Utilization of liquid smoke nanoencapsulation in fresh fish fillets as a preservation material

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Abstract. Liquid smoke of coconut shell was process to produce nanoencapsulation using 10% of arabic gum (A), 10% of maltodextrin (M) and mixed of 5% arabic gum + 5% of maltodextrin (AM). The products of all microencapsulation (A, M, AM) were then been analyzed in terms of : fenol content, pH, particel size, solution test, acid content, carbonyl content, and also their colour characteristic. After all, the nanoencapsulation were applied into white snapper for 2% w/w stand for. The fish sample then evaluated in terms of their color and TVBN values. The result of total phenolic compound of nanocapsule were found about :0.72% - 0.95%, carbonyl compound :1.812% - 2.380, acid value :11.04mg/g - 22.82mg/g and pH value of :4.1 – 4.6. The bacterial count on the snapper fillets between 5,04 Log CFU/g to 5,82 Log CFU/g. The result suggested that nancapsules had spherical and wrinkle shape with an average size of nanocapsules of 28.03 nm.

1. Introduction
Coconut shell liquid smoke has the potential to extend the shelf life of a product that has a high protein content including fish. This is because liquid smoke has antibacterial and antioxidant activities. Liquid smoke is nowadays becoming a superior popular product that is used as a preservation and flavoring agent for the product. However, the application of liquid smoke into fish fillet is still very rare and need to be developed. [1]. Therefore, it is necessary to find out a method to improve the stability of liquid smoke, such as in the form of nanoencapsulation. Nanoencapsulation is a technology for processing liquid into nano-size powder using a spray dryer. Nano technology is able to transform a material into a new material that has new, unique, and different properties. The choice of this technology is because the process is more stable and can carry good compounds. Nanoparticles are defined as particulate dispersions or solid particles with a size of 10-1000 nm [2]. Nanoencapsulation research produces properties as expected, namely storage will be better and provide protection against bioactive components such as vitamins, antioxidants, pigments, proteins and lipids and carbohydrates, so that it can improve functional and stability properties [3].

Some of the best ways to produce liquid smoke nanoencapsulation are to use alginate, arabic gum, and maltodextrin as encapsulants. This mixing will make it more stable in coating formation to reduce oxidation reactions. Alginate is used as a coating material because of its ability to form a gel [4]. Arabic gum can serve to protect the main ingredients from oxidation [5]. Whereas maltodextrin is easily soluble in water and can form films [6]. In a previous study, [7], used chitosan and maltodextrin for encapsulation of liquid smoke and succeeded in making nanometer-sized smoked flour products (nm).

2. Research Methods
2.1. Materials
Coconut Liquid Smoke was produced by condensation machine in the laboratory of processing Faculty of Fisheries and Marine Science meanwhile the raw material of nanoencapsulated used maltodextrin and arabic gum were distributed by industrial store in Semarang city. White snapper used
was obtained from the fish market, Semarang city. Spray dryer for microencapsulated process was done at Unika Soegiyopranoto University, Semarang, Indonesia.

2.2. Methods

2.2.1 Application of microcapsules on white snapper preservation

Whole white snapper done disposal of the contents of the stomach, gills, and head, and the fish red snapper are already clean fillet process done with the disposal of thorn and the skin of the white snapper. The fillet of white snapper given liquid smoke microencapsulated as antibacterial. Liquid smoke microencapsulated process microencapsulation using 10% of Arabic gum (A), 10% of maltodextrin (M) and mixed of 5% of Arabic gum + 5% of maltodextrin (AM). Fillet of white snapper that has been given treatment inserted into the plastic seal with labelling code and save by method cold storage with temperature 5 °C (± 4 °C). The measurement of TVBN was done during cold storage. While, total plate count (TPC) was measured during storage on 0 hours, 16 hours, 32 hours, and 48 hours.

