The application of ozonated water to maintain the quality of tuna meat: the effect of contact time, contact temperature and ozone dosage

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The application of ozonated water to maintain the quality of tuna meat: the effect of contact time, contact temperature and ozone dosage

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Abstract. Tuna is one of the common fish consumed by Indonesian people. Tuna is highly perishable. Therefore, the handling technique is needed to maintain tuna's quality. This research brought about the application of ozonated water in controlling quality of tuna by observing the effect of contact time, contact temperature and ozone dosage. The standard quality of tuna is detected from the number of aerobic mesophyll bacteria, pH value, water content and the protein content. The study was conducted by testing the ozone solubility in water and contacting tuna to ozonated water. Tuna was contacted to ozonated water for 40, 80, 120 minutes' contact time at 8°C and ozone dosage of 0.3 mg/L; contacted at 37°C, 25°C and 8°C for 120 minutes and ozone dosage of 0.3 mg/L; and contacted at 8°C for 120 minutes with ozone dosage of 0.3 mg/L and 0.24 mg/L. After the treatment process, tuna was saved in 8°C chiller for 168 hours or seven days. The results show that the longer duration contact, the lower contact temperature, and the higher ozone dose, the lower the rate of quality degradation of tuna. Contacting tuna for 120 minutes can eliminate bacteria up to 66.7%, pH value decreases to 5.58 shortly after tuna was contacted to ozonated water; water content increases to 1.20% and protein decreases to 0.67% after 168 hours of storage. Contacting tuna at 8°C can eliminate bacteria up to 91.2%, pH value decreases to 5.48 shortly after tuna was contacted; water content increases to 1.14% and protein decreases to 0.22% after 168 hours. Meanwhile highest ozone dosage (0.3 mg/L) can eliminate bacteria up to 66.7%, pH value decreases to 5.58 after tuna was contacted; water content increases to 1.20% and protein decreases to 0.67% after 168 hours of storage.
Keywords: aerobic mesophyll bacteria, ozonated water, pH, protein, tuna, water content

1. Introduction

Based on the records of the Ministry of Marine Affairs and Fisheries, Republic of Indonesia, Tuna, Tongkol and Cakalang: fish group became the most consumed fish with 16.45% from the total consumption of fish [1]. Fish in general, including tuna is a type of food that is easy to decay. This is because the content of water, protein and fat are high. Those components are good medium for bacterial growth. Therefore, a good preservation method is needed to maintain the quality of tuna to keep it fresh. Preservation is one way to keep the fish from decaying process, so that it can be stored longer until it is time to be consumed. Some types of preservation techniques commonly used by the community such as freezing, salting and smoking. However, these methods effected to the texture and taste of fish.

Ozone is a strong oxidant with a chemical potential of 2.07 eV which potential as a disinfectant that is capable of killing pathogenic microorganisms such as bacteria, viruses and fungi. Low concentrations of ozone between 0.01 ppm to 4.00 ppm are expected to be applied in agriculture, health, environment

and food industry. The superiority of ozone as a disinfectant is due to the nature of ozone which produces oxygen instead of leaving harmful chemical residues after reacting with other substances. Ozone is an environmentally friendly technology [2] and safe to be applied in food storage [3]. Ozone is considered to be a more effective substance as a disinfectant agent for bacteria when compared to chlorine gas because when applied in food ozone does not affect the taste and colour of the food. Based on the paper written by Karim, et.al., was carried out to investigate the quality indices (microbial and chemical) of tiger grouper (*Epinephelus fuscoguttatus*) stored for 20 days in slurry ice treated with 0.1 mg ozone/L and 3.3% sodium chloride (NaCl). Slurry ice contains 60% ice and 40% water was used as control. All samples were analysed for total aerobic, psychotropic bacteria, total volatile based nitrogen (TVBN) and trimethylamine (TMA) at interval of 5 days during 20 days' storage. The total bacteria count of tiger grouper treated in slurry ice with O_3. showed significantly ($p > 0.05$) was found between controls and treated samples for TVBN and TMA contents. The combination of the slurry ice and ozone may be recommended for chilling and storage of tiger grouper to improve its storage quality [4]. This research used tuna (*Thunnus sp*) as food material to be tested. Sample was in the form of fresh fillet. The quality of tuna fish was maintained by using ozonated water through the immersion process.

