

The effect of plasma generated ozone for cold storage the broiler chicken meat

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Abstract. *Yuliani M, Mahfudz LD, Nurwantoro, Nur M. 2019. The effect of plasma generated ozone for cold storage the broiler chicken meat. Nusantara Bioscience 11: 12-17.* The aim of this study was to determine the effect of combination treatment washing chicken meat with three ozone concentrations in the water during 10 min and stored at 2-7°C without ozonation and with ozonation, on the shelf life of chicken meat. The study was conducted with a complete random factorial design (CRD) factorial pattern 3 x 2 with 3 replications. The data were analyzed by using analysis of variance (ANOVA). If the results were significant, the analysis followed Duncan's New Multiple Range Test (DMRT). The results of statistical analysis showed that average pH values, color scores intensity b * for brightness (yellowness) and Peroxide value (PV) showed significant differences (P <0.05). H₂S test showed that combination treatment washing chicken meat with ozone concentrations 3ppm in the water during 10 min and stored at 2-7°C with ozonation was able to inhibit the start process of decomposition chicken meat until the 10th days. The conclusion of these results that combination treatment washing chicken meat with ozone concentrations 3ppm in the water during 10 min and stored at 2-7°C with ozonation can extend the shelf life of chicken meat until the 10th days.

Keywords: Carcass broiler, cold storage, level concentrations, ozonation, shelf life

INTRODUCTION

Poultry meat is a superior commodity that is right to be developed as a strategic commodity, especially in terms of the nutritional, health, and living standards of the community (Warsito et al. 2012). Fresh meat is easily damaged due to chemical, enzymatic and microbial reactions. Chicken meat is highly perishable towards the growth of spoilage microorganisms and pathogens that cause damage and disease in food (foodborne pathogens). To prevent the removal of chicken meat because it is damaged or cannot be fully utilized after being cut, it is necessary to have the right post-harvest technology in order to the meat to maintain its quality. One technology that can be applied is plasma (ozone) technology that is safe for food. Ozone is a strong oxidizing agent which has the potential as a disinfectant that can kill pathogenic microorganisms by oxidizing membranes (lipids) of microorganism cells (Khadre and Yousef 2001; Patel 2001).

Chicken meat is high in fat, so when it encounters strong oxidizing, there will be a process of fat oxidation, such as a rancid odor, so to overcome the nature of ozone as a strong oxidizing agent, it is necessary to conduct research with cold temperature storage treatment. According to Goncalves (2009), ozone at 1-3°C does not oxidize fat. Ozone is a powerful antimicrobial agent that is very effective in destroying various types of

microorganisms including viruses, bacteria, protozoa, and bacterial and fungal spores (Khadre and Yousef 2001) This agent inactivates bacteria by breaking down the membrane and cell wall, causing lysis of cells (Muhlisin et al. 2015). Utilization of ozone at low concentrations between 0.01 ppm - 4.00 ppm is safe to be applied in agriculture, health, environmental and industrial fields (Purwadi et al. 2006). Ozone has been used in preservation of various agricultural products, such as vegetables, fruits, fish (Manousaridis et al. 2005) and meat products (Sekhon et al. 2010; Stivarius et al. 2002; Muhlisin et al. 2015).

In general, meat damage due to bacterial contamination is more dominant than others (Hadiwiyoto 1983). The shelf life of meat is influenced by several factors such as pH and temperature (Farber et al. 1991 and Jamilah et al. 2003). There has not been much information and data reported related to the use of the ozonation method in preserving chicken meat. It is expected that by using the ozonation method the shelf life of chicken meat can be longer when compared to conventional storage methods that already exist. This study aimed to examine the effect of combination treatment washing chicken meat with three ozone concentrations (0, 1.5 and 3ppm) in the water during 10 min and stored at 2-7°C without ozonation and with ozonation to determine pH values, b * for brightness (yellowness) color scores, H₂S and Peroxide value (PV) in chicken meat.

