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Effect of chlorine dioxide as a decontaminant on physico-chemical and microbiological parameters of chicken meat under refrigeration storage

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Abstract
A study was conducted to evaluate the decontamination efficiencies of chlorine dioxide at 50, 75 and 100 ppm concentrations with a contact time of 10 min on naturally contaminated chicken carcasses and the treatments along with control were evaluated for Physico-chemical (pH and TBARS) and Microbiological (Total plate count, Psychrophilic count, coliform count and Salmonella count) of chicken meat on 0^{th}, 3^{rd}, 5^{th} and 7^{th} day under refrigeration storage. There was significant (P<0.05) increase in the physico-chemical and Microbiological qualities as storage progressed from 0-7 days in both control and chlorine dioxide treated samples. From the present study, it was concluded that chlorine dioxide can be used in decontamination of chicken carcasses as it effectively reduced bacteria on the surface of carcasses and among the chlorine dioxide treated samples 50, 75 and 100 ppm concentrations, 100 ppm of chlorine dioxide treatment showed much better results by greater reduction of mesophilic, psychrophillic and coliforms with acceptable quality. Based on the results it may be concluded that 100 ppm of chlorine dioxide treated chicken carcasses could be safely stored for 7 days under aerobic packaging at refrigerated temperature (4±1 °C) without any undesirable changes in quality.

Keywords: Chicken carcass, chlorine dioxide, decontamination, TBARS

1. Introduction
Increased demand for fresh poultry and a desire to transport to more distant markets have increased the need to extend the shelf life of poultry products (Jimenez *et al.*1999) [4]. However, fresh chicken is a highly perishable product even when stored in chilled conditions. Chicken and other types of poultry have higher numbers of bacterial pathogens and spoilage micro-organisms than other food (Snyder, 1998) [9]. The normal shelf life of chicken after slaughtering is less than 2 days (Ellis *et al.* 2004) [2]. Decontamination techniques for carcasses are targeted at reducing or eliminating bacteria that may be human pathogens as well as those that may cause meat spoilage (Huffman, 2002) [3]. The use of decontamination methods for meat remains important to ensure the safety of muscle foods. Several interventions have been attempted to reduce bacterial contamination on broiler carcasses during slaughter process. These include physical methods, such as application of hot water and steam. Antimicrobial agents, such as acetic acid, citric acid, hydrogen peroxide and chlorine have also been tested with various levels of success. Chlorine dioxide (ClO_2) is recognized as antimicrobial agent for its disinfectant properties since the early 1900's. This compound effectively kills microorganisms including bacteria, viruses or fungi on inanimate objects, foods, and other surfaces. Chlorine dioxide is approved by the USDA Food Safety Inspection Service as an antimicrobial chemical for poultry processing in scald tanks, pickers, carcass washers, and immersion chill tanks (USDA, 2006) [14]. However, its antimicrobial effect on carcass surface has not been attempted. Therefore the present study was initiated with the following objectives.

1. To determine the effectiveness of different concentrations of chlorine dioxide on the reduction of micro-organisms on poultry carcass.

2. Materials and Methods
The study was conducted to compare the efficacy of different concentrations of chlorine dioxide as decontaminant on chicken carcass and effects on various meat quality parameters during refrigeration storage. Different meat quality parameters were studied *viz.* pH, Thiobarbituric acid reactive substances value, total plate count, psychrophilic count, coliform count and Salmonella count.

2.1 Meat sample
Broiler birds were procured from local market of Hyderabad and slaughtered in experimental abattoir of ICAR-National Research Centre on Meat, Hyderabad. After removing the skin, each carcass was split into 2 halves. Then the visible fat and connective tissue residues were removed using a sterile, sharp stainless steel knife. These carcass were treated by dipping them in aqueous chlorine dioxide solutions at 50, 75, 100 ppm concentrations for about ten minutes and drained of excess water. The meat was packaged in LDPE bags and stored at 4±1 °C for a period of 7 days. This refrigerated meat was used for further studies.

