of the samples were evaluated in terms of water holding capacity (WHC) and color of chicken meat. Those parameters were analyzed for 0, 3, and 7 days. WHC was calculated as the percentage of the meat mass remained after centrifugation (CN-1050, AS-ONE, Japan) at 2800 × g for 10 minutes relative to the initial mass

of the meat [22]. The color was analyzed using CIE values for lightness (L^*), redness (a^*), and yellowness (b^*). The meat was scanned using an EPSON GT-X980 (Epson, Japan) at a 24-bit color depth and 300 dpi resolution. The background was removed from the resulting image of meat using Canva BG Remover. The removed background image was calculated using Python 3.9.7 to get CIE values [23].

Muscle structure

Muscle structure was photographed for the meat samples only after treatments in SEM Hitachi TM4000 II (Hitachi High-Tech, Japan) using a 5kV voltage with the magnification of $\times 100$ and $\times 1000$. Before that, the samples were pretreated using one-time fixation. In one-time fixation, chicken meat was fixed using only 2.5 % glutaraldehyde in buffer phosphate solution pH 7.2–7.4 for one night. After fixation, meat samples were washed with buffered phosphate pH 7.4 two times for 10 min. Then samples was dehydrated by immersion in ethanol serially (25, 50, 70, 80, 90, 99, and 99 %) for 13 min each, and dried for 2 days using a freeze dryer (Eyela FDU-1200, Tokyo Rikakikai Co. Ltd, Japan) [24–26]. Porosity was calculated using images from one-time fixed samples by thresholding operation in open source software ImageJ [27].

Trihalomethane detection

Trihalomethane (THMs) was determined using GC–MS Shimadzu QP-5050 (Shimadzu Corporation, Japan) using the method 8260D [28]. The chopped meat (2 g) was weighed in a 20 mL glass vial; then, the 10 mL matrix modifying solution was added and the glass vial was sealed. The headspace solid phase extraction was done by injecting SPME fiber into the vial. Then the sample was mixed for 2 min at room temperature and heated at 85°C for 40 min. During the heating, the sample was agitated for 10 min. After that, the fiber was removed and directly injected to GC injector manually [29]. Separations were performed on DB-5 capillary column (30 m \times 0.25 mm I.D. \times 0.25 μ m film thickness). Injection occurred in spitless mode with helium as carrier gas (1.0 mL/min). The initial temperature of the GC–MS was set to 40°C for 3 min and followed by an increase of 8°C/min to 80°C held for 3 min, and an increase of 6°C/min to 140°C. Then the temperature was increased 10°C/min to 200°C and held for 3 min.

Statistical analysis

This research used randomized complete block design, in which all treatments were applied in each replication. Each experiment was conducted in triplicate, with replications performed at different times. The data from all replicates were subjected to descriptive analysis to calculate means and standard deviations, shown as error bars in the figures. Additionally, repeated measures and one-way analysis of variance were performed to determine significant differences between treatments. Means that showed significant differences were further analyzed using Tukey's Honestly Significant Difference (HSD) test. The means within repeated measurements were analyzed using Bonferroni correction. Data analysis was performed using Microsoft Excel 365 and JASP [30].

Results and discussion

Figure 3 illustrates total microorganisms and *Enterobacteriaceae* before and after treatment. An air sanitation system with SAEW-Gas and SAEW-Mist showed no significant differences compared to the control ($P \geq 0.05$). However, SAEW-Gas and SAEW-Mist reduced total microorganisms by 0.23 ± 0.07 and 0.20 ± 0.084 log CFU/cm² and *Enterobacteriaceae* by 0.49 ± 0.46 and 0.32 ± 0.33 log CFU/cm², respectively, in all replications. In contrast, the total microorganisms and *Enterobacteriaceae* of the control samples increased. Gaseous HOCl has been proven to have bactericidal action. Gas is more effective against pathogens due to its ability to penetrate surface irregularities [31]. However, chlorine-consuming organic matter reduced HOCl effectiveness [13]. Additionally, the loss of ACC during misting also caused lower antibacterial activity of SAEW-Mist [14].

Immersion with 200 ppm of NaOCl solution reduced total microbial counts by 1.20 ± 0.70 log CFU/cm², and they were significantly different before and after treatment ($P < 0.05$). Still, the reduction was not significantly different from SAEW-Gas and SAEW-Mist treatments. Byun et al. [2] showed that *Salmonella enteritidis* biofilm on chicken skin treated with 200 ppm NaOCl was not significantly different from that on the untreated sample. On the other hand, chlorine levels used in poultry processing are limited

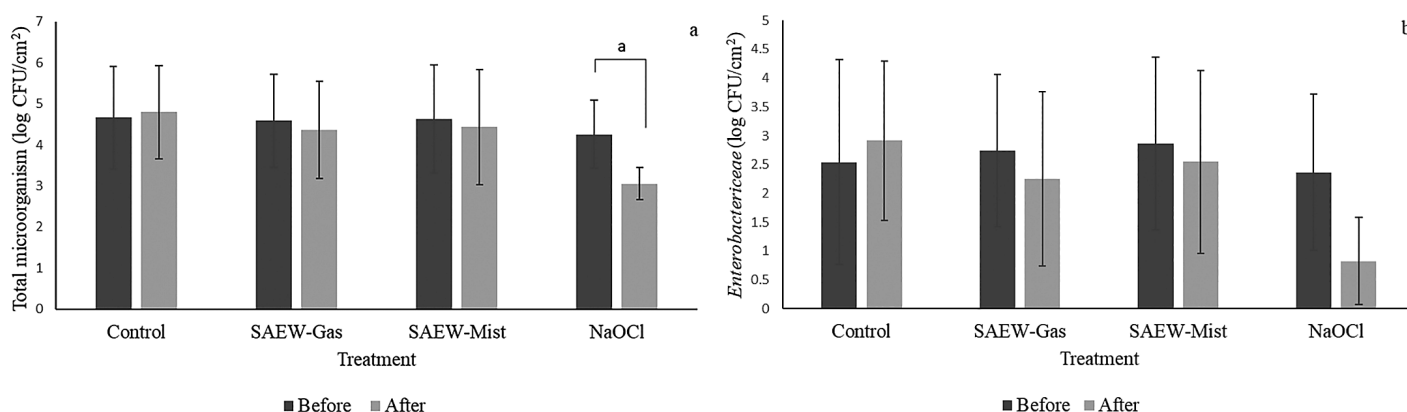


Figure 3. Total microorganisms (a) and *Enterobacteriaceae* (b) in raw chicken before and after treatment.

