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The effect of exposure time and water replacement in the application of ozonated water to maintain the quality of tuna

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Abstract. Fish was one of the common protein sources consumed by the Indonesian people. One type of fish that was widely consumed was tuna. Tuna contains protein as much as 18–30%. With high protein and water content, tuna was a highly perishable commodity. Therefore, the handling of tuna needs to be done to maintain its quality. The use of ozonated water for this issue was promising because ozone was a strong oxidant and does not produce toxic residues. This study used ozonated water to maintain the quality of tuna. The objective of this study was to evaluate the effect of exposure time and water replacement of the ozonated water on the quality of tuna, measured by the parameters of Total Mesophyll Aerobic Bacteria (TMAB), pH, water content, and protein content. Exposure time of 40, 80, and 120 minutes can deactivate the TMAB by 4.94, 60.99, and 65.43%, respectively. Meanwhile, water replacement every 60 and 40 minutes within 120 minutes can deactivate more TMAB, which values 82.59 and 89.38%. Exposure time of 120 minutes can also decrease the pH by 0.12, lower the water content by 0.63%, and decrease the protein content by 0.80%. Water replacement every 40 minutes within 120 minutes can decrease the pH by 0.08, lower the water content by 1.44%, and decrease the protein content by 2.20%.

Keywords: Ozone, pH, Protein, TMAB, Tuna, Water Content

1. Introduction

Fish contains protein as much as 18-30% and amino acids contained in it patterned close to the needs of amino acids in the human body. This makes the nutritional content of fish easy to be absorbed by the human body. With high protein and water content, fish was a highly perishable commodity. Therefore, the pre-treatment of fish needs to be done in order to get to the hands of consumers in good quality.

The dominant microorganisms that play an important role in the quality decrease of the fish was bacteria. Bacteria convert protein to amino acid during decomposition process. Amino acids were deaminated and decarboxylated which form more simple compounds by specific enzymes from microbes. The most commonly used decomposition products for assessing freshness of fish were the simplest ammonia and monoamine (methylamine, dimethylamine, trimethylamine) better known as basic volatile nitrogen. The accumulation of a volatile nitrogen base also caused the meat of the fish to become more alkaline thus increasing its pH value [1].

The use of ozone to maintain the quality of fish was one of the promising food preservation technologies. This was because ozone was a powerful and effective oxidizing agent to organic or inorganic pollutants. In addition, ozone does not produce toxic residues to the environment [2].

Research on fish preservation using ozonation technique has been conducted by previous researchers. Spraying ozone water can reduce the initial population of aerobic bacteria and *L. innocua* on salmon



fillets stored at 4°C [3]. The addition of ozone with a concentration of 0.1 mg/L in slurry ice was also able to slow the growth of grouper bacteria up to 0.24 log units [4]. This research aims to use immersion method to provide more comprehensive contact between tuna meat and ozonated water, than those of sprayed-water and ice slurry. With comprehensive contact, the number of deactivated bacteria was expected to be higher.

2. Methodology

2.1. Raw Materials

Material used in this research were tuna meat and ozonated water. Tuna was cut into pieces with a mass of 100 grams for each variation. Ozonated water was produced using a middle-scale ozonator with the capacity of 523.67 mg ozone/hour. Ozone was injected in water for 45 minutes to achieve the concentrations of 0.2404 mg/L.

2.2. Ozone Solubility in Water Test

Ozone solubility in water test was conducted using Merck Ozone Test Kit No. 100607 and read photometrically by spectrophotometer. Testing of ozone solubility in water was carried out with three different treatment variations. Treatment 1 used a circular semi-batch system and with the help of a stone bubbler, the volume of water used was 2 L. Treatment 2 used a circular and venturi injector semi-batch system, the system was equipped with a 4 L volume tank. Treatment 3 used the semi-batch system with circulation and venturi injector assistance, the system was not equipped with tank and used 4 L water.

2.3. Characterization Test of Raw Materials

This test was conducted to determine the quality of water before ozonated and tuna before and after soaked in ozonated water. The quality of tuna was measured by the parameters of Total Mesophyll Aerobic Bacteria (TMAB), pH, water content, and protein content. TMAB test was conducted using Total Plate Count (TPC) method based on SNI 2332.3: 2015. Procedure of pH measurement was based on SNI 01-2891-1992. 3 grams of tuna was first diluted with 15 mL of distilled water. Water content test was using Oven Method based on SNI 01-2891-1992. Protein content test was conducted using Kjeldahl Semi-micro Method based on SNI 01-2891-1992.

