



THE EFFECT OF ANTIOXIDANT AND ANTIBACTERIAL 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 liquid smoke nanocapsules on a catfish filet (*Pangasius sp.*). A combination of liquid smoke (corn cob and coconut shell) were processed into nanocapsules using three encapsulation i.e: gum arabic, maltodextrin, and alginate with a ratio of 1/6: 4/6: 1/6 each. Liquid smoke nanocapsules was containing total phenolic content, carbonyl, and Radical Scavenging Activity, there were 3.682 mg GAE/g, 3.439%, and 91.348%, respectively. 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 liquid smoke nanocapsules could effectively inhibit the oxidation of fat catfish showed with PV and TBA acceptable. 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, lipoxigenase damage, etc), protein and 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 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 fillet for 6 days at cold temperature storage (5°C) (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) 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 (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 nanocapsules was collected on a sealed bottle and stored at room temperature.

2.2.Characteristic of Liquid Smoke Nanocapsules

2.2.1. Analysis of Total Phenolic Content

A amount of 1 gram liquid smoke nanocapsules 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_2CO_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:

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

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 $0.5 \mu\text{g.kg}^{-1}$, 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 nanocapsules 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 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 nanocapsules as much as 1% of the weight of the fillet. After that, catfish fillet was roasted at a temperature of 90°C for 4 hours. Smoke catfish fillet was stored at room temperature ($28^\circ\text{C} \pm 2^\circ\text{C}$) 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 K₂CO₃ (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 10⁴, 10³, and 10⁵. 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 Nanocapsules

The content of total phenols, total carbonyl, and RSA of liquid smoke nanocapsules in a row was consecutively 3.682 mg GAE/g, 3.439% and 91.348% (Table 1). Total phenolic content of liquid smoke nanocapsules 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 Nanocapsules

Characteristics	Results
Total Phenolic Content (mg GAE/g)	3.68
Total Carbonyl (%)	3.44
Radical Scavanging Activity (%)	91.35
Polycyclic aromatic hydrocarbons (PAHs) (ppm)	
Naphtalen	286.40
Acenaphtane	106.35
Phenantrene	11.70
Phyrene	30.00
Benzo- α -Antrazene	67.10
Benzo- α -Phyrene	47.55

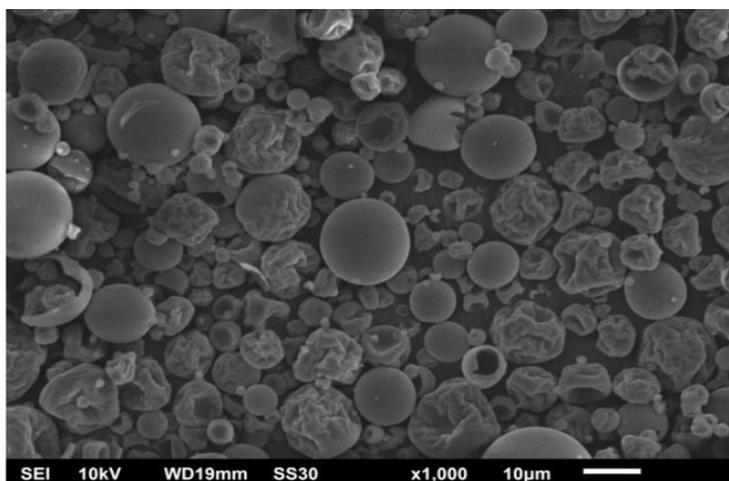


Figure 1. Microstructure of Liquid Smoke Nanocapsules

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 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.*, 2017). This character was used to protect the phenolic content and carbonyl component during nanoencapsulation process. Nanocapsules oxidative capability of liquid smoke was measured by Radical Scavenging Activity. The RSA of liquid smoke nanocapsules was 91.35%. 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- α -phyrene. Benzo- α -phyrene was known to be carcinogenic and mutagenic to human. Based Swastawati (2008), coconut shell liquid smoke had benzo- α -phyrene 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 nanocapsules PAH compounds.

Based on morphological observation of liquid smoke nanocapsules (Figure 1), it could be detected that the liquid smoke 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.

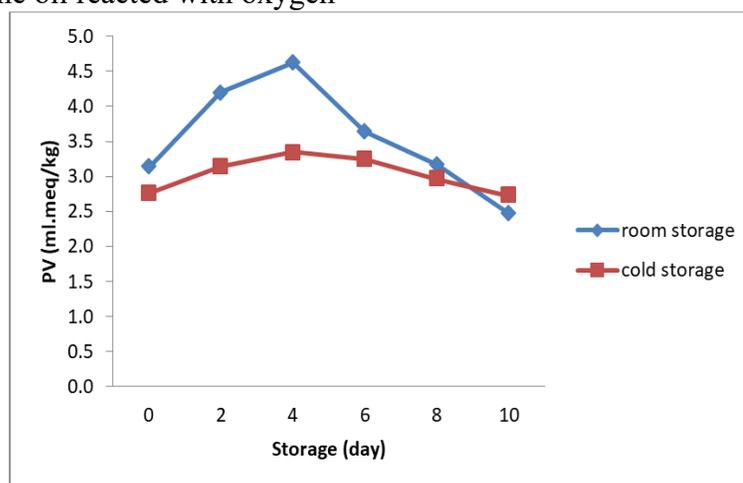


Figure 2. The Peroxide Value of Catfish Fillet Stored at Room Temperature and Cold Temperature

The combination of liquid smoke nanocapsules had a total phenolic content of 3.68% and the RSA of 91.35%, 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 4.70 meq/kg and 3.35 meq/kg, respectively. The peroxide value was decreased for 10 days of storage at room temperature and cold temperature, there were 2.47 meq/kg and 2.73 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 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 3.53 mg malonaldehyd/kg to 4.73 mg malonaldehyd/kg. Meanwhile the TBA value of catfish fillet at cold temperature storage were 3.29 mg malonaldehyd/kg to 4.05 mg malonaldehyd/kg. The TBA value decreased until 10 days of storage, there were 3.21 mg malonaldehyde/kg at room temperature and 2.80 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 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).

