EFFICACY OF OZONATED WATER AND CALCIUM HYPOCHLORITE TO REDUCE MICROBIAL POPULATION ON CHICKEN BREASTS

Thumdee, A[1] and Pilasombut, K[1]

[1] Division of Animal Production Technology and Fisheries, Faculty of Agricultural Technology,

King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand

Abstract - The aim of this experiment was to study the antimicrobial efficacy of ozonated water and calcium hypochlorite on chicken breasts. The experiment was arranged into 7 treatments included control, dipping breast in tap water, 50, 100, 150, 200 ppm of free available chlorine from calcium hypochlorite solution and 10 ppm of ozonated water. The samples were then stored in chilling room at 0-4 °C for 3 days. Total microbial count, psychotropic bacteria, total coliforms, fecal coliforms and *E. coli* were determined before dipping, after dipping and 3 days. The results presented that total microbial count and psychotropic bacteria of chicken breast were decreased after treated with different concentration of free available chlorine from calcium hypochlorite and ozonated water as compared to the control and the treatment with tap water. Antimicrobial treatments had no effect to total coliforms in chicken breasts, except chlorine 200 ppm could inhibit fecal coliforms and *E. coli.* Therefore, this experiment indicated that it has been beneficial to use ozone and calcium hypochlorite as antimicrobial agents and extending the product shelf life.

Key word - Calcium hypochlorite, Chicken breast, Ozonated water

I. INTRODUCTION

In Thailand, a traditional market is an open air place with various hygiene conditions. The temperature range is from 30 to 38 °C. Chicken stall owners displays various parts of chicken on the table without temperature control [9] . In Thailand, it has been reported that the occurrence of food poisoning caused more than 120,000 patients each year due to consumption of food contaminated with pathogenic microorganisms including *Salmonella* sp., *E. coli*, *S. aureus* and *Vibrio* sp. and so forth. Minami *et al*. [8] studied of pathogenic contamination of meat from Thai traditional markets and supermarkets in Bangkok and found that 48 and 57% of *Salmonella* contaminate in chicken meat, respectively. In addition, *Listeria monocytogenes* were isolated from chicken meat from open markets and supermarkets of 6 and 4%, respectively. Pilasombut *et al*. [11] investigated the microorganism contamination in beef from Thai traditional markets in Bangkok and found that 92.68 percent of the total samples having levels of microorganisms exceeding the the Thai Agricultural Standards 6700-2005. It was found that 75.1 % of samples having total coliform did not meet the standard. *E. coli* contamination was found in all samples between log 1.30-7.04 MPN /g. Many reports have studied that ozonated water and chlorine are antimicrobial agents that are active against various microorganisms, including spoilage and pathogenic bacteria [6,8]. Chlorinated water is used in some countries, at levels of 50 ppm or higher to control microorganisms and treat carcass immersion water to prevent cross-contamination [3,4]. Ozone is approved in the United States for use in treating water to be recycled in poultry chill tanks [4].

Therefore, the objective of this study was to apply different decontamination treatments, ozonated water and free available chlorine, for microbial population reduction of chicken breasts which contain initial microbial load from Thai traditional market.

II. MATERIALS AND METHODS

Chicken breasts were purchased from the Thai traditional market in Bangkok in early morning. Samples were transported immediately to laboratory after collected in an ice box. Ozone and chlorine were conducted to reduce microbial on chicken breasts. Piece of breast was dipped in antimicrobial solution for 5 min. The experiment was arranged into 7 treatments (3 replications)

included a control (no dipping), dipping breast in tap water, 50, 100, 150 and 200 ppm. of free available chlorine from calcium hypochlorite solution and 10 ppm of ozonated water (ENALY model O_3 generator). After that, samples were packed in foam trays and wrapped in oxygen-permeable cling film. The samples were then stored in chilling room at 0-4 °C for 3 days. Total microbial count and psychotropic bacteria were examined according to AOAC [2] and Diliello [5], respectively. The number of bacterial counts express as log $_{10}$ colony forming unit (CFU). Determination of total coliforms, fecal coliforms and *E.coli* counts were recorded as most probable number (MPN) [2]. The sampled time was at before dipped, after dipped for 10 min and 3 days.

