Application of liquid smoke from corncob and coconut shell to the fillet of catfish (*Pangasius* sp.)

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Abstract. Liquid smoke is known to have several compounds, such as phenols, acids and carbonyls, with antibacterial and antioxidant properties. This study aims to discover the liquid smoke toxicity and determine the effect of liquid smoke nanocapsule addition on the chemical characteristics of catfish (*Pangasius* sp.) fillet. The liquid smoke combination (corncob and coconut shell) was processed into nanocapsules and was applied on catfish fillet in various concentrations 0%, 0.5%, 1% and 1.5%. The fillet then was stored at room temperature for 24 hours. The application of liquid smoke (LS) nanocapsules in a concentration of 1.5% on catfish fillet maintained the moisture, lipid and protein content of catfish fillet, with the following values: 62.49%, 6.71% and 30.55%, respectively. The water activity, pH, thiobarbituric acid (TBA) and total volatile base nitrogen (TVBN) analyses values of catfish fillet with 1.5% liquid smoke nanocapsules were 0.707, 6.2, 3.593 mg malonaldehyde/kg and 82.237 mgN/100 g, respectively. This shows that 1.5% liquid smoke nanocapsules are able to maintain the chemical composition, as well as inhibit lipid oxidation in catfish fillet. However, it could not inhibit microbial growth, shown by the TVBN value, which exceeds the standard. According to the Scanning Electron Microscopy analysis, the morphology of catfish fillet with the addition of nanocapsules was more compact and solid compared to the control sample.

Key Words: toxicity, catfish fillet, chemical composition, nanocapsule.

Introduction. Catfish (*Pangasius hypophthalmus*) is a farmed freshwater fish in Indonesia with a high demand for its consumption (Tran et al 2017). According to the 2012 Asia Fish Model data, total catfish production (*Pangasius* sp.) in Indonesia reached 384000 tons, while the level of public consumption of catfish reached 243000 tons. It shows that the consumption of catfish reaches 63% of the total production. The rate of production and consumption of catfish is expected to increase until 2028. The level of consumption of catfish in Indonesia is mainly in the form of fillets. Nurilmala et al (2015) stated that Indonesians consume around 700 tons of catfish fillet per month. The high demand for catfish is due to the good taste and soft texture of the white meat of catfish (Rathod et al 2018).

Catfish is a great source of many nutrients. Catfish has a 75.3% moisture content, 7.8% crude fat, 17.2% crude protein, and 0.8% ash (Manthey-Karl et al 2016). Tenyang et al (2013) stated that catfish contains high levels of fat, mostly unsaturated fatty acids in the form of EPA and DHA. Hashim et al (2015) stated that besides EPA and DHA, the unsaturated fatty acids in catfish are omega-7 fatty acids, like palmitoleic acid and omega-9 fatty acids, such as oleic acid. Catfish also contains a high level of protein. Catfish contain high amounts of essential amino acids (AA), such as lysine, isoleucine, leucine, valine, glutamic acid, aspartic acid, alanine and arginine (Pratama et al 2018). The high nutrient content in catfish can lead to rapid degradation or damaged during storage. Ikasari & Suryaningrum (2015) stated that catfish fillets experience a decrease in panelist ratings of appearance, odor and texture during storage. This is also supported by the increasing value of total volatile base nitrogen (TVBN) in the filet. Therefore, preservation methods are necessary for maintaining their chemical characteristics.

One of the preservation methods is smoking. Smoking is a preservation technique using smoke compounds as a medium (Rizo et al 2015). Smoke can be condensed in liquid smoke, which is easy to apply to food. Liquid smoke contains active compounds that serve as food preservatives, such as phenols and acids (Lombok et al 2014) and also carbonyls (Budaraga et al 2017) that has antibacterial (Zuraida et al 2011) and antioxidant properties (Saloko et al 2014). Sometimes, liquid smoke is suspected to contain toxic compounds that appear during the pyrolysis process. However, sometimes, the components contained in liquid smoke have significant benefits. Besides being able to extend shelf life, the liquid smoke flavor is also very preferred. Liquid smoke has been widely applied to food products. Desniorita & Maryam (2015) applied 2% of liquid smoke to extend the shelf life of flour sauce for seven days. Maryam (2015) added 2% liquid powder smoke to sponge cake to produce a sponge cake with a flavor and taste liked by panelists. The sponge cake had a shelf life of 8 days. The liquid smoke compounds are volatile and not durable during storage. Therefore, nanoencapsulation should be carried out in order to protect the compounds.

