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BENZO (α) PYRENE POTENTIAL ANALYSIS ON SMOKED FISH (CASE STUDY: TRADITIONAL METHOD AND SMOKING KILN)

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ABSTRACT

Benzo(α)pyrene were determined by Gass Chromatography Mass Spectrofotometry method on nile, tilapia, milkfish, little tuna, narrow bared spanish mackerel, marine catfish, canine catfish and stingray processed by traditional method and the used of smoking kiln. Eight samples of smoked fish processed by traditional method showed a higher BaP levels ranging from 0.03 to 4.58 ppb. Whereas, BaP levels on smoked fish processed by smoking kiln were found in average lower than traditional method ranging from 0.01 to 3.04 ppb. Different fish species gave very significant different ($P < 0.01$) to BaP levels. Meanwhile, different method of smoking fish gave no significant different ($P > 0.05$) on carbonyl compounds (phenol, formaldehyde, and organic acid) levels on smoked fish processed by traditional method and smoking kiln, but different fish species gave very significant different ($P < 0.01$) to carbonyl compound level (phenol, formaldehyde, and organic acid).

Keywords: Benzo(α)pyrene, carbonyl, smoking kiln, smoked fish, traditional method

INTRODUCTION

Smoking is one of the fish preservation methods that combine drying and natural chemical decomposition from wood combustion, i.e. phenol, formaldehyde, organic acids and Polycyclic Aromatic Hydrocarbons (PAHs). PAHs are chemical compounds that compose of three or more aromatic rings. PAHs formed by wood combustion and occur during wood, charcoal, oil combustion levels (Basak *et al.* 2010; Wretling *et al.* 2010).

PAHs often function as a carcinogenic group that found in smoked products and always be identified for its composition intensively. Hence, several PAHs compounds represent carcinogenic especially for smoked fish. The EU Scientific Committee on Food (SCF) has identified 15 PAHs compounds as carcinogenic genotoxic i.e. benz(a)anthracene, benzo(b)fluoranthene, benzo(j)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, benzo(g,h,i)perylene, chrysene, cyclopenta(c,d)pyrene, dibenz(a,h)anthracene, dibenzo(a,e)pyrene, dibenzo(a,h)pyrene, dibenzo(a,i)pyrene, dibenzo(a,l)pyrene, indeno(1,2,3-cd)pyrene, and 5-methylchrysene. Especially for benzo (α) pyrene, has carcinogenic value higher than other PAHs compound, benzo (α) pyrene gives contribute 1-20% from total carcinogenic that found in foodstuff such as smoked products (Swastawati *et al.* 2007; European Commission 2002; Simon *et al.* 2006).

PAHs contamination from smoked fish can be significantly decreased by replacing of smoking position, where the fish are not placed directly from the smoke source (Visciano *et al.* 2008).

The utilization of traditional method and smoking kiln as an alternative smoking method has been implemented many years in Indonesia. Traditional smoking method is considered can lead contamination to PAHs, so that the process needs to control adequately (Leksono *et al.* 2009). The aim of this research was to compare the effect of smoking on the content of benzo(α)pyrene of smoked nile, milkfish, little tuna, narrow bared spanish mackerel, marine catfish, stingray, canine catfish, and tilapia processed by traditional method and smoking kiln.

MATERIALS AND METHODS

1. Materials and fish processing

Eight samples of fish namely nile, milkfish, little tuna, narrow bared spanish mackerel, marine catfish, stingray, canine catfish and tilapia were collected from Kobong's market, Semarang, Central Java. After collection, fish were deboned, beheaded, and washed, then they dipped into 5% brine. After dipped, then divided into two parts i.e. part 1 and 2. Part 1 was smoked by traditional method; fish were placed into trays and smoked by smouldering coconut shell in the smoking chamber. Part 2 were placed at trays then processed by smoking cabinet, which smoke developed in an external generator under controlled conditions of temperature and air access.

2. Benzo (α) pyrene analysis (GC-MS)

Ten g of sample were destructed by sodium sulphate and chloroform until homogenous. The sample was extracted into propylene carbonat and saponificated with NaOH solution. The extract was filtered with calcium chloride, celite and fluorisil. The filtrate then evaporated to 1 ml using a waterbath at 45°C, and passed a glass column (clean up) containing 10% of aluminium oxide in the lower part and 10 g of Na₂SO₄ in the upper part until the component was eluted. Samples were fractionated in the column in silica 60 resin columns, 70-230 mesh and added by 10% diethyleter and then evaporated. Finally, the sample was injected into Gas Chromatography to identificate benzo (α) pyrene.

