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## Submission Paper Carpathian Journal Dr. Fronthea Swastawati

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Semarang, August 2<sup>nd</sup>, 2018

Dear

Editor – In – Chief

**Carpathian Journal of Food Science and Technology**

Re: Submission paper

Dear Editor,

I hereby submit the paper entitled **"The Effect of Antioxidant and Antibacterial Nanoencapsulation Liquid Smoke on Catfish Fillet (*Pangasius sp.*) during Storage at Room Temperature and Cold Temperature"** to **Carpathian Journal of Food Science and Technology**. The paper is authored by Fronthea Swastawati, Ahmad Ni'matullah Al-Baari, Eko Susanto, Lukita Purnamayati, with myself as corresponding author.

We do state that this manuscript has been not submitted to other journal or elsewhere as well as had not been submitted earlier to Carpathian Journal of Food Science and Technology.

We also stated that there is no conflict of interest during its preparation and submission of this manuscript.

We hope you find this paper interesting and publishable in your esteemed journal. We look forward to your decision.

Best regards

Dr. Fronthea Swastawati

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THE EFFECT OF ANTIOXIDANT AND ANTIBACTERIAL  
NANOENCAPSULATION LIQUID SMOKE ON CATFISH FILLET (*Pangasius*  
sp.) DURING STORAGE AT ROOM TEMPERATURE AND COLD  
TEMPERATURE

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ABSTRACT

The purpose of this study was to determine the effect of antioxidant and antibacterial nanoencapsulation liquid smoke on a catfish file ( *Pangasius* sp.). A combination of liquid smoke (comcob and coconut shell) were processed into nanoencapsulation using three encapsulan i.e: gum arabic, maltodextrin, and alginate with a ratio of 1/6: 4/6: 1/6 each. Nanoencapsulation liquid smoke was known to have a total content of phenols, carbonyl, and Radical Scavenging Activity, there were 3.682 mg GAE/g, 3.439%, and 91.348%, respectively. Nanoencapsulation liquid smoke was applied to the catfish and stored at room temperature (28°C±2°C) and cold temperature (5°C). Observations were made on days 0, 2, 4, 6, 8, and 10 to parameter PV, TBA, TVBN and TPC. The results showed that nanoencapsulation liquid smoke could effectively inhibit the oxidation of fat catfish showed with PV and TBA acceptable. Nanoencapsulation liquid smoke was also capable of inhibiting the activity of microbes. indicated by the value of TVBN and TPC which were still below standard at all temperatures and long storage time.

1.Introduction

Catfish were easily damaged by changes fat content (oxidation process, lipoxygenase damage, etc), protein changes and changes the content of microorganisms (Masniyom, 2011). This damage is indicated by peroxide numbers, TBA (Valdes, *et al.*, 2015), TVBN (Tian, *et al.*, 2012; Castro, 2012) and TPC (Adilla, *et al.*, 2017) which increases during storage. Catfish have high nutrient content especially fat and protein. Catfish contain palmitic acid (24.05%), oleic acid (27.55%), and linoleic acid (7.63%). In addition, catfish also contains non essential amino acids, such as as glutamate (3.33%) and

essential amino acids, for example lysine (1.82%) (Nurilmala *et al.*, 2015). The high content of fatty acids and amino acids of catfish, resulting in catfish being damaged continuously during cold storage temperatures (Abbas *et al.*, 2005). Therefore, treatment were needed to inhibit catfish damage during storage.

Liquid smoke is one of the smoke condensation products in the form of liquid. Liquid smoke is widely used compared to traditional curing methods because it is easy to use and more economical. Liquid smoke also has several compounds such as phenol, acids and carbonyl that acts as an

antibacterial and antioxidant (Saloko, *et al.*,2014). Several studies has been done by other researcher using coconut shell liquid smoke to inhibit fish damage, such as tuna (Saloko, *et al.*,2014) tilapia (Ariestya, *et al.*,2016) , and catfish (Swastawati, 2008). Other research elaborated the use of corncobs liquid smoke in tilapia (Youssef, *et al.*,2015) and milkfish (Swastawati, *et al.*,2016); which shows the shelf life of tilapia meat for 6 days at cold temperature storage (5°C) tilapia (Ariestya, *et al.*,2016). Coconut shell liquid smoke increased the shelf life of mackerel fishballs for 32 hours at room temperature storage Zuraida, *et al.*,2011) While corncob liquid smoke was able to extend the shelf life of stingrays for 3 days at room temperature storage (Swastawati, *et al.*,2012) and tilapia meatballs for 15 days at cold temperature storage (4°C) (Youssef, *et al.*,2015). The existence of differences in the capability of coconut shell liquid smoke and corncobs liquid smoke increasing the shelf life of the product encourage the incorporation of these two liquid smokes in application of the product, which is expected to give effect in different shelf life at different storage temperatures. All the previous researcher were only use one raw material of liquid smoke. In this study, we apply combination of two raw materials i.e coconut shell and corncob (50:50) which is hope will give longer shelf life because these mixture of raw material were found to contain higher polyphenols (Anggraini, *et al.*,2017; Swastawati, *et al.*,2014; Lombok, *et al.*,2014; Yuniningsih and Anggraini, 2013).

Polyphenols were volatile bioactive components of liquid smoke. In addition, polyphenols have low and unstable water solubility (Conte, *et al.*,2016). Therefore, a system capable to improve the properties of polyphenols and maintaining polyphenols during storage was required. Nanoencapsulation technology changed

liquid smoke in liquid form to a nano-sized powder (nanoencapsulation) of 1 to 2000 nm Etheridge, *et al.*,2013) has an advantage in the delivery of bioactive components that were efficient in penetrating cells in desired products (Ezhilarasi, *et al.*,2012). Many research were limited to coconut shell encapsulation (Saloko, *et al.*, 2014; Ariestya, *et al.*,2016; Novianty, *et al.*, 2015;Ali, *et al.*, 2014; Saloko, *et al.*, 2012). Based on the above description, this study examined the effect of combination liquid smoke nanoencapsulation (coconut shell and corncob liquid smoke) on catfish fillet during storage of room temperature and cold temperature.

## 2. Materials and Methods

### 2.1. Materials

The materials used in this study were the corncob and coconut shell to produce liquid smoke. Each materials was processed into liquid smoke by pirolisator machine in laboratory of Fisheries and Marine Science Faculty, Diponegoro University, Semarang, Indonesia. Maltodextrin DE 10, arabic gum and Na-alginate were obtain from Multi Kimia Raya Semarang, Indonesia, meanwhile catfish were obtained from the local market in Semarang, Indonesia.

### 2.2. Nanoencapsulation of Liquid Smoke

Nanoencapsulation processed was carried out according to Saloko, *et al.*, (2013) with modification in core and coating materials. Coconut shell liquid smoke and corn cob liquid smoke was mixtured with ratio 1:1. Nanoencapsulation was processed by maltodextrin, gum arabic, and Na-alginate with a ratio of 1:4:1 was mixed with a combination of coconut shell and corncob liquid smoke. The solution was homogenized and centrifuged at 3000 rpm for 30 minutes at room temperature. Supernatant was separated and filtered to obtain a solution of pure nanoparticles. The



solution of nanoparticles was heated at 50°C in waterbath for 15 minutes and homogenized using a homogenizer at a speed of 4000 rpm for 2.5 minutes. The sample was dried with a spray dryer with inlet temperature about 130°C, while the outlet temperature about 70°C. The nanoencapsulation was collected on a sealed bottle and stored at room temperature.

## 2.2.Characteristic of Nanoencapsulation Liquid Smoke

### 2.2.1. Analysis of Total Phenol

A amount of 1 gram liquid smoke nanoencapsulation was diluted to a volume of 25 ml aquadest. 1 ml solution was diluted to 10 ml aquadest. Next 2.5 ml of it's solution was taken and diluted to 10 ml. After that, 1 ml solution was put into a test tube and 1 ml saturated Na<sub>2</sub>CO<sub>3</sub> (Merck, Germany) was added and left for 10 minutes at room temperature. Folin ciocalteu reagent (Sigma-Aldrich, USA) 0.5 ml and 7.5 ml of distilled water were added and homogenized by using a vortex for 30 minutes at room temperature. The absorbance of samples were measured at 760 nm wavelength. Phenolic content of samples was calculated as GAE in mg/g dry material (AOCS, 1990).

### 2.2.2. Analysis of Total Carbonyl

An amount of 1.6 mg of sample was diluted to 10 ml with carbonyl-free ethanol. 1 ml of solution was reacted with 2 ml solution of 2,4-dinitrophenyl-hydrazine (Sigma-Aldrich, USA) with a drop of concentrated hydrochloric acid in ethanol saturated. The mixture was heated in waterbath at temperature 50°C for 30 min. About 5 ml alcoholic solution of potassium hydroxide (Merchk, Germany) were added when the mixture was cool. Then 2 ml of distilled water was added and measured with a spectrophotometer with a wavelength of 480 nm. Results were calculated by comparing it with the standard curve of acetaldehyde 2,4-dinitrophenylhydrazone

(2,4-DNPH) and calculated equivalent of 13.7 ppm acetaldehyde (Sigma-Aldrich, USA) in the sample (Alice, *et al.*, 1961).

### 2.2.3. Radical Scavanging Activity

Radical Scavanging Activity (RSA) was measured by Li and Guo (2010) with modifications. Each sample was reacted with DPPH (Sigma-Aldrich, USA) 0.004 g/ml of ethanol. 0.1 ml of sample was added with 3.9 ml of DPPH and incubated at 28°C for 30 minutes. Scavanging activity on DPPH radical was measured at 515 nm wavelength. Percent of RSA was measured according to the following equation:

$$\% \text{ RSA} = \{(A_{\text{control}} - A_{\text{sample}}) \times A^{-1}\} \times 100\% \text{ control}$$

### 2.2.4. PAH Analysis

#### Solid-Liquid Extraction

Two grams of freeze-dried fish fillet mixed with a mixture of the 20 ml standard solution with 13 PAH was equal to 0.5 µg.kg<sup>-1</sup>, considered as internal standards which were homogenized in 40 ml of cyclohexane/ethyl acetate (50:50; v/v) and it was shaken during 30 minutes. The solution was centrifuged at 5000 rpm for 30 min at 0°C. After being homogenized, the liquid part was carefully isolated and evaporated to dryness under a gentle stream of nitrogen. The residue was dissolved in 6 ml of cyclohexane. PAH quantification was the result of the mean of measures carried out on three individual smoked fillets in the same conditions.

### 2.2.5. Scanning Electron Microscopy (SEM)

Morphology of liquid smoke nanoencapsulation was observed by using SEM. The sample was layered with gold and it was monitored by a magnification of 1,000 times at the voltage of 20 kV.

