Effect of ionized calcium on bacteria contamination in chicken carcass under slaughter process

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Abstract

The purpose of this study was to determine the effect of ionized calcium on bacteria cross contamination on chicken carcass and meat during the slaughter process. Compared to the control group, colony of *E. coli* was not observed on medium containing 0.5% ionized calcium. Cross contamination of bacteria on carcass surface of fresh chicken was increased as the number of scalding was increased. Cross contamination of bacteria on carcass surface of fresh chicken was lower in the 0.5% ionized calcium scalding treatment group than that in the control group. Bacteria colony count on chicken meat surface after cooling water treatment was increased as the storage period was increased. Bacteria colony count was lower in the 0.5% ionized calcium treatment group than that in the control group.

Keywords Ionized calcium · Bacteria · Chicken · *E. coli* · Scalding

Introduction

As chicken meat consumption continues to increase globally, it is essential to ensure microbial safety during slaughter of poultry. Preventing microbial contamination during the slaughter is very important to keep chicken meat fresh (Rouger et al. 2017). Decreasing contamination of raw poultry would significantly reduce the incidence of illness. Contamination occurs both in the farm and poultry slaughter plants (Hong et al. 2015; Choi et al. 2016). As most chicken meat is produced in small slaughter houses, meat quality is susceptible to degradation due to cross contamination of bacteria. Microbes contaminated on carcass and meat surface during the slaughtering process and distribution of chicken meat can proliferate during storage. They can cause lipid rancidity and change meat quality, leading to lower meat quality and shorter storage period. In addition, they are hygienic hazards (Chae et al. 2008; Lee et al. 2015).

During slaughtering process when chicken is scalded, washed, or cooled. Microbial cross contamination can

easily occur. The soft texture of fresh chicken meat makes it easy for microbes to proliferate. After bleeding, chicken is immersed in water at 55 °C for 3 min to remove feathers. In this scalding process, cross contamination of microbe can occur through the scalding water (Bailey et al. 1987; Chae et al. 2011). Because the body temperature of chicken is as high as 41 °C, microbial proliferation can increase. When fresh chicken meat is stored and transported, microbial proliferation can lower the freshness of meat. This is why cooling water treatment is necessary during slaughtering. If microbial cross contamination is high during cooling water treatment, it can cause problem in chicken meat quality, especially freshness maintenance (Patsias et al. 2006; Hong et al. 2008). A lot of efforts have been made to prevent microbial cross contamination on meat surface and maintain the freshness of chicken meat by adding various materials into cold treatment water (Gonzalez-Fandos and Dominguez 2007; Rand et al. 2007). In slaughter house, chicken meat is packaged individually to be shipped to retail market, where consumers buy chicken meat. If chicken meat with high initial microbial contamination is packaged after slaughtering, microbial proliferation can further increase during the distribution process (Chae et al. 2008).

Water soluble ionized calcium (CaO, Calcium oxide, ionized calcium) with purity of 99% or more has been developed from oyster and ark shells after they are burned to get rid of magnesium and phosphorus (Sawai 2003; Kang et al.

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2008). Ionized calcium (CaO) solution is basic through dehydration. It has anti-bacterial activity (Sawai et al. 2001; Sawai 2003). It can be used to substitute chlorine sterilization of vegetable (Kim et al. 2012). It can also be used to extend the storage period of food (Kim et al. 2007). However, whether ionized calcium could be used during chicken slaughtering to prevent microbial cross contamination of chicken meat remains unclear. Therefore, the objective of this study was to determine the effect of ionized calcium treatment on microbial cross contamination of carcass or chicken meat surface.

Materials and methods

Experimental materials

Broiler chickens (body weight, 1.8 kg) were supplied by a chicken breeding farm located in Ansong, Gyungki-do, Korea on the day of shipment. Fresh chicken meat was purchased from a slaughter house located in Choonchun, Kangwon-do, Korea. Ionized calcium was supplied by g2g Inc. (Seoul, South Korea).

Growth inhibition of *Escherichia coli*

When ionized calcium solution at different levels was used in treatment in a pre-test to study its inhibition effect on the growth of *E. coli*, it was found that 0.5% ionized calcium solution showed optimum inhibition effect. Therefore, 0.5% ionized calcium solution was used in this study to examine its inhibitory effect on the growth of *E. coli*. Treatment group and control group were used in six repeated tests. For the control group, 1 L of regular distilled water without any ionized calcium was used. For the treatment group, 1 L of 0.5% ionized calcium solution was used to prepare each test medium. To make test medium, 23 g of nutrient agar (Difco, USA) was mixed with 1 L of regular distilled water or ionized calcium solution. The medium was sterilized at 121 °C for 15 min and 15 mL was dispensed onto culture plate to prepare solid medium. Activated *E. coli* (KCTC 1039) was diluted to 10^6 . Diluted *E. coli* (100 µL) was spread onto the solid medium. After culturing at 37 °C for 24 h, the number of colonies was counted. The concentration of bacteria was expressed in log CFU/ml (Jang et al. 2009).

