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Antimicrobial Activity of Microencapsulation Liquid Smoke on Tilapia [*Oreochromis niloticus* (Linnaeus, 1758)] Meat for Preservatives in Cold Storage (± 5 C°)

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Abstract

Tilapia [*Oreochromis niloticus* (Linnaeus, 1758)] is the fresh fish, it generally classified as perishable food.One way to avoid meat quality degradation are cold storage and the addition of bioactive compound in liquid smoke microcapsules. The purpose of this study was to find the effectiveness of adding liquid smoke microcapsulation ratio and to determine the ability of microencapsulated bioactive coconut shell compounds to maintain the tilapia meat quality during cold storage. The preliminary research, adding dextrin 3 % in smoke liquid coconut shell get total phenol 0,98% and pH content 4,83. The main research, it applies microencapsulated liquid smoke coconut shell on meat tilapia 0 % , 1 % , and 1.5 % during cold storage and phase 0 d, 3 d, 6 d, 9 d of observation. The test parameters were Total Volatile Base Nitogen (TVBN), Total Plate Count (TPC), pH and Sensory evaluation. The results of the first study were adding dextrin 3 % can assist in maintaining phenol content values on 0.98% and pH 4.8. In the second study, the parameters 9 d of observation showed TVB concentration 0 % (37.787 mg · N · g^{-1}), 1 % (33.410 mg. N· g^{-1}) and 1.5 % (31.070 mg. N· g^{-1}) , TPC parameter 0 % (7.717 log · CFU · g^{-1}), 1 % (6.390 log· CFU· g^{-1}), and 1.5 % (6.263 log · CFU · g^{-1}), pH concentration parameter 0 % (8.873), 1 % (8.383), and 1.5 % (8.140). Coconut shell smoke liquid microcapsulated is proven to reduce quality deterioration on fresh fish meat and can be developed as a preservative in food.

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Keywords: Antibacteria; dextrin; liquid smoke; microencapsulated; tilapia meat.

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1. Introduction

Tilapia [*Oreochromis niloticus* (Linnaeus, 1758)] is a commodity exports potential in Indonesia. Agency of Maritime and Fisheries Affairs East Java (2015) said that total national production tilapia in Indonesia is a 912 613 $t \cdot yr^{-1}$. Tilapia is a favorite fish among people because it has low economic value , a savory and having the chemical content of protein 43.76 % , fat 7.01 %, levels of ash 6.80 %, and water 4.28 % on 100 g meat (Leksono, 2001). One way to avoid quality deterioration of meat tilapia is saved in cold storage or gave a preservative meat fish.

The fish quality deterioration can be prevented by a natural preservative with bioactive which prevent the activity of perishable bacteria called psikrofil bacteria. A preservative natural has any bioactive which is coconut shell liquid smoke. Liquid smoke has any bioactive compound such as phenol, carbonyl and organic acid that serves as antibacterial which prevent the quality of fish (Saloko et al., 2014). The coconut shell liquid smoke bioactive apply to fresh produce which previously keep the bioactive compound with microencapsulation processing.

Microencapsulation is a process to change a liquid product to powder sized micro product. According to Supriyadi and Sakha (2013), microcapsules is a technique for protecting the core material (core) which was originally a liquid into a solid so it's easy handling and can protect the core material from losing flavor.. Deladino et al. (2008) said that encapsules is a process a thin layer, usually polymer applied to fluids particles. This method used to protect the active and control the condition of the compound missing.

2. Material and methods

2.1. Materials

Coconut Liquid Smoke was produced by condensation machine in the laboratory of processing Faculty of Fisheries and Marine Science and white dextrin was distributed at industrial store in Semarang city. Tilapia used was obtain from fish market Kobong, Semarang city. Spray dryer for microencapsulated process at Bioproses laboratory, Chemical Enginering Gadjah Mada University, Yogyakarta, Indonesia

2.2. Preparation of microencapsulation process

The microencpsulated making processing does some modification method from Saloko et al. (2014) microencapsules are started by mixing coconut shell liquid smoke with dextrin concentration 1 %, 2 %, and 3 % on 1 000 mL. To homogenized dextrin in liquid smoke, use the magnetic stirer at 200 rpm for 30 min (1 rpm = 1/60 Hz). Next to get solution supernatant pure centrifuge process at 3 000 rpm for 3 min (1 rpm = 1/60 Hz). To get solution pure using paper strain then use screening process. After getting supernatant are followed by heating supernatant with waterbath temperatures 50 °C for 15 min. The microencapsulation process with a spray dryer temperatures inlet 90 °C (± 4 °C), with the temperature outlet about 70 °C. Process of using spray dryer more or less took 45 $min \cdot L^{-1}$ to 50 $min \cdot L^{-1}$ sample. Then spray dryer would process micro powder size and stored in toples airtight and kept temperatures the freezer under 0 °C so as to maintain the quality of the microencapsulation.