^a shows significant differences within treatment ($P < 0.05$)

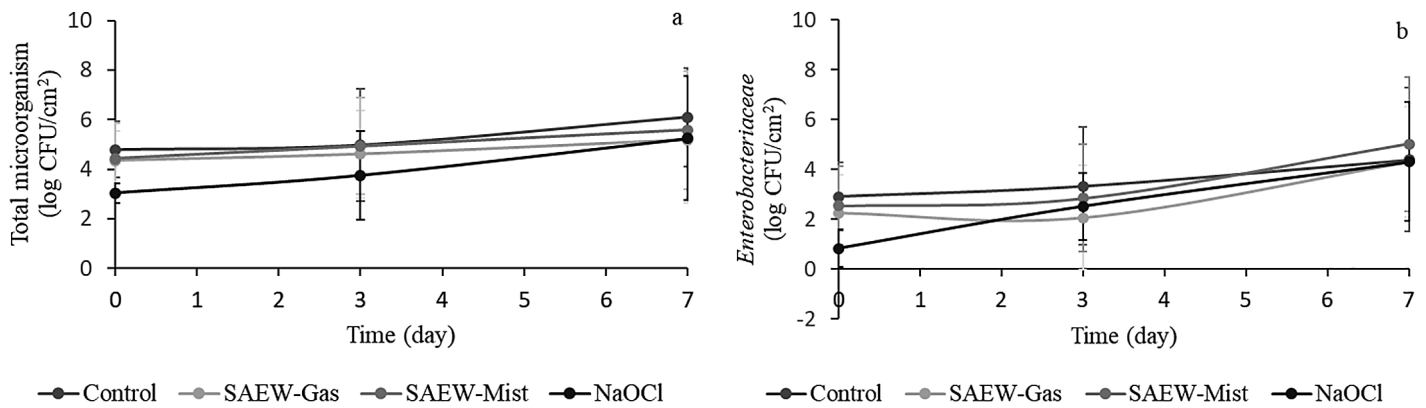


Figure 4. Total microorganisms (a) and *Enterobacteriaceae* (b) in raw chicken during storage.

There was no significant difference between all treatments ($P \geq 0.05$)

to a maximum of 50 ppm in the USA because of the formation of trihalomethanes. Kartikawati et al. [9] showed that immersion in 30 ppm of SAEW repeated three times with a total time of 9 min could reduce total microorganisms to 1.25 ± 0.14 log CFU/cm², which was slightly higher than NaOCl treatment.

Table 1. Growth rate and porosity of raw chicken

Treatment	Total microorganisms, log CFU/cm ² .day	<i>Enterobacteriaceae</i> , log CFU/cm ² .day	Porosity, %
Control	0.19 ± 0.12	0.21 ± 0.22	12.36 ± 5.16
SAEW-Gas	0.12 ± 0.20	0.31 ± 0.10	12.85 ± 3.54
SAEW-Mist	0.16 ± 0.14	0.36 ± 0.16	14.47 ± 4
NaOCl	0.32 ± 0.33	0.50 ± 0.26	15.17 ± 3.02

Values are means \pm standard deviations. There was no significant difference between all treatments ($P \geq 0.05$).

There were no significant differences in the growth of total microorganisms and *Enterobacteriaceae* between treatments (Figure 4). NaOCl treatment showed the highest growth rate of total microorganisms and *Enterobacteriaceae* of 0.32 ± 0.33 and 0.50 ± 0.26 log CFU/cm².d, respectively, with the highest porosity of 15.17 ± 3.02 %, even though they were not significantly different from those of other treatments ($P \geq 0.05$) (Table 1). During the storage, NaOCl treatment resulted in the highest growth rate, since the immersion system caused damage to the muscle

bundle (Figure 6), leading to higher porosity and growth rate. Immersing salmon in 100 ppm HOCl solution also resulted in higher total microorganisms than the control after 7 days of storage. The treatment likely caused increased cell lysis. Cell lysis released nutrients that became available for microbes [32]. Our previous research also showed that the muscle bundle was broken with an immersion system, which led to the increased growth rate [9]. SAEW-Mist had a higher growth rate of total microorganisms and *Enterobacteriaceae* than SAEW-Gas. However, the growth rate of total microorganisms for SAEW-Gas and SAEW-Mist was lower than that of the control. These results demonstrated that an air sanitation system could reduce microorganisms while slowing the growth rate.

Figure 5 shows that TVB-N and lipid oxidation in raw chicken increased in all groups during storage. TVB-N is widely used as an indicator of freshness and overall quality. This relates to protein and nonprotein breakdown by bacteria and enzymes [33]. However, the increases in these parameters were not significantly different between all treatments ($P \geq 0.05$). The highest TVB-N after 7 days of storage was observed in the negative control. In contrast, the lowest TVB-N on 0 to 7 days was in the NaOCl treatment, which was 6.63 ± 0.66 mg/100 g and increased to 11.75 ± 3.08 mg/100 g. Lower TVB-N in raw chicken may result from chlorine binding to protein, forming insoluble and less volatile precipitate forms [34]. The SAEW-Mist