2.4. Ozone Treatment of Tuna

The treatment of ozonated water to tuna was done to know the effect of ozonated water treatment to the quality of tuna. The ozonated water treatment was carried out with two variations, namely variation of exposure time and variation of water replacement.

2.4.1. The Effect of Exposure Time. This part was done by soaking 100 grams of tuna with 75 mL of ozonated water for exposure time of 40, 80, or 120 minutes. After the exposure time was over, the water was discharged. The tuna was then stored at 8°C for 168 hours.

2.4.2. The Effect of Water Replacement. This variation was done by soaking 100 grams of tuna with 75 mL of ozonated water for the exposure time of 120 minutes. During the time, replacement of water was done 3 times every 40 minutes, 2 times every 60 minutes, or 1 time every 120 minutes. After the exposure time was over, the water was discharged. The tuna was then stored at 8°C for 168 hours.

Sampling was done at hour 0, 1, 72, 120, and 168 for final characterization of tuna. Characterization was performed based on microbiological assessment by determining the Total Mesophyll Aerobic Bacteria (TMAB). In addition, quality assessments based on pH parameters, water content, and protein content were also performed.

3. Results and Discussion

3.1. Ozone Solubility in Water

Fig. 1 shows that the ability to dissolve ozone by a system with circulation (Treatment 2 and 3) was lower compared with a system without circulation (Treatment 1), although it had been assisted by the cavitation process by venturi injector. This was because the system with the circulation involves the pump in the system. The use of pumps generates additional heat which keeps the system temperature higher and the ozone becomes easier to decompose during the process.

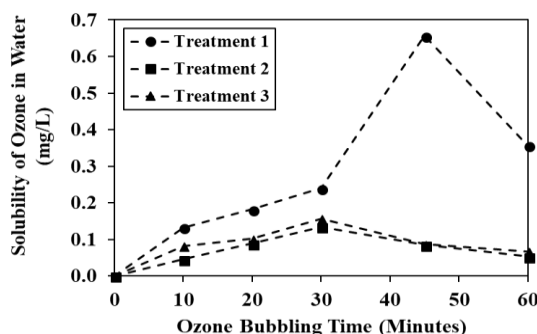


Figure 1. Ozone solubility in water for various dissolving procedures.

3.2. Initial Characteristic of Raw Materials

Characteristics of water before being ozonated and tuna before being soaked in ozonated water.

3.2.1. *Water.* Initial characteristics of water before ozonated is shown in table 1.

Table 1. Initial characteristics of water.

Parameter	Value	Standard
<i>Escherichia coli</i>	0.26 log(CFU/mL)	0.00 log(CFU/mL) (Government Regulation No. 82 2001)
Total Mesophyll Aerobic Bacteria (TMAB)	4.45 log(CFU/mL)	-
pH	5.06	6.00 – 9.00 (Government Regulation No. 82 2001)

The results indicate that the water quality was outside the standard value from microbiological and chemical aspects. The water contains 4.45 log(CFU/mL) of Total Mesophyll Aerobic Bacteria (TMAB), these numbers were expected to increase the burden of the bacterial deactivation process by ozone. Therefore, ozone does not only deactivate bacteria found in the tuna meat, but also those which was initially found in the water.

3.2.2. *Tuna.* Initial characteristics of tuna before soaked in ozonated water is shown in table 2. These parameters will be compared with the final characteristics of tuna after ozone treatment. The amount of Total Mesophyll Aerobic Bacteria (TMAB) in tuna was above the quality standard, then higher number of bacteria in the end of storage period was expected.

Table 2. Initial characteristics of tuna.

Parameter	Value	Standard
Total Mesophyll Aerobic Bacteria (TMAB)	7.61 log(CFU/gram)	5.70 log(CFU/gram) (SNI 01-2729: 2006)
pH	5.82	< 6.80 (Huss, 1988)
Water content	73.41 %	71.00 – 73.70 %
Protein content	22.87 %	-

3.3. The Effect of Exposure Time to the Quality of Tuna

The effect of exposure time was done by varying the time of contact between tuna and ozonated water for 40, 80, and 120 minutes. The ozonated water used has dissolved ozone content of 0.2404 mg/L.