## 2.3. Application Nanoencapsulation Liquid Smoke on Catfish

Catfish fillet with a size of 25 x 15 x 1 cm with a weight of approximately 100 grams, was smeared with liquid smoke nanoencapsulation as much as 1% of the weight of the fillet. Catfish fillet that has been smeared with liquid smoke nanoencapsulation then roasted at a temperature of 90°C for 4 hours. Smoke catfish fillet was stored at room temperature (28°C±2°C) and cold temperature (5°C) for 10 days and analyzed every 2 days.

#### 2.3.1. Peroxide Value (PV) Analysis

Peroxide value analysis was conducted by Memon *et.al.*, (2010). The sample was dissolved in a mixture of chloroform (Merck, Germany) and glacial acetic acid (Merck, Germany) and added with a solution of potassium iodide (Merck, Germany). The mixture was finally titrated with sodium thiosulfate solution (Merck, Germany) 0.01 M with 1% starch indicator.

#### 2.3.2. Thiobarbituric Acid (TBA) Analysis

TBA analysis was conducted by Molla *et.al.*, (2015), 2 ml of 20% trichloroacetic acid (Merck, Germany) and 2 ml of 0.67% thiobarbituric acid (Fluka Chemika, Switzerland) was added to 1 ml of the sample solution. The mixture was heated at 100°C for 10 minutes in waterbath. The mixture was centrifuged at 3000 rpm for 20 minutes. Supernatant containing TBARS absorbance was measured at 532 nm wavelength using a spectrophotometer.

#### 2.3.3. Total Volatil Base Nitrogen (TVBN) Analysis

Total Volatile Base Nitrogen (TVBN) was carried out according Indonesian National Standard 2354.8:2009 (BSN, 2009). Briefly, 25 g samples was weighed and mixed with 75 mL TCA (Merck, Germany) 7%. 1 ml filtrat was put in

conway cup of outer chamber which had previously been added 1 mL K<sub>2</sub>CO<sub>3</sub> (Merck, Germany). Another Conway cup of inner chamber was added 1 mL Boric acid and 2-3 drops of indicator (screen metal red) until the color was green. Blanko had been used 1 mL TCA 7%. Conway cup was incubated at 37°C until 2 hours. Conway cup in the inner chamber of blanko was titrated with HCl until the color was pink. Conway cup of samples titrated with boric acid until the color was equal with blanko.

#### 2.3.4. Total Plate Count (TPC) Analysis

Total Plate Count (TPC) was obtained by Indonesian National Standard 2332.3:2015 (BSN, 2015). Fish samples were diluted into Butterfields Phosphat Buffered (Merck, Germany) with concentration of 10<sup>4</sup>, 10<sup>3</sup>, and 10<sup>5</sup>. One milliliter of each sample solution was placed into petridisc containing plate count agar (PCA) (Merck, Germany). Petridisc containing samples was incubated with the opposite position at 35°C for 48 hours. The number of colony were calculated by hand tally counter for the amount 25-250.

### 3. Results and discussions

#### 3.1. Characterization of Liquid Smoke Nanoencapsulation

The content of total phenols, total carbonyl, and RSA of liquid smoke nanoencapsulation in a row was consecutively 3.682 mg GAE/g, 3.439% and 91.348% (Table 1). Total phenolic content of liquid smoke nanoencapsulation was influenced by the total phenolic content of liquid smoke and the composition of the coating material. Based on Hardianto and Yuniarta (2015) the total phenolic content of corn cob liquid smoke was lower than coconut shell liquid smoke.

TABLE 1. Characteristics of Liquid Smoke Nanoencapsulation

Characteristics	Results
Total Phenolic Content (mg GAE/g)	3.682
Total Carbonyl (%)	3.439
Radical Scavanging Activity (%)	91.348
Polycyclic aromatic hydrocarbons (PAHs) (ppm)	
Naphtalen	286.40
Acenaphtane	106.35
Phenantrene	11.70
Phyrene	30.00
Benzo- $\alpha$ -Antrazene	67.10
Benzo- $\alpha$ -Phyrene	47.55

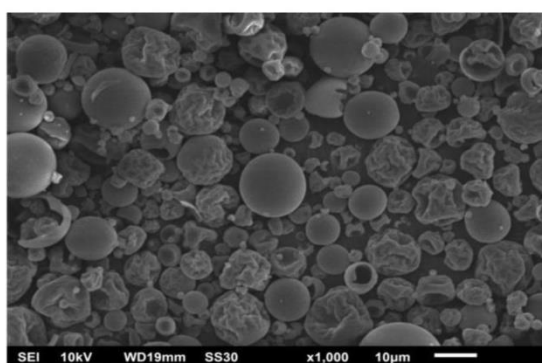


Figure 1. Microstructural of Liquid Smoke Nanoencapsulation

The composition of the coating material also affected the content of total phenols. The use of the coating material for one portion of alginate composition could trap phenolic content of liquid smoke during the spray drying process. This research was accordance with Novianty *et al.*, (2015) that the encapsulation process of liquid smoke with alginate 1% was able to trap the phenol content with the release of phenol for 20 minutes.

Total carbonyl content of liquid smoke nanoencapsulation was also affected by carbonyl content of liquid smoke. The carbonyl content of corncob liquid smoke was greater than coconut shell liquid smoke.

Because of the corncob liquid smoke contains cellulose degradation products that were more than the liquid smoke coconut shell (Hardianto and Yunianta, 2015). In addition, the alginate composition as a coating material can protect the carbonyl during the spray dryer. Alginate can form a gel (Novianty *et al.*,2015). Alginate was polysaccharide that contain of homopolymeric mannuronic (M) and guluronic (G) block. The gel characteristic of alginate was affect by M/G ratio (Fertah, *et al.*,2014). This character was used to protect the phenolic content and carbonyl component during nanoencapsulation process. Nanoencapsulation oxidative

capability of liquid smoke was measured by Radical Scavenging Activity. The RSA of liquid smoke nanoencapsulations was 91,348%. It was indicated that the coating materials was able to inhibit the oxidation of liquid smoke associated with total phenolic content and total carbonyl, where the component acts as an antioxidant and antimicrobial in food (Leha, 2010).

According to the table 1, it was known that liquid smoke nanoencapsulation contain PAH especially benzo- $\alpha$ -pyrene. Benzo- $\alpha$ -pyrene was known to be carcinogenic and mutagenic to human. Based Swastawati (2008), coconut shell liquid smoke had benzo- $\alpha$ -pyrene contents of 11.351 ppm, while corn cob liquid smoke was not detected (Swastawati, *et al.*,2007). According to the table 1, it showed that the coating material can trap nanoencapsulation PAH compounds.

Based on morphological observation of liquid smoke nanoencapsulation (Figure 1), it could be detected that the liquid smoke nanoencapsulation produced a perfect numerous circle. Novianty *et.al.*,(2015) showed that the concentration of 1% alginate microcapsules produced liquid smoke morphology with an unbroken sphere. This showed that alginate as a

coating material was capable of protecting the liquid smoke during nanoencapsulation process.

### 3.2. Peroxide Value (PV) Analysis

The combination of liquid smoke nanoencapsulation was applied to the catfish fillets and stored at room temperature and cold temperature. The antioxidant and antimicrobial effects were observed during storage. The number of peroxide value on a catfish fillet was presented in Figure 2. Based on the results obtained, the peroxide value of catfish fillets increased on days 0 to day 4. After that, the peroxide value decreased until 10 days at all storage temperatures. Peroxide value was the number that indicated the degree of damaged oil or fat by oxidation. The oil reacted with oxygen and form peroxides, especially when it contains unsaturated fatty acids (Panagan, *et al.*,2011). Catfish fillets had a fat content of 0.12 to 1.42% (Rario, 2015). Catfish fat contains omega-3 (Panagan, *et al.*,2011) as an unsaturated fatty acid, that potentially forms peroxides due to oxidation

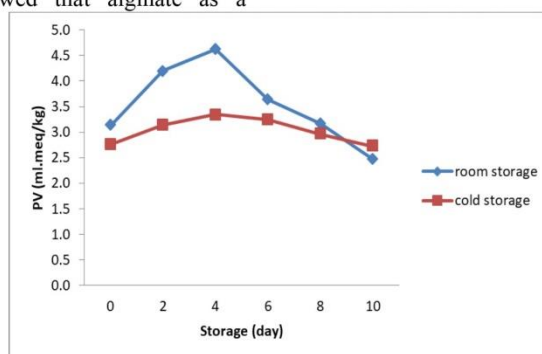


Figure 2. The Peroxide Value of Catfish Fillet Stored at Room Temperature and Cold Temperature

The combination of liquid smoke nanoencapsulation had a total phenolic content of 3.682% and the RSA of 91.348%, that can inhibit the oxidation process of catfish fillet. The result showed that the storage of catfish fillet for 4 days had a good peroxide value at room temperature and cold temperature, there were 4.695 meq/kg and 3.347 meq/kg, respectively. The peroxide value was decreased for 10 days of storage

at room temperature and cold temperature, there were 2.467 meq/kg and 2.725 meq/kg, respectively. The different results were shown by Adebowale *et.al.*, (2012) that the catfish storage at room temperature for 21 days obtained peroxide value for 5.12 meq/kg. A maximum limit for foodstuffs peroxide value was 5 meq/kg. This result showed that the catfish fillet after 10 days of storage was feasible for consumption.

### 3.3. Thiobarbituric Acid (TBA) Analysis

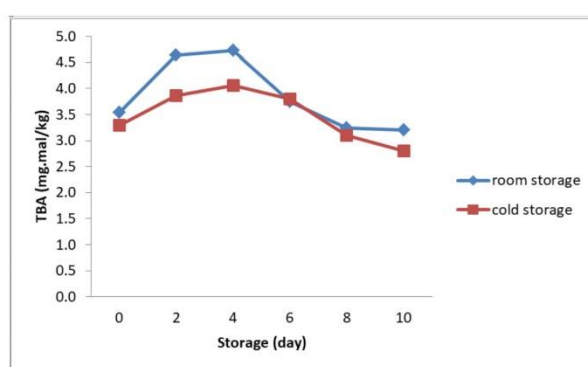


Figure 3. The TBA Value of Catfish Fillet Stored at Room Temperature and Cold Temperature

The TBA value of catfish fillet during storage was presented in Figure 3. TBA measured the amount of malonadehid which is the final product of fat oxidation (Piccolo, *et al.*, 2014). Based of figure 3, it could be seen that the TBA value of catfish fillet increased until 4 days of storage, for storage of room temperature from 3.534 mg malonaldehid/kg to 4.726 mg malonaldehid/kg. Meanwhile the TBA value of catfish fillet at cold temperature storage were 3.291 mg malonaldehid/kg to 4.052 mg malonaldehid/kg. The TBA value decreased until 10 days of storage, there were 3.206 mg malonaldehyde/kg at room temperature and 2.802 mg malonaldehid/kg at cold

temperature. Swastawati *et.al.*, (2012) applied the coconut shell liquid smoke on a stingray, showed the TBA value decreased after 6 days of storage. The maximum number of malonaldehyde was 5 mg/kg (Gunsen, *et al.*, 2011). This result showed that catfish fillets were still feasible for consumption either on the storage at room temperature or cold temperature until 10 days of storage. The combination of liquid smoke nanoencapsulation applied to the catfish fillet was able to inhibit the oxidation of fat. The decreasing of TBA value indicated that the secondary oxidation products formation which not detected with TBA value (Piccolo, *et al.*, 2014).