Bacterial cross contamination after scalding

Broiler chickens were electrically stunned and blood was depleted by cutting the carotid artery. Chickens were then scalded. Treatment was either normal scalding water

treatment or scalding water containing 0.5% ionized calcium. The temperature of water was maintained at 55 °C. The concentration of 0.5% for ionized calcium solution was chosen based on results of pre-test with different concentrations. The loss of solution at each scalding was compensated to maintain the same amount of scalding water. Sixty fresh chickens were assigned to normal scalding water group or 0.5% ionized calcium water group. Scalding was repeated 10 consecutive times (3 min each time) for six birds each time. Feathers were removed to obtain fresh chicken meat for measurement of cross contamination on carcass surface. Fresh chicken was scalded for 1, 5, 7, or 10 times. Aluminum foil was attached to the back, legs, and breast of the carcass surface (area of 10 cm²). A sterilized cotton swab absorbing 1 mL of sterile distilled water was used to collect the specimen by moving it up and down and from left to right direction on the area for which foil was attached. The cotton swab specimen was put in a 9 mL tube of sterile distilled water and diluted step by step (up to a dilution factor of 10^4), after which 1 mL of the diluted specimen was inoculated onto Petrifilm (RAC, Rapid Aerobic Count plate, 3M Co, St. Paul, MN, USA) and cultured at 35 °C for 24 h. Scalding water (1 mL each) used for 1, 5, 7, or 10 times of scalding was added to a tube containing 9 mL of sterile distilled water and diluted step by step up to a dilution factor of 10^4 and 1 mL of the diluted scalding water was cultured in the same way as described above. The number of bacteria (CFU/cm²) on fresh chicken surface or in scalding water (CFU/mL) was then counted.

Bacterial cross contamination after cooling water treatment

To determine the effect of ionized calcium treatment on freshness maintenance of chicken, one control group (ionized calcium, 0%) and three treatment groups (ionized calcium, 0.5, 0.7, and 0.9%) were used. Cooling water at 4 °C containing 0, 0.5, 0.7, or 0.9% of ionized calcium was prepared by dissolving 0, 40, 56, or 72 g of ionized calcium in 8,000 mL of regular potable water and maintained in a low temperature room during test. The volume of 8000 mL of cooling water was maintained by compensating the cooling water with ionized calcium at the same concentration each time when the cooling water was lost during the cooling process. A total of 80 fresh chickens were used for the four groups (20 chickens per group). Each chicken was treated in cooling water for 50 min. After cooling water treatment, chickens were packaged in commercial zipper bags and stored in a low temperature room for 0, 5, 7, and 9 days. Microbial cross contamination on the surface of chicken meat was measured for each storage period.

Statistical analysis

SPSS/Windows 21.0 (statistical package for the social science, SPSS Inc., Chicago, IL, USA) was used for all data analyses. One-way analysis of variance (ANOVA) was used to compare mean value of each test. Statistically significant difference was determined at 95% confidence level by Duncan's multiple test ($p < 0.05$).

Results and discussion

In the result of *E. coli* culture test on media containing regular distilled water without ionized calcium or with 0.5% ionized calcium, 8.01 colonies were formed on the media prepared with regular distilled water. However, no colony was observed on the media prepared with 0.5% ionized calcium (Table 1; Fig. 1). It has been reported that calcium oxide possesses sterilizing power (Sawai et al. 2001). After seashells are fired at high temperature at 1,300–1,500 °C to make high purity calcium oxide powder, the powder has been used to prepare liquid sterilizing coating and solid sterilizing additive. When these sterilizing agents are applied to various air purifying filters, it has 99.9% sterilizing power against *E. coli* and *Staphylococcus aureus* (Sawai et al. 2001; Chae et al. 2013). These results are consistent with results of this study.

When broiler chickens were scalded in regular scalding water to remove feathers, microbial cross contamination

Table 1 Detection of *E. coli* (CFU/mL) in distilled water and 0.5% ionized calcium medium

Basal medium	Distilled water	0.5% Ionized calcium
Nutrient agar	8.01	N.D

ND not detected

($n = 6$)

Fig. 1 Counts of *E. coli* with distilled water and 0.5% ionized calcium medium. Left: 0.5% ionized calcium. Right: distilled water medium

on the surface of fresh chicken was increased ($p < 0.05$) as the number of scalding was increased (Fig. 2). Microbial cross contamination on carcass surface of fresh chicken was increased as the number of scalding was increased. It was increased significantly from 5.21 CFU/cm² at the first scalding to 5.29, 5.54, and 5.65 CFU/cm² at the 5th, 7th, and 10th scalding, respectively. Our results are consistent with results of previous findings shown that microbial cross contamination on chicken meat surface is increased with increasing number of scalding (Bailey et al. 1987; Chae et al. 2011).

