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Impact of Smoking Materials on Smoked Fish Quality and Polycyclic Aromatic Hydrocarbon Contamination

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Abstract: *Smoking is the most widely practiced method applied practically for all species of fish. Smoking is a technique for preserving fish that has been processed with organic components derived from smoke. Smoke produces volatile fragrant compounds that impart specific properties to fish flesh. Fish smoking commonly takes place in conventional kilns with direct burning of wood, sawdust, coconut shell and coal combustion at high temperatures. The heat burnt organic matter may release chemical contaminants, polycyclic aromatic hydrocarbons (PAHs) via the pyrolysis process, leading to PAHs contamination in smoked fish. Therefore, this research is carried out to investigate the impact of smoking materials (wood and charcoal) on the quality of smoked fish (Clarias microcephalus) and levels of PAH contamination. The moisture content of smoked fish using wood and charcoal were below 6%.* Smoked fish using charcoal tended to have higher ash content, protein and lower total fat than smoked fish using wood. *The result of peroxide values of smoked fish using wood and charcoal were 15.19 ± 0.69 meq/kg and 8.45 ± 0.51meq/k, respectively. TBARS value were 3.76 ± 0.36 mg MDA/kg and 3.68 ± 0.1 mg MDA/kg for smoked fish used wood and charcoal, respectively. The results of PAH analysis, BaP and ∑PAH4 concentrations exceeded the maximum limit set by the Regulation Commission European Union (EU) 1881/2006 of 2 µg/kg and 12 µg/kg, respectively. BaP containing in smoked fish with skin was 3 to 4 times higher by comparison to smoked fish without skin, while ∑PAH4 of smoked fish with skin was 2 to 3 times higher. The concentration of BaP and ∑PAH4 in smoked fish using wood was higher than smoked fish using charcoal. As conclusion, smoked fish should be consumed with skin removal and smoke with charcoal as a heat resource in order to reduce PAH contamination. The design of the smoking kiln and the adjustment of the smoking process could be considered in further study to ensure smoked fish quality and to mitigate the health issues for consumers.*

Keywords: Smoking, *Clarias macrocephalus*, wood, charcoal, polycyclic aromatic hydrocarbons

1. INTRODUCTION

Freshwater aquaculture is widely implemented for economics and income in most countries. Fish is an important source in food production sectors with a variety of food development providing nutrients particularly protein sources, and income for fishermen across countries. In addition, fish is a very perishable food commodity that spoils quickly due to enzymatic and microbiological undertaking, producing an unpleasant taste, smell, and texture that lowers customer acceptability [1]. Previous studies have reported that fish spoilage is significantly influenced by high ambient temperatures, considerable distances between landing ports and consumer markets, and inadequate infrastructure for post-harvest handling, necessitating the implementation of preservation techniques such as chilling, freezing, salting, canning, drying, and smoking [2].

The smoking method was developed in ancient times by hanging fish over the fire, generating the smoke to attach

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to the fish surface providing a smoking flavor and enhancing the organoleptic profile [3]. Wood is generally used for smoke generation in the traditional smoking process. In contrast, smoking fish with a developed kiln commonly use charcoal and gas as fuel heat sources [4]. Smoke is defined as the result of thermal pyrolysis of fuel materials when access to oxygen is limited. The smoking temperature of the flame varies between 300 °C and 700 °C, and the kiln's temperature is approximately 80 °C may lead to release of polycyclic aromatic hydrocarbons (PAHs). PAHs is known as carcinogenic compounds, potentially causing health hazards. They are a group of environmental contaminants originating from incomplete combustion of organic matter when fuel materials are burnt [5].

The European Union Scientific Committee on Food has identified 15 PAHs as genotoxic carcinogens [6]. Benzo(a)pyrene (BaP) is a congener of the PAH family classified as carcinogenic for humans (group 1) and commonly used as an indicator of potential health risks associated with PAH exposure. Additionally, chrysene (CHR), benzo(a)anthracene (BaA) and benzo(b)fluoranthene (BbF) are identified as group 2B, which is possibly carcinogenic to humans [7]. The European Union Regulation Commission (EU) No. 1881/2006 has established the limit maximum levels for PAHs in smoked food for BaP and a total sum of four PAHs (∑PAH4), which are 2 µg/kg and 12 µg/kg, respectively [6]. The current study aimed to evaluate the impact of wood and charcoal on the quality of smoked *Clarias macrocephalus* and PAHs contamination using a stainless-steel kiln.