### 3.4. Total Volatile Base Nitrogen (TVBN)

TVBN analysis measured the declining of fish quality. TVBN measured the protein degradation which is formed dimethylamine, trimethylamine, and ammonia Saloko *et.al.*, (2014) that caused by bacterial activity (AOCS, 1990). The TVBN value of catfish fillet during storage was presented in Figure 4. The result showed that TVBN value increased during storage at 10 days. The TVBN value of catfish fillet increased in room temperature and cold temperature of storage, that is 15,075 mgN/100g to 22,576 mgN/100g for

room temperature and 10,954 mgN/100g to 21,510 mgN/100g for cold temperature. This indicated that the longer of storage time, the growth of bacteria in catfish fillet was also increased.

The maximum limit of TVBN value for fish was about 30-35 mgN/100g. This showed that until the 10th day of storage, TVBN value is still below standard, consequently the catfish fillet was fit for consumption. These results related to the total phenolic content of liquid smoke nanoencapsulation that the phenol content of liquid smoke was able as antimicrobial agents (Saloko *et.al.*, 2014).

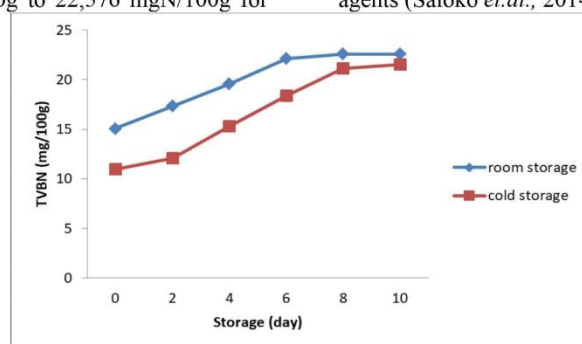


Figure 4. The TVBN Value of Catfish Fillet at Room Temperature and Cold Temperature

### 3.5. Total Plate Count (TPC)

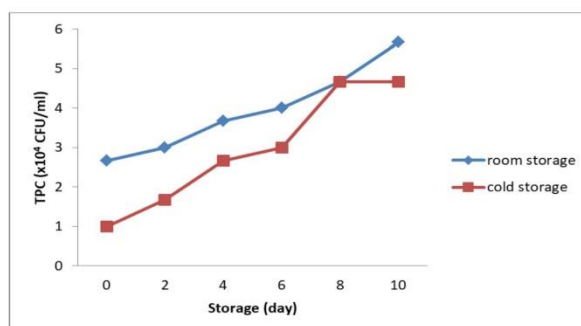


Figure 5. The TPC Value of Catfish Fillet at Room Temperature and Cold Temperature



TPC value of catfish fillet during storage was presented in Figure 5. The result showed that the number of microbial was increased during 10 days of storage. Both room temperature or cold temperature of storage, the number of microbial of catfish fillet were  $2.667 \times 10^4$  CFU/g to  $5.667 \times 10^4$  CFU/g at room temperature and  $1 \times 10^4$  CFU/g to  $4.667 \times 10^4$  CFU/g for cold temperature. Based on Indonesia National Standard, the TPC value of fish product was  $5 \times 10^5$  CFU/g (BSN, 2009). This result showed that until 10 days of storage, the catfish fillet was still feasible for consumption.

The combination of liquid smoke nanoencapsulation had total phenolic content that acted as an antimicrobial agent. Zuraida *et.al.*, (2011) the coconut shell liquid smoke was able to inhibit microbial growth of fish balls on 20 days of storage with TPC value 1.8 log CFU/g. Ariestya *et.al.*, (2016), also showed that the application of liquid smoke microcapsules on Tilapia meat could inhibit microbial growth with the TPC value 26 CFU/g at cold temperatures after 9 days of storage. The microbial growth inhibition because of the phenolic content of liquid smoke.

The liquid smoke nanoencapsulation application on catfish fillet was able to inhibit oxidation during storage, indicated by the PV and TBA value was under the limit standard until 10 days of storage. In addition, liquid smoke nanoencapsulation also able to inhibit microbial activity which was proved by the TVBN and TPC number was below the maximum limit. The result showed that the liquid smoke nanoencapsulation was act as antioxidant and antibacterial agent.

#### Conflict of Interest

The authors declare that there were no conflict of interest regarding the publication of this paper.

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August 2<sup>nd</sup>, 2018

Dear

Editor – In – Chief

**Carpathian Journal of Food Science and Technology**

We wish to submit an original research article entitled “**The Effect of Antioxidant and Antibacterial Nanoencapsulation Liquid Smoke on Catfish Fillet (*Pangasius sp.*) during Storage at Room Temperature and Cold Temperature**”. We confirm that this work is original and has not been published elsewhere, nor is it currently under consideration for publication elsewhere.

In this paper, we report on the effect of antioxidant and antibacterial from nanoencapsulation of liquid smoke to prolong smoked catfish quality. We believe that this manuscript is appropriate for publication by **Carpathian Journal of Food Science and Technology**, because it studied the application nanoencapsulation technology for liquid smoke as preservative agent in smoked catfish. The development of encapsulation especially in food science grow rapidly so that exploration on foodstuff variation still needs to be studied. This research is expected to provide a reference source for researchers, fish technology industries, academics, and everyone that have interesting in this field study.

We have no conflicts of interest to disclose. We also agree with the antiplagiarism rules. If you feel that the manuscript is appropriate for your journal, we suggest the following reviewers :

No Identity

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Please address all correspondence concerning this manuscript to me at [fronthea\\_thp@yahoo.co.id](mailto:fronthea_thp@yahoo.co.id) . Thank you for your consideration of this manuscript.

Sincerely,



Dr. Ir. Fronthea Swastawati, M.Sc

RE: Submission Paper Carpathian Journal Dr. Fronthea Swastawati

Dari: Liviu Giurgiulescu (giurgiulescu@yahoo.com)

Kepada: fronthea\_thp@yahoo.co.id

Tanggal: Sabtu, 4 Agustus 2018 22:01 WIB

Dear author,

Thank you for contribution. Your manuscript titled "**THE EFFECT OF ANTIOXIDANT AND ANTIBACTERIAL NANOENCAPSULATION LIQUID SMOKE ON CATFISH FILLET (*Pangasius* sp.) DURING STORAGE AT ROOM TEMPERATURE AND COLD TEMPERATURE**" was introduced in review process. Please consider for future conversation number CJFST.71.08.2018.

Regards,

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Semarang, August 2<sup>nd</sup>, 2018

Dear

Editor – In – Chief

**Carpathian Journal of Food Science and Technology**

Re: Submission paper

Dear Editor,

I hereby submit the paper entitled "**The Effect of Antioxidant and Antibacterial Nanoencapsulation Liquid Smoke on Catfish Fillet (*Pangasius* sp.) during Storage at Room Temperature and Cold Temperature**" to **Carpathian Journal of Food Science and Technology**. The paper is authored by Fronthea Swastawati, Ahmad Ni'matullah Al-Baari, Eko Susanto, Lukita Purnamayati, with myself as corresponding author.

We do state that this manuscript has been not submitted to other journal or elsewhere as well as had not been submitted earlier to Carpathian Journal of Food Science and Technology.

We also stated that there is no conflict of interest during its preparation and submission of this manuscript.

We hope you find this paper interesting and publishable in your esteemed journal. We look forward to your decision.

Best regards



Dr. Fronthea Swastawati

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Kepada: giurgiulescul@yahoo.com

Cc: fronthea\_thp@yahoo.co.id

Tanggal: Selasa, 17 Desember 2019 16.00 WIB

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Dear Mr. Liviu GIURGIULESCU

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Author : Fronthea Swastawati, Ahmad Ni'matullah Al Barri, Eko Susanto, Lukita Purnamayati

Title : The Effect of Antioxidant and Antibacterial Nanoencapsulation Liquid Smoke on Catfish (*Pangasius sp.*) During Storage at Room Temperature and Cold Temperature

We are looking forward about the current status of the manuscript.

Thank you.

10/20/2020

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Kepada: fronthea\_thp@yahoo.co.id  
Tanggal: Sabtu, 11 Januari 2020 05.00 WIB

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Dear author,

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Please send back ASAP final proof to finish December 2019 edition, any quick in answer will be appreciate.

Regards,

Liviu Giurgiulescu

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We strongly hope, that our manuscript

CJSFT.71.08.2018. Will be available to be published.

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Regards,

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Pada Sel, 7 Jan 2020 pada 4:32, [giurgiulesscu@yahoo.com](mailto:giurgiulesscu@yahoo.com)

[<giurgiulesscu@yahoo.com>](mailto:giurgiulesscu@yahoo.com) menulis:

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Sorry for late in answer. The manuscript is from 2018 and will be published in current issue of CJFST. Have in view that the manuscript is from 2018 we will provide DOI number for free, usually the service is 50 euro. Please be available in next email for final proof.

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**Sent:** January 3, 2020 23:18

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**Subject:** Trs: RE: Status of Submission Paper Carpathian Journal Dr. Fronthea Swastawati

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Editor in chief Carpathian Journal of Food Science and Technology.

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**Dari:** "Fronthea Swastawati" <[fronthea\\_thp@yahoo.co.id](mailto:fronthea_thp@yahoo.co.id)>

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**Terkirim:** Sel, 31 Des 2019 pada 4:14

**Judul:** Bls: RE: Submission Paper Carpathain Journal Dr. Fronthea Swastawati

Dear Ms. Liviu GIURGIULESCU,

Hope everythings goes well with you.

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Hopefully that our manuscript will be possible to publish in your journal.

I am looking forward of your response. Thank you.

Best regards,

Fronthea Swastawati.

