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The effect of wet-salting preservation method on the physicochemical and microbial quality of *Dasyatis* sp.

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KEYWORDS

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Microbial quality
Physicochemical
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ABSTRACT

Microbial *Dasyatis* sp. (stingray fish) was a popular fish in Indonesia. Commonly, this fish is processed into smoked fish and perishable products. Therefore, it needs a preservation method through the handling process. Wet salting was considered as an efficient and inexpensive preservation method. This study aimed to determine the effect of brine concentration on the physicochemical and microbial quality of *Dasyatis* sp. The research used a Completely Randomized Design with factor of brine concentration (i.e. 10%, 20% and 30%). The statistical analysis consists of variance analysis (ANOVA) and followed by LSD or DMRT test (α=5%). The fish sample was soaked in brine solution at different concentrations for one hour. Total crude protein, physicochemical (total volatile basic nitrogen/TVB-N, tri-methyl amine/TMA, and pH), and (total plate count/TPC) were analyzed. The results showed that the brine concentration effect of physicochemical and microbiological of *Dasyatis* sp ($p<0.05$). The best treatment was obtained at the application of 10% brine concentration, which had physicochemical parameters as follows: 6.92 pH, 6.110 mgN/100g TVB-N, 5.520 mgN/100g TMA, 16.78 % protein, and $0.537x10^5$ CFU/ml TPC.

Introduction

Fish is a food source that has a high protein content. *Dasyatis* sp. (local: stingrays fish) contains 16.86% protein (Sulistijowati et al., 2018) and is the most likely fish product by a local community. However, *Dasyatis* sp. is a perishable product (Gassem, 2019; Idakwo et al., 2016) and has a short shelf life (Masniyom, 2011). Several bacteria colony found in the skin and stinger of *Dasyatis* sp. such as *Oceanimonas* sp. and *Sediminibacterium* sp. (Silva et al., 2020). It was the cause of fish quality degeneration. *Oceanimonas* sp. produces enzymes that catalyze urea hydrolysis into ammonia (Yeganeh et al., 2015). Therefore, *Dasyatis* sp. needs further handling, such as food preservation.

Food preservation has a role in improving food quality and shelf life (Amit et al., 2017). Several methods of preserving food, mainly fish products, are curing, salting, drying, and chilling (Ahmed et al., 2018). The processing of *Dasyatis* sp. product is still limited to the curing process. The salting method becomes a potential candidate to be developed as a pre-treatment before the curing process. According to Bakhiet and Khogalie (2012) and Albarracin et al. (2011), the salting process before the curing process can reduce water activity, thereby inhibiting microbial growth (Solomon et al., 2017). The salting method is one of the traditional preserving fish used for long period (Bakhiet and Khogalie, 2012; Immaculate et al., 2016) and consists of wet-salting and dry-salting methods (Thorarinsdottir et al., 2011). The wet salting method is an efficient and inexpensive preservation method and one of the most preferred in food industries.

In the wet-salting method, brine concentration (Ahmed et al., 2018) and the duration salting process (Bellagha et al., 2007) affect the product quality.

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Table 1. Chemical analysis of fresh *Dasyatis* sp.

Parameter	Analysis Result	References
Protein (%)	19.20	16.86**
TVB-N (mgN/100g)	4.52	<30**
TMA (mgN/100g)	4.89	<15*
pH	6.78	<8**
TPC (10^5 CFU/ml)	2.4	<5*

Source: *SNI (Indonesian National Standart)
**Santoso et al., 2007

The effect of the duration salting process on the quality of *Dasyatis* sp. has been evaluated previously. According to Majid et al. (2020), *Dasyatis* sp. preservation with a wet-salting process method for one hour in 10% brine have significant total volatile basic nitrogen (TVB-N), tri-methyl amine (TMA), total plate count (TPC), pH, and protein values of 6.11 mgN/100g; 5.52 mgN/100g; 0.537 x10^5 CFU/mL; 6.93 and 16.78% respectively. However, this study did not evaluate the effect of brine concentration on the quality of *Dasyatis* sp. Therefore, this study aimed to analyze the effect of brine concentration on the physicochemical and microbial quality of *Dasyatis* sp.

Research Methods

Sample Preparation

1.6 kg of *Dasyatis* sp. was chopped into small pieces with length 15±2 cm, wide 10±2 cm, and weight 250±20 g. The samples were frozen and then transported to the Bioindustry Laboratory within a cooler box. Afterward, the sample was washed with water before being used.

Salting Process

The wet salting method adopted SNI 2721.3:2009 (Indonesian National Standard) and Immaculate et al. (2016). 6-10% of brine concentration will prevent spoilage bacteria's action. In this study, the fish was soaked in 10, 20, and 30% of brine solution for one hour. After salting, the wet salted fishes were towel-dried, and excess salt was eliminated in dry salted fishes before analysis. Protein content, pH, TVB-N, TMA, and TPC were analyzed in triplicates.