2. Material and Methods

This research used tuna (*Thunnus sp*) as food material to be tested. Sample was in the form of fresh fillet. The quality of tuna fish was maintained by using ozonated water through the immersion process. The ratio of fish mass to the volume of ozonated water was 100 grams of tuna: 75 mL of ozonated water.

Ozone gas is generated from an ozone generator device. Ozone is injected in water using a stone bubbler. The ozone injection is carried out for 60 minutes. After the injection of ozone, a spectrophotometer will be used to measure the ozone solubility in water by measuring the degree of absorbance of the ozonated water that has been mixed with the Ozone test kit reagent MERCK 100607.

The variations done in this study include variation of contact time, contact temperature and ozone dosage. Variations in contact time were made by contacting tuna fish with ozonated water through the immersion process for 40, 80, and 120 minutes. Meanwhile, the contact temperature variations were made by contacting the fish with water at the room temperature (25°C), refrigerator temperature (8°C) and incubator temperature (37°C) for 120 minutes. While the variation of ozone dosage is done by contacting the fish with ozonated water for 120 minutes with 2 types of ozone dose which are 0.24 mg/L and 0.30 mg/L. After the contacting process, the water was discharged and the samples were stored in the refrigerator (8°C) for seven days of storage and observed the quality based on the parameters of total aerobic mesophyll bacteria, water content, pH value and protein at hours' period 0, 1, 72, 120 and 168.

3. Result and Discussion

3.1. The effect of contact time to the quality of tuna

Fig. 1 shows that the longer the contact time, the lesser the total of aerobic mesophyll bacteria. Shortly after immersion, the total number of aerobic mesophyll bacteria for 120 minutes' contact is log 6.45 CFU/gram. The value is less when compared with the total mesophyll aerobic for contact time of 40 and 80 minutes which are log 6.47 CFU/g and log 6.46 CFU/g. At the end of storage period (hour-168), the 120 minutes' contact duration produces the best quality of tuna with total Aerobic Mesophyll Bacteria is log 8.34 CFU/g. The total elimination graph of the Aerobic Mesophyll Bacteria shortly after the sample was soaked with ozonated water can be seen in Fig. 2.

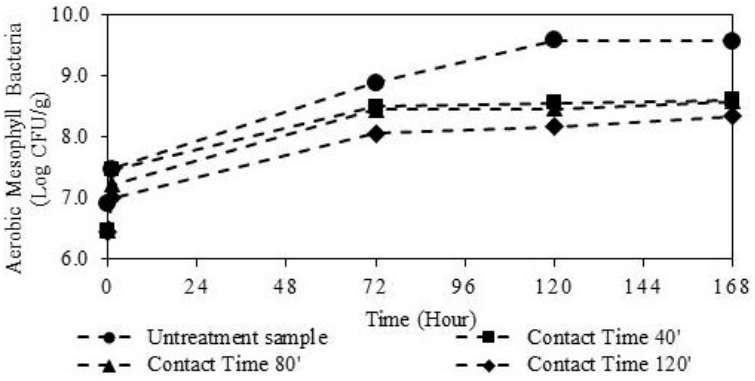

Figure 1. The total of aerobic mesophyll bacteria in tuna fish for the 168 hours' storage period on contact time variation (contact temperature: 25°C, ozone dose: 0.3 mg/L).