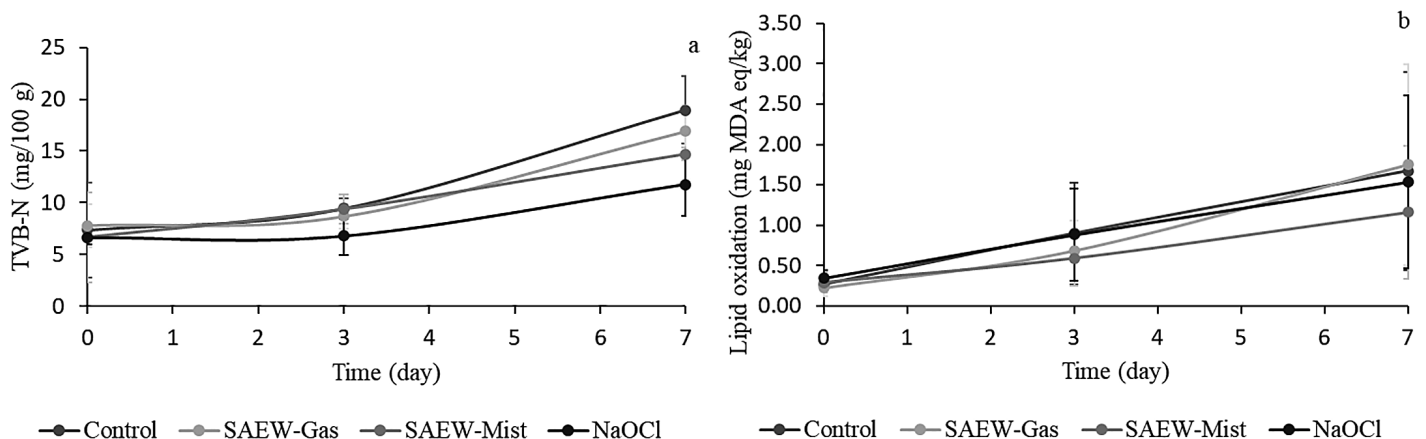


Figure 5. TVB-N (a) and lipid oxidation (b) in raw chicken during storage.

There was no significant difference between all treatments ($P \geq 0.05$)

treatment showed lower TVB-N values and smaller change during storage compared to SAEW-Gas. This indicates that chlorine was incorporated into proteins in the aqueous form more than in the gaseous one. Since chlorine-based sanitizing agents were used, treated samples had lower TVB-N values during storage. Additionally, all the sanitizing treatments reduced microorganisms in meat that prevent protein decomposition [6]. The 15 mg/100 g TVB-N threshold appears to be the most commonly used standard in the literature [35]. Therefore, SAEW-Mist and NaOCl treatments maintained acceptable quality even after 7 days of storage.

Lipid oxidation products are often evaluated through TBARS analysis [32]. After treatment, the lipid oxidation values of samples were 0.22 ± 0.33 to 0.34 ± 0.10 mg MDA eq/kg. NaOCl treated samples showed the highest values, while SAEW-Mist had the lowest. However, the differences among the treatments were not significant ($P \geq 0.05$). SAEW-Mist maintained the lowest lipid oxidation on both day 3 and 7. Meanwhile, SAEW-Gas resulted in the highest lipid oxidation after 7 days of storage. The phenomenon

of lipid oxidation can occur due to the presence of oxygen [36]. SAEW-Gas was generated using a forced-air system, which introduces additional oxygen. The destruction of lipids which is shown by non-round shape of the lipid in the SAEW-Gas treated sample is shown in Figure 6 at 1000x magnification. As an oxidant, chlorine interacts with lipids and increases lipid oxidation. Lipid oxidation threshold of 0.6–2.0 mg MDA/kg may cause off-flavors detectable by untrained consumers [36]. SAEW-Mist maintained lipid oxidation levels below this threshold after 3 days storage, unlike other treatments. Previous findings have shown that electrolyzed water can prevent lipid oxidation during storage [9,37].

WHC and color changes of raw chicken during storage are presented in Figure 7. WHC describes the ability of meat to retain water content. Water loss or retention affects the economic value of meat during processing and storage [21]. This property depends on interactions between muscle proteins and water molecules. Denaturation of proteins can disrupt these interactions and reduce WHC significantly [38]. The air sanitation systems with both SAEW

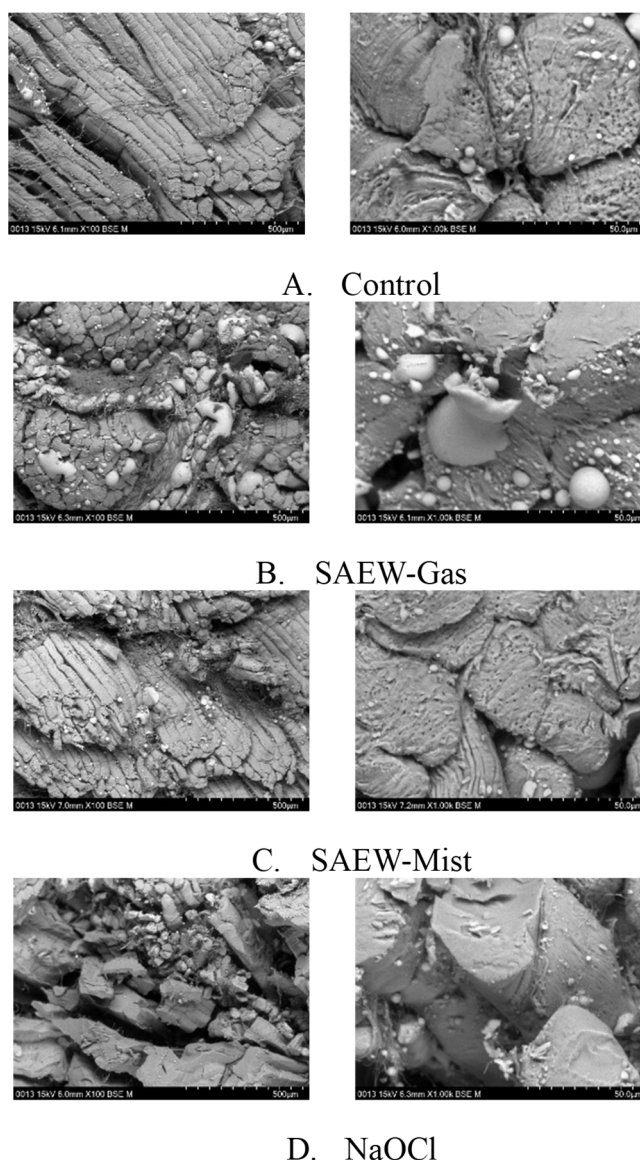


Figure 6. Muscle structure of raw chicken (Left: 100x magnification; Right: 1000x magnification)