2. METHODOLOGY

2.1 Raw materials

Clarias macrocephalus species (bighead catfish), called Trey Andoeng Tun as the local name, was purchased from the Kilo Lek Buon market, Tuek L'ak Ti Pir Commune, Tuol Kork District, Phnom Penh, Cambodia. Smoking materials used in this study included mangrove wood (*Barringtonia acutangular*) and charcoal. Palm oil was used as the fire starter.

2.2 Smoking kiln design

The smoking kiln was made of stainless steel with dimensions of 70cm × 70cm × 180cm. There are two blocks, including a fireplace and an ash collector block and a block of fish trays. The distance of each tray was 10 cm. The pipe was hung above the kiln and the wheel was stuck under the kiln.

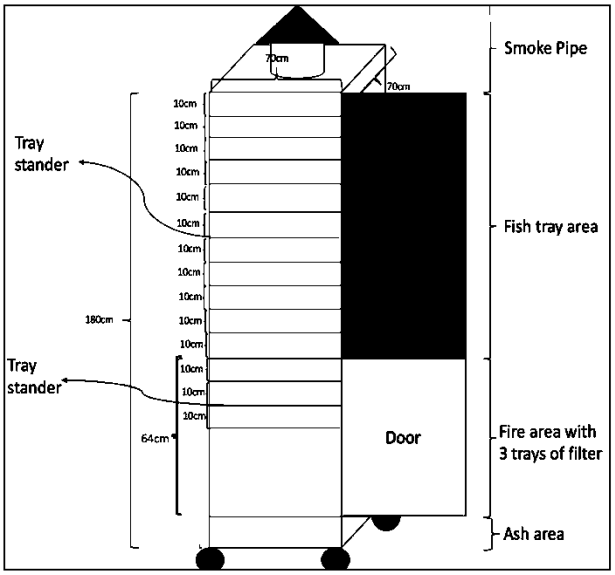

Fig.1. Smoking kiln design

2.3 Smoking fish

The smoking processes were conducted at the Institute of Technology of Cambodia. Fishes were prepared on the fish tray at a distance of 64 cm from the fireplace. The smoking batch of each condition was done in duplicate. Fresh *Clarias macrocephalus* was beheaded and gutted. Fishes were cleaned properly to remove undesired residues on the surface and slime of the fish. After that, the fish was divided into a group of three units of fish. Then, the fish was drained to remove water and to stick the tail together by using a drying oven for one hour at 60°C. It was then prepared on the smoking tray and placed into a kiln. The smoking temperature was thoroughly adjusted manually in the range of 70 to 80 °C with thermocouple. Smoking materials were applied in every one hour. The total smoking time was 26 hours. Smoked fish was cooled down at room temperature. The smoked fish sample was then homogenized using a grinder and stored in a refrigerator for proximate composition analysis and chemical contaminant determination.

2.5 Proximate composition analysis

2.5.1 Moisture content

Moisture content was analyzed in regard to AOAC Official Method 930.15. Three grams of sample was weighed into an aluminum capsule and dried in an oven at 105 ± 5 °C for 3 h to constant weight and placed in a desiccator for 30 min. The percentage of moisture content was determined by the following formula below:

$$\% \text{ Moisture content} = \left(\frac{m1-m2}{m1}\right) \times 100 \qquad \text{(Eq. 2.1.)}$$

Where m1=Mass of the sample before drying (g)
m2=Mass of the sample after drying (g)

2.5.2 Ash content

Ash content was analyzed according to the official method analysis (AOAC 942.05). Three grams of sample were weighed into a porcelain dish and burnt in the furnace for 4h at 550°C. After that the porcelain dish was placed in a desiccator for 30 min. The percentage of ash content is calculated by the formula below:

$$\% \text{ Ash content} = \left(\frac{m3-m1}{m2}\right) \times 100 \qquad \text{(Eq. 2.2.)}$$

