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Submission date: 13-Mar-2019 03:18PM (UTC+0700)
Submission ID: 1092543103
File name: 2._IFRJ_25_5_,_October_2018.pdf (417.17K)
Word count: 4238
Character count: 21825

International Food Research Journal 25(5): 1864-1869 (October 2018)
Journal homepage: http://www.ifrj.upm.edu.my

Concentration and application methods of liquid smoke for exotic smoked Skipjack (*Katsuwonus pelamis* L.)

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Article history

Received: 15 September 2017
Received in revised form: 3 October 2017
Accepted: 16 October 2017

Keywords

Smoked skipjack
Liquid smoke
PAH
Benzo(a)pyrene

Abstract

Smoked Skipjack (*Katsuwonus pelamis* L.) locally called "Cakalang fufu" is one of the Tuna products in North Sulawesi. It is potential to be exported, but the problems are the processing still traditional, and product contained high content of Polycyclic Aromatic Hydrocarbon (PAH). Therefore, it is important to rationalize the processing using liquid smoke. The aims of this study were to develop the best smoking method and the best liquid smoke concentration. Three concentrations of liquid smoke used (A) were: 0.4% (A1), 0.6% (A2), and 0.8% (A3). Three different application methods of liquid smoke (B) were: fresh fillets were dipped in liquid smoke with three concentrations (A1, A2, and A3) for 20 mins, then heated at 70-80°C for 4 hr (B1), fresh fillet were heated at 70-80°C for 1hr, dipped in liquid smoke with the same way as B1, and then heated again at 70-80°C for 4hr (B2), fresh fillet were steamed at 95-100°C for 30 mins, dipped in liquid smoke with the same way as B1 and then heated at 70-80°C for 4hr (B3). Analysis were done for water content, phenol content, pH, PAH, and sensory characteristics (appearance, flavor, taste, and texture). The results showed that the best formulation method was the fresh fillet heated at 70-80°C for 1hr, dipped in 0.8% liquid smoke, and then heated again at 70-80°C for 4 hr, which have water content 47.63%, phenol content 12.62%, pH 4.8, and sensory assessments for appearance 7.2, flavor 8.3, taste 7.7, and texture 6.46. Analysis of PAH showed that stock of liquid smoke, and liquid smoke with concentration of 0.8%, and liquid smoked fillet samples contained benzo(a)pyrene < 0.25 ppb.

Introduction

Smoking food is one of the oldest food processing method where is still frequently used nowadays, to give a smoke flavor to meat and fish. In Indonesia, total smoked fish production was 92,52 MT (SEAFDEC, 2010), while smoked fish production of North Sulawesi province was 31,408 MT, or 34% of Indonesian smoked fish production (NSFMASA, 2014). Some regions in Indonesia have a specific smoked fish, for example: smoked Skipjack called *Cakalang fufu* in North Sulawesi and *Cakalang asar* in Malucas, smoked Milkfish called *Bandeng asap* in East Java, smoked Eel called *Sogili fufu* in South East Sulawesi, and smoked Catfish called *Lele selai* in West Sumatera. In North Sulawesi smoked fish can be categorized as exotic indogenous food. Two famous smoked fish in North Sulawesi i.e. *Cakalang fufu* or smoked Skipjack (*Katsuwonus pelamis* L.) processed by hot smoking (temperature 80-100°C for 3-4 hr), and *Roa fufu* or smoked Halfbeak *(Hemiramphus far)* processed by semi-hot smoking (temperature 80°C for 1-2 hr and than 50-60°C for 10-15 hr).