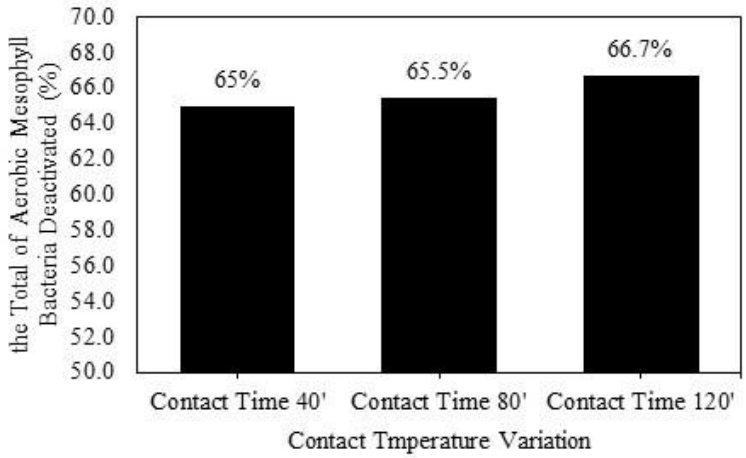

Figure 2. The Total of aerobic mesophyll bacteria deactivated after the immersion process at various contact time of ozonated water (contact temperature: 25°C, ozone dose: 0.3 mg/L).

In Fig. 2, the highest elimination rate of Aerobic Mesophyll Bacteria was found in 120 minutes' contact duration which is by 66.7%, So, it can be concluded that the longer the contact time, the higher the ability of the ozonated later to deactivate the bacteria.

Ozonated water of 120 minutes' contact time effected to reduce log 1.06 CFU/g aerobic mesophyll bacteria on tuna treated with 0.3 mg/L ozone dose. For comparison, ozone's antimicrobial efficacy when applied on beef treated with 2.3 mg/L aqueous ozone, reductions of only log 1.3 CFU/g [5].

3.2. pH Value

In Fig. 3, in general the pH of tuna has increased over time along with the time of the storage. This is because during fish storage, a protein decay process by bacteria occurs. This process produces alkaline compounds such as ammonia and trimethylamine and other basic volatile compounds which causes the fish pH to be increased [6] Furthermore, the accumulation of these volatile base compounds causes the meat of the fish to become more alkaline thus increasing its pH level [7].

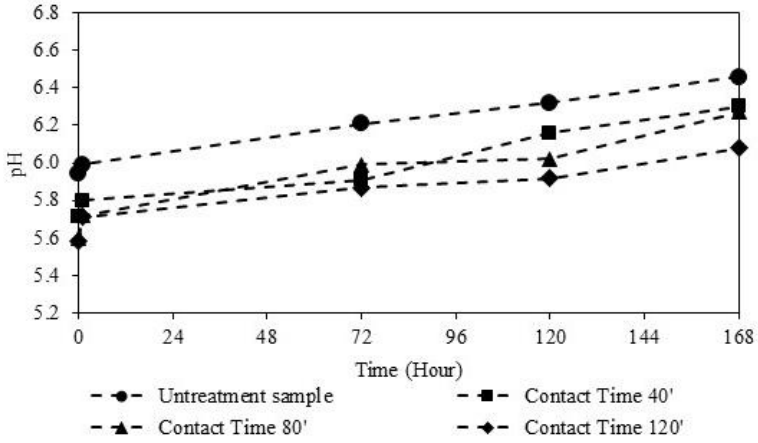

Figure 3. The changes of pH on the tuna fish for 168 hours' storage period on contact time variation (contact temperature: 25°C, ozone dose: 0.3 mg/L).

The highest increase of the pH level for treatment samples is at 168 hours' storage (7 days) is on the treatment of contact time of 40 minutes. The tuna pH level at 168 hours' storage at 40 minutes is at 6.30. Then the value is followed by contact time treatment of 80 minutes and 120 minutes with a pH level of 6.27 and 6.08. Therefore, it can be concluded that the longer the contact time of the ozonated water to the tuna, the increase of pH level in the tuna during the storage process will be more controlled.

3.2.1. Water Content. In Fig. 4, the water content of each sample increases with the length of storage as the effect of bacterial metabolism that produces CO_2 and water vapour [8]. The samples were treated with treatment of 40 minutes, followed by contact time of 80 minutes, and 120 minutes by 74.92%, 74.67% and 74.64% in the seventh day of the storage than the previous period in the first day of storage at 0, 73.68%, 73.45% and 73.44%. From Fig. 4, it can be seen that the longer the contact time of ozonated to the tuna then the increase in the water content during the storage period will be more controlled. This is in accordance to the Total Aerobic Mesophyll Bacteria from the fish in that minute is also the lowest, so the result of bacterial oxidation is in the form of low accumulated water.

