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BACTERIAL COUNTS AND OXIDATIVE PROPERTIES OF CHICKEN BREAST INOCULATED WITH *SALMONELLA* TYPHIMURIUM EXPOSED TO GASEOUS OZONE

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ABSTRACT

Poultry meats are highly perishable due to bacterial contamination; thus, elimination of bacterial contaminants is a challenge for food safety industry. This study was designed to evaluate the bacterial counts and oxidative properties of chicken breast contaminated with *Salmonella* Typhimurium (ST) exposed to gaseous ozone during refrigerated storage. Slices of chicken breast were inoculated with ST by immersing them in peptone water containing 1.0×10^7 cfu of ST per milliliter peptone water. Samples were placed in a container with normal air (no ozone) or in a container equipped with an ozone generator that produce a continuous flux of ozone (10×10^{-6} kg $O_3/m^3/h$) and negative ions. Gaseous ozone exposure significantly reduced ST and the total aerobic and anaerobic bacterial counts during storage compared with the nontreatment group ($P < 0.05$). Gaseous ozone exposure significantly reduced CIE L^* and increased CIE b^* surface color values after 3 and 2 days of storage, respectively ($P < 0.05$). Gaseous ozone exposure significantly inhibited catalase and glutathione peroxidase activity after 3 days ($P < 0.05$). A higher TBARS (2-thiobarbituric acid reactive substances) value ($P < 0.05$) was found in chicken breast subjected to ozone exposure after 3 days of storage. In conclusion, gaseous ozone exposure reduced the bacterial counts in chicken breast from the beginning of the study and affected the oxidative properties on the last day of the study.

PRACTICAL APPLICATIONS

This study shows that gaseous ozone exposure can be used as an antimicrobial agent for refrigerated meat. This information will be useful in the development of an effective ozone generator inside a refrigerator. Further studies regarding the appropriate ozone concentrations are needed.

INTRODUCTION

Poultry is a major source of meat worldwide. According to reports by the FAO (2006), poultry meat accounted for approximately 31% of global meat consumption. The popularity and consumption of poultry meat has been increasing in recent years. Poultry meat consumption, particularly in developing countries, is projected to grow by 38% by 2019 compared with the consumption from 2007–2009 (Bett *et al.* 2013). The reasons for the increasing popularity and consumption of poultry meat include its high nutritional value, low fat content and relatively low cost of production (which results in a low selling price) (Rimal 2005; Chouliara *et al.* 2007). However, consumers are concerned about the microbial safety of poultry meat because of the knowledge that poultry meats are highly perishable due to bacterial contamination (Hong *et al.* 2007; Rahman *et al.* 2012), and some bacterial contaminants in poultry meat have been recognized as foodborne human pathogen (Capita *et al.* 2001). *Salmonella*, *Listeria*, *Campylobacter* and *Escherichia coli* are naturally present bacterial contaminants in chicken intestine (Anang *et al.* 2007). Among these bacteria, *Salmonella*

spp. are recognized as a major foodborne pathogen and are the most commonly encountered bacteria in poultry products (Capita *et al.* 2001). *Salmonella* spp. are a major cause of gastroenteritis, and 35% of foodborne hospitalization cases in the U.S.A. are due to infection by this organism (Scallan *et al.* 2011). Various processing techniques have been used to eliminate bacterial contaminants and extend the shelf lives of the products they colonized (Hwang and Beuchat 1995; Kim and Day 2007; Rahman *et al.* 2012); however, Abbassi-Ghozzi *et al.* (2012) noted that *Salmonella* spp. remain at high prevalence in raw chicken (>50%) despite improvements in the hygienic processing of poultry.