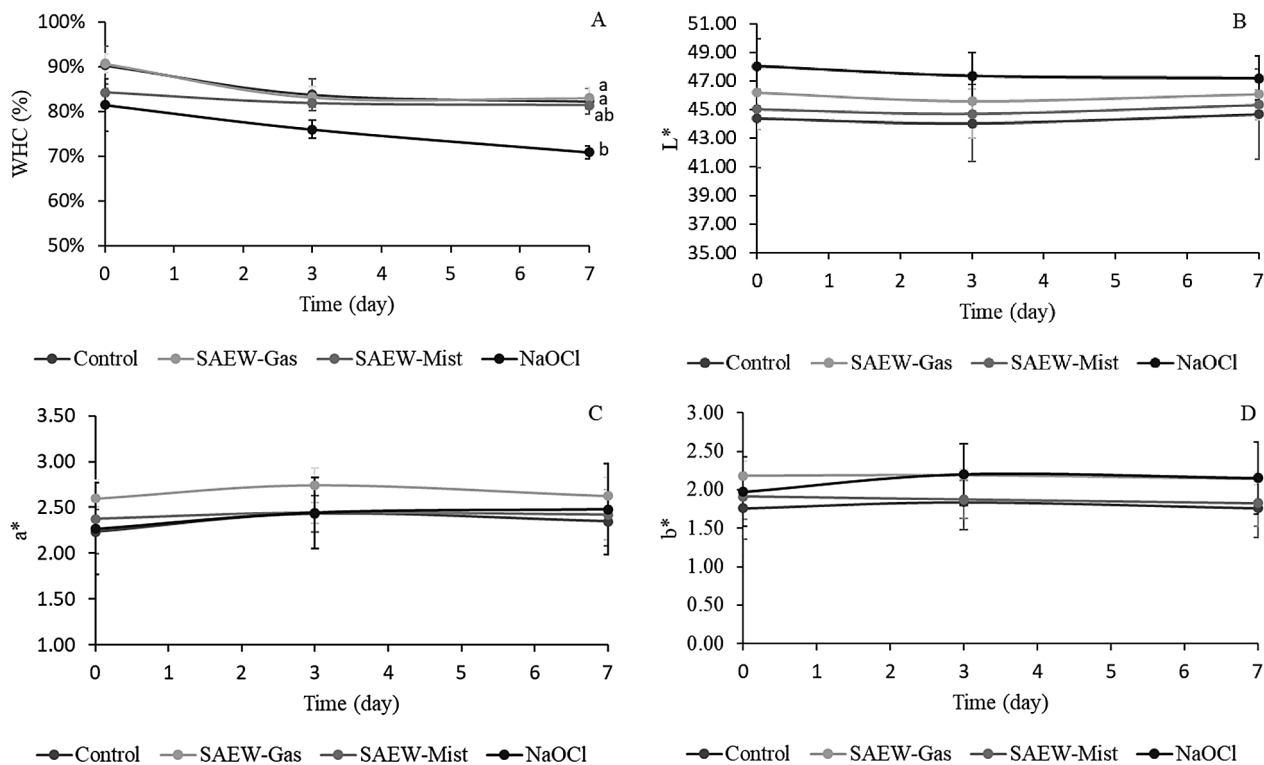


Figure 7. WHC (A) and color (B, C, and D) of raw chicken during storage. Values with different letters (a and b) are significantly different ($P < 0.05$)

Mist and Gas were not significantly different compared to the negative control. Immersion in 200 ppm NaOCl reduced WHC after treatment and during storage resulting in values that were significantly different from the control and SAEW-Gas treatment ($P < 0.05$). The lower WHC after NaOCl and SAEW-Mist treatment was due to increased moisture content absorption [39]. Carciofi and Laurindo [40] also showed that water uptake by poultry carcasses occurred within 10–15 minutes of immersion, which in turn may reduce the water holding capacity due to the higher amount of free water within the tissue. The porosity of raw chicken, as shown in Table 1, followed a similar trend to the WHC result. Kong et al. [41] also reported that larger gaps between muscle bundles are associated with lower WHC, further supporting this observation.

In terms of color, the NaOCl treated sample was the brightest with the L^* value of 48.02 ± 1.93 , although the difference was not significant compared with other treatments. Muscle proteins are generally classified into three main groups: sarcoplasmic proteins, myofibrillar proteins, and stromal or connective tissue proteins. Myofibrillar proteins, which form long, fibrous structures, constitute the majority of skeletal muscle proteins, contributing approximately 60 % to 70 % of the total protein content [42]. Che et al. [43] wrote that the denaturation of myofibrillar and sarcoplasmic proteins caused lower WHC and led to higher L^* values. The positive correlation between L^* values of poultry meat and WHC was also reported in previous research [44]. However, although SAEW-mist had lower WHC than SAEW-Gas, it showed higher L^* values. SAEW-Gas had higher TVB-N values, suggesting greater protein denaturation, which may contribute to increased

L^* values. SAEW-Mist exhibited color values similar to the control. At day 0, L^* , a^* , and b^* values were 45.03 ± 1.44 , 2.38 ± 0.20 , 1.91 ± 0.30 for SAEW-Mist, and 44.39 ± 3.42 , 2.23 ± 0.24 , 1.76 ± 0.40 for the control, respectively. SAEW-Gas showed the highest a^* value among all treatments, although it was not significantly different ($P > 0.05$). This may be attributed to the use of a forced-air system, which enhances surface myoglobin oxygenation and increases a^* values [45]. In addition, SAEW-Gas also had higher b^* values than SAEW-Mist. According to Kong et al. [41], increased b^* values may be associated with lipid oxidation.