Where m1: Mass of an empty porcelain dish (g)
m2: Mass of the sample before burning (g)
m3: Mass of the sample with porcelain dish after burning (g)

2.5.3. Protein content

Measurement of protein content was analyzed followed by the Kjedahl method according to Official Methods of Analysis (AOAO, 945.18-B). The homogenized sample (1g) was weighed on the filter paper and transferred to kjeldahl flask. After that, 20 ml of concentrated sulfuric acid (98 %) was added and 10 g of catalyst (potassium sulfate and copper sulfate) was added. The apparatus was preheated at 250 °C and heated up to 420 °C for 1 hour to 2 hours until the solution turned to light green. Next, the distillation was performed using (32%) sodium hydroxide and standard boric acid is used in excess amounts to capture the ammonia-forming solvated ammonium ion when the solvent passes through the condenser when the heat is applied. After that, the distillate solution was titrated with (0.25 M) sulfuric acid as the titrant, using methyl red as an indicator to determine the end-point of the reaction which changed from a green color to light pink and the volume of the titrant was recorded. The protein percentage was calculated according to the formula below:

$$\% N = \frac{(V-V_b)\times N\times M_N}{W} \times 100 \qquad \text{(Eq. 2.3.)}$$

$$\% P = \% N \times 6.25/1000 \qquad \text{(Eq. 2.4.)}$$

Where % N: Percentage of the weight of nitrogen
% P: Percentage of the weight of the protein
V: Volume of sulfuric acid for the sample (mL)
V_b: Volume of sulfuric acid for the blank (mL)
N: Normality of sulfuric acid (N)
W: Weight of the sample (g)
M_N: Atomic weight of nitrogen
6.25 =The protein-nitrogen conversion factor

2.5.4 Total fat content

The determination of fat content was analyzed based on Soxhlet extraction according to AOAC 963.15. One gram of sample was weighed and placed into a cellulose thimble and loaded into the main chamber of the Soxhlet extractor cup containing 70 mL of n-hexane. The solvent is immersed and heated at 130 °C for 60 min. It was simultaneously washed for 60 min and the oil was dropped down into the extraction cup and it was then recovered for 15 min. After that, the extraction cup containing oil was dried for 1 h in the oven at 105 °C and placed in a desiccator for 30 min. The dried cup containing extraction oil was weighed. The result of total fat content is determined by the formula below:

$$\% \text{ Total fat content} = \left(\frac{W3-W1}{W2}\right) \times 100 \qquad \text{(Eq. 2.5.)}$$

Where W_1: Mass of extraction cup (g)
W_2: Mass of sample (g)
W_3: Mass of cup and extraction oil (g)

2.6 Lipid oxidation

2.6.1 Peroxide value

The peroxide value was analyzed according to the modification of the AOCS official method Cd 8b-90. Three grams of sample was accurately weighed into a 250ml Erlenmeyer. Acetic acid: chloroform solution (3:2) was added 30 ml and 0.5 ml of potassium iodide was added. The prepared sample was left to rest for 5 minutes in the absence of the light. After that, 20 ml of distilled water was added and titrated with (0.1N) sodium thiosulphate solution and drops a few of (1%) starch. During titration, the solution will turn black and then titrate until the color changes to light yellow or transparent. The blank test was carried out under the same condition. The peroxide value was expressed in milliequivalent peroxide/ kg sample (meq/ kg) and calculated by the following formula:

$$\text{PV (meq/kg)} = \frac{[(a-b)\times M]\times 1000}{M_S} \qquad \text{(Eq.2.6.)}$$

Where a: Volume of thiosulfate solution used for the titration (mL)
b: Volume of thiosulfate solution used for the blank test (ml)
M: Molarity of sodium thiosulfate (mol/L)
MS: Mass of the sample (g)

