Improving The Quality of Smoked Shark Meat With Ozone Water Technique

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Abstract. The main problem of smoked shark process is the washing stage. The high content of urea in shark meat will cause ammonia odor in products that are not preferred consumers. The research aims to determine the effect of ozone immersion on improving the quality of smoke sharks. The research methodology used factorial complete randomization, with ozone immersion time (control; 5; 10; 15; 20 minutes) repeated 3 times. Data evaluation used analysis of variance (ANOVA) and Tukey differentiation test. The results show that the time for ozone immersion water has a very significant effect on total microbial, water content, urea content and sensory quality including the color, flavor and appearance of smoked shark meat. However, no significant effect on the acidity (pH) and texture of smoked shark meat. The ozone washing method can reduce urea content, deactivate microbes and improve the sensory quality of smoke sharks. The optimal water immersion time is 15 minutes showing optimal results, with total microbial results decreasing in 3 log cycles, from 1.7 x 105 CFU / gram to 9.0 x 102 CFU / gram, urea content decreasing 68.50%, pH 5.14; good appearance (4.28), bright brownish white (4.08), odorless ammonia (4.29), savory, non-acidic (4.12) and soft texture (2.63).

1. Introduction

The problem in the production of small industrial smoke sharks is that their products are less liked by consumers. The high fat, protein, pigment, urea and trimethylamine oxide (TMAO) content in shark and stingray meat causes unpleasant odors in fish products [1]. The appearance of shark meat is less preferred due to changes in the color of the meat which tends to become darker during storage, having stronger odor caused by the relatively greater content of free fatty acids which stimulates oxidation reactions [2]. Repeatedly washing shark meat in water method cannot eliminate the unpleasant ammoniac odor. [1] The study conducted on the use of ozone in the washing process of red mackerel aims to improve the color produced. [3] The study conducted on the characteristics of processed fish products by the ozonation process in tuna red meat (\textit{Euthynnus sp.}). Ozone is a strong oxidizing substance that can be used to kill bacteria (sterilization), remove color (decoloration), eliminate odors (deodorization) and decompose organic compounds (degradation). A relatively new process is fraternizing ozone gas into water, later is known as ozonation [4]. [1] Study on the use of ozone as a...
substitute for chlorine in the process of washing shrimp as a disinfectant. Furthermore, [5] study on the effects of ozone on microbiological tests and the color of catfish fillets. Considering the importance of using ozone in the food processing technology, it is necessary to apply this ozonation method to inactivate microbes and eliminate the strong ammonia odor in sharks' meat-smoking process.

This study aims to limit the effect of washing and so shark meat in ozone water for the improvement of the quality of sensory and chemical properties and for reduction of the total microbial content of smoked shark products.

2. Material and Methods

2.1. Material
This research uses headless frozen shark which was obtained from fishermen in Probolinggo Regency. Sample preparation is done by trimming process by sorting out the meat from the skin and bones. Next, the shark meat is cut into small pieces sized 8 cm x 3 cm x 1.5 cm and then stabbed with a stick. Shark meat is ready to be washed and immersed in ozone water with various immersion times (according to treatment). The shark meat is drained and fumigated until the shark flesh is brownish white.

2.2. Ozone Generation
Ozone is produced from pure oxygen using an ozone generator by Kriss-Tow Multifunction Ozonizer (model GES802). This tool measures 280x205x70mm with a weight of 0.66 kg. The outlet section, is connected by a porous hose and stone made of ore sand with ozone output constructions: 400 migram/hour. This system is used to distribute ozone which produces small bubbles in water and to increase ozone mass transfer and homogeneity. Ozonation is carried out in tanks measuring 20 cm x 20 cm x 25 cm

2.3. Ozonation Process
Pour 2 L of water into the tank. Turn on the ozone system and monitor the ozone concentration until it reaches 1.3 mg / L. Put 8 pieces of shark meat into an ozone tank. Flow the ozone gas to water from ozone generators for 5, 10, 15, and 20 minutes contact time (according to treatment). Remove and drain the shark meat that has been soaked in ozone solution and drain. Furthermore, the shark's meat is smoked until cooked, which is marked with brownish white meat. Then the shark is smoked until cooked. It is characterized by brownish white flesh. Each treatment is repeated 3 times. Samples are collected and evaluated for their total microbial content; chemical properties including water content, urea and acidity (pH) ; and sensory properties including color, flavour and odor, texture and appearance.

