Food Research 5 (5) : 250 - 258 (October 2021)
Journal homepage: http://www.myfoodresearch.com

FOOD RESEARCH

FULL PAPER

Effect of sanitizers and *Lactobacillus rhamnosus* R23 on the growth of *Salmonella* spp. in raw chicken fillets during temperature abuse storage

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Article history:
Received: 8 February 2021
Received in revised form: 8 March 2021
Accepted: 21 April 2021
Available Online: 30 October 2021

Keywords:
Lactobacillus rhamnosus R23,
Raw chicken,
Salmonella spp.,
Sanitizers,
Temperature abuse

DOI:
https://doi.org/10.26656/fr.2017.5(5).029

Abstract

One of the food products commonly contaminated by *Salmonella* is raw chicken. In wet markets in Southeast Asian countries, chicken carcasses frequently were handled and sold at abused temperatures, above the refrigeration temperatures (>5°C), thus could support the growth of *Salmonella*. One way to extend the shelf life of raw chicken carcasses at room temperature is by reducing the initial contamination using sanitisers such as ozone micro-bubble water (OMBW) or hypochlorite (NaOCl) solution. The other option is by adding bio-preservative agents such as lactic acid bacteria. This study aimed to evaluate the effect of sanitisers in reducing the initial contamination and the potential of lactic acid bacteria in inhibiting the growth of *Salmonella* in raw chicken fillets stored at abused temperatures. Chicken fillets were artificially inoculated with *Salmonella* (~5 log CFU/g) and rinsed for 5 minutes with sterile water, OMBW (1 and 2 ppm), or NaOCl solution (50 and 100 ppm). The results showed that washing the chicken fillets with NaOCl 100 ppm gave the most reduction of *Salmonella* counts. However, there were no significant effects regarding the inhibition of *Salmonella* growth during temperature abuse between those previously washed with OMBW or sterile water. The addition of *L. rhamnosus* R23 (6 log CFU/g and 8 log CFU/g) did not significantly inhibit the growth of *Salmonella* as compared to the control.

1. Introduction

Salmonella is a well-known pathogen causing food-borne diseases globally. Various strains of *Salmonella* have been found to contaminate various foods of animal origin. Poultry has been considered the main vehicle of *Salmonella* infection and is associated with the worldwide epidemic of *S.* Enteritidis (Regaldo-Pineda *et al.*, 2020). The presence of *Salmonella* in poultry animals, in particular chicken and turkey, is suggested as the main risk factor which allows easy transmission of the pathogen in table eggs and poultry meat to humans (Antunes *et al.,* 2015). Currently, it is estimated that 20 million human cases and 140,000 death per year occur due to *Salmonella* worldwide, and 30% of foodborne salmonellosis could be linked to poultry meat (Regaldo-Pineda *et al.*, 2020). Studies showed that about 30-50% of poultry carcass is contaminated by *Salmonella* at a concentration of 1-30 CFU/ carcass (Yang *et al.,* 2001). A study by Kusumaningrum *et al.* (2012) found that 52.5% of 40 samples of chicken carcasses from the open market and a supermarket in Bogor Indonesia were contaminated by *Salmonella*. The Indonesian National Standard SNI 3924 (2009) states that chicken carcasses can be stored fresh, chilled, or frozen; however, fresh carcasses must not exceed 4 hours after slaughter. Chilled carcasses must have an internal temperature of 0-4°C, while the frozen ones must have an internal temperature of -18°C. Ingham *et al.* (2007) recommended that poultry meat should not be stored for more than 8 hrs at 5-10°C or more than 2 hrs at 22°C to control *Salmonella*. The United States Food and Drug Administration (2001) defines that storage temperature at 5°C is considered safe to prevent the growth of pathogenic and non-psychotropic bacteria in potentially hazardous foods.

Temperature abuse is a condition where food

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eISSN: 2550-2166

products are not stored at the proper temperature or not according to the recommended temperature. This condition would lead to exposing the foods to the dangerous zone temperatures (5.5-60°C) where bacteria could grow rapidly to the numbers that could cause illness. The longer food products are stored in the danger zone, the faster the growth of pathogens is. An experiment by Ingham *et al.* (2007) observes the growth of *Escherichia coli* O157:H7 and *Salmonella* serovars in raw pork, poultry, and corned beef. Early counting showed a decrease of 0.2 log CFU/cut when stored at 5° C for 24 hrs, however, the numbers of log CFU/cut were increasing up to 0.2 log CFU/cut after exposure to temperature abuse. Brooks *et al.* (2008) observed an increase of 2 log of *Salmonella* in ground beef during temperature abused storage, i.e. 5 days at 0-2°C, 5 days at 10°C, followed by 10 days at 0-2°C. Another experiment by Oscar (2009) showed that storing raw chicken skin at temperature abuse of 5-50°C for 8 hrs could increase the numbers of *Salmonella.*