The food industry has developed sanitizing methods, such as acid and salt washing, gamma-ray irradiation and chlorine dioxide washes (Latha *et al.* 2009; Sheen *et al.* 2011; Lu and Wu 2012; Jouki 2013). In addition, ozone has attracted the attention of food scientists as an alternative sanitizer. Ozone has been commercially used for the disinfection of drinking water since the early 19th century (Uradzinski *et al.* 2005) and is regarded as a potential antibacterial and antiviral agent due to its broad-spectrum antimicrobial activity in water (Greene *et al.* 2012). Ozone inactivates bacteria by disrupting the cell membrane and cell wall, leading to cell lysis (Greene *et al.* 2012). Pascual *et al.* (2007) noted that the disruption of the cell wall by ozone is a faster bacterial inactivation mechanism than disinfectants, which require time to permeate the cell membrane. Ozone may also affect membrane-bound enzymes and damage proteins (Komanapalli and Lau 1996). In recent years, the use of ozone has increased after its designation as Generally Recognized as Safe (GRAS) by the Food and Drug Administration (FDA) in 1997 (Kim *et al.* 1999).

Ozone has been reported to eliminate foodborne pathogens such as *Salmonella*, *Listeria monocytogenes* and *Staphylococcus* (Restaino *et al.* 1995). Ozone is used in a wide variety of agricultural products, such as vegetables, fruits, fish (Greene *et al.* 2012) and meat products (Stivarius *et al.* 2002; Sekhon *et al.* 2010; Cardenas *et al.* 2011). However, the use of ozone in the meat industry is challenging due to its high oxidative properties, which can affect meat quality. Sekhon *et al.* (2010) noted that ozone damages fatty acids in the cell membranes and damages cellular proteins through oxidation. The ozonation of lipids leads to the formation of peroxides, which initiate the lipid oxidation of food (Greene *et al.* 2012). Ozone causes oxidative conditions to which the cells respond by stimulating the expression of antioxidant enzymes such as catalase (CAT), glutathione peroxidase (GSH-Px) and superoxide dismutase (Frischer *et al.* 1997; Lee *et al.* 2003).

Exposure to gaseous ozone during storage may eliminate foodborne pathogens in chicken meat that survived the sanitizing and hygienic processes. However, due to the high unsaturated fatty acid content of chicken meat, ozone exposure time is critical to prevent lipid oxidation that leads to the loss of meat quality. The present research evaluated the effect of gaseous ozone treatment on the microbial counts and oxidative properties of chicken breast contaminated with *Salmonella* Typhimurium (ST).

MATERIALS AND METHODS

Preparation of the Bacterial Inoculum

ST is an important pathogen that causes bacterial gastroenteritis in human via meat of cattle, pigs and chickens, or their by-products (Cho *et al.*, 2014). ST used in this study was isolated from ileocecal lymph nodes of pigs in Korea. This strain was classified as PFGE type A6 and phage type DT41. The ST strain was also resistant to ampicillin, tetracycline, trimethoprim-sulfamethoxazole and nalidixic acid. The ST was grown at 37C in Luria–Bertani (LB) broth (Difco, Detroit, MI) for 16 h, washed twice with 0.1% sterile peptone water and measured for optical density. The colony-forming units (cfu) of the ST were then enumerated on *Salmonella shigella* agar (BD Biosciences, Sparks, MD).

Sample Preparation

Skinless chicken breast fillets were obtained from a local market on the day of the experiment. The chicken breasts were cut into slices weighing 20.0 ± 1.0 g and were placed in a sterilized Petri dish (Ø60×15 mm, SPL Life Sciences, Pocheon, Korea). Slices of chicken breast were inoculated with ST by immersing them in peptone water containing 1.0×10^7 cfu of ST per milliliter peptone water. The immersed samples were aerated in a clean bench for 10 min to evaporate the peptone water.

The non-inoculated samples and samples inoculated with ST were placed in an air chamber or ozone chamber ($L \times W \times H = 25 \times 20 \times 20$ cm). The chambers were previously cleaned with 70% ethanol to eliminate potential contaminants during storage. The ozone chamber was equipped with an ozone generator (MA-2, NOAH environmental clean, Bucheon, Korea) that generates electric tension to produce a continuous flux of ozone (10×10^{-6} kg $O_3/m^3/h$) and negative ions. The ozone generator was set to run for 15 min and then to turn off for 45 min using an automatic timer plug (Theben 0260.0, Haigerloch, Germany), and this setting was used throughout the 3-day storage period. The chambers were placed in a refrigerator at a temperature of 4C, and daily observations were conducted to monitor the quality changes in the chicken breast samples.