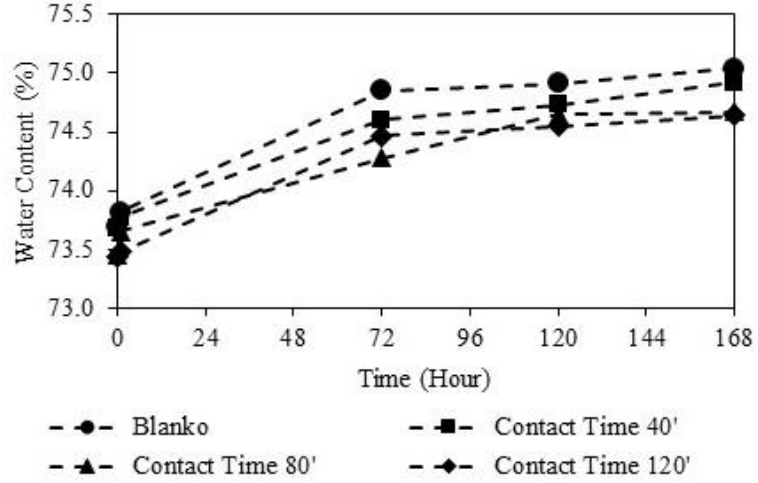

Figure 4. The changes of water content on the tuna fish for the 168 hours' storage period of contact time variation (contact temperature: 25°C, ozone dose: 0.3 mg/L).

3.2.2. Protein. Based on Fig. 5, the protein content of tuna decreases shortly after the fish is immersed in the ozonated water. The most declines happen in the contact temperature at 120 minutes which result in decreases of 2.25%. This is because ozone in the form of free radicals is a powerful and highly reactive oxidizer that can cause changes in the amino acid strain so that it can cause the occurrence of protein damage in the tuna fish. So, the longer the ozone reacts, the higher the protein damage that occurs shortly after the fish is contacted with the ozonated.

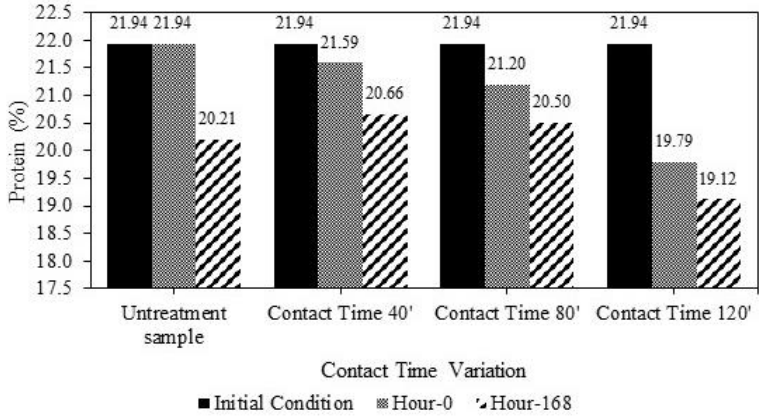

Figure 5. The protein content of the tuna fish at hours 0 and 168 on variations of contact time (contact temperature: 25°C, ozone dose: 0.3 mg/L).

In Fig. 5, in general the protein content decreased after 168 hours (7 days) of storage. This is because the protein will experience denaturation. Denaturation of proteins can be caused by bacterial activity found in fish through the decomposition process [9]. Denaturation of proteins during the 7-day storage is the highest in the treatment of 40 minutes' contact time with protein denaturation percentage of 0.93%. However, that number is lower than the blank (untreated tuna) which denaturation of 1.73%. This suggests that the ozonation process has a real effect to keep the protein from being denatured less during storage process.