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Pada Sab, 4 Agt 2018 pada 22:01, Liviu Giurgiuлесcul

<[giurgiuлесcul@yahoo.com](mailto:giurgiuлесcul@yahoo.com)> menulis:

Dear author,

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**Sent:** Thursday, August 2, 2018 12:22

**To:** [giurgulescu@yahoo.com](mailto:giurgulescu@yahoo.com)

**Subject:** Submission Paper Carpathian Journal Dr. Fronthea Swastawati

Semarang, August 2<sup>nd</sup>, 2018

Dear

Editor – In – Chief

**Carpathian Journal of Food Science and Technology**

Re: Submission paper

Dear Editor,

I hereby submit the paper entitled "**The Effect of Antioxidant and Antibacterial Nanoencapsulation Liquid Smoke on Catfish Fillet (*Pangasius sp.*) during Storage at Room Temperature and Cold Temperature**" to **Carpathian Journal of Food Science and Technology**. The paper is authored by Fronthea Swastawati, Ahmad Ni'matullah Al-Baari, Eko Susanto, Lukita Purnamayati, with myself as corresponding author.

We do state that this manuscript has been not submitted to other journal or elsewhere as well as had not been submitted earlier to Carpathian Journal of Food Science and Technology.

We also stated that there is no conflict of interest during its preparation and submission of this manuscript.

We hope you find this paper interesting and publishable in your esteemed journal. We look forward to your decision.

Best regards

Dr. Fronthea Swastawati

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THE EFFECT OF ANTIOXIDANT AND ANTIBACTERIAL  
NANOENCAPSULATION LIQUID SMOKE ON CATFISH FILLET (*Pangasius*  
sp.) DURING STORAGE AT ROOM TEMPERATURE AND COLD  
TEMPERATURE

Fronthea Swastawati<sup>1\*</sup>, Ahmad Ni'matullah Al-Baari<sup>2</sup>, Eko Susanto<sup>3</sup>, Lukita Purnamayati<sup>4</sup>

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Received:

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**Keywords:**

Catfish;

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**ABSTRACT**

The purpose of this study was to determine the effect of antioxidant and antibacterial nanoencapsulation liquid smoke on a catfish filet (*Pangasius* sp.). A combination of liquid smoke (corn cob and coconut shell) were processed into nanoencapsulation using three encapsulan i.e: gum arabic, maltodextrin, and alginate with a ratio of 1/6: 4/6: 1/6 each. Nanoencapsulation liquid smoke was known to have a total content of phenols, carbonyl, and Radical Scavenging Activity, there were 3.682 mg GAE/g, 3.439%, and 91.348%, respectively. Nanoencapsulation liquid smoke was applied to the catfish and stored at room temperature (28°C±2°C) and cold temperature (5°C). Observations were made on days 0, 2, 4, 6, 8, and 10 to parameter PV, TBA, TVBN and TPC. The results showed that nanoencapsulation liquid smoke could effectively inhibit the oxidation of fat catfish showed with PV and TBA acceptable. Nanoencapsulation liquid smoke was also capable of inhibiting the activity of microbes, indicated by the value of TVBN and TPC which were still below standard at all temperatures and long storage time.

**1. Introduction**

Catfish were easily damaged by changes fat content (oxidation process, lipoxygenase damage, etc), protein changes and changes the content of microorganisms (Masniyom, 2011). This damage is indicated by peroxide numbers, TBA (Valdes, *et al.*, 2015), TVBN (Tian, *et al.*, 2012; Castro, 2012) and TPC (Adilla, *et al.*, 2017) which increases during storage. Catfish have high nutrient content especially fat and protein. Catfish contain palmitic acid (24.05%), oleic acid (27.55%), and linoleic acid (7.63%). In addition, catfish also contains non essential amino acids, such as as glutamate (3.33%) and essential amino acids, for example lysine (1.82%) (Nurilmala *et al.*, 2015). The high

content of fatty acids and amino acids of catfish, resulting in catfish being damaged continuously during cold storage temperatures (Abbas *et al.*, 2005). Therefore, treatment were needed to inhibit catfish damage during storage.

Liquid smoke is one of the smoke condensation products in the form of liquid. Liquid smoke is widely used compared to traditional curing methods because it is easy to use and more economical. Liquid smoke also has several compounds such as phenol, acids and carbonyl that acts as an antibacterial and antioxidant (Saloko, *et al.*, 2014). Several studies has been done by other researcher using coconut shell liquid smoke to inhibit fish

damage, such as tuna (Saloko, *et al.*, 2014) tilapia (Ariestya, *et al.*, 2016), and catfish (Swastawati, 2008). Other research elaborated the use of corncobs liquid smoke in tilapia (Youssef, *et al.*, 2015) and milkfish (Swastawati, *et al.*, 2016); which shows the shelf life of tilapia meat for 6 days at cold temperature storage (5°C) tilapia (Ariestya, *et al.*, 2016). Coconut shell liquid smoke increased the shelf life of mackerel fishballs for 32 hours at room temperature storage Zuraida, *et al.*, 2011) While corncob liquid smoke was able to extend the shelf life of stingrays for 3 days at room temperature storage (Swastawati, *et al.*, 2012) and tilapia meatballs for 15 days at cold temperature storage (4°C) (Youssef, *et al.*, 2015). The existence of differences in the capability of coconut shell liquid smoke and corncobs liquid smoke increasing the shelf life of the product encourage the incorporation of these two liquid smokes in application of the product, which is expected to give effect in different shelf life at different storage temperatures. All the previous researcher were only use one raw material of liquid smoke. In this study, we apply combination of two raw materials i.e coconut shell and corncob (50:50) which is hope will give longer shelf life because these mixture of raw material were found to contain higher polyphenols (Anggraini, *et al.*, 2017; Swastawati, *et al.*, 2014; Lombok, *et al.*, 2014; Yuniningsih and Anggraini, 2013).

Polyphenols were volatile bioactive components of liquid smoke. In addition, polyphenols have low and unstable water solubility (Conte, *et al.*, 2016). Therefore, a system capable to improve the properties of polyphenols and maintaining polyphenols during storage was required. Nanoencapsulation technology changed liquid smoke in liquid form to a nano-sized powder (nanoencapsulation) of 1 to 2000 nm Etheridge, *et al.*, 2013) has an advantage in the delivery of bioactive components that were efficient in penetrating cells in desired products (Ezhilarasi, *et al.*, 2012). Many research were limited to coconut shell encapsulation (Saloko,

*et al.*, 2014; Ariestya, *et al.*, 2016; Novianty, *et al.*, 2015; Ali, *et al.*, 2014; Saloko, *et al.*, 2012). Based on the above description, this study examined the effect of combination liquid smoke nanoencapsulation (coconut shell and corncob liquid smoke) on catfish fillet during storage of room temperature and cold temperature.

## 2. Materials and Methods

### 2.1. Materials

The materials used in this study were the corncob and coconut shell to produce liquid smoke. Each materials was processed into liquid smoke by pirolisator machine in laboratory of Fisheries and Marine Science Faculty, Diponegoro University, Semarang, Indonesia. Maltodextrin DE 10, arabic gum and Na-alginate were obtain from Multi Kimia Raya Semarang, Indonesia, meanwhile catfish were obtained from the local market in Semarang, Indonesia.

### 2.2. Nanoencapsulation of Liquid Smoke

Nanoencapsulation processed was carried out according to Saloko, *et al.*, (2013) with modification in core and coating materials. Coconut shell liquid smoke and corn cob liquid smoke was mixed with ratio 1:1. Nanoencapsulation was processed by maltodextrin, gum arabic, and Na-alginate with a ratio of 1:4:1 was mixed with a combination of coconut shell and corncob liquid smoke. The solution was homogenized and centrifuged at 3000 rpm for 30 minutes at room temperature. Supernatant was separated and filtered to obtain a solution of pure nanoparticles. The solution of nanoparticles was heated at 50°C in waterbath for 15 minutes and homogenized using a homogenizer at a speed of 4000 rpm for 2.5 minutes. The sample was dried with a spray dryer with inlet temperature about 130°C, while the outlet temperature about 70°C. The nanoencapsulation was collected on a sealed bottle and stored at room temperature.

## 2.2.Characteristic of Nanoencapsulation Liquid Smoke

### 2.2.1. Analysis of Total Phenol

A amount of 1 gram liquid smoke nanoencapsulation was diluted to a volume of 25 ml aquadest. 1 ml solution was diluted to 10 ml aquadest. Next 2.5 ml of it's solution was taken and diluted to 10 ml. After that, 1 ml solution was put into a test tube and 1 ml saturated  $\text{Na}_2\text{CO}_3$  (Merck, Germany) was added and left for 10 minutes at room temperature. Folin ciocalteu reagent (Sigma-Aldrich, USA) 0.5 ml and 7.5 ml of distilled water were added and homogenized by using a vortex for 30 minutes at room temperature. The absorbance of samples were measured at 760 nm wavelength. Phenolic content of samples was calculated as GAE in mg/g dry material (AOCS, 1990).

### 2.2.2. Analysis of Total Carbonyl

An amount of 1.6 mg of sample was diluted to 10 ml with carbonyl-free ethanol. 1 ml of solution was reacted with 2 ml solution of 2,4-dinitrophenyl-hydrazine (Sigma-Aldrich, USA) with a drop of concentrated hydrochloric acid in ethanol saturated. The mixture was heated in waterbath at temperature 50°C for 30 min. About 5 ml alcoholic solution of potassium hydroxide (Merck, Germany) were added when the mixture was cool. Then 2 ml of distilled water was added and measured with a spectrophotometer with a wavelength of 480 nm. Results were calculated by comparing it with the standard curve of acetaldehyde 2,4-dinitrophenylhydrazone (2,4-DNPH) and calculated equivalent of 13.7 ppm acetaldehyde (Sigma-Aldrich, USA) in the sample (Alice, *et al.*, 1961).

### 2.2.3. Radical Scavenging Activity

Radical Scavenging Activity (RSA) was measured by Li and Guo (2010) with modifications. Each sample was reacted with DPPH (Sigma-Aldrich, USA) 0.004 g/ml of ethanol. 0.1 ml of sample was added with 3.9 ml of DPPH and incubated at 28°C for 30 minutes. Scavenging activity on DPPH radical

was measured at 515 nm wavelength. Percent of RSA was measured according to the following equation:

$$\% \text{ RSA} = \frac{[(A_{\text{control}} - A_{\text{sample}}) \times A^{-1}]}{A_{\text{control}}} \times 100\% \text{ control}$$

### 2.2.4. PAH Analysis

#### Solid-Liquid Extraction

Two grams of freeze-dried fish fillet mixed with a mixture of the 20 ml standard solution with 13 PAH was equal to 0.5  $\mu\text{g.kg}^{-1}$ , considered as internal standards which were homogenized in 40 ml of cyclohexane/ethyl acetate (50:50; v/v) and it was shaken during 30 minutes. The solution was centrifuged at 5000 rpm for 30 min at 0°C. After being homogenized, the liquid part was carefully isolated and evaporated to dryness under a gentle stream of nitrogen. The residue was dissolved in 6 ml of cyclohexane. PAH quantification was the result of the mean of measures carried out on three individual smoked fillets in the same conditions.