Table 2. Trihalomethane formation (chloroform) on raw chicken

Treatment	Chloroform, $\mu\text{g/kg}$
Control	ND
SAEW-Gas	3.43 ± 3.81
SAEW-Mist	ND
NaOCl	5.46 ± 5.62

Values are means \pm standard deviations and not significantly different ($P \geq 0.05$)

Chloroform was detected in NaOCl and SAEW-Gas at levels of 5.46 ± 5.62 and 3.42 ± 3.81 , respectively (Table 2). One of the trihalomethanes that is usually detected in chicken is chloroform. The ACC types of NaOCl solution is mostly ClO^- that drive to trihalomethane formation [6]. The high standard deviation indicates variations in results across different replications. These variations likely occurred due to differences in meat quality resulting from varying harvest times. As shown in Figure 4, the control group had a total *Enterobacteriaceae* count of 2.54 ± 1.78 log CFU/cm², reflecting variations in sample quality among replicates. *Enterobacteriaceae* are also

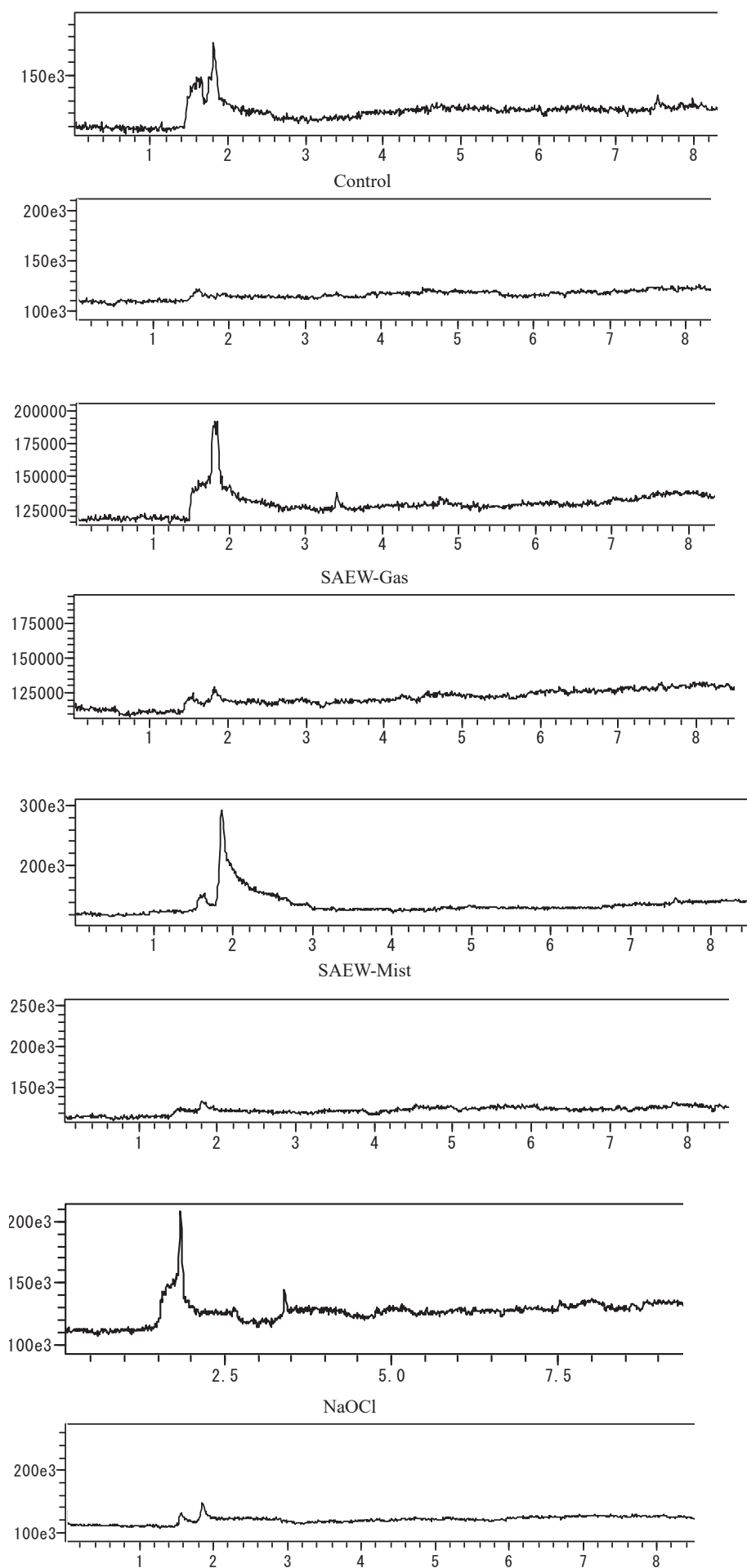


Figure 8. Trihalomethane detection in raw chicken from different replications

known as predominant histamine-producing bacteria in fish [46]. Meat quality can change depending on storage conditions and handling practices. Seasonal factors may also influence meat quality at the time of sampling [47]. Therefore, inconsistent meat conditions could affect the consistency of experimental results. The retention time of chloroform was recorded between 3.33 to 3.58. Some replications showed another peak with retention time 1.40 to 1.66. This peak was identified as propenamide (Figure 8). Protein through hydrolysis, rearrangement, and decarboxylation, can eventually give rise to formation of propenamide [48]. The presence of propenamide indicates the deterioration of meat. NaOCl and SAEW-Gas samples with propenamide peak also showed chloroform peaks. In contrast, control and SAEW-Mist samples showed no chloroform peak. This occurred even when propenamide peaks were present in those samples. The quality of the initial meat treated with a chlorine-based sanitation system may influence potential of trihalomethane formation. However, SAEW-Mist did not generate trihalomethanes. Therefore, SAEW-Mist can be considered a safer option for air sanitation systems.