Since an advanced in packaging and storing technologies, smoking fish was developed to produce a high value added products to satisfy consumer's taste (Agustinelli and Yohannes, 2015). According to Simon *et al.* (2005) over the past few decades traditional smoking of food has been replaced by use of smoke flavorings such as liquid smoke. However, Lingbeck *et al.* (2014) stated that smoke flavorings did not contain the same compounds as natural smoke since liquid smoke is filtered to remove toxic and carcinogenic impurities. Martinez and Machado (2016) stated that increasing phenol content in smoked meat could potentially enhance the smoky flavor of product. The problems of traditional smoked fish are: high contents of Polycyclic Aromatic Hydrocarbon (PAH) especially benzo(a)pyrene (BP), because of them have well-known genotoxic, mutagenic, and carcinogenic properties (Iwegbue *et al.*, 2015). According to Sikorski (1989) BP is an indicator of carcinogenetic and traditional smoked fish contained about 0.7-60 ng/g (wb) especially in skin layer. Benzo(a)pyrene is high in traditional smoked fish where the fish usually smoked directly above the fire (hot smoking). Content of PAH in some of cured fish products are: 716.84 µg/kg in smoked *Sardinella*

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aurita in Ghana (Essumang et al., 2012); 7.46 to 18.79 µg/kg in smoked *Lates mi[illegible]icus* in Kenya (Muyela *et al.*, 2012); and 0.56 to 1.46 µg/kg in all boiled, grilled, and fried fish in Western Cape, South Africa (Olatunji *et al.*, 2015). No data of PAH content in commercially smoked fish available in Indonesia yet. However, Swastawati *et al.* (2014) s[illegible]ied Benzo(α) pyrene (BP) in skipjack smoked with paddy chaff and coconut shell liquid smoke found BP of 9.55 and 8.72 ppm respectively. Beside PAH, traditional smoked fish also have low edible portion, no processing standard, flavor varied, difficulties in packaging, low performance, and short shelf life. Liquid smoke is an alternative, because easy to produce, use a simple equipment, can be found also in market, concentration can be controlled, quality of the product including flavor can be standardized, and smoked fish has high edible portion (100%). The aims of this study were to develop the best smoking method and the best liquid smoke concentration, based on PAH and sensory assessments.

Materials and Methods

Materials

Fresh Skipjack (*Katsuwonus pelamis* L) with a 1_{st} grade quality has been purchased at Bersehati fish market, Manado. The fish were put in cool box with fish: ice ratio = 1:2, and then transported by car for 30 minutes to laboratory. In laboratory, fish were washed and eviscerated, and fresh fillets (15 x 5 x 3 cm) were prepared. All treatments and procedures were done followed good manufacturing practices (GMP). Liquid smoke has been produced using smoke condensation equipment (Patent P00201405308), with coconut shell as fuel.

Treatments

Three liquid smoke concentrations (A) i.e. 0.4% (A_1), 0.6% (A_2), and 0.8% (A_3); and three application methods of liquid smoke (B) were used, namely: fresh fillet dipped in liquid smoked for 20 mins, and then heated in an oven at 70-80°C for 4 hr (B_1); fresh fillet was pre-heated at 70-80°C for 1 hr, and then dipped in liquid smoked for 20 mins, and heated again at 70-80°C for 4 hr (B_2); and fresh fillet was steamed for 30 mins, dipped in liquid smoked for 20 mins, and then heated at 70-80°C for 4 hr (B_3).

Research procedure

Research procedure can be seen in Figure 1. Before used, the initial total solid concentration of liquid smoke was determined, and then the concentration of treatments used (A_1, A_2, A_3) were determined based on the initial concentration, used formula of: ViCi = VtCt; where Vi is volume of initial liquid smoke, Ci is initial concentration of liquid smoke, Vt is volume of liquid smoke treatment, and Ct is concentration of liquid smoke treatment.