MATERIALS AND METHODS

Samples preparation

The experimental design was conducted with a complete random factorial design (CRD) factorial pattern 3 x 2 with 3 replications. First Factor: washing chicken meat with three ozone concentrations (0, 1.5 and 3ppm) in the water for 10 min. A consisted of = washing chicken meat with ozone concentration 0 ppm in the water during 10 min; B consisted of = washing chicken meat with ozone concentration 1.5 ppm in the water during 10 min; C consisted of = washing chicken meat with ozone concentration 3 ppm in the water during 10 min; Second Factor: stored at 2-7°C without ozonation and with ozonation. 1 First consisted of = stored at 2-7°C without ozonation and 2 consisted of = stored at 2-7°C with ozonation density 15g/m³. Rumah Pematangan Ayam Banjarsari Tembalang Semarang provided Eighteen carcasses of broiler. Chickens were 30-32 days old before slaughtering with approximately 1.93 kg. Chicken carcass was run into washing process, by preparing Ozone of different concentration of 0 ppm, 1.5 ppm, and 3 ppm then flowed into the water which will be used for the initial washing of all carcass broiler. After that the carcass broiler was drained for 10 minutes, all carcass broiler were stored in a refrigerated room (2-7°C) for the time of reception until used. Initial preparation of ozonation in a refrigerated space that is by flowing ozone gas (0 g/m³ and 15g/m³ cold storage ozone density) in a refrigerated room 3 m³ (Eurozon, Ecologyc 2000, Sestao, Vizcaya, Spain) during 90 minutes. The ozone generator then turned on every morning (08.00-09.30 am) and afternoon (15.00-16.30 pm). Ozone flowed continuously at a temperature of 2 -7 ° C and in a relative humidity of 90 ± 1%. This condition was important for the efficiency of the ozone bactericidal effect (Kim et al. 1999). Sampling was done with chicken carcass preparation (Soeparno 2007). Observation on the treatment of two conditions stored at 2-7°C and washing chicken meat with level ozone concentrations in the water. Data were collected on days 1, 4,10,14 and 17 treatments to determine pH values, b * for brightness (yellowness) color scores, H₂S and Peroxide value (PV) in chicken meat.

pH Value

The pH value was measured using a pH meter (Type Instruments Hanna HI 96107) with a combined electrode which penetrated the meat samples. The pH meter was calibrated with pH 4 and pH 7 standard solutions (Bintoro 2006).

Color analysis b * for brightness (yellowness)

The sample was prepared by weighing 10 g of chicken meat and put into color reader space. The target reading is L *, a *, b * and the color is measured. Then the color scale is read with the L * parameter for brightness (lightness), a * for brightness (redness) and b * for brightness (yellowness) (Pankaj et al. 2013).

H₂S test

H₂S Test was measured by weighing 5g of chicken meat and put into a petri dish and then cover it with filter paper, added 3 drops of 10% Pb acetate were dripped on filter paper covering the meat. After 2-3 minutes changes occur in filter paper was observed. If the dots appear brown to black means that the meat has undergone the initial process of decomposition chicken meat (AOAC, 2005).

Peroxide value

Five g of sample meat was put into a 250 ml Erlenmeyer and 30 ml of glacial acetic acid and chloroform with the ratio of 3:2 were added. After the sample was dissolved, 0.5 ml of saturated KI was added to this 250 ml Erlenmeyer in a closed state, allowed to stand for 1 minute while shaking. Then diluted with 30 ml of distilled water. Titrate with Na₂S₂O₃ 0.01 N until the yellow color was almost gone, 0.5 ml of 1% starch solution was added and titrated again until the blue color began to disappear. Calculated Peroxide Tables are expressed in milli-equivalents of peroxide in every 1000 g of sample (Muresan et al. 2010).

$$\text{Number of peroxide} = \frac{ml \text{ Na}_2 \text{ S}_2 \text{ O}_3 \times N \text{ Na}_2 \text{ S}_2 \text{ O}_3 \times 1000}{\text{sample weight} (g)}$$

Data analysis

The data was analyzed by using analysis of variance (ANOVA). If the results were significant, the analysis followed Duncan's New Multiple Range Test (DMRT) to determine differences between treatments. (Gomez and Gomez 2007).

