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Ozone immersion treatment to increase the shelf life of Tuna fish, milk fish, and shrimp in a cold storage system

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Abstract. The use of ozone in the preservation of fish with cold storage system is considered effective to extend the shelf life of fish by maintaining its nutritional value. Ozone which is as bacteriocidal with a combination of cold storage method, where the method can inactivate bacteria, so the combination of methods can extend the shelf life of fish. This study aims to determine the effect of ozone applications on the storage of tuna fish, milkfish, and shrimp. This research was conducted by dividing tuna fish, milkfish, and shrimp into 2 groups of control and immersion treatment which was ozone every day with dissolved ozone concentration 1.5 ppm - 2.5 ppm. Both treatments are stored into cold storage with temperature 2-8ºC for ± 2 weeks. Parameters observed: organoleptic value (SNI 2729: 2013), TPC (Total Plate Count), TVBN (Total Volatile Base Nitrogen) to control samples and ozone immersion. The results showed that tuna with ozone immersion treatment can be accepted organoleptically until day 15 and at day 16 value of TPC and TVBN respectively 21.40 mgN / 100g and 1.1 x 107 CFU / g, but the control treatment can only survive until day 4 with a value of TPC and TVBN 10.32 mgN / 100g and 5.3 x 106 CFU / g. This study shows that fish storage by immersion in ozone water with cold storage can maintain the freshness of the fish.

1. Introduction

Fish is known as one of the commodities that have high nutritional value because contains high level of protein [1]. Fish hold an important role in the fulfillment of nutritional source for humans. Fish as a source of unsaturated fatty acids, mineral and vitamins. Although fish rich of nutrients, but fish is one of the food that was easily broken, because the high temperature at tropic country include Indonesia and lack of hygiene sanitation implementation in the process of catching fish caused fish was going decay quick [2].

Various method used in the effort for preventing damage or decaying of fish, one of them with cooling. Cooling has a weakness, that is just maintaining freshness of fish for two or three days. This cooling method doesn't optimum for extending save capacity of fish, because of that ozone can be added in this system as alternative in remaining quality of the fish with safe [3]. Used of ozon for extending save capacity freshness fish is one of the preserving food technology that was pledge. In another side,

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ozone can't produce toxic residue at environment after treatment [4]. Ozone at industry of food manufacture valued as safed method with a minimum cost and free from chemistry substance in food safety management [5]. Beside of that ozon can be applicated for developing censoric quality, in this thing is organoleptic of fish [6].

Research application of ozone about saving of fish already much be done before, one of them do it by [4] that be using hake fish for sampling (Merluccius merluccius) with using method washing ozone. Concentrated ozone dissolved which used 2 ppm, after that saved in refrigerating room with temperature 2^{o}C. Calculation fish and ice is 2:1. Storage does for 12 days and must be done with analysis TVBN, TPC, and organoleptic at days 0,5,7, and 12. Result of that research show that hake fish with ozone treatment better for freshness compare with hake fish control. In another research that was be done by [7] application ozone about storage of sardine fish (sardina pilchardus) using slurry ice (ice:water = 2:3) with current ozone at temperature (-1)$^{\circ}$C and storage it for 22 days. Analysis of organoleptic, TVBN, and TPC are doing at days 0,2,5,8,12,15,19, and 22. Result of the research shows that freshness of fish that be storage at slurry ice with current ozone can be accepted until days 19.

In this research are doing storage of tuna fish, milkfish, and shrimps using current ozone during cold storage for knowing effect application of ozone about storage capacity of three sample of the fish during storage's period. Fish that be used is tuna fish, milkfish, and shrimp which is divide into two categories, that is ozone treatment and control. Ozone treatment doing when soaking fish in the water which is current with ozone during 2 hours every day during storage period. While control treatment is just storage it at refrigerating storage without ozone. Control sample and ozone's treatment sample after that analyzed from the organoleptic. Beside of that, two of that sample also analyzed it's TVBN and TPC

2. Material and method

This research held at month October – November 2017 at Teaching Industry, Diponegoro University, Semarang.