### 2.2.5. Scanning Electron Microscopy (SEM)

Morphology of liquid smoke nanoencapsulation was observed by using SEM. The sample was layered with gold and it was monitored by a magnification of 1,000 times at the voltage of 20 kV.

## 2.3. Application Nanoencapsulation Liquid Smoke on Catfish

Catfish fillet with a size of 25 x 15 x 1 cm with a weight of approximately 100 grams, was smeared with liquid smoke nanoencapsulation as much as 1% of the weight of the fillet. Catfish fillet that has been smeared with liquid smoke nanoencapsulation then roasted at a temperature of 90°C for 4 hours. Smoke catfish fillet was stored at room temperature (28°C±2°C) and cold temperature (5°C) for 10 days and analyzed every 2 days.

### 2.3.1. Peroxide Value (PV) Analysis

Peroxide value analysis was conducted by Memon *et.al.*,(2010). The sample was dissolved in a mixture of chloroform (Merck,

Germany) and glacial acetic acid (Merck, Germany) and added with a solution of potassium iodide (Merck, Germany). The mixture was finally titrated with sodium thiosulfate solution (Merck, Germany) 0.01 M with 1% starch indicator.

### 2.3.2. Thiobarbituric Acid (TBA) Analysis

TBA analysis was conducted by Molla *et.al.*, (2015), 2 ml of 20% trichloroacetic acid (Merck, Germany) and 2 ml of 0.67% thiobarbituric acid (Fluka Chemika, Switzerland) was added to 1 ml of the sample solution. The mixture was heated at 100°C for 10 minutes in waterbath. The mixture was centrifuged at 3000 rpm for 20 minutes. Supernatant containing TBARS absorbance was measured at 532 nm wavelength using a spectrophotometer.

### 2.3.3. Total Volatil Base Nitrogen (TVBN) Analysis

Total Volatile Base Nitrogen (TVBN) was carried out according Indonesian National Standard 2354.8:2009 (BSN, 2009). Briefly, 25 g samples was weighed and mixed with 75 mL TCA (Merck, Germany) 7%. 1 ml filtrat was put in conway cup of outer chamber which had previously been added 1 mL K<sub>2</sub>CO<sub>3</sub> (Merck, Germany). Another Conway cup of inner chamber was added 1 mL Boric acid and 2-3 drops of indicator (screen metal red) until the color was green. Blanko had been used 1 mL TCA 7%. Conway cup was incubated at 37°C

until 2 hours. Conway cup in the inner chamber of blanko was titrated with HCl until the color was pink. Conway cup of samples titrated with boric acid until the color was equal with blanko.

### 2.3.4. Total Plate Count (TPC) Analysis

Total Plate Count (TPC) was obtained by Indonesian National Standard 2332.3:2015 (BSN, 2015). Fish samples were diluted into Butterfields Phosphat Buffered (Merck, Germany) with concentration of 10<sup>4</sup>, 10<sup>3</sup>, and 10<sup>5</sup>. One milliliter of each sample solution was placed into petridisc containing plate count agar (PCA) (Merck, Germany). Petridisc containing samples was incubated with the opposite position at 35°C for 48 hours. The number of colony were calculated by hand tally counter for the amount 25-250.

## 3. Results and discussions

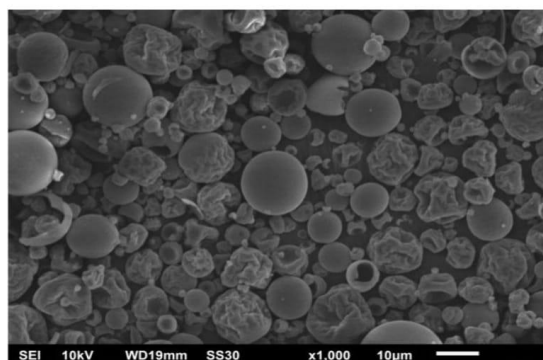
### 3.1. Characterization of Liquid Smoke Nanoencapsulation

The content of total phenols, total carbonyl, and RSA of liquid smoke nanoencapsulation in a row was consecutively 3.682 mg GAE/g, 3.439% and 91.348% (Table 1). Total phenolic content of liquid smoke nanoencapsulation was influenced by the total phenolic content of liquid smoke and the composition of the coating material. Based on Hardianto and Yunianta (2015) the total phenolic content of corn cob liquid smoke was lower than coconut shell liquid smoke.

**Table 1.** Characteristics of Liquid Smoke Nanoencapsulation

Characteristics	Results
Total Phenolic Content (mg GAE/g)	3.682
Total Carbonyl (%)	3.439
Radical Scavanging Activity (%)	91.348
Polycyclic aromatic hydrocarbons (PAHs) (ppm)	
Naphtalen	286.40
Acenaphtane	106.35
Phenantrene	11.70
Phyrene	30.00
Benzo- $\alpha$ -Antrazene	67.10
Benzo- $\alpha$ -Phyrene	47.55





**Figure 1.** Microstructural of Liquid Smoke Nanoencapsulation

The composition of the coating material also affected the content of total phenols. The use of the coating material for one portion of alginate composition could trap phenolic content of liquid smoke during the spray drying process. This research was accordance with Novianty *et al.*, (2015) that the encapsulation process of liquid smoke with alginate 1% was able to trap the phenol content with the release of phenol for 20 minutes.

Total carbonyl content of liquid smoke nanoencapsulation was also affected by carbonyl content of liquid smoke. The carbonyl content of corncob liquid smoke was greater than coconut shell liquid smoke. Because of the corncob liquid smoke contains cellulose degradation products that were more than the liquid smoke coconut shell (Hardianto and Yunianta, 2015). In addition, the alginate composition as a coating material can protect the carbonyl during the spray dryer. Alginate can form a gel (Novianty *et al.*, 2015). Alginate was polysaccharide that contain of homopolymeric mannuronic (M) and guluronic (G) block. The gel characteristic of alginate was affect by M/G ratio (Fertah, *et al.*, 2014). This character was used to protect the phenolic content and carbonyl component during nanoencapsulation process. Nanoencapsulation oxidative capability of liquid smoke was measured by Radical Scavenging Activity. The

RSA of liquid smoke nanoencapsulations was 91,348%. It was indicated that the coating materials was able to inhibit the oxidation of liquid smoke associated with total phenolic content and total carbonyl, where the component acts as an antioxidant and antimicrobial in food (Leha, 2010).

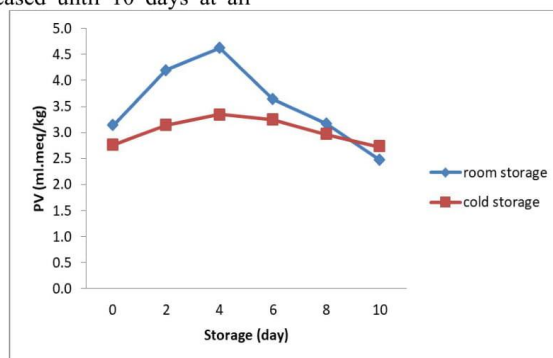
According to the table 1, it was known that liquid smoke nanoencapsulation contain PAH especially benzo- $\alpha$ -pyrene. Benzo- $\alpha$ -pyrene was known to be carcinogenic and mutagenic to human. Based Swastawati (2008), coconut shell liquid smoke had benzo- $\alpha$ -pyrene contents of 11.351 ppm, while corn cob liquid smoke was not detected (Swastawati, *et al.*, 2007). According to the table 1, it showed that the coating material can trap nanoencapsulation PAH compounds.

Based on morphological observation of liquid smoke nanoencapsulation (Figure 1), it could be detected that the liquid smoke nanoencapsulation produced a perfect numerous circle. Novianty *et al.*, (2015) showed that the concentration of 1% alginate microcapsules produced liquid smoke morphology with an unbroken sphere. This showed that alginate as a coating material was capable of protecting the liquid smoke during nanoencapsulation process.

### 3.2. Peroxide Value (PV) Analysis

The combination of liquid smoke nanoencapsulation was applied to the catfish fillets and stored at room temperature and cold temperature. The antioxidant and antimicrobial effects were observed during storage. The number of peroxide value on a catfish fillet was presented in Figure 2. Based on the results obtained, the peroxide value of catfish fillets increased on days 0 to day 4. After that, the peroxide value decreased until 10 days at all

storage temperatures. Peroxide value was the number that indicated the degree of damaged oil or fat by oxidation. The oil reacted with oxygen and form peroxides, especially when it contains unsaturated fatty acids (Panagan, *et al.*, 2011). Catfish fillets had a fat content of 0.12 to 1.42% (Rario, 2015). Catfish fat contains omega-3 (Panagan, *et al.*, 2011) as an unsaturated fatty acid, that potentially forms peroxides due to oxidation.



**Figure 2.** The Peroxide Value of Catfish Fillet Stored at Room Temperature and Cold Temperature

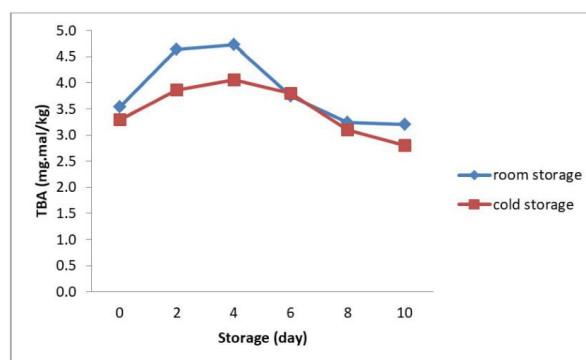
The combination of liquid smoke nanoencapsulation had a total phenolic content of 3.682% and the RSA of 91.348%, that can inhibit the oxidation process of catfish fillet. The result showed that the storage of catfish fillet for 4 days had a good peroxide value at room temperature and cold temperature, there were 4.695 meq/kg and 3.347 meq/kg, respectively. The peroxide value was decreased for 10 days of storage at room temperature and cold temperature, there were 2.467 meq/kg and 2.725 meq/kg, respectively. The different results were shown by Adebawale *et al.*, (2012) that the catfish storage at room temperature for 21 days obtained peroxide value for 5.12 meq/kg. A maximum limit for foodstuffs peroxide value was 5 meq/kg. This result showed that the catfish fillet after 10 days of storage was feasible for consumption.