III. RESULTS AND DISCUSSION

Total microbial contamination on chicken breasts

Initial number of microorganisms was observed before the treatments (Table 1). According to the report of Thai agricultural standards [14], the recommend limit of total microbial count in chicken is 5.70 log cfu/g. It was found that the average of total microbial count on chicken breasts exceeded the recommend limit of TAS-6700-2005. This data agree with Pilasombut *et al*.[10] who investigated the microorganism contamination in beef from traditional Thai markets in Bangkok and found that 92.68 percent of the total samples having levels of microorganisms exceeding the the TAS. 6001-2004. Minami *et al*. [8] was survey pathogenic contamination of meat from traditional markets and supermarkets in Bangkok and found that 48 and 57% of *Salmonella* contaminated in chicken meat, respectively. Moreover, *Listeria monocytogenes* were isolated from chicken from traditional markets and supermarkets of 6 and 4%, respectively.

Effect of antimicrobial treatments on total microbial count and psychotropic bacteria

Total microbial count and psychotropic bacteria of chicken breasts were decreased after treated with different concentration of free chlorine from calcium hypochlorite and ozonated water as compared to the control. The most effective method for reduction of total microbial count on chicken breasts was the ozonated water (1.60 log cfu/g) (Table 1). The microbial reduction of total microbial count of the breasts after treatment with tap water, 50, 100, 150 and 200 ppm of free available chlorine were 0.56, 0.74, 0.71, 1.01 and 1.09 log cfu/g, respectively, while number of total microbial count of control group increased. Similarly, the microbial reduction of psychotropic bacteria after treated with ozonated water was 1.07 log cfu/g, whereas the other treatments displayed 0.14, 0.63, 0.32, 0.4 and 0.98 log cfu/g, respectively. Their number of total microbial count and psychotropic bacteria of all groups increased with long-storage, especially in day 3. However, breasts treated with ozonated water and free chlorine (100, 150 and 200 ppm) revealed to inhibit total microbial count after stored for 3 days (Table 2). Bunsic and Sofos [4] reported that chlorine reacts with water to produce the forms of free available chlorine, hypochorous acid and hypochlorite ions. Hypochlorous acid is free form most lethal to microorganisms.

These results are in agreement with Al-Haddad *et al.*[1] who suggested that ozone is a bactericidal against *Pseudomonas aeruginosa* and *Salmonella infantis*. The use of gaseous ozone (2,000 ppm) and stored under gas packaging could reduce *Pseudomonas aeruginosa* 95% and *Salmonella infantis* 97% but indigenous coliforms were unaffected. Gonçalves *et al.* [7] found that calcium hypochlorite at 60, 70 and 100 mg/l reduced the viability of cell of *Listeria monocytognes*. In addition, Stivarius *et al.* [13] and Pohman *et al*. [12] who reported that using chlorine or ozone in ground beef was effective against aerobic plate count and *Salmonella* Typhimurium.

Table 1 Number of total microbial count and psychrotrophic bacteria in chicken breasts before and after treatments