Microbial cross contamination on carcass surface of fresh chickens was reduced from 6.65 CFU/cm² after scalding with 0% ionized calcium solution (the control group) to 5.23 CFU/cm² after scalding with 0.5% ionized calcium solution (Fig. 3). This result proved that 0.5% ionized calcium scalding treatment during chicken slaughtering could reduce microbial cross contamination on the carcass surface of fresh chickens. Various efforts have been made to prevent microbial cross contamination on carcass surface to maintain freshness of chicken. It has been reported that mixture of citric acid and sodium chlorite can inhibit the growth of microbe on the surface of chicken thigh meat. Diffusion of calcium and hydroxyl ion is associated with solvent used to

Fig. 2 Contamination of bacteria in chicken carcasses after scalding under slaughter process (CFU/cm²). ^{A,B}Curves not sharing a common letter differ significantly ($n = 6$)

Fig. 3 Contamination of bacteria in chicken carcasses after scalding with ionized calcium under slaughter process (CFU/cm²). Bars show standard error of mean values. * $p < 0.05$ show significant difference between control and 0.5% ionized calcium group

Fig. 4 Counts of bacteria colony in chicken carcasses during storage at 4 °C after cooling water by ionized calcium. Bars show standard error of mean values ($n = 6$). ^{a,b,c}differ between control and ionized calcium groups ($p < 0.05$). ^{X,Y,Z}differ among storage days ($p < 0.05$)

dissolve calcium hydroxide (Kim and Yi 2002). To enhance the effect of calcium hydroxide, various solvents have been used to increase its anti-bacterial activity (Stuart et al. 1991). However, no study has reported its effect on fresh chicken meat. Cross contamination of microbes on carcass surface of fresh chicken produced in slaughter houses is a very serious issue. Results of this study are highly beneficial to satisfy the preference of consumers for clean and safe meat product for their well-being (Hwang et al. 2004).

Changes in bacteria count (CFU/cm²) on chicken meat surface on each day during storage at 4 °C after cooling water treatment using solutions containing different concentrations of ionized calcium are shown in Fig. 4. Bacteria count on chicken meat surface was lower in ionized calcium treated group than that in the control group. It was increased over time in all treatment groups. Difference between groups was significant. In the control group, the count was 3.22 CFU/cm² on day 0. It was increased to

3.61, 4.81, and 5.61 CFU/cm² on days 5, 7, and 9, respectively. In the 0.5% ionized calcium treated group, the count was 2.74 CFU/cm² on day 0. It was increased to 2.71 when it actually decreased from 2.74 CFU/cm².

In 0.7% ionized calcium treated group, the count was 2.37 CFU/cm² on day 0. It was increased to 2.61, 4.17, and 5.17 CFU/cm² on days 5, 7, and 9, respectively. Among the two ionized calcium treatment groups, bacterial counts with the same storage time were not significantly different. The reason why bacterial count in ionized calcium treated group was lower than that in the control group might be due to the fact that the pH of chicken meat in ionized calcium treated group was lower than that in the control group. The pH value is a very important factor in bacteria proliferation. When pH becomes higher, the speed of proliferation of harmful bacteria is also faster. Harmful bacteria can decompose protein during meat storage and produce ammonia which increases the pH of chicken breast meat (Kim et al. 2013). Our results showed that using a solution containing 0.5% ionized calcium for cool water treatment for 50 min, while slaughtering had potential to increase freshness and storage of chicken meat by keeping bacteria count on chicken meat surface under 10⁵ CFU/cm².

Conclusions

In conclusion, 0.5% ionized calcium treatment compare to control treatment without ionized calcium had anti-bacterial activity against *E. coli* and other bacteria. Bacterial cross contamination on the carcass surface of fresh chickens was reduced from 6.65 to 5.23 CFU/cm² after scalding in 0.5% ionized calcium solution. After 9 days of storage at 4 °C after cooling with ionized calcium solution, the number of bacteria on the surface of chicken meat was decreased by 8.91%. Therefore, ionized calcium treatment can be used to prevent microbial cross contamination on chicken meat surface. Additional tests are needed to confirm the promising potential of such treatment.

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Compliance with ethical standards

Conflict of interest The author had no conflicts of interest to declare.

