Effect of ozone exposure time and ozonated water replacement to control the quality of chicken meat

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Abstract. Survei Sosial Ekonomi Nasional in 2015 stated that Indonesia ranks as the third country with the largest consumption of chicken meat per year (Badan Pusat Statistik, 2015). Ozone is able to effectively disinfect micropolutant (Loeb, et al., 2012) and Escherichia coli (Driedger, et al., 2001) in drinking water. The objectives of this study are to evaluate the performance of the ozone exposure time and water replacement in controlling the quality of chicken meat. Ozone generator used has production and number of solubility in water around 523,67mg/hr and 0,21mg/L. The variations evaluated in this study are ozone exposure time (40, 80, and 120 minutes) and the replacement of ozonated water (twice, thrice and no replacement). The evaluated parameters were Escherichia coli, total mesophyll hyll aerobic (TBMA), protein content, pH, and water content. On the seventh day storage at 120 minutes of ozone exposure time, it was able to disinfect Escherichia coli and TBMA as much as 1.700 and $9,4 \times 10^8$ CFU/g, while on the replacement water every 40 minutes 1,700 and $1,1 \times 10^9$ CFU/g more than blank. While the levels of protein, moisture content, and pH on the variation of exposure time and replacement of water respectively of 17,45%; 79,85%; 4,95 and 15,70%; 80,61%; 4,91.

1. Introduction

Indonesia is one of the countries with the largest consumption of chicken meat in the world. Based on data from the Survei Sosial Ekonomi Nasional in 2015, Indonesia ranks as the third country with the largest consumption of chicken meat in the world that is as much as 1.559.000 tons per year. The increase of national chicken meat consumption is supported by the growth of the average population of Indonesia about 1,34% per year and the increase of people's nutritional knowledge as indicated by the increase of chicken meat consumption per capita in 2014 which is 13,04% (Badan Pusat Statistik, 2015). The big number of consumptions creates a big issues about the quality of chicken meat consumed by society to the government.

Quality parameters of chicken meat consumed daily can be seen from the physical and microbiological quality (Standar Nasional Indonesia: 3924, 2009). Physically, fresh chicken meat has a pale white color, fine meat fibers, soft texture, no fat between meat fibers, yellow in fat, and a slightly fishy odor that does not even smell. While microbiologically, chicken meat consumable have microbiological quality requirement of Escherichia coli bacteria as much as $<10$ CFU/g and total mesophyll aerobic bacteria $<10^1$ CFU/g (Standar Nasional Indonesia, 2009). Other parameters that may affect the quality of chicken meat are temperature, humidity, moisture content, and pH. If the value of those parameters of chicken meat is not in accordance with acceptable quality limits, then the chicken meat has been damaged or the process of decay.

One method that can be used to maintain the quality of chicken meat is to inhibit the growth of microorganisms through ozonation method. Ozonation method has been commonly applied to drinking water treatment for the purpose of disinfection and micro pollutant oxidation (Loeb, et al., 2012). Studies on the disinfection of microorganisms using ozonation methods have been done on
E. coli bacteria (Driedger et al., 2001). Based on the results of these studies ozone is able to effectively decrease the activity of bacteria and microorganisms. This study aims to evaluate the performance of ozonated water in controlling the quality of chicken meat. The independent variables to be varied in this study are the ozone exposure time and the frequency of water replacement. The exposure time of ozone varied with three different periods of 40, 80, and 120 minutes. While the replacement of water varied into thrice, twice, and no replacement in total of 120 minutes. The dependent variables to be analyzed were Escherichia coli, TBMA, protein content, pH and water content in each variation. This method will be evaluated whether its performance is able to control the quality parameters of chicken meat mentioned above.

2. Methods
This study consists of three main procedures such as preparation, characterization of chicken water and chicken meat also ozone treatment to chicken meat. Ozone generator used in this study to produces ozone is a medium scale commercial ozone generator with production and solubility in water of 523,67 mg/hr and 0,21 mg/L.

2.1. Preparation
Prior to the study, the cleaning of tools used such as bottle, refrigerator, and ozone generator must be done. This step is done in order to avoid the contamination from other impurities or microorganisms that could affect the data results of the study. Cleaning is done by washing the entire research tool with soap to clean, followed by spraying alcohol 70% on every tool that will be used.

