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FULL PAPER

Quality characteristics and amino acid profile and fatty acid profile of smoked catfish treated using coconut shell liquid smoke

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Abstract

Smoked catfish is one of the processed fish products that is often found in various regions in Indonesia. Smoking method with liquid smoke can be alternative as a substitute for traditional smoking so that the resulting product has good quality. This study aimed to evaluate changes in the quality and amino acid profile of catfish processed using liquid smoke. Fish were immersed in liquid smoke with a concentration of 5%. Parameters analyzed in this study included levels of proximate, total bacteria, amino acid profile and fatty acid profile. The results showed that the use of liquid smoke had a significant effect ($p<0.05$) on the composition of proximate, total bacteria, amino acids profile, and fatty acid profile. Fresh and smoked catfish have a complete amino acid content. The dominant essential amino acids lysine and leucine were 25,738.98 mg/kg and 27,977.04 mg/kg, respectively. The highest fatty acid profile in fresh and smoked catfish were unsaturated fatty acids at 4.11 mg/kg and 4.33 mg/kg, respectively. Catfish processed by smoking liquid smoke changes in characteristics including proximate composition, amino acid profile, and fatty acid profile. The application of liquid smoke on smoked catfish also can reduced the total bacteria values.

1. Introduction

Catfish (*Clarias* sp.) is one of the fisheries farming commodities that have the potential to be developed and processed into value-added products. The production of catfish farming in Indonesia in 2016 was 764,796 tons, then in 2017, it increased to 1,125,526 tons. Catfish production in 2018 decreased to 1,027,032 tons (Ministry of Marine Affairs and Fisheries Republic of Indonesia, 2019). Catfish have good nutritional content in the term of crude protein (17.2%), fat (7.8%), water (75.3%) and ash (0.8%) (Manthey-Karl *et al.,* 2016). Fish in general are also known to contain fatty acids.

Omega-3 polyunsaturated fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic (DHA) play a role in lowering cholesterol and the several diseases risk. Catfish have been reported to contain high levels of fat, mostly unsaturated fatty acids, namely omega-7 fatty acids (palmitoleic acid) and omega-9 fatty acids (oleic acid). Volatile compounds from the aldehyde, ketone, and alcohol groups have been known to originate from various reactions involving fatty acids (Peinado *et al.,* 2016; Lazo *et al.,* 2017). Catfish also contain essential amino acids such as lysine, isoleucine, leucine, valine, glutamic acid, aspartic acid, alanine, and arginine (Pratama *et al.,* 2018).

Amino acids have physiological roles in the human body such as regulation of food intake, gene expression, and protein phosphorylation (Chalamaiah *et al.,* 2012; Machado *et al.,* 2020). Dietary supplements with the amino acid methionine can regulate lipid metabolism in the body and lysine has been reported to improve growth performance in the body (Martinez *et al*., 2017; Yang *et al*., 2017). Catfish is easy to experience quality deterioration which can cause the nutritional content in fish to be easily damaged. Smoking can be an effort to preserve and diversify products.

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Fish smoking accounts for 12.42% of all fish processing units in Indonesia (Ministry of Marine Affairs and Fishery Republic of Indonesia, 2021). This shows that smoked fish has a role in fulfilling community nutrition. The smoking method that involves the application of heat can remove moisture content and it can inhibit the activity of microorganisms and enzymes, and improve aroma, color, texture, and taste (Irwandi, 2016). Traditional fish smoking process in Indonesia is still does not meet the standards of smoked fish in Indonesia (Swastawati, 2018). Liquid smoke has been developed to improve the smoking process.