Nanoencapsulation is a technology that traps the bioactive compound, so it can be transferred appropriately on food until reaching the cells. One of the nanoencapsulation methods is by spray drying, which produces nanoparticles (Ezhilarasi et al 2013). The nanoparticles are in the form of powder so that they can be easily applied to food. Saloko et al (2014) applied liquid smoke nanocapsules from coconut shells to fresh tuna and produced tuna that can survive for 48 hours at room temperature. There is no research yet on the application of nanocapsules from liquid coconut shell smoke combined with corncobs. Therefore, this study aimed to determine the concentration of liquid smoke nanocapsules from coconut shells and corncob, which can preserve the chemical characteristics of catfish fillets.

Material and Method

Research materials. The materials used in this study are comprised of: catfish, coating materials, liquid smoke produced from corncobs and coconut shells. Catfish or "patin fish" (*Pangasius hypophthalmus*) were purchased from a local market in Semarang, Central Java, Indonesia. The research samples were five fresh live catfish, weighing approximately 2 kg each. Liquid smoke was obtained by the pyrolysis of corncobs and coconut shells. The process was conducted in a laboratory of Diponegoro University. The coating materials for the nanoencapsulation process were Na-alginate provided by PT Selalu Lancar Maju Karya, Jakarta, Indonesia. Maltodextrin DE 10 and Arabic gum were purchased from CV Multi Kimia Raya, Semarang, Indonesia. Other chemicals used were Kjeldahl tablets, acetic acid, sodium hydroxide, methyl red indicator, benzene, total volatile base nitrogen (TVB-N), chloric acid (TVB), phenolphthalein, silicon anti-foaming indicator and sodium hydroxide. The experiment was conducted from April to November 2017.

Liquid smoke production process. The liquid smoke was produced by pyrolysis process with a set of pyrolysis tools: a drum unit made of stainless steel with a 100 kg capacity, a unit condenser, a pipe to release smoke, a set of distillation and ignition tools. Corncobs and coconut shells were introduced in the pyrolysis set. The pyrolysis process was conducted at 400°C for 5 hours. Liquid smoke produced from the process was distillated to be applied on the catfish fillets.

Liquid smoke nanoencapsulation. The liquid smoke (combination of corncobs and coconut shells) nanoencapsulation process was performed according to Saloko et al (2013), using maltodextrin, Arabic gum and Na-alginate as a coating material with a ratio of 0.167:0.667:0.167. The solution was homogenized and centrifuged at 3000 rpm for 30 minutes at room temperature. The supernatant was separated and filtered to obtain a pure nanoparticle solution. The nanoparticle solution was heated at 50°C in a water bath for 15 minutes and homogenized using a homogenizer at 4000 rpm for 2.5 minutes. The sample was then dried using a spray dryer at 130°C for the inlet temperature, the outlet temperature was 70°C. The nanocapsules obtained were then collected into a sealed glass bottle and stored at room temperature.

The application of liquid smoke nanocapsules on catfish fillet. Catfish fillets with an approximate size of 25x15x1 cm and a weight of around 100 g were smeared with liquid smoke in a concentration of 0%, 0.5%, 1% and 1.5% of the fillet weight. The fillet was heated in an oven at 90°C for 4 hours. It was stored at room temperature for 24 hours.

Chemical composition. Moisture content analysis was carried out using the thermogravimetric method, protein content was determined using the Kjeldahl method, and fat content was determined using the Soxhlet extraction with petroleum ether.

pH and Aw analysis. A digital pH meter (pH meter TPX-90i Chemical Laboratories Co., Ltd.), was used to analyze the pH, while a digital Aw meter (Rotronic HYGROPALM) was used to analyze the Aw.

TVBN analysis. The TVBN analysis was conducted according to established methods (Badan Standarisasi Nasional 2009; Achmadi et al 2013). About 10 g of sample were mixed with ceramics mortar then 90 mL of perchloric acid 6% was added. The solution was homogenized for 2 minutes, then filtered and settled at room temperature for 30 minutes. Afterwards, the solution was filtered with filter paper number 2-3. About 50 mL of filtrate was poured into a distillation tube and several drops of phenolphthalein and silicone anti-foaming indicator were added. Distillation was carried out by adding 10 mL of NaOH 20%. An Erlenmeyer containing 100 mL of H_3BO_4 3% and 3-5 drops of Tashiro indicator (purple solution) were prepared. Distillation lasted 10 minutes, until 100 mL of distillate and 200 mL of a final volume (green solution) were obtained. The distilled sample was then titrated using HCl 0.02 N. Titration was terminated when a purple solution was formed.