Material that be used is tuna fish, milkfish, and shrimp with average heavy 350-500 gram and average long 24-27 cm. Treatment sample from waters to research room are does with lifting alive fish using container sterofoam with ice, this thing for protecting chain of cooling. That fish was killed, after that divide into two categories treatment that is control treatment and ozone treatment. At ozone treatment fish remain in the box using water with ratio 1:1 (w/v). Fish was storage inside current water and at the air.

Ozone was produced from ozone generator. That ozone injected inside current water. First ozone injection that is with fussing ozone gasses for 60 minutes (1 hour at morning and 1 hour at noon). Observing organoleptic was done for 16 days and trial (TPC and TVBN) for 4 days.

2.1. Organoleptic

Testing organoleptic freshness fish and shrimp was done based on 2729 : 2013. Testing organoleptic are doing by 7 people trained panelist using score sheet organoleptic freshness fish and shrimp with range score 1-9, 1 for bad score and 9 for good score.

2.2. TVBN analysis

Testing TVBN is doing based on Indonesian Standard National 2354.8 : 2009. Test is doing with weigh 25 gram sample then mixed with 75 ml TCA (7%). As much 1 ml filtrate fill in Conway cup which was filled with 1 ml K2CO3, then fill with borat acid 2-3 drops and fenolftalein indicator until become green. Blanko solid which is 1 ml TCA 7% incubated at temperature 37^{0} C for 2 hours. That liquid titration with HCI until become pink.

$$TVBN\ \left(\frac{mgN}{100g}\right) = \frac{(Vc - Vb) \times N\ \times 14{,}007\ \times 2 \times 100}{w} \quad (1)$$

Where :
V_c = Volume of HCl solution at sample titration
V_b = Volume of HCl solution at blank titration
N = Concentration of HCl solution
W = Weight of sampel (g)

2.3. TPC analysis

Testing microbiology that known with Total Plate Count (TPC), used for evaluating fish quality from microbiology side. Testing TPC based on Indonesian National Standard 01-2332.3-2013. That testing are doing with take sample 10 gram chop until aceptic situation, then mix it with 90 ml peptone. Next, homogenous with stomacher. Dilution is doing until 10^{-2}. Media for testing this TPC using Plate Count Agar (PCA), from every dilution incubation it for 48 hours at 30^0 C.

$$N = \frac{\Sigma C}{[(1 \times n1) + (0{,}1 \times n2)] \times (d)} \quad (2)$$

Where :
N = number of colonies of products, expressed in colonies per ml or colony per g
$\sum C$ = number of colonies in all calculated bowls
n_1 = the amount of cup in the first dilution is calculated
n_2 = the amount of cup on the second dilution is calculated
d = the first dilution used

3. Result and discussion

3.1. Organoleptic

Testing organoleptic about sample tuna fish, bandeng fish and shrimp is one of the step for knowing freshness level during storage. This testing doing every day until 16 days. Result of organoleptic research tuna fish, bandeng fish and shrimp during cold storage served on figure 1.

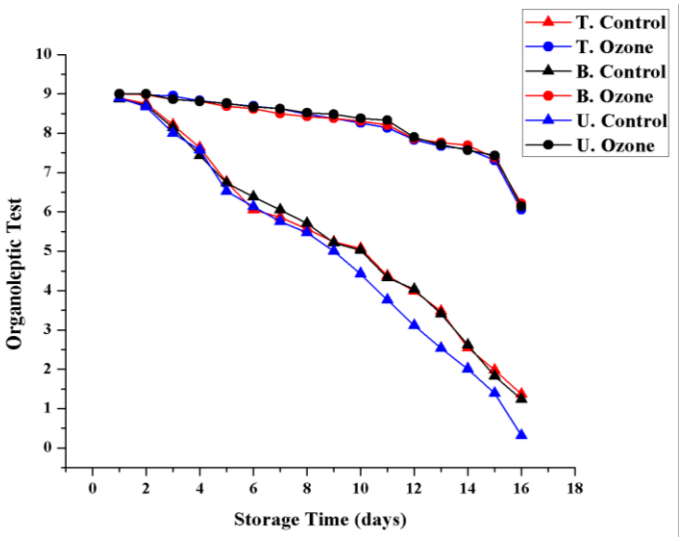

Figure 1. Average value of organoleptic.