Liquid smoke is a compound that evaporates simultaneously from a hot reactor through the pyrolysis technique (decomposition with heat) and condenses in the cooling system. Liquid smoke that has undergone redistillation can be directly applied to food products. Liquid smoke has several advantages: easy to apply, more efficient to use, reduces environmental pollution, and reduces carcinogenic compounds. Liquid smoke can be applied in several ways, namely spraying, soaking, immersing, or mixing directly into food (Simon *et al*., 2005). Liquid smoke contains complex chemical components, consisting of aldehydes, ketones, alcohols, carboxylic acids, esters, furans, pyran derivatives, phenols and their derivatives, and hydrocarbons (Swastawati *et al*., 2019). Non-volatile flavor compounds influence the taste characteristics of a product and are usually derived from compounds of free amino acids, peptides, and nucleotides (Chen and Zhang, 2006; Pratama *et al*., 2013). Liquid smoke compounds can also act as antioxidants that can prevent oxidative damage to fish fat (Swastawati, 2008; Ernawati *et al*., 2012). Among processed fish products, smoked fish plays an important role in the diet of most of the world's population. The quality of smoked fish is influenced by raw materials, salting methods, smoke composition, smoking methods, and smoked agents (Siskos *et al.,* 2007; Alcicek *et al.,* 2010; Adeyeye, 2016). Food processing methods can affect composition and nutrition. food. The purpose of this study was to evaluate changes in the quality, amino acid profile, and fatty acid profile of smoked fish processed by the liquid smoke method.

2. Materials and methods

2.1 Materials

Catfish (*Clarias* sp.) were purchased from Local Fish Market in Semarang, Central Java, Indonesia. Fish were procured deceased and put into a Styrofoam icebox in fresh condition and were brought to laboratory for smoking process with liquid smoke addition. Coconut shell liquid smoke was purchased from PT. Asap Cair Multiguna in Semarang, Central Java, Indonesia.

2.2 Smoking process

Fresh catfish were cleaned using clean water and gutted. Then the fish that were gutted, immersed in a 5% liquid smoke for 1 hr, drained for 30 mins, then heated gradually, at 40–45°C for 1 hr; 60 – 70°C for 1 hr; and 90°C for 1 hr.

2.3 Proximate analysis

The proximate analysis such as water content (AOAC 925. 09 2005), ash content (AOAC 941.12 2005), protein content (AOAC 920.87 2005), fat content (AOAC 960. 39 2005), and carbohydrates (by difference method).

2.4 Total plate count

An antibacterial assay was performed according to National Standardization Agency for Indonesia number 2332.3: 2015. The sample was weighed (25 g), put in a sterile container containing 225 mL of Butterfield's phosphate buffered solution and was homogenized for 2 mins. This homogenate is a solution of 10^1. Approximately 1 mL of the homogenate was taken and put into 9 mL of Butterfield's phosphate buffered solution to obtain a 10^2 dilution. The 10^3 dilutions were carried out by taking 1 mL of the sample from the 10^2 dilutions into 9 mL of Butterfield's phosphate-buffered solution. Each dilution was shaken using a vortex. The same was done for diluents 10^4, 10^5 and so on according to the sample conditions. Samples of 0.1 mL from each dilution were pipetted into a petri dish that already contained PCA media and leveled using a bent glass rod. This is done in duplicate for each dilution. The plates were incubated in an inverted position in an incubator for 48 hrs at 37°C (National Standardization Agency for Indonesia, 2015).

2.5 Amino acid profile

Amino acid profile of smoked fish was carried out based on the AOAC procedure (2005) using high-performance liquid chromatography (HPLC) with instrument parameters: Ultra Techsphere column, mobile phase flow rate of 1 mL/min and the detector used was fluorescene.

2.6 Fatty acid profile

Analysis of fatty acid profiles using Gas Chromatography (GC 210A SHIMADZU) was carried out based on AOAC method (2005), with equipment specifications: GC Cyanopropyl methyl sil (capillary column) GC column parameters (60 mm × 0.25 mm × 0.25 μm), nitrogen and hydrogen carrier gases, initial temperature 190°C (hold 15 mins), increasing temperature 10°C/min, final temperature 230°C (hold 20 mins).

2.6 Data analysis

The proximate and total plate count data were analyzed using a t-test to see the difference in quality between fresh fish and smoked fish treated with liquid smoke. If $p<0.05$ then the treatment was significantly different. Amino acid and fatty acid profiles data were discussed descriptively.