2.6.2 Thiobarbituric Acid Reactive Substances (TBARS)

To make solution 2-thiobarbituric acid (TBA) concentration, 300 mg of TBA was weighed and transferred to a beaker with approximately 45 mL of deionized water. The beaker was placed in an ultrasonic bath and heated to 60 °C. When TBA was dissolved, the solution was transferred to a volumetric flask of 50 mL and filled with water to the mark. A fresh solution of TBA was prepared every day. Butylated-hydroxytoluene (BHT) solution was prepared by adding 400 mg BHT into 50 mL of methanol. A standard solution of Malondialdehyde (MDA) (8.33 mmol/L) was prepared by transferring 200 µL of 1,1,3,3-tetramethoxypropane (TEP) to a 100 mL volumetric flask. The solution which was prepared by mixing ethanol and deionized water in a ratio of 2:3 was taken into the flask until marked. A mixture ratio of 1:1.5 methanol to (5%) Trichloroacetic acid (TCA) was used to dilute the standard solution to stock solution (41.65 nmol/mL). From the stock solution, different concentrations (1.11, 2.22, 3.33, 5.55, 6.66, 7.77, 9.99 and 11.11 nmol/mL) of MDA were prepared.

2.6.2.1 Calibration standard curves

Standard MDA solutions (0.75 mL) were transferred to 10 mL test tubes and mixed with 0.6% TBA (1.5 mL). The tubes were tightly closed and heated in a water bath at 90 °C for 60 minutes. The tubes were then cooled at room temperature for 30 minutes and absorbance was read at 532 nm using a UV visible spectrophotometer model SPECORD M40 (Carl Zeiss Jena, Germany). The calibration curve was constructed by plotting the absorbance values versus the concentration values. For each point of the calibration curve, replicates were analyzed according to the above protocol.

2.6.2.2. TBARS assay

Determination of TBARS was performed according to the methods of the AOCS Cd 19-90. The sample (0.1 g) was mixed with 0.5 L of BHT in (2 mL) Eppendorf. Then, 0.75 mL of 5% TCA was added to the tubes, vortexed for 15 min and centrifuged for 15 min at 14500 rpm at 4 °C. A volume of 0.75 ml of supernatant was mixed with 1.5 mL of 0.6% TBA, and heated at 90 °C for 60 min, then cooled down. The absorbance was read at 532 nm. The results were calculated against the standard curve. The TBARS value was calculated according to the following equation (Eq. 2.7):

$$\text{TBARS (mg MDA/kg)} = \frac{(A-b)\times V}{a\times m} \quad \text{(Eq. 2.7.)}$$

Where A: Absorbance corresponding to the tested sample
b: Intercept of the calibration curve
a: The slope of the calibration curve
m: Weight of the sample
V: Volume correction

2.6.3 Analysis of polycyclic aromatic hydrocarbons

2.6.3.1 Chemicals and reagents

Four PAHs standard and internal standard materials of Benzo[a]anthracene (BaA), Benzo[a]pyrene (BaP), Benzo[b]fluoranthene (BbF) and Chrysene (Chr) and Benzo[a]anthracene-D12 (BaA-D12) were purchased from Dr. Ehrenstorfer (Germany). The standards were prepared at concentrations of 100 ppm was used for dilution. Acetonitrile was purchased from Biosolve (Valkenswaard, The Netherlands). Dichloromethane and water HPLC grade were contributed by VWR International (West Chester, PA, USA).

2.6.3.2 Sample preparations and PAH extraction

Smoked *Clarias macrocephalus* (Trey Andoeng) was homogenously ground and categorized into two groups: with and without skin. Five grams of the ground samples were spiked with 200 ng of BaA-D12 as an internal standard and extracted with 10 mL of acetonitrile, then thoroughly mixed in a 50 mL falcon tube with ceramic homogenizers (Agilent, USA) by shaking and vortex. The mixtures were then centrifuged at 4,000 rpm for 10 minutes. Six milliliters of supernatant were transferred into a 15 mL tube containing a QuEChERS dispersive kit (5982-5156, Agilent, USA). These samples were mixed by vortex and centrifuged again at 4,000 rpm for 10 minutes. The supernatants were transferred to a vial through a 0.45 µm syringe filter and stored in a refrigerator for subsequent analysis.