2.4. Total of Microbes Analysis (TPC)
Samples were evaluated for their total microbes by the Total Plate count (TPC) method. Weigh 10 grams of sample and crush them aseptically. Put 10 grams of the crushed sample into an Erlemeyer containing 90 mL of sterile 0.1 N phosphate buffer solution will get a 10-1 dilution rate factor. Samples were evaluated for their total microbes by the Total Plate count (TPC) method. Weigh 10 grams of sample and destroy it aseptically. Put 10 grams of the crushed sample into an Erlemeyer containing 90 mL of sterile 0.1 N phosphate buffer solution will get a 10-1 dilution rate factor. Preparation of microbial suspension at a 10^2 level dilution factor, by piping 1 ml of the smoke shark suspension from a 10^-1 level dilution factor and dissolving into 9 mL of sterile phosphate buffer solution. In the same way a sample suspension is made at a dilution factor of levels 10^-3, 10^-4, 10^-5 made in the same manner. Analysis of the total number of microbes in each dilution factor was carried out by taking 1 ml and pouring it into a sterile petri dish. Add sterile PCA media to grow microbes using plate pouring technique on PCA, homogeneous and incubated at 37 ° C for 24 to 48 hours.
Count the number of microbial colonies growing on petridish dishes and count with the Standat Plate count (SPC) method. The total number of microbial colonies is presented as the average number of colony forming units (CFU / ml). The total number of microbial colonies is presented as the average number of colony forming units (CFU/Gram).

2.5. Chemical Analysis
The evaluation of determined chemical properties of smoked shark meat includes water content, acidity (pH) and urea content. The analysis of water content of shark meat employs Gravimetry Method (AOAC 925.10-1995), while the analysis of acidity uses a pH meter.

2.6. Sensory Evaluation
The sensory quality testing of smoked sharks engages 25 trained panelists. The tested sensory attributes includes appearance, color, flavor and odor, texture, which are evaluated using a hedonic quality scale in accordance to the 5 specified criteria scales. The smoke shark sensory attribute scale is shown in Table 1.

Table 1. Criteria scores for the sensory attributes of smoked sharks

| Score  | Appearance  | Color          | Odor            | Flavor                | Texture   |
|--------|-------------|----------------|-----------------|-----------------------|-----------|
| 1      | Awful       | Dark brownish white | Very strong ammoniac | Savory, very strongly acidic | Very soft |
| 2      | Bad         | Slightly dark brownish white | Strong ammoniac | Savory, strong acidic    | Soft      |
| 3      | Not bad/ fair | Brownish white | Mild ammoniac | Savory, slightly acidic | Slight soft |
| 4      | Good        | Bright Brownish White | Bare ammoniac | Savory, Barely Acidic | A bit hard |
| 5      | Very good   | Clear brownish white | Less ammoniac | Savory, non-acidic      | Hard      |

2.7. Statistical analysis
This research used a non-factorial complete randomized trial design with 5 treatments (control-without ozonation, immersion in ozonation water for 5 minutes, 10 minutes, 15 minutes, 20 minutes). Data were evaluated using the SPSS 16.0 program with the analysis of variance (ANOVA) model, and continued with the average difference test between treatments using the Tuckey test. All data are presented as mean and standard deviation.

3. Results and Discussion

3.1. Total of Microbes content of smoked shark meat
Washing and immersion of the ozonation method resulted in a decrease in total microbes by 3 log cycles. Based on the results of statistical analysis with ANOVA (P < 0.01), the total number of smoke shark microbes and the treatment of the immersion time in ozone water have a very significant effect.
The Tuckey test showed that the ozonation process with various immersion times showed a decrease in the total number of smoke shark microbes. Smoked shark meat products in the control treatment (without ozone immersion) contain the highest total microbial amount of $1.7 \times 10^5$, while the 20 minute ozone immersion water treatment contains the lowest total microbial count of $1.5 \times 10^2$. The results of the calculation of total microbes (TPC) of smoked shark meat by ozonation treatment is shown in Table 2.

| The time of ozone immersion (minutes) | Total of Microbes (CFU/Gram) |
|--------------------------------------|-----------------------------|
| Control (not soaked)                 | $(1.7 \times 10^5)^a \pm 0.44$ |
| 5                                    | $(4.7 \times 10^4)^b \pm 0.56$ |
| 10                                   | $(1.7 \times 10^3)^b \pm 0.63$ |
| 15                                   | $(9.0 \times 10^2)^b \pm 0.48$ |
| 20                                   | $(1.5 \times 10^2)^b \pm 0.35$ |

* Value followed by different alphabetical notation showed significant difference.
If followed by the same aphabeted notation shows no significant difference

