

## Chemical and microbial changes of stingray fish (*Trygon sephen*) during soaking in salt solution before smoking process

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### Abstract

The salting process at a fisherman's level is still traditional and does not yet have a standard for salt concentration and soaking time, thus affecting the freshness and quality of fish for further processing. For this reason, it is necessary to conduct research that can maintain the quality of fish before the smoking process to ensure results can be recommended for fellow fishermen. This research aimed to find the best quality of low-salted stingray fish (*Trygon sephen*) treated with different salt solution concentrations and soaking time durations before the smoking process. This study used three levels of salt solution concentration (10%, 20%, and 30% w/v, respectively) as the first factor and soaking time duration (1 hr, 2 hrs, and 3 hrs) as the second factor. Chemical and microbial analyses of samples were performed during treatment to investigate their quality. The results showed that stingray fish soaked in 10% salt solution for 1 hr was the best treatment. In this treatment, the pH, total plate count (TPC), total volatile base nitrogen (TVB-N), and trimethylamine nitrogen (TMA-N) values were 6.92,  $4.98 \times 10^5$  CFU/g, 6.12 mg N/100 g, and 5.56 mg N/100 g, respectively. Meanwhile, protein loss in this treatment was 2.66. In conclusion, treatment with 10% salt solution for 1 hr could be used in stingray fish considering that this treatment resulted in good quality with the lowest protein loss. Furthermore, this treatment is used as a reference for the smoking process because the concentration of the salt solution is low and the soaking time duration is short.

## 1. Introduction

Fisheries play a vital role in supporting the nutritional protein supply of Indonesian people. However, the need for protein supply of people in Indonesia has not been fulfilled due to the uneven distribution of fish availability per capita. Processing technology can preserve fish and thus the fish can be distributed from the production centre to other regions.

Stingray (*Trygon sephen*) is one of Indonesia's important economic freshwater fish, although not the main catch. Stingray has a high content of nutrition resulting in the high intensity of the muddy and fishy odour when it is not preserved properly. The presence of this nutrition can strongly affect the flavour and colour of processed stingray meat during storage and affect consumer acceptability (Tam *et al.*, 2003). Traditionally, stingray has been used almost exclusively for smoked

consumption, and some parts have been introduced to the fillet industry. Altintzoglou *et al.* (2014) suggested that the quality of fresh cod fillets is perceived as superior to the quality of fillets preserved using other methods. Due to the high current production volume, this commodity could be processed to prolong its shelf-life prior to distribution and marketing. Thus, specific processes to prevent spoilage and prolong the shelf-life of the processed products should be carried out, such as using salting treatment.

Salting is an ancient form of preservation that is still used today. Salting methods vary greatly across countries, but in general, two methods are commonly employed such as dry salting and wet or pickle curing (Barat *et al.*, 2003). The proportions of salt in fish vary from 10% to 35%. The salt withdraws moisture as it penetrates the flesh and denatures the protein. Nketsia-

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Tabiri and Sefa-Dedeh (1995), reported that muscle protein denaturation facilitated the diffusion of water from fish. Salt uptake depends on many factors, including species, muscle type, fish size, fillet thickness, weight, composition, physiological state, salting method, brine concentration, fish-to-salt ratio, and temperature (Gallart-Jornet *et al.*, 2007).

In the traditional salting process, the type and quality of raw materials are highly variable and control environmental conditions are difficult to control. Processes and procedures are always different according to places, people, and circumstances; thus, the operation cannot be repeated with identical results. Therefore, it is necessary to develop a strategy to improve the salting process. The traditional salting process has resulted in protein loss of up to 5%, depending on salt concentration and salting time (Bellagha *et al.*, 2007).

Several studies reporting the effects of the salting process on the chemical and microbiological properties of several types of fish are available (Thorarinsdottir *et al.*, 2004; Carneiro *et al.*, 2016; Hasan *et al.*, 2019; Goswami and Manna, 2020; Ye *et al.*, 2020; Alak *et al.*, 2021; Auttanak *et al.*, 2021; Crobotova *et al.*, 2021). However, there is little information on the optimum salting concentration and soaking time on stingray fish. Therefore, this study aims to determine the optimum salt concentration and soaking time in stingray fish by evaluating the changes in chemical and microbiological parameters before the smoking process.

