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ORIGINAL RESEARCH PAPER

FATTY ACID PROFILE OF SMOKED TUNA PROCESSED WITH LIQUID SMOKES

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Abstract: The research aimed to analyze the effect of several different liquid smokes on the fatty acid profile of smoked tuna. The liquid smokes were processed from coconut shells, sago bark, and *Eucalyptus* sp. Fatty acids were analyzed using gas chromatography and the lipid quality of smoked tuna was determined by computing the Atherogenic Index (AI) and Thrombogenic Index (TI). The results showed that the fatty acids profile of smoked tuna contained 28 fatty acids, 11 saturated and 17 unsaturated fatty acids. The polyunsaturated fatty acid (PUFA) content of smoked tuna was greater than the saturated fatty acid and monounsaturated fatty acid contents. The high ratio of PUFA-ω3/PUFA-ω6 and the low value of the AI and TI of the smoked tuna with liquid smoke from sago bark showed that its lipid quality was better than the lipid quality of control tuna and the other smoked tuna.

Keywords: *coconut shell, Eucalyptus, fatty acid profile, sago bark, smoked tuna*

INTODUCTION

Tuna is an important commodity in Maluku Province, Indonesia. It has a high nutritional value. Besides its protein content, tuna is also a source of lipid/fat. It contains omega-3 (ω3) fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) which are important to human health [1]. PUFAs (Long-chain polyunsaturated fatty acids) are essential in preventing and healing the cardiovascular diseases, such as hypertension, arthritis, inflammation, autoimmune disorders and cancer [2].

Mollucas (Maluku people) usually consume fresh tuna and in another processed tuna, like "Ikan Asap" (smoked tuna). Its flesh is tasty and has specific smoked flavors and aromas, and also interesting color combinations ranging from yellow to bronze. However, the limited shelf-life of this traditional smoked product is an obstacle in distribution to a larger market. It needs a lot of efforts to make the smoking process more modern, safer and free of contamination. The use of efficient and effective technology is necessary to achieve this aim. One potential technological improvement is using liquid smoke.

Potential sources of liquid smokes are readily available in Maluku and have already been used to some extent, mainly wastes from agriculture processes such as sago bark, coconut shells, nutmeg, and others. This technology is simple and applicable. Fish flesh is dipped or soaked into the liquid smoke, then heated process to cure the fish and infuse it specific smoked flavors and aromas.

Recently, there are several studies about the application of liquid smoke technology in smoked fish production, for example, its antimicrobial activities and aptitude for impeding the oxidative breakdown of fish lipids [1 – 6]. Some results show that there is an increase in the shelf-life of smoked fish from one-two days up to five days [7 – 9], or even more than one week if combined with vacuum packaging [10 – 12]. To further develop these findings, research on the effect of liquid smoke technology on the nutritional value, especially the profile of fatty acids in this smoked fish, needs to be done. Therefore, the aim of this research was to analyze the effect of several liquid smokes on the fatty acid profile of smoked tuna when the liquid smokes are made from coconut shells, sago barks, and *Eucalyptus* sp.

MATERIAL AND METHODS

Materials and Equipment

The species of tuna used in this research is *Thunnus alalunga*. Fresh tuna was bought from fishermen in Rumah Tiga market, Ambon. The materials used for the liquid smoke were coconut shells, sago bark, and *Eucalyptus* sp. The equipment used in this research area pyrolysator (making the liquid smoke - in the laboratory of Fishery Processing Technology, Fishery and Marine Science Faculty, Pattimura University), redistillation apparatus, analytical balance (Ohauss Adventurer AR2140, USA), water bath (Memmert WNB7, Germany), centrifuge (Hettich Zentrifugen EBA20, Germany), gas chromatography (Shimadzu GC 2010 Plus, USA)

Methods

Liquid smoke is made by separately pyrolyzing the plant materials mentioned earlier in a pyrolator at a temperature of 400 °C, following the methods of Tranggono *et al*. [13]; the pyrolysis products were redistilled using a water bath at a temperature between 100 and 125 °C.
The redistillates of liquid smokes obtained then applied in the smoked fish production process [14]. The steps were as follows: fresh tuna were cleaned, washed, and filleted, then dipped into the liquid smoke of either coconuts shell, sago bark, or *Eucalyptus* at a concentration of 4 % for 10 minutes. As a control, another sample was prepared by the same treatment without dipping the fish into the liquid smoke. The control and dipped fish were then heated in an oven for about 45 minutes until done. Then the fishes were vacuum-packed in plastic and stored at room temperature for further analysis.