Nowadays, NaOCl is the most commonly used sanitiser in food industries because of the wide spectrum inhibition and easily dissolves in water. However, NaOCl could produce toxic byproducts that become public concerns. Another alternative is ozone in the form of ozone microbubble water (OMBW). Ozone is considered a safe sanitizer for foods because it does not produce hazardous residues, and is considered as Generally Recognized As Safe (GRAS). Ozone (O_3) is a strong antimicrobial compound with high oxidative activity and does not produce hazardous residues because it is decomposed into oxygen. In contrast to hypochlorous acid (HOCl), ozone does not effectively penetrate the cell but causes damage to the cell wall structure on the outside of the cells. Tirawat *et al.* (2014) reported that OW at a concentration of 0.5 mg/L for 10 mins exposures times gave a 3.93 log reduction of *Salmonella enterica* serovar Typhimurium *in vitro*.

Ozone micro-bubble water (OMBW) is a technique to increase the solubility of ozone in water that produces microscopic size of ozone bubbles in the water. The sizes of the bubbles may vary between 50-200 μm in diameter. During the production, micro-bubbles will slowly float up to the water or any liquid surface. During the floatation, more ozone will be dissolved than that in normal conditions. As a result, micro-bubbles have increased surface area, are highly stable in water, and have high efficiency for a surface cleaner. OMBW reduced viable cells of *E. coli* O157:H7 (5.0-7.4 log), better than that of the ozone water and resulted in decontamination on the surface of leafy vegetables (Chuajedton *et al.,* 2017). The *in vitro* experiments on 13 types of bacteria, such as *E. coli* O157:H7, *Salmonella enterica* serovar Enteritidis JCM1652, *Cronobacter sakazakii* JCM1233T, *Listeria monocytogenes* ATCC13932, *Staphylococcus aureus* JCM2413, and *Lactobacillus plantarum* JCM1149, showed that a reduction of 5.0 to 7.4 log CFU/mL of viable cells was observed after 3 mins of exposure to OMBW (5.44 mg/L) at 25°C (Inatsu *et al.,* 2011).

The use of lactic acid bacteria (LAB) as bio preservative in food products has been reported due to their wide antagonistic characteristics against pathogens. Sakaridis *et al.* (2014) reported a decrease of 0.51 log CFU/cm^2 *Salmonella* spp. and *L. monocytogenes* in raw chicken carcass samples that were inoculated with 10^6 log CFU/cm^2 LAB and stored at 7°C for 6 days. In addition, the sensory evaluation showed no effect on the odor and appearance of the chicken carcasses after incubation with the LAB.

This study is aimed to determine the best sanitizing agents to reduce the initial loads of *Salmonella* spp. in raw chicken carcasses and the effectiveness of LAB to inhibit the growth of *Salmonella* spp. in raw chicken carcasses during temperature abuse storage. The research results are expected to help carcass producers in providing quality and safe chicken carcass.

2. Materials and methods

2.1 Raw chicken fillet and microbial cultures

Raw chicken fillets were obtained from local retail in Bangkok (Thailand) and Bogor (Indonesia). *Salmonella enterica* serovar Enteritidis, *Salmonella enterica* serovar Derby, *Salmonella enterica* serovar London, and *Salmonella enterica* serovar Newport were obtained from the Laboratorium of Food Science and Technology - Kasetsart University collection. Meanwhile, *Lactobacillus rhamnosus* R23, *Salmonella enterica* serovar Hadar, *Salmonella enterica* serovar Heidelberg and *Salmonella enterica* serovar Kentucky were from SEAFAST CENTER IPB collection.

2.2 Effect of sanitizing agents on Salmonella spp. and the microbial viable counts in chicken fillet

2.2.1 Preparation of Salmonella culture

Each culture of *S. enterica* ser. Enteritidis, *S. enterica* ser. London, *S. enterica* ser. Newport, and *S. enterica* ser. Derby from culture stocks on Tryptic Soy Agar (TSA) was inoculated into 9 mL of sterile Tryptic Soy Broth (TSB) separately and incubated at 35°C for 24 hrs. A loop full of each culture was streaked on Xylose Lysine Deoxycholate (XLD) Agar and incubated at 35°C for 24 hrs. A single colony of each culture were

harvested and inoculated into 9 mL of Tryptic Soy Broth (TSB) and incubated at 35°C for 24 hrs. A total of 10 mL (2.5 mL from each culture) was added into 90 mL of sterile TSB to form a cocktail mix of *Salmonella* and incubated at 35°C for 18 hrs.

2.2.2 Preparation of chicken fillet

Raw chicken breast from local retail was packed in sterile plastic bags and stored in a cooling box to be transported to the laboratory. The chicken breast was aseptically filleted, cut and weighed to obtain 300 g samples. The initial microbial analysis showed the absence of *Salmonella* in the chicken fillets.