2.3. Chemical analyses of microencapsules

Some tests carried out to see the effectiveness of the ratio encapsulan added to maintain bioactive content on coconut shell liquid smoke microencapsulated is by testing total phenol and pH. Testing total phenol done by using the method Ali et al. (2014) 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 Na_2Co_3 saturated and leave on 10 min at room temperature. Homogenized reagen folinciocalteau 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.

2.4. Application of microcapsules on tilapia preservation

Whole tilapia fish done disposal of the contents of the stomach, gills and head and the fish tilapia are already clean fillet process done with the disposal of thorn and the skin of the tilapia. Then fillet of tilapia get cutting process until like as a minced meat. Mince meat of tilapia given liquid smoke microencapsulated as antibacterial. Liquid smoke microencapsulated given treatment with a concentration 0 %, 1 %, and 1,5 % at 50 g minced meat of tilpia. Minced meat of tilpia that has been given treatment inserted into the plactic seal with labeling code and save by method cold storage with temperature 5 °C (± 4 °C). During the cold storage be tested total volatile base nitrogen (TVBN), total plate count (TPC), pH, and sensory evaluation on 0 d, 3 d, 6 d, and 9 d.

2.5. Total Volatile Base Nitrogen

TVBN analyze done by using method BSN (2006) where testing started by mixing 5 g sample with a solution of TCA 7 % by comparison 1:3 and homoginizing sample with a stirrer for 1 min and filter solution by the use of paper strain which resulted solution clear. Next do preparation an inner conway admit boric acid 1 mL and on the outer admit solution K_2CO_3 1 mL on the right side of outer and on the left side of admit sample clear 1 mL that not mixed. And do incubation for 2 h with the temperature 37 °C, then performing a titration in the inner by using 0.02 N HCl to have a little bit pink color. And performing calculations:

$$\text{TVBN (mg N \%)} = \frac{\text{Sample titration (mL)} - \text{blanko (mL)} \times \text{N HCl} \times 14.007}{\text{Sample weight (g)}} \times 100 \quad (1)$$

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 10 to 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 tub 1 000 and add to different tube for already BFP solution 9 mL, and votex 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 0.1 mL and city using rod bent. The incubation media at an incubator temperatures 37 °C for 24 h (BSN, 2015).

2.7. pH

Testing on the sample pH microencapsulated by using a method of testing pH BSN (2004) 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.

2.8. Sensory evaluation

Minced meat tiapia already given microencapsulation 0 %, 1 % and 1.5 % done testing sensory and testing fondness with a value of 1 to 9 with 30 the panel that will be the final result was obtained liked and dislike. Who conducted the process is inserting minced meat tilapia already given treatment into plastic seal already given code, next the panel can assess the panel in fondness and testing descriptive (BSN, 2006).

2.9. Stastical analysis

Statistical analysis for preliminary research is a copletely radomized design with 3 treatment is dextrin concentration applicated in liquid smoke. Concentration is 1 %, 2 %, and 3 % dextrin. Statistical analysis on main research using a completely randomized design with factorials with 4 × 3 design. Factor A during storage, Factor B is concentration of microcapsulated for best dextrin applied to mince meat tilapia, and interaction for treatment and during storage. The differences between the mean values of multiple groups wre analyzed by one way analysis of variance (ANOVA) with Tukey methods range test. ANOVA data with a $p < 0.05$ was classfied as statiscally significant. SPSS 16.0 software and microsoft Excel 2007 programs were used.

3. Result and discussions

3.1. Compound of liquid smoke microcapsules

This analysis conducted to determine the ratio of the influence of the addition of encapsulated on total phenol compound and pH in the final outcome coconut shell liquid smoke microencapsulated. Based on the content of the highest value total of phenol and the lowest value pH that can determine the ratio of the best concentration encapsulated (dextrin) on liquid smoke during the process. The results of the analysis can be seen in Table 1 .