Bacterial Count Measurements

For the determination of bacterial counts, 1 g of chicken breast sample was weighed in a sterile bag (Nasco

Whirl-Pak, Janesville, WI) and homogenized with 9 mL of 0.1% sterilized peptone water in a stomacher (Lab blender 400, Seward Laboratory, Worthing, U.K.) for 2 min. Serial dilutions were prepared using 0.1% sterilized peptone water. For the determination of ST and total aerobic bacterial counts, 1 mL of the serial dilution was placed in LB broth (Difco) and plate count agar (PCA; Difco). The plates were incubated aerobically at 37C for 24–48 h. For the determination of total anaerobic bacterial counts, 1 mL of the serial dilution was placed in the PCA (Difco) and incubated anaerobically at 37C for 24–48 h. Microbial population was counted using a colony counter (C-C03, Chang Shin Scientific, Busan, Korea) and expressed as log cfu/g sample.

Instrumental Color

The surface color values of CIE L^* (lightness), a^* (redness) and b^* (yellowness) of the chicken breast samples were measured using a color difference meter (CR-400, Konica Minolta Sensing, Inc., Tokyo, Japan) and an Illuminant C on each day of storage. A white plate ($Y = 93.6$, $x = 0.3134$ and $y = 0.3194$) was used for calibration prior to measurements. Three measurements of each sample were performed on the surface immediately after the samples were removed from the chamber.

Lipid Oxidation

Lipid oxidation of the chicken breast samples was determined using the 2-thiobarbituric acid reactive substances (TBARS) analysis according to the method of Sinnhuber and Yu (1977). Each 0.5 g sample was mixed with 3 drops of antioxidant solution, 3 mL of thiobarbituric acid solution and 17 mL of 25% (w/v) trichloroacetic acid, followed by incubation in a water bath at 100C for 30 min. The mixture was cooled, and a 5 mL volume was removed and centrifuged at 1,000 x g for 30 min. The absorbance of the supernatant was measured at 532 nm using a spectrophotometer (UVmini-1240, Shimadzu, Kyoto, Japan). The results were expressed as milligram of malonaldehyde (MA) per kilogram of sample.

Determination of Antioxidant Enzyme Activity

Catalase. The CAT activity was measured by recording H_2O_2 disappearance characterized by an absorbance decrease at 240 nm according to a method described by Aebi (1984), with modifications. A 5 g sample was mixed with 25 mL of 50 mM phosphate buffer (pH 7.0) at 13,500 rpm using a homogenizer (Ultra-Turrax T25 basic, IkaWerke GmbH & Co., Staufen im Breisgau, Germany) for 15 s. The mixture was centrifuged at 1,000 x g at 2C for 15 min. The supernatant of the mixture was removed and filtered with Whatman filter paper No. 1 (GE Healthcare Life Sciences, Amersham, Buckinghamshire, UK). Then, 100 μL of the filtered supernatant was mixed with 2.9 mL of 30 mM H_2O_2. The decrease in absorbance at 240 nm was recorded every 30 s for 3 min. The catalase activity was calculated as follows:

$$\text{Catalase activity (units/g meat)} = \frac{3.45 \times \text{dilution factor (6)}}{\text{t-min} \times 0.1}$$

The number 3.45 represents the decomposition of 3.45 μmol of hydrogen peroxide in 3.0 mL of reaction mixture to produce a decrease in absorbance at 240 nm from 0.45 to 0.40 absorbance units. Further, t-min corresponds to the time in minutes required for absorbance at 240 nm to decrease from 0.45 to 0.40 absorbance units, and 0.1 (in mL) represents the volume of the meat extract. The catalase activity was expressed as units/g sample.

