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Original Research

The Use of Chlorine Dioxide Solution as an Effective Decontaminant for Broiler Chicken Carcass

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Abstract

Ensuring the microbial safety of poultry meat and cuts is essential as there was an upsurge in the consumption of meat and value-added products over the years worldwide. Chlorine dioxide (ClO_2) is a powerful decontaminant and is effective against wide range of microorganisms. The present study investigates the effect of ClO_2 as an effective decontaminant for broiler chicken carcass. Broiler chicken carcass was washed with hot water (T1), carcass washed with 100 ppm ClO_2 solution for 5 minutes (T2) and carcass washed with 200 ppm ClO_2 solution for 5 minutes (T3) were the three treatments taken. The untreated chicken carcass is taken as control (C). Surface swabs were collected from broiler carcass and assessed the microbial quality. pH, water holding capacity and sensory evaluation of the samples were also evaluated. Total viable count, Coliform count, and Yeast and mould count were significantly lower ($p<0.05$) for T3. T3 also recorded higher values for pH and water holding capacity. On sensory evaluation, mean overall acceptability of the control, T2 and T3 show no significant differences ($p<0.05$), but T1 showed significantly lower overall acceptability scores as compared to others. This study shows that broiler chicken carcasses can be effectively decontaminated after processing by washing with ClO_2 solution at 200 ppm concentration for 5 minutes. This method can be used as a tool to increase the shelf life and reduce the illness caused by various pathogens without altering the sensory qualities.

Key words: Broiler Chicken, Chlorine Dioxide, Carcass Decontamination, Poultry Processing

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Introduction

Poultry is the second most widely consume meat in the world, accounting for 30% of meat production Worldwide. The growth of poultry production and processing industry remains relatively positive and shows an upward trend due to its competitive prices with respect to other meat and strong consumer preference. The growth is 6-8% in layers and 10-12% in broiler per year against the growth of agriculture as a whole which is around 2.5%. Global production of poultry meat has increased rapidly over the last 50 years, growing more than 12- fold between 1961- 2015. (FAO, 2016).

Poultry provides a perfect medium for the growth of microorganisms. The spoilage bacteria found on poultry include *Pseudomonas, Staphylococcus, Micrococcus, Acinetobacter* and *Moraxella* (Mullerat *et al*., 1994). The contamination of raw poultry meat with bacterial pathogens has important implications for public health (Purnell *et al*., 2004). There is much interest amongst poultry farmers, meat plant operators, retailers and government agencies in strategies to minimise the microbiological burden on the product. Such intervention can be targeted at any point from farm to fork. Potential causes of contamination of poultry during the slaughtering and processing procedures include contact of the carcass with body parts that contain a high microbial load (e.g.: Feathers, feet and intestinal contact) (Hecer and Geudas, 2011), use of contaminant equipment and physical manipulation of the meat (Graham *et al*., 2002). The prevention of microbial contamination and diffusion, abolishing the food- born toxicities and infections have appeared with cleaning and disinfection processes (Firildak *et al*., 2015). Development of various methods to decontaminate carcasses during poultry processing has become an urgency as a result of stricter regulations governing the use of chlorine for this purpose.

There are a variety of approaches to minimize the incidence of pathogens in retail poultry and these can be targeted at various control points, from farm to table. Since pathogens present on the live chickens can still be detected on carcasses after processing (Newell *et al*., 2011), it is clearly desirable that birds delivered to the processing plant should have reduced numbers of pathogens and it is logical to target primary intervention at the poultry farm. Methods to improve biosecurity on poultry farms and thus prevent the contamination of chickens with Salmonella and Campylobacter have been reported but can be difficult to implement and expensive (Gibbens *et al*., 2001). Improving the hygiene of crates in order to prevent contamination of broilers in transit to the processing plant has been investigated (Slader *et al*., 2002) but Campylobacter can still be isolated from a large proportion of crates. Therefore, additional approaches are essential for use in combination with these methods, including treatments during processing that reduce the contaminant on the poultry carcass.