2.2 Experimental design
Forty broiler birds were procured from local market of Hyderabad and slaughtered in experimental abattoir of ICAR-National Research Centre on Meat, Hyderabad by adopting traditional method and five trials were conducted each containing eight birds. After deskinnng and evisceration process, eight carcasses were subjected to four treatments, two carcasses under each treatment as T0 (without any treatment as control) and other six carcasses were treated by dipping in aqueous solution of chlorine dioxide of three different concentrations designated as T1, T2 and T3 with 50, 75 and 100 ppm respectively. Physico-chemical and microbiological characteristics of chicken meat were analyzed on 0^{th}, 3^{rd}, 5^{th} & 7^{th} day under refrigeration storage.

2.3 Analytical procedures
2.3.1 Physico-chemical parameters
2.3.1.1 pH
The pH of meat sample was determined as per Trout *et al*.,(1992).The homogenate was prepared by blending 10 g sample with 90 ml distilled water using an Ultra Turrax tissue homogenizer (Model IKA T-25, Janke and Kenkel, IKA Labor Technik, Germany) for one minute. Then the pH was recorded by immersing combined glass electrode of digital pH meter (Thermo Orion, Model 420 A^+, USA) into the meat homogenate.

2.3.1.2 Thiobarbituric acid reactive substances (TBARS) value
The Thiobarbituric acid reactive substances (TBARS) value of samples was determined by the extraction method (Krauze, & Bailey, 1970) [5] with slight modifications. Four gram sample was homogenized with 20% trichloroacetic acid solution (20 ml) and the slurry was centrifuged at 3000 g for 10 min. Two ml of supernatant was mixed with equal volume of freshly prepared 0.1% thiobarbituric acid in glass test tubes and heated in water bath at 100 ºC for 30 min followed by cooling under tap water. The absorbance of the mixture was measured at 532 nm using UV-VIS spectrophotometer (Model: UV-1700 Pharma Spec, SHIMADZU, Japan) and the TBARS values were calculated using a TBARS standard curve and expressed in mg of malonaldehyde/kg.

2.3.2 Microbiological analysis
2.3.2.1 Total plate count
23.5g plate count agar obtained from Hi-Media Laboratories Pvt Ltd., Mumbai (Code No.M091) was suspended in 1000 ml distilled water and boiled to dissolve the media completely and sterilized by autoclaving at 15 lb pressure at 121 °C for 15 min. Final pH of the media was adjusted to 7.0±0.2. Duplicate sets of petri dishes were inoculated aseptically with 1 ml aliquots from appropriate dilutions. About 20ml of plate count agar, melted and maintained at 44-46 °C, was poured gently. The plates were incubated at 37±1 °C for 48 h. Plates showing 30-300 colonies were counted. The number of colonies were multiplied with reciprocal of the dilution and expressed as $\log_{10}$ cfu/g.

2.3.2.2 Psychrophilic count
The plates were prepared similar to that of total plate count but incubated at 4±1 °C for 10-14 days. The number of colonies were multiplied with reciprocal of the dilution and expressed as $\log_{10}$ cfu/g.

2.3.2.3 Coliform count
A quantity of 41.5 g of violet red bile agar (hi-Media laboratories Pvt. Ltd., Mumbai (Code M 049) was suspended in 1000 ml of distilled water. It was then boiled to dissolve the medium completely and cooled to 45 ºC. The final pH of the medium was 7.4±0.2 at 25 ºC. Pour plate with overlay technique was followed for inoculation of suitable sample dilutions and the plates were incubated at 35±2 ºC for 24 hours. The colonies that appeared on the plates were counted and expressed as $\log_{10}$ cfu/g.

2.3.2.4 Salmonella Count
A quantity of 72.66 gm of Hektoen Enteric agar (hi-Media laboratories Pvt. Ltd., Mumbai (Code M 049) was suspended in 1000 ml of distilled water and boiled to dissolve the medium completely and cooled to 45 ºC. The final pH of the medium was adjusted to 7.30-7.70 and it was not autoclaved. Pour plate with overlay technique was followed for inoculation of suitable sample dilutions and the plates were incubated at 30-35 ºC for 24-48 hours. The colonies that appeared on the plates were counted and expressed as $\log_{10}$ cfu/g.

2.4 Statistical analysis
The experiment was repeated three times in duplicate and the data generated for different meat quality parameters were compiled and analyzed using SPSS (version 20.0 for Windows; SPSS, Chicago. 111, U.S.A.). The data were subjected to analysis of variance, (oneway ANOVA between different groups and storage periods), least significant difference and Duncan's multiple range tests for comparing the means to find the difference between the groups and different storage periods. The smallest difference (D_5%) for two means was reported as significantly different (P<0.05).