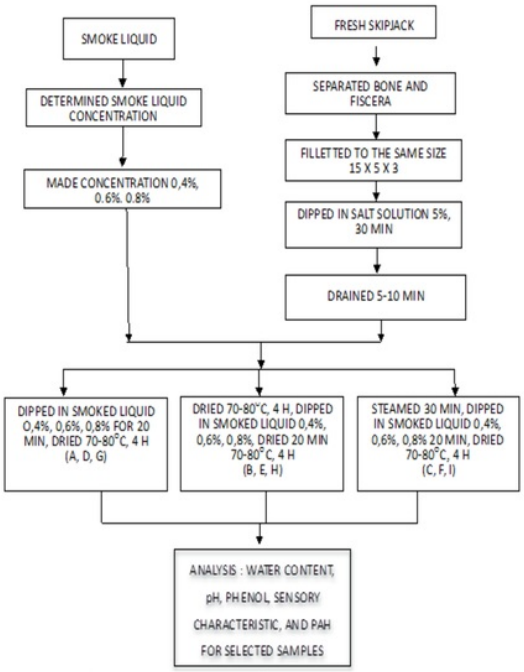

Figure 1. Scheme of research procedure

Samples analysis

Analysis have been done for water content (AOAC, 2005), phenol content using spectrophotometer (Standards National Indonesia, 2004), pH using pH meter (Adwa AD 1000 pH/mV and temperature meter), and sensory characteristics for appearance, flavour, taste, and texture. Hedonic assessment was used for sensory characteristics, and Triangle test has been used to assess the differences between liquid smoked products and the local conventional (traditional) product, using 18 to 21 semi-trained panelists (Meilgaard *et al.*, 1999). Score sheet have been used for both of hedonic and triangle assessments. Analysis of PAH using HPLC (Basak *et al.*, 2010) was done only for liquid smoke stock, the best concentration of liquid smoke (0.8%), and liquid smoked fillet.

Statistical analysis

There are two factors implementing on this study, [illegible]mely: liquid smoke concentration, with three sub-factors i.e.: A_1, A_2, and A_3; a[illegible] application methods of liquid smoke, with three factors i.e.: B_1, B_2, B_3.

Completely Randomize Design (CRD) Factorial 3x3 was designed. Replication of treatments was done twice. Analysis of Variance has been used to analyze the data, and continued by Least Significant Difference (LSD) test for a significant treatment (Steel and Torrie, 1980).

Results and Discussion

Water content

Water contents of smoked Skipjack were varied from 47.6% for sample that heated dipped in 0.8% liquid smoke and then heated again, to 60.6% for fillet that steamed, dipped in 0.6% liquid smoke, and then heated. Except for sample that steamed before dipped, the range of water contents was similar to the traditional local products. Water contents of traditional smoked fish products varied between 46-59% (Palinggi 1994; Berhimpon *et al*., 1995). Figure 2 showed that all steamed samples have high water content, because of after steamed the water content increase and the protein denatured.

Analysis of variance showed that liquid smoke concentration and interaction between two treatments were non-significant effect (P≥0.05) on water content, while application method of liquid smoke has a highly significant effect (P≤0.01). This condition affected by increased water holding capacity (WHC) in treatment B_2 and B_3 caused by protein denatured. This condition also found by Brdar *et al*. (2015) work with smoked fresh and frozen fish.

Phenol content

Phenol contents were varied from 1.98 mg% for sample of fresh fillet dipped in 0.4% liquid smoke, and then heated, to 12.6% for sample that steamed, dipped in 0.8% liquid smoke and then heated. From Figure 2, can be seen that fresh fillet that directly dipped in liquid smoke, contained lower phenol contents, followed by fillet which heated before dipped, and the higher was fillet that steamed first before dipped and heated. It can be seen also that increasing of phenol content, caused in decreasing of pH. Phenol is representative of all smoke components that exist in smoked fish. Increased in phenol content, followed also by increased in other smoke components (Berhimpon *et al.,* 1995). Phenol contents of traditional hot smoked skipjack of North Sulawesi, Indonesia smoked directly above fire for 3hr were 5.1-9.1mg% (Berhimpon *et al*., 1995). Kang *et. al.* (2012) found phenol content of the cold smoked salmon for 24 hr were 184.21±21.75 mg GAE/100g. Traditional smoked catfish in Riau, Indonesia smoked with different firewoods have phenol content range of

