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FULL PAPER

Effect of different chilled storage method and ozone treatment on the quality of steamed whole blue swimming crab (*Portunus pelagicus*)

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Abstract

Pasteurized canned blue swimming crab (*Portunus pelagicus*) meat is an important export fisheries product from Indonesia. Ozone treatment in food is known to kill bacteria and other microorganism. The purpose of this study was to determine the effects of ozone treatment in chilled storage on the quality of steamed whole blue swimming crab. A total of four combinations of chilled storage with ozone treatment were tested: storage in cool box with ice (CBI), storage in cool box with ice and ozone treatment (CBI-O), storage in refrigerator with ozone treatment (REF-O), storage in refrigerator (REF). Steamed whole blue swimming crab were stored in each storage for 6 days. Melted ice was replaced every 12 hrs. Samples were taken every day for central temperature measurement and sensory analysis. Water holding capacity (WHC), total volatile base nitrogen (TVBN), moisture, pH, and total plate count (TPC) analysis were carried out every 3 days. The results showed that whole steamed blue swimming crab were rejected after 3 days in CBI, after 4 days in cool box with ozone treatment CBIO and after 6 days were still accepted in both refrigerated storages. Ozone treatment could prolong the freshness of steamed blue swimming crab but reduce the typical aroma.

1. Introduction

Blue swimming crab (*Portunus pelagicus*) is one of the important Indonesian fishery commodities and has a high nutritional content and it has a high selling value. Generally, blue swimming crab are traded in the form of canned blue swimming crab that have been pasteurized (Hutajulu *et al*., 2019). Blue swimming crab is a food that quickly declines in quality; therefore, it must be processed quickly and accurately. According to Jumiati and Zainuddin (2019), the decrease in the quality of blue swimming crab was caused by the activity of enzymes and bacteria contained in the blue swimming crab. The final process of processing crab is storing steamed and boiled crab meat that has been peeled, storage methods affect the quality and prevent the deterioration of the quality of the blue swimming crabs.

Steaming is the first step in processing blue swimming crab which aims to inhibit quality deterioration. The thermal process in blue swimming crabs has an influence on sensory and chemical characteristics such as TVBN, TPC, pH and water holding capacity. Dima *et al.* (2020) reported that the process of steaming blue swimming crab increased TVB-N and pH due to the absence of a diffusion medium. Furthermore, the WHC value in blue swimming crab decreased after the steaming process. This requires a food product preservation technology such as ozonation technology (Suharyono *et al*., 2011).

Ozone is known as oxygen which can react with the reduction of inorganic compounds and organic materials. According to Sachadyn-Król (2020), the application of

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ozonation in the food industry is used to extend shelf life so as to increase production profitability. In addition, ozone is used to decontaminate meat, seafood, poultry and vegetable products. According to Rijal and Nur (2015), the application of ozone to food products can eliminate fungi, protozoa, bacteria, and viruses. This can be seen from the success of ozonation in influencing rice characteristics such as moisture, carbohydrates, and fat with different irradiation times.

The addition of ozone during the storage process in foodstuffs has been widely carried out. Based on Melantina *et al*. (2022), ozone was used to preserve fresh fish so that it could slow down the decline in quality during storage and prolong the length of time for preservation. The longer the preservation time, the protein content would increase. The aim of this study was to determine the effects of ozone treatment in chilled storage on the quality of steamed whole blue swimming crab.

2. Materials and methods

2.1 Materials

This study used whole fresh blue swimming crab caught by fishermen in District of Tugu, Semarang. Whole fresh blue swimming crab immediately steamed after landed and then taken to the laboratory for storage treatments. The "deozone" ozone generator used was the product of PT. Dipo Technology Semarang with an ozone capacity of 120-130 grams/hr. The gas flow rate set in the range of 3–4 lpm using a "wiebrock" flowmeter. Two "Modena" refrigerator with a volume capacity of 137.5 liters and set at a temperature of 1-7°C were used for chilled storage treatment with refrigerator and two insulated plastic boxes with volume 112 L were used for chilled storage treatment with cool box and ice. In both cool boxes with ice treatments, the ratio of steamed crab with ice was 1:2, melted ice was replaced every 12 hrs. Digital thermometers were used to observe the temperature in each chilled storage treatment.

2.2 Experimental procedure

Fresh whole blue swimming crab were cleaned with water and steamed for approximately 30 mins. Steamed crab was cooled by aeration and drained. Steamed crab meat was stored in chilled storage with four treatments: refrigerator (REF), cool box with ice (CBI), cool box with ice and ozone (CBI-O) and refrigerator with ozone (REF-O) (Figure 1). Ozone delivery was carried out twice a day with an interval of every 12 hrs. The given ozone density ranges from 19–20 mg/l. Ozone density in the cooling chamber was the product of the ozone capacity and the gas flow rate, divided by the volume of the cold storage room (SNI 8759:2019, Badan Standardisasi Nasional (2019)). Quality assessment of crab meat were carried out at the 0, 1st, 2nd, 3rd, 4th, 5th, and 6th days of storage for central temperature of whole steamed crab, sensory value, pH and water holding capacity (WHC), while quality assessment for moisture content, total volatile base nitrogen (TVBN), and total plate count (TPC) were carried out at 0, 3rdand 6th days of storage.