## 2. Materials and methods

### 2.1 Materials

Fresh stingray fish (*Trygon sephen*) were obtained from the Central of Fish Auction Center in Brondong, Lamongan, East Java, Indonesia. The fish with a weight of  $250 \pm 20$  g/fish were stored in a cool box with a temperature of 20°C. All chemical reagents used for the parameters studied were reactive grade from Sigma-Aldrich.

### 2.2 Salting process

The stingray fish were cleaned and their gut removed and subjected to different salting concentrations (10%, 20%, and 30% salt solution, respectively) for 1, 2, and 3 hrs, drained and then analysed for their chemical and microbial components.

### 2.3 Analysis of chemical and microbial

#### 2.3.1 Proximate analysis

Proximate (moisture, protein, ash, and fat contents) analyses were carried out according to AOAC (2005). The moisture content was analysed by gravimetry at

105°C. Protein content (% N $\times$ 6.25) was quantified by Kjeldahl. Ash content was determined in a muffle oven at 550°C. Crude fat content was determined by exhaustive Soxhlet extraction with n-hexane. The results were expressed in mass fraction (%) on a dry basis. The carbohydrate content was calculated by reducing moisture, ash, fat, and protein content (by difference). Protein loss was calculated from the differences in the protein content of fresh stingrays before and after the treatment.

#### 2.3.2 Determination of total plate count

Total Plate Count (TPC) was determined according to Arifan *et al.* (2019) with slight modification. Fish samples were taken from each stage of treatment and homogenized with 225 mL of sterile physiological saline. Serial dilutions were carried out with the same diluents. The diluted suspensions were spread plated on a plate count agar. The plates were incubated for 24 to 48 hrs at 36°C. The number of colonies developed on the plates was counted as total bacterial count and expressed as CFU/g.

#### 2.3.3 Determination of pH, total volatile base nitrogen and trimethylamine nitrogen

pH value was determined according to Goulas and Kontominas (2005). The pH meter to be used must be calibrated first using a pH solution of 4.7 and 10. Then, the pH of the fish measured is homogenized and made in the form of a solution. Finally, measurements are carried out with a pH meter by placing the electrodes directly into the fish flesh's suspension in distilled water.

TVB-N and TMA-N assays were conducted according to Conway's method (Jinadasa, 2014). The determination of TVB-N is based on the extraction of TVB using the alkaline solution and the titration of the recovered ammonia. All tests were performed in triplicate and the average values were recorded. The principle of TMA-N determination was similar to TVB-N determination except for the addition of formaldehyde to the sample solution. Formaldehyde was added in order to fix any ammonia present in the sample.

### 2.4 Experiment design and data analysis

This study used a randomized block design with two factors: (1) salt solution concentration (three levels), 10%, 20%, and 30% and (2) soaking duration in salt solution (three levels), 1, 2, and 3 hrs. The experiment was repeated three times. Data were analysed by variance (ANOVA) using SPSS 16.00 software for Windows program at a 95% confidence level. Duncan's test was carried out to determine if there was a significant difference between treatments.

### 3. Results and discussion

#### 3.1 Characteristics of stingray fish

Stingrays for this study were obtained from Fish Auction Center in fresh condition. Fish freshness was one of the considerations for choosing fish when used as a subsequent product. Product quality depends on the quality of raw materials, methods, and storage time. The proximate analysis of fresh stingray fish is shown in Table 1. The fish quality for this study was compared with references, as shown in Table 2.

Table 1. The proximate analysis of fresh stingray fish

No.	Parameters	Composition
1	Protein (%)	19.20±0.04
2	Fat (%)	0.10±0.03
3	Moisture (%)	79.09±0.24
4	Ash (%)	0.18±0.02
5	Carbohydrate (%)	1.43±0.18

Values were expressed as mean±standard deviation.

Table 2. The quality of fresh stingrays for the study was compared with references (fresh fish)

No	Parameters	Composition	Fresh Fish
1	TVB-N (mg N/100 g)	4.52	< 30 <sup>a</sup>
2	TMA-N (mg N/100 g)	4.89	< 15 <sup>a</sup>
3	TPC (CFU/g)	2.4×10 <sup>5</sup>	< 5×10 <sup>5b</sup>
4	pH	6.78	< 8 <sup>c</sup>
5	Protein (%)	19.2	18-20 <sup>c</sup>

<sup>a</sup>Borgstorm (2005), <sup>b</sup>Nasional (2013)-SNI 01-2729-2013, <sup>c</sup>Rehbein and Oehlenschlager (2009).