2.2.3 Salmonella inoculation

Chicken breast fillets (300 g) were inoculated with 5 mL of culture suspension containing 10^8 log CFU/mL of *Salmonella* cocktail mix to achieve 10^5-10^6 log CFU/g of Salmonella in the chicken fillets. The cocktail mix was placed in the middle part of the fillet and spread aseptically on the fillet's surfaces using a sterile hockey stick. Samples were kept in laminar flow for 10 mins prior to further treatment.

2.2.4 Preparation of sanitizer solution for washing

Ozone microbubble water was prepared using Microbubble generator (Model: Microstar FS101-1, Fuki Manufacturing Co., Ltd., Japan) and Ozone generator (Model: ED-0GR6, Ecodesign, Inc., LTD., Japan) and placed in 40 L distilled water in a sterile acrylic tank. The acrylic tank was previously sanitized using 70% ethanol. The ozone concentration was measured immediately before use using an N,N-diethyl-p-phenylenediamine (DPDs) measuring photometer with a DPD tablet No.4 (ProMinent® HD-MMP 01, Germany). Sodium hypochlorite (NaOCl) solution was freshly prepared before use by diluting a bleach solution containing 6% sodium hypochlorite (v/v) (Haiter, Kao Industrial, Thailand) in distilled water to achieve the required concentration. The free chlorine concentration was measured immediately before use using an N,N-diethyl-p-phenylenediamine (DPDs) measuring photometer with a DPD tablet No.1 (ProMinent® HD-MMP 01. Germany).

2.2.5 Washing process

Chicken fillets samples were washed separately in 3 L (1:10) of sterile water or OMBW (1 ppm and 2 ppm), NaOCl (50 and 100 ppm) in a shaking water bath (80 rpm) for 5 mins and then aseptically drained for 5 mins (Phaephiphat and Warapa 2018, with some modification).

2.2.6 Storage of samples at 4°C and abused temperatures

Chicken fillet samples were stored at 4±1°C for 24 hrs, transferred to temperature abuse at 10±1°C for 4, 6, and 8 hrs, and then continued to 30±1°C for 2 hrs.

2.2.7 Microbial analysis

A total of 25 g of chicken fillet samples were diluted into 225 mL of 0.1% peptone water (Merck, Darmstadt, Germany) and then homogenized in a stomacher (Seward, UK) for 120 s. Serial dilutions were prepared using 10 mL of each homogenized suspension and then were enumerated on the specific media. The total *Salmonella* was enumerated on Xylose Lysine Deoxycholate (XLD) Agar using the spread plate technique after incubation at 37°C for 48 hrs. Meanwhile, the total viable counts (TVC) was enumerated on Plate Count Agar (PCA) following incubation at 37°C for 48 hrs.

2.3 Effect of lactic acid bacteria as a biopreservative on chicken filled contaminated with Salmonella spp.

2.3.1 Preparation of LAB culture

A total of 1 mL of the stock culture of *Lactobacillus rhamnosus* R23 was inoculated into 9 mL of sterile De Man, Rogosa, and Sharpe Broth (MRSB) and incubated at 37°C for 48 hrs. 10 mL of culture was then transferred into 90 mL of fresh MRSB and incubated at 37°C for 32 hrs.

2.3.2 Preparation of chicken fillet and Salmonella culture

The preparation of *Salmonella* culture was done as above (2.2.1). The strains used in this step were *S. enterica* ser. Enteritidis, *S. enterica* ser. Hadar, *S. enterica* ser. Kentucky, and *S. enterica* ser. Heidelberg . Samples preparation and inoculation of *Salmonella* cocktail into chicken fillet was done as before (2.2.3). The inoculated samples were washed in 3 L (1:10) of 100 ppm NaOCl solution in a shaking water bath (80 rpm) for 5 mins. Samples were then drained for 5 mins, aseptically.

2.3.3 Lactic acid bacteria inoculation

Approximately 3 mL of *L. rhamnosus* R23 suspension containing 10^8 log CFU/mL and 10^{10} log CFU/mL was placed in the middle part of the chicken fillet surfaces and spread using sterile hockey stick to achieve 10^6 log CFU/g and 10^8 log CFU/g of LAB in chicken fillet. Samples were then kept in the laminar flow for 10 mins before further treatment. The samples were then stored at 4±1°C for 24 hours, transferred to 10±1°C for 2, 4, and 6 hrs, and then stored at 30±1°C for

2 hrs (Sakaridis *et al.,* 2014, with modification)

2.3.4 Microbial analysis

Enumeration of total *Salmonella* and TVC was done as (2.2.7). The total LAB was enumerated by placing 25 g of chicken fillet in 225 mL of KH_2PO_4 solution and then homogenized using a stomacher (Seward, UK) for 120 s. Serial dilutions were prepared by using 10 mL of each homogenized suspension and enumeration was done on De Man, Rogosa, and Sharpe Agar (MRSA) after incubation at 37°C for 48 hrs.