TVBN (mg/100 g)=[(Vc-Vb)xNx14.007x100]/W

Where: TVBN - total volatile base nitrogen; Vc - HCl volume for sample titration; Vb - HCl volume for blank titration; N - HCl normality; W - sample weight (g); 14.007 - the nitrogen weight.

TBA analysis. The thiobarbituric acid (TBA) analysis was measured according to Swastawati et al (2012). About 5 g of sample were added to 50 mL of distilled water and the mix was homogenized. The solution was poured into a distillation tube and 2.5 mL of HCl 4 N was added until a pH of 1.5 was reached. It was heated for 10 minutes until 50 mL of distillate was obtained. The distillated mixture was then filtered and 5 mL of distillate was transferred into a 50 mL Erlenmeyer in sterile conditions. 5 mL of TBA reagent was added and heated for 35 minutes. Afterwards, the solution was cooled and measured spectrophotometrically at 528 nm. Lastly, the TBA value was calculated.

Morphology. The catfish fillet morphology was observed using Scanning Electron Microscopy (SEM) with 10 kV of voltage.

Statistical analysis. This study used a completely randomized design with one factor of liquid smoke nanocapsule concentration. The experiment was conducted in triplicate. The SPSS 17 software was used to analyze the parametric data. If there were significant differences, the analysis was continues with the Tukey test.

Results and Discussion

Toxicity of liquid smoke. Liquid smoke is the result of pyrolysis using high temperature. According to Dhabbah (2015), fire effluents are sometimes able to produce acute toxicity in the human body. Toxicity is affected by the components contained in liquid smoke. The dominant components of liquid smoke produced by pyrolysis at a temperature of 100-400°C are acetic acid and phenols (Budaraga et al 2016a; Yuniningsih & Anggaini 2013). Some negative results are observed by Budaraga et al (2016b) who analyzed the toxicity of liquid smoke from cinnamon, and reported mortality in mice at concentrations higher than 1500 ppm. The toxicity assay on liquid smoke should be conducted before applying on food, and will be the object of a future study, in the case of liquid smoke from corncob and coconut shells.

Moisture, crude protein and crude fat of smoked catfish fillet. The catfish fillet smeared with liquid smoke nanocapsules in various concentration was analyzed for its chemical composition. The analysis results are presented in Table 1.

Table 1

Moisture, crude protein and crude fat content of smoked catfish fillet

Concentration	*Moisture (%)*	*Crude protein (%)*	*Crude fat (%)*
0%	65.303±0.405^{d}	28.107±1.17^{a}	7.573±0.297^{d}
0.5%	61.147±0.301^{a}	28.530±0.306^{a}	4.677±0.282^{a}
1.0	63.597±0.325^{c}	29.853±0.525^{ab}	5.737±0.208^{b}
1.5%	62.490±0.248^{b}	30.547±0.759^{b}	6.713±0.337^{c}

Note: different superscript letters in the same column show significant differences (P<0.05).

The moisture content of catfish fillet with different liquid smoke nanocapsule concentrations decreased due to the heat, which reduced the moisture level in fish fillet. The moisture content of catfish fillet is around 61.147-65.303%, in accordance with Isamu et al (2012), who processed smoked tuna (*Katsuwonus pelamis*) and the moisture level obtained after smoking was around 63-68%. The moisture level in stingray (*Dasyatis blekeery*) is around 61-73% (Swastawati et al 2012). The moisture level of stingray fillet decreases inversely proportional to the protein and lipid content. Protein and lipid contents of catfish increase along with the decline of the moisture level. According to Swastawati et al (2012), the decline in moisture level and the increase of protein and lipid contents of smoked fish are because of heating in the smoking process.

Aw. Water activity (Aw) holds an important role in fish deterioration due to microbial growth (Abbas 2009). The Aw value in this research is presented in Table 2.