Table 2. shows a very significant decrease in total of microbes in the mean total number of smoke shark microbes by the Tuckey Test ($P < 0.01$) between control and treatment of immersion time in ozone. The time of ozone immersion for 5 minutes showed a significant decrease in total microbes by 1 log cycle. The same thing is also shown at the time of immersion of ozone for 10 minutes, 15 minutes and 20 minutes which each shows a very significant decrease in total microbes, counting from 2 cycles log to 3 cycles log. Ozone has a strong oxidation ability and is able to kill pathogenic microbes without leaving residue in the meat of smoked sharks. Ozoneated water can prevent the decontamination of shark meat and act as a disinfectant in water used in food processing. According to [6], short contact times at low ozone concentrations have been able to inactivate batteries, yeasts, fungi, viruses and parasites. The reduction in the total number of microbes that affect ozonation in smoke sharks is in line with research conducted by [7] which shows that washing fresh broccoli in ozone water can eliminate a small amount of total microbes. The same thing also showed a decrease of 1.0 log total cycle of aerobic bacteria in grated carrots [8] and fresh carrots there was a decrease of 0.5 log cycle of *E. coli* and *Salmonella* bacteria [9]. Ozonated water can effectively reduce microbial contamination. This is due to their strong oxidative strength against a broad spectrum of microorganisms (for example, fungal spores and vegetative cells, bacteria, and viruses). The mechanism of action of ozone as a disinfectant is by damaging microbial cells. [11] who reported that ozone was very effective at damaging the components in the cytoplasm in *E. coli* bacterial cells. [12] The results of studies using ozone microbubbles show the most effective way to deactivate the growth of *E. coli* O157: H7 at exposure temperatures of $13 \ ^\circ C$ for 30 minutes, compared to controls (distilled water).

### 3.2. Chemical properties of smoked shark meat

The chemical parameters of the observed smoke shark meat were water content and acidity (pH). The average value of water content and acidity (pH) of each treatment can be seen in Table 3.

**3.2.1 Water Content.** The water content of smoked shark meat principally defines its color and shelf life. Based on ANOVA statistical analysis ($P < 0.01$), treatment of immersion length in ozone water has a very significant effect on the water content of smoked shark meat. Statistical test results among the treatments by using the Tuckey test showed that between the controls and the varying immersion
time in ozone water it was significantly different (P <0.01). Smoked shark with ozone solution immersion treatment has varying water content. The control treatment (without ozone immersion) has a low water content (66.70% ± 0.54). Traditional smoked fish products have a water content of between 46-59% [13]. Table 3 shows that all samples treated at various times soaking ozone solution have high water content (68.60 ± 0.73). Water will be absorbed in the shark's meat tissue during the ozone solution immersion treatment. This situation is influenced by increased water holding capacity (WHC) in the treatment of immersion in liquid smoke due to hydrolysis and denaturation of smoked fish protein [14].

Table 3. Water content (%), pH and urea content of shark meat influence the time of ozone immersion

| The time of ozone immersion (minutes) | Water content (%wb) | pH | Urea content (MGram/Gram) |
|--------------------------------------|---------------------|----|--------------------------|
| Control (no immersion)               | 66.70 ± 0.54        | 5.26 ± 0.22 | 19.53 ± 0.59 |
| 5                                    | 68.60 ± 0.73        | 5.13 ± 0.07 | 6.82 ± 0.33 |
| 10                                   | 68.58 ± 0.39        | 5.13 ± 0.09 | 5.12 ± 0.52 |
| 15                                   | 68.50 ± 0.34        | 5.14 ± 0.08 | 3.02 ± 0.64 |
| 20                                   | 68.09 ± 0.36        | 5.13 ± 0.09 | 1.82 ± 0.53 |

* Value followed by different alphabetical notation showed significant difference. If followed by the same alfabed notation shows no significant difference

3.2.2. Acidity (pH). The acidity (pH) value of smoked shark meat ranges from 5.13 to 5.26 the pH range of smoked sharks immersed in ozone solution has the same value as conventional Skipjack [14] and which is immersed in smoked liquid [13]. Based on the results of statistical analysis with ANOVA (P> 0.05), it showed that the acidity (pH) of smoked shark meat by ozonation treatment has no significant effect. The average test results between treatments also did not show significant difference (P <0.05). Ozone is an unstable compound. Ozone compounds will decompose to produce free radical groups, especially hydroxyl ions which increase with temperature and decrease pH [15].

