

The Chemical Composition, Microbiology and Micronutrients Changes of Fresh Barracuda Fish and Smoked Barracuda Fish using Different Smoking Methods

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Abstract

Fish play an essential role in human nutrition and ensure about 20% of protein intake for one-third of the world's population, especially in developing countries. Fish is consumed because of its nutritional benefits, such as protein, essential amino acids, fats, and micronutrients (vitamins and minerals). Micronutrients can prevent disease disorders due to micronutrient deficiencies. But behind its nutritional advantages, fish are very easy to spoil. Fish preservation and processing methods explore ways to stop or slow down spoilage. One method of preserving and processing fish that can be applied is smoking. This study aimed to evaluate the moisture content, total fat, heavy metals, vitamin A, and microbiology of fresh and smoked barracuda fish with different smoking methods, namely traditional smoking, and liquid smoke. Fresh barracuda fish is smoked using the traditional smoking method and liquid smoke. Fresh and smoked barracuda fish were then analyzed, including water content, total fat content, heavy metals (Cd, Hg, Sn, As), histamine, micronutrients (vitamins A and D), and microbial contamination. The levels of heavy metals, histamine, and microbial contamination have met the quality standard of smoked fish (SNI 2725: 2013). Vitamin A in fresh barracuda and smoked barracuda was < 15.85 mcg/100 g, while vitamin D was not detected in either fresh barracuda or smoked barracuda.

Keywords: *heavy metals, histamine, microbiology contamination, smoked fish, vitamin*



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INTRODUCTION

Fish play an important role in human nutrition and ensure about 20% of protein intake for one-third of the world's population, especially in developing countries (Bene et al., 2007). Fish is consumed in various parts of the world because of its nutritional benefits, such as protein, essential amino acids, vitamins, minerals, and fats (Geoffroy et al., 2018). Fish protein has a nutritional value which is very important for pregnant women for proper fetal development and will promote proper mental development and immunity to disease among growing children (NAFDAC, 2003). Micronutrients such as vitamins and minerals can also prevent disease disorders

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due to micronutrient deficiencies (Mohanty et al., 2016). But despite its nutritional advantages, fish are very susceptible to spoilage at high environmental temperatures in the tropics within 12-20 hours (Clucas, 1981). Fish preservation and processing methods explore ways to stop or slow down spoilage (Olokor et al., 2007). One method of preserving and processing fish that can be applied is smoking.

Smoking is a traditional fish preservation method that aims to extend the shelf life, improve the taste of the final product, give color and taste to the product, and has a bacteriostatic and antioxidant role (Kristinsson et al., 2008). Smoked fish quality is influenced by raw materials, salting method, salt concentration, smoke composition, and smoking method (Adeyeye, 2016; Alcicek & Atar, 2010; Stolyhwo & Sikorski, 2005). The proximate and fat composition of smoked fish is highly dependent on its content and composition in the fresh fish used for smoking. Other factors that can affect the fat composition of smoked fish are the preparation of smoking raw materials when smoking and storage of smoked fish (Goulas & Kontominas, 2005). Food processing methods have been found to affect the composition of foods as well as to lose nutrients in processed foods. The nature of the diet and the effect of the processing method on the fish to be processed should be considered.

Therefore, this study aimed to evaluate the moisture content, total fat, heavy metals, vitamin A, and microbiology of fresh and smoked barracuda fish with different smoking methods, namely traditional smoking, and liquid smoke.

LITERATURE REVIEW

Fresh barracuda fish weighing as much as ± 10 kg were procured from the Fish Auction Hall in Demak, Central Java, Indonesia, as the raw material. Barracuda fish samples were taken to Diponegoro University, Semarang, Indonesia, for smoking by two different methods.

Smoking Process

Fresh barracuda fish were cleaned and washed using clean water. Then, the fish were gutted. All of the fish that were gutted were then washed and drained. The traditional smoking method used a smoking furnace for approximately ± 15 minutes. The liquid smoke method was carried out by immersing the fish in a 5% liquid smoke solution for 30 minutes, draining it for 30 minutes, then heating it gradually, at a temperature of 40-45°C for 1 hour; 60-70°C for 1 hour; and 90°C for 1 hour (Swastawati et al., 2017).

Moisture and Total Fat Content

The proximate analysis consisting of moisture was carried out according to the AOAC 925.09 2005 method, and total fat content was carried out to the AOAC 960.39 2005. (AOAC, 2005).