2.6.3.4 Polycyclic aromatic hydrocarbon determination

PAHs in standard solutions and extracted samples were injected (10 µL) onto a Shimazu C18 column (4.6 mm x 250 mm, 5 µm) of an HPLC/FLD (Shimazu, Japan) using a gradient mobile phase with 1 mL/ min. The mobile phase A consisted of deionized water and phase B was acetonitrile. Phase B commenced at 60% for 5 minutes, then gradually increased to 85% over 25 minutes, briefly escalated to 100% which was held for 5 minutes, and then finally decreased back to 60% for 5 minutes. The PAHs were detected at the excitation and emission wavelength of 264 nm and 407 nm, respectively. PAHs were identified by comparison of sample chromatograms with standards. Quantification was performed by the method of external standards.

2.7 *Statistical analysis*

The results presented are mean values of each determination ± standard deviation (SD) of duplication. Analysis of variance was performed by one-way ANOVA. The differences between the mean values of the treatments were determined for the significance differences ($p \leq 0.05$) followed by the Statistical Package for the Social Sciences (SPSS, Version 26.0 2019; IBM, USA).

3 RESULTS AND DISCUSSION

3.1 *Proximate composition of smoked fish using wood and charcoal*

Clarias macrocephalus smoked using wood and charcoal in stainless steel kiln for 26 hours were subjected to proximate composition analysis. The results were expressed as a mean ± standard deviation of the dry basis of samples.

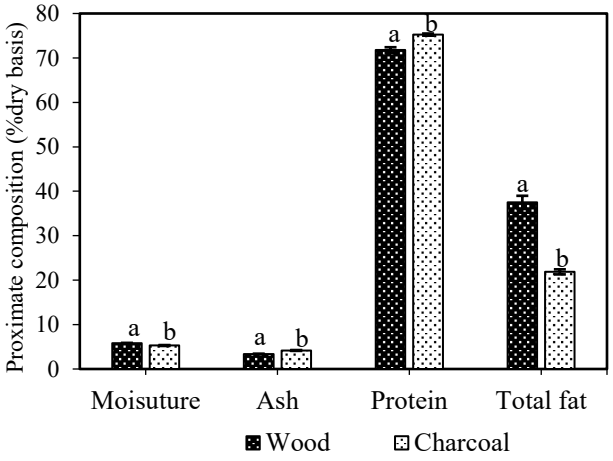

Fig. 2. Proximate composition of smoked fish

Moisture content of both smoked fish products using wood and charcoal were less than 6%, about 5.81 ± 0.06 and 5.28 ± 0.09%, respectively. The study by Olayemi Folorunsho et al. [8] also found that the moisture of fish dropped to its lowest point of 7.3% with a long time of 15 hours of smoking. Faturoti et al. [9] cited that the moisture content of smoked-dried Catfish in African had moisture content of 6.27% to 10.92%. According to Codex Alimentarius [10], moisture content level of smoked fish should be below 10% as an optimal value. Charcoal is often considered a better fuel than plain wood for several reasons, partially burned in a low-oxygen environment along with a lower moisture content than wood, thus it ignites more easily and burns hotter to produce a steady heat over an extended period [11].

Ash content was in the range of 3.37 ± 0.01 and 4.17 ± 0.08% in smoked fish using wood and charcoal, respectively. The data obtained in this study is in agreement with the previous study as reported by Ikape [12], which found that ash content of smoked fish using different smoke sources such as fuel wood, cow dung and corn husk was significantly difference from each other due to the different compositions in smoked materials remain residual after drying in open spaces enhances the possibility of insect and bird infections and permits the accumulation of dust and residue carried by Mohammed et al. [13].

Protein contents found in smoked fish using wood and charcoal were 71.79 ± 0.61% and 75.25 ± 0.24%, respectively. The protein content of smoked fish using charcoal was higher in comparison to smoked fish using wood ($p \leq 0.05$). Charcoal fires produce a steady, high heat that can reduce moisture more quickly, leading to a more concentrated protein content in the final product [14]. Omodara et al. [14] reported that smoking with charcoal could raise the protein in smoked fish due to more extensive drying compared to wood smoking. Moreover, charcoal combustion may reach higher temperatures that effectively cooking the fish more thoroughly and possibly denaturing proteins to a different extent which remain high and concentrated. Okereke et al. [15] suggested that the product dehydration concentrates the proteins during the fish's heat treatment. The extent of the protein effect may depend on factors such as the type of fish, the smoking method, and the season [16].