3. Results and discussion

3.1 Proximate content and total bacteria

Proximate composition is an important component of food ingredients to be known in the food industry. Proximate information on a food ingredient can be used as data in product development, quality control or to determine the shelf life and superiority of the product (Thangaraj, 2016). Processing methods influence the nutritional value of fish, and there are considerable differences in water, protein, fat, and ash content (Turkkan *et al.*, 2008). The smoking process reduces the value of the water content and increases the value of the protein and fat content. The results of the t-test analysis showed that the water content of fresh catfish (72.62%) and catfish treated with liquid smoke was significantly different ($p<0.05$). The water content of smoked catfish with the addition of liquid smoke is less than that of fresh catfish. The water content of fish meat treated with liquid smoke will decrease due to the osmosis process. The amount of free water contained in fish meat is decreasing due to the entry of smoke components (Herawati *et al*., 2017). The smoking process in red tail Brycon fillet (*Brycon cephalus*) can reduce the water content from 72.9% to 58.5% (Franco *et al*., 2010). The moisture content of smoked fish treated by immersing redistilled liquid smoke ranged from 48.19–57.2% (Swastawati *et al*., 2016). The addition of different concentrations of liquid smoke nanocapsules can reduce the water content in catfish fillets, from 65.303% to 62.490% (Swastawati *et al*., 2019). The water content value in smoked catfish treated with liquid smoke has met the Indonesian smoked fish standard (SNI 2725: 2013), which is the maximum water content value of 60%. The value of water content can be influenced by factors during the smoking process, including smoking temperature, the size of fish, liquid smoke raw materials, and the speed of smoke flow in the smoking device. These factors affect the amount of smoke that comes into contact with fish, so it also affects the heat provided and the amount of water lost from fish products (Irwandi, 2016).

The second largest proximate composition of catfish is protein. The protein content of fresh and smoked catfish was 16.99% and 35.63%, respectively (Table 1). Changes in protein levels in catfish are influenced by the processing and the addition of liquid smoke. The moisture content of stingrays treated with liquid smoke was different (coconut shell and corn cob), respectively 33.73% and 32.54% (Swastawati *et al*., 2012). Protein content of ground beef jerky of African catfish with the addition of 0% liquid smoke; 1%, 3%, 5% each by 39.48%, 41.06%, 41.76%, 41.94% (Ramadayanti *et al*., 2019). The difference in protein content is caused by the constituent components of liquid smoke, especially cellulose, hemicellulose, lignin, carbonyl and phenol. The type of raw material greatly affects the protein content of smoke can change due to interaction with smoke components (Effendi, 2012).

Smoked catfish have a higher fat content than fresh catfish (Table 1.). Liquid smoke immersion can maintain the quality of the ingredients while maintaining the fat content in the product without breaking down the fat composition. Fat content and water content in smoked fish are related, if the water content is lower than the fat content in smoked fish will increase (higher). Liquid smoke can bind the water content in the material so that the water content in smoked fish decreases and causes the fat content to increase (Hutomo *et al.,* 2015). The high-fat content value of smoked catfish can be influenced by heat and smoke sticking which can react with enzymes in fish tissue causing an increase in the rate of change in fat content (Stolyhwo and Sikorski, 2005). The fat content in smoked catfish has met the Indonesian smoked fish standard (SNI 2725: 2013), which was a maximum of 12.5% (National Standardization Agency for Indonesia, 2013).

Ash content is a parameter of the nutritional value of foodstuffs and is related to the minerals contained in a material (organic and inorganic salts). Mineral components in a material can be determined by determining the remnants of burning mineral salts, known as ashing (Sudarmadji *et al*., 2003). The smoking method with liquid smoke had a significant effect ($p<0.05$) on the ash content of catfish. The ash content of smoked catfish (2.90%) was higher than that of fresh fish (1.68%). Swastawati *et al*. (2007) reported the ash content of mackerel with rice husk liquid smoke immersion of 2.38%. The value of ash content is inversely proportional to the water content.