2.2.2. Nanocapsule Particle Size Measurement

Measurement of nanocapsule particle size was done by suspending it in distilled water. Nanocapsules were measured using a laser particle size distribution analyzer (Malvern Zetasizer Nanoseries Nano ZS Ver 6.20, Malvern Instruments Ltd., Malvern, UK). The size distribution is determined by the range value. [7]. The morphology of nanocapsules was investigated by scanning electron microscopy. The SEM was conducted with an S-3400N scanning microscope. By conduction double-sided tape, electron sprayed micro particle were mounted on metal stubs, and then were coated with gold under an argon atmosphere [8].

2.2.3. Phytochemical Screening Test

Testing total phenol done by using the method [9] as 1 mL liquid smoke weighed or 1 g liquid smoke nanocapsules or microcapsules diluted be 25 mL, taken 1 mL diluted longer be 10 mL (factors dilution = 250×). Taken 2.5 mL diluted longer be 10 mL (factors dilution = 1 000×). The dilution 1mL into tube reaction then added Na2CO3 saturated and leave on 10 min at room temperature. Homogenized reagentfolincioalteau 1 mL and 7.5 mL aquadest, with vortex then incubation for 30 min at room temperature. These sample measured at wavelengths 770 nm. Phenol levels samples counted based on a curve standard obtained.

2.2.4. pH

Testing on the sample pH microencapsulated by using a method of testing pH [10] with calibration way beforehand on the tool pH meters with buffer solution in accordance with the instructions of work a tool every time doing testing. Next try pH solution aquadest on until the device pH meters can read value by recitation that remain. Sample of 5 g homogenized with aquadest 10 mL then insert electrodes already calibrated earlier on the sample after it waited until the pH meter stop and show figures that remain, then obtained indigo pH of microencapsulation.

2.2.5. Total Volatile Base Nitrogen

The TVB-N content was determined according to the method of [11] in [7] with a slight modification. TVB-N sample extract in TCA 7% was absorbed in boric acid solution during incubation at 35 °C for 2 h. Then, it was titrated with 0.02 N HCl and the value was expressed as mg N per 100 g fish muscle.to have a little bit pink color. And performing calculations:

\[
TVBN (\text{mg N %}) = \frac{\text{Sample titration (mL)} - \text{blanko (mL)}}{\text{Sample weight (g)}} \times N \text{ HCl} \times 14.007 \times 100
\]

2.2.6. Total Plate Count

Preparation on a procedure is sample dissolving rough with solution Butterfield’s Phosphate Buffered (BFP) by comparison between 1:9 sample BFP with a solution in plastic sterile. Then do dilution in
samples from dilution 10to 10 000 000, this dilution for get result total coloni in sample. First step homogenizing mix solution BFP and those using stomacher bag for 30 s to till the homogeneous, samples named dilution 10. Dilution 100 through the way to a solution in 1 mL dilution 10 to test tube was completed first with solution BFP 9 mL and vortex solution to homogeneous. Then take 1 mL solution dilution 100 and add some 1ml test tube that there are already BFP 9 mL solution. And homogeneous solution with vortex, this solution it is called solution 1 000. Then take 1mL solution for tube 1 000 and add to different tube for already BFP solution 9 mL, and vortex this solution. This solution called 10 000. Then take again 1 mL solution dilution 10 000, then add to different tub for already BFP solution 9 mL, vortex this solution dillution. This solution dilution called 100 000. Media nutrient that already dissolved on aquadest and sterilization first, then pour about 10 mL on a petri dish and lead sample dilution as many as 2 last 0.1mL and city using rod bent. The incubation media at an incubator temperature 37 °C for 24 h [12].