3.3.1. Total Mesophyll Aerobic Bacteria (TMAB). Fig. 2 shows the percentage of TMAB successfully deactivated by ozonated water for the variations of exposure time.

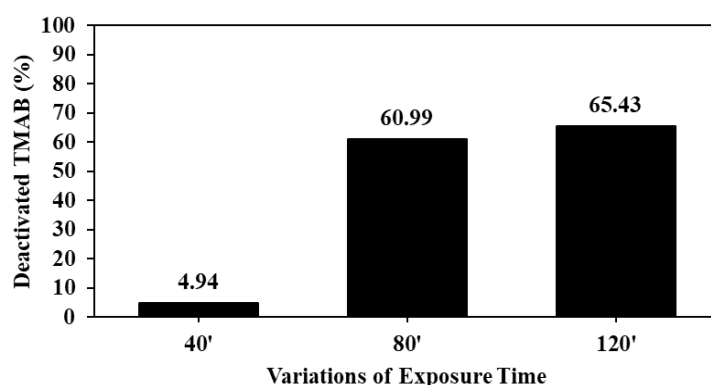


Figure 2. The amount of deactivated TMAB after the contact with ozonated water for the variations of exposure time (ozone dose: 0.2404 mg/L).

Ozone was a dominant antibacterial component [5]. Ozone can oxidize the components of bacterial cells including unsaturated fatty acids, glycoproteins, and glycolipids resulting in leakage of bacterial cell contents that cause lysis [6].

Fig. 2 indicates that the longer the contact between ozonated water and the tuna, the more opportunities for ozone to deactivate the bacteria. Chen *et al.* [7] stated that ozonated water concentration and treatment time were taken as the main factors related to the trend in bacteria reduction in oyster. This was also similar to the research by Aguayo that the use of 3 minutes immersion time was more effective than the 1 minutes in reducing bacteria on tomato slices, possibly because more time allows the O₃ react with other components [8].

Variations of exposure time of 40 minutes resulted in a relatively low bacterial deactivation compared with the exposure time of 80 and 120 minutes. This was due to the possibility of oxidation reactions between ozone and initial bacteria coming from water. In Section 3.2.1, the test results on the number of TMAB in the water indicate a substantial amount of 0.26 log(CFU/mL). For the exposure time of 120 min, the number deactivated bacteria gives the best result compared to the other variations.

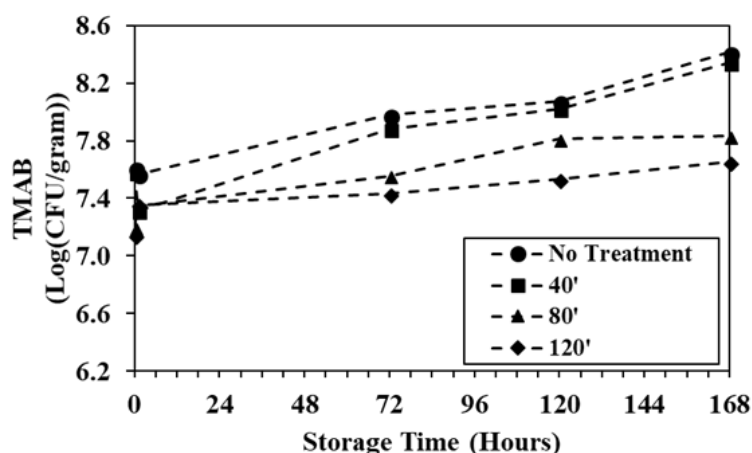


Figure 3. The amount of TMAB during 168 hours of storage in the variations of exposure time (ozone dose: 0.2404 mg/L).

After contact with the water, the tuna was stored in the refrigerator for 168 hours. Fig. 3 shows the growth of TMAB amount during storage period in log(CFU/gram). Fig. 3 shows that bacteria continue to grow during storage period. This indicates the occurrence of the process of decomposition in fish.