Based on picture no. 1 organoleptic value of tuna fish, bandeng fish and shrimp at ozone treatment until end of the day that is days 15 storage showed average for tuna fish 7,24 ≤ μ ≤ 7,38 ; milkfish 7,31 ≤ μ ≤ 7,46 ; and shrimp 7,34≤ μ ≤7,52. For control treatment at days 14 showed average for tuna fish 6,73 ≤ μ ≤ 6,79 ; milkfish 6,69 ≤ μ ≤ 6,78 ; and shrimp 6,41 ≤ μ ≤ 6,64. This thing show that fish which

remain ozone treatment still suitable consumed until days 15, whereas control treatment survive until days 4. That result shows that fish which is storage with ozone treatment can be survive 4 times longer than storage without ozone. Explain that is ozone treatment can get product with sensoric aspect better, prevent form of mushroom and decaying process [8]. This is because ozone molecule characteristic can be malfunctioned bacteria, mushroom, parasite and virus. Ozone molecule effect intraceluler enzyme, nucleat acid, and another cell component from that microbe. Ozone which is injected inside cooling system.

Ozone gas which is current inside submerged water and recent through the air cold storage give an effect about fish storage capacity. Based on organoleptic research, can be concluded that storage capacity of fish for remaining ozone treatment better than control treatment. Explain that using ozone can prolong storage capacity of fish which is conservation technology which in agreed [9].

Used of ozone during cold storage with various kind of method was done at fishing product for increasing freshness quality. Research from [7] explain that quality of the lemuru fish which is cold storage with mixed ozone can survive with good until days 8 with categories E (highest quality) and A (good quality) along can be accepted until 19 days. Whereas control treatment just can be accepted until days 5 storage. Shown of fish gills and eye is the first indicator which is limit of eye accepting.

3.2. TVBN analysis (total volatile base nitrogen)

During storage, compound of nitrogen inside fish in condition the freshness increasing. Testing TVBN value as one of the indicator freshness of fish. Based on result of research show that added ozone at cold storage give an effect according to TVBN value of the fish. Factors that gives an effect TVBN value of fish with ozone treatment that is time of storage, temperature cold storage, and ozone concentration. Graphic of TVBN value tuna fish, bandeng fish, shrimp for storage days 0,4,8,12 and 16 shown at ficture 2.

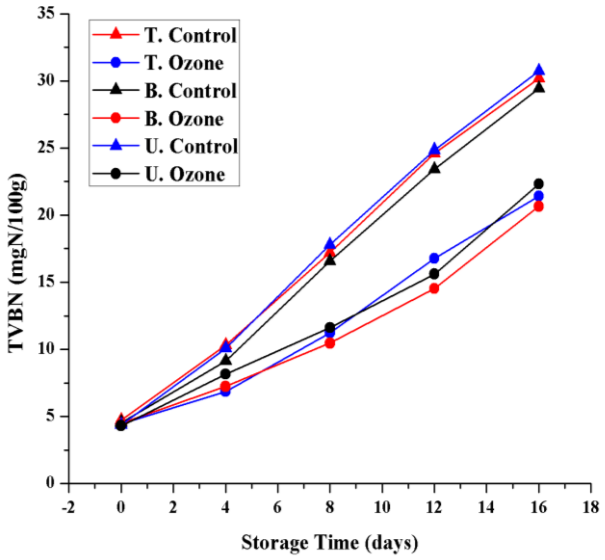

Figure 2. Average value of TVBN.

TVBN value tuna fish, bandeng fish, shrimp at two treatment increasing. Increasing of TVBN value at cold storage with added ozone relative slowly compare with control treatment. TVBN value ozone treatment every 4 days increase about 5 mgN/ 100 g. Ozone treatment with concentration 1,5 ppm -2,5 ppm with cold storage days 16 not more than limit of TVBN value. According to [10] explain that limit of TVBN for storage freshness fish is 25mg/ 100g. [4] is doing research hake fish (Merluccius merluccius) with method washing it with ozone water (2 ppm) storage at temperature 0^0 C for 12 days have a value TVBN approach it's limit that is 24 mgN/ 100g. Nerantzaki t al. 2005 doing research trout fish whish is storage at 4^0 C with ozone water (1mg/L) for 90 minutes have a storage capacity until 12

