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Effect of Brine Time on Quality of Wet-salted Fish

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ABSTRACT

Stingrays or *Dasyatis sp.* is one kind of fishery commodities. This product required careful handling through the handling process. Fish preservation is an alternative that can be applied. This study aimed to protect the fresh fish quality throughout the handling process before it was processed further. This research method used the wet-salting method. The principle of this method was to keep the product for a long time in brine for 0h (control/fresh fish), 1h, 2h, and 3h. The analysis achieve was TVB-N, TMA, TPC, total crude protein, and pH. The effect of time in brine on the result showed significant variation with *p-value* < 0.05. PH value was significantly correlated with TVB-N and total protein, while, TPC was significantly correlated with total crude protein. Fish at 1h in 10% brine gave better results than the two other treatments.

Keywords: salt concentration, stingrays, time in brine, wet-salting method, quality.

1. Introduction

Fresh fish is a very perishable product. In the handling process, preservation is needed to maintain its quality (Gassem, 2019). One type of fish that needs careful handling is stingrays. Stingray can be used as smoked fish. However, stingray easily spoilage in particular after death. This condition is made worse in the handling process. According to Hui (2006), spoilage indicative change quality to unacceptability and change physical characteristics. Some factors that contribute to spoilage of fish are high protein content, moisture content, fat content, and unhygienic handling (Ugochukwu, 2017). PH, protein content, microorganisms, TVB-N (Total Volatile Base Nitrogen) value, and TMA (*Trimethylamine*) value are some indicators Fish Spoilage.

It can be minimized by application preservation techniques. Salting is one method for preserving fish techniques. There are two kinds of salting methods; dry-salting and wet-salting method. This method is a traditional method and has been practiced by people to control fish quality for a long time. Some factors can affecting salting fish quality is freshness fish, kind of salting method (Bellagha, 2007), salting period (Jeyasanta et al., 2016), and salt concentration (Ahmed et al., 2018). In this study, wet-salting is used. These method keeps the fish in brine for a long time (Ugochukwu , 2017). The point of this process is to inhibit fatty oxidation, salt penetration more optimal, and control the salt solution concentration easier (Rahmani et al., 2007). In this process, the fish should be sliced into small pieces. According to Jeyasanta et al., (2016), fish wet-salted were better quality than dry salted.

Therefore, this study was carried to determine the best brine time to inhibit fish spoilage during the handling process, so that the quality of fish can still be preserved. In this study, wet-salting is used. This principle method keep the fish in brine for a long time (Ugochukwu, 2017). This brine is made

from salt and some water. In this process, the fish should be sliced into small pieces. It aims to prevent the formation of microorganisms that affect fish spoilage. Therefore, this study was carried to determine the best brine time to inhibit fish spoilage during the handling process, so that the quality of fish can still be preserved.

2. Materials and Methods

Materials

This study was conducted at the Bioindustri Laboratory, Agro-industrial technology, Brawijaya University. Stingrays or *Dasyatis sp.* were obtained from Brondong, Lamongan. NaCl 94.55-78.56%, PCA (Plate count agar), peptone-water, distilled water, K_2CO_4, TCA, H_3BO_3, HCl, NaOH, HNO_3, indicator PP was purchased from Sigma Aldrich.

Methods

Sample Preparation

Stingrays or *Dasyatis sp.* were obtained from fish markets in Brondong, Lamongan. The samples were transported to the bio industry laboratory within a cooler box. The fish was sliced into small pieces (length 15$\pm$2 cm, wide 10$\pm$2, and weight 250$\pm$20 gram) to make the salt solution easier adsorb by fish meat. Then the sample divided into four groups and will be treated with brine. Fresh fish were analyzed as control (0h).

Wet-salting Process

In this process, a 10% brine was made. 250 mL of sterile water in a volumetric flask (Pyrex) was added 25 gram of salt (NaCl 94.55-78.56%) and homogenized. The fresh sample was placed into glass jars. The Samples were added 50 ml 10% brine and kept for 1h, 2h, and 3 h. Then, samples were analyzed.

TVB-N and TMA Analysis

25 gram sample was weighed and added 75 mL TCA 7%. The sample homogenous used sterile blenders (Electrolux EBR3646). Then, the sample was screened to separate filtrate using Whatman filter paper. TMA and TVB-N value was measured using the method illustrated by AOAC (1999), and Antonocopoulus, 1973 respectively.

TPC (Total Plate Count)

10 gram Stingrays or *Dasyatis sp* were minced in sterile blenders (Electrolux EBR3646) with 90 mL 0.1% sterile peptone water. Similar method were observed by Binici & Kaya, (2018). Then sample diluent 10^{-4} was poured on the PCA. Plates were incubated on incubator (Vision VS1203P3L) for 48 hours at 35 ± 1 °C and the total of microorganism was counted and expresses as colony-forming units per gram (log 10 cfu/g).

PH measurement

The pH was measured using pH-meter (pHTestr 30 Eutech). 10 gram Stingrays or *Dasyatis sp* were minced in sterile blenders (Electrolux EBR3646) with 90 mL sterile distilled water. Samples were homogenized and transferred to pH meter for reading. It taken for 3 times.

Protein Analysis

This analysis used the Kjeldahl method. 2 gram dry samples were added 0.5 mL tablet Kjeldahl and 15 ml H_2SO_4. Then it was destruction for 1 h until the clear solution formed and be chilled. 25 mL distilled water and 100 mL NaOH 40% was added in the solution until a brown solution formed. It was the distillation. Extract that contains 20 mL H_3BO_3 was added indicator PP 3% and titration with HCl 0.1N until pink solution formed. The formula for measure the protein content was used.

