

Fatty acid profile and changes in quality of smoked barracuda fish (*Sphyraena jello*) with different concentrations of liquid smoke

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Abstract

Barracuda fish (*Sphyraena jello*) is an Indonesian marine capture fishery with high economic value and is in high demand among consumers because it has a delicious taste and complete nutritional profile. One of the nutrients contained in barracuda fish is fatty acids. Fatty acids are easily oxidized which causes a rancid odour in fish. The method of processing with smoking using liquid smoke can prevent oxidation in fish caused by the phenol content in liquid smoke which acts as an antioxidant. This study aimed to examine the effect of different concentrations of liquid smoke on the fatty acids of smoked barracuda fish. The experimental design used was RAL (completely randomized design), and analysis of variance (ANOVA) was used to analyze the data, and then the honestly significant difference test was performed. The treatments included the addition of liquid smoke with a concentration of 0% as control, 4%, 6% and 8% with three repetitions. The parameters tested were fatty acid profile, cholesterol content, moisture content, lipid content, phenol content, and organoleptic test. The results showed that applying liquid smoke can retain fat and fatty acids in smoked barracuda fish. The highest fatty acids in smoked barracuda fish were palmitic acid with a value of 1.084-1.618% (%w/w) for saturated fatty acids, oleic acid with a value of 0.469-0.764% (%w/w) for monounsaturated fatty acids, and EPA with values 0.104-0.143 (%w/w) for polyunsaturated fatty acids. The addition of liquid smoke with a concentration of 8% has the highest fatty acid content, producing a total value of 4.759%. The variation in liquid smoke concentration in smoked barracuda had a significant effect ($P < 0.05$) on cholesterol levels, moisture content, protein, phenol, and organoleptic values of smoked fish.

1. Introduction

Barracuda fish (*Sphyraena jello*) is an Indonesian marine capture fishery with high economic value and is in high demand among consumers because it has a delicious taste and complete nutritional profile. In the span of 2014-2018 the production of barracuda fish experienced fluctuations in the amount of production with a total production range of 13,045 tons to 28,932 tons. Every year barracuda fish production has increased with an average increase of 11% (Ministry of Marine Affairs and Fisheries, 2018). Another nutrient content of barracuda fish is fatty acids. Barracuda fish has a high fatty acid content, where unsaturated fatty acids are more dominant than saturated fatty acids in barracuda fish. Fatty acid found in the majority of barracuda fish is docosahexaenoic acid (DHA) of 20.6-21.1%. An essential fatty acid and an omega 3 fatty acid is DHA. Palmitic acid ranks as the second-largest fatty acid

(15.34-16.02%). Palmitic fatty acid is a saturated fatty acid (Rustaiyan, 2013). The content of fatty acids in barracuda fish in addition to having good benefits also has side effects that are susceptible to oxidation. According to Josef *et al.* (2019), Fish are very susceptible to oxidation because they contain fatty acids. The content of unsaturated fatty acids makes meat. Fish are easily oxidized, causing a rancid odor. Fish is one of the most perishable foods due to the well-known lipid oxidation induced by unsaturated carbon bonds in fatty acids, which is the primary driver of food degradation (Xu *et al.*, 2015).

One way of handling fat oxidation is by processing and adding antioxidant compounds. The processing method used is smoking with liquid smoke. The smoking process with liquid smoke is used because the liquid smoke contains phenol which acts as an antioxidant. Antioxidants are compounds that can inhibit oxidation

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(Shahidi and Zhong, 2015; Gulcin, 2020). This fat damage can be prevented by the addition of antioxidants that can inhibit the oxidation reaction. Synthetic antioxidants are added to food to prevent rancidity, most of the synthetic antioxidants are phenolic compounds. One of the ingredients that contain antioxidants is liquid smoke (Pratiwi *et al.*, 2019). Besides phenol, liquid smoke also contains carbonyl compounds and other organic acids.

The content of substances owned in addition to the phenol content is carbonyl substances and organic acids. These substances play a role in forming the characteristics of taste, aroma, color, typical of smoked products and include antioxidant and antimicrobial substances (Lingbeck *et al.*, 2014). These substances can affect the quality and durability of smoked fish products. Condensation from wood containing carbonyl, organic acids, and phenol produces liquid smoke. These three substances help smoked fish products have better antimicrobial and antioxidant qualities. The structure of the characteristics of the produced smoked fish is influenced by carbonyl substances in liquid smoke. (Swastawati *et al.*, 2018). This study aimed to determine whether liquid smoke can act as an antioxidant in smoked barracuda fish (*Sphyraena jello*) by examining the impact of various liquid smoke concentrations on the fatty acid profile and quality of the fish.