Table 1. Compound of liquid smoke microencapsules

Concentration dextrin on liquid smoke (%)	Total Phenol (%)	pH values
1	0.42[a]	5.19[c]
2	0.69[b]	5.02[b]
3	0.98[c]	4.83[a]

Note: Means within colums followed by different superscripts are significally different at $p < 0.05$ by Tukey Methods range test

The addition of dextrin 3 % in liquid smoke having the values of phenol highest and having the values of lowest pH is 0.98 % and 4.83 % this is proven that the more concentration encapsulated dextrin which added will be more effective in protecting compound bioactive in liquid smoke microcapsulated. Things are dealing with the functioning main dextrin that is as substance carrier, as a coating. Function dextrin another is dextrin layer can form a thin film that would coat the compound content contained in the liquid smoke so can reduce oxidation reaction during the process of spray dryer that uses high temperatures on the material. According to Prasetyo and Vincentius (2005) dextrin serves as agent fastener foam or the shaper of a thin layer spur speed drying and preventing damage due to the heat by means of lining flavor components in material.

Phenol chosen in testing because the bioactive compound which needed as antibacterial in coconut shell liquid smoke microencapsulated is a total value of phenol and pH values considered able to represent the compound organic acids in liquid smoke microencapsulated. 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. Saloko et al. (2014) said various compound phenolic can help in destroying bacterial cells. Rahayu et al. (2012) added an idea phenol and derivatives can be bacteriostatic and bacteriacide because it can nonactivation of essential enzims, koagulation SH group and NH group protein. Mechanisms antimicroba phenol activity and derivatives covering reaction cell membranes causing increasing permeability cell membranes resulting in the intracellular material cells, essential enzims can inactivation and

vandalism or generic material can inactivation.

pH values chosen as representing organic acids content in liquid smoke microencapsulated. The organic acids in liquid smoke microencapsulated can serve as a compound antibacterial, antioxidants and can provide think that if applied both in the fresh and fisheries products containing proteins. Saloko et al. (2013) explained that organic acids such as acetic acid and formic acid as antioxidant agents together with phenolic compound. Acids contained in coconut liquid smoke influencethe food stuffs in their flavor, pH, and shelf life. Tranggono et al. (1999) and Khadir et al. (2012) reported found than acetic acid was dominant in coconut liquid smoke. Coconut shell liquid smoke consist mostly of acetic and propionic acids.

In Table 1. explained that an increase of phenol also affected by the value acid it is affected because acid compound of can affect the values of phenol. The acid can be increase phenol value. According to Darmadji et al. (2012) with the acid compound of will affect of the phenol content. Acid content can be increase phenol. Solubility phenol will increase due to improving deposits of the acid (approaching) acid. If the acid not high enough so it is possible that pheno l increase little so as to affect phenol levels.

Increase acid decrease ph value to the process microencapsulated also deals with the use of temperature inlet on spray dryer. The use of high temperatures will speed up the microcapsulated process while the use of temperature too low would cause temperature drying less stable so will result in oxidation reaction lasted more than using high temperatures. It is expressed by Ali et al. (2014) the efficiency of encapsulated at low temperatures at the minimum (± 140 °C), this would cause lost compound of phenol during the period drying unstable formed before particles. Saloko et al. (2013) adds to the temperature increase nanoencapsulation treatment of 40 °C to 50 °C or more will raise their pH values and it is dependent in concentration on the material encapsulated and liquid smoke added.

3.2. Total Volatile Base Nitrogen

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 tilapia 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. This is in accordance with opinion Pasaraeng et al. (2013) a protein height could caused the process degradation or the deamination protein, peptides and amino acids contained in the body of fish by activity bacteria that produce a compound base-base volatile including ammonia, dietilamin, and trimetilamin. Fluctuation of TVBN values during cold storage can be seen in Table 2.

Table 2. Fluctuation of TVBN values during cold storage

Storage day to-	Liquid smoke microencapsulated concentration		
	0 %	1 %	1.5 %
0	13.307^{b}	12.770^{b}	10.733^{a}
3	21.073^{d}	18.773^{c}	17.587^{c}
6	29.797^{g}	25.997^{f}	24.267^{e}
9	37.787^{i}	33.410^{h}	31.070^{g}

Note: Means within colums followed by different superscripts are significally different at $p < 0.05$ by Tukey Methods range test

On meat tilapia treatment amounted the addition of liquid smoke microcapsulated could help reduce the increase in TVBN values it because work phenol content that can help as bacterioside or bacteriostatic so can help in reducing the activity of perishable bacteria so can raise the age of keep fisheries products. This is in accordance with the opinions Pszczola (1995) some phenolic compounds in liquid smoke help lowered pH and destroy cells on bacteria. Saloko et al. (2013) added liquid smoked application in a proteinacious food products is not only act as a

coloring and flavoring agent but also possess antibacterial and antioxidative properties. Zuraida et al. (2011) mentioning coconut shell liquid smoke has been reported to contain bioactive compounds such as phenols, carbonyls, and organic acids. Therefore, the coconut shell liquid smoke is potential in increasing shelf life of proteinaceous food products.