Protein Content Analysis

The total crude protein of *Dasyatis* sp. was analyzed by the Kjeldahl method (AOAC, 1999) 0.5 mL tablet Kjeldahl and 15 mL H_2SO_4 (Merck) were added to 2 g dry samples. The sample was then destructed for one hour. 25 mL distilled water, and 40% NaOH solution (Sigma Aldrich) were added to the sample until it turned brown. After that, the sample was distilled, and 20 mL, H_3BO_3 3% (Sigma Aldrich), and PP indicator (Sigma Aldrich) were added. The sample was titrated by HCl 0.1 N (Sigma Aldrich) until it turned pink.

Physicochemical Analysis

10 g samples of *Dasyatis sp.* were homogenized in sterile blenders with 90 mL distilled water. The pH value was measured using a pH meter (pHTestr 30 Eutech) (Binici and Kurt, 2017). Total volatile basic nitrogen (TVB-N) was determined on steam distillation using the Kjeldahl distillation apparatus and titration. Tri-methyl amine (TMA) was determined by the Conway micro diffusion method (Cobb et al., 1973).

Microbiological Analysis

Plate count agar (PCA, Merck) was used to obtain the total plate count (TPC). 10 g samples of *Dasyatis* sp. were homogenized in sterile blenders with 90 mL 0.1% peptone water (Merck). The homogenates were serially diluted in the same diluent. 1 mL was taken from the prepared dilutions, and four parallel inoculations were performed using the pour plate method. Plates were incubated on an incubator (Vision VS1203P3L) for 48 hours at 35 ± 1 °C (Binici and Kurt, 2017), and the number of viable microorganisms was counted and expressed as colony-forming units per gram (log 10 CFU/g).

Statistical Analysis

Statistical analysis was performed using SPSS 26.0. The design of the experiment was a Completely Randomized Design. The difference in the means between the groups was analyzed using the LSD test. Duncan's multiple range tests were applied to make multiple means comparison with p-value < 0.05.

Results and Discussion

Table 1 shows the chemical analysis of fresh *Dasyatis* sp. It indicates that the parameters tested

are in close agreement with previous studies. According to some studies, the limit acceptable of protein content, TMA, TVB-N, pH, and TPC in fresh stingray fish was 16.86%, < 15 mgN/100 g, < 30 mgN/100 g, < 8, and < 5×10^5 CFU/ml, respectively. Analysis result of this study shows the parameter on chemical analysis of fresh *Dasyatis* sp. acceptable.