The total fat content of smoked fish using wood and charcoal resulted in values of 37.49 ± 1.48% and 21.85 ± 0.56%, respectively. The total fat content of smoked fish using wood had a higher value than smoked fish using charcoal (significantly different at $p \leq 0.05$). The study by Tiwo et al. [17] on smoked catfish (*Clarias gariepinus*) found fat content in dry matter of 11.82±0.06% to 14.18±0.42%. Wood-smoked fish tended to have a higher total fat than charcoal-smoked fish. Several chemical substances found in wood smoke can adhere to the fish's surface which is contributed to raise fat content. Charcoal fires is hotter and more radiative that could cause more fat to drip out of the fish or be oxidized during smoking, resulting in a slightly lower retained fat percentage [14]. The fat content of smoked fish depends on many factors, including the species, how and where the lipids are stored, and the smoking time and conditions [18].

3.2 *Lipid oxidation of smoked fish*

Lipid oxidation in the smoked *Clarias macrocephalus* sample was evaluated by the determining peroxide value (PV) and the 2-thiobarbituric acid reactive substances

(TBARS) value. The data obtained was calculated as a mean ± standard deviation.

3.2.1 *Peroxide value of smoked fish (PV)*

Peroxide value is the primary product of lipid oxidation characterized by hydroperoxide production. Its value was determined and expressed in (meq/kg).

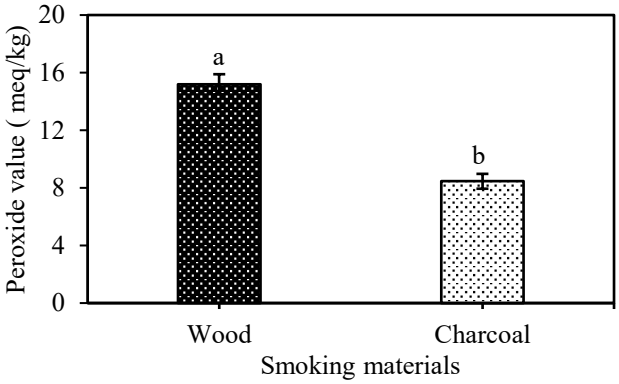

Fig. 3. Effects of smoking materials on peroxide value of smoked fish

Peroxide value were significantly higher in smoked fish with wood (15.19 ± 0.69 mep/kg) than that of smoked fish with charcoal (8.45 ± 0.51meq/kg) as shown in **Fig. 2** ($p \leq 0.05$). A product with PV below 5 meq/kg is classified as a low oxidation state, that between 5 and 10 meq/kg at moderate oxidation and above 10 meq/kg is classified as a high oxidation state [19]. Previous study by Adeyemi et al. [20] indicated the high PV values on day 0 for wood smoked (26.71 ± 1.87 meq/kg), and charcoal smoked (27.47 ± 0.20 meq/kg) state that they were exceeded the acceptable limit of lipid oxidation, which is an accordance of the findings in our current research study. Wood smoke also contains more reactive oxygen species and organic volatiles, which can initiate lipid oxidation and increase peroxide formation. Charcoal, in contrast, burns more cleanly with fewer reactive volatiles, leading to less oxidative stress on fats in the product [20]. Catfish is rich in polyunsaturated fat and these fatty acids are very sensitive to oxidation reactions due to temperature [21], which may align with our study that found high peroxide value in *Clarias macrocephalus*.

3.2.2 *Thiobarbituric Acid Reactive Substances (TBARS)*

The production of secondary lipid oxidation products in food contributes to the deterioration of their nutritive value. Freshly produced smoked fish was evaluated for TBARS value expressed in (mg MDA/kg) as shown in **Fig. 3** below.