Table 1 and Table 2 show that the fish used in this study have a good freshness level. Fish quality is one aspect to consider to determine whether the fish can still be consumed or not. Fish is a perishable product, and its freshness should always be maintained for consumers. However, fish has a highly perishable matrix due to high levels of free amino acids and other non-nitrogenous substances utilized to provide nutrients for microbial growth (Monteiro *et al.*, 2018). Due to the abundant endogenous enzymes and psychrophilic bacteria in fish bodies and the fragile organization of fish, fish and fish products are easily perishable. They are affected by pre-harvest and post-mortem factors, thus decreasing their value. Therefore, it is significant to evaluate fish freshness effectively and timely (Wu *et al.*, 2019).

The type of fish, harvest time, and location are the most critical factors affecting the chemical composition of fish. Microbiological changes have a significant role in determining the freshness of fish. Besides, the formation of unwanted tastes and odours is another determining factor (Vasconi *et al.*, 2020).

According to Esposito *et al.* (2018), seafood freshness is a quality key parameter due to the short shelf life of this highly perishable foodstuff in relation to safety, nutritional value, availability, and edibility. The freshness quality of fish plays an important role in human health and the acceptance of consumers as well as in international fishery trade (Cheng *et al.*, 2015).

#### 3.2 Total plate count

TPC result showed the number of microbes contained in stingrays were as shown in Figure 1, which indicated the freshness level of the sample.

TPC values of stingray soaked in various salt solutions for 1 hr ranged from 2.64×10<sup>5</sup> to 4.98×10<sup>5</sup> CFU/g, well below the standard (SNI 01-2729-2013) 5×10<sup>5</sup> CFU/g for fresh fish, except for the control. Furthermore, for soaking times of 2 and 3 hrs, only stingrays soaked in 30% salt solution met the standards for fresh fish. These results indicate that the TPC of stingrays soaked in the salt solution for 3 hrs has increased. According to Jeyasanta *et al.* (2016), fish soaked in a low concentration of salt solution (10% to 33%) still had an increase in the number of bacteria during the salting process. Thus, soaking in a salt solution only inhibits the growth of spoilage microorganisms.

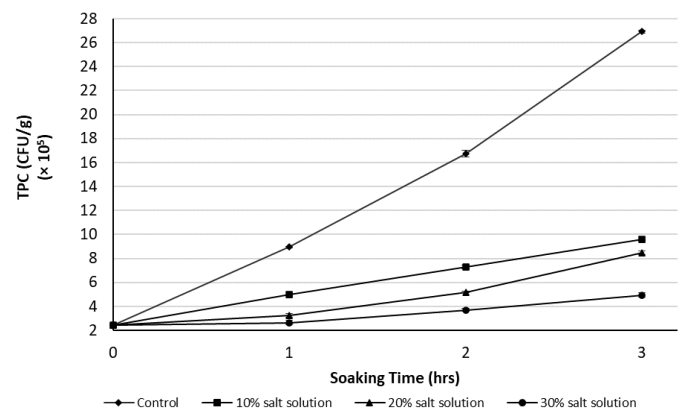


Figure 1. The effect of salt solution concentration and soaking time on the total plate count of stingray

Based on the analysis of variance (ANOVA), the TPC value for all treatments did not show a significant difference ( $\alpha = 0.05$ ). Although it was not significantly different when referring to the freshness of the fish, all treatments at the 1 hr of fish were still considered fresh because the TPC was less than 5×10<sup>5</sup> CFU/g. Duflos *et al.* (2002) reported that the number of microbes in fish flesh only provides general information about the level of contaminants and therefore it should not be used as the only freshness indicator.

#### 3.3 pH value

The pH of fish has been suggested as a good index of

freshness. The profile of pH in this study was represented in Figure 2.

Based on Figure 2, the pH value of stingray soaked in 10%, 20%, and 30% salt solution for 1-hour ranges from 6.92-7.16, for 2 hrs is 6.99-7.19, and for 3 hrs is 7.04-7.24. These results indicated that the pH values of all fish treatments were still within the standard range of fresh fish (Nasional, 2013). The pH value of fresh fish was initially 6.77. However, after that, it increased slowly along with the duration of soaking time in the salt solution.