Determination of ozone density in chilled storage rooms:

Cool box room dimensions =
$40\times40\times70$ cm = 112000 cm^3 = 112 l (1 m^3 = 1000 l)

Cool box room dimensions =
$60\times35\times36$ cm = 75600 cm^3 = 75.6 l

Ozone density in cold storage:

Generator capacity 1 (blue) = 120 g/hr (with built-in flow of 20 lpm)

Generator capacity 2 (light blue) = 132 g/hr (with built-in flow of 20 lpm)

Cold storage with cool box used generator 2 and cold storage with box used generator 1.

Density on cool box cold storage:

$$\text{Density} = \frac{\text{Capacity} \times \text{time}}{\text{Volume}}$$

$$\text{Density} = \frac{132\ \left(\frac{\text{g}}{\text{hr}}\right) \times 5\ \text{mins}}{112\ \text{l}}$$

$$\text{Density} = \frac{132\ \text{g} \times 5\ \text{mins}}{60\ \text{mins} \times 112\ \text{l}}$$

$$\text{Density} = 0.09821\ \frac{\text{g}}{\text{l}} = 98.21\ \text{mg/l}$$

Considering the default flow rate is 20 lpm, to get a density in the range of ±20, then 98.21 mg/l divided by 5 gives 19.64 mg/l, thus the required flow rate is 20 divided by 5 which is 4 lpm.

Density on cool box iced:

$$\text{Density} = \frac{120\ \left(\frac{\text{g}}{\text{hr}}\right) \times 5\ \text{mins}}{75.6\ \text{l}}$$

$$\text{Density} = \frac{120\ \text{g} \times 5\ \text{mins}}{60\ \text{mins} \times 75.6\ \text{l}}$$

$$\text{Density} = 0.132\ \frac{\text{g}}{\text{l}} = 132\ \text{mg/l}$$

Considering the default flow rate was 20 lpm, to get a density in the range of ±20, then 132 mg/l divided by 6.67 gives 19.79 mg/l, thus the required flow rate was 20 divided by 6.67 which is 3 lpm.

2.3 Central temperature

The central temperature of the steamed blue

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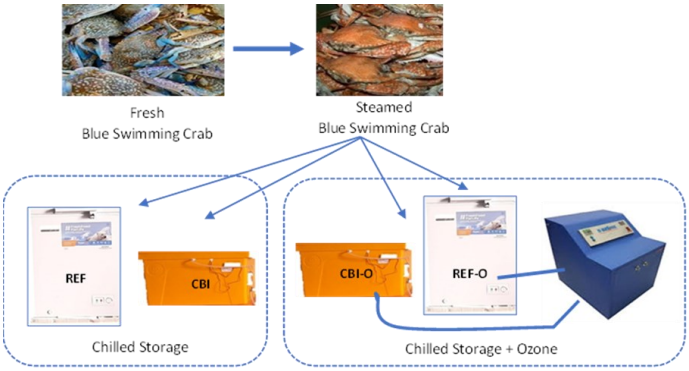

Figure 1. Four chilled storage treatments of steamed blue swimming crab.

swimming crab meat sample was measured using a digital thermometer during the storage period. Central temperature measurements were carried out every day for 6 days.

2.4 Moisture content

Moisture content analysis of steamed blue swimming crab used the sample drying method in an oven at a temperature of 105°C and determined the moisture content with the difference in weight between dry and wet ingredients according to the Association of Official Analytical Chemist to constant weight (AOAC, 2005). Based on the method described by Holma *et al.* (2013), an empty crucible that would contain the sample was weighed, using an electronic scale. The blue swimming crab (2±0.5 g) were weighed and put into the crucible. The crucible was then reweighed. The sample in the crucible was then placed in an air oven at a temperature of 105°C. The samples were then left for more than 18 hrs. The crucible was removed and put into a desiccator. Then it was weighed again to get the value of the dry sample. The sample was put back into the oven and reweighed every 2 hrs until a constant weight of the dry sample was obtained.

2.5 Water holding capacity

A sample of 10 g of steamed blue swimming crab meat was placed in a 50 mL plastic centrifuge tube and heated for 15 mins in a water bath (90°C). Then, the sample was cooled to room temperature and centrifuged at 9000×*g* at 4°C for 20 mins. The supernatant was removed and the WHC of the remaining samples was calculated as follows:

WHC (%) = 1 - ([weight of sample before heating - weight of sample after heating and centrifugation]/total moisture content in sample × 100) (Jin *et al*., 2014).

2.6 pH

Measurement of pH began with making a thick blue swimming crab slurry. 10 grams of blue swimming crab muscle and 10 mL of deionized water were homogenized using a sterile blender. The pH of the blue swimming crab thick slurry was measured using a pH meter (Arulkumar *et al.,* 2017).

2.7 Total plate count

TPC analysis refers to the testing procedure according to SNI 2332.3:2015 (Badan Standardisasi Nasional, 2015). The 25 g steamed crab sample was homogenized with 225 mL of butterfield's phosphate buffered solution for 2 mins in a sterile plastic. 10 mL of the homogenate was added to 90 mL of Butterfield's phosphate buffered solution to obtain a 10^{-2} dilution, then dilution is carried out up to 10^{-3}. The 1 mL dilution sample was spread on nutrient agar using an L-rod. Incubate the petri dishes in an inverted position at 37°C for 48 hrs. Total colonies were calculated by multiplying the number of colonies in the nutrient agar plate by the dilution factor.

2.8 Total volatile base nitrogen

TVBN measurements were carried out according to SNI 2354.8:2009 (Badan Standardisasi Nasional, 2009). This method was carried out using volatile base extraction and steam distillation. The distillate was accommodated in perchloric acid (6% w/v), then the distillate was titrated with standard hydrochloric acid 0.02 mol/L using phenolphthalein.

