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Physico-Chemical Characteristics, Fatty Acid Profile and Polycyclic Aromatic Hydrocarbon of Skipjack Tuna *(Katsuwonus pelamis)* Smoked in Smoking Material of Nutmeg Shells for Different Duration in Bitung Municipality, North Sulawesi Province

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Abstract : This study was aimed to analyze the physico-chemistry, the profile of fatty acid, the polycyclic aromatic hydrocarbon of skipjack tuna smoked with nutmeg shell as smoking material. Smoking was carried out in different duration, 3, 6, 9, 12 and 15 hours, respectively. Results showed that 15-hour smoking duration gave the lowest water content and A_w and the highest protein level ($P<0.05$).Moreover, the fatty acid profile of the skipjack smoked for 15 hours exhibited the lowest *Saturated fatty acid* (SFA)and*Monounsaturated fatty acid* (MUFA) and significant difference ($P<0,05$), the highest *Polyunsaturated fatty acid* (PUFA).

Keywords : nutmeg shell, skipjack smoking, fatty acid profile,polycyclic aromatic hydrocarbon.

Introduction

Smoked fish is one of the oldest fish preservation methods, and direct heat curing is a traditional method commonly practiced in tropical countries. Nevertheless, there are various smoking methods in different countries or different areas of the same country. These different methods could result from different fish species and different end products expected [1]. Several phenolic, formalidehyde, and other compounds of the smoke stay in the fish flesh and function as preservative to extend the storage duration of the end product and typical good flavor, savory with peculiar aroma produced by the smoking process [2,3,4]. Skipjack tuna (*Katsuwonuspelamis*) locally called "cakalang" is one of the most popular fishes preserved using traditional smoking method in Bitung Municipality, North Sulawesi.Several factors could influence the characteristics of the smoked fish, such as smoking materials[3,5,6], fish shape smoked[7], fish sampling location, sampling season, and smoking method[8].

Indonesia possesses numerous natural wood and agricultural waste sources, such as teak wood, bangkirai, leucaena, coconut shell, rice straw, corn cob, potentially used as smoking materials[9].Also, there are many types of timber and other agricultural wastes distributed in all areas of Indonesia, and particularly North Sulawesi, such as nutmeg shell, coconut coir, and hazelnut skin.Beside smoking material potency, Indonesia also possesses high fisheries resources potency with high fishspecies diversity. If both potencies is synergized it will certainly be able to give functional and economic products, such as smoked fish. The smoking process in Bitung municipality still uses coconut coir as smoking material and nutmeg shell as wastes of nutmegstripping. Thus, netmeg shell could be used as smoking material to obtain specific smoked fish product.

Chemical compounds of wooden smoke are generally phenol (functioning as antioxidant), organic acid, alcohol, carbonyl, hydrocarbon and nitrogen compounds, such as nitrooxide[10], aldhehyde, ketone, ester, ether, adhering on the surface and penetrating the fish flesh[11].Types of wooden smoking material result in different complex chemical components, as mixtures of various volatile and non-volatile compound structures with various sensory characteristics, such as phenol, syringol and guaiacol and their derivates[12].

Information on smoked skipjack characteristics using nutmeg shell as smoking material under different smoking duration has not been available yet. Hence, this study was aimed at providing information on physico-chemical characteristics, polycyclic aromatic hydrocarbon, and fatty acid profile of the skipjack tuna smoked with nutmeg shell material under 3, 6, 9, 12, and 15-hour smoking duration.

Materials and Method

Fresh skipjack tuna (*Katsuwonus pelamis*) samples were collected from skipjack producer group and nutmeg shells were obtained from nutmeg farmers around Bitung Municipality, North Sulawesi. The fish used had mean weight of 2.5 kg/ind., cleansed and gill and intestine discarded. The fish were divided by half and clipped within bamboo sticks.Fifty individuals of skipjack tuna were smoked using nutmeg shell smoking materials for 3, 6, 9, 12 and 15 hours. Smoking process was done in a 6m x 4m x 60cm-smoking chamber. The smoking treatment needed 300 kg of nutmeg shell per turn to have the smoked fish product.The smoking processes were run in3, 6, 9, 12, and 15 hours, respectively, until the fish were cooked and the fish color turned to golden silver to golden yellow. The smoked fish products were then characterized their physico-chemistry, fatty acid profile, and polycyclic aromatic hydrocarbon. Their physico-chemical characteristics, water content, protein, fat and ash, were determined following AOAC (2005) method, and $a_w$following Fuentes *et al*., (2010).Fatty acid profile and polycyclic aromatic hydrocarbon determination used Gas Chromatography (GC 210A SHIMADZU).