RESULTS AND DISCUSSION

pH value

The observation of the average pH value of meat can be seen in Table 1. The results of observational statistical analysis of the combination treatment washing chicken meat with three ozone concentrations (0, 1.5 and 3ppm) in the water during 10 min and stored at 2-7°C without ozonation and with ozonation on the pH value of chicken meat show a real difference. Combination treatment without ozonation in the average pH value increases the length of storage. The higher the level of ozone concentrations, the increase of pH value seems slower. Results of study 1-day Combination treatment washing chicken meat with ozone concentrations 3ppm in the water during 10 min and stored at 2-7°C without ozonation is able to slow the increase in pH value. Combination treatment washing chicken meat with ozone concentrations 1.5ppm in the water during 10 min and stored at 2-7°C without ozonation was not different from the control treatment. The results of study on the 7th-day Combination treatment washing chicken meat with ozone concentrations 1,5ppm and 3ppm in the water during 10 min and stored at 2-7°C without ozonation did not experience a difference and was different from the control treatment.

Combination treatment washing chicken meat with ozone concentrations in the water during 10 min and stored

at 2-7°C with ozonation overall can inhibit the increase of average pH. The results showed that the combination treatment with ozone concentrations 3ppm in the water during 10 min and stored at 2-7°C with ozonation could inhibit the increase in pH values until the 10th day. The combination treatment washing chicken meat with ozone concentrations 3ppm in the water during 10 min and stored at 2-7°C with ozonation on day 10 was not different from the combination treatment washing chicken meat with ozone concentrations 1.5ppm in the water during 10 min and stored at 2-7°C without ozonation

Generally, the average pH value has increased along with the shelf life of chicken meat. Samples in all treatments showed significant differences ($P < 0.05$) on days 1, 4, 7, 10, 14 and 17. Standard normal pH values in broiler chicken meat ranged from 5.96 to 6.07 (Van Laack et al. 2000) Sanjaya (2007) states that the pH value of rotten chicken meat is always higher than that of fresh chicken meat, pH value of rotten chicken 6.16 (raw). The results of the combination treatment washing chicken meat with ozone concentrations in the water and stored at 2-7°C without ozonation and with ozonation on days 14 and 17 showed the mean pH value of combination treatment conditions of ozonation refrigerated storage was higher than the combination treatment of cooled storage conditions without ozonation. This is due to the fact that ozone is a strong oxidizing agent so that the breakdown of proteins becomes volatile compounds ammonia. Overall the results of the combination treatment study of cooled storage conditions (without ozonation and with ozonation) on days 14 and 17 showed the average pH value of chicken meat has increased pH value. The increase in pH value is caused by the breakdown of protein into ammonia volatile compounds. This ammonia compound can interact with water in meat which causes the formation of ammonium hydroxide which is alkaline so that the pH increases (Amin 2012). According to Kasmadiharja (2008), the increase in pH value will affect the rate of decay that occurs in producing alkaline compounds such as NH_3 , H_2S , trimethylamine, and other volatile compounds that cause an increase in pH value. However, the accumulation of lactic acid formed during storage causes the pH value of the meat to decline again after 3 months of storage (Takasari 2008).

Color b* for brightness (yellowness) analysis of chicken meat

The results of combination treatment washing chicken meat with three ozone concentrations (0, 1.5 and 3ppm) in the water during 10 min and stored at 2-7°C without ozonation and with ozonation on the statistical analysis of color chicken meat showed a significant difference in the color intensity of b* for brightness (Yellowness) during the storage period in all samples. The average value of color intensity b* for brightness (Yellowness) effect of combination treatment washing chicken meat with ozone concentrations in the water and stored at 2-7°C without ozonation and with ozonation can be seen in Table 2. Based on the results of the study showed the increase and decrease in color intensity b* for brightness (Yellowness), during the storage period.