### 3.3. Thiobarbituric Acid (TBA) Analysis

The TBA value of catfish fillet during storage was presented in Figure 3. TBA measured the amount of malonaldehyd which is the final product of fat oxidation (Piccolo, *et al.*, 2014). Based of figure 3, it could be seen that the TBA value of catfish fillet increased until 4 days of storage, for storage of room temperature from 3.534 mg malonaldehyd/kg to 4.726 mg malonaldehyd/kg. Meanwhile the TBA value of catfish fillet at cold temperature storage were 3.291 mg malonaldehyd/kg to 4.052 mg malonaldehyd/kg. The TBA value decreased until 10 days of storage, there were 3.206 mg malonaldehyd/kg at room temperature and 2.802 mg malonaldehyd/kg at cold temperature. Swastawati *et al.*, (2012) applied the coconut shell liquid smoke on a stingray, showed the TBA value decreased after 6 days of storage. The maximum number of malonaldehyde was 5 mg/kg (Gunsen, *et al.*, 2011). This result showed that catfish fillets

were still feasible for consumption either on the storage at room temperature or cold temperature until 10 days of storage. The combination of liquid smoke nanoencapsulation applied to the catfish fillet

was able to inhibit the oxidation of fat. The decreasing of TBA value indicated that the secondary oxidation products formation which not detected with TBA value (Piccolo, *et al.*, 2014).



**Figure 3.** The TBA Value of Catfish Fillet Stored at Room Temperature and Cold Temperature

### 3.4. Total Volatile Base Nitrogen (TVBN)

TVBN analysis measured the declining of fish quality. TVBN measured the protein degradation which is formed dimethylamine, trimethylamine, and ammonia Saloko *et.al.*, (2014) that caused by bacterial activity (AOCS, 1990). The TVBN value of catfish fillet during storage was presented in Figure 4. The result showed that TVBN value increased during storage at 10 days. The TVBN value of catfish fillet increased in room temperature and cold temperature of storage, that is 15,075 mgN/100g to 22,576 mgN/100g for room

temperature and 10,954 mgN/100g to 21,510 mgN/100g for cold temperature. This indicated that the longer of storage time, the growth of bacteria in catfish fillet was also increased.

The maximum limit of TVBN value for fish was about 30-35 mgN/100g. This showed that until the 10th day of storage, TVBN value is still below standard, consequently the catfish fillet was fit for consumption. These results related to the total phenolic content of liquid smoke nanoencapsulation that the phenol content of liquid smoke was able as antimicrobial agents (Saloko *et.al.*, 2014).

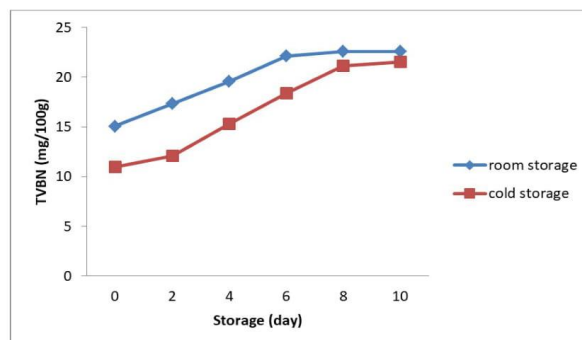


Figure 4. The TVBN Value of Catfish Fillet at Room Temperature and Cold Temperature

### 3.5. Total Plate Count (TPC)

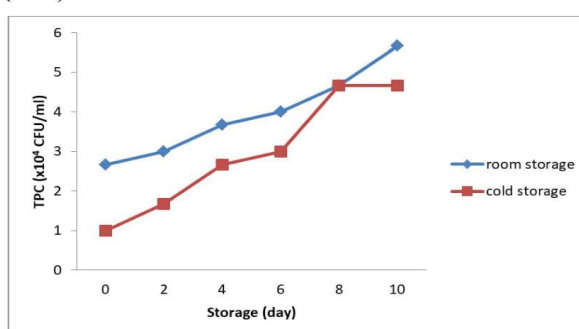


Figure 5. The TPC Value of Catfish Fillet at Room Temperature and Cold

Temperature TPC value of catfish fillet during storage was presented in Figure 5. The result showed that the number of microbial was increased during 10 days of storage. Both room temperature or cold temperature of storage, the number of microbial of catfish fillet were  $2.667 \times 10^4$  CFU/g to  $5.667 \times 10^4$  CFU/g at room temperature and  $1 \times 10^4$  CFU/g to  $4.667 \times 10^4$  CFU/g for cold temperature. Based on Indonesia National Standard, the TPC value of fish product was  $5 \times 10^5$  CFU/g (BSN, 2009). This result showed that until 10 days of storage, the catfish fillet was still feasible for consumption.

The combination of liquid smoke nanoencapsulation had total phenolic content that acted as an antimicrobial agent. Zuraida

*et.al.*, (2011) the coconut shell liquid smoke was able to inhibit microbial growth of fish balls on 20 days of storage with TPC value 1.8 log CFU/g. Ariestya *et.al.*, (2016), also showed that the application of liquid smoke microcapsules on Tilapia meat could inhibit microbial growth with the TPC value 26 CFU/g at cold temperatures after 9 days of storage. The microbial growth inhibition because of the phenolic content of liquid smoke.

### 4. Conclusions

The liquid smoke nanoencapsulation application on catfish fillet was able to inhibit oxidation during storage, indicated by the PV and TBA value was under the limit standard until 10 days of storage. In addition, liquid

smoke nanoencapsulation also able to inhibit microbial activity which was proved by the TVBN and TPC number was below the maximum limit. The result showed that the liquid smoke nanoencapsulation was act as antioxidant and antibacterial agent.

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# THE EFFECT OF ANTIOXIDANT AND ANTIBACTERIAL NANOENCAPSULATION LIQUID SMOKE LIQUID SMOKE NANOCAPSULES ON CATFISH FILLET (*Pangasius* sp.) DURING STORAGE AT ROOM TEMPERATURE AND COLD TEMPERATURE

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## ABSTRACT

The purpose of this study was to determine the effect of antioxidant and antibacterial of nanoencapsulation liquid smoke liquid smoke nanocapsules on a catfish filet (*Pangasius* sp.). A combination of liquid smoke (corn cob and coconut shell) were processed into nanoencapsulation using three encapsulation i.e: gum arabic, maltodextrin, and alginate with a ratio of 1/6: 4/6: 1/6 each. Nanoencapsulation liquid smoke liquid smoke nanocapsules was known to have a total content of phenols containing total phenolic content, carbonyl, and Radical Scavenging Activity, there were 3.682 mg GAE/g, 3.439%, and 91.348%, respectively. Nanoencapsulation liquid smoke liquid smoke nanocapsules was applied to the catfish and stored at room temperature (28°C±2°C) and cold temperature (5°C). Observations were made on days 0, 2, 4, 6, 8, and 10 to parameter PV, TBA, TVBN and TPC. The results showed that nanoencapsulation liquid smoke liquid smoke nanocapsules could effectively inhibit the oxidation of fat catfish showed with PV and TBA acceptable. Nanoencapsulation liquid smoke liquid smoke nanocapsules was also capable of inhibiting the activity of microbes, indicated by the value of TVBN and TPC which were still below standard at all temperatures and long storage time.

## 1.Introduction

Catfish were easily damaged by the changes of fat content (oxidation process, lipoxygenase damage, etc), protein changes and changes the content of microorganisms (Masniyom, 2011). This damage is indicated by peroxide numbers, TBA (Valdes *et al.*, 2015), TVBN (Tian *et al.*, 2012; Castro, 2012) and TPC (Adilla *et al.*, 2017) which increases during storage. Catfish have high nutrient content especially fat and protein. Catfish contain palmitic acid (24.05%), oleic acid

(27.55%), and linoleic acid (7.63%). In addition, catfish also contains non essential amino acids, such as as-glutamate (3.33%) and essential amino acids, for example lysine (1.82%) (Nurilmala *et al.*, 2015). The high content of fatty acids and amino acids of catfish, resulting in catfish being damaged continuously during cold storage temperatures (Abbas *et al.*, 2005). Therefore, treatment were needed to inhibit catfish damage during storage.

Liquid smoke is one of the smoke condensation products in the form of liquid. Liquid smoke is widely used compared to traditional curing methods because it is easy to use and more economical. Liquid smoke also has several compounds such as phenol, acids and carbonyl that acts as an antibacterial and antioxidant (Saloko, *et al.*, 2014). Several studies has been done by other researcher using coconut shell liquid smoke to inhibit fish damage, such as tuna (Saloko, *et al.*, 2014) tilapia (Ariestya, *et al.*, 2016), and catfish (Swastawati, 2008). Other research elaborated the use of corncobs liquid smoke in tilapia (Youssef, *et al.*, 2015) and milkfish (Swastawati, *et al.*, 2016); which shows the shelf life of tilapia ~~meat fillet~~ for 6 days at cold temperature storage (5°C) ~~tilapia~~ (Ariestya, *et al.*, 2016). Coconut shell liquid smoke increased the shelf life of mackerel fishballs for 32 hours at room temperature storage (Zuraida, *et al.*, 2011). While corncob liquid smoke was able to extend the shelf life of stingrays for 3 days at room temperature storage (Swastawati, *et al.*, 2012) and tilapia meatballs for 15 days at cold temperature storage (4°C) (Youssef, *et al.*, 2015). The existence of differences in the capability of coconut shell liquid smoke and corncobs liquid smoke increasing the shelf life of the product encourage the incorporation of these two liquid smokes in application of the product, which is expected to give effect in different shelf life at different storage temperatures. All the previous researcher were only use one raw material of liquid smoke. In this study, we apply combination of two raw materials i.e coconut shell and corncob (50:50) which is hope will give longer shelf life because these mixture of raw material were found to contain higher polyphenols (Anggraini, *et al.*, 2017; Swastawati, *et al.*, 2014; Lombok, *et al.*, 2014; Yuniningsih and Anggraini, 2013).

Polyphenols were volatile bioactive components of liquid smoke. In addition, polyphenols have low and unstable water solubility (Conte, *et al.*, 2016). Therefore, a system capable to improve the properties of

polyphenols and maintaining polyphenols during storage was required. Nanoencapsulation technology changed liquid smoke in liquid form to a nano-sized powder (nanocapsules ~~encapsulation~~) of 1 to 2000 nm (Etheridge, *et al.*, 2013) has an advantage in the delivery of bioactive components that were efficient in penetrating cells in desired products (Ezhilarasi, *et al.*, 2012). Many research were limited to coconut shell encapsulation (Saloko, *et al.*, 2014; Ariestya, *et al.*, 2016; Novianty, *et al.*, 2015; Ali, *et al.*, 2014; Saloko, *et al.*, 2012). Based on the above description, this study examined the effect of combination liquid smoke nanocapsules ~~encapsulation~~ (coconut shell and corncob liquid smoke) on catfish fillet during storage of room temperature and cold temperature.

## 2. Materials and Methods

### 2.1. Materials

The materials used in this study were the corncob and coconut shell to produce liquid smoke. Each materials was processed into liquid smoke by pirolisator machine in laboratory of Fisheries and Marine Science Faculty, Diponegoro University, Semarang, Indonesia. Maltodextrin DE 10, arabic gum and Na-alginate were obtain from Multi Kimia Raya Semarang, Indonesia, meanwhile catfish were obtained from the local market in Semarang, Indonesia.