Chlorine dioxide, sodium hypochlorite, lactic acid, peracetic acid are most commonly used disinfectants for commercial poultry processing (Duan *et al*., 2017). Chlorine dioxide has large bactericidal effects with several advantages over chlorinated water, including lower toxicities, more stable forms, making them promising candidates for replacing chlorinated water in the field of chicken processing (SCVPH, 2003; Burfoot and Mulvery, 2011). It is legally permitted in China and USA for sanitizing fruit and vegetables in water and recommended by the World Health Organization (Zhu *et al*., 2013). This paper supports research

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data to support technical feasibility to use Chlorine dioxide as an effective decontaminant for broiler chicken carcass.

Materials and Methods

Selection and Processing of Broiler Chicken Carcass

Broiler chicken (1500 Nos) were procured from the University Poultry and Duck Farm, Kerala Veterinary and Animal Sciences University. Broiler chicken were scientifically slaughtered in an automatic poultry processing line at Centre of Excellence in Meat Science and Technology, Mannuthy. Eight carcasses were randomly selected from the processing line for the experiment. The carcass was pre-chilled for 10-15 minutes at 7-12ºC after evisceration and immediately used for the experiment. Selected broiler chicken carcasses were randomly divided into four groups of two chicken carcass each.

Treatments

Four different treatments were prepared. Untreated broiler carcass was taken as control (C). Broiler chicken carcass washed with hot water at 80ºC for 20 seconds was the first treatment (T1). Washing of broiler chicken carcass with 100 ppm chlorine dioxide solution for 5 minutes was the second treatment (T2). Washing of broiler chicken carcass with 200 ppm chlorine dioxide solution for 5 minutes third treatment (T3).

Preparation of Chlorine Dioxide Solution

CloDox®, stabilized chlorine dioxide powder was procured from Neospark, Drugs and Chemicals Private Limited, Hyderabad, Telangana, India for the preparation of chlorine dioxide solution. 120 gm of CloDox Powder was mixed in 5 Litres of water as per the direction from manufacturer to prepare 4000 ppm chlorine dioxide stock solution. Stock solution was used for the preparation of 100 and 200 ppm levels of chlorine dioxide solution by diluting with distilled water for the experiment. Freshly prepared chlorine dioxide solution was used for each experiment.

Sample Collection

Surface swabs were collected from the broiler carcass after treatment for microbiological evaluation. The (Hi-Media) PW-003 sterile cotton swabs with a screw cap, were moistened with peptone water before collection. A 1 cm^2 area of the carcass surface was marked. Each marked area was swabbed lengthwise and breadthwise using the sides of the swab and from corner to corner. These swabs were transferred to a screw-capped test tube containing 10 ml of sterile maintenance medium.

Microbial Culture

Total Viable Count

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Total viable count (TVC) of each sample was estimated by pour plate technique, as described by Morton (2001). Plate count agar (Hi media M091, Mumbai) was made use of.

Yeast and Mould Count

The method described by Beuchat and Cousin (2001) was followed for estimation of yeast and mould count. Sabouraud Dextrose Agar (HiMedia, Mumbai) was used and count estimated by pour plate technique.

***Escherichia coli* Count**

Eosin methylene blue is a selective and differential medium for *E. coli* organism. It is a blend of two different stains (Eosin and Methylene Blue in a ratio of 6:1). In 1000 ml of distilled water, 35.96 g of Eosin methylene blue agar (Hi-Media M317, Mumbai) was suspended, boiled to dissolve the agar completely, and sterilized by autoclaving at 121ºC and 15 pounds' pressure for 15 minutes. The final pH of the medium was adjusted to 7.0 ± 0.02 at 25ºC. The selected tenfold dilution of each sample, one millilitre of the inoculum was transferred on to duplicate petri-dish of uniform size. To each of the inoculated plates about 10 to 15 millilitre sterile agar (Hi-Media M317) maintained at 45ºC was poured and mixed with the inoculum by gentle rotary movement i.e., clockwise, anticlockwise, forward and backwards.