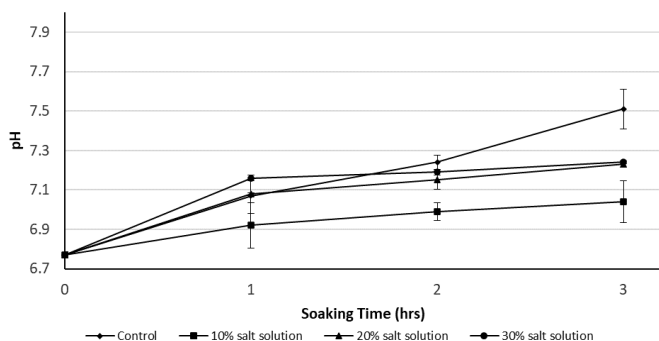


Figure 2. The effect of salt solution concentration and soaking time on the pH value of stingray

This pH value was predicted to be related to the pH of myofibrillar proteins, major proteins in fish muscle, leading to decreased water holding capacity of fish muscle. Ramirez-Suarez and Morrissey (2006) proposed that pH increases due to denatured proteins, resulting in a decrease in amino acid groups. During post mortem period, the decomposition of nitrogenous compounds leads to increases in pH in the fish flesh.

The increase in pH was caused by the formation of volatile alkaline compounds such as Total Volatile Bases (TVB), Trimethylamine (TMA), and ammonia during the fermentation process. According to Lee *et al.* (2016), an increase in pH is caused by autolysis. The autolysis process results in enzymes or microbes breaking into simple compounds (Nurjanah *et al.*, 2020).

### 3.4 Total volatile bases-nitrogen

One of the indicators of fish spoilage is the total volatile bases nitrogen (TVB-N) results. In Figure 3 TVB-N displays the profile of the stingray that was used for this study.

As shown in Figure 3, fish that were not soaked in the salt solution and left at room temperature for 3 hrs had the highest TVB-N values (7.58 mg/100 g, 10.53 mg/100 g, and 14.11 mg/100 g, respectively) than fish soaked in the salt solution.

Based on the analysis of variance (ANOVA), the TVB-N for all treatments did not show a significant

difference ( $\alpha = 0.05$ ). This value shows that the fish is still fresh and suitable to be consumed. Fresh fish stored for 3 hrs had a TVB-N of 14.11 mg/100 g, while fish treated with a salt solution soaked in 10%, 20%, and 30% salt solution have TVB-N of 8.64 mg/100 g, 8.91 mg/100 g, and 10.92 mg/100 g, respectively. This result is supported by Turan *et al.* (2007) that the TVB-N value of fishery products soaked in a salt solution containing 26.4 g NaCl/100 mL water increased during storage. Therefore, all fish samples, either untreated or treated until 3 hrs, are still good or of high quality, because the levels of TVB-N are still below 25 mg/100 g (Ozyurt *et al.*, 2009; Amegovu *et al.*, 2012).

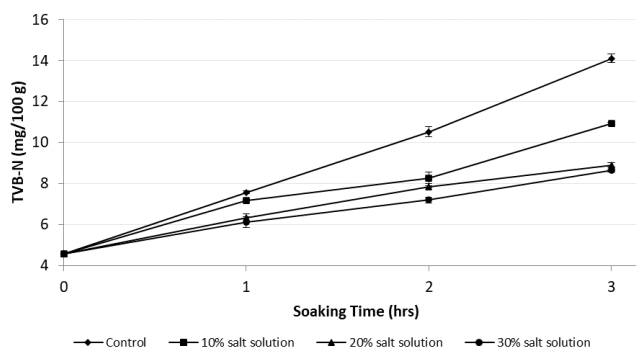


Figure 3. The effect of salt solution concentration and soaking time on the TVB-N content of stingray

Sallam *et al.* (2007) reported that the significant increase of TVB-N was influenced by microbial, autolysis reactions, and changes in Trimethylamine (TMA) to Trimethylamine Oxide (TMAO). TVB is one of the most widely used seafood quality measurements and appears as the most common chemical indicator of marine fish spoilage (Liu *et al.*, 2010).