2.2. Characterization of water and chicken meat
Chicken meat used, as study sample is broiler type chicken meat bought from supermarket in South Jakarta, Indonesia. Samples were cut into uniform size and weight for each observation times. The chicken meat samples weighted around 100 grams and soaked in 100 mL of ozonated water for each sampling time and all parameters. The initial characterization of chicken meat was conducted to determine the effect of ozone to the dependent variables compared from the quality of chicken meat before and after ozone treatment. The parameters tested were amount of Escherichia coli, TBMA, protein content, pH, and water content. While water characterization conducted in order to determine the content in process water so the level of pollution in water and the source of bacteria in chicken meat could be known. Characterization of process water also needs to be performed to assess the performance of ozone in disinfecting bacteria in water before contacting the chicken meat samples.

2.3. Ozone treatment to chicken meat
In this study, chicken meat used for each samples are 100 g soaked in 100 mL of ozonated water with total exposure time of 120 minutes. The independent variables of this study are ozone exposure time and replacement of ozonated water. Ozone exposure time variation is done in three different times. Exposure time variation is evaluated for 40, 80, and 120 minutes. Mean while in the variation of water replacement, ozone treatment is performed with different frequency of replacement of water with the same total duration, 120 minutes. The ozonated water replacement variation is done thrice, twice, and no replacement. While the evaluated variables in this study are number of Escherichia coli, total mesophyll bacteria, protein content, pH and water content. Samples of chicken meat are stored in refrigerator with temperature 4-7 °C for seven days.

3. Result and Discussion
In this section, the result of ozone treatment and its performance to control the quality of chicken meat will be discussed further.

3.1. Characterization of water and chicken meat
Based on the regulation in the Peraturan Pemerintah Republik Indonesia No.82 in 2001, on the Management of Water Quality and Control of Water Pollution, raw water for drinking must contain
*Escherichia coli* maximum 100 CFU/100ml. *Escherichia coli* become one of the most important parameters determining the quality of water because the presence of it could indicate the contamination of human waste in water. Comparing the process water with existing standards, it could be conclude that the process water is feasible for further processing into process water for food products. Initial character for process water is shown in Table 1 below.

**Table 1. Characteristics of Process Water**

| Parameter                  | Unit          | Information |
|----------------------------|---------------|-------------|
| *Escherichia coli*         | CFU/100 ml    | <1,8        |
| Total Mesophyll Aerobic    | CFU/100 mL    | 2,8×10⁴    |
| pH                        | -             | 5,06        |

Standar Nasional Indonesia No. 3924 in 2009 states the the terms of chicken meat worth to be consumed if the amount of *Escherichia coli* <10 CFU/g, TBMA <10⁷ CFU/g, protein content 16-22%, pH 5,3-6,4 (Jia, 2017), and water content 68%-75% (Soeparno, 1994). If the standards mentioned before is compared with the initial character of samples, all parameters of quality fall below the maximum number set in SNI except the number of *Escherichia coli*. So it could be conclude that the chicken meat used, as samples in this study are not in a good quality. The initial character of chicken meat used in this study is shown in Table 2 below.

**Table 2. Characteristics of Chicken Meat Before Ozone Treatment**

| Parameter                  | Unit | Information |
|----------------------------|------|-------------|
| *Escherichia coli*         | CFU/g| 9.000       |
| Total Mesophyll Aerobic    | CFU/g| 520.000     |
| Protein Content            | %    | 21,36       |
| pH                        | -    | 6,39        |
| Water Content              | %    | 79,18       |

3.2. *Ozone treatment to chicken meat*

The quality of worth consume chicken meat can be seen from several parameters, such as physical, chemical, and microbiological parameters. Preservation of chicken meat using ozone is done to determine the effect of ozone treatment in controlling the quality of chicken meat.

3.2.1. Effect of ozone exposure time to control the quality of chicken meat

Ozone exposure time variation is done in three different times. Exposure time variation is evaluated for 40, 80, and 120 minutes.