The color of fresh chicken meat is yellowish white. Color in a chicken ging yellowish white is caused by provitamin A content found in meat fat. Along with the storage time, the value of the intensity of the color b* has increased so that it begins to fade yellowish color on chicken meat. This is caused by the effect of the treatment of exposure to ozone gas to oxidize the provitamin A content found in meat fat so that the intensity value of the color b* has increased which causes the yellowish-white color of chicken to turn pale white. The color of chicken meat is influenced by the level of fat oxidation that occurs during meat storage. The high-fat content of chicken meat can affect color levels. Meanwhile, color change is strongly influenced by the level of nutrients in the feed given, not due to genetic factors (Zhao et al. 2013).

Color is an indicator of meat quality, although color does not affect nutritional value (Nugrahen 2012). This color is influenced by animal-specific differences such as age, sex, meat content, pre-cutting conditions, and processing variables (Totosaus et al. 2007). According to Muhlisin et al. (2015), exposure to ozone gas during 3 days storage does not affect the value of brightness and yellowish color on chicken breast meat. The storage time of chicken meat in the refrigerator for up to six days shows that the color changes become reddish white (Sari et al. 2012).

Table 1. The average results of the study of pH values

Day	Treatment					
	A		B		C	
	1	2	1	2	1	2
1	5.93 ^a	5.80 ^b	5.92 ^a	5.92 ^a	5.76 ^b	5.79 ^b
4	6.05 ^a	5.81 ^c	5.94 ^b	5.92 ^b	5.83 ^c	5.79 ^c
7	6.10 ^a	5.95 ^c	6.09 ^{bc}	5.98 ^b	5.97 ^c	5.94 ^c
10	6.17 ^a	5.98 ^c	5.94 ^d	6.04 ^b	5.97 ^c	5.93 ^d
14	6.22 ^c	6.25 ^b	6.30 ^a	6.25 ^b	6.14 ^d	6.30 ^a
17	6.31 ^c	6.71 ^a	6.36 ^b	6.34 ^b	6.15 ^d	6.38 ^b

Note: ^{ab}) Superscripts with different letters on the same line show significant differences ($P < 0.05$). Treatment of A, B, C = washing chicken meat with three ozone concentrations (0; 1.5 and 3 ppm) in the water. 1, 2 = stored at 2-7°C without ozonation and with ozonation

Table 2. The average results of the research are the color intensity values b* (Yellowness)

Day	Treatment					
	A		B		C	
	1	2	1	2	1	2
1	32.66 ^b	32.66 ^b	41.66 ^a	32.00 ^b	32.66 ^b	32.33 ^b
4	30.00 ^c	32.00 ^b	33.66 ^b	32.33 ^b	34.33 ^a	33.33 ^b
7	26.66 ^b	27.00 ^b	30.66 ^a	27.66 ^b	29.66 ^a	27.66 ^b
10	32.33 ^a	25.66 ^b	29.00 ^a	27.66 ^b	30.00 ^a	28.33 ^a
14	30.33 ^b	28.00 ^c	30.66 ^b	34.66 ^a	34.00 ^a	34.66 ^a
17	30.66 ^b	30.33 ^b	33.66 ^a	33.00 ^a	32.66 ^a	31.33 ^b

Note: ^{ab}) Superscripts with different letters on the same line show significant differences ($P < 0.05$). Treatment of A, B, C = washing chicken meat with three ozone concentrations (0; 1.5 and 3 ppm) in the water. 1, 2 = stored at 2-7°C without ozonation and with ozonation

Table 3. The average results of H₂S level data research

Treatment	Day 0	Day 4	Day 7	Day 10	Day 14	Day 17
A1	-	+	++	+++	+++	+++
B1	-	-	+	++	+++	+++
C1	-	-	-	-	+++	+++
A2	-	-	-	+	+++	+++
B2	-	-	-	+	+++	+++
C2	-	-	-	-	+++	+++