2.9 Sensory analysis

Sensory analysis was carried out using the SNI 4224:2015 (Badan Standardisasi Nasional, 2015) scoresheet assessment sheet, with a sensory scale of 1-9 with color, aroma, aroma, and texture parameters. Samples were divided into four groups according to storage treatment. The panelists were undergraduate students of Fisheries Products Technology Study Program, Faculty of Fisheries and Marine Science, Universitas Diponegoro.

3. Results and discussion

3.1 Central temperature

Central temperature measurement of the whole blue swimming crab was aimed to measure the effectiveness of chilling with different methods during storage. Temperature is an important factor that affects the shelf life and freshness of fishery products, so keeping the temperature low could maintain freshness and extend the shelf life of fish. Mercier *et al.* (2017) reported that temperature is the most important factor affecting the durability of a food product. The temperature factor must be considered to determine the compatibility of food products during storage. The results of the central temperature measurement are presented in Table 1.

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Table 1. Central temperature of steamed blue swimming crab.

Storage Time (days)	Storage Treatments			
	CBI	CBI-O	REF-O	REF
0	32.50±0.00^{Aa}	30.72±0.00^{Aa}	31.11±0.00^{Ab}	30.61±0.00^{Ab}
1	11.3±2.03^{Ba}	11.60 ±1.72^{Ba}	1.33±1.00^{Bb}	1.80±1.49^{Bb}
2	10.76±3.64^{Ba}	11.52±4.32^{Ba}	1.06±1.06^{Bb}	1.39±1.83^{Bb}
3	7.96±3.00^{Ba}	8.65±1.04^{Bc}	1.63±0.75^{Bb}	1.96±1.81^{Bb}
4	9.70±0.80^{Ba}	9.94±2.10^{Ba}	0.02±0.35^{Bb}	0.96±1.00^{Bb}
5	8.35±0.64^{Ba}	10.15±2.59^{Ba}	0.74±2.20^{Bb}	1.17±1.26^{Bb}
6	9.22±0.70^{Ba}	8.91±0.32^{Ba}	1.33±3.68^{Bb}	1.69±1.38^{Bb}

Values are presented as mean±SD of three replicates. Values with different uppercase superscripts within the same column are statistically significantly different ($p<0.05$) while values with different lowercase superscripts within the same row are statistically significantly different ($p<0.05$).

Based on data obtained, there was a significant difference between the central temperature of steamed blue swimming crab in cool box iced, refrigerator with ozone and cool box iced, refrigerators without ozone additions on storage for 6 days of storage which are marked by the same superscript on ANOVA analysis with 95% confidence interval. The central temperature of the steamed blue swimming crab in cool box iced storage without and with the addition of ozone was higher than the central temperature of the steamed blue swimming crab in the refrigerator storage without and with the addition of ozone. Chilled storage treatment of whole blue swimming crab in refrigerator with the addition of ozone was the best treatment because the central temperature of the steamed blue swimming crab was lower so that it could maintain the quality of the steamed crab. The central temperature of the steamed blue swimming crab tended to decrease during the 0, 1st, 2nd, 3rd, 4th, 5th, and 6th days of storage, respectively, by 31.23°C, 6.51°C, 6.18°C, 5.05°C, 5.16°C, 5.10°C and 5.29°C. This is in accordance with the research of Pratiwi *et al.* (2017), who reported that the temperature and storage time in refrigerator at 4°C, and ice at 10°C significantly affected the quality of fish. Refrigerator storage gave better results than ice storage. The fish stored in the refrigerator was rejected (already smelled not fresh) on the 15th days of storage while in the ice storage it was rejected earlier at the 5th days of storage. Afsah-Hejri *et al.* (2020) reported that O_3 did not affect the temperature of food products, but only reacted with atmospheric water in the storage room and lowers the RH of the air. O_3 can be used as a safe and environmentally friendly technology for food preservation and contaminant control.

3.2 Moisture content

Moisture content is the amount of water contained in a material. Moisture content analysis was carried out to determine the amount of free water contained in crab meat. According to Cheng *et al.* (2015), moisture is very important for the freshness quality of fish, which can affect the texture and muscles found in fish meat. The results of the measurement of moisture are presented in Table 2.

Based on the data obtained, it shows that there was no significant difference of moisture content in meat of blue swimming crabs in four storage treatments during storage. The moisture of blue swimming crabs tended to increase during storage, but chilled storage in refrigerator could reduce the rising pattern of moisture content during storage. This is also in accordance with the research of Goliat *et al*. (2016) on the increasing moisture of tilapia until the 6th day of observation. The increase in initial moisture of fish with increasing ice storage time could be due to the fact that fish absorb water during the first days of storage. The increase in moisture during storage also occurred in the study of Murthy *et al.* (2015), they reported that during ice storage for whole fish and shelled fish, the moisture increases with the storage period. The increase in moisture was relatively higher in shredded fish and reached 84.55% at the end of 13 days of ice storage, while in whole fish the moisture reached 82.95% at the

Table 2. Moisture content of steamed blue swimming crabs during storage (%).

Storage Time (days)	Storage Treatments			
	CBI	CBI-O	REF-O	REF
0	75.29±1.83^{Aa}	75.77±2.40^{Aa}	74.87±0.56^{Aa}	75.22±1.11^{Aa}
3	74.35±0.85^{Aa}	75.69±0.42^{Aa}	75.28±1.75^{Aa}	76.21±0.75^{Aa}
6	76.66±1.71^{Aa}	76.46±0.88^{Aa}	75.31±0.40^{Aa}	74.89±1.64^{Aa}

Values are presented as mean±SD of three replicates. Values with different uppercase superscripts within the same column are statistically significantly different ($p<0.05$) while values with different lowercase superscripts within the same row are statistically significantly different ($p<0.05$).