According to Husni et al. (2014) the limits of safety consumption fisheries products fresh namely by TVBN value 30 mg · N per 100 g to 35 mg · N per 100 g. The condition of fish in the state of fresh and save in cold storage.

3.3. Total Plate Count

TPC values used as parameter because one cause a setback quality fish is because perishable bacteria activity. The activity of bacteria is sometimes can be stopped by using cold temperatures, however at a temperature of 0 °C to 5 °C psycothropic bacteria could still grow and meat tilapia is also proper media to the development of bacteria. According to Husni et al. (2014) the number of bacteria on meat fish in early storage not too different but the increases with long storage. Bacteria increasing the total content on meat fish indigo red because meat media fish is suitable for the growth of bacteria that cause bacteria can grown on fish meat. Susanto et al. (2011) added the use of low temperatures could hinder the development of bacteria but cannot stop the bacterial growth. This is because bacteria should be conforming to the area before breed. Bacteria growing on storage cold is that bacteria microbes psycrophil because it grows good at temperature –7 °C to 10 °C. A change in TPC values on meat tilapia for storage is presented in Table 3.

Table 3. Fluctuation of TPC values during cold storage

Storage day to-	Liquid smoke microencapsulated concentration		
	0 %	1 %	1.5 %
0	2.867^{b}	2.700^{b}	2.307^{a}
3	5.013^{e}	3.887^{d}	3.350^{c}
6	6.250^{g}	5.257^{ef}	5.077^{ef}
9	7.717^{h}	6.390^{g}	6.263^{g}

Note: Means within colums followed by different superscripts are significally different at $p < 0.05$ by Tukey Methods range test

The results of TPC analyze during storage experienced a rise in TPC value (log · CFU · g^{-1}), on the 6 d to 9 d storage price hikes of perishable bacteria because it was because the bacteria already beginning to adjust media to grow .Value TPC on meat nila fish without treatment on the day-6 until day-9 century range 6.250 log · CFU · g^{-1} to 7.717 log · CFU · g^{-1}, while in flesh indigo but the addition of liquid smoke microencapsulated 1 % on the 6 d until the 9 d century namely 5.257 log · CFU · g^{-1} to 6.390 log · CFU · g^{-1}, and on meat nila fish treatment the addition of liquid smoke microencapsulated 1.5 %, 6 d until the 9 d century namely 5.077 log · CFU · g^{-1} to 6.263 log · CFU · g^{-1}. The difference between a rise in TPC values flesh of indigo the number of without treatment by treatment because the total phenol in liquid smoke microencapsulated can help in pursuing bacteria activities. Including antibacterial phenol compound that serves can penetrate the cell membrane bacteria and destroying bacteria, nevertheless this is dependent on the phenol contained in a bio preservative. According to Wisley and Wheeler (1993) compound of phenol used as bacteriostatic or bactericide depends (concentration) levels. When used in concentration high, phenol work with destructive membrane cytoplasm overall to settle cell proteins, however if in concentration 0.1 % to 2 % phenol can damage membrane its cytoplasm cause to leak these important and non active a number of cells bacteria. ICMSF (1986) said microbiological threshold of ready to eat fish products is 6.00 log · CFU · g^{-1}.

3.4. pH

The results of the pH analysis is presented in Table 3. pH values keeps increasing for storage. The increase in pH value of acid approaching bases because pH values influenced by degradation proteins by a number of bacteria. Degradation protein this will provide a number of base-base nitrogen that will affect the rise in value pH

approaching a base and would be near reaction decay. According to Husni et al. (2015) reduction in the pH value at the beginning of storage this induced in the establishment of the lactic acid the results of reaction solving glycogen by an enzyme that was found in flesh of . Husni et al. (2014) added an increase of pH because the establishment of the compound is alkaline for example ammonia the results of the process reshuffle a protein on fish by an enzyme and bacteria. An increase in pH on meat fish indigo red for storage shows the existence of the activity of an enzyme proteolytic there are on a network flesh of that produces ammonia.