Note: ⁻No Initial Decomposition, ⁺Initial Decomposition

Table 4. The results of the research are data on Peroxide Figures value

Day	Treatment					
	A		B		C	
	1	2	1	2	1	2
1	0.80 ^a	0.66 ^{ab}	0.60 ^{ab}	0.46 ^b	0.40 ^b	0.53 ^b
4	1.20 ^a	1.06 ^b	0.86 ^b	0.86 ^b	0.80 ^b	0.86 ^b
7	1.40 ^a	1.20 ^a	1.06 ^b	1.00 ^b	0.86 ^b	0.93 ^b
10	3.73 ^{bc}	2.20 ^c	4.40 ^b	1.80 ^d	4.20 ^b	5.46 ^a
14	8.20 ^a	3.60 ^f	7.13 ^b	6.46 ^c	5.53 ^d	5.00 ^e
17	10.20 ^d	11.00 ^c	12.93 ^b	8.40 ^e	17.33 ^a	6.06 ^f

Note: ^{ab}Superscripts with different letters on the same line show significant differences ($P < 0.05$). Treatment of A, B, C = washing chicken meat with three ozone concentrations (0; 1.5 and 3 ppm) in the water. 1, 2 = stored at 2-7°C without ozonation and with ozonation

Asmara et al. (2006) stated the color of fresh chicken meat is yellowish white. Color yellowish chicken meat caused by provitamin A contained in the fat of meat and pigments oxymyoglobin. Oxymyoglobin pigments are important pigments in fresh meat, these pigments are only present on the surface and describe the color of the meat that consumers want. The color of chicken meat due to imperfect blood loss is caused by hemoglobin pigment (Lawrie 2003). Esmer et al. (2011) state the redness loss in meat and changes in color to brownish red with metmyoglobin formation. Seydim et al. (2006) stated that during refrigerated storage, this loss of redness was due to myoglobin oxidation to metmyoglobin.

H₂S test

The effect of a combination treatment washing chicken meat with three ozone concentrations (0, 1.5 and 3ppm) in the water during 10 min and stored at 2-7°C without ozonation and with ozonation on observations of average H₂S levels can be seen in Table 3. The results of H₂S test showed a combination treatment washing chicken meat with ozone concentrations 0ppm in the water and stored at 2-7°C without ozonation (control treatment) began to occur at the beginning of decomposition of chicken meat on day 4. The combination treatment washing chicken meat with ozone concentrations 3ppm in the water and stored at 2-7°C with ozonation was able to inhibit the start process of decomposition of chicken meat until the 10th day. The effect of combination treatment washing chicken meat with ozone concentrations in the water against H₂S test results experience the initial process of decomposition along with storage time, but the higher the level ozone

concentrations in the water of leaching can slow down the initial process of decomposition.

The H₂S test is basically to see H₂S which is released by the spoilage bacteria found in the meat. The bacteria that produce H₂S are Pseudomonas bacteria. The results of protein metabolism will produce products that are closely related to indicators of decay. Pseudomonas will produce ammonia during the metabolism of amino acids which causes the pH of the meat to increase during decay (Ray 2000). Through H₂S test the initial process of chicken meat decomposition can be detected through the activity of H₂S producing bacteria which appear on the filter paper area to change color to brown. This suggests that H₂S producing bacteria has grown up on meat and undergo initial decomposition. H₂S which is released by the decomposing bacteria will bind Pb acetate to Pb sulfite (PbSO₃) then produce brown on filter paper. Pseudomonas bacteria also produce enzymes that are able to break down fat components and protein components from food so as to cause foul odor and cause mucus.

Decay will damage meat is characterized by the formation of foul-smelling compounds such as ammonia, H₂S, indole, and amine which are the result of protein breakdown by microorganisms. Decay in meat is characterized by a foul odor, mucus formation, changes in texture, formation of pigments (changes in color), and changes in taste (Adams and Moss 2008). Anggreani (2005) states that the beginning of decay occurs due to bacteria, starting with the fermentation of glucose and glycogen found in chicken meat, then the next material protein that will be fermented after the carbohydrate in chicken meat starts to run out and the result of the breakdown of proteins from microorganisms will form ammonia compounds H₂S, indole and amine. Meat decomposition is also intensive bacterial decomposition of organic ingredients that form odorous gases so that it affects the nutritional value of meat (Wanniatie et al. 2014). Decreased meat protein during storage is caused by the occurrence of proteolysis process by bacterial activity which causes the formation of NH₃ gas which results in decreased meat protein content (Hadju 2006).