3.2.3. Urea content. The urea content of smoked shark meat is around 1.27 Mg/Gram. Up to 19.97 Mg/Gram. Based on ANOVA statistical analysis (P <0.01) treatment of immersion time in ozone water has a very significant effect on the urea content of smoked shark meat. Statistical test results among the treatments by using the Tuckey test showed that between the controls and varying immersion time in ozone water it was significantly different (P <0.01). The control treatment (without ozone immersion) contained high urea (19.53 ± 0.59), while the 20 minute ozone immersion time showed low urea levels (1.82 ± 0.53). The treatments between ozone immersion time of 15 minutes and 20 minutes did not show any significant difference (P> 0.01). Table 3 shows that the reduction of urea content in smoked shark meat in each treatment was due to the chemical reaction between ozone and urea during immersion. The decrease in urea levels is caused by ozone oxidizing urea to ammoniac [16]. Furthermore, ammoniac will be decomposed by ozone to be NO3 gas, and a small portion to be NO2. Ammoniac nitrogen can be removed by ozonation [17]. Ozone as an oxidizing agent can reduce ammonia and nitrite into nitrate compounds [18].

3.3. Sensory properties of smoked shark meat
The sensory nature of food basically influences the quality of products and consumer preferences. The sensory quality of observed smoked shark meat products includes appearance, color, smell, taste and
The results of statistical evaluations of each of the sensory quality attribute of smoked shark meat are presented in Table 4.

### 3.3.1. Color.

The color of smoked shark meat is the main parameter to determine the consumers’ attractiveness. The determination of the color attribute of the smoked shark meat practices the hedonic quality method with the 5 scales criteria. The results of the evaluation of 20 panelists on the color of the hedonic quality of smoked shark meat ozonation treatment ranged from dark brownish white (1.88) to bright brownish white (4.32). The results of statistical evaluation with ANOVA (P <0.01) have a very significant effect. The results of the difference test analysis of the average among treatments by using the Tuckey method (P > 0.01) were not significantly different between the controls and the time to soak in ozone; between the time of ozone immersion for 5 minutes and 10 minutes; between is 15 and 20 minutes ozone immersion time. However, between controls; 5, 10 minutes and 15 to 20 minutes ozone immersion time it shows a real difference. The ozone immersion treatment for 15 minutes and 20 minutes produces a bright white brown product color. The effect of ozone on the color of smoked shark meat is able to inhibit browning reactions. The hydroxyl radical group produced by ozone will react by opening the aromatic ring, and interacting with ozone will oxidize some organic acids, aldehydes, and ketones. The mechanism of action of ozone begins with ozone molecules experiencing 1-3 addition of double bonded dipolar cyclones, the formation of ozonide compounds from alkenes, and ozone with aldehydes or ketones of oxide which have a limited time [19]. This event causes oxidative damage to ozonides and forms carbonyl compounds, while ozone oxidative action leads to carboxylic acids or ketones. Ozone attacks the OH radical groups, especially the double bonds of organic compounds. Significant discoloration of smoked shark meat is also caused by browning reactions. The effect of browning can be related to the presence of enzyme activity at the beginning of ozone exposure and the occurrence of non-enzymatic browning in the smoking process. [15] reported that ozone can stimulate non-enzymatic reactions are maillard reactions.

### Table 4. Sensory properties of smoked shark meat influence the time of ozone immersion

| The time of ozone immersion (minutes) | Color   | Odor    | Flavor  | Texture | Appearance |
|--------------------------------------|---------|---------|---------|---------|------------|
| Control (0)                          | 1.88±0,16 | 1.35±0.27 | 2.02±0.01 | 2.83±0.16 | 2.55±0.20 |
| 5                                    | 3.12b±0.53 | 3.02b±0.44 | 3.05b±0.29 | 2.75b±0.28 | 3.12bc±0.08 |
| 10                                   | 3.33b±0.35 | 3.12b±0.18 | 3.52b±0.23 | 2.80b±0.51 | 3.33b±0.35 |
| 15                                   | 4.08a±0.28 | 4.29a±0.53 | 4.12a±0.05 | 2.63a±0.33 | 4.28a±0.28 |
| 20                                   | 4.32a±0.20 | 4.75a±0.10 | 4.25a±0.23 | 2.65a±0.52 | 4.50a±0.40 |

* Value followed by different alphabetical notation showed significant difference. If followed by the same alfabed notation shows no significant difference.