The longer the exposure time the greater the number of bacteria deactivated after the contact and the less number of bacteria present at the end of the storage period. 120 minutes of exposure time can deactivate TMAB 60.49% more than the exposure time of 40 minutes. At the end of storage period (168 hours), the TMAB amount for 120 minutes' exposure time was 7.65 log(CFU/gram), while for the exposure time of 40 minutes was 8.34 log(CFU/gram).

3.3.2. pH. Fig. 4 shows pH changes before the contact, after the contact, and after 168 hours of storage for the variations of exposure time.

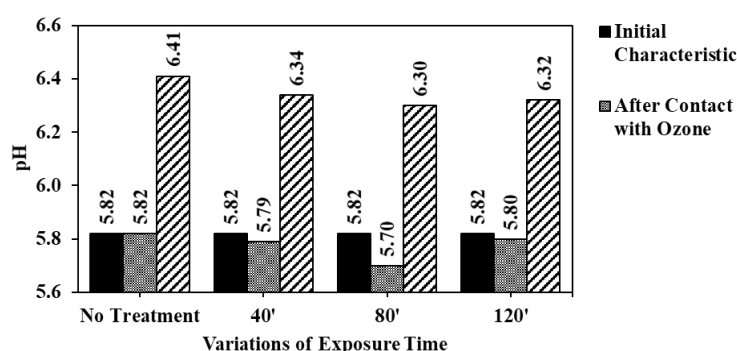


Figure 4. pH level changes in the variations of exposure time (ozone dose: 0.2404 mg/L).

Fig. 4 shows that there was a decrease in pH after the contact. The value was larger for treated sample than for no treated sample, which might be caused by the generation of weakly acidic H_2O_2 by ozone in water [9]. The longer the exposure time, the greater the pH decrease occurs in the tuna after the contact. Exposure time of 80 minutes can decrease the pH by 0.12, while the pH decrease by exposure time of 40 minutes was 0.03. This was because the longer the contact between the ozonated water with the tuna, the more chance for ozone to react with the protein in the fish.

After being stored for 168 hours, the pH has increased. This increase in pH was caused by the emergence of basic compounds such as ammonia, trimethylamine, and other volatile compounds. The

accumulation of these volatile base compounds caused the meat of the fish to become more alkaline thus increasing its pH value [1, 10, 11]. Based on the pH characteristics, tuna was still safe to consume because the pH level was less than 6.8 [12].

3.3.3. Water Content. For the variations of exposure time, the water content contained in tuna for 168 hours of storage after being soaked with ozonated water is shown in Fig. 5.

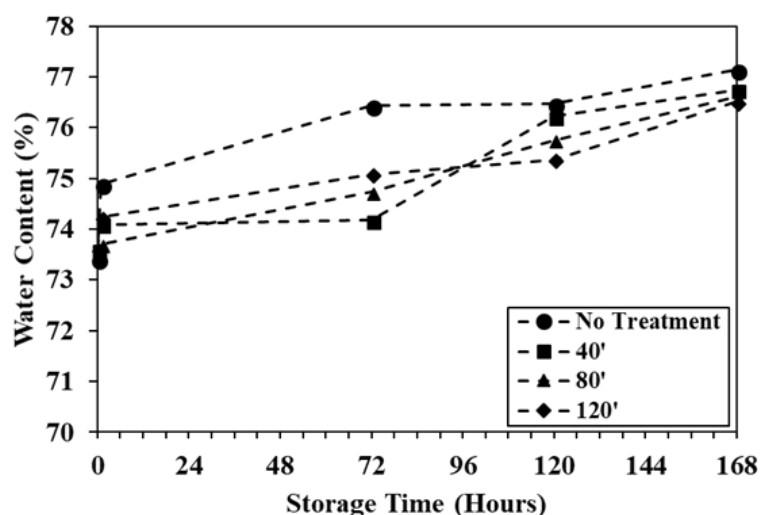


Figure 5. Water content changes during 168 hours of storage in the variations of exposure time (ozone dose: 0.2404 mg/L).

Fig. 5 indicates that there was an increase in water content during the storage period of 168 hours. Contamination and early growth of microbes in fish were preceded by aerobic bacteria [13]. Aerobic bacteria perform metabolism by converting carbohydrates into CO_2 and H_2O [14]. This was the cause of the increase of water content during storage.