- **Effect of ozone exposure time to number of *Escherichia coli***

The number of *Escherichia coli* bacteria before the ozone treatment in this study was 9.000 CFU/g. The performance of ozone in disinfecting *Escherichia coli* can be seen from the changes of the number of *Escherichia coli* exactly after being treated by ozone. According to Figure 1, the longer the ozone exposed with the sample, the more *Escherichia coli* disinfected. *Escherichia coli* were at least in variation of 120 minutes exposure time with a total of 240 CFU/g. While *Escherichia coli* contained in sample contact time 40 and 80 minutes is 2.400 and 1.100 CFU/g.
Figure 1. Effect of Ozone Exposure Time to Number of *Escherichia coli* after Ozone Treatment (Ozone dose: 0.21 mg/L)

Along with storage time, the number of *Escherichia coli* tends to increase and then decrease as shown in Figure 2. This phenomenon is related to the life cycle of *Escherichia coli*. *Escherichia coli* has five growth phases, namely adaptation phase, logarithmic, growth reduction, fixed growth and defunct. At 120 hours storage time, *Escherichia coli* began to enter a logarithmic phase where its amount increased significantly. The largest amount of *Escherichia coli* was found in blank sample with total of 24,000 CFU/g. At 168 hours of storage, *Escherichia coli* began to enter the phase of fixed growth and defunct. This is due to the nutrients in growth media has decreased due to pH change, and the presence of toxic metabolic results that inhibit bacterial growth.

Figure 2. Effect of Storage Time to Number of *Escherichia coli* in Ozone Exposure Time Variation (Ozone dose: 0.21 mg/L)
Effect of ozone exposure time to total mesophyll aerobic bacteria

Based on the initial characterization of the sample before being given any treatment, the TBMA amount is as much as 520,000 CFU/g or log5.72 CFU/g. To simplify the visualization of graphic, log is used to evaluate TBMA. After contact with ozone at contact time variation, the number of TBMA decreases as shown in Figure 3. Based on these data, the number of TBMA just after the ozone treatment is at least 120 minutes with bacteria log 5.30 CFU / g. While at exposure time of 40 and 80 minutes only as much as log 5.67 and log 5.59 CFU/g.

Figure 3. Effect of Ozone Exposure Time to TBMA after Ozone Treatment (Ozone dose : 0.21 mg/L)

As seen from the storage time in Figure 4, the number of TBMA increases along with the increasing of storage time. The presence of TBMA was at most on the 168th hour of storage time, whereas the number of TBMA at exposure times of 40, 80, and 120 minutes was log8.79; log 8.61 and log8.41 CFU/g. The enhancement of number of TBMA is caused by the source of bacterial nutrition that still contained in the sample.

Figure 4. Effect of Storage Time to TBMA in Ozone Exposure Time Variation (Ozone dose : 0.21 mg/L)
Effect of ozone exposure time to protein content

Protein content contained in blank about 21.36%. Figure 5 shows the longer of the ozone exposure time, the more protein denatured. Protein content exactly after exposed with ozone has the least value at 120 minutes exposure time about 17.91%, while at exposure time of 40 and 80 minutes as much as 19.37 and 18.54%. The protein denaturation process is caused by the direct reaction of ozone. Ozone direct reaction causes oxidative damage by breaking the double bond on the protein chain, so the protein splits into the simplest chain (amino acids).

![Protein Content vs Exposure Time](image)

**Figure 5.** Effect of Ozone Exposure Time to Protein Content (Ozone dose : 0.21 mg/L)

Figure 5 above also shows that the longer samples are stored, the fewer protein content present in the sample. This is caused by bacterial activity. Several types of TBMA bacteria is a protease bacteria that has the ability to produce protease enzymes. The protease enzyme is capable of causing proteolysis to occur. The more TBMA bacteria, the more protease enzymes are formed which results in a reduction in protein levels. Protein levels on the 168th hour of storage time in ozone exposure time of 40, 80, and 120 minutes showed 17.84%; 17.85%; and 17.45%. The percentage decrease in protein content is at least 120 minutes in contact. This is in accordance with the hypothesis presented in the preceding paragraph.

Effect of ozone exposure time to pH

Based on the pH change data shown in Figure 6 below, there is a decrease in pH exactly after ozone treatment in ozone exposure time variation although it is not significant. In the initial pH characterization of the sample before any treatment was 6.39. This decrease in pH value is due to the performance of ozone in disinfecting bacteria. Gram-positive bacteria are more stable to ozone treatment so that their metabolism will produce lactic acid.
The tendency of pH decrease over storage time is caused by anaerobic bacterial metabolism. In anaerobic bacterial respiration, the product produced from bacterial metabolism by breaking one mole of glucose through the glycolysis pathway is 2 pyruvic acids, 1 ATP, 1 NADH, and 1 NADPH. The pyruvic acid is then converted to lactic acid by a fermentation process. Lactic acid produced by the process of glycolysis is what causes the pH of the sample to become more acid during storage time.