2.3.5 pH measurement

A total of 25 g of chicken fillet was homogenized in 225 mL of distilled water by stomacher for 120 s. The determination of the pH valued was carried out using a pH meter (Eutech pH 700, Eutech Instruments Pte Ltd., Singapore).

2.3.6 Experimental design

This study was conducted using a completely randomized design, with four repetitions in the first stage and three repetitions in the second stage. Every replication was done in duplicate. Statistical analysis was performed by Analysis of Variant (ANOVA) and post hoc test with Duncan Multiple Range Test (DMRT) at a significant level of 5% using SPSS 22.0 software.

3. Results and discussion

3.1 Effect of sanitizing agents on Salmonella in chicken fillet

Washing of raw chicken fillet with OMBW, NaOCl solution, and sterile water at different concentrations could reduce the number of *Salmonella* spp. Washing chicken fillet with 100 ppm NaOCl solution was the most effective to reduce the *Salmonella* as shown by the most reduction numbers of *Salmonella* (i.e. 1.3 log CFU/g), followed by 50 ppm NaOCl, 1 ppm OMBW, 2 ppm OMBW, and sterile water. As compared to OMBW, washing chicken carcasses with 50 ppm NaOCl did not show a significant reduction of *Salmonella*. Washing chicken fillet with 100 ppm NaOCl showed a significant reduction ($P<0.05$) of *Salmonella* as compared to washing with OMBW or sterile water (Table 1). This result showed that at the concentration used in this study, NaOCl caused more damage to *Salmonella* cells than ozone. NaOCl works by penetrating the cell membrane of bacteria and disturbing the components inside the cell such as enzymes, RNA, and proteins. Meanwhile, ozone works by interfering with the cell membrane without penetration into the cell (Gonçalves, 2009). Generally, there are 2 mechanisms of microorganism inactivation by ozone. First, ozone will oxidize the sulfhydryl groups and amino acids of enzymes, peptides, and proteins to produce a smaller peptide. Second, ozone is able to oxidize the polyunsaturated fatty acid to form acid peroxide. That reaction leads to subsequent leakage of cellular contents and cell lysis (Kim *et al.,* 1999; Gonçalves, 2009). The experiment by Phaephiphat and Warapa (2018) showed that washing fresh sweet basil using 50 and 100 ppm NaOCl was more effective to reduce *S. enterica* ser. Typhimurium compared to washing with 1 ppm OMBW at 30°C. According to Phaephiphat and Warapa (2018), washing using a high concentration of ozone is less effective because of its instability in water, especially at room temperature. Ozone is more stable to dissolve in water if applied at low temperature or in a cool environment. A study by Luiz *et al.* (2017) showed that washing fish meat with ozone water at different combinations of ozone concentration and temperatures (21°C × 0.35 ppm; 20°C × 0.45 ppm; 21°C × 0.60 ppm; 20°C × 0.80 ppm; 19°C × 1.7 ppm; 6°C × 5.1 ppm; 4°C × 7.2 ppm; and 2°C × 9.1 ppm) for 3 mins did not eliminate the *Salmonella* Typhimurium (ATCC 14028) contamination. The existence of organic components also could reduce the effectiveness of ozone because chemical interactions between ozone and organic components could hinder the contact of ozone and cells (Kim *et al.,* 1999).

3.2 Effect of sanitizing agents on microbial count in chicken fillet

The initial count in raw chicken fillets was 5-6 log CFU/g and was found to decrease after washing with all

Table 1. *Salmonella* in chicken fillet after washing with sterile water and various sanitizing agents

Sanitizers	Initial count (Log CFU/g)	Viable count after washing (Log CFU/g)	Log reduction (Log CFU/g)	Percentage of reduction (%Log CFU/g)
Sterile Water (Control)	5.3±0.7	4.9±0.8	0.4±0.3^{a}	7.6
1 ppm OMBW	5.1±0.0	4.2±0.2	0.9±0.2^{b}	17.7
2 ppm OMBW	5.1±0.0	4.4±0.1	0.7±0.1^{ab}	13.7
50 ppm NaOCl	5.6±0.9	4.6±1.2	0.9±0.3^{b}	16.1
100 ppm NaOCl	5.6±0.9	4.2±0.8	1.3±0.2^{c}	23.2

Values are presented as mean±SD, n = 4. Values with different superscripts in the same column are significantly different at the 5% level ($p<0.05$).

treatments. Washing chicken fillet with 100 ppm NaOCl exhibited the highest reduction of TVC, i.e. 1.5 log CFU/g, as compared to other treatments. The results showed a significant reduction of TVC ($P<0.05$) in chicken fillets washed with NaOCl (50 ppm and 100 ppm) as compared to the control. When compared to washing with 1 ppm and 2 ppm OMBW, the reduction of TVC in chicken fillet washed with 50 ppm NaOCl was not significantly different ($P>0.05$), while washing with 100 ppm NaOCl showed a significant difference ($P<0.05$) (Table 2). This result is in line with research by Pan and Nakano (2014) suggesting that a 2-log reduction of TVC in fresh vegetables was achieved by washing with 100 ppm NaOCl for 10 mins. According to Rocky *et al.* (2017), the antibacterial activity of sodium hypochlorite will increase along with the temperature increase. Rocky *et al.* (2017) reported that there was a reduction of 0.83 log CFU/g of TVC in quail meats after being washed with 50 ppm chlorine at 30°C while washing with tap water did not significantly reduce the microbe contamination.