Compared to an immersion sanitation system using NaOCl, the air sanitation system using SAEW-Gas and SAEW-Mist showed a lower microbial reduction. However, those treatments resulted in a slower microbial growth rate during storage. SAEW-Mist caused fewer chemical and physical changes throughout the storage period. This treatment also did not generate trihalomethanes. Moreover, the air system with mist reduces water usage and minimize waste. It also prevents cross-contamination and

improves hygienic production conditions during the chilling process [12]. Therefore, atomized SAEW (SAEW-Mist) can be proposed for air sanitation in chicken processing. It is especially suitable for the chilling step as part of an integrated sanitation system.

Conclusions

This study demonstrated that air sanitation systems using SAEW-Gas and SAEW-Mist were able to reduce total microorganisms and *Enterobacteriaceae*, although the reductions were not significantly different from the control groups. However, both treatments effectively slowed microbial growth during storage. SAEW-Mist showed better consistency in maintaining microbial, chemical, and physical quality. Compared to air sanitation system, the NaOCl immersion system caused higher porosity and faster microbial growth due to cell lysis and protein denaturation. SAEW-Mist also showed lower levels of TVB-N and lipid oxidation throughout storage, remaining below the threshold for off-flavor detection. Furthermore, SAEW-Mist produced no detectable trihalomethanes, unlike NaOCl and SAEW-Gas, which had potential of trihalomethane formation. The SAEW-Mist treatment offers additional benefits, including reduced water usage, elimination of liquid waste, and lower risk of cross-contamination. These advantages support the potential of SAEW-Mist as a safe, effective, and environmentally friendly alternative for air sanitation during the chilling step in chicken processing. Therefore, incorporating SAEW-Mist into integrated sanitation systems may improve product safety and quality while minimizing environmental impact.

REFERENCES

1. FAO. (2023). Meat Market Review: Emerging trends and outlook 2023. Roma, 2023. Retrieved from <https://www.fao.org/3/cc9074en/cc9074en.pdf> Accessed April 10, 2025.
2. Byun, K.-H., Han, S. H., Yoon, J., Park, S. H., Ha, S.-D. (2021). Efficacy of chlorine-based disinfectants (sodium hypochlorite and chlorine dioxide) on *Salmonella* Enteritidis planktonic cells, biofilms on food contact surfaces and chicken skin. *Food Control*, 123, Article 107838. <https://doi.org/10.1016/j.foodcont.2020.107838>
3. Golden, C. E., Rothrock, M. J., Mishra, A. (2021). Mapping foodborne pathogen contamination throughout the conventional and alternative poultry supply chains. *Poultry Science*, 100(7), Article 101157. <https://doi.org/10.1016/j.psj.2021.101157>
4. Yan, P., Chelliah, R., Jo, K. hee, Oh, D. H. (2021). Research trends on the application of electrolyzed water in food preservation and sanitation. *Processes*, 9(12), Article 2240. <https://doi.org/10.3390/pr9122240>
5. Bing, S., Zang, Y., Li, Y., Zhang, B., Mo, Q., Zhao, X. et al. (2021). A combined approach using slightly acidic electrolyzed water and tea polyphenols to inhibit lipid oxidation and ensure microbiological safety during beef preservation. *Meat Science*, 183, Article 108643. <https://doi.org/10.1016/j.meatsci.2021.108643>
6. Hernández-Pimentel, V. M., Regalado-González, C., Nava-Morales, G. M., Meas-Vong, Y., Castañeda-Serrano, M. P., García-Almendárez, B. E. (2020). Effect of neutral electrolyzed water as antimicrobial intervention treatment of chicken meat and on trihalomethanes formation. *Journal of Applied Poultry Research*, 29(3), 622–635. <https://doi.org/10.1016/j.japr.2020.04.001>
7. Vetchapitak, T., Rana, M. S., Sasaki, S., Taniguchi, T., Sugiyama, S., Soejima, J. et al. (2021). A new disinfectant technique for *Campylobacter jejuni* and spoilage bacteria on chicken skin using a high-pressure pulsed jet spray apparatus. *Food Control*, 125, Article 107989. <https://doi.org/10.1016/j.foodcont.2021.107989>
8. Rahman, S. M. E., Park, J., Song, K., Al-Harbi, N. A., Oh, D.-H. (2011). Effects of slightly acidic low concentration electrolyzed water on microbiological, physicochemical, and sensory quality of fresh chicken breast meat. *Journal of Food Science*, 77(1), M35–M41. <https://doi.org/10.1111/j.1750-3841.2011.02454.x>
9. Kartikawati, M., Kitamura, Y., Kokawa, M., Hamatani, M., Soejima, T. (2023). Effect of slightly acidic electrolyzed water immersion at different frequencies on quality of raw chicken legs. *Journal of Poultry Science*, 60(2), Article 2023027. <https://doi.org/10.2141/jpsa.2023027>
10. Demirok, E., Veluz, G., Stuyvenberg, W. V., Castañeda, M. P., Byrd, A., Alvarado, C. Z. (2013). Quality and safety of broiler meat in various chilling systems. *Poultry Science*, 92(4), 1117–1126. <https://doi.org/10.3382/ps.2012.02493>
11. Liu, C., Zheng, W., Li, Z., Zhou, L., Sun, Y., Han, S. (2022). Slightly acidic electrolyzed water as an alternative disinfection technique for hatching eggs. *Poultry Science*, 101(3), Article 101643. <https://doi.org/10.1016/j.psj.2021.101643>