### 2.2. Nanoencapsulation of Liquid Smoke

Nanoencapsulation processed was carried out according to Saloko, *et al.*, (2013) with modification in core and coating materials. Coconut shell liquid smoke and corn cob liquid smoke was mixtured with ratio 1:1. Nanoencapsulation was processed by maltodextrin, gum arabic, and Na-alginate with a ratio of 1:4:1 was mixed with a combination of coconut shell and corncob liquid smoke. The solution was homogenized and centrifuged at 3000 rpm for 30 minutes at room temperature. Supernatant was separated and filtered to obtain a solution of pure nanoparticles. The solution of nanoparticles was heated at 50°C in

waterbath for 15 minutes and homogenized using a homogenizer at a speed of 4000 rpm for 2.5 minutes. The sample was dried with a spray dryer with inlet temperature about 130°C, while the outlet temperature about 70°C. The nanoencapsulation was collected on a sealed bottle and stored at room temperature.

## 2.2. Characteristic of Nanoencapsulation Liquid Smoke Liquid Smoke Nanocapsules

### 2.2.1. Analysis of Total Phenolic Content

A amount of 1 gram liquid smoke nanoencapsulation was diluted to a volume of 25 ml aquadest. 1 ml solution was diluted to 10 ml aquadest. Next 2.5 ml of it's solution was taken and diluted to 10 ml. After that, 1 ml solution was put into a test tube and 1 ml saturated Na<sub>2</sub>CO<sub>3</sub> (Merck, Germany) was added and left for 10 minutes at room temperature. Folin ciocalteu reagent (Sigma-Aldrich, USA) 0.5 ml and 7.5 ml of distilled water were added and homogenized by using a vortex for 30 minutes at room temperature. The absorbance of samples were measured at 760 nm wavelength. Phenolic content of samples was calculated as GAE in mg/g dry material (AOCS, 1990).

### 2.2.2. Analysis of Total Carbonyl

An amount of 1.6 mg of sample was diluted to 10 ml with carbonyl-free ethanol. 1 ml of solution was reacted with 2 ml solution of 2,4-dinitrophenyl-hydrazine (Sigma-Aldrich, USA) with a drop of concentrated hydrochloric acid in ethanol saturated. The mixture was heated in waterbath at temperature 50°C for 30 min. About 5 ml alcoholic solution of potassium hydroxide (Merck, Germany) were added when the mixture was cool. Then 2 ml of distilled water was added and measured with a spectrophotometer with a wavelength of 480 nm. Results were calculated by comparing it with the standard curve of acetaldehyde 2,4-dinitrophenylhydrazone (2,4-DNPH) and calculated equivalent of 13.7 ppm acetaldehyde

(Sigma-Aldrich, USA) in the sample (Alice, et al., 1961).

### 2.2.3. Radical Scavenging Activity

Radical Scavenging Activity (RSA) was measured by Li and Guo (2010) with modifications. Each sample was reacted with DPPH (Sigma-Aldrich, USA) 0.004 g/ml of ethanol. 0.1 ml of sample was added with 3.9 ml of DPPH and incubated at 28°C for 30 minutes. Scavenging activity on DPPH radical was measured at 515 nm wavelength. Percent of RSA was measured according to the following equation:

$$\% \text{ RSA} = \{(A_{\text{control}} - A_{\text{sample}}) \times A^{-1}\} \times 100\% \text{ control}$$

### 2.2.4. PAH Analysis

#### Solid-Liquid Extraction

Two grams of freeze-dried fish fillet mixed with a mixture of the 20 ml standard solution with 13 PAH was equal to 0.5 µg.kg<sup>-1</sup>, considered as internal standards which were homogenized in 40 ml of cyclohexane/ethyl acetate (50:50; v/v) and it was shaken during 30 minutes. The solution was centrifuged at 5000 rpm for 30 min at 0°C. After being homogenized, the liquid part was carefully isolated and evaporated to dryness under a gentle stream of nitrogen. The residue was dissolved in 6 ml of cyclohexane. PAH quantification was the result of the mean of measures carried out on three individual smoked fillets in the same conditions.

### 2.2.5. Scanning Electron Microscopy (SEM)

Morphology of liquid smoke nanoencapsulations was observed by using Scanning Electron Microscopy (FEI, Inspect S50). The sample was layered with gold and it was monitored by a magnification of 1,000 times at the voltage of 20 kV.

## 2.3. Application Nanoencapsulation-Liquid Smoke Liquid Smoke Nanocapsules on Catfish

Catfish fillet with a size of 25 x 15 x 1 cm with a weight of approximately 100 grams, was

smeared with liquid smoke nano~~capsules~~~~eneapsulation~~ as much as 1% of the weight of the fillet. ~~After that, Catfish fillet that has been smeared with liquid smoke nanoeneapsulation then was~~ roasted at a temperature of 90°C for 4 hours. Smoke catfish fillet was stored at room temperature (28°C±2°C) and cold temperature (5°C) for 10 days and analyzed every 2 days.

### 2.3.1. Peroxide Value (PV) Analysis

Peroxide value analysis was conducted by Memon *et al.*, (2010). The sample was dissolved in a mixture of chloroform (Merck, Germany) and glacial acetic acid (Merck, Germany) and added with a solution of potassium iodide (Merck, Germany). The mixture was finally titrated with sodium thiosulfate solution (Merck, Germany) 0.01 M with 1% starch indicator.

### 2.3.2. Thiobarbituric Acid (TBA) Analysis

TBA analysis was conducted by Molla *et al.*, (2015), 2 ml of 20% trichloroacetic acid (Merck, Germany) and 2 ml of 0.67% thiobarbituric acid (Fluka Chemika, Switzerland) was added to 1 ml of the sample solution. The mixture was heated at 100°C for 10 minutes in waterbath. The mixture was centrifuged at 3000 rpm for 20 minutes. Supernatant containing TBARS absorbance was measured at 532 nm wavelength using a spectrophotometer.

### 2.3.3. Total Volatil Base Nitrogen (TVBN) Analysis

Total Volatile Base Nitrogen (TVBN) was carried out according Indonesian National Standard 2354.8:2009 (BSN, 2009). Briefly, 25 g samples was weighed and mixed with 75 mL TCA (Merck, Germany) 7%. 1 ml filtrat was put in conway cup of outer chamber which had

previously been added 1 mL K<sub>2</sub>CO<sub>3</sub> (Merck, Germany). Another Conway cup of inner chamber was added 1 mL Boric acid and 2-3 drops of indicator (screen metal red) until the color was green. Blanko had been used 1 mL TCA 7%. Conway cup was incubated at 37°C until 2 hours. Conway cup in the inner chamber of blanko was titrated with HCl until the color was pink. Conway cup of samples titrated with boric acid until the color was equal with blanko.

### 2.3.4. Total Plate Count (TPC) Analysis

Total Plate Count (TPC) was obtained by Indonesian National Standard 2332.3:2015 (BSN, 2015). Fish samples were diluted into Butterfields Phosphat Buffered (Merck, Germany) with concentration of 10<sup>4</sup>, 10<sup>3</sup>, and 10<sup>5</sup>. One milliliter of each sample solution was placed into petridisc containing plate count agar (PCA) (Merck, Germany). Petridisc containing samples was incubated with the opposite position at 35°C for 48 hours. The number of colony were calculated by hand tally counter for the amount 25-250.

## 3. Results and discussions

### 3.1. Characterization of Liquid Smoke Nano~~eneapsulation~~~~Nanocapsules~~

The content of total phenols, total carbonyl, and RSA of liquid smoke ~~nanoeneapsulation nanocapsules~~ in a row was consecutively 3.682 mg GAE/g, 3.439% and 91.348% (Table 1). Total phenolic content of liquid smoke ~~nanoeneapsulation nanocapsules~~ was influenced by the total phenolic content of liquid smoke and the composition of the coating material. Based on Hardianto and Yunianta (2015) the total phenolic content of corn cob liquid smoke was lower than coconut shell liquid smoke.

**Table 1.** Characteristics of Liquid Smoke ~~Nanoeneapsulation~~~~Nanocapsules~~

Characteristics	Results
Total Phenolic Content (mg GAE/g)	3.682

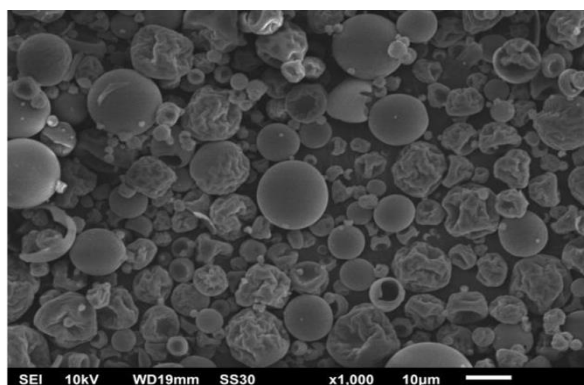
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Total Carbonyl (%)	3.4 <del>439</del>
Radical Scavenging Activity (%)	91.3 <del>548</del>
Polycyclic aromatic hydrocarbons (PAHs) (ppm)	
Naphtalen	286.40
Acenaphtane	106.35
Phenantrene	11.70
Phyrene	30.00
Benzo- $\alpha$ -Antrazene	67.10
Benzo- $\alpha$ -Phyrene	47.55

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**Figure 1.** Microstructure of Liquid Smoke ~~Nanoencapsulation~~ Nanocapsules

The composition of the coating material also affected the content of total phenols. The use of the coating material for one portion of alginate composition could trap phenolic content of liquid smoke during the spray drying process. This research was accordance with Novianty *et al.*, (2015) that the encapsulation process of liquid smoke with alginate 1% was able to trap the phenol content with the release of phenol for 20 minutes.