3.3 Effect of sanitizing agents on chicken fillets contaminated with Salmonella spp. during storage

Storage of chicken carcasses at the refrigeration temperature of 4±1°C for 24 hrs resulted in a 0.5 - 0.8 log CFU/g decrease in *Salmonella* in the samples previously washed with NaOCl solutions or sterile water. This result is in line with research by Ingham *et al.* (2007) who reported a 0.2 log CFU/cut reduction of *Salmonella* in chicken carcass stored at 5°C for 24 hrs.

Chicken fillet previously washed with OMBW showed an insignificant increase in *Salmonella* spp. count (i.e. 0.2 log CFU/g) after storage at 4±1°C for 24 hrs. Further storage of chicken fillet previously washed with 1 ppm OMBW at moderate temperature abuse (10±1°C) exhibited a small increase in *Salmonella* (i.e. 0.2 log CFU/g) after 8 hrs, while chicken fillet previously washed with 50 ppm and 100 ppm NaOCl solutions showed a decrease of *Salmonella* spp. by 0.7 log CFU/g after storage at 10° C for 8 hrs. Washing chicken fillets with sterile water also reduced the number of *Salmonella* by 0.4 log CFU/g after being stored at 10°C for 8 hrs, yet the amount of reduction was not significantly different ($P>0.05$) from the other treatments. These results showed that sanitizing agents did not significantly affect the growth of *Salmonella* spp. during storage at 10°C. In chicken fillet samples sanitized with 1 ppm OMBW and stored at 30°C (extreme temperature abuse) for 2 hrs, there was an increase in *Salmonella* count by 0.2 log CFU/g from the condition before storage. On the other hand, in chicken fillet samples sanitized with 2 ppm OMBW, no changes in *Salmonella* count were observed at the end of storage at 30°C for 2 hrs. In chicken fillet samples sanitized with 50 ppm and 100 ppm NaOCl, the *Salmonella* count decreased by 0.2-0.6 log CFU/g after storage at 30°C for 2 hrs (Figure 1). The sanitizer residues, neither ozone nor NaOCl was detected after storage at 4°C for 24 hrs (data not displayed). The absence of residues supports the growth of *Salmonella* during the storage period, especially when samples were exposed to favourable temperature. According to Allende *et al.* (2008), sanitizer agents will reduce the initial contamination, but during storage at refrigeration temperature, the surviving bacteria could grow in absence of sanitizers.

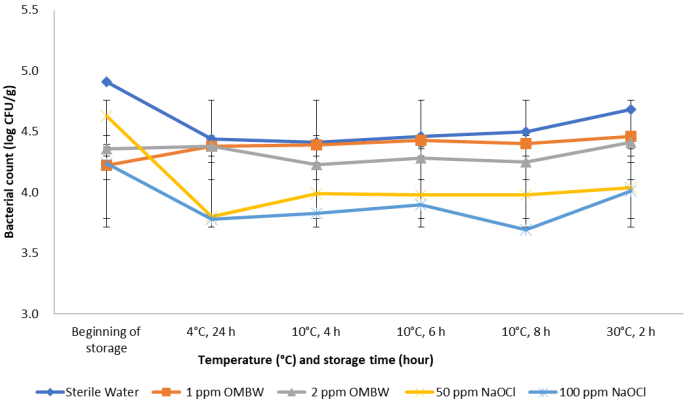

Figure 1. Change in *Salmonella* counts during the temperature abuse storage of chicken fillet.

TVC of chicken fillet sanitized with NaOCl solutions or sterile water decreased by 0.2-0.3 log CFU/g after storage at 4°C for 24 hrs. In samples sanitized with 1 ppm and 2 ppm OMBW, the TVC numbers increased by 0.1-0.3 log CFU/g. The changes of TVC in chicken fillet

Table 2. Total viable count (TVC) in chicken fillet after washing with sterile water and various sanitizing agents

Sanitizers	Initial count (Log CFU/g)	After washing (Log CFU/g)	Log reduction (Log CFU/g)	Percentage of reduction (%Log CFU/g)
Sterile Water (Control)	5.5±0.8	5.3±0.8	0.2±0.1^{a}	3.6
1 ppm OMBW	5.2±0.1	4.5±0.1	0.6±0.0^{b}	11.5
2 ppm OMBW	5.2±0.1	4.8±0.0	0.3±0.0^{ab}	5.8
50 ppm NaOCl	5.8±1.0	5.1±0.7	0.8±0.0^{b}	13.8
100 ppm NaOCl	5.8±1.0	4.4±0.5	1.5±0.4^{c}	25.9