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Heavy Metals

Analysis of heavy metals (Cd, Hg, Sn, As) was performed following the methods reported by National Standardization Agency for Indonesia number 2354.5:2011, the testing method using an atomic absorption spectrophotometer (National Standardization Agency for Indonesia, 2011).

Histamine Levels

Histamine analysis was carried out according to the histamine testing from Shimadzu (2013) and Hitachi Technical Data. The analysis begins with the preparation of a standard histamine series of at least six concentration points in the linearity range of 0.1-500 mg/L into a 50 ml volumetric flask. The sample weighed 5 g of the test portion and added 10 ml of TCA 5%, then vortexed. The test sample solution was quantitatively transferred into a 25 ml volumetric flask and sonicated. TCA 5% was added up to the mark and homogenized. The test sample solution was transferred into a 2 ml tube and then centrifuged. Supernatant 1 ml was added with 0.4 ml of 1 N NaOH and calibrated with distilled water, then homogenized. The test sample solution was filtered with a 0.45 µm syringe filter into a 2 ml tube. The series solution and the test sample 200 µL were put into each 2 ml vial, then added 900 µL MPA, 440 µL OPA, and 50 µL AABA, then vortexed. The solution was injected into the HPLC system. Histamine levels are calculated by the following formula:

$$\text{Histamine levels (mg/kg)} = \frac{\frac{(\text{Rasio spl}-a)}{b} \times FP \times V}{W_{spl}}$$

- a = Intercept of the standard calibration curve
- b = Slope of the standard calibration curve
- FP = Dilution factor
- V = Final volume of test solution (ml)
- W_{spl} = Weight of the test portion weighing (g)

Vitamine A and D Levels

Analysis of vitamins A and D was carried out according to the AOAC 2001.13.2011 method procedure (AOAC, 2011). The procedure begins by making a standard series of vitamins A and D, at least six points of concentration in a 10 ml volumetric flask. The solid sample was weighed in a 100 ml glass beaker, then a solution of ethanol 95%, KOH 50%, and pyrrolic acid was added and stirred until homogeneous. The solution mixture was heated in a water bath at 80°C for 45 minutes. Then the solution was cooled to room temperature. The solution was put into a 100 ml volumetric flask and added with glacial acetic acid, and diluted with THF: ethanol (1:1) solution to the mark, then homogenized. The solution was filtered using a 0.45 m GHP/PTFE filter syringe into a 2 ml amber vial, then 20 µL was injected into the HPLC system, with a maximum wavelength of 325 nm for vitamin A and 264 nm for vitamin D. Vitamin levels were calculated using a standard calibration curve, with the equation of the line: $Y = bx + a$, with the following formula:

$$\text{Vitamin Levels (}\mu\text{g/100 g or } \mu\text{g/100 ml)} = \frac{\frac{(\text{Luas area spl}-a)}{b} \times V \times FP \times 100}{W_{spl} \text{ atau } V_{spl}}$$

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- a = Intercept of the standard calibration curve
b = Slope of the standard calibration curve
FP = Dilution factor
V = Final volume of test solution (ml)
 W_{spl} = Weight of the test portion weighing (g)
 V_{spl} = Sample pipetting volume (ml)

Microbiological Contamination

Microbiological analysis of fresh barracuda and smoked barracuda included determination of total plate count (TPC), *Escherichia coli*, and *Salmonella* sp. TPC determination was carried out on fresh and smoked barracuda fish samples using the Petri dish count method based on Indonesian National Standard number 01-2323.3-2015. Analysis of *Escherichia coli* using the most probable number (MPN) method was based on 01-2332.1-2015. Determination of *Salmonella* sp. was based on the method of Indonesian National Standard number 01-2332.2-2006.

RESULTS AND DISCUSSIONS

Moisture and Total Fat Content

The moisture and total fat content of fresh barracuda and smoked barracuda using different smoking methods are shown in Table 1.