Sample Preparation

Ten grams of sample were homogenized in10 ml of concentrated HCL, heated in a water bath at 70°C until boilingabout 30 minutes, cooled, extracted with 25 ml of diethyl ether, then vortex. Add with 25 ml of petroleum benzene, then vortex. Separate the clear upper part, poured into a 100 ml flask, and evaporate in the water bathat 60°C by flowing nitrogen gas (N_2).Add ± 3 ml of 0.5 N Sodium methanolic,heated in the water bath at 60° C for about 10 minutes, and after cooled, add 3 ml of 20% BF_3-$CH_3$0Hsolution. Reheat it in the water bath at 60°C for about 10 minutes and cooled.Methyl-esther formed was then extracted with 1 ml of n-Heptane (vortex) and added with 2 ml of saturated NaCl, so that 2 layers were formed.One-μl ofthe upper layer was injected into GCat the running condition of 140^{0}Cinitial temperature programmed by 10°C/min. increment and final column temperature reached 260^{0}C.

Polycyclic Aromatic Hydrocarbon (PAH) Analytical Procedure:

Twenty grams of samples were put into a 250 ml-ball flask, added with 30 ml of 50% KOH, heated in the water bath at 70^{0}Cfor 30 minutes, and cooled.The samples were extracted with 25 ml of Dichlor Methan (CH_2CL_2) twice (the extractant was put together), evaporated up to reaching the end volumn of 5 ml, and pass it up through Catridge Florisil previously activated with methanol, eluted with 10 ml of methanol, then evaporated to drought.Redissolve in 1 ml of CH_2CL_2 (Dichlor methane), and inject 1 μl into GC at running condition of 180^{0}C programmed by 10^{0}C/min- increment andreached column final temperature of 315^{0}C.

Statistical Analysis

Data was analyzed using One-Way ANOVA was used to analyze the effect ofsmoking duration on the physico-chemical composition, the fatty acid profile, and the polycyclic aromatic hydrocarbonof the skipjack tuna (*Katsuwonus pelamis*)smoked using nutmeg shell.The statistical analysis used version 20-SPSS Software (Chicago,IL, USA). The value was expressed as mean and standard deviation at P<0.05.

Results and Discussion

Mean and standard deviation of the variables under different smoking duration are presented in Table 1.

Table 1. Chemical content with smoking duration.

Smoking Duration	Chemical parameters				
	A_w	Water content (%)	Protein (%)	Fat (%)	Ash (%)
3	0.94±0.010	56.34±0.15	40.21±0.020	1.30±0.10	1.35±0.015
6	0.93±0.015	43.98±0.010	50.76±0.015	1.68±0.010	1.79±0.010
9	0.92±0.010	38.01±0.38	55.74±0.020	1.99±0.006	2.81±0.015
12	0.89±0.010	32.50±0.010	60.22±0.021	3.07±0.010	3.18±0.021
15	0.85±0.015	28.89±0.015	63.02±0.036	2.54±0.010	3.97±0.10

Note: The same alphabetic notations in the same column indicate no difference between the treatments (P>0.05)

The $a_w$values of the smoked fish ranged from 0.85 to 0.94, in which the lowest $a_w$occurred in 15-hour smoking, 0.85, and the highest one in 3-hour smoking, 0.94, since longer smoking duration could cause higher evaporation of the fish water content. According [13], smoking duration, smoking room humidity, and smoked material-watercontent interaction could affect the a_w of the end product.

Water content of the smoked skipjack tuna under different smoking duration ranged from 28.89% to 56.34%. The highest water content, 56.34%, occurred in 3-hour smoking and the lowest one, 28.89%, in 15-hour smoking.It could result from smoking duration and number of smoking material used.According[14], the smoking duration and the smoking material used is assumed to be able to increase in the smoke component sticking on the fish so that the taste and the aroma could also be different among smoking durations and cause the loss of water content of the smoked fish as a result of heat and water removal from the fish tissue through absorption of various smoke chemical compounds.Furthermore, [15]found that the product of smoking process could remove the water content of the fish flesh up to certain level so that it could stop the microbial activities.Different smoking duration could influence the water content of the fish flesh, in which the higher the smoking durationthe more water content is released[14].

Protein level of the smoked skipjack under different smoking duration ranged from 40.21% to 63.02%. The highest protein content of the smoked skipjack, 63.02%, was recorded in 15-hour smoking and the lowest, 40.21%, in 3-hour smoking. [16]showed that the longer the fish was smoked, the higher protein level and the lower the water content would be.[17]reported that mean protein level of the skipjack tuna smoked with beech wood ranged from 15.4 % to 31.5%. The present study shows that the fish protein level of all smoking duration is higher than that previously reported, andaccording[18], it could result from the absorption and conversion ability of important nutrition protein of the species.