Values are presented as mean±SD, n = 4. Values with different superscripts in the same column are significantly different at the 5% level ($p<0.05$).

eISSN: 2550-2166

samples sanitized with different sanitizer agents were not significantly different (P>0.05) after storage at 4°C for 24 hrs, therefore the numbers tend to be stable. The increase of TVC was observed after samples were stored at 30°C for 2 hrs because of favourable temperature that supports the growth of contamination bacteria, yet the growth was less than <1 log CFU/g (Figure 2).

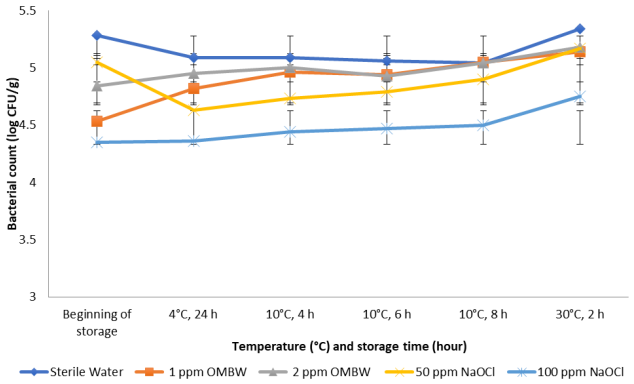

Figure 2. Change in the total viable count (TVC) during temperature abuse storage of chicken fillet

3.4 Effect of lactic acid bacteria as *a biopreservative on chicken fillet contaminated with Salmonella spp.*

Sanitizing chicken carcasses can reduce the initial contamination of bacteria. However, those surviving the process may grow during storage. To inhibit the growth of pathogens during chicken carcasses storage, biopreservative agents such as lactic acid bacteria (LAB) can be added. LAB is known to have antibacterial activity against some pathogens. One of the LAB that was known to possess the antibacterial characteristic is *L. rhamnosus* R23 isolated from breast milk. A study by Nuraida, Susanti, Hana *et al.* (2012) showed that *L. rhamnosus* isolated from breast milk was able to inhibit the growth of other pathogens such as *E. coli*, *B. cereus*, *S. aureus*, and *S. enterica* ser. Typhimurium. Nuraida, Hana, Hartini *et al.* (2012) also reported that *L. rhamnosus* R23 could inhibit the growth of *E. coli* enteropathogenic (EPEC) K1.1 in mice.

Lactobacillus rhamnosus R23 inoculated in chicken fillet containing *Salmonella* spp. (5-6 log CFU/g) survive during storage at 4°C for 24 hrs and stay viable during 6 hrs storage at 10°C which is shown by relatively stable LAB counts. Statistical analysis showed no significant difference (p<0.05) of the LAB population during cold storage. After storage at 30°C for 2 hrs, the LAB count increased by 0.3-0.4 log CFU/g. In chicken fillet previously washed with sterile water and inoculated with 6 Log CFU/g LAB, the final population of LAB was 6.4 log CFU/g, while in those inoculated with 8 Log CFU/g LAB, the final population was 8.1 log CFU/g. In chicken fillet previously sanitized with 100 ppm NaOCl and inoculated with 6 log CFU/g LAB and 8 log CFU/g LAB, the final population of LAB was 6.2 log CFU/g and 8.2 log CFU/g, respectively, after 2 hrs incubation at 30°C (Figure 3). Nuraida *et al.* (2014) showed that the viability of *L. rhamnosus* R23 in yogurt relatively stable during storage at low temperature (<10°C) for 32 days. The viability of *L. rhamnosus R23* was not affected by *Salmonella* in samples. This result is similar to Riyanti (2012) who reported that the growth of mixed strains of *L. rhamnosus* was not affected by the presence of mixed strains *C. sakazakii*.

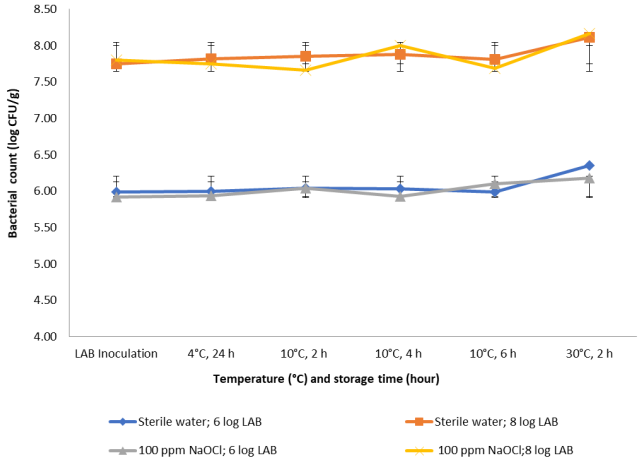

Figure 3. LAB growth in the presence of *Salmonella* spp. during temperature abuse storage of chicken fillet.