Glutathione Peroxidase (GPS-Px). GPS-Px activity was measured by homogenizing 5 g of sample with 25 mL of 50 mM of phosphate buffer and 1 mM ethylenediaminetetraacetic acid using a homogenizer (Ultra-Turrax T25 basic, IkaWerke GmbH & Co., Staufen im Breisgau, Germany) at 13,500 rpm for 30 s. The mixture was centrifuged at 1,000 x g for 10 min at 4C followed by filtration using Whatman filter paper No 1. A volume of 100 μL of the supernatant was incubated with 4,900 μL of the assay mixture containing 5 units/mL glutathione reductase (Sigma G3664 St. Louis, Missouri, USA) in phosphate buffer, 10 mM glutathione (Sigma G4251, St. Louis, Missouri, USA), 1.5 mM NADPH (Sigma N1630, St. Louis, Missouri, USA), 1.5 mM H_2O_2 (Sigma H1009, St. Louis, Missouri, USA) and 100 mM NaN_3. The GPS-Px activity was measured by recording the absorbance decrease of the incubation mixture at 340 nm over 3 min. The GPS-Px was expressed as units/g sample.

Statistical Analysis

Statistical analysis was performed using the statistical package SPSS 19.0 (SPSS, Inc., 2010). All values are reported as mean ± standard deviation for each treatment group. Analysis of variance (ANOVA) and Duncan's test were performed at the level of 5% to compare the mean of oxidative properties (Table 2) and instrumental surface color (Table 3). The difference in bacterial counts between the ozone treatment and control groups (Table 1) was determined using a paired sample *t*-test with a confidence interval of 95% ($P < 0.05$).

TABLE 1. BACTERIAL COUNTS ON CHICKEN BREAST INOCULATED WITH *SALMONELLA* TYPHIMURIUM (ST) EXPOSED TO GASEOUS OZONE DURING STORAGE

Bacteria	Inoculation with ST	Ozone treatment	Storage (days) 0	1	2	3
ST	Yes	No	*7.64 ± 0.38^{aB}	7.84 ± 0.29^{aB}	8.05 ± 0.18^{aAB}	8.30 ± 0.04^{aA}
		Yes	7.64 ± 0.38^{aA}	7.24 ± 0.14^{bA}	7.32 ± 0.04^{bA}	7.51 ± 0.03^{bA}
Total aerobic bacteria	Yes	No	8.05 ± 0.19^{aB}	8.08 ± 0.27^{aB}	8.49 ± 0.17^{aA}	8.82 ± 0.36^{aA}
		Yes	8.05 ± 0.19^{aA}	7.26 ± 0.18^{bC}	7.53 ± 0.24^{bBC}	7.75 ± 0.27^{bAB}
Total anaerobic bacteria	Yes	No	7.98 ± 0.06^{aC}	8.16 ± 0.21^{aC}	8.56 ± 0.11^{aB}	9.18 ± 0.28^{aA}
		Yes	7.98 ± 0.06^{aA}	7.43 ± 0.14^{bB}	7.60 ± 0.29^{bB}	8.17 ± 0.02^{bA}

* Mean values ± standard deviation; the unit is log cfu/g.
Means in the same column followed by different superscript lowercase letters are significantly different ($P < 0.05$).
Means in the same row followed by different superscript uppercase letters are significantly different ($P < 0.05$).

RESULTS AND DISCUSSION

Bacterial Counts

Table 1 shows the bacterial counts obtained daily from the chicken breast inoculated with ST exposed to gaseous ozone during the 3-day storage. The ST count (7.24 log cfu/g) in the ozone treatment group at 1 day was reduced by 0.4 log cfu/g compared with the initial count in same group, whereas there was a 0.2 log cfu/g increase in the nontreatment group ($P > 0.05$). The ST counts in the ozone treatment group at each time point were significantly lower than those in the nontreatment group. After 1 day, the ST counts in ozone treatment and nontreatment groups increased throughout the experiment period compared with the initial counts (ozone treatment, $P > 0.05$; ozone nontreatment, $P < 0.05$); however, it was clear that the increase of ST counts in the ozone treatment group gradually lagged behind the counts in the nontreatment group over time ($P < 0.05$). This finding indicates that ozone exposure continuously eliminated ST on the surface of the chicken breast samples. Similar to the ST results, gaseous ozone exposure significantly reduced total aerobic and anaerobic bacterial counts compared with those of the nontreatment groups at each time point during 3 days of storage. Total aerobic (7.26 log cfu/g) and anaerobic bacterial counts (7.43 log cfu/g) in the ozone treatment groups were reduced by 0.83 and 0.73 log cfu/g, respectively, at 1 day ($P < 0.05$). After 1 day, the counts of total aerobic and anaerobic bacteria in all groups gradually increased ($P < 0.05$); however, the increase in the counts of the ozone treatment groups lagged behind those in the nontreatment groups during 3 days of storage ($P < 0.05$). At the end of the study, the total aerobic and anaerobic bacterial counts were significantly reduced by 1.01 and 1.07 log cfu/g by ozone treatment.