2. Materials and methods

2.1 Materials

Material used in this research was barracuda fish (*Sphyraena jello*) obtained from the Rejomulyo Fish Market, Semarang, Central Java, Indonesia with a size of around 300 g. The liquid smoke used was obtained from PT Asap Cair Multiguna.

2.2 Smoking process

The Barracuda fish liquid smoking process was based on previous research (Ghazali *et al.*, 2014; Swastawati *et al.*, 2018). Barracuda Fish were cleaned and gutted, then cut into fillets. each fillet was separated and marinated in a mixed solution of salt and liquid smoke with different concentrations with the same amount of fillets. The concentrations of liquid smoke used were 0%, 4%, 6% and 8%. After that fillets were drained and baked in the oven for 4 hrs at a gradual temperature (50°C and 70°C for 1 hr each, 90°C for two hrs) until the barracuda fish is cooked.

2.3 Fatty acid analysis

The procedure for analyzing fatty acid levels uses fatty acid analysis (AOAC, 2005). The principle of the

test method used is to convert fatty acids into their derivatives such as methyl esters, so that they can be detected by chromatography. The stage of analyzing the fatty acid profile is to extract the fat from the material then methylate it to get the methyl ester from the fat. The extraction step was carried out using the Soxhlet method and then weighing the results of the fat extraction in the form of oil of 0.02-0.03 g. The next step is methylation by refluxing the extracted fat over a water bath with the addition of 1 mL of 0.5 N NaOH into methanol and heated for 20 mins at 80°C. The next step, 2 mL of saturated NaCl and 1 mL of isooctane were added to the sample for homogenization, then the isooctant layer was pipetted into a test tube containing 0.1 g of anhydrous Na₂SO₄ and allowed to stand for 15 mins. Before being put into gas chromatography, the solution was filtered with a microfilter to separate the liquid phase. A flame ionization detector (FID) will be used to detect the fatty acids in the methyl ester, and the response will be recorded through the chromatogram (peak).

2.4 Cholesterol analysis

Analysis of cholesterol levels was based on the Liebermann - Buchard Color Reaction method. The sample was weighed to a maximum of 0.1 g, placed in a centrifuge tube, and mixed well with 8 mL of a 3:1 mixture of petroleum benzene and ethanol. The stirrer was cleaned with 2 mL of a 3:1 solution of ethanol and petroleum benzene before being centrifuged for 10 mins (3,000 rpm). In a water bath, the supernatant was evaporated after being placed into a 100 mL beaker glass. The residue was placed into a graduated tube and gradually evaporated with chloroform (up to a volume of 5 mL). Next, 2 mL of acetic anhydride and 0.2 mL of concentrated H₂SO₄ were added to the residual. The remedy was vortexed and left in a dim environment for 15 mins, then the absorbance was read on spectrophotometry with a wavelength (λ) of 420 nm and the standard used was 0.4 mg/mL. Cholesterol levels are calculated using the following formula:

$$\text{Cholesterol Content} = \frac{\text{Sample Absorbance}}{\text{Standard Absorbance}} \times \frac{\text{Standard Concentration}}{\text{Sample Weight}}$$

2.5 Moisture analysis

Determination of moisture content was carried out using the gravimetric method. The standard value of moisture content in fish is a maximum of 60-65% (BSN, 2006). The process of testing the moisture content is first to adjust the oven temperature to the temperature to be used until it reaches a stable state. After that, the empty dish was baked for at least 2 hrs and then cooled to room temperature for 30 mins. The weight of the empty dish was taken (A) and 2 g of the sample was placed into the

dish with the weight recorded (B). The dish containing the sample was heated in the oven at 95100°C for 16-24 hrs until the weight is constant. Before the final weight (C) was taken, the dish was placed in the dessicator for 30 mins to cool. The moisture content of was calculated using the formula:

$$\text{Moisture Content} = \frac{B - C}{B - A} \times 100\%$$

2.6 Lipid analysis

Sample (W_1) of 2 g was weighed on filter paper and placed into a lipid sleeve. The lipid sleeve was placed into the lipid flask attached to a Soxhlet tube and measured its fixed weight (W_2). The lipid sleeve was inserted into the soxhlet extractor chamber and immersed in the lipid solvent. The test tube was installed in the Soxhlet extraction apparatus, then heated with an electric heater at 40°C for 6 hrs. The collected lipid solvent was distilled until all the solvent evaporated. The lipid bottle was dried in the oven at 105°C, left to cooled in a dessicator at room temperature before measuring its final constant drying weight (W_3). The final result of lipid content was calculated using the formula:

$$\text{Lipid Analysis} = \frac{W_3 - W_2}{W_1} \times 100\%$$

2.7 Phenol analysis

Phenol content was determined by weighing 5 g of the mashed sample into a 100 mL Erlenmeyer flask. The volumetric flask was used to dilute 5 g of the sample with distilled water to 100 mL. Centrifugation of the solution until a clear solution is obtained. A test tube containing 1 mL of supernatant was filled with 0.5 mL of Follin Dennis solution (Follin 1:1), 1 mL of saturated Na_2CO_3 solution, and left to still for 10 mins. Approximately 10 mL of distilled water were added, and the mixture was then stirred in the vortex until it was homogeneous. A spectrophotometer with a 730 nm wavelength was used to read the sample's absorbance. The standard curve equation was used to calculate the phenol content. Gallic acid served as the reference for the standard curve. The concentrations of the standard preparations of gallic acid were 0, 25, 50, 100 and 200 mg/L.

3. Results and discussion

3.1 Fatty acid profile

The results of the fatty acid profile test (Table 1) showed that smoked barracuda fish using liquid smoke contained saturated fatty acids or saturated fatty acid (SFA) which were classified as complete (lignoceric acid, stearic acid, palmitic acid, pentadecanoic acid,

Table 1. Fatty acid profile of smoked barracuda fish (*Sphyaena jello*).

Fatty Acids	0% (% w/w)	4% (% w/w)	6% (% w/w)	8% (% w/w)
Saturated Fatty Acids (SFAO)				
Lignoceric Acid	0.056	0.069	0.061	0.081
Stearic Acid	0.344	0.383	0.372	0.503
Palmitic Acid	1.084	1.221	1.384	1.618
Pentadecanoic Acid	0.033	0.037	0.041	0.045
Myristic Acid	0.133	0.139	0.186	0.158
Lauric Acid	0.016	0.013	0.073	0.001
Arachidic Acid	0.009	0.013	0.014	0.021
Heptadecanoic Acid	0.042	0.046	0.046	0.062
Total	1.717	1.921	2.177	2.489
Monounsaturated Fatty Acids (MUFA)				
Oleic Acid	0.469	0.542	0.616	0.764
Palmitoleic Acid	0.144	0.163	0.223	0.219
Pentadecenoic Acid	0.013	0.014	0.015	0.022
Eicocenoic Acid	0.011	0.013	0.014	0.018
Total	0.637	0.732	0.868	1.023
Polyunsaturated Fatty Acids (PUFA)				
Linolenic Acid	0.015	0.018	0.025	0.018
Linoleic Acid	0.033	0.034	0.04	0.044
Arachidonic Acid	0.097	0.119	0.115	0.132
DHA	0.68	0.826	0.791	0.922
EPA	0.104	0.124	0.143	0.131
Total	0.929	1.121	1.114	1.247
Total Fatty Acids	3.283	3.774	4.159	4.759

myristic acid, lauric acid, arachidic acid, and heptadecanoic acid). The highest saturated fatty acid in smoked barracuda fish based on the test results was palmitic acid with respective values of 1.084%, 1.221%, 1.384% and 1.618%. The highest palmitic acid in the addition of 8% liquid smoke concentration and the lowest in the control treatment. Hadinoto and Kolanus (2017) reported a comparable effect of adding liquid smoke can raise the value of palmitic acid in presto fish.

The test results showed that smoked barracuda fish contained monounsaturated fatty acids (MUFA) consisting of oleic acid, palmitoleic acid, pentadecenoic acid and eicosenoic acid. The detected monounsaturated fatty acid with the highest content was oleic acid with values of 0.469%, 0.542%, 0.616% and 0.764%, respectively. This is similar to the research conducted by Widiastuti *et al.* (2019), The monounsaturated fatty acids detected in fresh cuttlefish consisted of palmitoleic, oleic and eicosenoic acids. Monounsaturated fatty acid (MUFA) in smoked cuttlefish is oleic acid (C18:1) with a value of 22.68%. Oleic acid is the most common unsaturated fatty acid.

Testing the fatty acid profile of smoked barracuda fish with the addition of liquid smoke detected several polyunsaturated fatty acids (PUFA). The fatty acids detected were linolenic acid, linoleic acid, arachidonic acid, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). Leiwakabessy and Wenno (2019) reported similar effect that smoked block dried tuna had of omega 3 fatty acids, namely linolenic acid, EPA and DHA.