The addition of LAB into chicken fillet previously washed with sanitizing agents did not affect the growth of *Salmonella* spp. during 24 hrs storage at 4°C. No inhibition activity of LAB was detected in this experiment during chicken fillet storage at 10°C for 8 hrs (Figure 4). This result is in line with Agilar dan Klotz (2010) who reported that the inhibition activity of LAB significantly decreases along with the decrease in temperature. The phenomenon is associated with the decrease in metabolic activity of LAB to produce antibacterial compounds. The low growth of *Salmonella* at 10°C was caused by the decrease of metabolism activity due to the inhibition of enzymatic activities at low temperature, rather than due to antibacterial compounds.

Chicken fillet washed with sterile water and inoculated with 6 log CFU/g and 8 log CFU/g LAB stored for 2 hrs at 30°C exhibited an increase of *Salmonella* count by 0.2 log CFU/g. The same results were also found in chicken fillet previously sanitized with 100 ppm NaOCl and inoculated with 6 log CFU/g. However, in samples inoculated with 8 log CFU/g LAB, there was an increase in *Salmonella* count by 0.3 log CFU/g at the end of the storage period. The number of *Salmonella* in chicken fillet previously washed with sterile water without the addition of LAB increased by

0.1 log CFU/g after storage at 30°C for 2 hrs, however, there was no significant difference (P>0.05) in the number of S*almonella* increases in all samples (Table 3).

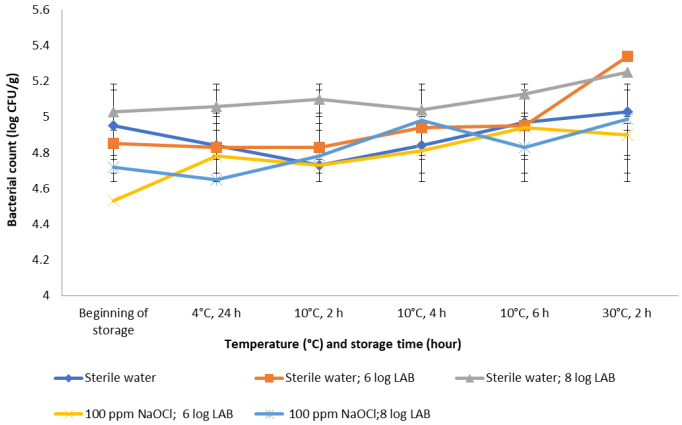

Figure 4. The growth of *Salmonella* spp. during temperature abuse storage of chicken fillet

LAB could inhibit the growth of pathogens by producing antimicrobial compounds, such as organic acids (lactic acid and acetate acid), hydrogen peroxide, carbon dioxide, diacetyl, and bacteriocin (Servin, 2004). Researches by Sakaridis *et al.* (2014) and Ingham (2009) reported that inhibition activity could occur without any cell growth because the production of antimicrobial compounds continues through the metabolism process during storage. It is likely that in this study, the metabolism activity of LAB was not sufficient to inhibit the growth of *Salmonella* during storage at temperature abuse.

Lactobacillus rhamnosus is a homofermentative LAB that produces mainly lactic acid (Nuraida *et al.,* 2014). The lactic acid produced in this research did not significantly reduce the pH of samples. During storage at temperature abuse, the pH of samples was observed at 5.64-5.89, thus allowing Salmonella to grow. It was presumed that the growth rate of *Salmonella* at 30°C was faster than LAB causing *Salmonella* to grow first in the samples. Veys *et al.* (2016) reported that the growth of *Salmonella* as much as 6 Log CFU/g was observed after incubation at 37°C for 10 hrs with a growth rate of 0.82 log CFU/hr and lag time 0.85 hr. At 25°C, the lag time was 1.85 hrs, and the growth rate was 0.63 log CFU/hr. Subagiyo *et al.* (2015) reported that in the first 6 hrs, LAB is still in the lag phase when incubated at 25°C-35°C. These findings explain that the first 2 hrs of incubation at 30°C in this research was not sufficient for LAB to grow optimally to inhibit *Salmonella* growth.

According to Russel *et al.* (2004), one of the factors that influence antimicrobial activities is the number of contaminating microbes. Fewer microbial cells will increase the likelihood of contact between the antimicrobial components and the microbial cells. Thus, the inactivation is more effective. Uddin *et al.* (2019) reported that the number of *Salmonella* spp. in chicken carcasses sold at supermarkets in Dhaka (Bangladesh) ranged between 0.47-3.36 CFU/g. These salmonellae numbers were much lower than those added to the chicken meat in this study (5-6 log CFU/g). It is likely that the high number of salmonellae resulted in the ineffectiveness of LAB to inhibit the pathogen.