3.3. The Effect of Contact Temperature to The Quality of Tuna

3.3.1. The Total of Aerobic Mesophyll Bacteria. In Fig. 6, it is known that the Total Aerobic Mesophyll Bacteria at the incubator temperature is the highest compared with the treatment at other temperatures. This is because the temperature at the incubator of 37°C is the optimum temperature for the growth of aerobic mesophyll bacteria. Total aerobic mesophyll bacteria at the incubator temperature after immersion process was reached to Log 6.93 CFU/g

Fig. 7, shown the percentage of the elimination of aerobic mesophyll bacteria shortly after the sample is contacted with the ozonated water on various temperature; room temperature (25°C), refrigerator temperature (8°C) and incubator temperature (37°C). It can be seen that the ability to eliminate the bacteria with the highest number is when the sample is contacted with the ozonated water at the temperature of refrigerator (8°C) which is at 91.20%. At the lower ozone particle was difficult to decompose. So, ozone concentration in water solution was still high and ability to eliminate a lot of bacteria. Therefore, it can be concluded that the lower the temperature of the treatment the higher the ability to eliminate bacteria will be.

Ozonated water of 120 minutes' contact time and 8°C contact temperature effected to reduce log 1.06 CFU/g aerobic mesophyll bacteria on tuna treated with 0.3 mg/L ozone dose. For comparison, ozone's antimicrobial efficacy when applied on mussel flesh treated with 1 mg/L aqueous ozone at 90 minutes of ozonation treatment process, reduction in total count by 0.7 log cfu/g was achieved by ozonation (day 0 of storage) [10].

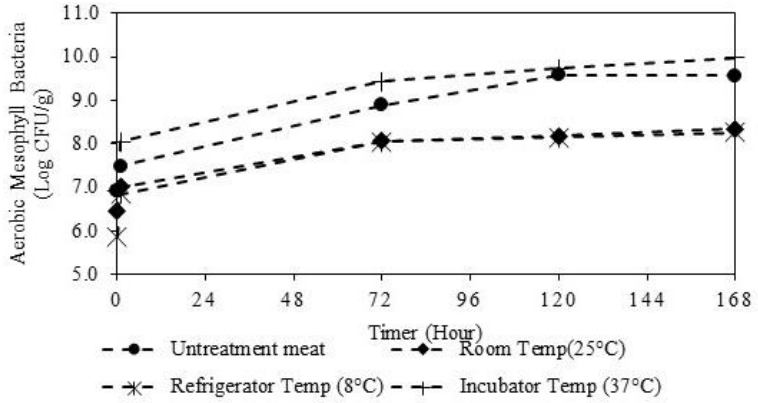

Figure 6. The total of aerobic mesophyll bacteria in tuna fish for the 168 hours' storage period on contact temperature variation (ozone dose: 0.3 mg/L).

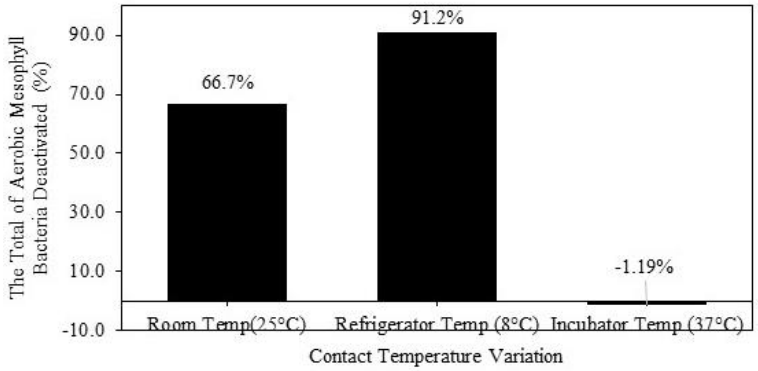

Figure 7. The total of aerobic mesophyll bacteria deactivated after the immersion process of the tuna fish with ozonated water on various contact temperature (ozone dose: 0.3 mg/L).

3.3.2. pH Value. Fig. 8, it is known that the pH level of the tuna fish during the storage period is on its lowest in the refrigerator temperature treatment (8°C). As is known in the previous points the lower the temperature causes the growth of bacteria will be inhibited. Bacterial content and low water content leads to slow protein decomposition. Therefore, the result of the accumulated base volatile amount becomes low so that the pH level on the refrigerator (8°C) is lower.