Table 1 Moisture and total fat content of fresh barracuda and smoked barracuda

	Treatments		
	Fresh barracuda fish	Traditional smoking methods	Liquid smoke methods
Moisture content (%)	74.35±0.275 ^c	72.02±0.424 ^b	67.75±0.262 ^a
Total Fat (%)	0.32±0.000 ^a	0.73±0.007 ^c	0.64±0.014 ^b

Data are the average of two replication ± standard deviation
Data followed by different letters show significant differences (≤ 0.05)

Different smoking methods were able to reduce the water content of fresh barracuda fish, from 74.35 to 72.02% in the traditional method and 67.75% in the liquid smoke method. The decrease in water content can reduce microbial activity and extend the shelf life of the product (Cardinal, 2001). Smoked barracuda fish in both smoking methods has a water content that still exceeds the Indonesian national standard for smoked fish, which is a maximum of 60% (BSN, 2013). Both smoked fish smoked using traditional smoking methods, and liquid smoke provided higher levels of fat compared to raw materials, which were 0.73 and 0.64%, respectively (Table 1). The increase in fat is caused by the water content lost during the smoking process. Smoking, heating, and high

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salt concentrations can cause chemical and physical changes that increase protein digestibility. This change also reduces thermolabile compounds and polyunsaturated fatty acids (Arason et al., 2014). The increase in total fat content in smoked barracuda correlated with a decrease in water content. An inverse correlation between fat and water content for many fish species has been widely reported (Ljubojevic et al., 2016).

Heavy Metals

The heavy metals analyzed in this study are Hg, Cd, As, and Sn. The heavy metal content of fresh and smoked barracuda fish is showed in Table 2.

Table 2 The heavy metals content of fresh and smoked barracuda fish

Logam berat (mg/kg)	Treatments		
	Fresh barracuda fish	Traditional smoking method	Liquid smoke method
Hg	0.90 ± 0.000 ^b	0.18 ± 0.007 ^a	0.16 ± 0.021 ^a
Cd	ND	ND	ND
As	6.21 ± 0.346 ^a	7.46 ± 0.721 ^a	6.795 ± 0.417 ^a
Sn	ND	ND	ND

ND = Not detected

Data are the average of two replication ± standard deviation

Data followed by different letters show significant differences (≤ 0.05)

Heavy metal levels of Hg in fresh barracuda fish were 0.90 mg/kg, while in traditional smoked and liquid smoked fish were 0.18 mg/kg and 0.16 mg/kg, respectively. The highest concentration of heavy metal As was detected in smoked fish using the traditional smoking method, which was 7.46 mg/kg, and the lowest was 6.21 mg/kg in fresh fish. There was a slight variation in As levels in both fresh and smoked fish, and it was not statistically significant ($p > 0.05$), while heavy metals Cd and Sn were not detected in the three samples. The levels of Hg, Cd and Sn metals are below the maximum limit of the Indonesian national standard, which is a maximum of 0.5 mg/kg while (National Standardization Agency for Indonesia, 2013). Cadmium (Cd) is known as an endocrine disruptor and can cause the development of prostate cancer and breast cancer in humans (Saha & Zaman, 2012).

Histamine Levels

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Histamine analysis results showed that histamine was not detected in fresh or smoked barracuda fish samples (traditional smoking and liquid smoke). This can be caused by histamine levels that are too small because the fresh barracuda fish used as raw material is still in fresh condition. Fresh fish does not contain free histamine but contains the amino acid L-histidine. Histamine is formed in fish by certain bacteria capable of producing the enzyme histidine decarboxylase, which can convert free histidine into histamine (FAO/WHO, 2013). Histamine was not detected in barracuda fish samples smoked using liquid smoke due to the presence of phenolic compounds that act as antibacterials (Dien et al., 2019).

Various countries have set legal limits for the consumption of foods containing histamine. The maximum limit exceeds 50 mg/kg (FDA, 1998), the maximum limit exceeds 100 mg/kg (European Commission, 2003; South African Bureau of Standards, 2001), and the maximum limit for histamine consumption exceeds 200 mg/kg (Australian Food Standards Code, 2001).

Vitamin A and D

Vitamin A in fresh barracuda fish was < 15.85 mcg/100 g, while in smoked barracuda fish smoked using traditional methods and liquid smoke, no vitamin A was detected (Table 3). This is caused by the heating in the smoking process, so that it can cause the loss of important nutrients. Smoking fish contributes to the physical loss of lipids and micronutrients due to the dripping of fat and more water from the fish (Roos et al., 2003). Vitamin A in G. barracuda fish smoked using a smoking drum, and the kiln was 11.41 mg/100 g and 13.93 mg/100 g, respectively (Adeyeye et al., 2017). Vitamin A has antioxidant activity, improves vision and bone growth. Fish species, in general, can easily convert carotenoids into vitamin A (Aremu et al., 2013).