3 Result and Discussion
3.1 pH
The pH of control and chlorine dioxide treated chicken breast and thigh samples increased during the storage (7days) at refrigeration temperature, which might be due to accumulation of metabolites that resulted due to microbial growth (Shin *et al.*, 2011) [8]
Quio *et al.* (2002) [7] reported an increase in the pH of chicken breast meat due to accumulation of amines and ammonia by psychotropic bacteria. Aksu *et al.* (2006) [1] reported a positive correlation between pH and total aerobic bacteria growth on fresh chicken carcass during the storage at refrigeration temperature (4 $\pm$1 ^{0}C).

Table 1: Effect of different levels of Chlorine dioxide on pH of poultry carcass (Mean ± SE).

Portion of carcass	Treatments	0th day	3rd day	5th day	7th day
Breast	C	5.81±0.02^{bA}	5.86±0.02^{bA}	5.94±0.02^{bB}	6.13±0.02^{cC}
	T_1	5.78±0.02^{abA}	5.84±0.02^{abA}	5.91±0.02^{bB}	6.11±0.02^{bcC}
	T_2	5.75±0.02^{abA}	5.81±0.02^{abA}	5.88±0.02^{abB}	6.08±0.02^{abC}
	T_3	5.63±0.10^{aA}	5.78±0.02^{aAB}	5.85±0.02^{aB}	6.04±0.01^{aC}
Thigh	C	6.02 ±0.02^{bA}	6.12±0.02^{bB}	6.16±0.02^{bB}	6.28±0.02^{cC}
	T_1	5.99±0.02^{abA}	6.07±0.02^{bB}	6.13±0.02^{abC}	6.25±0.02^{bcD}
	T_2	5.97±0.02^{abA}	6.03±0.02^{abB}	6.10±0.02^{abC}	6.23±0.02^{abD}
	T_3	5.94±0.02^{aA}	6.00±0.03^{aA}	6.07±0.02^{aB}	6.19±0.02^{aC}

C: 0 ppm ClO_2, T_1: 50 ppm ClO_2, T_2: 75 ppm ClO_2, T_3: 100 ppm ClO_2.

3.2 Thiobarbituric Acid Reactive Substances (TBARS) Value

The mean TBARS values significantly (P<0.05) increased in both control and chlorine dioxide treatment (50, 75 and 100 ppm) groups stored at refrigeration temperature (4±1 ^{0}C). The TBARS values significantly differed between control and treated samples in both breast and thigh portions of carcass on the 7th day of storage (P<0.05). The TBARS values of treated samples with chlorine dioxide in present study were 0.093 and 0.096 mg/kg in breast and thigh respectively, which were far less than threshold level of TBARS (1-2 mg malondialdehyde) for spoilage suggested by Tims and Watts. (1958) [12].

Table 2: Effect of different levels of Chlorine dioxide on TBARS value (mg malonaldehyde/kg) of poultry carcass (Mean ± SE).

Portion of carcass	Treatments	0th day	3rd day	5th day	7th day
Breast	C	0.016±0.001^{aA}	0.025±0.001^{bB}	0.090±0.001^{bC}	0.112±0.001^{dD}
	T_1	0.016±0.001^{aA}	0.023±0.001^{bB}	0.088±0.001^{bC}	0.093±0.001^{cD}
	T_2	0.016±0.001^{aA}	0.022±0.001^{bB}	0.053±0.001^{aC}	0.069±0.001^{bD}
	T_3	0.016±0.001^{aA}	0.017±0.001^{aA}	0.042±0.001^{aB}	0.063±0.001^{aC}
Thigh	C	0.018±0.001^{aA}	0.026±0.001^{aB}	0.092±0.001^{cC}	0.126±0.001^{dD}
	T_1	0.018±0.001^{aA}	0.024±0.001^{abB}	0.092±0.001^{cC}	0.096±0.001^{cC}
	T_2	0.018±0.001^{aA}	0.023±0.001^{abB}	0.062±0.001^{bC}	0.083±0.001^{bD}
	T_3	0.018±0.001^{aA}	0.020±0.001^{aA}	0.046±0.001^{aB}	0.072±0.001^{aC}

C: 0 ppm ClO_2, T_1: 50 ppm ClO_2, T_2: 75 ppm ClO_2, T_3: 100 ppm ClO_2.