Preparation and analysis of fatty acid

Fatty acids were analyzed by using gas chromatography (GC), following the procedure of Integrated Laboratory IPB Bogor, where the composition of the fatty acids are determined as "fatty acid methyl ester" (FAME). FAME formation in the sample is preceded by a hydrolysis process and with esterification. A total of 20-30 samples were placed into a test tube with teflon liner cap which was added with 1 mL NaOH (0.5 N) and heated in a water bath over 20 minutes. Then, 2 mL of BF3 16 % and 5 $mg \cdot mL^{-1}$ internal standard were added and heated again for 20 minutes. The samples were cooled and 2 mL of saturated NaCl and 1 mL hexane were added. The hexane layer was displaced with a pipette into a tube containing 0.1 g Na_2SO_4 anhydride and incubated for about 15 minutes. Then, 5 µL of sample mixed with the liquid phase of FAME standard was injected into a gas chromatography column (cyanopropyl methyl oil, Sigma-Aldrich, USA; 100 m x 0.25 mm I.D., 0.20 um). After that, 5 µL of sample was injected. The operational conditions of gas chromatography were: injector temperature 200 °C, capillary detector (FID Detector 2010 Plus) temperature 230 °C, initial column temperature 190 °C/15 minutes and the final temperature 230 °C/20 minutes, with flow rate column of 10 °C/minute, carrier H_2 gas, H_2 gas flow rate 30 mL/minute, N_2 gas 20 mL/minute, and air flow rate 200 – 250 mL/minute. Then the result was compared with the standard.

$$\mathbf{Cx} = \frac{\mathbf{Ax.R.Cs}}{\mathbf{As}} \quad (1)$$

where *Cx* is the concentration of the component, *Cs* is the concentration of the internal standard, *Ax* is the area of the component, *As* is the area of the internal standard, and *R* is the detector response of the component relative to the standard.
For the external standard method, the preparation was the same but the standard was not added into the sample. The component concentration can be calculated:

$$\mathbf{Concentration} = \frac{\frac{\mathbf{Ax}}{\mathbf{As}} \mathbf{x\,C\,standard\,x\,V} \frac{\mathbf{sample}}{\mathbf{100}} \mathbf{x\,100\%}}{\mathbf{gram\,(sample)}} \quad (2)$$

The measurement was conducted for three times (triple).

Analysis of the lipid quality of the smoked tuna

The lipid quality of smoked fish can be determined by analyzing the fatty acid composition, i.e., by computing the Atherogenic Index (AI) and Thrombogenic Index (TI), which were calculated with equations adopted from Ghaeni *et. al*. [15]:

$$IA = \frac{[(4xC14:0) + C16:0 + C18:0]}{[\Sigma MUFA + \Sigma PUFAn3]} \quad (3)$$

$$IT = \frac{(C14:0 + C16:0 + C18:0)}{0.5xMUFA + 0.5xPUFAn6 + 3xPUFAn3 + \frac{PUFAn3}{PUFAn6}} \quad (4)$$

Statistical Analysis

The Data analysis was performed by *Duncan's Multiple Range Test* (DMRT) ($\alpha = 0.05$) using SPSS 16.0 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

The results of the fatty acid profile of smoked tuna treated with several liquid smokes and analyzed by gas chromatography are shown in Table 1.

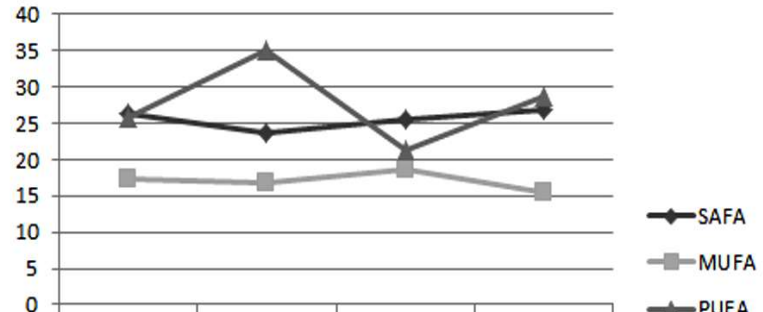
Table 1 shows that the fatty acid of smoked tuna consists of 28 fatty acids, comprising 11 saturated fatty acids and 17 unsaturated fatty acids. Mostly fatty acids showed significantly ($p < 0.05$) different in various smoked treatments, except lauric acid (C12:0), pentadecanoic acid (C15:0), heptadecanoic acid (C17:0), arachidic acid (C20:0), and tricosaenoic acid (C23:0). Similar to the fatty acid composition of other fishery products, saturated fatty acids of smoked tuna are dominated by palmitoleic acid (C16:0) and stearenoic acid (C18:0); the unsaturated fatty acids by oleic acid (C18:1n9), arachidonoic acid (C20:4n6), eicosapentaenoic acid/EPA (C20:5n3), and docosahexaenoic acid/DHA (C22:6n3) [7, 16].