The highest increase of the pH level at 168 hours' storage (7 days) is on the treatment of contact temperature of incubator temperature (37°C). The tuna pH level at 0 day (after immersion) at incubator temperature (37°C) is at 5.91. Then the value is followed by contact Temperature treatment of room (25°C) and refrigerator (8°C) with a pH level of 5.58 and 5.48. Therefore, it can be concluded that the lower the contact temperature of the ozonated water to the tuna the increase of pH level in the tuna after immersion process and during the storage process will be more controlled.

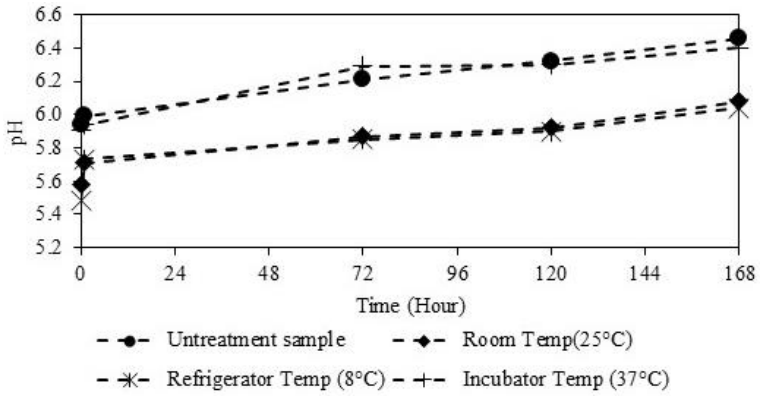

Figure 8. The change of the pH level on the tuna fish for 168 hours' storage period on variation of the contact temperature (ozone dose: 0.3 mg/L).

3.3.3. Water Content. Fig. 9, it is known that in the sample subjected to the ozonation treatment, the highest water content is at the incubator temperature (37°C), then the room temperature (25°C) and refrigerator temperature (8°C), i.e. 73.49%, 73,44%, and 73.23% shortly after the fish was contacted with ozonated water and 74.97%, 74.64% and 74.40% respectively in storage 7 days. Thus, it can be concluded that the lower contact temperature, the increase in water content will be more controlled.

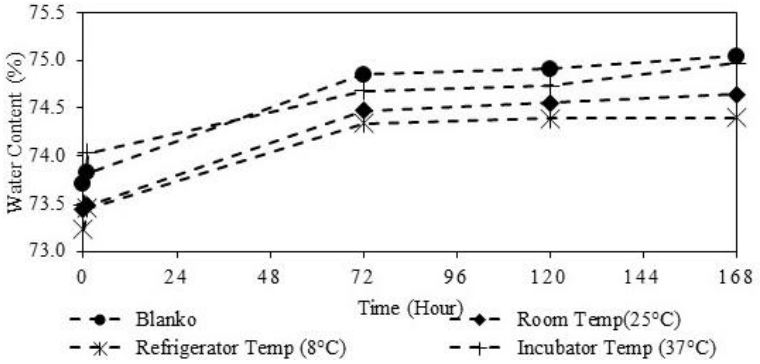

Figure 9. Changes in tuna water content for 168 hours' storage period on various of contact temperature (ozone dose: 0.3 mg/L).

3.3.4. Protein. In figure 10, it is known that the protein content decreases in each treatment against the storage time. moreover, in Fig. 10, it is found that shortly after the fish immersion process, the decrease in protein occurs at most in room temperature variation (25°C) as a result of bacterial activity and protein oxidation by the ozone. Denaturation of proteins during the 7-day storage is the highest in the treatment of incubator temperature (37°C) with protein denaturation percentage of 1.79%. Moreover, the higher temperature while immersion process made the protein denaturation was higher after 7 days of storage.