3. Phenol analysis

Samples was homogenized by distilled water, and placed into iodide tubes 0.1N bromine solutions and 5 ml of HCl concentrated and left it until 30 minutes. Then, it was added 5 ml of 20% KI solutions and 3 ml of 5% amylop indicator. Titrated with Na-Thiosulphate 0.1 N until the blue colour disappeared.

4. Formaldehyde analysis

Standard solutions were prepared as follows: 0.027 ml of 37% formaldehyde was added in 500 ml distilled water and then divided into different concentrate (0, 0.05, 0.1, 0.5, 0.75, 1.0, 1.5, and 2.0). Concentrates were placed into tubes and added 5 ml chromatic acid on different concentrate. The concentrates was heated during 30 minute on 100°C. Twenty ml samples was homogenized with distilled water, heated and then filtered. Two ml filtrate was

added by 5 ml chromatic acid and heated during 20 minutes. Absorbance measured with spectrophotometer on λ 520 nm.

5. Organic acid analysis

Organic acids can be analyzed by using a highly versatile reversed phase column with the UV absorbance detection at 210 nm. The simultaneous analysis of 10 organic acid standards and the analyses of the organic acids in smoked fish were performed. As components other than organic acids that are detected by this analytical system, this system issuitable for samples containing organic acid as a main component and with little contaminants. One of the simple methods to distinguish target components from contaminants is to compare the elution positions with the standards when the column temperature is changed.

6. Statistical analysis

The collected data from two replications was subjected to independent sample *t*-test to compare differences quality between traditional method and smoking kiln.

RESULTS AND DISCUSSION

1. Benzo (a) pyrene

The PAHs compound that represented by Benzo(a)Pyrene found in smoked fish samples can be seen in Table 1. The lowest levels of Benzo(a)Pyrene was fond in smoked nile processed by smoking kiln, whereas the highest Benzo(a)Pyrene was found in the smoked marine catfish processed by traditional method.

Tabel 1. Benzo(a)pyrene compound in smoked fish

Fish	Traditional (ppb)	Smoking klin (ppb)
Nile	0.03	0.02
Tilapia	0.24	0.11
Milkfish	1.90	3.04
Stingray	0.32	0.01
Little tuna	0.72	0.20
Narrow barred spanish mackerel	0.05	0.14
Marine catfish	4.58	1.67
Canine catfish	0.85	0.51

There was no significant effect ($P>0.05$) of different method of smoking fish to the content of benzo(a)pyrene compound on all smoked fish samples, either by traditional method or smoking kiln, but different types of fish were found gives very significant different ($P<0.01$) to benzo(a)pyrene content of all smoked fish. PAHs compound are bioavailable in the aquatic ecosystem to fish via the food chain, as waterborne, compounds and from sediments (Jonsson *et al.* 2004).

In accordance with other authors (Hadiwiyoto *et al.* 2000), hot smoking method (traditional) has caused increasing of benzo (a) pyrene value. Smoked red snaper, bared spanish mackerel, and little tuna by hot smoking and liquid smoke contained benzo (a) pyrene values of 0.52 ppb, 0.3 ppb, 0.58 ppb, 0.32 ppb, 0.63 ppb, 0.34 ppb,

respectively. Study by Wretling *et al.* 2010 found that benzo(a)pyrene content of smoked herring were in the range of 0.4-14.4 µg/kg; smoked salmon n.d.-8.4 µg/kg; smoked rainbow trout <0.3-0.9 µg/kg and smoked mackerel <0.3-3.7 µg/kg.

2. Phenol, formaldehyde, and organic acid

Table 2. The levels of phenol, formaldehyde, and organic acid in smoked fish using traditional method and smoking kiln

Smoked Fish	Total Phenol (mg/100g)		Formaldehyde (mg/100g)		Organic Acid (mg/100g)	
	Traditional	Smoking Kiln	Traditional	Smoking Kiln	Traditional	Smoking Kiln
Nile	0.14±0.0009	0.15±0.0006	50.74±0.59	27.54±0.24	0.28±0.004	0.24±0.004
Tilapia	0.12±0.0002	0.15±0.0005	ND	ND	0.19±0.003	0.51±0.003
Milkfish	0.10±0.0002	0.09±0.0005	ND	ND	0.25±0.004	0.53±0.004
Stingray	0.08±0.0007	0.26±0.0006	ND	ND	0.19±0.003	0.19±0.004
Little tuna	0.16±0.0009	0.08±0.0003	25.34±0.43	45.43±0.62	0.32±0.004	0.34±0.004
Bared spanish mackerel	0.11±0.0003	0.12±0.0002	20.99±1.09	ND	0.21±0.004	0.48±0.004
Marine catfish	0.13±0.0005	0.10±0.0002	21.02±0.58	ND	0.18±0.003	0.34±0.004
Canine catfish	0.17±0.0005	0.17±0.0002	21.88±1.23	31.07±1.25	0.20±0.003	0.28±0.003

Note: Mean ± standard deviation. ND : Not detected

Smoke from wood combustion, producing aldehyde, keton, phenol, formaldehyde, and organic acid that has important role to antibacterial, antioxidant, increasing color perform, taste, and odour (Goulas *et al.* 2005). The levels of phenol, formaldehyde, and organic acid in eight samples of smoked fish are given in Table 2.