TABLE 2. OXIDATIVE PROPERTIES OF CHICKEN BREAST INOCULATED WITH *SALMONELLA* TYPHIMURIUM (ST) EXPOSED TO GASEOUS OZONE DURING STORAGE

Oxidative properties	Inoculation with ST	Ozone treatment	Storage (days) 0	1	2	3
Catalase/CAT activity (U/g sample)	No	No	*54.9 ± 8.2^{aA}	53.8 ± 7.7^{aA}	50.7 ± 8.2^{aA}	51.8 ± 7.3^{a}
		Yes	54.9 ± 8.2^{aA}	51.8 ± 8.6^{aA}	49.7 ± 7.6^{aA}	44.5 ± 4.3^{bB}
	Yes	No	55.9 ± 7.6^{aA}	53.2 ± 4.4^{aA}	51.8 ± 7.3^{aA}	50.7 ± 9.3^{aA}
		Yes	55.9 ± 7.6^{aA}	52.8 ± 8.1^{aA}	50.7 ± 6.9^{aB}	43.5 ± 5.9^{bC}
Glutathione peroxidase (GSH-Px) activity (U/g sample)	No	No	0.215 ± 0.02^{aA}	0.216 ± 0.02^{aA}	0.204 ± 0.01^{aA}	0.198 ± 0.01^{aA}
		Yes	0.215 ± 0.02^{aA}	0.208 ± 0.03^{aA}	0.189 ± 0.06^{aAB}	0.167 ± 0.05^{bB}
	Yes	No	0.210 ± 0.01^{aA}	0.212 ± 0.01^{aA}	0.199 ± 0.01^{aA}	0.193 ± 0.02^{aA}
		Yes	0.210 ± 0.01^{aA}	0.196 ± 0.02^{aA}	0.186 ± 0.02^{aB}	0.176 ± 0.02^{bB}
TBARS (mg MA/kg sample)	No	No	0.32 ± 0.11^{aA}	0.32 ± 0.03^{aA}	0.30 ± 0.05^{cA}	0.32 ± 0.12^{bA}
		Yes	0.32 ± 0.11^{aB}	0.37 ± 0.04^{aB}	0.38 ± 0.05^{abB}	0.60 ± 0.08^{aA}
	Yes	No	0.28 ± 0.07^{aA}	0.33 ± 0.03^{aA}	0.32 ± 0.08^{bcA}	0.32 ± 0.05^{bA}
		Yes	0.28 ± 0.07^{aB}	0.35 ± 0.09^{aB}	0.42 ± 0.11^{aB}	0.62 ± 0.10^{aA}

* Mean values ± standard deviation.
Means in the same column followed by different superscript lowercase letters are significantly different ($P < 0.05$).
Means in the same row followed by different superscript uppercase letters are significantly different ($P < 0.05$).

TABLE 3. INSTRUMENTAL SURFACE COLOR OF CHICKEN BREAST INOCULATED WITH *SALMONELLA* TYPHIMURIUM (ST) EXPOSED TO GASEOUS OZONE DURING STORAGE