Table 2

Aw, pH, TVBN and TBA of smoked catfish fillet

Concentration	*Aw*	*pH*	*TVBN mgN/100 g*	*TBA mgmalonaldehyde/kg*
0%	0.914±0.004^{d}	6.733±0.057^{c}	95.167±0.511^{d}	10.563±0.025^{c}
0.5%	0.890±0.003^{c}	6.533±0.057^{b}	88.363±0.565^{c}	4.960±0.223^{b}
1.0	0.777±0.009^{b}	6.433±0.057^{b}	81.417±0.281^{a}	4.007±0.111^{a}
1.5%	0.707±0.007^{a}	6.200±0.100^{a}	82.237±1.521^{b}	3.593±0.225^{a}

Note: Aw – water activity; TVBN - total volatile base nitrogen; TBA - thiobarbituric acid. Different superscript letters in the same column show significant differences (P<0.05).

Based on Table 2, the higher liquid smoke nanocapsule concentration applied on catfish fillet is, the lower the Aw value is. On 1.5% nanocapsule concentration, the Aw value was 0.707, compared with that of 0% concentration, 0.914. This result is similar to others, which show that the Aw value of hot smoked rainbow trout (*Oncorhynchus mykiss*) is around 0.7-0.9 (Esmailnia 2015). This indicates that liquid smoke nanocapsules are capable to inhibit microbial growth. Aw is closely related with the moisture content. According to Lisa et al (2015), the relation between the moisture content and water activity is indicated by a directly proportional tendency: the Aw increases with the moisture value.

pH. The pH value of the catfish fillet decreases along with the increasing of nanocapsule concentration. The pH value of smoked catfish with 1.5% nanocapsule concentration was 6.2. This result was significantly different from the pH value of the fillet with 0% nanocapsule concentration, of 6.7. Liquid smoke contains acid compounds (Swastawati et al 2007). This indicates that nanocapsules are able to trap acid compounds in liquid smoke and can be absorbed into the tissues.

TVBN value. TVBN measures the bacterial activity able to degrade proteins (Ariestya 2016). Based on Table 2, the TVBN of catfish fillet decreased along with the increase of liquid smoke nanocapsule concentration after 24 hours of storage. The TVBN value of catfish fillet with 1.5% liquid smoke nanocapsules was 82.237 mgN/100g. This result is higher compared with those of other studies, where 8% liquid smoke concentration on pomfret (*Colossoma macropomum*) produces a TVBN value of approximately 23 mgN/100g (Achmadi et al 2013). The smoke was obtained from palm oil and shells.

The increasing value of TVBN caused by bacterial activity on muscle tissues produce ammonia, trimethylamine and dimethylamine. The nanocapsule concentration and the TVBN values are inversely proportional. This because liquid smoke contains phenols able to inhibit microbial activities. Saloko et al (2014) state that phenols from liquid smoke are antimicrobial agents able to inhibit microbial activities. The maximum limit for TVBN in fish is 35 mgN/100g (Gunsen et al 2011). In this study, the TVBN exceeded the maximum limit for standard consumption. This is allegedly due to phenols, which partially evaporated during the nanoencapsulation process with spray dryer in high temperature, thus not inhibiting microbial activities.

TBA value. TBA indicates the lipid oxidation degree (Bilgin et al 2008). Based on Table 2, the liquid smoke nanocapsule concentration and TBA values are inversely proportional. This indicates that liquid smoke nanocapsules are able to inhibit lipid oxidation in catfish fillet. The TBA of smoked catfish with 1.5% nanocapsule concentration was 3.593 mg paraformaldehyde/kg. This result is significantly different from the one in the control sample. The maximum limit of TBA is 5 mg paraformaldehyde/kg (Gunsen et al 2011). The catfish fillet with nanocapsule addition produces TBA values below the maximum limit. It shows that the application of liquid smoke nanocapsules in catfish fillet works well in inhibiting lipid oxidation.

Morphology. The morphology of catfish fillet are visible in Figure 1. According to the figure, the muscle tissues of catfish without liquid smoke nanocapsule addition were fragile and easily damaged, whereas the muscle tissues with liquid smoke addition were compact and solid. This indicates that the addition of liquid smoke nanocapsules positively affects the catfish muscle tissue texture. This is related to the decline of the moisture content in catfish fillet with the addition of nanocapsules. The decreasing level of moisture affects the texture, making it more compact and solid. Martinez et al (2011) state that liquid smoke affects the texture of muscle tissue. Furthermore, liquid smoke compounds, especially carbonyl, are able to affect the texture of the product.