3. Result and Discussion

Table 1. Charasteristic of liquid smoke with encapsulated different material.

| Sample                   | Total Phenol (%) | Carbonyl (%) | Acid value (mg/g) | Solubility (%) | pH  |
|--------------------------|------------------|--------------|-------------------|----------------|-----|
| Malto-dextrin            | 0.95± 0.001      | 2.380± 0.008 | 16.23± 1.519      | 93.520± 0.097  | 4.1 |
| Arabic Gum               | 0.72± 0.002      | 1.812± 0.005 | 22.82± 0.026      | 91.603± 0.250  | 4.5 |
| Malto-dextrin + Arabic   | 0.81± 0.002      | 2.016± 0.005 | 11.04± 0.484      | 95.370± 0.220  | 4.6 |

3.1. Characteristic of liquid smoke encapsulation

Phenol compound are an important substances of smoke constituent responsible for antibacterial and antioxidant properties. Phenol constituting a compound antibacterial which could deny work bacteria in the flesh of fish containing protein by means of unload reaction in to the cell membrane bacteria so that it can be nonactive work of bacteria. Oxidation process of catfish fillet can inhibit with combination of nanocapsules liquid smoke with a total phenolic content of 3.68% [13]. Various compound phenolic can help in destroying bacterial cells [7]. Added an idea phenol and derivatives can be bacteriostatic and bactericidal because it cannonactivation of essential enzyme, coagulation SH group and NH group protein [14]. Mechanisms antimicrobial phenol activity and derivatives covering reaction cell membranes causes the increasing permeability of membrane cells resulting in the intracellular material cells, essential enzyme become inactivation.

The carbonyl value of the sample A was approximately 1.812%, while in sample M and AM were found 2.380% and 2.016% respectively. The colour of nanoparticles were white yellow wish in the formation of powder. The carbonyl compound plays an important role in the formation of colour and affect the texture of food. Colour of the product generally contribute by carbonyls it reacts with amino groups [15].

Nanocapsule of liquid smoke has highest values of pH 4.6 from AM and lowest values in A 4.1. pH values chosen as representing organic acids content in liquid smoke nanocapsule. pH from nanocapsule The temperature during spray dryer affect increase and decrease of nanocapsule pH values [16].
The acids content of sample AM decrease significantly compared to sample A and M of 22.82 and 16.23, respectively. Organic acid is a result from hemisellulosa and sellulosa of wood during pyrolisis process. Organic acid in nanocapsule liquid smoke affected of colour, flavour, texture and microbial. The most prevalent organic acid present in smoke has been identified in acetic acid. The content of organic acid in liquid smoke reached 9.2% [7].

| Table 2. The colour of liquid smoke nanoencapsulation |
|-----------------------------------------------|
| Sample                        | L     | a     | b         |
| Malto-dectrin                  | 88.43±| 5.43± | 3.93± 0.155 |
| ArabicGum                     | 88.09±| 5.44± | 7.13± 0.095 |
| Malto-dectrin + Arabic Gum    | 77.76±| 5.54± | 9.71± 0.223 |

The liquid smoke encapsulation colour is expressed as L (lightness), a (redness), and b (yellowish). Sample has a bright yellow colour, indicated by the largest L value. The value of L, a, b for sample each was 20.40; 2.05 and 1.55. This showed that sample had a bright yellow colour. While sesame oil had a reddish yellow colour which was indicated by the smallest L value and the largest value of a. Sesame oil had a darker colour with values L, a, b, each of which was 39.96; 24.86 and 28.94. The colour of the mixture of sample and sesame oil produces an increasing intensity of reddish yellow colour indicated by the decreasing value of L.

3.2. Nanocapsule Particle Size Measurement

![Size measurement of liquid smoke nanoencapsulated AM](image-url)
The mean particle sized of nanocapsules is shown in figure 1. The average sized of particle formed from LS2, LS3, and LS5 were 25.00 nm, 30.05 nm, and 29.05 nm, respectively (Figure 2-3). Nanoparticle in a mixture of low molecular weight, a particle sized distribution ranging between 153 and 500 nm [17]. Interaction between phenolic groups of liquid smoke and amino acid may lead to decrease in the cross linked density [18].
The nature of nanocapsules were influenced by its morphology. In this study the used of microscopy scanning electron (SEM) was aimed to determine the structure of nanocapsules. Figure 4 shows that the addition of arabic gum with maltodextrin was resulting on a crack of the nanocapsules. This crack can occure due to the used of high drying temperature. The cracks in microcapsules can causes a decrease in the rate of release of active constituent [19]. Figure 4 obtained a rounded nanocapsule shape, this is due to the occurrence of water evaporation during the spray dryer process. Rapid evaporation of solvents during the spray dryer process result in a round and wavy microcapsules. [20]