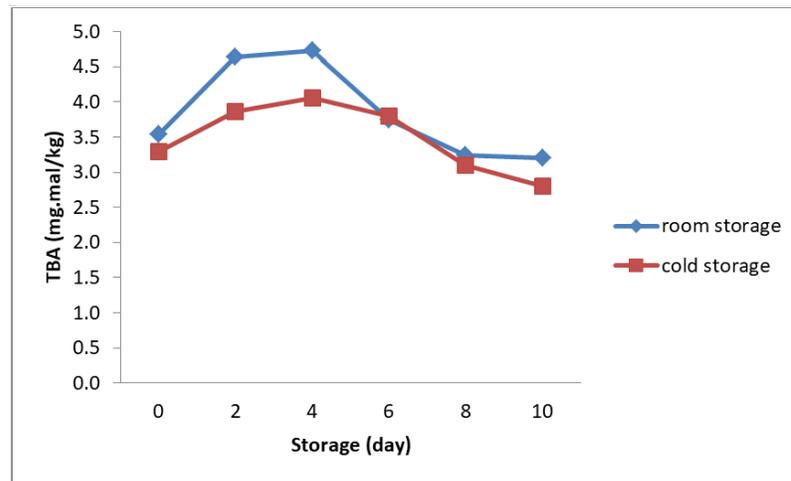


Figure 3. The TBA Value of Catfish Fillet Stored at Room Temperature and Cold Temperature

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 15.08 mgN/100g to 22.58 mgN/100g for room

temperature and 10.95 mgN/100g to 21.51 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 nanocapsules that the phenol content of liquid smoke was able as antimicrobial agents (Saloko *et al.*, 2014).

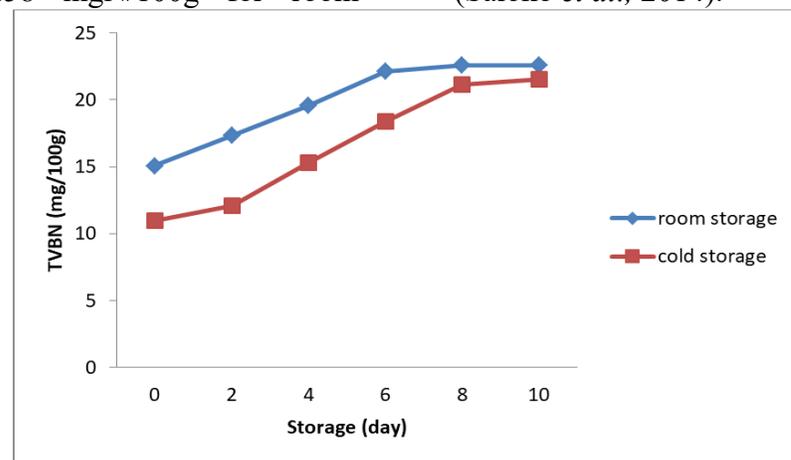


Figure 4. The TVBN Value of Catfish Fillet at Room Temperature and Cold Temperature

3.5. Total Plate Count (TPC)

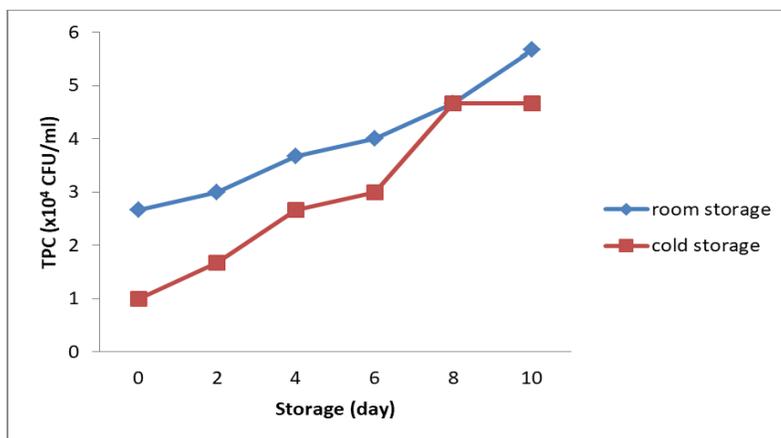


Figure 5. The TPC Value of Catfish Fillet at Room Temperature and Cold

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 2.67×10^4 CFU/g to 5.67×10^4 CFU/g at room temperature and 1×10^4 CFU/g to 4.67×10^4 CFU/g for cold temperature. Based on Indonesia National Standard, the TPC value of fish product was 5×10^5 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 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 nanocapsules application on catfish fillet was able to inhibit oxidation during storage, indicated by the PV and TBA value were under the limit standard until 10 days

of storage. In addition, liquid smoke 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 nanocapsules was act as antioxidant and antibacterial agent.

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