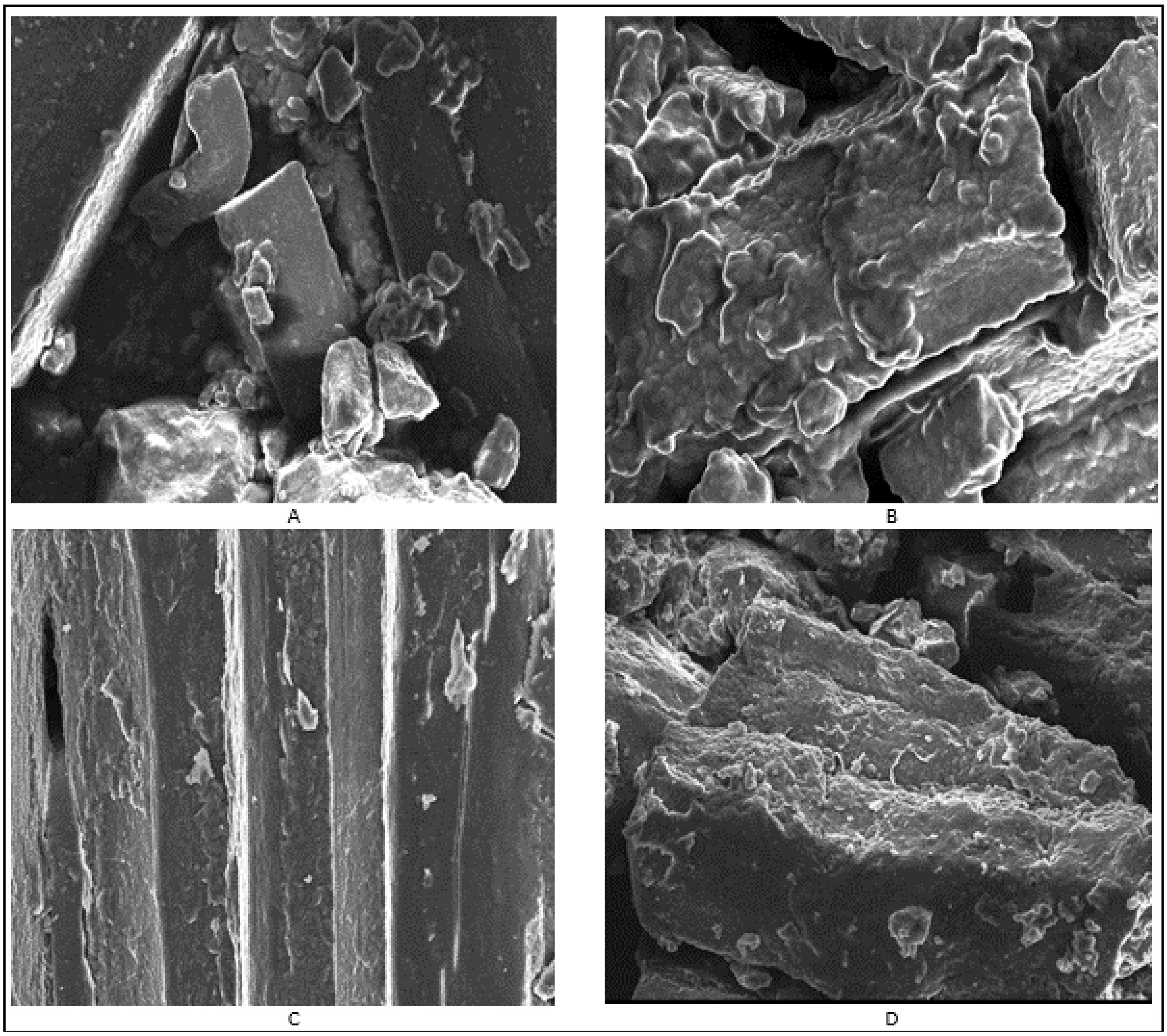

Figure 1. The Morphology of catfish muscle tissue; A - 0% liquid smoke nanocapsule concentration; B – 0.5% liquid smoke nanocapsule concentration; C – 1.0% liquid smoke nanocapsule concentration; D – 1.5% liquid smoke nanocapsule concentration.

Conclusions. In terms of toxicity, liquid smoke still needs to be tested. The 1.5% concentration of liquid smoke nanocapsule applied to catfish fillet is able to maintain the crude protein and crude fat content, as well as to inhibit lipid oxidation. However, microbial growth is still active, as shown by the TVBN value, which exceeds the Indonesian standard. Scanning Electron Microscopy analysis shows that the morphology of catfish fillet with the addition of nanocapsules was more compact and solid compared to the control sample.

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