3.3. Total Volatile Base Nitrogen

Table 3. Fluctuation of TVBN values of snapper fillet during storage

| Encapsulans       | Storage time to (ours) | 0     | 16      | 32    | 48          |
|-------------------|------------------------|-------|---------|-------|-------------|
| Maltodextrin 2%   | 13.39±0.05             | 22.26±0.05 | 28.09±0.02 | 33.37±0.05 |
| Gum arab 2%       | 12.83±0.02             | 21.48±0.05 | 31.27±0.02 | 37.08±0.04 |
| Gum arab+maltodextrin 2% | 13.83±0.07 | 20.45±0.06 | 29.31±0.01 | 34.24±0.05 |

TVBN analyze was carried out because of a decrease in the quality of fish can look of the value of base-base nitrogen recommend. Base-base nitrogen was caused by the process degradation protein occurring in tissue fish, generally the process degradation protein was caused by perishable bacteria activities in the body of fish. Some activity perishable bacteria activatable a working enzyme and help speed up degradation protein so as to produce a setback the quality of on meat fish. Reaction degradation protein that produces base-base nitrogen the can look of the value of TVBN on meat white snapper without treatment have started to undergo decay in storage all 6 d with value 29.797 mg N·g–1 while in flesh of indigo the number of but the addition of microencapsulated smoke the water began subjected to the process decay in storage 9 d century with the concentration 1 % is 33.410 and on concentration 1.5 % is a 31.070. Bacterial activity on muscle tissues caused increasing of TVBN value, this because liquid smoke contains phenols able to inhibit microbial activities. High protein levels can undergo deamination from peptides and amino acid contained in the body of the fish. This process causes the formation of volatiles, diethylamine and trimethylamine [21].
3.4. Total Plate Count

Table 4. TPC value of white snapper fillets during storage

| Encapsulated            | Storage time (Log CFU/g) |
|-------------------------|--------------------------|
|                         | 0           | 16       | 32       | 48       |
| maltodextrin 2%         | 4.46       | 4.83     | 4.93     | 5.04     |
| gum Arabic 2%           | 4.77       | 4.91     | 4.92     | 5        |
| gum Arabic +malto 2%    | 5.17       | 5.27     | 5.41     | 5.56     |

Table 4 shows that after 48 hours of storage it was found that all sample are still suitable for consumption in terms of the TPC value, with the tendency that gum arabic encapsulation was the best treatment proven by the lowest TPC value. Based on the national standard the maximum limit of TPC value in fresh fish is $5 \times 10^5$ colonies/g (maximum log value is 5.7 CFU/g) [22]. Bacteria growth in white snapper fillets is inhibited. This showed that liquid smoke nanoencapsulated has antimicrobial activity. According to [23] antimicrobial activity of encapsulated liquid smoke is caused by the presence of phenol and acid compounds found in liquid smoke. [24] Phenols can react with cell membranes causing increased in cell membrane permeability, inactivation of essential enzymes and destruction or inactivation of genetic material. This is reinforced by the opinion of [25] which states that organic acids can inhibit the growth of bacteria in food so that organic acids were act as a function of preservative.

4. Conclusion

Different concentration of nanoencapsulation were found gave very significant effect (p<0.05) to the quality characteristics of white snapper fillets. These showed by the number of TVBN, pH and TPC value. The shape of mixture of arabic gum and maltodextrin nanocapsuled has texture that does not clump. Therefore, it can facilitate a better process of evaporation of water from the fish meat.

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