The value of the fatty acid profile of saturated, monounsaturated, and polyunsaturated fatty acids increased as a result of the addition of liquid smoke, due to liquid smoke's antioxidant properties. Antioxidants function as substances to slow down or prevent the oxidation process. Among the components that contain antioxidants is liquid smoke. Liquid smoke contains phenolic compounds that can inhibit the oxidation process (Namaskara *et al.*, 2017). phenolic compounds also inhibit the oxidation of fat by preventing the formation of free radicals that have an impact on the prevention of the formation of oxidative off flavors (Angraini and Yuniningsih, 2014).

3.2 Cholesterol analysis

The results of cholesterol analysis test of smoked barracuda fish (Figure 1), indicate that the addition of liquid smoke at 4%, 6% and 8% had different cholesterol values with 0% control. Smoked barracuda fish with the addition of 8% liquid smoke has the lowest cholesterol value with a value of 210.89 mg/100 g, followed by the addition of 6% liquid smoke at 232.42 mg/100 g and 4%

at 273.85 mg/100 g. The highest cholesterol value in the control treatment was 0% with a value of 315.90 mg/100 g. These results indicate that the higher the level of liquid smoke in the product, the lower the cholesterol value in the product. This is in agreement with the research of Daniswara *et al.* (2019), The effect of adding liquid smoke is seen in lowering cholesterol levels in smoked white leg shrimp: the higher the concentration of liquid smoke, the lower the cholesterol content. The diagram of cholesterol analysis of smoked barracuda fish is shown in Figure 1.

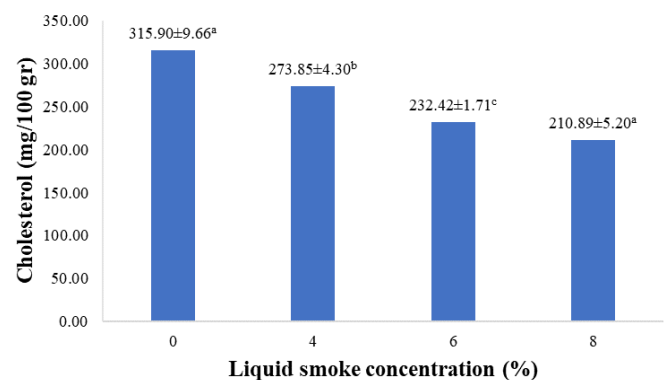


Figure 1. Cholesterol content in smoked barracuda fish (*Sphyraena jello*). Values are presented as mean±SD. Values with different superscripts are statistically significantly different ($p < 0.05$).

Liquid smoke can reduce the value of cholesterol in the smoke fish due of the phenolic chemicals it contains. In liquid smoke, phenol molecules have antibacterial and antioxidant properties. Phenol can break the oxidation of fatty acids with oxygen so that fatty acids in fish will increase. The decrease in cholesterol content can be caused by the administration of liquid smoke in the smoking process, because there are phenolic compounds in the liquid smoke which can break the chain of fat oxidation in the initiation stage. The phenolic properties contained in liquid smoke are antioxidant and antibacterial (Swastawati, 2007).

3.3 Moisture analysis

The results of the moisture content of smoked barracuda fish (Figure 2) imply that the moisture content of smoked barracuda fish could impacted by liquid smoke addition. The results of the analysis of the moisture content of smoked barracuda in 0%, 4%, 6% and 8% treatments were 57.44%, 56.14%, 54.65% and 47.06, respectively. The moisture content of smoked barracuda fish decreases as liquid smoke concentration in the fish increases. Ardianto *et al.* (2014), showed similar results in arabushi with the addition of liquid smoke, showing that the concentration of liquid smoke when immersing in liquid smoke was able to reduce the moisture content of arabushi.

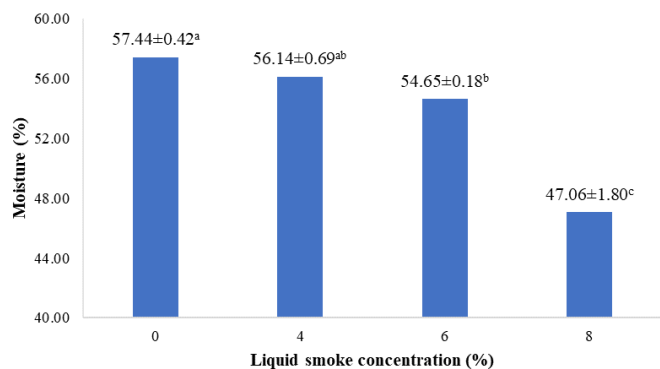


Figure 2. Moisture content in smoked barracuda Fish (*Sphyraena jello*). Values are presented as mean±SD. Values with different superscripts are statistically significantly different ($p<0.05$).