3. Phenol

One of the most important factor affecting the quality of smoked fish is the content of phenol. Recent studies by many researchers performed on phenolic compounds have shown that their deposition depends on the smoking conditions and research conducted so far has suggested that phenolic compounds play a key role in smoke perception (Cardinal *et al.* 2006). In this study the levels of phenol of smoked fish processed by traditional method and smoking kiln were not significant different ($P>0.05$). Phenol level increased due to temperature and smoking time. Birkerland *et al.* 2005 found that there was no significant difference in mean total phenol between different groups processed with similar method or between group processed with different methods. The content of phenolic compound in smoked salmon processed with a gentle and tough processing method at 20°C were found in the range of 0.7-1.0 mg phenol/100g sample). The higher temperature and the longer smoking time caused in increasing the amount of phenol absorbed into the fish. This may be caused by the accumulation of total phenols deposition in smoked fish. Study by Cardinal *et al.* 2006 found that smoked salmon cold smoking at 16°C containing phenol of 0.76-0.82 mg/100g sampel.

4. Formaldehyde

The combine effect of some chemical component of smoke i.e. formaldehyde, organic acids or carboxylic acids, phenols giving a characteristics flavour, colour, and appearance and also increasing the shelf-life of the smoked fish products (Swastawati *et al.* 2012; Visciano *et*

al. 2008). Traditional smoking method involves the combustion which wood as a source of smoke is located directly below the trays containing fish, whereas smoking kiln provide the smoking chamber located in external smoke generator under controlled conditions of temperature and air circulation. The formaldehyde levels of smoked fish (Table 2) showed that different smoking method gave significant different effect ($P>0.01$) to the fish samples. Study by Noordiana *et al.* (2011) resulted that the average formaldehyde content in mackerel, threadfin bream, lizard fish (Bombay duck), yellowtail scad, jewfish, hardtail, black pomfret, squid, white prawn and bridshrimp were about $1.37 \mu\text{g g}^{-1}$, $0.38 \mu\text{g g}^{-1}$, $15.75 \mu\text{g g}^{-1}$, $0.72 \mu\text{g g}^{-1}$, $0.87 \mu\text{g g}^{-1}$, $0.68 \mu\text{g g}^{-1}$, $0.49 \mu\text{g g}^{-1}$, $0.69 \mu\text{g g}^{-1}$, $1.08 \mu\text{g g}^{-1}$, respectively. Smoking process caused the amount of formaldehyde due to its decomposition into fish flesh. However, specific discussion on the function of formaldehyde in smoked fish is still very view. It is known that formaldehyde is a naturally occurred in wood smoke and smoked foods including smoked fish.

5. Organic acid

In the smoking process, organic acids is one of the important compound that gave a specific colour to the product together with phenolic compounds. Organic acids is antimicrobial substantif which are responsible for colour and taste together with many different components such as aldehydes, ketones, alcohols, hydrocarbons, esters, phenols and ethers (Gomez-Estaca *et al.* 2011). In this study, no significant difference was found between the two smoking method of traditional and the use of smoking kiln ($P>0.05$) from the organic acid point of view (Table 2). Smoking kiln can maintain the organic acid better than that of additional smoking method. Organic acid together with phenol and formaldehyde has an important role in increasing the quality of smoked fish, as antibacterial agent and antioxidant activity. Darmadji (1996), reported that the acidity plays an important role to inhibit the microorganism growth in smoked fish. On pH 4, smoke from coconut shell combustion can inhibit microorganism growth. 16 hours after smoking process and storage at 30°C , the amount of colony of bacteria found was 2.4×10^5 CFU/gram, whereas the samples storage at 4°C resulted 1.3×10^5 CFU/gram. Smoked catfish processed by coconut shell combustion traditionally, has total viable amount of 53.33 CFU/gram (Swastawati 2008).

CONCLUSION

Traditional method and smoking kiln can be applied as a method of fish processing and preservation although both of them still producing benzo(a)pyrene. There is a tendency that smoking kiln has more advantages in terms of maintaining quality of the product and safety point of view, since the smoking processed can be controlled properly, although smoking time needed was longer than that of traditional method.

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