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end of 13 days. Based on research of Rieuwpassa *et al.* (2017), during storage at low temperatures, fish would experience damage, for example denaturation of myofibril proteins and thus cause an increase in moisture due to the reduced ability of myofibrils to hold water in their tissues.

3.3 Water holding capacity

WHC is the power to bind water to a material by other molecules through high-energy bonds such as proteins, carbohydrates and salts. According to Kaale *et al.* (2014), WHC was an important quality parameter because it affects profitability and quality, because it affects changes in weight during storage and shrinkage during cooking, juiciness, and softness of food products. The low WHC is caused by post-mortem structural changes in the muscles. These changes could be in the form of shrinkage of the myofilaments lattice, myosin denaturation and an increase in extracellular space. Listrat *et al.* (2016) reported that a decrease in WHC usually occurs during cold storage and cooking. WHC is strongly influenced by the rate and extent of the decrease in pH. The results of the WHC measurements are presented in Table 3.

Based on the data obtained, there is a significant difference between the WHC data of blue swimming crabs for cold box ice storage, refrigerator with added ozone and refrigerator with storage on cool box ice with added ozone for 6 days of storage which is marked by different superscripts on ANOVA analysis with 95% confidence interval. Steamed blue swimming crabs in refrigerator storage with the addition of ozone had a higher WHC value than other treatments on the observations of the 0, 1, 2, 3, 4 and 6 days with an average WHC value of 34.84%. The blue swimming crabs in the ice cool box storage without the addition of ozone produced a WHC value that was greater than the ice cool box storage with the addition of ozone on the observations of the 0, 1, 2, and 6 days with an average WHC value of 29.52% and 28.95%, respectively. Blue swimming crab in refrigerator storage without the addition of ozone resulted in the smallest WHC values on the 0, 1st, 2nd, 3rd, 4th and 6th day observations, with an average of 27.37%. The best treatment was in refrigerator and ozone storage because with these storage treatments the WHC value of steamed blue swimming crab was higher than other treatments so that the ability of blue swimming crab products to hold or bind water were stronger. The WHC values in all treatments resulted in fluctuating values with the length of storage, on the 0, 1st, 2nd, 3rd, 4th, 5th, and 6th days, were 27.2%, 31.5%, 33.84%, 29%, 29.33%, 25.5% and 34.83%, respectively. According to Prabawa *et al.* (2021), the decrease in WHC occurs due to reduced hydrophilic properties, thereby reducing the ability to bind water so that the amount of water released is increasing. It was influenced by storage time and storage temperature. According to Hughes *et al.* (2014), myofibrils are linked to each other to cell membrane through protein bonds that comprise the cytoskeleton in muscle cells. If this bond is intact, the fluid will come out into extracellular space, when cytoskeleton and intermediate filaments are damaged, so that cell membrane has been damaged, the fluid remains in muscle cell.

3.4 pH

The pH value is one indicator of the quality of steamed blue swimming crab. The difference in pH in fishery products is caused by the activity of lactic acid bacteria and the accumulation of organic acids (Tumonda *et al.,* 2017). The initial pH value of steamed crab was 7.3, then it increased with the length of storage time. The increase in pH in the steamed blue swimming crab indicated the level of quality deterioration. The results of measuring the pH of steamed blue swimming crabs in ozone storage for 6 days are presented in Table 4.

Based on the results obtained, there is a significant difference between steamed blue swimming crabs and storage in cool box with ice, cool box with ice + ozone,

Table 3. Water holding capacity (%).

Storage Time (days)	Storage Treatments			
	CBI	CBI-O	REF-O	REF
0	20 ± 0^{Aa}	26.67 ± 5.77^{Aa}	31.2 ± 9.89^{Aa}	30.93 ± 30.93^{Aa}
1	32 ± 5.29^{Aa}	34.67 ± 1.15^{ABa}	33.33 ± 6.11^{Aa}	26 ± 5.29^{Aa}
2	33.33 ± 11.55^{Aa}	36.67 ± 3.06^{ABa}	34.67 ± 8.33^{Aa}	30.67 ± 9.45^{Aa}
3	30 ± 7.21^{Aa}	23.33 ± 5.03^{ABa}	34 ± 7.21^{Aa}	28.67 ± 3.06^{Aa}
4	29.33 ± 2.31^{Abc}	28 ± 3.46^{ABab}	39.33 ± 4.62^{Aa}	20.67 ± 5.03^{Aa}
5	32.67 ± 1.15^{Ab}	23.33 ± 4.16^{ABab}	20.00 ± 2^{Aa}	26 ± 10.39^{Aab}
6	29.33 ± 11.72^{Aa}	30 ± 22.54^{Ba}	51.33 ± 42.16^{Aa}	28.67 ± 3.06^{Aa}

Values are presented as mean±SD of three replicates. Values with different uppercase superscripts within the same column are statistically significantly different ($p<0.05$) while values with different lowercase superscripts within the same row are statistically significantly different ($p<0.05$).

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Table 4. pH of blue swimming crab during chilled storage.