### 3.3.2. Flavor and Odor.

Flavor is a sensory impression of smoked fish meat which is determined chemically by taste and smell. Taste is also an attribute of hedonic quality in determining the level of consumer preferences. The taste of the smoke shark combined savory and sour taste. Panelist evaluation results on the taste of smoked shark meat ranged from savory to slightly acidic (2.02) to savory and non-acidic (4.25) ANOVA statistical evaluation results (P <0.01), taste of hedonic quality of smoked shark meat showed a very strong influence. Likewise, the average difference test between the treatments by the Tuckey method (P <0.01) showed a very significant difference between the controls and the time of ozonation immersion (Table 4). The treatment of ozone immersion can reduce acidity. 5 and 10 minutes immersion time of ozone water produces a taste: between 15 and 10 minutes of soaking it is not significantly different. The length of immersing in ozone water (Table 4) produces shark meat with a non acidic savory taste.
The smoking product will produce a specific smoke odor. The shark smoking process will produce a product with a distinctive odor which is favored by consumers. But often smoked shark products are accompanied by a very strong ammoniac odor which consumers dislike. This odor arises because of the high content of urea and dissolved protein in shark meat. The very strong ammoniac smell is the main problem faced by smoked shark producers. The ozonation of the shark meat washing and soaking stages turned out to be able to eliminate the pungent ammoniac odor. The results of the panel evaluation to the odor of the hedonic quality of smoked shark meat by treatment with ozone water immersion ranged from a very strong ammoniac odor (1.35) to less ammonia odor (4.75). ANOVA statistical test results (P <0.01) showed a very real effect. Likewise in the test of the difference among mean by the Tuckey method (P <0.01), between the control treatment and various time of ozone immersion showed a significant difference (Table 4). However, between 5 and 10 minutes time of immersion in water with ozone; between 15 and 20 minutes time of ozone immersion water it was not significantly different (Table 4). The longer the damping time in ozone-containing water, the weaker the ammoniac smell. The products with 15 and 20 minutes immersion in water resulted in no ammonia odor. Ozone acts as a potential oxidizing agent and is able to eliminate odors in food. The ammonia odor of shark meat comes from the change in trimethylamine oxide (TMAO) to trimethylamine (TMA) because it is reduced by spoilage bacteria. [13] In the post rigor phase, TMAO will turn into trimethylamine (TMA), which is said to be a fishy odor and stinging ammonia.

3.3.3. Texture. The results of sensory evaluations by panelists on the texture of smoked shark meat ranged between soft and rather soft. Based on Table 4 it shows that the statistical evaluation with ANOVA (P> 0.01) on the hedonic texture of smoked shark meat did not significantly influence. Test data by panelists showed the hedonic texture value of smoked shark meat ranged from 2.65 to 2.83 with the criteria of being somewhat soft (Table 4). The texture of the smoked shark meat is influenced by the pH value and the moisture content of the smoked shark meat. The acidity (pH) of smoked shark meat will affect its texture. The value of smoke shark meat at pH 5 can affect the water binding capacity of the smoked shark meat which causes changes in the texture of the meat. In accordance to Forrest's opinion in [14] a decrease in low pH (acid) will result in the water binding capacity and meat protein to be a low liquid which causes changes in the texture of the meat. In addition, the texture of the meat is also influenced by the immersion treatment which gives an opportunity for water to absorb in the shark meat.

3.3.4. Appearance. Appearance is quality attribute representing the smoked shark meat appearance as a whole. The value of hedonic quality of smoked shark meat appearance ranged from not bad (2.55) to very good (4.50). The data of appearance attribute tends to be quite similar to the assessment of color. ANOVA statistical evaluation results (P< 0.01) showed a very significant influence. Likewise, in the average difference test between treatment by using Tuckey Test (P<0.01) it showed very significant difference (Table 4), except between control (without ozonation) and 5 minutes of ozone immersion it tends to slightly good (2.55). Meanwhile for 15 and 20 minutes immersion it results in not significantly different hedonic quality appearance with value which tends to be very good (4.50).

4. Conclusion
The time of immersion in ozone water in the process of smoking shark meat showed a very significant effect on total microbial, water content, and parameters of hedonic quality attributes such as color, taste and appearance. However, there was no significant effect on the acidity (pH) and texture of the smoked shark meat. The time of 15 minutes ozone immersion showed optimal results. The immersion treatment of shark meat in ozone water for 15 minutes showed optimal results. This treatment can reduce the total number of microbes by 3 log cycles, from 1.7 X 105 to 9.0x102 CFU / mL, reduce urea levels to 1.82 mg / gram, water content 68.50%, pH 5.14, and improve the sensory quality of smoked shark meat with good performance criteria (4.28), bright brownish white (4.08), no ammonia odor (4.29), savory and non-acidic taste (4.12) and soft texture (2.63). Washing ozonation
methods can inactivate total microbes, reduce urea content, and improve the sensory quality of smoked shark products.

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