Fat content of the skipjack tuna smoked in different duration ranged from 1.30% to 3.07%. Table 1 shows that the lowest fat content, 1.30%, occurs in 3-hour smoking and the highest, 3.07%, in 12-hour smoking. [19]reported that long smoking process would cause the water content reduce with smoking duration. [17]found that decrease in material water content made protein, fat, and vitamin content increase. [20]suggested that fat content of the fish flesh varied depending upon species, age, spawning, and type of fish flesh.

Ash content of the skipjack smoked in different smoking duration ranged from 1.35% to 3.97%. Table 1exhibits that the lowest ash content, 1.35%, is 3-hour smoking and the highest, 3.97%, in 15-hour smoking.It could result from long smoking process, distance to the smoke source and high smoking temperature reducing much fat from the fish flesh.As a result of long smoking duration, much fat is removed from the fish flesh, and then will lose.The present study indicates that the ash content is in the average range reported by Toisuta *et al* (2014) for smoked skipjack tuna, 1.36 % – 5.66%.

Table 2. Fatty acid profile of the skipjack tuna smoked using the nutmeg shell for 3, 6, 9, 12, and 15 hours.

Fatty Acids	Smoking Duration				
	3 hours	6 hours	9 hours	12 hours	15 hours
Kapric acid (C10:0)	2.16±0.091 b	1.94±0.03 a	2.28±0.11c	2.40±0.02b	2.971±0.02d
Lauricacid (C12:0)	1.02±0.052 ab	0.99±0.02 a	1.04±0.05ab	1.13±0.01b	1.287±0.01c
Myristic acidt (C14:0)	29.67±1.371b	26.17±0.41 a	28.2±0.19bc	30.84±0.28d	32.03±0.24e
Pentadecylic acid (C15:0)	0.363±0.02 a	0.45±0.01bc	0.29±0.06 ab	0.43±0.06ab	0.46±0.26c
Palmiticacid (C16:0)	16.15±0.22 b	13.49±0.22a	14.49±0.69a	18.69±0.03 d	14.70±0.12c
Heptadecanic acid(C17:0)	0.32±0.03 a	0.49±0.01bc	0.47±0.02b	0.44±0.11ab	0.57±0.02cd
Stearic acid (C18:0)	28.17±0.29ab	36.49±0.64d	32.95±1.58c	27.44±0.11a	25.62±0.17a
Arachidic acid (C20:0)	0.28±0.01a	0.32±0.01b	0.27±0.01a	0.36±0.01d	0.35±0.01c
Total SFA	78.11±2.01	80.33±1.34	80.11±3.67	81.72±0.11	76.99±0.44
Palmitoleic acid (C16:1n-7)	13.52±0.52a	9.44±1.47b	10.88±0.68bc	7.39±0.01bc	9.10±0.01c
Oleicacid (C18:1n-9) Elaidat acid(C19:1)	2.40±0.06d 0.94±0.022a	0.30±0.003ab 1.27±0.005a	0.21±0.01a 1.24±0.09a	1.67±0.07c 1.22±0.06a	0.82±0.08b 1.20±0.02a
Cis-eicosonoic acid (C20:1)	2.08±0.09a	2.14±0.04a	2.03±0.10a	2.28±0.01b	2.18±0.05a
Total MUFA	18.94±0.31a	13.15±1.09a	14.36±0.52a	12.57±0.09a	15.31±0.09a
Linoleic acid (C18:2n-6)	0.43±0.002a	0.54±0.01a	0.44±0.02a	0.55±0.01a	0.56±0.32a
Linolenicacid (C18:3n-3)	0.69±0.03b	0.66±0.01b	0.60±0.03a	0.79±0.01d	2.48±0.02c
Gama Linolenic acid (C18:3n-6)	0.257±0.14b	0.46±0.01c	0.43±0.02bc	0.29±0.003b	0.09±0.09 a
Arachidonic acid (C20:4)	2.23±0.11b	1.62±0.02a	2.82±0.22d	2.68±0.02c	2.88±0.06ab
Eicosapentaenoic acid (EPA)	0.33±0.02b	0.25±0.01a	0.57±0.02d	0.42±0.01c	0.34±0.01b
Total PUFA	3.95±0.03 a	3.53±0.06 a	4.86±0.31 b	4.72±0.03b	6.35±0.45 a

Note : The alphabetic notation at the same row indicates no difference between the treatments (P>0.05)

Fatty acid profile examinations(Table 2) show that smoking duration affects Saturated Fatty Acid (SFA) content consisting of kapric acid (C10:0), lauric acid (C12:0), miristic acid (C14:0), pentadecanoic acid (C15:0), palmitic acid (C16:0), heptadecanoic acid (C17:0), stearic acid (C18:0) and arachidic acid (C:20:0). The lowest total SFA, 76.99%, was found in the skipjack tuna smoked for 15 hours and significantly different (P<0.05) from the highest, 81.72%, in 12-hour smoking. Ilow (2013) reported that total SFA of several smoked marine fish ranged from 24.2% to 28.0%, and therefore, this study found higher total SFAthan that in previous study. Moreover, [21,22] found that different smoking duration and smoking method could also influence the chemical characteristics of the smoked fish product.