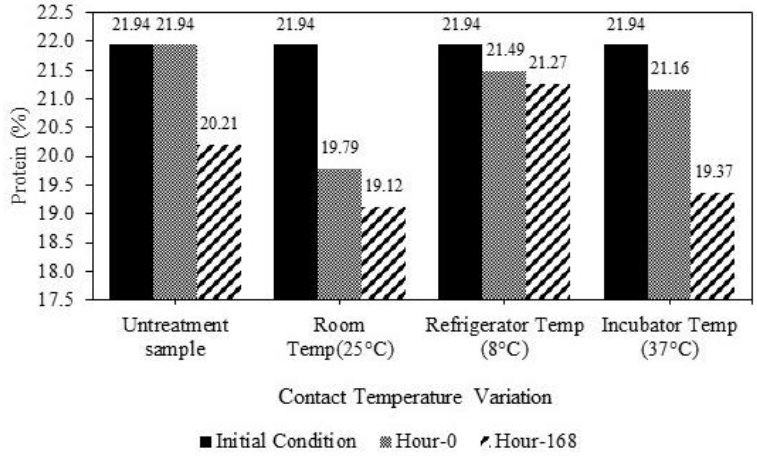

Figure 10. The protein content of the tuna fish at hours 0 and 168 on contact temperature variations (ozone dose: 0.3 mg/L).

3.4. The Effect of Ozone Dosage to The Quality of Tuna

3.4.1. The total of aerobic mesophyll bacteria. In Fig. 11, it is concluded that a high ozone solubility can increase the ability of the ozonated water in eliminating the existing bacteria. This is in accordance with the nature of ozone that can act as a disinfectant. So, the higher the ozone solubility or the ozone concentration, the ability to disinfect will be higher also.

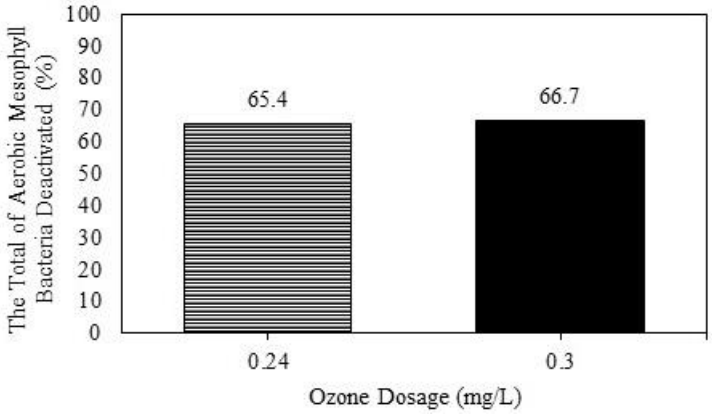

Figure 11. The total of aerobic mesophyll bacteria deactivated after the immersion of the tuna fish with ozonated water on variation of ozone dosage (contact temperature: 25°C).

3.4.2. pH Value. In Fig. 12, an ozone dose of 0.3 mg/L has a greater ability to suppress the pH level in the tuna when compared with an ozone dose of 0.24 mg/L. the increasing of the pH in 7 days' storage at 0.3 mg/L ozone dose is of 0.50 in which the number is smaller than the increase of pH level at the dose of 0.24 mg/L which is of 0.52. This is of course related to the low content of aerobic mesophyll bacteria when there is a larger ozone doses that cause the protein denaturation by bacteria to be lower. This causes that the total accumulation of the volatile bases such as ammonia and trimethylamine to be low, so then the pH level will be lower.

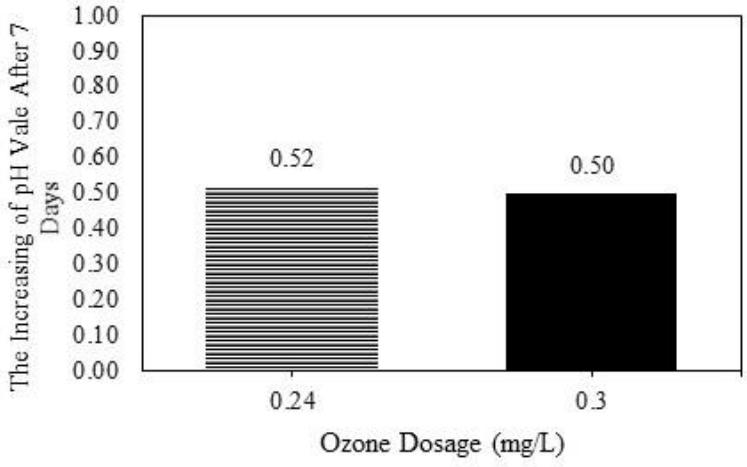

Figure 12. The increasing of the pH level of the samples after 7 days of storage on variation of ozone dosage (contact temperature: 25°C).