Microbiological damage to fishery products depends on the speed of microbial growth, especially spoilage bacteria. The application of liquid smoke in the smoking process can reduce the total bacteria value (TPC) to 1×10^1 CFU/g (Table 1). The decrease in total bacteria was caused by the bacteriostatic nature of liquid smoke

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because it contains phenol so that it can inhibit bacteria. Huong *et al.* (2013) reported a log TPC value of smoked mackerel treated by liquid smoke is lower than the traditional method (3.33 log CFU/g).

Table 1. The proximate composition and total bacteria of fresh and smoked catfish.

Parameter	Fresh catfish	Smoked catfish treated by liquid smoke
Proximate (%)		
Water	72.62±0.14	51.99±0.32
Protein	16.99±0.08	35.63±0.50
Fat	6.08±0.09	8.90±0.08
Ash	1.68±0.02	2.90±0.77
Carbohydrate	1.17±0.03	0.57±0.14
Microbiology		
Total bacteria (CFU/g)	$4.8\times10\pm2.83^6$	$1\times10\pm0.00^1$

Values are presented as mean±SD of three replications.

3.2 Amino acid profile

The protein quality of a product is determined by its amino acid composition. High-protein is a protein that contains all types of amino acids and in proportions suitable for growth (Almatsier, 2006). Amino acid analysis was carried out to determine the profile and amino acid content of fresh and smoked catfish meat. The results showed that in catfish, 15 types of amino acids were detected, consisting of 8 essential amino acids and 7 types of non-essential amino acids. The highest content of essential amino acids in fresh and smoked catfish include leucine, arginine, and lysine (Table 2). Leucine is an important molecule that can stimulate muscle protein synthesis and also has a therapeutic value associated with stress, trauma and burns (Vijayan *et al.*, 2016). Lysine plays an important role in strengthening circulation and maintaining normal cell growth (Suryaningrum *et al.*, 2010). The three amino acids increased after the fumigation process with liquid smoke were 9.546.24 mg/kg to 26,329.8 mg/kg (arginine), 12,701.86 mg/kg (lysine) and 12,047.39 mg/kg to 27,977.04 mg/kg (leucine). The length of time treatment and the smoking method can play a role in changes in the amino acid content of fish (Oluwaniyi *et al.*, 2010). A decrease in water content will cause an increase in amino acids (Lopes *et al.*, 2015). The proteolytic reactions that occur during the smoking process can also lead to increased formation of free amino acids (Liu *et al.*, 2009). The heating process will cause chemical changes in the amino acid residues, which can cause changes in the structure, digestibility, and functional properties of the protein (Deng *et al.*, 2014). The amino acids profile of smoked fish treated using liquid smoke affected by phenol and organic acids in liquid smoke. This is because the combination of phenol functional components and high organic acid content synergistically prevents and controls the growth of microbes that cause spoilage in which proteins and amino acids are converted into basic ammonia compounds (Himawati, 2010).

The main function of amino acids in protein synthesis and energy production and special functions are found in each type. As well as the function of histidine as a histamine precursor in the framework of growth, Lysine as a cross-linking process protein biosynthesis. Valine plays a role in growth, nervous and digestive systems. Isoleucine plays a role in the formation of muscle and hemoglobin while threonine plays a role in maintaining protein balance plays a role in the formation of collagen and elastin. In addition, Phenylalanine works as a precursor of tyrosine, catecholamines, and melanin while methionine functions as a precursor cysteine. The highest glutamate amino acid in fresh skipjack tuna has a role in the reaction transaminases (Linder, 2006; Yuliarti, 2009; Mohanty *et al.*, 2014).

Table 2. Amino acid profile of fresh and smoked catfish.