Bacteria	Inoculation with ST	Ozone treatment	Storage (days) 0	1	2	3
Lightness (CIE *L**)	No	No	*55.6 ± 0.5^{aA}	56.6 ± 3.4^{abA}	54.3 ± 2.1^{aA}	56.0 ± 5.9^{aA}
		Yes	55.6 ± 0.5^{aB}	57.3 ± 2.0^{aA}	54.1 ± 3.1^{aB}	49.4 ± 2.0^{cC}
	Yes	No	56.5 ± 1.5^{aA}	54.5 ± 2.6^{bcB}	54.0 ± 3.2^{aB}	51.6 ± 1.5^{bC}
		Yes	56.5 ± 1.5^{aA}	53.2 ± 2.4^{cB}	53.6 ± 2.3^{aB}	47.1 ± 2.0^{cC}
Redness (CIE *a**)	No	No	2.5 ± 0.6^{aA}	1.4 ± 0.8^{aB}	1.1 ± 0.8^{aB}	0.8 ± 0.5^{aB}
		Yes	2.5 ± 0.6^{aA}	1.2 ± 0.1^{aB}	0.9 ± 0.7^{aB}	0.8 ± 0.3^{aB}
	Yes	No	2.6 ± 0.7^{aA}	1.2 ± 0.4^{aB}	1.5 ± 0.7^{aB}	0.3 ± 0.7^{aC}
		Yes	2.6 ± 0.7^{aA}	1.3 ± 0.9^{aB}	1.7 ± 0.8^{aB}	0.4 ± 0.5^{aC}
Yellowness (CIE *b**)	No	No	2.0 ± 0.6^{aC}	2.1 ± 1.7^{aC}	4.6 ± 2.4^{bB}	4.6 ± 1.9^{aA}
		Yes	2.0 ± 0.6^{aB}	2.1 ± 0.8^{aB}	7.1 ± 1.1^{aA}	6.9 ± 1.6^{aA}
	Yes	No	2.1 ± 0.3^{aC}	2.7 ± 1.2^{aBC}	3.9 ± 1.3^{bAB}	4.6 ± 1.8^{bA}
		Yes	2.1 ± 0.3^{aB}	2.2 ± 1.3^{aB}	6.6 ± 2.7^{aA}	7.8 ± 1.5^{aA}

* Mean values ± standard deviation.
Means in the same column followed by different superscript lowercase letters are significantly different ($P < 0.05$).
Means in the same row followed by different superscript uppercase letters are significantly different ($P < 0.05$).

Gaseous ozone exposure significantly reduced ST, total aerobic and anaerobic bacterial counts at each time point compared with the nontreatment groups. The reduction patterns of these three counts were similar for 3 days, and the difference in reduction in bacterial count between the ozone treatment groups and the nontreatment groups was gradually increased over time. This pattern suggested that the effect of gaseous ozone increased with an increase in storage time. Similar findings have been reported by Cardenas *et al.* (2011), who found that an ozone concentration of 154×10^{-6} kg O_3/m^3 decreased the total aerobic bacteria by less than 1.0 $\log_{10}$ during the first 4 h of treatment, with a greater reduction, ranging from 1.0 to 2.0 log cfu/g, found after 24 h of exposure. An ozone concentration of 270×10^{-6} kg O_3/m^3 was effective at inhibiting bacteria found naturally in fish, such as *Pseudomonas putida*, *Lactobacillus plantarum* and *Enterobacter* spp. (Da Silva *et al.* 1998). The concentration of ozone in our study was quite low (10×10^{-6} kg O_3/m^3) compared with other studies, which reported values of 154×10^{-6} kg O_3/m^3 (Cardenas *et al.* 2011) and 270×10^{-6} kg O_3/m^3 (Da Silva *et al.* 1998).

Oxidative Properties

The oxidative properties, including CAT and GSH-Px activities and lipid oxidation (TBARS) in breast meat are presented in Table 2. Gaseous ozone exposure had no significant effect on CAT or GSH-Px activities in either inoculated or non-inoculated chicken breast during 0–2 days of storage. During that period, CAT and GSH-Px activities were slightly decreased but were not significantly different. At 3 days, the gaseous ozone exposure significantly affected CAT and GSH-Px. The activities of CAT and GSH-Px in chicken breast subjected to gaseous ozone exposure were significantly lower than that of nontreated samples regardless of ST inoculation. Whiteside and Hassan (1988) reported that exposure to ozone could inactivate CAT and that the inactivation effect of ozone depends on exposure time and pH level. The inactivation of CAT by ozone was primarily due to the damage in protein moieties, which subsequently led to heme release (Lee *et al.* 2003).