References

- Bailey MJ, Tavlas GS, Uian M (1987) The impact of exchange-rate volatility on export growth: some theoretical considerations and empirical results. *J Pol Model* 9:225–243
- Chae HS, Ahn CN, Yoo YM, Jang AR, Jeong SG, Ham JS, Cho SH (2008) Effect of water uptake rate of chicken on lipid oxidation, color of meat, and microbes of chicken during storage. *Kor J Poult Sci* 35:247–253
- Chae HS, Na JC, Choi HC, Kim MJ, Bang HT, Kang HK, Kim DW, Suh OS, Ham JS, Jang AR (2011) Effect of gas mixture ratio of modified atmosphere packaging on quality of chicken breast. *Korean J Food Sci An* 31:100–106
- Chae SY, Kim JR, Lee JL (2013) Development of anti-bacterial coating materials using the ionized oyster shell powder. *Theories Appl Chem Eng* 19:736–739
- Choi EJ, Chung YB, Kim JS, Chun HH (2016) Effects of freezing and thawing treatments on natural microflora, inoculated *Listeria monocytogenes* and *Campylobacter jejuni* on chicken breast. *J Food Hyg Safe* 31:42–50
- Gonzalez-Fandos E, Dominguez JL (2007) Effect of potassium sorbate washing on the growth of *Listeria monocytogenes* on fresh poultry. *Food Control* 18:842–846
- Hong YH, Kim MK, Song KB (2008) Effects of aqueous chlorine dioxide treatment on microbial safety and quality of samgae chicken. *Korean J Food Pres* 15:769–773
- Hong SH, Park NY, Jo HJ, Ro EY, Ko YM, Na YJ, Park KC, Choi BG, Min KJ, Lee JK (2015) Risk ranking determination of combination of foodborne pathogens and livestock or livestock products. *J Food Hyg Safe* 30:1–12
- Hwang WM, Lee SM, Hwang HS, Han JH (2004) Survey on the contamination of microorganisms in chicken meat from slaughterhouse in Incheon area. *Korean J Vet Public Health* 28:59–65
- Jang AR, Park SO, Shin JH, Choi WK, Park BS (2009) Antibacterial activity of ethanol extracts from house fly-maggot against various strains of Methicillin-resistant *Staphylococcus aureus* and Vancomycin-resistant *enterococci* strain. *Ann Anim Res Sci* 20:49–57
- Kang NS, Choi Y, Park YS, Sohn ES, Pyo S, Kang SC, Eum HA, Sohn EH (2008) Development of ionic calcium as food additives; market and technology trends analysis. *Korean Soc Biomat* 12:141–147
- Kim TH, Yi YH (2002) The effect of citric acid and sodium chlorite mixtures on the growth of microorganisms from boiler thigh surface. *Korean J Food Sci An* 22:44–49
- Kim YS, Choi YM, Noh DO, Cho SY, Suh HJ (2007) The effect of oyster shell powder on the extension of the shelf life of tofu. *Food Chem* 103:155–160
- Kim JG, Nimtkeatkai H, Choi JW, Lee SG (2012) The effects of calcinated calcium solution washing and heat treatment on the storage quality and microbial growth of fresh-cut broccoli. *J Bio-Environ Control* 21:411–418
- Kim DS, Song YR, Muhlism M, Seo TS, Jang TS, Lee SK, Pak JI (2013) The effects of Doenjang (Korean traditional fermented soy bean paste) powder on the quality and shelf-life of chicken sausages during storage. *Korean J Poult Sci* 40:315–325
- Lee MS, Kim SH, Moon SH, Kim YH (2015) Inhibitory effects of natural additives on pathogenic microorganisms growth during storage of commercial chicken. *Korean J Food Sci Technol* 47:574–578
- Patsias A, Chouliara I, Badeka A, Savvaadis IN, Kontominas MG (2006) Shelf-life of a chilled precooked chicken product stored in air and under modified atmospheres: microbiological, chemical, sensory attributes. *Food Microbiol* 23:423–429
- Rand JL, Hofmann R, Alam MZB, Chauret C, Cantwell R, Andrews RC, Gangnon GA (2007) A field study evaluation for mitigating biofouling with chlorine dioxide or chlorine integrated with UV disinfection. *Water Res* 41:1939–1948
- Rouger A, Tresse O, Zagorec M (2017) Bacterial contaminants of poultry meat: Sources, species, and dynamics. *Microorganisms*. 5:50–65
- Sawai J (2003) Quantitative evaluation of antibacterial activities of metallic oxide powders (ZnO, MgO and CaO) by conductimetric assay. *J Appl Microbiol* 54:177–182
- Sawai J, Satoh M, Horikawa M, Shiga H, Kojima H (2001) Heated scallop-shell powder slurry treatment of shredded cabbage. *J Food Prot* 64:1579–1583
- Stuart KG, Miller CH, Brown CE, Newton CW (1991) The comparative antimicrobial effect of calcium hydroxide. *Oral Surg Oral Med Oral Pathol* 72:101–104