Based on Fig. 5 it can be said that the longer the exposure time, the lower the water content in the tuna during the storage period. The exposure time of 120 minutes resulted in water content 0.63% lower than the blank, while the exposure time of 40 minutes was 0.39% lower at the end of shelf life (168 hours). This was related to the number of TMAB, where exposure time of 120 minutes was the most effective in keeping bacterial growth, so bacterial activity was insignificant and makes the lowest water content produced.

3.3.4. Protein Content. The changes in protein content of tuna for variations of exposure time were shown in Fig. 6. Even though insignificant, Fig. 6 shows that the longer the exposure time, the greater the decrease of protein content in tuna after contacted with ozonated water. The percentage decrease in protein content in the exposure time of 120 minutes was 0.80%, greater than the decrease by the exposure time of 40 minutes which was 0.20%. This indicates that ozone can damage proteins. Secondary structures of proteins linked to hydrogen bonding characteristics between $\text{C}=\text{O}$ and $\text{N}-\text{H}$ groups on polypeptides. This $\text{C}=\text{O}$ bond was sensitive to ozone attacks [15]. During 168 hours of storage, protein also decreased due to the activity of bacteria that broke down proteins into alkaline compounds such as ammonia [1].

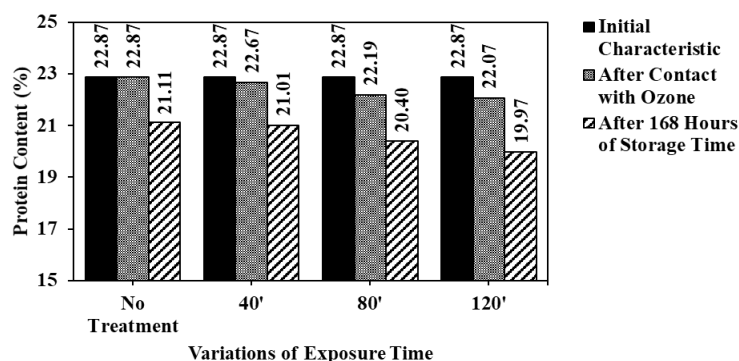


Figure 6. Protein content changes in the variations of exposure time (ozone dose: 0.2404 mg/L).

3.4. The Effect of Water Replacement to the Quality of Tuna

The effect of water replacement was done with total duration of 120 minutes. The concentrations of ozonated water differed in each time the water replacement was done, because the ozonator performance decrease after repeated use (shown in table 3).

Table 3. Ozonated water concentrations in each water replacement.

Variations of Water Replacement	Ozonated Water Concentrations in Each Water Replacement (mg/L)		
	1 st Replacement	2 nd Replacement	3 rd Replacement
120' x 1	0.2404	-	-
60' x 2	0.2404	0.1440	-
40' x 3	0.2404	0.1992	0.1462

3.4.1. *Total Mesophyll Aerobic Bacteria (TMAB)*. Fig. 7 shows the percentage of TMAB successfully deactivated by ozonated water for the variations of water replacement.

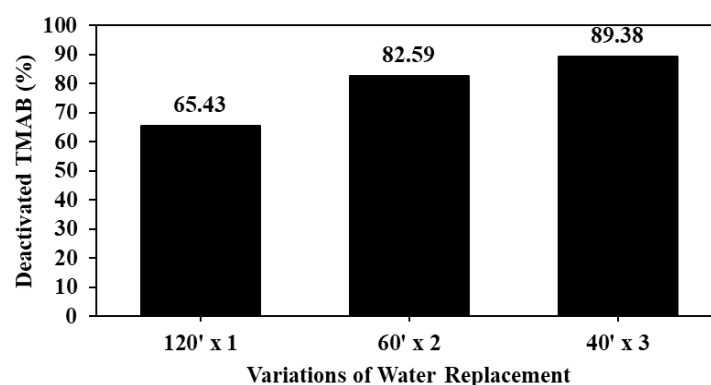


Figure 7. The amount of deactivated TMAB after the contact with ozonated water for the variations of water replacement (ozone dose: 0.2404 mg/L).

Fig. 7 shows that the 3 times replacement of water reduced the number of bacteria by 89.38%, more effective than the 1 time and 2 times replacement, respectively 65.43% and 82.59%. This was caused

by the replacement of water increased the amount of ozone involved in the oxidation reaction with the bacteria, which results to more bacteria were deactivated.