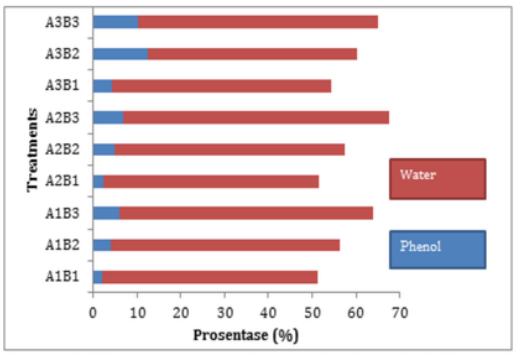

Figure 2. Water content and phenol of smoked Skipjack with different treatments. A1B1: dipped (0.4%) then heated; A1B2: heated, dipped (0.4%) then heated; A1B3: steamed, dipped (0.4%) then heated; A2B1: dipped (0.6%) then heated; A2B2: heated, dipped (0.6%) then heated; A2B3:steamed, dipped (0.6%) then heated; A3B1:dipped (0.8%) then heated; A3B2: heated, dipped (0.8%) then heated; A3B3:steamed, dipped (0.8%) then heated.

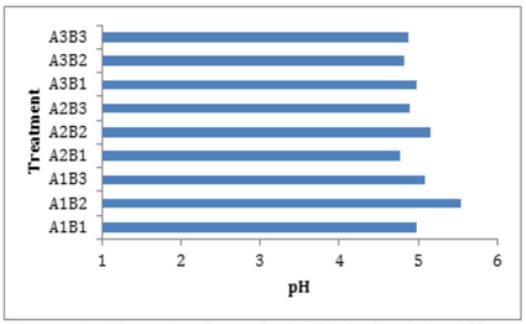

Figure 3. The pH of smoked Skipjack with different treatments. A1B1: dipped (0.4%) then heated; A1B2: heated, dipped (0.4%) then heated; A1B3: steamed, dipped (0.4%) then heated; A2B1:dipped (0.6%) then heated; A2B2: heated, dipped (0.6%) then heated; A2B3:steamed, dipped (0.6%) then heated; A3B1:dipped (0.8%) then heated; A3B2: heated, dipped (0.8%) then heated; A3B3:steamed, dipped (0.8%) then heated.

0.048±0.002 – 0.060±0.003% (Laksono *et al*., 2014).

pH

Data of pH in Figure 3, shown that pH were varied from 4,8 for sample that dipped in 0.6% liquid smoke and then heated, to 5.5 for sample that heated, dipped in 0.4% liquid smoke and then heated. The range of pH is similar to the pH of conventional smoked Skipjack (Berhimpon *et al.*, 1995). From Figure 2b, can be seen that all treatments almost have a similar pH. Analysis of variance showed that all treatments have no significant effect (P≥0.05) on pH. In conventional smoked Skipjack, variation of pH in smoked fish can be caused by variation in smoking time. In this research all samples dipped with the

Table 1. Polycyclic aromatic hydrocarbon (PAH) contents of liquid smokes and smoked Skipjack

PAH	Smoked Skipjack (ppb)	Liquid smoke Stock (ppb)	Liquid smoke 0.8% (ppb)	Standard ERC (ppb)	Toth and Potthast (ppb)
Benzo(a)pyrene	< 0.25	< 0.25	< 0.25	5	1
Benzo(a)anthrancene	< 0.25	< 0.25	< 0.25	-	-
Benzo(b,k)fluoranthene	< 0.5	< 0.5	< 0.5	-	-
Benzo(g,h)perylene	< 1.5	< 1.5	< 1.5	-	-

same time, and the concentration of liquid smoke did not also give a significant effect.