12. Akekseeva, Y. A., Komlatsky, V. I., Khoroshailo, T. A., Vulykh, N. V. (November 18–20, 2020). *Modern methods for cooling raw meat*. IOP Conf. Series: Earth and Environmental Science, Volume 677. IV International Scientific Conference: AGRITECH-IV-2020: Agribusiness, Environmental Engineering and Biotechnologies 18–20 November 2020, Krasnoyarsk, Russian Federation, 2020. <https://doi.org/10.1088/1755-1315/677/3/032098>
13. Fukuzaki, S. (2023). Uses of gaseous hypochlorous acid for controlling microorganisms in indoor spaces. *Journal of Microorganism Control*, 28(4), 165–175. https://doi.org/10.4265/jmc.28.4_165
14. Zhao, Y., Xin, H., Zhao, D., Zheng, W., Tian, W., Ma, H. et al. (2014). Free chlorine loss during spraying of membraneless acidic electrolyzed water and its antimicrobial effect on airborne bacteria from poultry house. *Annals of Agricultural and Environmental Medicine*, 21(2), 249–255. <https://doi.org/10.5604/1232-1966.1108585>
15. Nasiłowska, B., Włodarski, M., Kaliszewski, M., Bogdanowicz, Z., Krzowski, Ł., Kopczyński, K. et al. (2024). Decontamination effect of hypochlorous acid dry mist on selected bacteria, viruses, spores, and fungi as well as on components of electronic systems. *International Journal of Molecular Sciences*, 25(13), Article 7198. <https://doi.org/10.3390/ijms25137198>
16. Miura, M., Gotoh, K., Tanamachi, C., Katayama, H., Fuketa, H., Tomoike, H. et al. (2024). Microbiological analysis concerning the antibacterial effect of atomized Ionless® hypochlorous acid water in a nursery school environment. *Journal of Infection and Chemotherapy*, 30(2), 123–128. <https://doi.org/10.1016/j.jiac.2023.09.024>
17. James, C., Daramola, B., Dudkiewicz, A., Reyers, F., Purnell, G., Turner, R. et al. (2014). Qualitative Risk Assessment to support a policy decision on partially-eviscerated poultry production. University of Lincoln and the Grimsby Institute. UK, 2014. Retrieved from https://www.food.gov.uk/sites/default/files/media/document/883-1-1628_FS101044_Effile_Final_Report_Revisedv2.pdf Accessed April 15, 2025.
18. Takaisangyou. (2019). 画像での製造工程を見たい方は、下記の工程名をクリックしてください Retrieved from <https://www.takaisangyo.co.jp/process> Accessed April 16, 2025 (In Japan)
19. USDA. (2015). Laboratory Guidebook Notice of Change: Quantitative Analysis of Bacteria in Foods as Sanitary Indicators. Retrieved from https://www.fsis.usda.gov/sites/default/files/media_file/2021-03/MLG-3.pdf Accessed April 16, 2025.
20. Dias Costa, R., Silva, V., Leite, A., Saraiva, M., Lopes, T. T., Themudo, P. et al. (2023). *Salmonella* spp., *Escherichia coli* and enterobacteriaceae control at a pig abattoir: Are we missing lairage time effect, pig skin, and internal carcass surface contamination? *Foods*, 12(15), Article 2910. <https://doi.org/10.3390/foods12152910>
21. Kim, H.-J., Shin, D.-J., Kim, H.-J., Cho, J., Kwon, J.-S., Kim, D. et al. (2022). Assessment of chicken thigh meat quality of Ross 308 broiler of animal welfare certified farm. *Animal Bioscience*, 35(12), 1957–1966. <https://doi.org/10.5713/ab.22.0044>
22. Zahir, H. (2021). Impact of different thawing methods on physico-chemical characteristics, electrophoretic profile and sensory evaluation of frozen beef longissimus dorsi muscle. *Journal of Animal and Poultry Production*, 12(1), 7–14. <https://doi.org/10.21608/jappmu.2021.149200>
23. Pereira, L. M., Lins, R. G., Gaspar, R. (2022). Camera-based system for quality assessment of fresh beef based on image analysis. *Measurement: Food*, 5, Article 100013. <https://doi.org/10.1016/j.meafao.2021.100013>
24. Běhalová, H., Tremlová, B., Kalčáková, L., Pospiech, M., Dordevic, D. (2020). Assessment of the effect of secondary fixation on the structure of meat products prepared for scanning electron microscopy. *Foods*, 9(4), Article 487. <https://doi.org/10.3390/foods9040487>
25. Murtey, M., Ramasamy, P. (2021). Life science sample preparations for scanning electron microscopy. *Acta Microscópica*, 30(2), 80–91.
26. Yang, T., Liu, R., Yang, L., Yang, W., Li, K., Qin, M. et al. (2022). Improvement strategies for quality defects and oxidation of pale, soft and exudative (PSE)-like chicken meat: Effects of domestic cooking and core temperature. *RSC Advances*, 12(12), 7485–7496. <https://doi.org/10.1039/d2ra00392a>
27. Hojat, N., Gentile, P., Ferreira, A. M., Siller, L. (2022). Automatic pore size measurements from scanning electron microscopy images of porous scaffolds. *Journal of Porous Materials*, 30(1), 93–101. <https://doi.org/10.1007/s10934-022-01309-y>
28. EPA. (2018). Method 8260D: Volatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS). Retrieved from <https://www.ehponline.org/viewfullarticle.php?url=/full/2018/11/10/1016/j.toxrep.2020.01.019> Accessed March 11, 2021.
29. Simões, C., Mendes, S., Martins, A., Gil, M. M. (2020). Risk assessment of trihalomethanes exposure by consumption of IV gamma products: Evidences from a Portuguese regional survey. *Toxicology Reports*, 7, 288–295. <https://doi.org/10.1016/j.toxrep.2020.01.019>
30. Goss-Sampson, M. A. (2020). Statistical Analysis in JASP A Guide for Students (JASP v.014). JASP. Retrieved from <https://jasp-stats.org/wp-content/uploads/2020/11/Statistical-Analysis-in-JASP-A-Students-Guide-v14-Nov2020.pdf> Accessed April 8, 2025.
31. Malka, S. K., Park, M.-H. (2022). Fresh produce safety and quality: Chlorine dioxide's role. *Frontiers in Plant Science*, 12, Article 775629. <https://doi.org/10.3389/fpls.2021.775629>
32. Chung, W. H., Chaklader, M. R., Howieson, J. (2024). Efficacy evaluation of chlorine dioxide and hypochlorous acid as sanitisers on quality and shelf life of Atlantic salmon (*Salmo salar*) fillets. *Foods*, 13(19), Article 3156. <https://doi.org/10.3390/foods13193156>
33. Huang, X., Zhu, S., Zhou, X., He, J., Yu, Y., Ye, Z. (2021). Preservative effects of the combined treatment of slightly acidic electrolyzed water and ice on pomfret. *International Journal of Agricultural and Biological Engineering*, 14(1), 230–236. <https://doi.org/10.25165/j.ijabe.20211401.5967>
34. Fukayama, M. Y., Tan, H., Wheeler, W. B., Wei, C. I. (1986). Reactions of aqueous chlorine and chlorine dioxide with model food compounds. *Environmental Health Perspectives*, 69, 267–274. <https://doi.org/10.1289/ehp.8669267>
35. Hopkins, D., Holman, B., Bekhit, A. E.-D., Giteru, S. (2020). Total volatile basic nitrogen in meat products: Occurrence, method of determination and use as a freshness indicator. Australian Meat Processor Corporation. Sydney, 2020. Retrieved from https://www.ampc.com.au/getmedia/c8521bea-98ab-4145-9794-fe4580db6fa2/Final-Report_2.pdf?ext=.pdf Accessed April 8, 2025.
36. Hadidi, M., Orellana-Palacios, J. C., Aghababaei, F., González-Serrano, D. J., Moreno, A., Lorenzo, J. M. (2022). Plant by-product antioxidants: Control of protein-lipid oxidation in meat and meat products. *Lebensmittel-Wissenschaft und Technologie*, 169, Article 114003. <https://doi.org/10.1016/j.lwt.2022.114003>
37. Rosario-Perez, P. J., Rodríguez-Sollano, H. E., Ramirez-Orejuel, J. C., Severiano-Pérez, P., Cano-Buendía, J. A. (2023). Neutral electrolyzed water in chicken breast — A preservative option in poultry industry. *Foods*, 12(10), Article 1970. <https://doi.org/10.3390/foods12101970>
38. Barbut, S. (2024). Measuring water holding capacity in poultry meat. *Poultry Science*, 103(5), Article 103577. <https://doi.org/10.1016/j.psj.2024.103577>
39. Carroll, C. D., Alvarado, C. Z. (2008). Comparison of air and immersion chilling on meat quality and shelf life of