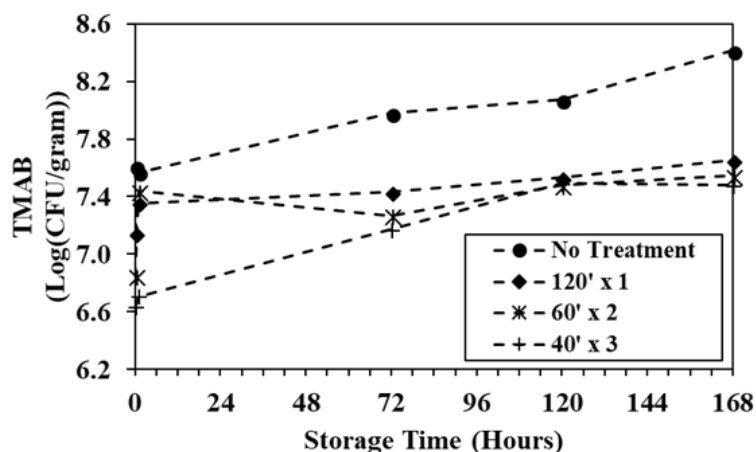


Figure 8. The amount of TMAB during 168 hours of storage in the variations of water replacement (ozone dose: 0.2404 mg/L).

Fig. 8 shows the growth of TMAB during storage period (168 hours) in log(CFU/gram). The more frequent the water replacement is, the greater the number of TMAB deactivated after the contact and the less number of TMAB present at the end of the storage period. 3 times replacement of water can deactivate TMAB 23,95% more than the variation with 1-time replacement. At the end of the storage period (168 hours), the TMAB amount for the variation of 3 times replacement was 7.48 log(CFU/gram), while for the variation of 1-time replacement was 7.65 log(CFU/gram).

3.4.2. *pH*. The pH changes in tuna for the variations of water replacement were shown in Fig. 9.

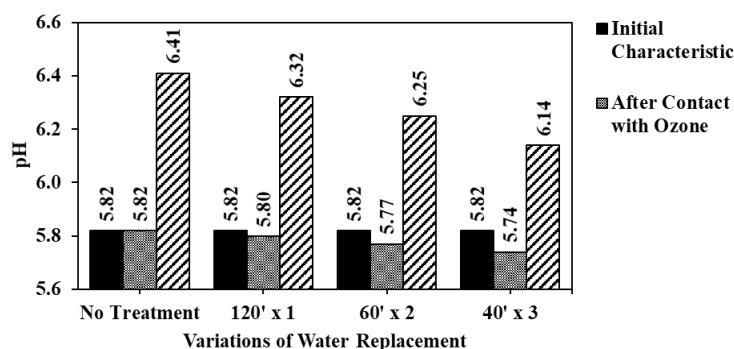


Figure 9. pH level changes in the variations of water replacement (ozone dose: 0.2404 mg/L).

Fig. 9 shows that the more frequent the replacement of water was done, the greater the pH decrease that occurred in the tuna after the contact. Replacement of water 3 times can decrease the pH by 0,08, while the decrease of pH at variation of 1-time replacement was 0.02. This was because the more frequent replacement of water was done, the more ozone reacts with the protein in the fish, so the more acidic compounds were produced.

The increase in pH during storage was lower on variations with the water more replaced. This was related to the lower number of bacteria, so the decomposition activity of proteins into alkaline compounds was also less. Based on the pH characteristics, tuna was still safe to consume because the pH level was less than 6.8 [12].

3.4.3. Water Content. For the variations of water replacement, the water content contained in tuna after the contact with ozonated water and during 168 hours of storage is shown in Fig. 10.