3.3 Total plate count

As the concentration of chlorine dioxide increased the total plate count decreased and a significant difference (P<0.05) between the control and treated samples was observed. All the levels of chlorine dioxide effectively reduced the bacterial proliferation on carcass, but 100 ppm chlorine dioxide treatment was more effective and reduced the count from 4.38 to 3.36 in breast and 4.62 to 3.59 in thigh portion i.e about 1 log reduction compared to the control samples. Similar reductions were observed by Lillard *et al.* (1989) [6] and Villarreal *et al.* (1990) [15] who reported that the chlorine dioxide treatment reduced the total plate count by 1.2 log on chicken carcass.

Table 3: Effect of different levels of Chlorine dioxide on total plate counts (log cfu/gm) of poultry carcass (Mean ± SE).

Portion of carcass	Treatments	0th day	3rd day	5th day	7th day
Breast	C	4.38±0.03^{dC}	4.92±0.03^{dB}	5.22±0.23^{dB}	6.08±0.03^{Da}
	T_1	3.72±0.03^{cC}	3.72±0.03^{cC}	4.92±0.03^{cB}	5.41±0.03^{cA}
	T_2	3.62±0.03^{bC}	3.62±0.03^{bC}	4.74±0.03^{bB}	5.28±0.03^{bA}
	T_3	3.36±0.03^{aC}	3.36±0.03^{aC}	4.56±0.03^{aB}	5.04±0.40^{aA}
Thigh	C	4.62±0.03^{dD}	5.01±0.03^{dC}	5.68±0.03^{dB}	6.39±0.03^{dA}
	T_1	3.98±0.03^{cD}	4.32±0.03^{cC}	5.18±0.03^{cB}	5.98±0.03^{cA}
	T_2	3.88±0.03^{bD}	4.12±0.03^{bC}	5.08±0.03^{bB}	5.80±0.03^{bA}
	T_3	3.59±0.03^{aD}	4.01±0.03^{aC}	4.97±0.03^{aB}	5.42±0.03^{aA}

C: 0 ppm ClO_2, T_1: 50 ppm ClO_2, T_2: 75 ppm ClO_2, T_3: 100 ppm ClO_2.

3.4 Psychrophilic count

As the concentration of chlorine dioxide increased the Psychrophilic count decreased significantly (P< 0.05). All the levels of chlorine dioxide effectively reduced the bacterial count on carcass but 100 ppm chlorine dioxide treatment was more effective and reduced the count from 3.06 to 2.34 in breast and 3.23 to 2.50 in thigh portion i.e about 0.75 log reduction compared to the control samples. Similarly Thiessen *et al.*, (1984) [11] reported that chilled water in a poultry processing plant treated with 1.39 mg/liter of chlorine dioxide resulted in reducing the psychrophilic count by more than 1 log.

Table 4: Effect of different levels of Chlorine dioxide on Psychroplilic counts (log cfu/gm) of poultry carcass (Mean ± SE).

Portion of carcass	Treatments	0th day	3rd day	5th day	7th day
Breast	C	3.06 ± 0.22^{dA}	3.44 ± 0.21^{dB}	3.79 ± 0.21^{dC}	4.32 ± 0.22^{dD}
	T_1	2.60 ± 0.21^{cA}	2.99 ± 0.22^{cB}	3.44 ± 0.21^{cC}	4.04 ± 0.22^{cD}
	T_2	2.52 ± 0.23^{bA}	2.85 ± 0.22^{bB}	3.31 ± 0.22^{bC}	3.86 ± 0.21^{bD}
	T_3	2.34 ± 0.23^{aA}	2.74 ± 0.21^{aB}	3.20 ± 0.22^{aC}	3.69 ± 0.22^{aD}
Thigh	C	3.23 ± 0.21^{dA}	3.50 ± 0.22^{dB}	3.97 ± 0.22^{dC}	4.46 ± 0.23^{dD}
	T_1	2.78 ± 0.22^{cA}	3.02 ± 0.21^{cB}	3.62 ± 0.22^{cC}	4.05 ± 0.13^{cD}
	T_2	2.71 ± 0.22^{bA}	2.88 ± 0.21^{bB}	3.52 ± 0.22^{bC}	3.84 ± 0.22^{bD}
	T_3	2.50 ± 0.23^{aA}	2.80 ± 0.22^{aB}	3.47 ± 0.22^{aC}	3.79 ± 0.21^{aD}