Storage Time (days)	Storage Treatments			
	CBI	CBI-O	REF-O	REF
0	7.3 ± 0^{A}	7.3 ± 0^{A}	7.3 ± 0^{A}	7.3 ± 0^{A}
1	7.25 ± 0.19^{Aa}	7.6 ± 0.18^{ABab}	7.575 ± 0.49^{Abab}	7.85 ± 0.05^{Abb}
2	7.57 ± 0.20^{Aa}	7.67 ± 0.20^{Ba}	7.77 ± 0.05^{ABCa}	7.77 ± 0.27^{Bca}
3	8.15 ± 0.26^{Ba}	8.25 ± 0.12^{Ca}	8.22 ± 0.37^{ABCa}	8.27 ± 0.15^{Bca}
4	8.02 ± 0.05^{Ba}	8.17 ± 0.05^{Cb}	7.72 ± 0.09^{BCc}	7.52 ± 0.05^{Cd}
5	8 ± 0.11^{Ba}	8.07 ± 0.22^{Ca}	8.05 ± 0.19^{Ca}	7.92 ± 0.09^{Cda}
6	8.17 ± 0.12^{Ba}	8.25 ± 0.12^{Ca}	8.17 ± 0.17^{Ca}	8.1 ± 0.18^{Da}

Values are presented as mean±SD of three replicates. Values with different uppercase superscripts within the same column are statistically significantly different ($p<0.05$) while values with different lowercase superscripts within the same row are statistically significantly different ($p<0.05$).

refrigerator + ozone, and refrigerator for 6 days of storage which were marked by different superscripts on ANOVA analysis with 95% confidence interval. Changes in pH at each storage treatment fluctuated with the longer the storage process causing the pH of the steamed blue swimming crab to be higher. Anam *et al.* (2019), the pH of soymilk beverage with the application of ozone has a different pH, in which there was an increase and a decrease. This is related to the buffering capacity of the liquid.

3.5 Total plate count

TPC is a method used to determine the number of bacteria in a product. TPC in steamed blue swimming crabs indicated the level of quality deterioration which has implications for damage to nutritional components. Steamed blue swimming crabs with storage of cool box iced, cool box iced and ozone, refrigerator and ozone, and refrigerator for 6 days had an increasing TPC value. The results of the steamed blue swimming crab TPC measurement are presented in Table 5.

Based on the results of data processing, there is significant differences between steamed blue swimming crabs stored in cool box iced, cool box iced and ozone, refrigerator and ozone, and refrigerator on day 3 to day 6 which were marked by different superscripts. The TPC value in the steamed blue swimming crab sample on day 0 was 1.30×10^{3} CFU/mL. The TPC value of steamed blue swimming crab storage in the cool box iced had the highest TPC value compared to other treatments, where the TPC value of steamed blue swimming crab on the 3rd and 6th days of cool box iced storage was 1.88×10^{4} CFU/mL and 7.4×10^{5} CFU/mL. The average TPC value of steamed blue swimming crab on the 3th and 6th days of cool box iced and ozone storage was 2.12×10^{4} CFU/mL and 5.73×10^{4} CFU/mL, while the average TPC value of steamed crab on the 3th and 6th days of refrigerator storage was 2.23×10^{4} CFU/mL and 3.51×10^{5} CFU/mL. Storage of steamed blue swimming crab in a refrigerator and ozone is the best practice because it is able to inhibit the growth of bacteria or has the lowest TPC value during the storage process. The average TPC value of steamed blue swimming crab on the 3th and 6th days of refrigerator and ozone storage was 1.92×10^{4} CFU/mL and 4.02×10^{4} CFU/mL. This shows that the use of ozone was able to give a real effect on the storage time of steamed blue swimming crab. According to Zhao *et al.* (2019), ozone can extend the shelf life of fishery products by means of microbial inactivation. The application of ozone to swabbed fish was able to reduce TPC, where the TPC value of swabbed fish not washed with ozone was 7.5×10^{4} CFU/g and swabbed fish washed with ozone was 2.1×10^{3} CFU/g (Sopher *et al.*, 2007). Based on the research by Susan *et al.* (2018), the application of ozone to tomatoes was able to inhibit the growth of bacteria from a TPC value of 4.8×10^{5} CFU/g on day 12, while tomatoes without application of ozone were only able to last 6 days with a TPC of 9.7×10^{8} CFU/g.

3.6 Total volatile base nitrogen

TVBN is an indicator of the freshness of fishery products based on the accumulation of basic compounds (ammonia, trimethylalamine, and other volatile compounds) through bacterial activity. The higher the TVBN, the higher the total bacteria. Nitrogen bases will

Table 5. Total plate count (Log CFU).

Storage Time (days)	Storage Treatments			
	CBI	CBI-O	REF-O	REF
0	3.11 ± 0.00^{A}	3.11 ± 0.00^{A}	3.11 ± 0.00^{A}	3.11 ± 0.00^{A}
3	4.27 ± 0.09^{AB}	4.30 ± 1.87^{B}	4.26 ± 0.18^{B}	4.34 ± 0.05^{B}
6	5.47 ± 0.84^{B}	4.71 ± 0.25^{B}	4.52 ± 0.35^{B}	4.82 ± 0.59^{B}

Values are presented as mean±SD of three replicates. Values with different superscripts are statistically significantly different ($p<0.05$).

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increase during the decay process (Sholahuddin, 2020). TVBN measurement results are presented in the Table 6.