Table 3 Vitamin content of fresh and smoked barracuda fish

Vitamin (mcg/100 g)	Perlakuan		
	Fresh barracuda fish	Traditional smoking method	Liquid smoke method
Vitamin A	< 15.85	< 15.85	< 15.85
Vitamin D	ND	ND	ND

ND = Not detected

Data are the average of two replication

Vitamin D is a fat-soluble vitamin and has two main forms, namely vitamin D2 and vitamin D3. Vitamin D is synthesized in the skin and partly comes from food sources (Macdonald, 2012). Fresh and smoked barracuda fish samples in this study, no vitamin D was detected (Table 3). Previous studies have found that fatty fish, such as salmon, bluefish, mackerel, and tuna, are good sources of vitamin D (Lu et al., 2007). Vitamin D levels in the skin of various types of fish, such as trevally, Atlantic salmon, yellowfin tuna, bream, blackfish, and rainbow trout, are found in the range of 1.8

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to 30 g/100g (Pierens & Fraser, 2015). The level of vitamin content may vary due to several factors such as environment, season, climate, age, nutrition, and species (Mattila et al., 1995). Vitamin D deficiency can be a risk factor for chronic diseases, including cancer, autoimmune, and cardiovascular disease (Grant & Garland, 2002; Munger et al., 2006; Zittermann, 2006). Vitamin D insufficiency (vitamin D serum < 50 nmol/L) has a worldwide prevalence (Holick & Chen, 2008).

Microbial contamination

Microbial quality of smoked fish samples using traditional smoking and liquid smoke methods were 4.9×10^2 colonies/g and 6.0×10^2 colonies/g, respectively. The total number of microbes in barracuda fish after the smoking process decreased when compared to fresh barracuda fish (8.2×10^2 colonies/g). The results of the analysis of variance and Duncan's further test (Table 3) showed that smoked barracuda fish smoked using traditional smoking methods and liquid smoke did not significantly affect the total microbial count. The decrease in the total number of microbes in the sample was caused by the presence of bactericidal and bacteriostatic smoke components (Swastawati et al., 2007). Smoked fish products smoked by different methods (traditional and liquid smoke) have met quality standards because the total plate number value is below the maximum limit of the Indonesian national standard number 2725: 2013, which is 5.0×10^4 colonies/g or with a log value of 4.69 (BSN, 2013).

Table 3 Microbial contamination of fresh and smoked barracuda fish

Microbial contamination	Treatments		
	Fresh barracuda fish	Traditional smoking method	Liquid smoke method
TPC (colony/g)	8.2×10^{2b}	4.9×10^{2a}	6.0×10^{2a}
<i>Escherichia coli</i> (APM/g)	< 1.8	< 1.8	< 1.8
<i>Salmonella</i> sp (negative/g)	Negatif	Negatif	Negatif

Data are the average of two replication

Escherichia coli is an indicator of sanitation. Sanitation facilities affect the presence of *Escherichia coli* bacteria in food. The content of *Escherichia coli* bacteria in smoked fish samples using different smoking methods is < 1.8 MPN/g. This is influenced by the heating process during the smoking process, resulting in the death of *Escherichia coli* bacteria. *Escherichia coli* is a mesophilic bacterium with a growth temperature of 7°C to 50°C and an optimum temperature of around 37°C (Adams & Moss, 2008). Both smoked fish products have met the quality standard of smoked fish on the Indonesian national standard number 2725: 2013 for *Escherichia coli* a maximum of < 3 APM/g (National Standardization Agency for Indonesia, 2013).

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Fresh and smoked barracuda fish samples (traditional methods and liquid smoke) were found to be negative/g on the pathogenic bacteria *Salmonella* sp., according to the Indonesian national standard number 2725: 2013 (National Standardization Agency for Indonesia, 2013). *Salmonella* sp. can grow at an optimum temperature of 37°C and a maximum of 45.6°C. *Salmonella* is sensitive to heat and dies at 70°C, so smoking at 70°C or more is sufficient to kill *Salmonella* bacteria in all parts of the food being cooked (Hu & Kopecko, 2003; Jay et al., 2005).

CONCLUSIONS

Barracuda fish has good potential to be processed into smoked fish. Smoked barracuda fish is smoked with different methods, namely the traditional smoking method and liquid smoke. It is safe for consumption because of the heavy metal content, histamine has met the quality standard of smoked fish. Smoked fish samples also contain microbiological content, such as ALT, *Escherichia coli* bacteria, and pathogenic bacteria *Salmonella* sp. below the limit determined by the Indonesian national standard number 2725:2013. Vitamin A levels in fresh barracuda fish and smoked barracuda fish were < 15.85 mcg/100 g, while vitamin D was not detected in either fresh barracuda fish or smoked barracuda fish.

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