Total carbonyl content of liquid smoke ~~nanoencapsulation~~ nanocapsules was also affected by carbonyl content of liquid smoke. The carbonyl content of corncob liquid smoke was greater than coconut shell liquid smoke. Because of the corncob liquid smoke contains cellulose degradation products that were more than the liquid smoke coconut shell (Hardianto and Yunianta, 2015). In addition, the alginate

composition as a coating material can protect the carbonyl during the spray dryer. Alginate can form a gel (Novianty *et al.*, 2015). Alginate was polysaccharide that contain of homopolymeric mannuronic (M) and guluronic (G) block. The gel characteristic of alginate was affect by M/G ratio (Fertah, *et al.*, 2014~~7~~). This character was used to protect the phenolic content and carbonyl component during nanoencapsulation process. ~~Nanoencapsulation~~ Nanocapsules oxidative capability of liquid smoke was measured by Radical Scavenging Activity. The RSA of liquid smoke ~~nanoencapsulations~~ nanocapsules was 91.~~3~~~~5~~~~48~~%. It was indicated that the coating materials was able to inhibit the oxidation of liquid smoke associated with total phenolic content and total carbonyl, where the

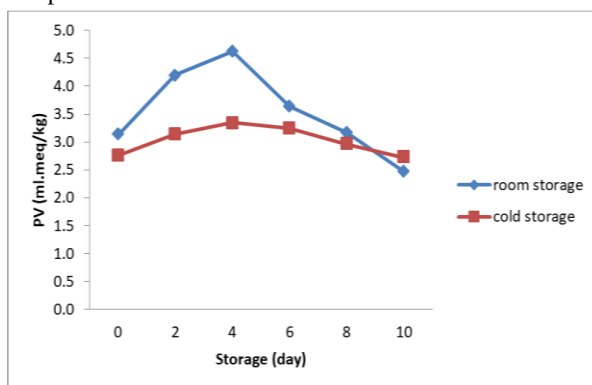
component acts as an antioxidant and antimicrobial in food (Leha, 2010).

According to the table 1, it was known that liquid smoke nanoencapsulation contain PAH especially benzo- $\alpha$ -pyrene. Benzo- $\alpha$ -pyrene was known to be carcinogenic and mutagenic to human. Based Swastawati (2008), coconut shell liquid smoke had benzo- $\alpha$ -pyrene contents of 11.351 ppm, while corn cob liquid smoke was not detected (Swastawati, *et al.*, 2007). According to the table 1, it showed that the coating material can trap ~~nanoeencapsulation~~ ~~nanocapsules~~ PAH compounds.

Based on morphological observation of liquid smoke ~~nanoeencapsulation~~ ~~nanocapsules~~ (Figure 1), it could be detected that the liquid smoke ~~nanoeencapsulation~~ ~~nanocapsules~~ produced a perfect numerous circle. Novianty *et.al.*, (2015) showed that the concentration of 1% alginate microcapsules produced liquid smoke morphology with an unbroken sphere. This showed that alginate as a coating material was capable of protecting the liquid smoke during nanoencapsulation process.

### 3.2. Peroxide Value (PV) Analysis

The combination of liquid smoke nanoencapsulation was applied to the catfish fillets and stored at room temperature and cold temperature. The antioxidant and antimicrobial effects were observed during storage. The number of peroxide value on a catfish fillet was presented in Figure 2. Based on the results obtained, the peroxide value of catfish fillets increased on days 0 to day 4. After that, the peroxide value decreased until 10 days at all storage temperatures. Peroxide value was the number that indicated the degree of damaged oil or fat by oxidation. The oil reacted with oxygen and form peroxides, especially when it contains unsaturated fatty acids (Panagan, *et al.*, 2011). Catfish fillets had a fat content of 0.12 to 1.42% (Rario, 2015). Catfish fat contains omega-3 (Panagan, *et al.*, 2011) as an unsaturated fatty acid, that potentially forms peroxides due to oxidation.



**Figure 2.** The Peroxide Value of Catfish Fillet Stored at Room Temperature and Cold Temperature

The combination of liquid smoke ~~nanoeencapsulation~~ ~~nanocapsules~~ had a total phenolic content of 3.682% and the RSA of 91.3548%, that can inhibit the oxidation process of catfish fillet. The result showed that the storage of catfish fillet for 4 days had a good peroxide value at room temperature and cold temperature, there were 4.70695 meq/kg

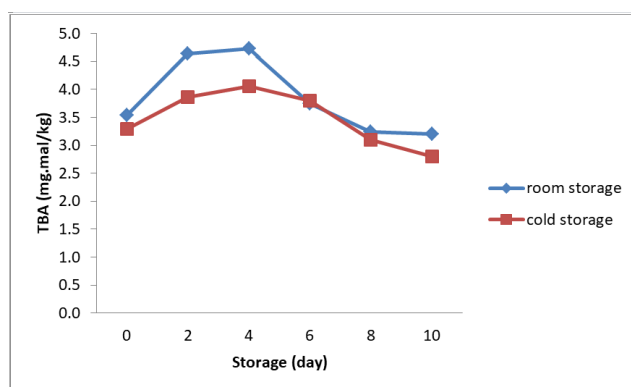
and 3.3547 meq/kg, respectively. The peroxide value was decreased for 10 days of storage at room temperature and cold temperature, there were 2.467 meq/kg and 2.7325 meq/kg, respectively. The different results were shown by Adebawale *et.al.*, (2012) that the catfish storage at room temperature for 21 days obtained peroxide value for 5.12 meq/kg. A

maximum limit for foodstuffs peroxide value was 5 meq/kg. This result showed that the catfish fillet after 10 days of storage was feasible for consumption.

### 3.3. Thiobarbituric Acid (TBA) Analysis

The TBA value of catfish fillet during storage was presented in Figure 3. TBA measured the amount of malonaldehyd which is the final product of fat oxidation (Piccolo, *et al.*, 2014). Based of figure 3, it could be seen that the TBA value of catfish fillet increased until 4 days of storage, for storage of room temperature from 3.534 mg malonaldehyd/kg to 4.7326 mg malonaldehyd/kg. Meanwhile the TBA value of catfish fillet at cold temperature storage were 3.294 mg malonaldehyd/kg to 4.052 mg malonaldehyd/kg. The TBA value decreased until 10 days of storage, there were

3.2196 mg malonaldehyd/kg at room temperature and 2.802 mg malonaldehyd/kg at cold temperature. Swastawati *et al.*, (2012) applied the coconut shell liquid smoke on a stingray, showed the TBA value decreased after 6 days of storage. The maximum number of malonaldehyd was 5 mg/kg (Gunsen, *et al.*, 2011). This result showed that catfish fillets were still feasible for consumption either on the storage at room temperature or cold temperature until 10 days of storage. The combination of liquid smoke ~~nanoencapsulation nanocapsules~~ applied to the catfish fillet was able to inhibit the oxidation of fat. The decreasing of TBA value indicated that the secondary oxidation products formation which not detected with TBA value (Piccolo, *et al.*, 2014).



**Figure 3.** The TBA Value of Catfish Fillet Stored at Room Temperature and Cold Temperature

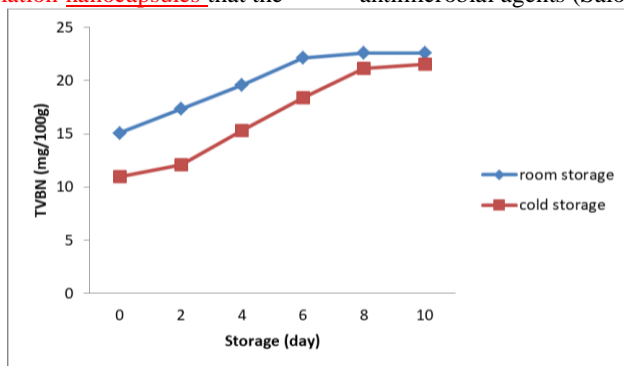
### 3.4. Total Volatile Base Nitrogen (TVBN)

TVBN analysis measured the declining of fish quality. TVBN measured the protein degradation which is formed dimethylamine, trimethylamine, and ammonia Saloko *et al.*, (2014) that caused by bacterial activity (AOCS, 1990). The TVBN value of catfish fillet during storage was presented in Figure 4. The result showed that TVBN value increased during storage at 10 days. The TVBN value of catfish fillet increased in room temperature and

cold temperature of storage, that ~~was~~ 15.0875 mgN/100g to 22.5876 mgN/100g for room temperature and 10.954 mgN/100g to 21.519 mgN/100g for cold temperature. This indicated that the longer of storage time, the growth of bacteria in catfish fillet was also increased.

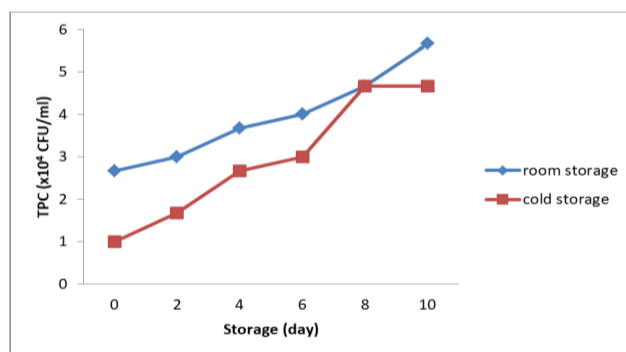
The maximum limit of TVBN value for fish was about 30-35 mgN/100g. This showed that until the 10th day of storage, TVBN value is still below standard, consequently the catfish fillet was fit for consumption. These results

related to the total phenolic content of liquid smoke ~~nanocapsulation~~ ~~nanocapsules~~ that the phenol content of liquid smoke was able as antimicrobial agents (Saloko *et al.*, 2014).



**Figure 4.** The TVBN Value of Catfish Fillet at Room Temperature and Cold Temperature

### 3.5. Total Plate Count (TPC)



**Figure 5.** The TPC Value of Catfish Fillet at Room Temperature and Cold

Temperature TPC value of catfish fillet during storage was presented in Figure 5. The result showed that the number of microbial was increased during 10 days of storage. Both room temperature or cold temperature of storage, the number of microbial of catfish fillet were  $2.667 \times 10^4$  CFU/g to  $5.667 \times 10^4$  CFU/g at room temperature and  $1 \times 10^4$  CFU/g to  $4.667 \times 10^4$  CFU/g for cold temperature. Based on Indonesia National Standard, the TPC value of fish product was  $5 \times 10^5$  CFU/g (BSN, 2009). This result showed that until 10 days of storage, the catfish fillet was still feasible for consumption.

The combination of liquid smoke ~~nanocapsulation~~ ~~nanocapsules~~ had total phenolic content that acted as an antimicrobial agent. Zuraida *et al.*, (2011) the coconut shell liquid smoke was able to inhibit microbial growth of fish balls on 20 days of storage with TPC value 1.8 log CFU/g. Ariestya *et al.*, (2016), also showed that the application of liquid smoke microcapsules on Tilapia meat could inhibit microbial growth with the TPC value 26 CFU/g at cold temperatures after 9 days of storage. The microbial growth inhibition because of the phenolic content of liquid smoke.

#### 4. Conclusions

The liquid smoke ~~nanonecapsulation nanocapsules~~ application on catfish fillet was able to inhibit oxidation during storage, indicated by the PV and TBA value ~~wasere~~ under the limit standard until 10 days of storage. In addition, liquid smoke ~~nanonecapsulation nanocapsules~~ also able to inhibit microbial activity which was proved by the TVBN and TPC number was below the maximum limit. The result showed that the liquid smoke ~~nanonecapsulation nanocapsules~~ was act as antioxidant and antibacterial agent.

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