The TVBN values of steamed blue swimming crabs with storage of cool box iced, cool box iced + ozone, refrigerator + ozone, and refrigerator on day 3 to day 6 showed significant differences which were indicated by different superscripts. Based on the TVBN graph, it shows that steamed blue swimming crabs stored in an ice cool box have the highest TVBN value, while steamed blue swimming crabs with ozone and refrigerator storage have the lowest TVBN values. The longer the storage time of the steamed blue swimming crab, the higher the TVBN value, which can be an indicator of the decline in the quality of the steamed blue swimming crab. Yunizal *et al.* (1998) in Bentalen *et al.* (2017) showed that the indicator of fish freshness can be seen from the number of TVBN, where the higher the TVBN, the lower the freshness of the fish. This is due to the reshuffling of proteins by the activity of microorganisms into volatile compounds during the storage process. Based on the research of Yuliani *et al.* (2018), the TPC value in tuna, milkfish, and shrimp stored with ozone in cold storage has a smaller value than the control. Tuna with ozone storage and cold storage had a TVB-N value of 21.40 mgN/100 g on day 15, while the TVB-N value of control samples was 10.32 mgN/100 g on day 4.

3.7 Sensory analysis

Sensory test is a form of testing that uses the human senses to determine the physical acceptance of product quality characteristics. Sensory evaluation is carried out at various processing levels such as after landing to after processing (Bernardi *et al.,* 2013). The sensory test parameters of steamed crabs consist of texture, taste, color, and smell. The results of sensory observations are presented in Table 7.

Based on the results obtained, there were significant difference between steamed blue swimming crabs and storage in a cool box iced, cool box iced + oxon, refrigerator + ozone, and refrigerator for 6 days of storage which is marked by a different superscript on the 95% confidence interval ANOVA analysis. Steamed blue swimming crabs with storage in the refrigerator + ozone and cool box iced+ozone has sensory values that can be accepted by consumers until after the sixth day, but the sensory values of refrigerator+ozone were higher. Steamed blue swimming crabs with cool box iced storage only lasted on the fourth day, while steamed blue swimming crabs with refrigerator storage lasted until the fifth day. This shows that the use of refrigerator + ozone in the storage of steamed blue swimming crabs can increase the shelf life and sensory quality. Okpala (2016) reported that the technological advances encourage the application of ozone in the food industry because ozone was able to extend shelf life and maintain product sensory quality. In addition, ozone can come into contact with food briefly.

4. Conclusion

Different chilled storage treatments of whole blue swimming crab gave different effects to whole steamed blue swimming crab. Central temperature of whole blue

Table 6. Total volatile base nitrogen (mgN/100 g).

Storage Time (days)	Storage Treatments			
	CBI	CBI-O	REF-O	REF
0	14.39 ± 0.00^{A}	14.39 ± 0.00^{A}	14.39 ± 0.00^{A}	14.39 ± 0.00^{A}
3	23.50 ± 12.67^{Aa}	23.12 ± 12.96^{Aa}	20.66 ± 5.83^{Aa}	17.68 ± 2.65^{Aa}
6	40.33 ± 17.14^{Aa}	23.10 ± 1.80^{Ba}	22.11 ± 4.31^{Aa}	23.84 ± 5.97^{Aa}

Values are presented as mean±SD of three replicates. Values with different uppercase superscripts within the same column are statistically significantly different ($p<0.05$) while values with different lowercase superscripts within the same row are statistically significantly different ($p<0.05$).

Table 3. Water holding capacity (%).

Storage Time (days)	Storage Treatments			
	CBI	CBI-O	REF-O	REF
1	8.75 ± 0.34^{Aa}	8.58 ± 0.28^{Aa}	8.91 ± 0.28^{Aa}	8.66 ± 0.28^{Aa}
2	8.33 ± 0.33^{Aa}	8.5 ± 0.57^{AEa}	8.83 ± 0.28^{Aab}	8.41 ± 0.31^{Aa}
3	7.25 ± 0.31^{BCa}	7.75 ± 0.31^{ABCab}	7.91 ± 0.28^{BCDEab}	$7.50\pm0.44B^{Cab}$
4	6.83 ± 0.28^{Ca}	$7.05\pm0.04B^{CDbcd}$	7.66 ± 0.33^{CDEc}	7.25 ± 0.50^{Cd}
5	5.83 ± 0.44^{DEa}	7.33 ± 0.33^{CDbc}	7.58 ± 0.04^{Dec}	6.41 ± 0.21^{Da}
6	4.50 ± 0.57^{Ea}	7.16 ± 0.28^{Dbc}	7.41 ± 0.31^{Ec}	5.25 ± 0.31^{Ea}

Values are presented as mean±SD of three replicates. Values with different uppercase superscripts within the same column are statistically significantly different ($p<0.05$) while values with different lowercase superscripts within the same row are statistically significantly different ($p<0.05$).

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swimming crab in refrigerator were lower than blue swimming crab in cool box. Ozone treatment (CBI-O and REF-O) showed better preservation results in TPC, TVBN and sensory value compare to non-ozone treatment. The results showed that whole steamed blue swimming crab were rejected after 3 days in CBI, after 4 days in CBI-O and after 6 days were still accepted in both refrigerated storage (REF and REF-O). Ozone treatment could prolong the freshness of steamed blue swimming crab but reduce the typical aroma.

Conflict of interest

The authors declare no conflict of interest.

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References

Afsah-Hejri, L., Hajeb, P. and Ehsani, R.J. (2020). Application of ozone for degradation of mycotoxins in food: A review. *Comprehensive Reviews in Food Science and Food Safety*, 19(4), 1777-1808. https:// doi.org/10.1111/1541-4337.12594

Anam, C., Perwitasari, A.A., Jannah, H.N., Yaqin, N., Yulianto, E., Sasmita, E., Restiwijaya, M., Kinandana, A.W., Aryanto, F. and Nur, M. (2020). Effect of ozone treatment on microbiological and physicochemical properties of soymilk beverage. *IOP Conference Series: Earth and Environmental Science,* 443(1), 012100. https:// doi.org/10.1088/1755-1315/443/1/012100

AOAC. (2005). Official methods of analysis. USA: AOAC International.