Estimation of CFU

The inoculated plates were left at room temperature and allowed to solidify, and incubated by inverting the plates and maintained at 37ºC for 24 h. At the end of incubation, plates showing between 30 and 300 colonies were selected and counts taken with the help of a colony counter (Infra instruments, Chennai). The number of colony forming units (CFU) per sample was calculated by multiplying the mean count in duplicate plates with the dilution factor and was expressed as $\log_{10}$ CFU/cm^2.

pH Value

Approximately 10 g of deboned skin-on breast sample was homogenized at 6000 rpm for 30s with 90 mL of distilled water, using the homogenate for pH determination by a pH meter (Hanna HI9025c, Portugal)

Water Holding Capacity

Water holding capacity of meat sample was estimated after 72 h of ageing using filter paper press method as per Grau and Hamm (1957).

Sensory Evaluation

The sensory evaluation of chicken meat was conducted in a portioned booth by a semi- trained panel (n=10) consisting of faculty and post- graduate students from the Department of Livestock Products Technology, College of Veterinary and Animal Sciences, Mannuthy. They were briefly told about the nature of the

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experiment without disclosing the identity of samples. All panelists observe each meat samples coded with three-digit numbers along with a score card (AMSA, 1983). The panelists were asked to rate the samples immediately after slaughter and dressing for colour, odour and overall acceptability on an eight-point hedonic scale (AMSA, 1983).

Statistical Analysis

Six trials were conducted for each experiment. The data generated from various trials under each experiment were pooled and analysed by statistical method of one way-ANOVA and Mean±S.E using SPSS Statistics 24.0 software package developed as per the procedure of Snedecor and Cochran (1995) and means were compared by using Dunkan's multiple range test (Dunkan, 1995).

Results and Discussion

Microbiological Quality

The results of microbiological quality evaluations of C-Control (untreated broiler chicken carcass), T1, T2, T3 are given in Table 1 and Fig. 1.

Table 1: Microbiological quality (Mean ± SE, cfu/cm^2) of broiler chicken carcasses treated with hot water and chlorine dioxide solutions

Treatments	Control	T1	T2	T3
Total plate count	4.62 ± 0.03^a	3.90 ± 0.04^b	3.89 ± 0.02^b	3.55 ± 0.04^c
Coliform count	3.20 ± 0.06^a	2.37 ± 0.06^b	2.55 ± 0.09^b	2.05 ± 0.10^c
Yeast and mould count	3.70 ± 0.02^a	3.26 ± 0.06^b	3.36 ± 0.06^b	3.05 ± 0.09^c

Mean of microbiological attributes, bearing lower- case alphabets in row, as superscripts indicates significant difference (p<0.05)

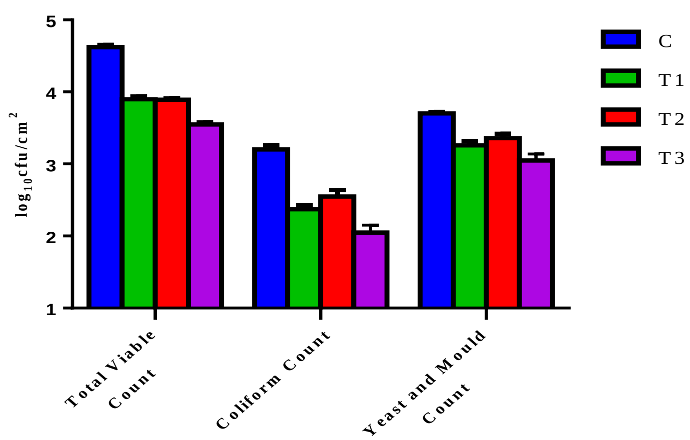

Fig. 1: Microbiological quality (Mean ± SE, cfu/cm^2) of broiler chicken carcasses treated with hot water and chlorine dioxide solutions.

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Total Viable Count

The mean TVC (log cfu/ cm^2) of the control carcass (untreated broiler chicken carcass) was 4.62±0.12. The mean TVC of T1, T2 and T3 were 3.90±0.04, 3.89±0.02 and 3.55±0.04 respectively. The mean values of the total viable count (TVC) of control and all the three treatment samples differ significantly ($p<0.05$) except between T1 and T2. Total viable count (TVC) describes a measure of bacteria in the sample that can survive in the conditions on the surface of muscle or carcasses or in processed meat, be harvested by the sampling procedure used and grow in the presence of air on an agar plate. Since pathogens present on the live birds can be detected on carcasses after processing (ACMSF, 2005), it is clearly desirable that carcasses contaminated with bacteria not only from the processing plant but also from the birds itself. A major mechanism of chlorine dioxide killing of bacterial spores is by damage to the spore's inner membrane resulting in a major permeability change in the germinated spore's plasma membrane that is derived from the spore's inner membrane (Young and Setlow, 2003).