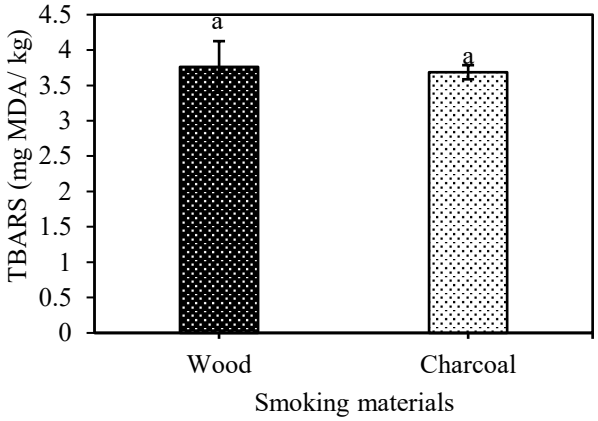

Fig. 4. Effects of smoking materials on TBARS value of smoked fish

TBARS value of smoked *Clarias macrocephalus* were 3.76 ± 0.36 mg MDA/kg and 3.68 ± 0.1 mg MDA/kg in smoked fish using wood and charcoal, respectively. There were no significant differences of both treatment on smoked fish in terms of TBARS values ($p \geq 0.05$). The suggestion by Alparslan et al. [22] said that good quality fish should contain TBARS less than 5 mg MDA/kg. Al-Kahtani et al. [21] reported that meat products that have less than 3 mg MDA/kg is considered as good for human consumption. Ortiz et al. [23] found that the duration of exposure during the drying process significantly influences the formation of secondary lipid oxidation compounds in the dried fish samples.

3.3 *Presence of polycyclic aromatic hydrocarbons in smoked fish using wood and charcoal*

The contaminated PAHs, BaP and ∑PAH4 (BaP, CHR, BaA and Bbf), in smoked fish samples from wood and charcoal burning were determined by HPLC analysis and expressed as µg/kg dry matter. Two types of smoked fish samples were considered in the current study, smoked fish with skin and without skin. The results showed that BaP were between 3.70 ± 1.02 to 8.00 ± 1.05 µg/kg and ∑PAH4 of 127.92 ± 46.25 to 478.00 ± 60.87 µg/kg. BaP and ∑PAH4 in all tested smoked fish samples were exceeded maximum limit set by the regulation commission European Union (No 1881/2006) in the concentration of 2 µg/kg and 12 µg/kg, respectively [6].

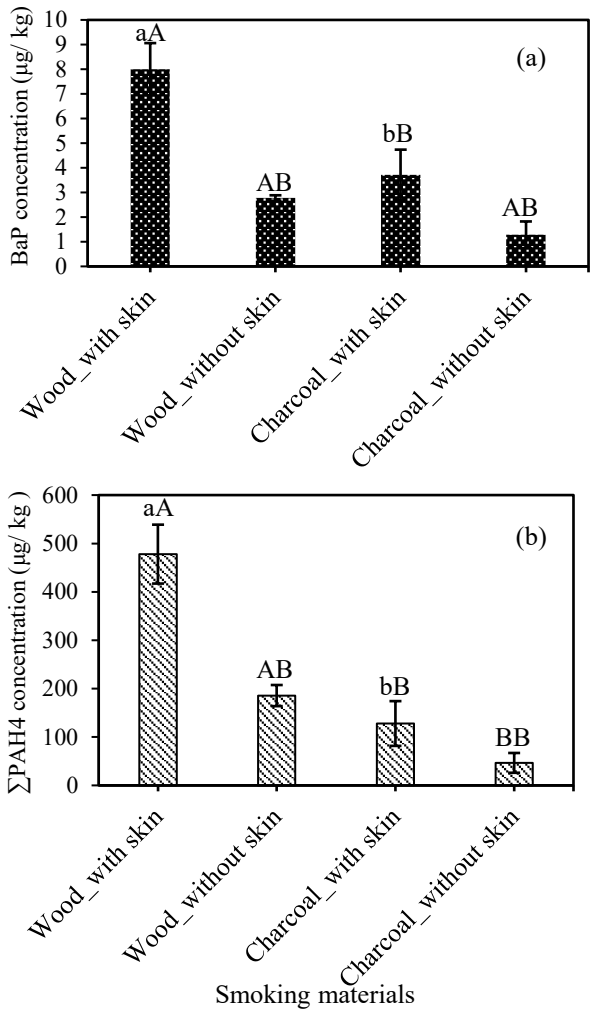

Fig. 5. Concentration of (a) BaP and (b) ∑PAH4 in smoked fish with skin and without skin using wood and charcoal as the smoking materials