The TVB-N concentration in freshly caught fish is typically reported to vary between 5 and 20 mg/100 g (Boran and Köse, 2007). The TVB-N increased during storage (Ozyurt *et al.*, 2009). This condition is due to the hydrolyzation of amino acids into  $\text{NH}_3$ . Bellagha *et al.* (2007) explained that salt was used to preserve the fish. It does not protect the bacteria but only inhibits their growth. This bacterial activity can decompose amino acids to become  $\text{NH}_3$ . TVB-N level of about  $\geq 10$  mg TVB-N/100 g flesh could be regarded as the limit of acceptability (Ozogul *et al.*, 2005). Amegovu *et al.* (2012) reported that the growth of spoilage bacteria in fish post-mortem must be handled because these bacteria cause damage to fish flesh.

### 3.5 Trimethylamine-nitrogen

The profile of TMA-N of stingray soaked in the salt solution can be seen in Figure 4.

Figure 4 shows the stingrays soaked in salt solution

at concentrations of 10%, 20%, and 30% for 3 hrs, partial dehydration occurred, and high levels of TMA-N were found. The TMA-N level also increased gradually with salting, but the rate of increase of these values decreased with increasing salt solution concentration.

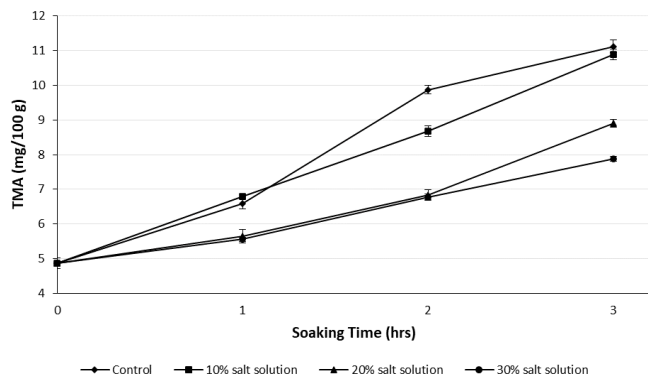


Figure 4. The effect of salt solution concentration and soaking time on the Trimethylamine-N (TMA-N) of stingray

Based on the analysis of variance (ANOVA), the TMA-N for all treatments did not show any significant difference ( $\alpha = 0.05$ ). Although not significantly different, treatment with 30% salt solution for 1 hr, 2 hrs, and 3 hrs had the lowest value compared to the others, i.e., 56 mg/100 g, 6.77 mg/100 g, and 7.88 mg/100 g, respectively. The TMA value for the controlled fish was 11.12 mg/100 g, above the TMA value recommended for fresh fish in international trade. The TMA changes due to the degradation of trimethylaminoxid (TMAO) by microorganisms. According to Erkan (2005), the TMA content of salted cod, which is stable during storage, is high in amine content and is associated with a fishy odour.

TMA can be inhibited by salt concentration and soaking time. The salt content can inhibit bacterial growth and evaporate volatile compounds. This argument is supported by Kolodziejska *et al.* (2002), that reducing enzyme and bacterial activity could be attributed to the preservative effect of salts. Sallam *et al.* (2007) reported that the TMA remains low and does not increase significantly because of the high salt content. The increase in the amount of TMA-N is parallel with the increase in TVB-N during soaking in the salt solution.

The increase in TMA was also influenced by bacterial activity during salting. Dissaraphong *et al.* (2006) said that TMA is caused by the formation of alkaline compounds and bacteria during the fermentation process. According to Jeyasanta *et al.* (2016), the formation of TMA-N is related to many factors such as differences in species, bacterial growth, processing methods and storage condition.

### 3.6 Protein loss

Protein is one of the nutrients found in fish. The processing of fish will result in changes in these nutrients, both in quality and quantity. Changes in protein content and the amount of protein loss in stingrays during the soaking process in the salt solution can be seen in Figure 5 and Figure 6.

Based on Figure 5, it can be seen that salt solution concentration and soaking time affect the protein content. The protein content decreased along with the increase of salt solution concentration and soaking time. The protein content of stingray soaked in 10%, 20%, and 30% salt solution for 1 hr was 16.54%, 16.26%, and 16.24%, respectively, for 2 hrs was 15.70%, 15.63%, and 14.31%, respectively, and for 3 hrs were 14.59%, 13.9%, and 12.80%, respectively. The lowest total protein content was at the salt solution of 30% for 3 hrs. This result indicated the salt solution concentration and the duration of soaking time affect the protein content of stingrays, although not significantly ( $\alpha = 0.05$ ).