- marinated broiler breast fillets. *Poultry Science*, 87(2), 368–372. <https://doi.org/10.3382/ps.2007-00213>
40. Carciofi, B.A.M., Laurindo, J.B. (2007). Water uptake by poultry carcasses during cooling by water immersion. *Chemical Engineering and Processing: Process Intensification*, 46, 444–450. <https://doi.org/10.1016/j.cep.2006.05.020>
 41. Kong, D., Quan, C., Xi, Q., Han, R., Koseki, S., Li, P. et al. (2022). Study on the quality and myofibrillar protein structure of chicken breasts during thawing of ultrasound-assisted slightly acidic electrolyzed water (SAEW). *SSRN Electronic Journal*. <https://doi.org/10.2139/ssrn.4129782>
 42. Dara, P. K., Geetha, A., Mohanty, U., Raghavankutty, M., Mathew, S., Chandragiri Nagarajarao, R. et al. (2021). Extraction and characterization of myofibrillar proteins from different meat sources: A Comparative study. *Journal of Bioresources and Bioproducts*, 6(4), 367–378. <https://doi.org/10.1016/j.jobab.2021.04.004>
 43. Che, S., Susta, L., Barbut, S. (2023). Effects of broiler chilling methods on the occurrence of pale, soft, exudative (PSE) meat and comparison of detection methods for PSE meat using traditional and Nix colorimeters. *Poultry Science*, 102(10), Article 102907. <https://doi.org/10.1016/j.psj.2023.102907>
 44. Lee, S.-K., Chon, J.-W., Yun, Y.-K., Lee, J.-C., Jo, C., Song, K.-Y. (2022). Properties of broiler breast meat with pale color and a new approach for evaluating meat freshness in poultry processing plants. *Poultry Science*, 101(3), Article 101627. <https://doi.org/10.1016/j.psj.2021.101627>
 45. Zhou, G. H., Xu, X. L., Liu, Y. (2010). Preservation technologies for fresh meat — A review. *Meat Science*, 86(1), 119–128. <https://doi.org/10.1016/j.meatsci.2010.04.033>
 46. Refai, M.A.E., El-Hariri, M., Ahmed, Z.A.M., El Jakee, J. (2020). Histamine producing bacteria in fish. *Egyptian Journal of Aquatic Biology and Fisheries*, 24(7), 1–11.
 47. Kutay, H., Şahan, Z., Polat Açık, İ., Durmuş, M. (October 4–6, 2023). *Factors affecting meat quality in farm animals*. BIO Web of Conferences, 3rd International Conference on Research of Agricultural and Food Technologies (I-CRAFT-2023). Adana, Turkey, 2023. <https://doi.org/10.1051/bioconf/20248501066>
 48. Lingnert, H., Grivas, S., Jägerstad, M., Skog, K., Törnqvist, M., Åman, P. (2002). Acrylamide in food: Mechanisms of formation and influencing factors during heating of foods. *Scandinavian Journal of Nutrition*, 46(4), 159–172. <https://doi.org/10.1080/110264802762225273>

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