In fact, during and after slaughtering, the bacteria from animal microbiota, the slaughterhouse environment, and the equipment used contaminate carcasses, their subsequent cuts, and processed meat products. Some of these bacterial contaminants can grow or survive during food processing and storage. The resulting bacterial communities present in poultry meat can include pathogenic species such as Salmonella and Campylobacter, the two main pathogens responsible for human gastroenteritis due to poultry meat consumption (Rouger *et al*., 2017). Because the TVC includes the organisms responsible for spoilage of meat, it will also give an indication of the keeping quality of the meat. According to Balamatsia *et al.* (2007), total viable count of broiler chicken carcasses immediately after slaughtering is 4.9 log_{10} cfu/cm^2. Similar type of results was reported by various researchers on broiler chicken carcass (Capita *et al.*, 2002; Chouliara *et al.*, 2007; Chaiba *et al.*, 2007 and Al-Nehlawi *et al.*, 2013).

The observed results in this experiment indicate the reduction in the mean value of TVC is due to the strong antimicrobial activity of the hot water as well as chlorine dioxide in the solution. There was 0.7 log cfu/cm^2 and 1 log cfu/cm^2 reduction in the mean value of TVC in the broiler carcasses after treating with hot water at 80 ºC and 200 ppm chlorine dioxide solution for 5 minutes. Similar results were reported by Thomson *et al*. (1974) that the broiler carcasses were washed with hot water (65.6ºC or 71.1ºC), after treatment the microbial counts on skin were reduced about 1.0 log and shelf life was extended 1 to 2 days. James *et al.* (2000) reported that the chicken breast portion is treated with atmospheric pressure steam reduced the total bacterial counts by 1.6-3.0 log units.

Duan *et al.* (2017) reported that the washing of 50 and 100 ppm chlorine dioxide is effective for the decontamination of chicken carcasses. Doyle and Waldroup (1996) found that ClO_2 added to chill water in two commercial broiler processing plants reduced total viable count by 90%. The result demonstrated that

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washing of poultry carcass with hot water as well as the 200 ppm Chlorine dioxide solution for 5 minutes can be potentially useful to improve microbiological quality.

Coliform Count

The mean value of coliform count (log cfu/ cm^2) of control was 3.20±0.06 and that of T1, T2, T3 were 2.37±0.06, 2.55±0.09 and 2.05±0.10 respectively (Table 1). The mean values of the coliform count of control and all the treatment muscle samples differ significantly ($p<0.05$) except between T1 and T2. The lowest coliform count was observed in T3 whereas the highest was observed in the control. This study showed 1.2 log cfu/cm^2reduction in the mean value of coliform count in T3 as compared with control (C). These results are in agreement with Sinhamahapatra *et al.* (2004) which concluded that washing the poultry carcass with 70 ºC for 1 min treatment reduced coliform count 1.3 log cfu/cm^2. Similar type of results with hot water treatment also observed by Northcutt *et al.* (2005), Corry *et al.* (2007) and Berrang *et al.* (2000) in poultry carcasses. The reduction in coliform count in the treated carcasses is mainly due to the strong antimicrobial nature of hot water and chlorine dioxide. As a disinfectant, ClO_2 works through oxidation and disrupts the cell wall, resulting in death of the microorganism by breaking up the cell. Chlorine dioxide also penetrates bacterial cell walls and alters the protein involved in the structure of microorganisms, resulting in rapid destruction of the bacteria Ahamed *et al*. (2016).

Hence, the experiment showed that hot water treatment and Chlorine dioxide solution are equally effective as decontaminating agent to reduce the total coliform counts in broiler chicken carcasses.