- **Effect of ozone exposure time to water content**

In the initial characterization of the sample before any treatment was given, the water content of the blank was 79.18%. In this study, changes found in the water content of the sample. However, the direct ozonation process but rather the ozone performance does not cause this change in water content to the bacterial activity contained in the sample. The changes in water content is shown in Figure 7 below.
Water content increased along with storage time is caused by the effect of ozone to protein in chicken muscle (myofibrils). Myofibrils have the ability to bind water. When meat is ozone-treated, the protein breaks out so it loses the ability to bind water. Therefore the water content also increases by the storage time. Water content also increased due to the respiration of aerobic bacteria that contaminate chicken meat. The process produces carbon dioxide and water vapor. But bacteria to multiply so that the addition of moisture content in the sample is not too significant also use the addition of water to the sample.

3.2.2. Effect of ozonated water replacement to control the quality of chicken meat
In the variation of water replacement, ozone treatments are carried out with different frequency of water replacement with total duration of 120 minutes. The variations of ozone replacement are done thrice, twice, and no replacement. The difference in solubility value between residual ozone (after contact) with new dissolved ozone in water is very significant about 99%.

- **Effect of ozonated water replacement to number of Escherichia coli**
In the initial characterization of *Escherichia coli* contained in the sample was as much as 9,000 CFU/g. The effect of water replacement to on the amount of *Escherichia coli* can be analyzed based on the number of *Escherichia coli* disinfected right after the ozone treatment is given. The more often water replaced, the more *Escherichia coli* disinfected as shown in Figure 8. The number of *Escherichia coli* is at least in thrice water replacement of 200 CFU/g.

![Figure 8. Effect of Water Replacement Time to Number of Escherichia coli after Ozone Treatment (Ozone dose: 0.21 mg/L)](image)

Based on Figure 9, the number of *Escherichia coli* increases along with the storage time, but decreases at some point. This is due to the life cycle of *Escherichia coli* as mentioned in the previous subsection. At 120th hour of storage time, *Escherichia coli* begins to enter the logarithmic phase with the least amount of *Escherichia coli* present in a thrice water replacement sample of 2,000 CFU/g. At 168th hour of storage, *Escherichia coli* began to enter the phase of growth reduction and defunct with the least amount of *Escherichia coli* was on thrice water replacement with a total of 1,100 CFU/g.
Figure 9. Effect of Storage Time to Number of *Escherichia coli* (Ozone dose : 0.21 mg/L) in Water Replacement Variation

- **Effect ozonated water replacement to total mesophyll aerobic bacteria**

Based on the initial character of the sample before any treatment is given, the TBMA amount is as much as 520,000 CFU/g or log\(5,2\) CFU/g. The more often the water is changed, the more TBMA is disinfected. As can be seen in Figure 10, the number of TBMA is at least at thrice water replacement variation of log\(5,33\) CFU/g.

Figure 10. Effect of Water Replacement Time to TBMA after Ozone Treatment (Ozone dose : 0.21 mg/L)

Analysis of the effect of water replacement can also be seen from the growth of TBMA bacteria along with the storage time as shown in Figure 11. The longer the sample is stored, the more the amount of TBMA contained in the sample. This is due to the source of bacterial nutrition that still available. The presence of TBMA is at most on the 168\(^{th}\) hour of storage time whereas the amount of TBMA in thrice water replacement is log\(7,98\) CFU/g. The insignificant difference in the number of TBMA between different contact times at the same storage time is due to the decomposition of
ozone. The dissolved ozone in the water will decrease as mentioned in the previous TBMA chapter.

![Figure 11. Effect of Storage Time to TBMA (Ozone dose : 0.21 mg/L) in Water Replacement Variation](image1)

- **Effect of ozonated water replacement to protein content**

  Under no treatment conditions, the blank contained protein of 21.36%. As can be seen in Figure 12, the more frequent of water replacement, the more denaturation of the protein occurred. Protein content right after the ozone treatment has the least value on thrice replacement i.e. 17.23%. The protein denaturation process is caused by a direct reaction of ozone. The direct reaction of O3 causes oxidative damage capable of breaking double bonds on the protein chain, so the protein splits into the simplest chains (amino acids).