The quantity and concentration of liquid smoke were responsible for the variation in the moisture content of smoked barracuda fish. The content of the moisture content decreases with increasing liquid smoke concentration. This is due to the fact that liquid smoke has the ability to bind any free water that was present in the fish prior to processing. Smoking lowers the pH of flesh, moisture loss, and reactions between phenols, polyphenols, and carbonyl compounds and the amino groups and SH of proteins, respectively (Martinez *et al.*, 2007).

3.4 Lipid analysis

The lipid content results of smoked barracuda fish revealed that each treatment led to appreciable variations (Figure 3). Based on these results, smoking barracuda using liquid smoke can reduce the loss of lipid content in the product and maintain the quality of the lipid content contained. The use of liquid smoke in smoking barracuda fish has the potential to maintain the durability of the product. This is because liquid smoke contains phenolic compounds that function as antioxidants to prevent lipid oxidation. According to Ernawati *et al.* (2012), liquid smoke has phenolic compounds that serve as

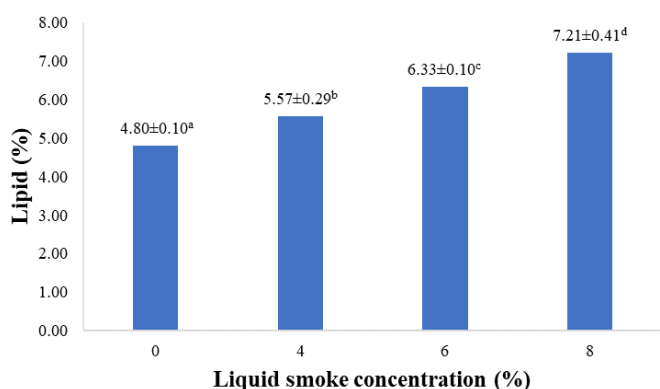


Figure 3. Lipid content in smoked barracuda fish (*Sphyraena jello*). Values are presented as mean±SD. Values with different superscripts are statistically significantly different ($p<0.05$).

antioxidants and can slow down the oxidation of unsaturated fatty acids in products by preventing the formation of hydroperoxides at the propagation stage.

3.5 Phenol analysis

Based on the diagram of the results of the analysis of phenol levels (Figure 4) shows that the lowest phenol content is found in the control treatment (0%) and the treatment that includes the addition of liquid smoke has the highest concentration of phenol at 8%. These results indicate that the higher the concentration of liquid smoke added, the higher the phenol content in the product. This is in accordance with the research results of Widiastuti *et al.* (2019) who reported on the phenol content in smoked cuttlefish with control treatments of 0%, 6%, 12% and 18% respectively were 110 ppm, 175 ppm, 2114 ppm and 5669 ppm, respectively. The addition of 18% liquid smoke was found to have the highest phenol content. The findings demonstrated that the amount of phenol produced increased with the amount of liquid smoke given. This is reinforced by the research of Megawati *et al.* (2014), who showed that Smoked milkfish with the most liquid smoke present contained the highest phenol content.

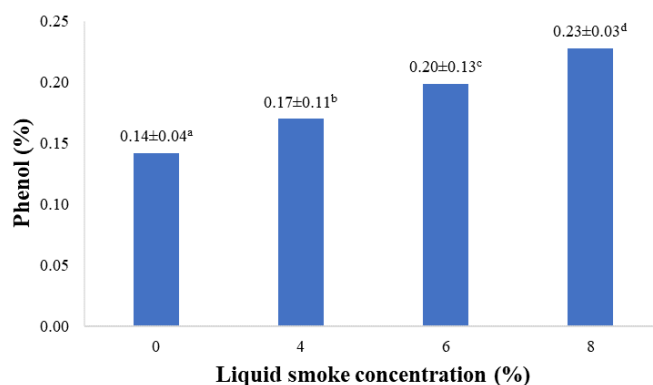


Figure 4. Phenol content in smoked barracuda fish (*Sphyraena jello*). Values are presented as mean±SD. Values with different superscripts are statistically significantly different ($p<0.05$).

4. Conclusion

The addition of different concentrations of liquid smoke affected the fatty acid profile and quality of smoke barracuda fish. The higher liquid smoke concentration, the higher fatty acid profile content, lipid content and phenol content. Furthermore, the higher liquid smoke concentration, the lower the cholesterol content and moisture content.

Conflict of interest

The authors declare no conflict of interest.

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