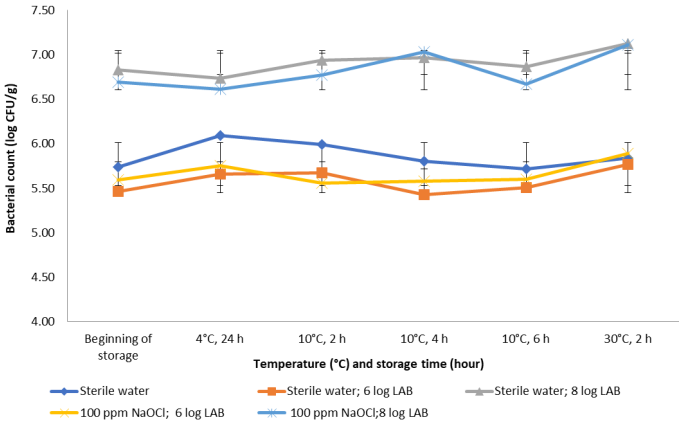

Figure 5. Total viable count (TVC) during temperature abuse storage of chicken fillet contaminated with *Salmonella* spp.

The viable bacteria enumeration showed an increase in samples that were inoculated with LAB, while in samples not added with LAB, the TVC decreased at the end of the storage period at low temperature (4°C for 24 hrs and 10°C for 6 hrs). After storage of the chicken fillet at 30°C for 2 hrs, the TVC of samples inoculated

Table 3. Effect of lactic acid bacteria (LAB) on *Salmonella* spp. growth in chicken fillet during temperature abuse storage

Temperature and time of storage	Change in viable count of *Salmonella* (log CFU/g)				
	Sterile water	Sterile water, 6 Log LAB inoculation	Sterile water, 8 Log LAB inoculation	100 ppm NaOCl, 6 Log LAB inoculation	100 ppm NaOCl, 8 Log LAB inoculation
4°C, 24 h	-0.1±0.2^{Aa}	0.0±0.7^{Aa}	0.0±0.1^{Aa}	0.3±0.4^{Aa}	-0.1±0.4^{Aa}
10°C, 4 h	-0.2±0.3^{Aa}	-0.2±0.4^{Aa}	0.1±0.1^{Aa}	0.2±0.4^{Aa}	0.1±0.2^{Aa}
10°C, 6 h	-0.1±0.4^{Aa}	-0.1±0.5^{Aa}	0.0±0.3^{Aa}	0.3±0.4^{Aa}	0.3±0.3^{Aa}
10°C, 8 h	0.0±0.3^{Aa}	-0.2±0.5^{Aa}	0.1±0.1^{Aa}	0.4±0.5^{Aa}	0.1±0.3^{Aa}
30°C, 2 h	0.1±0.7^{Aa}	0.2±0.3^{Aa}	0.2±0.1^{Aa}	0.2±0.2^{Aa}	0.3±0.5^{Aa}

Values are presented as mean±SD, n = 3. Values with different lowercase superscripts in the same column are significantly different at the 5% level ($p<0.05$) while values with different uppercase in the same row are significantly different at the 5% level ($p<0.05$).

eISSN: 2550-2166

with LAB showed an increase of 0.30 – 0.42 log CFU/g. The samples previously washed with 100 ppm NaOCl and inoculated with 8 log LAB showed the highest increase in TVC, while in samples with no LAB added, the TVC only increase by 0.10 log CFU/g (Figure 5). The results showed that the addition of LAB in the chicken fillet would increase the TVC.

4. Conclusion

Washing chicken fillet with 100 ppm NaOCl solution was the best treatment to reduce the initial *Salmonella* population as compared to OMBW. Washing the chicken fillets with sanitizers before storage did not inhibit the growth of *Salmonella* spp. significantly during temperature abuse. The addition of LAB after washing the chicken fillets with sterile water or NaOCl solution also did not inhibit the growth of *Salmonella* spp. during temperature abuse. The best way to reduce the *Salmonella* population during storage is by reducing the initial contamination. For future research, it is suggested to enhance the effectiveness of sanitizing agents such as increasing exposure time and washing temperature. The use of LAB cultures, which can produce a bacteriocin, and/or in combination with other methods could also be evaluated to effectively inhibit the growth of *Salmonella* during storage at temperature abuse. The effect of sanitizer and LAB on specific strains of *Salmonella* would support the behaviour of *Salmonella* during storage at temperature abuse.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgement

The authors would like to express their gratitude to The Kasetsart University of Thailand for partially funded the research, and to the Master of Science in Food Security and Climate Change (MS-FCC) Programme through Erasmus+ Programme of the European Union and Southeast Asian Regional Center for Graduate Study and Research in Agriculture (SEARCA) in supporting Mr. Nalle during his internship at Kasetsart University.

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