For smoked fish analyzed without skin resulted 1.28±0.54 to 2.17±0.11 µg/ kg for BaP and 46.55±20.44 to 185.65±21.82 µg/ kg for ∑PAH4. BaP concentration of smoked fish analyzed without skin showed insignificant differences ($p \geq 0.05$) between smoked fish using wood and charcoal, whereas ∑PAH4 showed a significantly difference ($p \leq 0.05$). Smoked fish samples were exceeded maximum limit set by the regulation commission European Union (No 1881/2006) in the concentration of 2 and 12 µg/ kg for BaP and ∑PAH4, respectively [6], except smoked fish using charcoal analyzed without skin was under criteria limited. The highest concentration of BaP and ∑PAH4 was detected on smoked fish samples that were analyzed with skin, had 3 to 4 times of BaP and 2 to 3 times of ∑PAH4 higher compared to smoked fish analyzed without skin. Similarly, the reported by Douny et al. [24] found the highest concentrations of BaP and ∑PAH4 in two samples of C. macrocephalus were equal to 102.4 and 570.7 µg/ kg, respectively, and the concentration of BaP was approximately 4 times higher and ∑PAH4 about 2 times higher in the fish with skin compared to the fish without skin. According to the previous research, the skin prevents PAH contamination by acting as a barrier that prevents smoke particles from penetrating, thus PAHs accumulats greatly on the skin of smoked fish. Due to their solubility in fat, PAHs may migrate underneath fatty tissue after accumulating on the skin's surface [25]. In this study, BaP and ∑PAH4 of smoked fish using wood had higher concentrations than smoked fish using charcoal within two types of smoked fish analysis. Osineye et al. [26], reported that direct use of fuel wood shows higher levels of PAHs compared to charcoal. This is similar to the result reported by Silva et al. [27] discovered more PAHs were detected in fish smoke-dried with other fuel sources when compared with those smoke-dried with charcoal. The pyrolysis of hardwood to charcoal results in low PAH contents in the charcoal, generating essentially smaller PAH level since charcoal may generate low molecular weight PAHs called light PAHs when meat is charcoal-grilled [28]. The formation of PAHs within food products is influenced by numerous factors. The Codex Alimentarius commission [29] identified some variables that influence levels of PAHs within food products such as fat content of the food and where it goes during processing, fuel type, smoking method (hot or cold smoking), smoke generation process, airflow of the smoke, distance between food and the heat source, position of the food from the heat source, smoking duration, temperature during smoking and the design of the smoking kiln.

4 CONCLUSIONS

Smoked *Clarias macrocephalus* were produced using smoke material of wood and charcoal. Moisture contents were below 6%. The variations of proximate composition demonstrate how the smoking material and condition affect the quality of smoked fish. Lipid oxidation of smoked fish, a primary product which is peroxide value resulted within moderated oxidative stage for smoked fish using charcoal, whereas the secondary productis thiobarbituric acid reactive substances, resulting in a range of consumption allowance. In terms of PAHs contaminants formation, BaP and ∑PAH4 exceeded the maximum permitted level defined by European Commission approximately 1 to 4 times and 4 to 40 times, respectively. Smoked fish using charcoal analyzed without skin was in limit allowance of BaP concentration. Smoked fish analyzed with skin had higher concentrations of BaP and ∑PAH4 than smoked fish analyzed without skin. Smoked fish using wood obtained a higher concentration of BaP and ∑PAH4 compared to smoked fish using charcoal. Therefore, through consumption of smoked fish without

skin and use charcoal as a smoke material could help to reduce the PAHs contaminants. Our findings suggest that in order to effectively reduce the rate of PAH contamination of the final product and guarantee consumer safety, the smoking process and smoking kiln design need to be improved.

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