days based on limit of TVBN. This research before ever done by [11] that fish after treated with ozone water with concentration 0,5 ppm, 1 ppm, 1,5 ppm have a standard value under Indonesian Standard Nationality (SNI: 235.8: 2009) for fresh fish. TVBN indicated freshness muscle where is capacity 30 mgN/100 g not suitable to consumed by human [4]. Deforming chemistry that happened to the fish during process retreat of capacity that is growing up bacterial, although there was a study that showing decay because of enzyme. TVBN value shells with ozone treatment for 60 and 90 days at days 12 continuously increase become 24,2 mg/100 g and 26,9 mg/100g [12]. Doing a research with salmon fish with fresh treatment, cooling and freezing without ozone have a TVBN value as much as 17,0 mgN/100 g, 22,7 mgN/100 g, 14,8 mgN/100 g [13].

Current ozone treatment can blocked enzyme process and bacterial, pending fish decompotition during cold storage until days 16. During cold storage there was happened degradation protein become compounds more simple like trimetilatina and ammonia because of enzyme activities and microbiology. Explain that increasing TVBN after storage few days caused by microbiology activities from fish sample [14]. TVBN that be produced by degradation ammonia and ammonia volatile from muscle of fish based on microbiology activities. With the growing up of microba and still be used as indicator broken of fish. Excessively amount of microba inside of fish forming degradation process protein become alkali nitrogen compound quickly so concentration TVBN also increase acute at saving days 16 [15].

3.3. TPC analysis (total plate count)

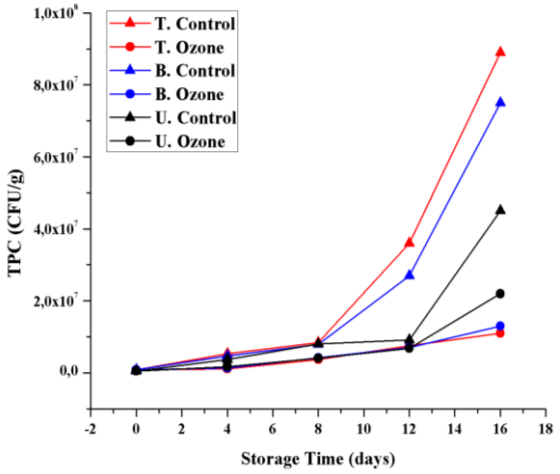

Figure 3. Average value of TPC

Data of testing TPC that is getting can be seen at picture 3. Based on that picture shown that TPC value get from tuna fish, bandeng fish, and shrimp with treatment without added ozone treatment (control) and current with ozone show that more longer storage more high TPC value. Testing of TPC value is doing storage for 16 days. That result show that three sample of the fish without added ozone have an age more shorter than treatment without ozone. Tuna fish, bandeng fish and shrimp which is current it with ozone water have a value TPC smaller than without using ozone. This thing can be look from TPC value which is current in ozone at days 12 smaller than TPC value without added ozone at days 8. That result show that treatment with ozone water can prolong storage capacity of fish, this is because ozone have a characteristic bacteriosidal or substance that can kill bacteria. This research along with research of [14] that explain amount of TPC fish which is deviated with ozone treatment have a value smaller than fish that gives treatment without ozone. Using spray ozone method with concentration 1,5 -2 ppm at temperature 4^0 C also can suppress grow up of bacteria [16].

Ozone mechanism in inactivated microorganism can't be understood better. Soluble ozone can be reacted with microbe directly by molecule ozone or not directly with free radical which was formed consequence ozone decompotition [17]. In general microbe have differential sensitivities about oxygen

pressure in the air environment or oxidation-reduction potential from growth medium. That things linked with medium ability or substract for catching or release electron. Combination from pH, Aw, oxidation-reduction potential, content of nutrient substance, and obstacle compound effect growth of microbe [3].

Based on [7] that using of ozone in storage fish can increase age's capacity until 60 %. And decreasing 2-3 log unit with using ozone 0,6 ppm for 30 minutes. Then after enter second week reach 10^6 until 10^7 [18] explain that limit of TPC for fresh sea fish is 6 log CFU/g after 9 days storage.

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