![Figure 12. Effect of Water Replacement Time to Protein Content (Ozone dose : 0.21 mg/L)](image2)

Figure 12 above also shows that the longer the sample is stored, the fewer protein content present in the sample. This may be due to the activity of protease bacteria in samples capable of producing
protease enzymes. Protease enzymes are capable of causing proteolysis so that the reduction in protein levels is increasing. The protein content on the 168th hour for thrice water replacement was 15.70%.

Effect of ozonated water replacement to pH
In the initial character of chicken without any treatment has a pH of 6.39. Right after the ozone treatment in the water replacement variation, the pH of the sample has decreased. pH right after ozone treatment for water replacement of thrice, twice, and no replacement is 6.23; 6.28; and 6.33. This decrease in pH is due to the performance of ozone in disinfecting bacteria. Gram-negative bacteria have walls that are more susceptible to ozone, so in the disinfecting stage the bacteria first lysate. Then leaving gram-positive bacteria whose metabolism will produce lactic acid. This is what makes the pH of the sample just after the ozone treatment decreases.

![Figure 13](image_url)

Figure 13. Effect of Water Replacement Time to pH (Ozone dose : 0.21 mg/L)

Based on Figure 13, the longer sample stored, the pH will decrease. The most acidic pH on the 168th hour of storage was in a thrice water replacement sample of 4.91. The tendency to decrease the pH value over time of sample storage is caused by anaerobic bacterial metabolism. In anaerobic bacterial respiration, the product produced from bacterial metabolism by solving one mol of glucose through the glycolysis pathway is 2 pyruvic acids, 1 ATP, 1 NADH, and 1 NADPH. The pyruvic acid is then converted to lactic acid by fermentation process so that the pH decreases.

Effect of ozonated water replacement to water content
In the initial character of the sample before any treatment was given, the water content of the sample was 79.18%. In this study changes found in the water content of the sample. However, a direct ozonation process but rather a result of ozone performance against bacteria contained in the sample does not cause this change in water content. The change in sample water content is shown in Figure 4.14 below.
Increased moisture content along with storage time is caused by the effect of ozone on protein in chicken muscle (myofibrils). Myofibrils have the ability to bind water. When meat is ozone-treated, the protein breaks out so it loses the ability to bind water. Therefore the water content increases with time. Increased moisture levels also occur due to respiration of aerobic bacteria that contaminate chicken meat that produces carbon dioxide and water vapor. But bacteria to multiply so that the addition of moisture content in the sample is not too significant also use the addition of water to the sample.

4. Conclusion

Based on data of evaluation obtained from this research, the conclusion that can be taken is as follows:

a. Methods of soaking chicken meat with ozonated water proved to control the quality of chicken meat better than with no ozone treatment.
   - On the 168th hour of storage, the number of *Escherichia coli* and TBMA for 40 minutes contact time was 2.000 and $6.2 \times 10^8$ CFU/g
   - On the 168th hour of storage, the number of *Escherichia coli* and TBMA for blanks was 2.800 and $1.2 \times 10^9$ CFU/g.

b. The effect of ozone exposure time to chicken meat quality are as follow:
   - The longer the contact time, the more bacteria disinfected in the sample of chicken meat.
   - On the 168th hour of storage, the number of *Escherichia coli* and TBMA for 40 minutes exposure time was 1.100 and $2.6 \times 10^8$ CFU/g while for 120 minutes of exposure time was 1.100 and $2.6 \times 10^8$ CFU/g. While the protein content, water content, and pH at 120 minutes exposure time were 17.45%; 79.85%; and 4.95.

c. The effect of ozonated water replacement to the quality of chicken meat are as follow:
   - The more frequent of ozonated water is replaced, the more bacteria disinfected in the sample of chicken meat.
   - On the 168th hour of storage, the number of *Escherichia coli* and TBMA for no replacement variation of the ozonated water was 1.100 and $2.6 \times 10^8$ CFU/g while for the thrice replacement of ozonated water was 1.100 and $9.5 \times 10^7$ CFU/g. While the protein content, water content, and pH at thrice water replacement variation were 15.70%; 80.61%; and 4.91.
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