Asam amino	Concentration of amino acids (mg/kg)	
	Fresh catfish	Smoked catfish treated by liquid smoke
Essential		
L-Phenylalanine	7,014.54	18,747.82
L-Isoleucine	6,274.35	14,715.84
L-Valine	6,745.91	16,085.10
L-Arginine	9,546.24	26,329.80
L-Lysine	12,701.86	25,738.98
L-Leucine	12,047.39	27,977.04
L-Threonine	7,828.28	19,665.25
L-Histidine	9,930.03	9,930.03
Non-essential		
L-Serine	7,028.87	17,805.43
L-Glutamic acid	22,850.94	50,876.69
L-Alanine	8,868.65	20,916.45
Glysine	8,622.36	26,296.08
L-Aspartic acid	13,955.26	31,348.77
L-Tyrosine	5,250.44	13,591.09
L-Proline	5,528.39	15,215.36

3.3 Fatty acid profile

Fatty acids are important nutritional compounds that can affect the health of people who consume them, it depends on various factors such as the source and type of fatty acids consumed. The fat content in fish is influenced by the environment in which the fish grows and develops as well as its eating habits. The types of unsaturated fatty acids that play an important role in human health are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Both are also found in fresh and smoked catfish. Fresh and smoked catfish had EPAs of 0.019% and 0.039% and DHA of 0.160% and

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0.302%, respectively (Table 3). The content of EPA and DHA in the sample with the addition of liquid smoke was higher. This is related to the smoke component which acts as an antioxidant. Various phenolic compounds such as syringol and guaiacol can function as hydrogen donors and in very small amounts are effective in preventing lipid oxidation (Bortolomeazzi *et al*., 2007). EPA and DHA fatty acids are very important for humans to prevent coronary heart disease. DHA is an important component of brain development, eye and heart health. EPA is known to be useful in treating brain disorders and treating cancer (Ozogul *et al*., 2007).

Oleic acid in samples of fresh and smoked catfish had a high quantity of 2.437% and 2.171%, respectively (Table 3). Products with the addition of liquid smoke contain several higher fatty acids than fresh fish products. The addition of liquid smoke can maintain the quality of fatty acids. Liquid smoke can be used as a natural antioxidant in food processing, including fish and its processed products, this is because the content of 2-methoxy phenol, 2,6 dimethoxyphenol, and formic acid in liquid acid can act as an antioxidant and antibacterial (Swastawati, 2008; Zuraida *et al*., 2011; Ernawati *et al*., 2012; Swastawati *et al*., 2014).

4. Conclusion

Catfish processed by liquid smoke method changes in characteristics including proximate composition, total bacteria, amino acid profile, and fatty acid profile. Smoked catfish in this research has met the Indonesian smoked fish standard (SNI 2725:2013).

Conflict of interest

The authors declare no conflict of interest.

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Table 3. Fatty acids profile of fresh and smoked catfish.

Fatty acid	Concentration of fatty acid (%)	
	Fresh catfish	Liquid smoke method
Butyric acid (C4:0)	ND	ND
Caprylic acid (C8:0)	ND	ND
Caproic acid (C10:0)	ND	ND
Lauric acid (C12:0)	0.025	0.068
Tridecanoic acid (C13:0)	ND	ND
Myristic acid (C14:0)	0.065	0.118
Palmitic acid (C16:0)	1.471	1.882
Palmitoleic acid (C16:1)	0.149	0.120
Heptadecanoic acid (C17:0)	ND	0.018
Stearic acid (C18:0)	0.401	0.546
Oleic acid (C18:1n-9)	2.437	2.171
Linoleic acid (C18:2n-6)	1.059	1.174
Arachidic acid (C20:0)	ND	0.019
Eicosenoic acid (C20:1)	0.031	0.043
Linolenic acid (C18:3n-3)	0.101	0.174
Tricosanoic acid (C20:5)	ND	ND
Arachidonic acid (C20:4n6)	0.095	0.181
Heneicosanoic acid (C21:0)	ND	ND
Behenic acid (C22:0)	ND	ND
Docosadienoic acid (C22:2)	ND	ND
EPA (C20:5n3)	0.019	0.039
DHA (C22:6n3)	0.160	0.302
Omega-3 fatty acids	0.225	0.408
Omega-6 fatty acids	1.276	1.527
Omega-9 fatty acids	2.437	2.171
Unsaturated fatty acid	4.117	4.333
Polyunsaturated fatty acid	1.501	1.981
Monosaturated fatty acid	2.616	2.352
Saturated fatty acid	1.963	2.686

ND: Not detected

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