Polycyclic aromatic hydrocarbon (PAH)

The content of PAH and the standards for food, can be seen at Table 1. The results showed that the content of benzo(a)pyrene in liquid smoke stock, liquid smoke 0.8% concentration, and smoked skipjack dipped in liquid smoke 0.8%, were <0.25 ppb. The data showed that stock of liquid smoke produced by smoke condensation equipment (Berhimpon *et al.*, 2013) had low contents of PAH, especially benzo(a) pyrene i.e. < 0.25 ppb. The liquid smoke has varied in total solid of 20-40% depend on kind of fuels. The liquid smoke was than soluted to concentration of 0.8% and used in this research. As long as the liquid smoke used in this research has PAH content lower than 0.25 ppb, can be guaranteed that all products were smoked with 0.8% liquid smoke will be much lower than 0.25 ppb. This value is still lower than that of level recommended by the European Regulation Commission No. 1881/2006, where the maximum level of benzo(a)pyrene for oil and fats intended for direct consumption or as an ingredient in food are restricted to a level of up to 2 ppb, for smoked fish and meat 5 ppb, and bivalve mollusks to 10 ppb. A lower level of up to 1ppb was also recommended for children foods. Toth and Potthast (1984), stated that in Germany the content of benzo(a)pyrene in meat products is limited to 1 ppb. Until now Standards National Indonesia (SNI, 2013) did not recommended benzo(a)pyrene to be regulated yet.

Sensory analysis

Appearance

The scores of appearances can be seen in Figure 4. The lowest score was in sample that steamed, dipped in 0.6% liquid smoke and then heated. The highest score was in sample that heated, dipped in 0.8% liquid smoke and then heated. Analysis of variance shown that only application methods of liquid smoke

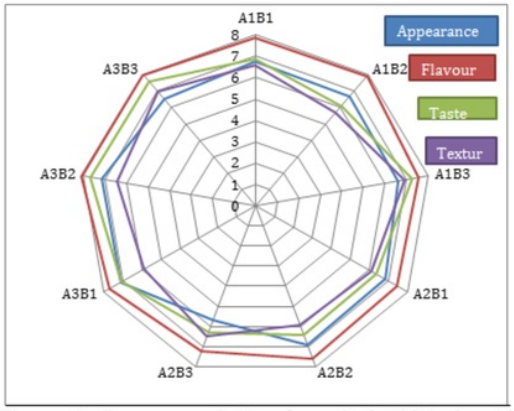

Figure 4. Sensory analysis of smoked Skipjack with different treatments. A1B1: dipped (0.4%) then heated; A1B2: heated, dipped (0.4%) then heated; A1B3: steamed, dipped (0.4%) then heated; A2B1:dipped (0.6%) then heated; A2B2: heated, dipped (0.6%) then heated; A2B3:steamed, dipped (0.6%) then heated; A3B1:dipped (0.8%) then heated; A3B2: heated, dipped (0.8%) then heated; A3B3:steamed, dipped (0.8%) then heated.

has a significant effect ($P \leq 0.05$) on appearance, while all other treatments including interaction did not give any significant effect ($P \geq 0.05$). For all concentrations of liquid smoke, fillet that steamed first before dipped, trend to have a low appearance. The best appearance was sample that heated before dipped in 0.8% liquid smoke, and then heated.

Flavor

The scores of flavors can be seen also in Figure 4. It can be seen that the scores of flavors in generally higher than the scores of appearance. Although, the higher score of flavor was for sample that heated first, then dipped in 0.8% liquid smoke and then heated, followed by samples that steamed, dipped in 0.8% liquid smoke and then heated. However, all samples shown satisfy values of flavor. This probably because of panelists could recognize that the flavor of smoked skipjack that smoked with liquid smoke was better than flavor of conventional smoked skipjack. Analysis of variance showed that all treatments including interaction did not give any significant effect ($P \geq 0.05$). From Figure 4 can be seen that the value of flavor were the highest among the sensory parameters assessed. The result also similar to that found by Agustinelli and Yeannes (2015) worked with cold smoked mackerel. Furthermore they stated that sample of smoked mackerel at 28°C and the sample of liquid smoked salmon resulted with an equal preference level, followed by sample of cold smoked at 22°C.