Arulkumar, A., Paramasivam, S., Rameshthangam, P. and Rabie, M.A. (2017). Changes on biogenic, volatile amines and microbial quality of the blue swimmer crab (*Portunus pelagicus*) muscle during storage. *Journal of Food Science and Technology*, 54 (8), 2503-2511. https://doi.org/10.1007/s13197-017-2694-5

Badan Standardisasi Nasional. (2009). Cara uji kimia bagian 8: Penentuan kada total volatile base Nitrogen (TVBN) dan trimetil amin nitrogen (TMAN) pada produk perikanan (SNI 2354.8:2009). Indonesia: Badan Standardisasi Nasional [In Bahasa Indonesia].

Badan Standardisasi Nasional. (2015). Cara uji mikrobiologi bagian 3: Penentuan Angka Lempang Total pada produk perikanan (SNI 2332.3:2015). Indonesia: Badan Standardisasi Nasional [In Bahasa Indonesia].

Badan Standardisasi Nasional. (2015). Daging rajungan rebus dingin (SNI 4224:2015). Indonesia: Badan Standardisasi Nasional [In Bahasa Indonesia].

Badan Standardisasi Nasional. (2019). Alat penyimpanan produk hortikultura pascapanen menggunakan teknologi ozon – Syarat mutu dan metode uji (SNI 8759:2019). Indonesia: Badan Standardisasi Nasional. [In Bahasa Indonesia].

Bentalen, S.G., Onibala, H. and Salindeho, N. (2017). Mutu Ikan Cakalang (*Katsuwonus pelamis* L) Asap Yang Direndam Dengan Larutan Kulit Buah Manggis (*Gracinia mangostana* Linn). *Media Teknologi Hasil Perikanan*, 5(1), 13-18. https:// doi.org/10.35800/mthp.5.1.2017.14908 [In Bahasa Indonesia].

Bernardi, D.C., Mársico, E.T. and Freitas, M.Q.D. (2013). Quality Index Method (QIM) to assess the freshness and shelf life of fish. *Brazilian Archives of Biology and Technology*, 56(4), 587-598. https:// doi.org/10.1590/S1516-89132013000400009

Cheng, J.H., Sun, D.W., Zeng, X.A. and Liu, D. (2015). Recent advances in methods and techniques for freshness quality determination and evaluation of fish and fish fillets: A review. *Critical Reviews in Food Science and Nutrition*, 55(7), 1012-1225. https://doi.org/10.1080/10408398.2013.769934

Dima, J.B., Sequeiros, C., Barón, P. and Zaritzky, N. (2020). Biochemical Composition and Quality Parameters of Raw and Cooked Refrigerated Patagonian Crabmeat (*Ovalipes trimaculatus*). *Journal of Aquatic Food Product Technology*, 29 (10), 1013–1028. https:// doi.org/10.1080/10498850.2020.1828524

Goliat, C., Kapute, F. and Valeta, J. (2016). Effect of prolonged storage in ice on nutrient composition and sensory quality of whole fresh pond raised tilapia fish (*Oreochromis shiranus*). *American Journal of Food and Nutrition*, 4(5), 127-130.

Holma, K.A. and Maalekuu, B.K. (2013). Effect of traditional fish processing methods on the proximate composition of red fish stored under ambient room conditions. *American Journal of Food and Nutrition*, 3(3), 73-82.

Hughes, J.M., Oiseth, S.K., Purslow, P.P. and Warner, R.D. (2014). A structural approach to understanding the interactions between colour, water-holding

capacity and tenderness. *Meat Science*, 98(3), 520-532. https://doi.org/10.1016/j.meatsci.2014.05.022

Hutajulu, J., Kusumo, T., Saputra, A., Mualim, R., Handri, M., Sugriwa, E., Nainggolan, C. and Syamsuddin, S. (2019). Financial analysis in the exploitation of blue swimming crab *Portunus pelagicus* in Banten Bay, West Java, Indonesia. *Aquaculture, Aquarium, Conservation and Legislation*, 12(2), 724-734.

Jin, S.K., Go, G.W., Jung, E.Y., Lim, H.J., Yang, H.S. and Park, J.H. (2014). Effect of mechanically deboned chicken meat hydrolysates on the physicochemical properties of imitation fish paste. *Asian-Australasian Journal of Animal Sciences*, 27 (1), 115-122. https://doi.org/10.5713/ajas.2013.13284

Jumiati dan Zainudin, M. (2019). Analisis Good Manufacturing Practice (Gmp) Dan Mutu Daging Rajungan Pada Miniplant Pengupasan Di Kabupaten Tuban. *Pena Akuatika: Jurnal Ilmiah Perikanan dan Kelautan*, 18(1), 19-27. https://doi.org/10.31941/penaakuatika.v18i1.709 [In Bahasa Indonesia].