Yeast and Mould Count

The mean value of yeast and mould count (log cfu/cm^2) of control and all the three treatment samples differ significantly ($p<0.05$) except between T1 and T2. The lowest mean value of yeast and mould count was observed in T3 (3.05±0.09) whereas the highest mean value of yeast and mould count was observed in control (3.70±0.02). According to Zhu *et al*. (2013) the protein and DNA leakage, and the damage of genomic DNA structure were not correlated with inactivation rate of *Saccharomyces cerevisiae* in comparison with bacteria. The inactivation mechanism of *Saccharomyces cerevisiae* by ClO_2 is related to the leakage of K^+, Ca_2^+, and Mg_2^+, the inhibition of enzyme activities of metabolic pathway, and the disruption of the cell integrity

The results clearly indicate that there was a 0.5 and 0.7 log cfu/cm^2 reduction in the yeast and mould count of broiler chicken carcass treated with hot water at 80ºC for 20 seconds (T1) and 200 ppm of chlorine dioxide solution respectively. As yeast and moulds growth and meat spoilage are potentially damaging for the export meat industry (Frazier and Westhoff, 2007), treating or washing with hot water or chlorine dioxide or similar decontaminating agents can be advocated.

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Physico-chemical Attributes

pH

The mean pH value of Control, T1, T2 and T3 are presented in Table 2. All the three treatment samples showed no significant difference in pH value from control. The mean pH value of control was 5.86 ± 0.05. The mean pH value of the broiler chicken carcass washed with hot water at 80°C for 20 seconds (T1) and 100 ppm and 200 ppm of chlorine dioxide solution for 5 minutes (T2 and T3) were 5.92 ±0.07, 5.99 ±0.04, and 6.05 ±0.04 respectively. The results clearly indicated that there are no significant change in the pH value of the decontaminated carcasses after the application of chlorine dioxide and hot water. This is in agreement with results of Richmond *et al.* (2017) where no significant difference in pH was observed after treatment of quail carcasses with chlorinated water.

Table 2: Physcio-chemical attributes (Mean ± SE) of broiler chicken carcasses treated with hot water and chlorine dioxide solutions

Treatment	Control	T1	T2	T3
pH	5.86±0.05	5.92±0.07	5.99±0.04	6.05±0.04
WHC	32.22±0.15^{b}	26.13±0.06^{a}	31.94±0.13^{b}	32.24±1.11^{b}

Mean of pH and WHC, bearing different lower- case alphabet in row, as superscripts indicates significant difference (p<0.05)

Water Holding Capacity (WHC)

The mean water holding capacity of T1differ significant ($p<0.05$) with control and all other treatment carcasses. The lowest water holding capacity was observed in broiler chicken carcass washed with hot water at 80°C for 20 seconds (T1). The results clearly indicated that there is no effect of chlorine dioxide treatment up to the level of 200 ppm on WHC on broiler chicken carcasses but hot water treatment at 80°C for 20 seconds critically affecting the WHC. This might be due to the denaturation of protein present in the poultry carcass at higher temperature and longer exposure time.

Sensory Evaluation

Colour Scores

The mean colour of the control, T2 and T3 samples were shown non-significant differences ($p<0.05$) (Table 3). But compare to other treatments T1 sample showed significance differences. The colour difference in the hot water treatment as compared to the untreated carcasses and carcasses treated with chlorine dioxide is due to the epidermis damage and subsurface cooking. Similar observations were reported by Purnell *et al*. (2004) in poultry carcasses treated with hot water for decontamination.

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Table 3: Sensory attributes (Mean ± SE) of broiler chicken carcasses treated with hot water and chlorine dioxide solutions

Sensory Attributes	Control	T1	T2	T3
Colour Scores	6.49±0.12^{b}	5.98±0.18^{a}	6.84±0.12^{b}	6.89±0.11^{b}
Odour Scores	6.54±0.11	6.73±0.08	6.80±0.10	6.63±0.16
Acceptance Scores	6.66±0.11^{b}	5.55±0.11^{a}	6.75±0.10^{b}	6.71±0.14^{b}

Mean of sensory scores, bearing different lower-case alphabets in row, as superscripts indicates significant difference (p<0.05).