Table 1 shows the presence of some ω3 fatty acids, such as α-linolenic acid (18:3n3), EPA (C20:5n3), and DHA (C22:6n3). It also shows the high quality of lipid nutrition of smoked tuna, like in the lipid of other fishery products as well. The presence of long-chain polyunsaturated fatty acids (PUFAs) such as EPA and DHA show the unique feature which distinguishes the lipid between aquatic animal and land animal [3, 13]. Lipids of smoked fish are a good source of PUFA ω3 fatty acids [14]. The saturated fatty acid (SAFA), monounsaturated fatty acid (MUFA), and PUFA of smoked tuna processed with liquid smokes are shown in Figure 1.

Table 1. *Fatty Acid Composition of Smoked Tuna Processed with Several Liquid*

Fatty Acid	Smokes			
	Without Liquid Smoke (Control)	Sago Bark Liquid Smoke	Coconut Shell Liquid Smoke	Eucalyptus Liquid Smoke
Lauric Acid-C12:0	0.04a	0.04a	0.06a	0.06a
Myristic Acid-C14:0	1.69b	1.85b	1.10c	2.40a
Pentadecanoic Acid-C15:0	0.46a	0.49a	0.26a	0.50a
Palmitic Acid-C16:0	15.18b	14.34b	16.78a	14.64b
Heptadecanoic Acid-C17:0	0.69a	0.77a	0.47a	0.78a
Stearic Acid-C18:0	6.89a	5.39b	5.77b	7.08a
Arachidic Acid-C20:0	0.38a	0.35a	0.35a	0.43a
Heneicosanoic Acid-21:0	0.07ab	0.05b	0.05b	0.08a
Behenic Acid-C22:0	0.29ab	0.21c	0.24bc	0.33a
Tricosaenoic Acid-C23:0	0.12a	0.07a	0.09a	0.11a
Lignoseric Acid-C24:0	0.37a	0.22c	0.29b	0.35ab
Miristoleic Acid-C14:1	0.02b	0.03a	0.00c	0.02b
Palmitoleic Acid-C16:1	3.16b	2.89c	1.35d	3.65a
Elaidic Acid-C18:1n9t	0.10b	0.20a	0.07b	0.1b
Oleic Acid-C18:1n9c	12.82b	11.93c	16.25a	10.52d
Linoleic Acid-C18:2n6c	0.95c	0.93c	5.32a	1.11b
γ- linolenic Acid-C18:3n6	0.08b	0.07c	0.04d	0.11a
Eicosenoic Acid-C20:1	0.50b	1.23a	0.60b	0.44b
Linolenic Acid-C18:3n3	0.42b	0.24c	0.61a	0.33bc
Eicosedienoic Acid-C20:2	0.18b	0.29a	0.15b	0.22ab
Eicosetrienoic Acid-C20:3n6	0.00d	0.10b	0.08c	0.16a
Erucic Acid-C22:1n9	0.08b	0.00c	0.08b	0.11a
Eicosetrienoic Acid-C20:3n3	0.09b	0.53a	0.11b	0.09b
Arachidonic Acid-C20:4n6	2.23c	2.94a	1.53d	2.55b
Docosadienoic Acid-C22:2	0.00b	0.03a	0.03a	0.03a
Eicosapentanoic Aci-C20:5n3	3.56c	5.27a	2.17d	4.46b
Nervonic Acid-C24:1	0.72a	0.59c	0.46b	0.65ab
Docosahexanoic-Acid-C22:6n3	18.41c	24.67a	11.27d	19.58b
SAFA	26.19a	23.78b	25.46a	26.76a
MUFA	17.40b	16.87b	18.81a	15.49a
PUFA	25.92c	35.07a	21.31d	28.64b
n-3 PUFA	22.48c	30.71a	14.16d	24.46b
n-6 PUFA	2.31c	3.11a	1.65d	2.82b
n-3/n-6	9.72a	9.87a	8.59b	8.68b
Fatty Acid Total	69.51b	75.72a	65.58c	70.89b

[a-d]Different letters in the same row show significant differences ($p < 0.05$) according to Duncan's Multiple Range Test.