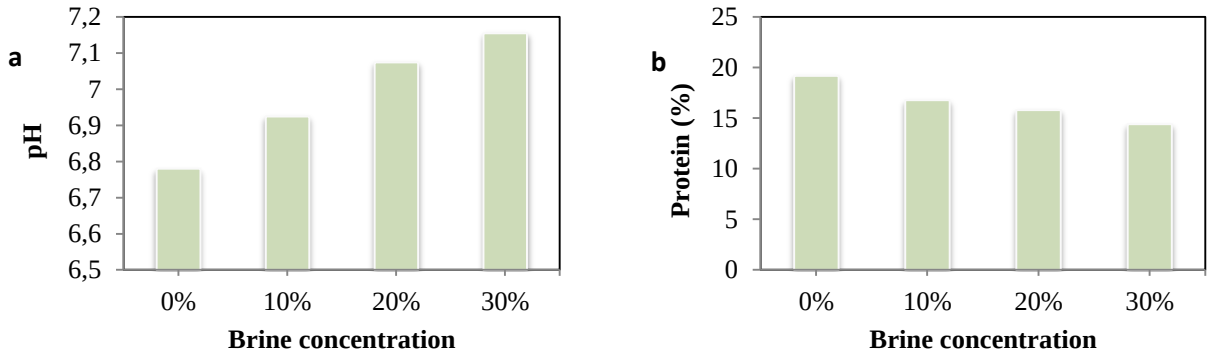

Figure 1. Physicochemical analysis results: (a) pH and (b) Protein

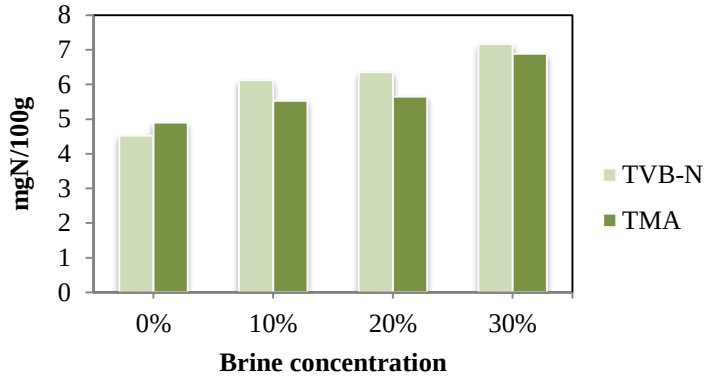

Figure 2. Physicochemical analysis results: TMA and TVB-N

The result of this study showed that brine concentration affects physicochemical (pH, protein, TVB-N, and TMA) and total microbial (TPC) ($p<0.05$). Figure 1a shows the result of the pH analysis. The addition of 30%, 20%, and 10% brine concentrations to pH were 7.155, 7.075, and 6.925, respectively. The pH value after salting performance increases, and it is acceptable. According to Kayim and Can (2010), the pH values of fresh fish are neutral (7.00). The increase of pH value after the fish dies is caused by amino acids dissociation in the presence of salt (Mueda, 2015). A previous study by Immaculate et al. (2016) showed that the increasing pH value is caused by the decomposition of nitrogenous compounds and the autolysis process. The Asian Food handling newsletter reported that the pH of 7.35 is an acceptable upper limit.

Figure 1b shows the protein analysis results. The total protein content after salting indicates a gradual reduction parallel to increasing the brine concentration. The presence of salt to fish has effect on the protein loss, mainly in the wet salting process. It can be influenced by some factor such as the type of fish, the length of time for salting, and salt concentration (Binici and Kaya, 2017). According to Pourashouri et al. (2015) and Latifa et al. (2014), the protein in salted fish was lower than that of the fresh fish. The fish protein is breakdown into amino acids and peptides during storage, thus affecting the protein losses (Lee et al., 2018). According to Mukit et al. (2016), a decrease of protein from 18.29 ± 0.62% to 17.52 ± 0.40 was due to various factors, including protein denaturation throughout the salting process, protein dissolved (Ahmed et al., 2018), and the presence of salting-out (Oliyaei et al., 2019). The presence of salt can affect protein losses (Duong-Ly and Gabelli, 2014); at approximately of 1-5% (Bate-smith and Bendall, 1956).

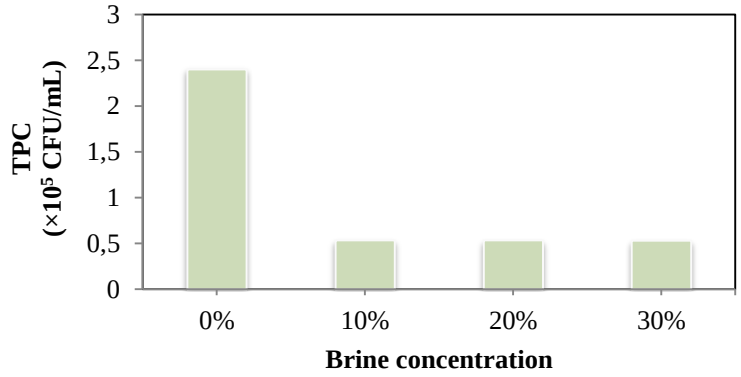

Figure 3. Microbiological analysis results

TVB-N and TMA values in this research increase continuously with salt concentration (Figure 2). This is not in agreement with the study of Besas and Dizon (2016) and Immaculate et al. (2016). In this research, increasing brine concentration causes an increase in the TVB-N and TMA values. The formation of TMA and TVB-N are influenced by various factors, including bacterial growth, fish species, processing methods, and storage conditions (Immaculate et al., 2016).

The content of lipoprotein compounds in the matrix fish is disrupted through enzymatic activity. This has effect on increasing the value of TMAO, which also causes an increase in the value of TVB-N and TMA. The activity of spoilage bacteria and enzymatic activity is known to increase TVB-N and TMA value (Singapurwa et al., 2017).

Figure 3 shows the result of TPC analysis on *Dasyatis* sp. The findings show that fresh *Dasyatis* sp. had TPC ($2.4x10^5$ CFU/mL) higher than *Dasyatis* sp at 10%, 20%, and 30% brine concentrations ($0.537x10^5$ CFU/mL, $0.534x10^5$ CFU/mL, and $0.532x10^5$ CFU/mL respectively). The salting method on *Dayastis* sp. was found to effectively inhibiting microorganisms' growth. The osmotic pressure on the salting method can transport water on microbial cells. This affects microbial destruction. This is supported by Immaculate et al. (2016) that high brine concentration can inhibit spoilage microorganisms' growth.

Conclusion

The physicochemical and total microbial of *Dasyatis* sp was significantly changed during the salting process. It was possibly affected brine concentration and salting duration. The brine concentration of 10% was the most reliable alternative compared to that of at concentration 20% and 30%. The addition of 10% salt concentration found to be more effective to decrease microbes $0.5327x\ 10^5$ CFU/mL with16.78% protein content, 6.110 mgN/100g TVB-N, 5.520 mgN/100g TMA, and 6.92 pH.

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