Total Monounsaturated Fatty Acid (MUFA) consists of palmitoleic acid (C16:1), oleic acid (C18:1), elaidic acid, cis eicosenoic acid (C20:1). The highest MUFA content, 18.94%, was recorded in 3-hour smoking, but not significantly different (P>0.05)from those in 6, 9, 12 and 15-hour smoking. This study indicates lower total MUFA content than previous findings. According to Ilow (2013), total MUFA content for several smoked marine fish ranged from 26.0% to 39.8%.

Total Polyunsaturated Fatty Acid (PUFA) consists of linoleic acid (C18:2n-6), linolenic acid (C18:3n-3), gama linoleic acid (C18:3n-6), arachidonic acid (C20:4n-6), and eicopentanoic acid (EPA).The highest total

PUFA content of the smoked skipjack tuna was recorded in 15-hour smoking, 6.35%, but not significantly different (P>0.05) from that of other smoking duration, and the lowest one, 3.53%, occurred in 6-hour smoking.Ilow (2013) reported that total PUFA content of smoked marine fishes ranged from 31.9% to 45.4%, but the present study shows lower PUFA content than that in previous study.

According [23], unsaturated fatty acids could not sustain to heat, and its instability increased with saturation level. Stephen *et al*. (2010) reported that mean content of omega-3 fatty acid, particularlyeicosapentaenoic acid(EPA) anddocosahexanoic acid (DHA),was 1.67% and 2.50%, sucessively.

Polycyclic Aromatic Hydrocarbon (PAH) content of the smoked skipjack tuna under 3, 6, 9, 12, and 15-hour smoking is presented in Table 3.

Table 3. PAH profile of the smoked skipjack tuna using nutmeg shell smoking material under 3, 6, 9, 12, and 15-hour smoking duration.

PAH COMPOUNDS	SMOKING DURATION				
	3 HOURS	6 HOURS	9 HOURS	12 HOURS	15 HOURS
Naphthalene (ppb)	0.136	0.165	0.272	0.368	1.113
Acenapthene (ppb)	0.084	1.516	1.524	1.788	0.352
Phenentrene (ppb)	0.077	0.166	0.053	0.336	0.468
Fluoranthene (ppb)	0.667	0.360	0.372	0.069	0.319
Pyrene (ppb)	0.799	1.895	0.816	1.019	0.288
Benzo(a)anthracene (ppb)	0.009	0.003	0.006	0.011	0.002
Perylene (ppb)	0.019	0.010	0.037	0.034	0.025

Table 3shows that smoked skipjack tuna using nutmeg shell under 3, 6, 9, 12 and 15-hour smoking duration contains7 polycyclic aromatic hydrocarbon (PAH) compounds,naphthalene, acenaphthene, phenentrene, fluoranthene, pyrene, benzo(a)anthracene and perylene.The detected PAH compound could also result from nutmeg shell smoke and smoking duration reaction.[24]explained that number and diversity of produced PAH compounds were affected by smoking process conditions. Benzo(a)pirene (BaP) is usually used as compound indicator of carcinogenic PAH. The BaP content in the flesh or processed meat product cannot exceed 5 μg/kg of end product (EC, 2006). Thus, smoked skipjack tuna using the smoking material of nutmeg shell under 3, 6, 9, 12, and 15-hour smoking duration is a safe food material to consume due to its lower BaP content than established by EC,2006; 5 ppb.[25]reported that strong correlation was found between fish fat and PAH compounds[26]also claimed that PAH content highly varied in various fish species smoked with sawdust, firewood and charcoal. [27]found that *acenapthene, phenentrene, anthracene* and *fluorantene* were low molecular weight PAH compounds and did not belong to carcinogenic compounds.

Conclusion

This study concluded that the skipjack tuna smoked with the nutmeg shell for 15 hours was good to consume due to low water content, a_w, fat and SFA, high protein contentand PUFA compared with that in other smoking duration.The carcinogenic compound level, benzo(a)piren (BaP), in all smoking duration was less than 0.01 ppb indicating that it is safely consumable.

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