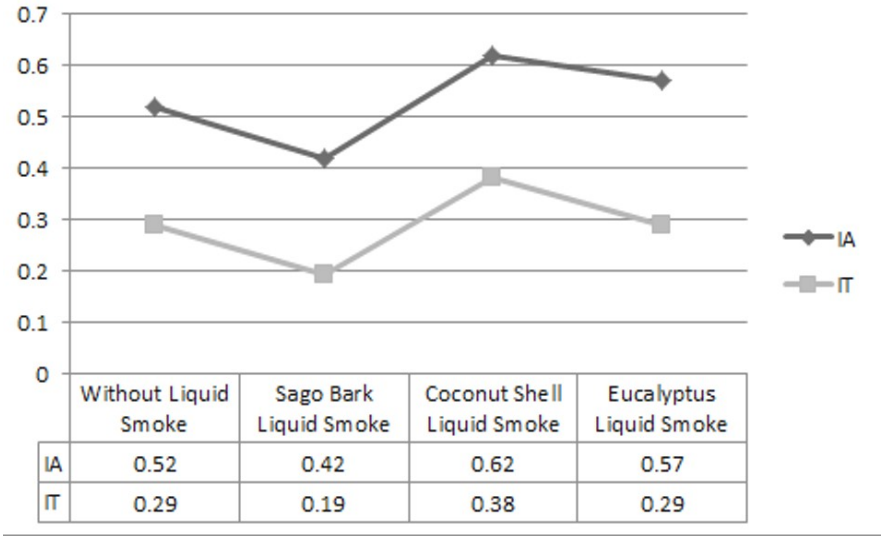
	Without Liquid Smoke	Sago Bark Liquid Smoke	Coconut Shell Liquid Smoke	Eucalyptus Liquid Smoke
SAFA	26.19	23.78	25.46	26.76
MUFA	17.4	16.86	18.66	15.49
PUFA	25.92	35.08	21.25	28.64

Figure 1. *Fatty acid profile (%) of smoked tuna processed with several liquid smokes*

Figure 1 shows that PUFA in smoked tuna is greater that SAFA and MUFA except in smoked tuna treated with coconut shell liquid smoke. SAFA-content of the control, sago bark, coconut shell, and *Eucalyptus* treatment were 26.19 %, 23.78 %, 25.46 % and 26.76 %, respectively. The MUFA content of control, sago bark, coconut shell, and *Eucalyptus* treatment were 17.4 %, 16.86 %, 18.66 % and 15.49 %, respectively. The PUFA of these treatments were 25.92 %, 35.08 %, 21.25 % and 28.64 %, respectively. SAFA in fish usually ranged from 15-20 %, MUFA from 35-60 %, and PUFA from 24-40 % [16]. PUFA in smoked tuna was greater than MUFA though both of them is unsaturated fatty acid. PUFA in tuna used in this research naturally is greater than MUFA so it resulted that PUFA was greater than MUFA. Some species of tuna such as yellowfin tuna, tuna, and frigate tuna have PUFA contents that are greater than SAFA and MUFA [11].

Figure 1 also shows the different values of PUFA content among the four treatments. PUFA content of smoked tuna with sago bark and *Eucalyptus* treatment were increased over the control treatment, while in the coconut shell treatment PUFA content was decreased. This could be caused by the physical characters of fatty acids, unsaturated or the saturated, which can be converted by heat during the smoking process [17].

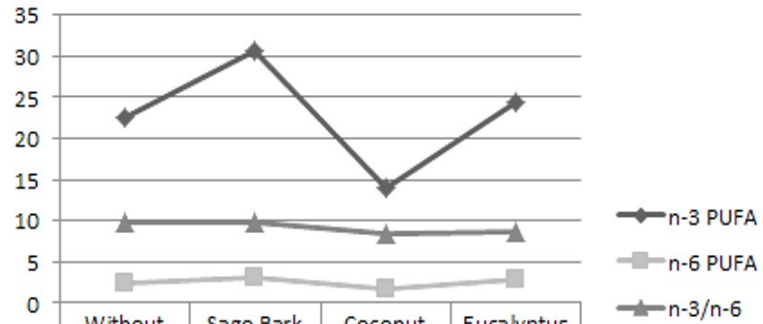
The boiling point of saturated fatty acids is higher than unsaturated fatty acids for the same chain. Unsaturated fatty acids with a great amount of covalent bond have low boiling points, therefore, will have lower viscosity and boiling points than the saturated fatty acid with same chain length [5]. The differences in fatty acids of smoked tuna may be caused by the type of liquid smokes used in this research, components of which, such as organic acids, carbonyl, or phenol and its derivates, may have different penetration rates. For instance, the penetration rate of the components from sago bark is faster than the penetration rate of the components from coconut shells, *Eucalyptus*, canary shells, and nutmeg shells [17]. Organic acid components identified in several liquid smokes are propanoic acid, butanoic acid, myristic acid, lauric acid, palmitic acid, and other fatty acids [18, 19]. These organic acids probably give effect to the trend seen here of fatty acid compositions of smoked tuna (Figure 2).