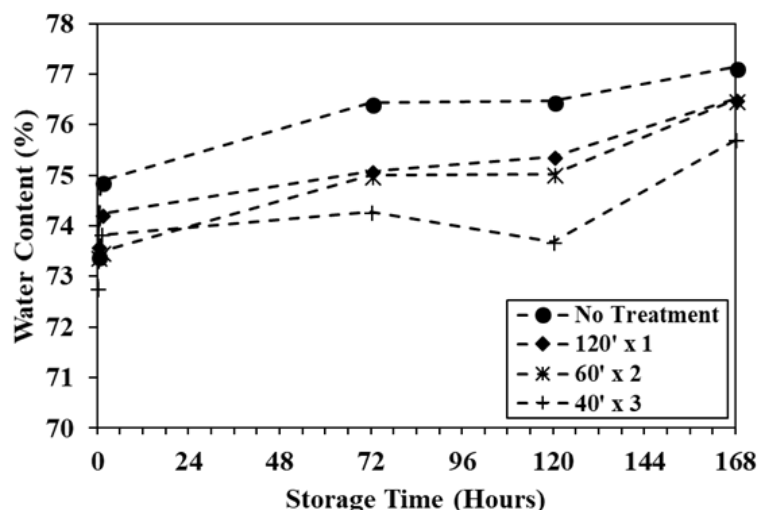


Figure 10. Water content changes during 168 hours of storage in the variations of water replacement (ozone dose: 0.2404 mg/L).

Based on Fig. 10 it can be seen that the more frequent the replacement of water was done, the lower the water content in the tuna. This was because the less number of bacteria, so the water resulted from bacterial metabolism activity was also less. Replacement of water 3 times resulted in water content of 1.44% lower than the blank, while the variation of 1-time replacement were lower by 0.63% at end of the storage period. This was related to the number of TMAB, where the 3 times replacement was the most effective in restraining bacterial growth, so bacterial activity was not significant and makes the lowest results in water content.

3.4.4. Protein Content. Changes in protein content in tuna after soaked in ozonated water for the variations of water replacement were shown in Fig. 11.

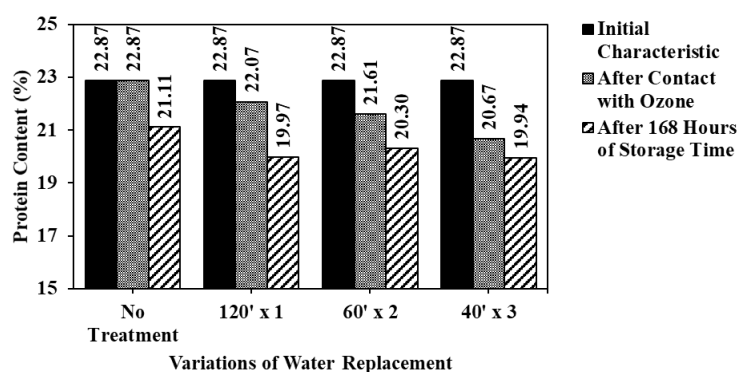


Figure 11. Protein content changes in the variations of water replacement (ozone dose: 0.2404 mg/L).

At 1-time replacement variation, the decrease in protein content due to oxidation by ozone was lower than that of bacterial activity. After the contact with ozonated water, the protein content decreased by 0.80%, while during the storage period the protein content decreased by 2.10%. This was because the water used in ozonation was in acidic conditions (pH 5.06), so the ozone was more stable and does not

easily decompose to OH^\bullet . Ozone that works in molecular form was more selective and has a lower reaction rate constant than the form of OH^\bullet .

However, when more water replacements were done, the decrease in protein content due to oxidation by ozone becomes greater. At 2 times replacement variation, the protein content decreased by 1.26%. This value approaches the amount of protein decreased after the shelf life, which was 1.31%. Then, in the 3 times water replacement, the decrease of the protein content due to the ozonated water (2.20%) becomes greater than the decrease due to storage factor (0.73%). This was due to the more water replacement, the more ozone molecules that work to oxidize proteins, even though ozone was not in its radical form.

4. Conclusions

The longer the exposure time, the greater amount of TMAB became deactivated after tuna was soaked in ozonated water. Exposure time of 120 minutes can deactivate 65.43% of TMAB in tuna. Longer exposure time was also resulted in lower water content during storage and greater pH and protein decrease in tuna after contact with ozonated water.

The more frequent the water replacement is, the greater the number of TMAB deactivated after the contact with ozonated water. Variation of 3 times water replacement can deactivate 89.38% of TMAB. More frequent the water replacement was also resulted in lower water content during storage and greater pH and protein decrease in tuna after contact with ozonated water.

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