Kaale, L.D., Eikevik, T.M., Rustad, T. and Nordtvedt, T.S. (2014). Changes in water holding capacity and drip loss of Atlantic salmon (*Salmo salar*) muscle during super chilled storage. *LWT-Food Science and Technology*, 55(2), 528-535. https://doi.org/10.1016/j.lwt.2013.10.021

Listrat, A., Lebret, B., Louveau, I., Astruc, T., Bonnet, M., Lefaucheur, L. and Bugeon, J. (2016). How muscle structure and composition influence meat and flesh quality. *Production Animales*, 28(2), 125-136. https://doi.org/10.20870/productions-animales.2015.28.2.3020

Melantina, D., Swastawati, F. and Syakur, A. (2022). Aplikasi teknologi ionisasi tegangan tinggi untuk pengawet ikan tongkol (*Euthynnus affinis*). *Jurnal Ilmu dan Teknologi Perikanan*, 4(1), 9-14. https://doi.org/10.14710/jitpi.2022.12061

Mercier, S., Villeneuve, S., Mondor, M. and Uysal, I. (2017). Time–temperature management along the food cold chain: A review of recent developments. *Comprehensive Reviews in Food Science and Food Safety*, 16(4), 647-667. https://doi.org/10.1111/1541-4337.12269

Murthy, L.N., Rao, B.M., Asha, K.K. and Prasad, M.M. (2015). Nutritional composition, product development, shelf-life evaluation and quality assessment of pacu *Piaractus brachypomus* (Cuvier, 1818). *Indian Journal of Fisheries,* 62(1), 101-109.

Okpala, C.O.R. (2016). Lipid autoxidation in ozone-processed crustacea under cold storage: A treatise. *Lipid Technology*, 28(5-6), 93-95. https://doi.org/10.1002/lite.201600026

Prabawa, S., Putri, D.R., Kawiji, K. and Yudhistira, B. (2021). Pengaruh variasi waktu ozonisasi dan suhu penyimpanan terhadap karakteristik fisika, kimia, dan sensoris pada daging ayam broiler (*Gallus domesticus*). *Jurnal Ilmiah Rekayasa Pertanian dan Biosistem*, 9(2), 168-184. https://doi.org/10.29303/jrpb.v9i2.277 [In Bahasa Indonesia].

Pratiwi, A.I., Husni, A., Budhiyanti, S.A., and Aji, B.R. (2017). Karakteristik mutu wader pari hasil budidaya pada berbagai suhu penyimpanan. *Jurnal Pengolahan Hasil Perikanan Indonesia*, 20(1), 123-130. https://doi.org/10.17844/jphpi.v20i1.16499 [In Bahasa Indonesia].

Rieuwpassa, F.J., Sehangunaung, A.O. and Dalawir, I.Y. (2017). Analisis Kadar Air dan Total Plate Count Surimi Ikan Tongkol (*Euthynnus* sp.) an Ikan Layang (*Decapterus russelli*) Selama Penyimpanan Beku. *Jurnal Ilmiah Tindalung*, 3(1), 43-47. [In Bahasa Indonesia].

Rijal, S. and Nur, M. (2015). Analisa Pengaruh Ozonisasi Hasil Lucutan Plasma Berpenghalang Dielektrik pada Beras terhadap Perubahan Amilografi, Kekerasan, dan Warna. *Youngster Physics Journal*, 4(1), 61-66. [In Bahasa Indonesia].

Sachadyn-Król, M. and Agriopoulou, S. (2020). Ozonation as a method of abiotic elicitation improving the health-promoting properties of plant products—A review. *Molecules*, 25(10), 2416. https://doi.org/10.3390/molecules25102416

Sholahuddin, M.A. (2020). Honey application as a halal replacement material for fillet fish product in transportation. *Journal of Halal Product and Research*, 3(1), 9-19. https://doi.org/10.20473/jhpr.vol.3-issue.1.9-19

Sopher, C.D., Battles, G.T. and Knueve, E.A. (2007). Ozone applications in catfish processing. *Ozone: Science and Engineering*, 29(3), 221-228. https://doi.org/10.1080/01919510701312965

Suharyono, A.S., Hasanudin, U. and Kurniadi, M. (2011). Proses Sterilisasi Sari Buah Terong Ungu (*Solanum melongena*) Dengan Sistem Ozonisasi. *Jurnal Kimia Terapan Indonesia*, 13(1), 1-7. [In Bahasa Indonesia].

Susan, A.I., Sasmita, E., Yulianto, E., Arianto, F., Restiwijaya, M., Kinandana, A.W. and Nur, M. (2018). Ozone application to extend shelf life of vegetables by microbial growth inhibition. *MATEC Web of Conferences*, 197, 02004. https://doi.org/10.1051/matecconf/201819702004

Tumonda, S., Mewengkang, H.W. and Timbowo, S.M.

(2017). Kajian mutu ikan cakalang (*Katsuwonus pelamis* L) asap terhadap nilai kadar air dan pH selama penyimpanan. *Media Teknologi Hasil Perikanan*, 5(2), 64-68. https://doi.org/10.35800/mthp.5.2.2017.14937 [In Bahasa Indonesia].

Yuliani, Y., Puspita, M., Nur, M., Agustini, T.W., Yulianto, E., Susan, A.I., Zahar, B. and Suraya, B. (2018. Ozone immersion treatment to increase the shelf life of Tuna fish, milk fish, and shrimp in a cold storage system. *IOP Conference Series: Materials Science and Engineering,* 434, 012021. https://doi.org/10.1088/1757-899X/434/1/012021

Zhao, Y.M., de Alba, M., Sun, D.W. and Tiwari, B. (2019). Principles and recent applications of novel non-thermal processing technologies for the fish industry—A review. *Critical Reviews in Food Science and Nutrition*, 59(5), 728-742. https://doi.org/10.1080/10408398.2018.1495613