	Without Liquid Smoke	Sago Bark Liquid Smoke	Coconut Shell Liquid Smoke	Eucalyptus Liquid Smoke
IA	0.52	0.42	0.62	0.57
IT	0.29	0.19	0.38	0.29

Figure 2. *Index of lipid quality of smoked tuna processed with several liquid smokes*

PUFAs-n3 of fish, mainly EPA and DHA have been mostly researched because of their effect on preventing cardiovascular diseases; arachidonic acid is a PUFA-n6, which needed in body and brain growth processes, and essentially maintains skin tightness [20]. PUFA-n3 and PUFA-n6 profiles of smoked tuna processed with several liquid smokes are showed in Figure 2. PUFA-n3 and PUFA-n6 concentrations in smoked tuna processed with liquid smoke from sago bark were higher than those in control, coconut shells, and *Eucalyptus* treatments. This same trend was seen in the PUFA-n3/PUFA-n6 ratios shown in Figure 3; PUFA-n3/PUFA-n6 ratio of smoked tuna treated with liquid smoke from sago bark is 9.88 %, higher than those in control, coconut shells, and *Eucalyptus* treatments, 9.73 %, 8.55 % and 8.67 %, respectively. The high value of the ratio of PUFA-n3/PUFA-n6 shows that the lipid quality of smoked tuna processed with liquid smoke from sago bark is better than when processed with liquid smokes from coconut shells and *Eucalyptus*, as well as the control treatment.



	Without Liquid Smoke	Sago Bark Liquid Smoke	Coconut Shell Liquid Smoke	Eucalyptus Liquid Smoke
n-3 PUFA	22.48	30.72	14.1	24.48
n-6 PUFA	2.31	3.11	1.65	2.82
n-3/n-6	9.73	9.83	8.55	8.67

Figure 3. *PUFA fatty acid profile of smoked tuna processed with several liquid smokes*

This conclusion is also supported by the values of AI and TI for the smoked tuna shown in Figure 2. This figure shows that AI and TI values of smoked fish processed with sago bark liquid smoke (0.42 and 0.19, respectively) were lower than coconut shells (0.62 and 0.38), *Eucalyptus* (0.57 and 0.29), and the control (0.52 and 0.29). The low AI and TI values of smoked tuna processed with liquid smoke from sago bark showed that the lipid quality of the smoked tuna was better than those processed with coconut shells or *Eucalyptus* liquid smokes, as well the control treatment.

Foodstuffs are suggested to have the highest quality when the values of AI and TI are relatively lower [21]. In other words, the values of AI and TI are taken to determine the lipid quality of foodstuffs including fish and other fishery products [7, 8, 14].

The AI value describes the relation between the number of main saturated fatty acids and unsaturated acids, which are known as pro-atherogenic and anti-atherogenic, respectively. Pro-atherogenic fatty acids are thought to be involved in lipid adhesion processes in immune cells and blood circulation, while anti-atherogenic fatty acids hold up plaque aggregation and minimize esterified fatty acid percentage, cholesterol, and phospholipids; so that the coronary disease can be prevented. The TI value describes the relation between saturated fatty acids (pro-thrombogenic) and unsaturated fatty acids, known as anti-thrombogenic, by their tendency to prevent the blood coagulation [14, 21, 22].

Overall, the result of this research suggests that the low values of saturated fatty acids and the high value of unsaturated fatty acids in the smoked tuna processed with liquid smoke from sago bark, and also its low AI and TI values, shows that it is better for human health compared to liquid smokes from coconut shells and *Eucalyptus*, or smoked tuna without liquid smoke use. Foods with lower saturated fatty acids provide more benefits, mostly for heart health, compared to those with higher saturated fatty acids [23 – 25].

CONCLUSION

The fatty acids profile of smoked tuna with several liquid smokes contains 28 fatty acids, consist of 11 saturated fatty acids and 17 unsaturated fatty acids. The polyunsaturated fatty acid (PUFA) content of smoked tuna was greater than the content of saturated fatty acids (SAFA) and monounsaturated fatty acids (MUFA). The high ratio of PUFA-ω3/PUFA-ω6 and the low value of the Atherogenic Index (AI) and Thrombogenic Index (TI) of smoked tuna with liquid smoke from sago bark showed that its lipid quality was better than of the control tuna (with no liquid smoke) and the smoked tuna with the liquid smoke made from either coconut shells or *Eucalyptus*.

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