Taste

The scores of taste can be seen in Figure 4. The lowest score was for sample that heated, dipped in 0.4% liquid smoke then heated again, while the highest score was in sample that heated, dipped in 0.8% liquid smoke and then heated, and followed by samples that steamed, dipped in 0.8% liquid smoke and then heated. Analysis of variance shown that an only application method of liquid smoke has no significant effect ($P \geq 0.05$) on taste, but smoke concentrations gave a highly significant effect ($P \leq 0.01$), and interaction gave a significant effect ($P \leq 0.05$). For all concentrations of liquid smoke, sample which steamed first before dipped, and also heated first before dipped, trend to have a high taste. The same as appearance, the best taste was for sample that heated before dipped in 0.8% liquid smoke and then heated.

Texture

The scores of textures can be seen in Figure 4. The same as appearance, the score of texture little bit lower than score of flavor and taste. However, all sensory characteristics (appearance, flavor, taste, and texture) showed that panelist like the all assessed samples. The lowest score was in sample that heated and then dipped in 0.4% liquid smoke then heated, while the highest score was in sample that steamed, then dipped in 0.8% liquid smoke and then heated, followed by samples that steamed, dipped in 0.4% and then heated. Samples that heated, dipped in 0.8% liquid smoke and then heated also have a high score. Analysis of variance shown that liquid smoke concentration did not give significant effect ($P \geq 0.05$), while application methods of liquid smoke has a significant effect ($P \leq 0.05$) on texture.

Triangle test

Triangle test have been done for appearance, flavor, taste, and texture, to find the difference between new products compared to traditional (conventional) product, and the differences between each treatments.

The results shown that for appearance, sample that was heated first, dipped in 0.6% liquid smoke and then heated again, had a highly significant ($P \leq 0.01$) different and better compare to the conventional smoked skipjack and other treatments. The sample has a yellowish-brown color and bright. Sample which heated, then dipped in 0.8% liquid smoke, and then heated also had a highly significant ($P \leq 0.01$) different with the conventional smoked skipjack and other treatments, although this sample had a little bit dark brown color. According to Berhimpon *et al.* (2013), components of smoke will penetrate to middle layer during storage, and the dark brown color in surface will change to more light.

Figure 5. Traditional smoked Skipjack (left) and smoked skipjack using liquid smoke 0.8% in vacuum bag (right).

For flavor, sample heated first and followed by dipped in 0.8% liquid smoke and then heated again, has a different and better flavor compared to the conventional smoked skipjack and the other treatments. The flavor recognized as a real smoked skipjack flavor and more preferred by panelist. Taste also has a same result as flavor. Sample heated and followed by dipped in 0.8% liquid smoke and then heated, has a different and better taste compared to the conventional smoked skipjack and the other treatments. The sample taste was more delicious compare to the conventional smoked skipjack and the other treatments. Texture also has a same result; the sample heated first, then dipped in 0.8% liquid smoke and then heated again, has a good texture, more dry and solid. Agustinelli and Yeannes (2015) worked with cold smoked mackerel at 28°C, found that liquid smoked salmon had a same preference level compared with cold smoked mackerel. The picture of conventional (traditional) smoked skipjack and smoked skipjack heated first, and dipped in 0.8% liquid smoke and then heated again, can be seen in Figure 5.

Conclusion

The best formula and the best application method of liquid smoke for fresh Skipjack fillet was fresh fillet heated at 70-80°C for 4hr, dipped in 0.8% smoked liquid for 20 mins, and then heated again at 70-80°C for 4 hr. The second best was fresh fillet steamed for 30 mins, dipped in 0.8% smoked liquid for 20 mins, and then heated at 70-80°C for 4hr. Polycyclic Aromatic Hydrocarbon (PAH) content of liquid smoke stock, 0.8% liquid smoke that used to dip the samples, and sample treated with a best formula and best application method, safe for consumer because had a benzo(a)pyrene contents less than 0.25 ppb.

Acknowledgement

This research was funded by grants from Directorate General of Strengthening Research and

Development, Indonesian Ministry of Research, Technology and Higher Education.

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