C: 0 ppm ClO_2, T_1: 50 ppm ClO_2, T_2: 75 ppm ClO_2, T_3: 100 ppm ClO_2.
$^{A-D}$ Means within a row, not sharing a common superscript (Uppercase), differ significantly ($P<0.05$)
$^{a-d}$ Means within a column, not sharing a common superscript (lowercase), differ significantly ($P<0.05$)

3.5 Coliform count

The coliform count of control, 50, 75 and 100 ppm chlorine dioxide treated samples differed significantly ($P<0.05$) with each other in both breast and thigh portions during entire period of refrigeration storage.

Similar to the present study findings Stivarius *et al.* (2002) [10] reported 1 log cfu /gm reduction of Coliform count in ground beef by washing beef trimmings before grinding. Thiessen *et al.*, (1984) [11] also reported that Chilled water in a poultry processing plant treated with 1.39 mg/liter of chlorine dioxide resulted in reducing the coliform count (<1 log cycle).

Table 5: Effect of different levels of Chlorine dioxide on Coliform counts (log cfu/gm) of poultry carcass (Mean ± SE).

Portion of carcass	Treatments	0th day	3rd day	5th day	7th day
Breast	C	1.96 ± 0.01^{dA}	2.21 ± 0.01^{dB}	2.43 ± 0.01^{dC}	2.77 ± 0.01^{dD}
	T_1	1.67 ± 0.01^{cA}	1.92 ± 0.01^{cB}	2.21 ± 0.01^{cC}	2.59 ± 0.01^{cD}
	T_2	1.62 ± 0.01^{bA}	1.83 ± 0.01^{bB}	2.13 ± 0.02^{bC}	2.48 ± 0.01^{bD}
	T_3	1.50 ± 0.01^{aA}	1.76 ± 0.01^{aB}	2.05 ± 0.01^{aC}	2.37 ± 0.01^{aD}
Thigh	C	2.07 ± 0.01^{dA}	2.25 ± 0.01^{dB}	2.55 ± 0.01^{dC}	2.87 ± 0.01^{dD}
	T_1	1.78 ± 0.01^{cA}	1.94 ± 0.01^{cB}	2.32 ± 0.01^{cC}	2.68 ± 0.01^{cD}
	T_2	1.74 ± 0.01^{bA}	1.85 ± 0.01^{bB}	2.28 ± 0.01^{bC}	2.60 ± 0.01^{bD}
	T_3	1.61 ± 0.01^{aA}	1.80 ± 0.01^{aB}	2.23 ± 0.01^{aC}	2.43 ± 0.01^{aD}

C: 0 ppm ClO_2, T_1: 50 ppm ClO_2, T_2: 75 ppm ClO_2, T_3: 100 ppm ClO_2.
$^{A-D}$ Means within a row, not sharing a common superscript (Uppercase), differ significantly ($P<0.05$)
$^{a-d}$ Means within a column, not sharing a common superscript (lowercase), differ significantly ($P<0.05$)

3.6 Salmonella count

On enumeration on specific media of *salmonella* though there colonies which were found to be negative on performing biochemical tests for *salmonella*.

The absence of salmonella may be attributed to the hygienic handling and sanitary conditions adopted slaughter process. Similarly Thiessen *et al.* (1984) [11] reported that chilled water in a poultry processing plant treated with chlorine dioxide (ClO_2) at various concentrations from 0 to 1.39 mg/liter resulted in reduction of the bacterial count to the point, where *salmonellae* could not be isolated from the chill water or the chilled broiler carcasses.

4. Conclusion

From the present study, it was concluded that chlorine dioxide can be used in decontamination of poultry carcasses as it effectively reduced bacteria on the surface of carcasses and among the chlorine dioxide treated samples, 50, 75 and 100 ppm concentrations, 100 ppm of chlorine dioxide treatment showed much better results by greater reduction of mesophilic, psychrophillic and coliforms with acceptable quality and sensory attributes. Based on the results it may be concluded that 100 ppm of chlorine dioxide treated poultry carcasses could be safely stored for 7 days under aerobic packaging at refrigerated temperature (4±1°C) without any undesirable changes in quality.

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