Odour

There was a non-significant decrease (P>0.05) in odour scores of chicken sample subjected to washing in hot water at 80^{o}C for 20 seconds and chloride dioxide solution from 100 ppm (T1) to 200 ppm (T2) concentration. The mean odour score of control sample was 6.54 ± 0.11. The mean odour score of the chicken sample treated in hot water, 100 ppm and 200 ppm of chlorine dioxide solution for 5 minutes, i.e. T1, T2 and T3 were 6.73 ± 0.08, 6.80 ± 0.10 and 6.63 ± 0.16 respectively. The odour scores of control (C), T1, T2 and T3 did not differ significantly.

Similar kind of observations were reported by Stivarius *et al.* (2002) in ground beef samples and beef carcasses respectively. On the contrary, Garcia-Zepeda *et al.* (1994) found that vacuum packaged beef chucks treated with 200 ppm chlorine dioxide were showed actually higher in odour acceptability scores when compared to a control. They hypothesized that the decrease in off-flavour aromatic notes were caused by the ability of chlorine to dissipate faster off treated meat surfaces, leaving no residual aroma. Therefore, our findings show that chlorine dioxide can be effective against contamination in the excised muscle, with little effect on sensory colour and odour characteristics.

Overall Acceptability

The mean overall acceptability of the control sample and T2 and T3 samples are show non-significant differences (p<0.05), but T1 sample (washing of broiler chicken carcass with hot water at 80^{o}C for 20 seconds) showed significantly lower overall acceptability scores as compared to other treatment including control. Since, the overall acceptability score is basically the reflection of all the other sensory attributes of the sample, the observed results might be due to the combined effect of colour and odour scores in the control and treated samples. In summary, the results in the current study indicate that hot water at 80 ^{o}C and chlorine dioxide solution treatment of broiler chicken carcasses immediately after processing, will reduce the level of contamination effectively. Since, 200 ppm level of chlorine dioxide solution improve the microbial quality of broiler chicken carcass without affecting the physico-chemical and sensory characteristics, it can be advisable to the poultry processor for decontaminating the carcasses after processing.

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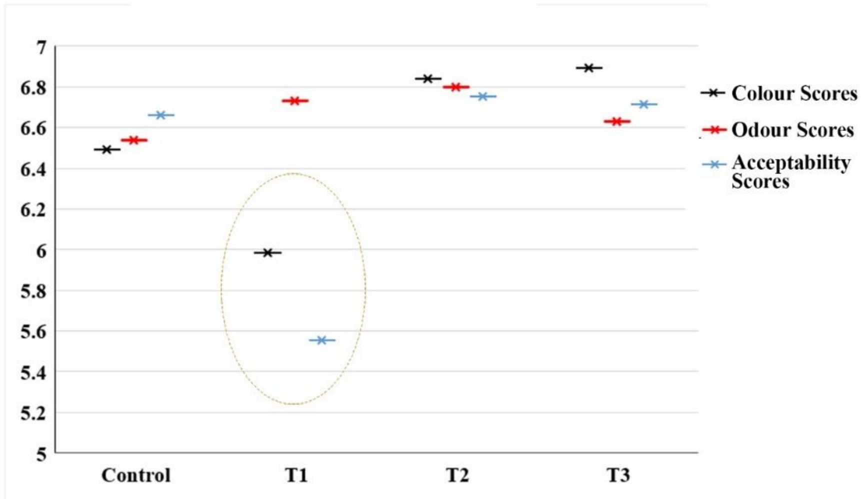

Fig. 4: Sensory attributes (Mean ± SE) of broiler chicken carcasses treated with hot water and chlorine dioxide solutions.

Conclusion

The control of process and product hygiene in poultry and meat processing plants is of the utmost concern from both a public-health and commercial point of view. The pathogenic and harmful bacteria that are present in the interior organs, on the skin surface and the feather of chickens, can be easily contaminated to the meat during processing steps. The bacterial contamination can be mostly seen in the production stages such as boiling, tearing the feathers, and removing of the interior organs. In addition to this, cross contamination from the skeleton, the process water and the equipment can also lead to increase in the contamination level. Results in the current study indicate that hot water at 80 ºC and chlorine dioxide solution treatment of broiler chicken carcasses immediately after processing, will reduce the level of contamination effectively. Since 200 ppm level of chlorine dioxide solution improve the microbial quality of broiler chicken carcass without affecting the physico-chemical and sensory characteristics, it can be advisable to the poultry processor for decontaminating the carcasses after processing.

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