Peroxide value

The effect of a combination treatment washing chicken meat with three ozone concentrations (0, 1.5 and 3ppm) in the water during 10 min and stored at 2-7°C without ozonation and with ozonation on observing the average value of peroxide can be seen in Table 4. The results of statistical analysis showed that the peroxide value of chicken meat combination treatment washing chicken meat with ozone concentrations in the water and stored at 2-7°C without ozonation and with ozonation showed significant differences. The results showed that the average peroxide value increased along with storage time. The combination treatment washing chicken meat with ozone concentrations in the water and stored at 2-7°C without ozonation and with ozonation can be slow the oxidation process of chicken meat ($P < 0.05$). This is indicated by the average value of peroxide for 10 days of storage of not more than 10 mek O₂/kg (SNI 2000). The treatment of combination treatment

washing chicken meat with ozone concentrations 3ppm in the water and stored at 2-7°C with ozonation can slow down the oxidation process. This is according to Nam et al. (2001) Cold or frozen storage conditions can inhibit the transport of free radicals and slow the transport of the oxidation process which results in a decrease in meat quality. The bactericidal effect of ozone depends on several factors, such as temperature, relative humidity, pH and the presence of organic matter (Kim et al. 1999).

The results showed combination treatment washing chicken meat with ozone concentrations in the water and stored at 2-7°C without ozonation had an average peroxide value which is higher than the treatment combination treatment washing chicken meat with ozone concentrations in the water and stored at 2-7°C with ozonation. This is due to the occurrence of lipid oxidation reactions in chicken meat. Lipid oxidation is a major cause of deterioration in meat quality because it affects the storage or storability of meat exposed to air (Gray and Pearson 1994).

One-third of unsaturated fatty acids in chicken meat is oleic acid. Fatty acids consist of carbon, hydrogen, and oxygen are one of the constituent components of lipids. This acid is widely found in simple fats and compound fats. Some of the important fatty acids in nutrition are palmitic, stearic, linoleic and oleic acids (Nursanyoto 1993). Yuanita (2006) states that the oxidation process in food containing fat will occur to a certain level during storage, but the process is affected by time, temperature and contact with air. Free oxygen in the air will oxidize the double bonds of unsaturated fatty acids in food, and oxidation of fatty acids followed by the formation of hydrogen peroxide (H₂O₂) which causes rancidity. Tao (2015) states that oxidation of fat begins with hydrolysis of triglycerides to produce fatty acids and glycerol. Furthermore, unsaturated fatty acids are oxidized to produce hydroperoxide, then aldehydes, ketones, and the peak is the formation of malonaldehyde. The higher the peroxide value of a food, the higher the fat damage due to food processing. Increased Peroxide Tables significantly during heating indicate that an oxidation reaction has occurred in the product. The oxidation process can occur if there is contact between oil or fat with oxygen. This oxidation occurs in non-saturated bonds in fatty acids. The increase in peroxide Tables is one indicator and warning that the product will smell rancid and damaged (Ketaren 1986).

To conclude, the results showed that the overall pH value increased with the length of storage, but the higher the level ozone concentrations of washing chicken meat in the water can slow the increase in pH value, the intensity of the color score b * yellowness in chicken meat showed an increase and decrease during the storage period and the value of peroxide (PV) shows the average value of peroxide numbers increases with the length of storage. The combination treatment washing chicken meat with three ozone concentrations (0, 1.5 and 3ppm) in the water during 10 min and stored at 2-7°C without ozonation and with ozonation can slow down the oxidation process of chicken meat. H₂S test the combination treatment of chicken meat washing with level ozone concentration of 3ppm in water for 10 minutes and stored at 2-7°C with ozonation was able

to inhibit the decomposition of chicken meat until the 10th day. The conclusion of the study was the effect of a combination treatment washing chicken meat with ozone concentrations 3ppm in the water during 10 min and stored at 2-7°C with ozonation can extend the shelf life of chicken meat until the 10th days.

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