The TBARS values of all groups of samples ranged from 0.28 to 0.42 mg MA/kg sample from 0 to 2 days of storage. Within that storage period, gaseous ozone exposure did not affect the TBARS values of the chicken breasts ($P > 0.05$). The ozone significantly increased lipid oxidation at 3 days of storage regardless of ST inoculation. This finding was similar to and may be related to the data for CAT and GSH-Px activities, in which the activity of those enzymes was decreased due to gaseous ozone exposure at 3 days of storage. Organisms have developed a defense system to regulate oxidative stress, including lipid peroxidation, through an enzymatic system including CAT and GSH-Px (Laguerre *et al.* 2007). In the present study, CAT and GSH-Px, which are used in defense against lipid oxidation, were weakened by the ozone exposure. Thus, the resulting lipid oxidation was expressed as an increase of the TBARS values. The other possible explanation for the higher TBARS values in chicken breast subjected to gaseous ozone exposure might be that ozone itself attacked the lipids in the cells. The ozone may have promoted lipid oxidation by causing irreversible damage to fatty acids in the cell membrane and to cellular proteins (Beuchat 1991; Luck and Jager 1998; Sekhon *et al.* 2010). Ozone (O_3) is categorized as a nonradical derivative reactive oxygen species (ROS) that is responsible for the initiation of the lipid oxidation reaction

(Choe and Min 2005). Moreover, ozone gas is unstable and decays naturally into diatomic oxygen (Finch and Fairbairn 1991). Environments containing high oxygen promote lipid oxidation (McMillin 2008). At 3 days of storage, the TBARS values of non-inoculated and inoculated chicken breast samples subjected to gaseous ozone exposure were 0.60 and 0.62 mg MA/kg sample, respectively. Considering that TBARS values up to 0.6 mg MA/kg sample of fresh meat are acceptable (Tarladgis *et al.* 1960), the chicken breast sample subjected to gaseous ozone was at a critical acceptance threshold. However, the chicken breast sample that was not subjected to gaseous ozone was considered fresh until 3 days of storage.

Instrumental Surface Color

The effect of gaseous ozone on the surface color of chicken breast is shown in Table 3. There was no significant effect of gaseous ozone exposure on the CIE a^* value (redness) of chicken breast during storage. Additionally, inoculation with ST did not affect ($P > 0.05$) the CIE a^* value. The CIE a^* value of all samples was significantly decreased at 1 day of storage, followed by a stable value thereafter. Our finding contradicts Cardenas *et al.* (2011) and Stivarius *et al.* (2002), who found lower a^* values in beef subjected to ozone exposure. This contradiction might be related to the myoglobin content in muscle. Chicken breast meat is categorized as white muscle due to the lower content of myoglobin (Judge *et al.* 1989). Ozone and other ROS are strong oxidants of myoglobin (Bekhit *et al.* 2013). Myoglobin oxidation produces metmyoglobin, which causes the discoloration of meat characterized by reducing red coloration (Mancini and Hunt 2005). This effect of ozonation is not observed in white muscle.

The gaseous ozonation had no effect on L^* (lightness) or b^* (yellowness) until 2 days and 1 day of storage, respectively. Our results agree with Cardenas *et al.* (2011), who reported that exposure to ozone for 3 and 24 h at 0 and 4C did not affect the L^* and b^* values of beef. At 3 days of storage, the chicken breast subjected to gaseous ozone exposure had a significantly lower L^* value than the non-ozone-treated samples. The lower L^* value might be related to the drier surface of the chicken breast subjected to gaseous ozone. After 2 days of gaseous ozone exposure, the chicken breast had a higher yellowness ($P < 0.05$) than that of chicken breast without ozone exposure regardless of ST inoculation.

In conclusion, ozone exposure (10×10^{-6} kg $O_3/m^3/h$) significantly reduced the growth of ST, total aerobic and anaerobic bacteria in ST-inoculated chicken breast samples during 3 days of storage. Ozone exposure significantly increased the TBARS values. However, the TBARS values of the samples were regarded as acceptable until the end of storage. A lower level of redness was observed in samples under ozone exposure. The CAT and GSH-Px activities decreased with an increase in storage time. Ozone exposure slightly reduced the CAT activity from day 2 to day 3 of storage and the GSH-Px activity on day 3 of storage. In this study, we did not include chicken breast samples that were not inoculated with ST to evaluate the growth of bacteria, and this might weakened the statistical analysis of bacterial counts.

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