Treatment (ppm)	Number of microbial count (log CFU/g)			Log reduction (Before-After)
	Before	After	3 days	
Total microbial count				
Control	5.12 ± 0.35	5.98 ± 0.45	5.97 ± 0.91	increase
Tap water	5.94 ± 0.78	5.38 ± 0.50	5.42 ± 0.59	0.56
Cl 50	6.26 ± 0.57	5.52 ± 0.70	6.07 ± 0.25	0.74
Cl 100	6.00 ± 0.62	5.29 ± 0.43	5.54 ± 0.59	0.71
Cl 150	6.36 ± 0.93	5.35 ± 0.41	5.42 ± 0.54	1.01
Cl 200	5.95 ± 0.17	4.86 ± 0.26	4.94 ± 0.41	1.09
Ozone 10	6.31 + 0.65	5.34 + 0.23	5.42 + 0.54	1.60
Psychotropic bacteria				
Control	6.32 ± 0.79	6.10 ± 0.29	7.32 ± 0.09	increase
Tap water	5.89 ± 0.28	5.75 ± 1.22	6.90 ± 0.20	0.14
Cl 50	6.32 ± 0.32	5.69 ± 0.59	6.98 ± 0.03	0.63
Cl 100	5.65 ± 0.05	5.33 ± 0.33	6.75 ± 0.28	0.32
Cl 150	5.62 ± 0.34	5.17 ± 0.26	6.79 ± 0.26	0.45
Cl 200	6.73 ± 0.91	5.75 ± 0.35	6.55 ± 0.32	0.98
Ozone 10	6.97 ± 0.90	5.92 ± 0.49	6.67 ± 0.33	1.05

Note: Cl means chlorine
Log reduction = number of microbial before treated – number of microbial after treated

Effect of antimicrobial treatments on total coliforms, fecal coliforms and E. coli

The results found that antimicrobial treatments have no effect to inhibit total coliforms. Ozonated water at concentration of 10 ppm was not inhibiting total coliforms, fecal coliforms and *E. coli*. However, after treatment with 200 ppm of free available chlorine from calcium hypochlorite resulted in reductions of fecal coliforms and *E. coli* on chicken breasts (Table 2). Al-Haddad *et al.* [1] reported that the use of gaseous ozone had unaffected to coliforms. On the other hand, Stivarius *et al.* [12] and Pohman *et al*. [11] found that both ozone and chlorine were effective inhibit coliforms and *E. coli*.

IV. CONCLUSION

Ozonated water (10 ppm) and sodium hypochorite solution (50-200 ppm) can be used for microbial decontamination and prolonging shelf-life of chicken breasts up to 3 days under refrigeration. Inhibitory effect of both antimicrobial agents on total coliforms was not observed. However, free available chlorine from calcium hypochlorite solution at 200 ppm could inhibit fecal coliforms and *E. coli.*

Table 2 Number of total coliform bacteria, fecal coliform bacteria and *E.coli* in chicken breasts before and after treatments

Treatment (ppm)	Number of microbial count (log MPN/g ± S.D.)		
	Before	After	3 days
Total coliform bacteria			
Control	3.69±0.49	4.07±0.30	4.69±0.54
Tap water	3.39±0.07	3.51±0.84	4.36±0.74
Cl 50	3.56±0.86	3.38±0.83	4.20±0.14
Cl 100	2.97±0.34	4.07±0.30	4.49±0.63
Cl 150	4.14±0.39	3.98±0.38	4.36±0.74
Cl 200	3.04±0.96	3.07±0.30	4.03±0.20
Ozone 10	3.89±0.83	3.93±1.11	4.06±1.13
Fecal coliform bacteria			
Control	3.61±0.19	3.52±0.75	4.60±0.57
Tap water	3.23±0.43	3.44±0.74	4.11±0.40
Cl 50	3.60±0.31	3.65±0.36	3.89±0.34
Cl 100	3.24±0.42	3.10±0.33	4.36±0.73
Cl 150	3.39±0.67	3.21±0.17	4.02±0.29
Cl 200	3.81±0.42	3.36±0.56	3.81±0.30
Ozone 10	3.52±0.80	3.91±1.12	3.52±0.52
E.coli			
Control	3.59±0.21	3.47±0.91	4.60±0.57
Tap water	3.00±0.37	2.97±0.41	4.11±0.40
Cl 50	3.70±0.34	3.78±0.40	3.89±0.35
Cl 100	3.24±0.42	3.34±0.02	4.36±0.74
Cl 150	3.85±0.19	3.33±0.03	4.02±0.29
Cl 200	4.02±0.36	3.37±0.01	3.80±0.52
Ozone 10	4.00±1.08	4.04±0	3.52±0.52

Note: Cl means chlorine

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