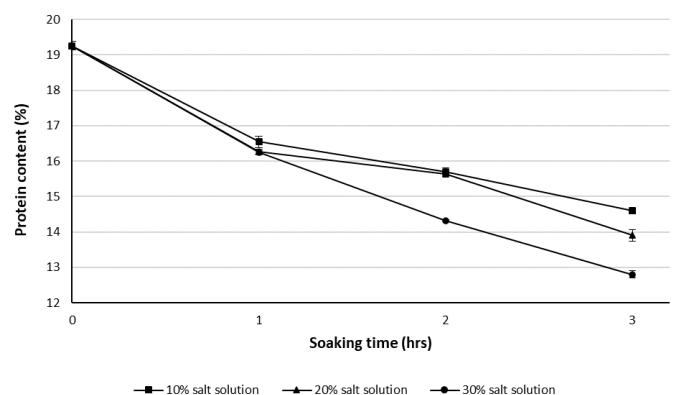


Figure 5. The effect of salt solution concentration and soaking time on the protein content of stingray

Meanwhile, Figure 6 shows the loss of protein content of stingray soaked in 10%, 20%, and 30% salt solution for 1 hr (2.66%, 3.50%, and 4.61%), for 2 hrs (2.94%, 3.57%, and 5.3%), and for 3 hrs (2.96, 3.89%, and 6.40%), respectively. Thus, the lowest protein loss was at the salt solution of 10% and soaking time for 1 hr, i.e., 2.66%. The higher salt concentration causes higher protein loss. This condition may be due to the salting out of fish during soaking.

Chaijan (2011) reported that the salt concentration could extract the protein present in the cell wall. Protein solubility is expected when salts are initially added. High salt concentrations promote the aggregation and precipitation of proteins. This phenomenon is considered to occur due to the disruption of the hydration barriers between protein molecules, as salt causes water surrounding the protein to move into the bulk solution. Additional mechanisms for increasing the affinity of



neighbouring protein molecules, such as direct binding between the salt and protein molecules, may also be involved in salting out (Doran, 2013).

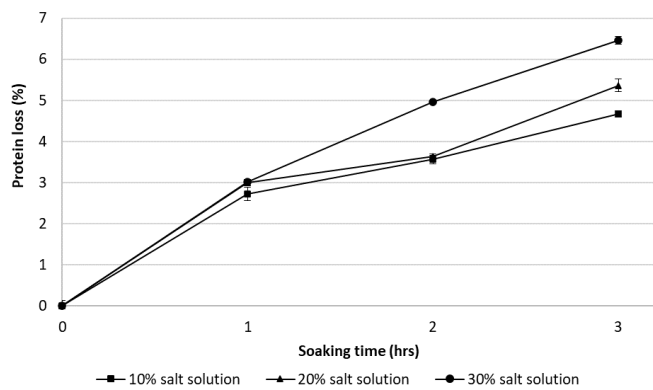


Figure 6. Protein loss of stingray fish after soaked in different salt concentration

On the other hand, the protein that did not denature could be dissolved in the salt solution, leading to higher protein loss during soaking. Within the concentration range of interest, the amount of dissolved protein increased with an increase in the concentration of the salt solution. As the soaking time increased, a significant increase in protein loss could be observed. However, at a more extended period of soaking (3 hrs), protein loss remained almost unchanged because most proteins had already denatured. The protein denaturation was high compared to the protein loss at the same time. Protein denaturation is more sensitive to the salt solution than the dissolution of protein (Niamnuy *et al.*, 2008). It means that soaking fish products in a salt solution can change the quality and quantity of protein.

#### 4. Conclusion

In conclusion, the chemical and microbial changes of stingrays during salting were influenced by salt solution concentration and the duration of soaking time. Stingrays soaked in 10% salt solution for 1 hr have resulted in excellent fish quality due to the condition of fish which is still fresh and the least amount of protein loss. This suggested that a salting process with low salt concentration and short soaking time can be recommended for fishermen before the smoking process.

#### Conflict of interest

The authors declare no conflict of interest.

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