Table 4. Fluctuation of pH values during cold storage

Storage day to-	Liquid smoke microcapsulated concentration		
	0 %	1 %	1.5 %
0	6.440^{ab}	6.273^{a}	6.093^{a}
3	7.313^{d}	7.107^{c}	7.040^{c}
6	8.087^{f}	7.733^{e}	7.333^{d}
9	8.873^{h}	8.383^{g}	8.140^{f}

Note: Means within colums followed by different superscripts are significally different at $p < 0.05$ by Tukey Methods range test

The result of the pH values keeps increasing. Meat of tilapia without treatment is starting to increase to decay in storage start from 3 d to the 6 d century with pH values that is pH 8.087 to pH 8.873 while in treatment 1% start approaching bases on the 6 d until to 9 d with pH values that is pH 7.733 to pH 8.383 and in treatment 1.5 % start approaching bases on the 6 d until the 9 d century with pH values that is pH 7. 333 to pH 8.140. According to Yunizal and Wibowo (1998) after the rigor mortis over and decay bacteria held so pH flesh of strengthened approaching neutral up to pH 7.5 to pH 8.0 or more high if decay have very severe. Severity decay caused by levels of those compounds that is alkaline. In this condition pH will surged to a slowly and with ever more many compounds bases purine and pyrimidine formed will be more speed up the increase in pH fish.

3.5. Sensory evaluation

Table 5. Sensory evaluation of minced meat tilapia during cold storage

During Storage (d)	Concentration of liquid smoke microencapsulated (%)	Spesification		
		Appearance*	Texture*	Odor*
0	0	8.57 ± 0.50	8.60 ± 0.49	8.53 ± 0.50
	1	8.83 ± 0.38	8.72 ± 0.45	8.37 ± 0.49
	1.5	8.63 ± 0.49	8.67 ± 0.41	8.80 ± 0.48
3	0	6.90 ± 0.40	6.67 ± 0.66	6.58 ± 0.27
	1	7.37 ± 0.54	7.60 ± 0.49	7.20 ± 0.48
	1.5	7.73 ± 0.64	7.93 ± 0.74	7.87 ± 0.79
6	0	5.93 ± 0.48	5.97 ± 0.58	5.90 ± 0.63
	1	7.27 ± 0.52	7.30 ± 0.64	7.00 ± 0.67
	1.5	7.87 ± 0.78	8.07 ± 0.52	7.87 ± 0.52
9	0	5.17 ± 0.70	5.23 ± 0.71	5.10 ± 0.76
	1	6.90 ± 0.48	7.20 ± 0.65	6.70 ± 0.75
	1.5	7.53 ± 0.56	7.87 ± 0.51	7.67 ± 0.66

*Note: means (± standars deviation) derived from 3 replication with 3 sample per replication

Sensory evaluation on meat tilapia the number of in terms of the visibility, smell, and texture to have a fondness. It was because the visibility of flesh of indigo but the addition of liquid smoke microencapsulated become colored

more brownish and still visible a vein the nerves on the flesh of indigo minced meat compared to the white ones. In texture on the day-0 of a sample of without treatment and by the addition of microencapsulated 1 % and 1.5 % occurring almost similarity between them still compact. Level fondness types of buyers in the smell of tilapia meat the addition of 1.5 % is highly like until the last day of storage. It was because smell ammonia caused by the results degradation protein a little apparents with an odor liquid smoke microencapsulated. This is also expressed by Saloko et al. (2014) stated that the treatment of fish with liquid smoke eyedrops to change not only in its physicochemical but also in sensorial attributes.

The results of the visibility of flesh of indigo by concentration of the 1 % and 1.5 % is like consumers until the 9 d. Susanto et al. (2011) expressing rate of reduction in the value of organoleptic due to the difference the number and activity compound antibacterial in each natural materials so as to cause effects differing at a reduced rate organoleptic value. The texture flesh of indigo by concentration of the 1 % and 1.5 % is like consumers until the 9 d it was because the texture flesh of indigo still compact and a slightly elastic. According to Indonesian Standarization Agency (2006) fillet of tilapia in storage all 4 d is fresh. While on the 6 d century having the texture and somewhat solid, compact and elastic. While in the 10 d in cold temperature have started to pulpy, less compact and less elastic.

4. Conclusion

From this research it can be concluded that additional coconut shell liquid smoke microencapsulated can be as antibacterial impact on the quality of a deterioration tilapia meat during cold storage with the parameters TVBN analyze, TPC analyze, pH anayze and sensory evaluation. Additional coconut shell liquid smoke microencapsulated has any bioactive compound phenol and organic acids can help in impeding the activity of bacteria and help in pursuing a deterioration quality of tilapia.

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