Statistical Analysis

Data were analyzed with SPSS 26.00. The LSD test or least significant difference was used for all measures to determine the differences between groups (p value<0.05). A Pearson correlation test was applied to analyze the correlation between parameters.

3. Results and Discussion

Quality of Fresh Stingray

The result showed fresh stingray or *Dasyatis* sp from the local fish market had total crude protein, pH; TVB-N, TMA, and TPC 19.20%; 6.78; 4.52 mgN/100 g; 4.89 mgN/100 g; and 2.4 × 10^5 CFU/ml respectively. These parameters are often used as quality and spoilage indicators and showed that sample has good quality. According to literature, the good quality of fresh fish have total crude protein 16.3-21.7% (Tim Penelitian dan Pengembangan Perkreditan dan UMKM, 2010), pH <8 or neutral (Santoso et al., 2007), TVB-N <30 mgN/100g (Santoso et al., 2007), TMA <15 mgN/100g, and TPC <5×10^5 CFU/ml (Santoso et al., 2007).

Quality of Treated Stingray

As shown in Table 1, Stingray or *Dasyatis sp.* samples with brine time 1 h had significantly lower TVB-N (Total Volatile Base Nitrogen), that from 2 h and 3 h. According to Jeyasanta et al., (2016), TVB-N is produced from the degradation of protein and nitrogen components by microorganism activity. TVB-N can be used as an index shelf life of a fishery product. The low content of TVB-N indicates that the product has good quality. Based on the results of control testing (fresh stingray at 0 h), the value of TVB-N was 4.52 mgN/100g. This value increased to 6.11 mgN/100g, 6.34 mgN/100g, and 7.16 mgN/100g. The increase can occur due to several factors including delay salting period, fish species, salt, and acetic acid concentration (Szymczak & Kołakowski, 2016). The same thing was observed by Jeyasanta et al., (2016). According to Silva et al., (2006), TVB-N value >2-3.6 mgN/100g indicates that the fish is fresh, while a TVB-N value >50 mgN/100g indicates the fish would be rejected for human consumption. The correlation was found between TVB-N and ph. In this study, TVB-N value increased gradually with a delay in mass time and an increase in ph. Based on ph-tvbn2, the pH value depends on the production of volatile nitrogen base.

Results on TPC indicated there was no significant difference. TPC showed increase to 0.537 CFU/ml, 0.54×10^5 CFU/ml, and 0.541×10^5 CFU/ml (1h, 2h, and 3h respectively). Similar result to Besas & Dizon (2012) TPC increase in 10% salt concentration during salt period. This result lower than stingray or *Dasyatis* sp from the local fish market which had TPC 2.4×10^5 CFU/ml. According to Farid et al., (2016) in comparison with fresh fish, total bacteria were decrease because the presence of high salt concentration, so pathogenic microorganism effect and growth is controlled. The correlation was found between TPC and Protein. In this study, the TPC value increased gradually with brine time and decreased total crude protein. According to Wattimena et al., (2017), the activity of microorganisms can increase protein denaturation in fish, resulting in a decrease in protein content.

Table 1. Parameters of *Dasyatis* sp. after salting process

Treatment (hours)	Parameters				
	TVB-N (mgN/100 g)	TMA (mgN/100 g)	TPC (10^5 CFU/ml)	pH	Protein (%)
1	6.11[a]	5.52[a]	0.537[a]	6.93[a]	16.78[a]
2	6.34[b]	5.65[a]	0.54[a]	6.99[b]	16.52[b]
3	7.16[c]	6.71[b]	0.541[a]	7.04[b]	16.42[c]

Remarks: *Mean values within a column followed by the same letters are not significantly different at $p < 0.05$ according to Duncan's Multiple Range Test

Result in Table 1, brine time 1 h had a significantly higher pH than 2 h and 3 h. Similar results were observed by Jeyasanta et al., (2016). pH value is used as an indicator of product quality (Farahita et al., 2012). According to the Asian Food Handling Newsletter, the pH of fresh fish is 7.35. Increased pH value is due to the fish spoilage process. During the decomposition process, nitrogen decomposition will occur by increasing the pH value. The pH value is increased with the delay of salting the fishes. The correlation was found between pH and total crude protein. The decomposition process occurs when the fish die. pH values increase continuously because of protein decomposition by enzymatic digestion (Mueda, 2015).

Similar to the results of TVB-N, the 1 h brine time had significantly higher total crude protein than 2 h and 3 h. The protein content of the salted sample was decreased. The salting time is one of the factors affecting the decrease in protein content (Mukit et al., 2016). Decreased protein levels are due to protein denaturation process. Protein will dissolve into the brine, and it is causing protein losses (Ahmed et al., 2018). The correlation was found between a total crude protein with pH and TPC.

According to Nahar et al., (2017), pH influenced protein solubility. In pH increasing, the activity of microorganisms can increase the hydrolysis of protein components. It begins a decrease of total crude protein content.

4. Conclusions

In this study, the correlation was found in pH with TVB-N and protein, TPC with protein. Stingrays or *Dasyatis sp.* treated with brine and kept it for 1 h more significant than 2 h and 3 h. The result showing, fish was kept for 1 h in 10% brine having significant TVB-N, TMA, TPC ph, and protein value (6.11 mgN/100g; 5.52 mgN/100g; 0.537 x 10^5 CFU/mL; 6.93 and 16.78% respectively).

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