3.4.3. Water Content. In Fig. 13, it was concluded that the ozone level of 0.3 mg/L had the ability to suppress deeper water content of the tuna when compared with ozone levels of 0.24 mg/L. It is characterized by an increase in water content at a dose of 0.3 mg/L of 1.2%, whereas at 0.24 mg/L ozone dose increased the water levels by 2.91%. This is because the number of bacteria that is successfully eliminated by 0.3 mg/L ozone level is higher when compared with the dose of 0.24 mg/L so that the total bacteria will be lower and the result of bacterial oxidation in the form of accumulated water will be lower.

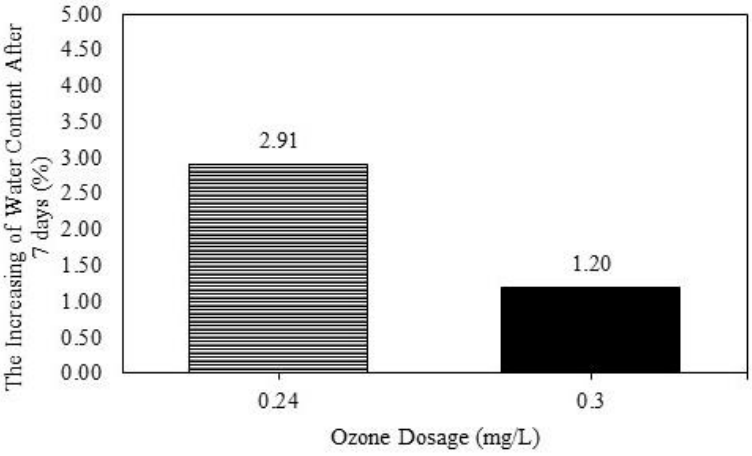

Figure 13. Escalation in the water content of the samples after 7 days of storage on variation of ozone dosage (contact temperature: 25°C).

3.4.4. Protein. In Fig. 14, it is known that with larger ozone doses, the protein denaturation will be lower. This is related to the protein denaturation activity which done by the bacteria. The higher the total of the bacteria will lead to higher protein denaturation. In the previous points it was found that the elimination of Total Aerobic Mesophyll Bacteria at ozone doses of 0.30 mg/L was higher when compared to the ozone dose of 0.24 mg/L.

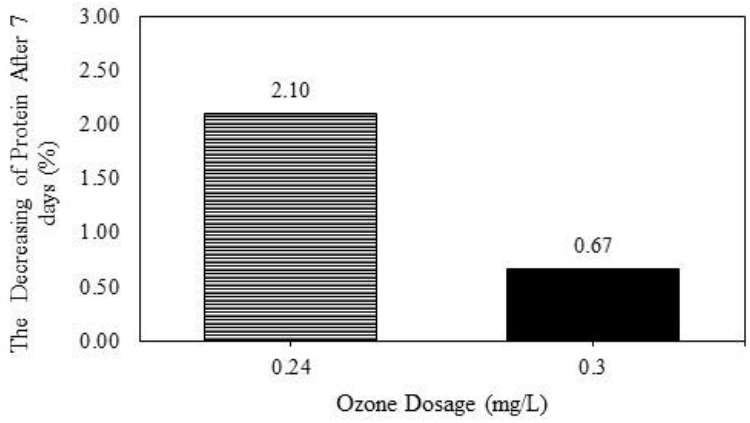

Figure 14. The decline in the protein level of the sample after 7 days of storage on variation of ozone dosage (contact temperature: 25°C).

4. Conclusion

Ozonated water with a concentration of 0.30 mg/L is effective to deactivate the total aerobic mesophyll bacteria, decrease the pH level, decrease the water content, shortly after the ozonation process takes place. The longer the contact time, the lower the contact temperature and the higher the ozone dosage in the ozonated water will result to a more effective result in maintaining the quality of the tuna.

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