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## The Usage of Chitosan from Shrimp Waste as Natural Preservative for Fish *Cilok* (Traditional Food in Indonesia)

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### ABSTRACT

*Cilok* is one of the traditional food in Indonesia. *Cilok* is a popular treat among Indonesians due to its good taste and cheap price. To make it last longer, *cilok* is sometimes added with preservative. However, such attempt will endanger consumers if dangerous preservatives are used, such as formaldehyde and borax. Their usage in food preservation may negatively impact consumers' health. The aim of this research was to develop an alternative, natural preservative for fish *cilok*. The preservative would be chitosan. The method used is an experiment that is by making a chitosan solution from the shrimp skin waste. In the experiment, chitosan was extracted from shrimp waste. A 1,5% chitosan concentrate was used. Its preservation potency was tested by giving three treatments towards fish *cilok*: fish *cilok* with 1,5% chitosan coating; fish *cilok* mixed with 1,5% chitosan solution and fish *cilok* without chitosan. The three treatments were observed and an organoleptic test was performed according to the preservation time in a room temperature on Day 1, 2, 3, 4 and 5. The parameters tested during the experiment were taste, color, odor and texture of fish *cilok*. The research results showed that fish *cilok* coated with 1,5% chitosan solution lasted longer than fish *cilok* without or added with chitosan.

**Keywords:** *chitosan, natural preservative, fish cilok*

### INTRODUCTION

*Cilok* is one of popular treats in Indonesian community. It is children's favourite; one can find them easily in sideroads or schools. According to Yanuarlan (2015), the term *cilok* is an acronym of Sundanese words *aci dicolok* (skewered *aci*), a meatball shaped food made from tapioca starch (*aci*) and served using wood stick/skewer.

*Cilok* is not only made from tapioca starch, but also mixed with beef, chicken or fish meat. It is a favourite treat for both adults and children, since it is relatively cheap and easy to find. Fish *cilok* tastes best, but decays quickly. In order to make it last longer, some fish *cilok* makers or vendors use synthetic preservatives such as

borax or formaldehyde which are dangerous to human health.

*Cilok* with borax as its preservative may endanger children's health. According to Safira (2014), one of children's favourite snacks is *cilok*. Children love its because it has delightful shape and chewy texture. One needs to be careful when buying *cilok* in roadside vendors because some of them might add dangerous food additive to *cilok* they sell.

Side effects resulted from consuming food which contains borax are irritations in digestion canals, shell and eyes, stomachache, headache, severe pain in upper part of stomach. Borax consumption for a long period may result in liver disease, body circulation system failure and death. Consuming 5-10 gram borax may result in shock and death on children.

Several misuses of borax are applied on foods such as meatballs, *cilok*, *lontong* (rice cake) and *gendar* cracker (Deviana, 2015).

Food preservatives should be made from natural substances which do not produce negative side effects on health. Therefore, new innovations in producing natural food preservatives should be attempted. One of them is the usage of chitosan. Chitosan is made from shrimp waste, especially shell and head parts because they contain chitin.

Chitosan is widely used in industries as a preservative alternative to formaldehyde and borax. Preservatives are additional substances added in food to reduce activities of microorganism, slowing down the decay process. Chitosan contains positively charged polycations which are able to reduce the growth of bacteria and molds (Harjanti, 2014).

Chitosan can be derived from chitin contained in shrimp shell. Shrimp shell contains beneficial nutrients such as proteins, calcium and chitin. Proteins and calcium can be used as additives in animal feed. Shrimp waste used in this research were head and shell parts which cover 35%-50% total weight of shrimp.

According to Marganov (2003), shrimp shell contains proteins (25%-40%), calcium carbonate (45%-50%) and chitin (15%-30%). Taolee (in Agustina, 2015) explains that chitosan is a derivation from chitin with structure of  $[\beta\text{-}(1\text{-}4)\text{-}2\text{-}\text{amine} - 2\text{-dioxide-Dglucose}]$ , a result of deacetylated chitin.

Chitosan is a polycationic polymer. Its hydroxyl and amine groups in the polymer chain allow chitosan to effectively bind heavy metal cations as well as cations from proteins and fats. Chitosan can also form an absorbing membrane during the binding of organic and anorganic compounds. These make chitosan more useful than chitin (Sanjaya et. al., 2007). As chitosan possesses a high economic value, therefore it is important to conduct a research for producing chitosan from

shrimp shells, reducing the environmental contamination loads (Agustina et. al., 2015).

Chitosan is an organic compound derived from chitin. It is polyelectrolyte, non-poisonous, and biodegradable. Its hydroxyl and amine groups make it suitable as preservative and color stabilizer. Chitosan can be used as a preservative since it can slow down the growth of destructor microorganisms. Chitosan can be coated on food, minimizing the interaction between the product and its surrounding environment (Soegiarto et. al., 2013).

According to a study by Killay (2013), chitosan could be used as an anti-bacteria additive in comestibles. Such finding is supported by Hafdani (2011) who argues that chitosan is antimicrobial as it could delay the growth of pathogenic bacteria and decomposing microorganisms, including fungi.

Chitosan could also be used as a preservative for fish. Fish decays rather quickly. The decay process is caused by activities of enzymes, microorganisms and oxidation within fish body. A spoiled fish is indicated by unpleasantly sharp smell, inelastic flesh, cloudy eyes and sticky slime in gills and body (Arifin & Nugroho, 2016).

A good way for preserving fish is to soak it in water mixed with 1.5% chitosan. Another is to spray fish with 2.5% chitosan. Such attempts may prolong fish preservation for another 5 hours (Silvia et. al., 2014).

Chitosan can also be used to preserve fruits. A study by Trisnawati et.al. (2013) shows that chitosan can be used to preserve lanzones through coating. Coating can be done by dissolving chitosan powder in acetic acid solution. Next, soak fruits into the mix. Coating may reduce the loss of water, oxygen, flavour and dissolved ingredients in several products, making it effective in preserving food quality.

Helander (2001) states that chitosan compound disrupts outer membranes of gram-negative bacteria. Chitosan compound, as an antimicrobial agent, can be added to comestibles since it is safe for human. Chitosan cannot be digested by human therefore it does not have calorie values and is discharged with feces.

A factor influential on the capability of chitosan as a preservative is the number of amine groups it contains. The number of amine group depends on the removed acetyl group. The removed acetyl group is called the degree of deacetylation. Therefore, the higher the degree of deacetylation is, the better the capability of chitosan as a preservative will be. The application of chitosan as a preservative is gained in the best condition of the degree of deacetylation (70.34%). The best soaking time is 45 minutes in 2% chitosan (Harjanti, 2014).

Chitosan has several benefits such as possessing a structure familiar to cellulose viber contained in fruits and vegetables. Various hypotheses regarding the working mechanism of chitosan as an anti-bacterial agent address its affinity which is strong with microbes' DNA, allowing it to bond wih DNA and then disrupt mRNA and protein synthesis (Killay, 2013). Therefore, a further research on the usage of chitosa as a natural preservative is needed. The aim of this research was to describe the creation process of chitosan and its effectiveness as a natural preservative for fish cilok.

## RESEARCH METHOD

This research is experimental, conducted in a laboratory for producing chitosan from shrimp waste and creating fish *cilok*. Fish *cilok* is added with chitosan in order to discover effectiveness of chitosan as a natural preservative for fish *cilok*.

This research was conducted in a laboratory of FMIPA IKIP PGRI Jember for producing chitosan and test its

effectiveness as a preservative. It was conducted during May – November 2018. This research needs to be done because to determine the effectiveness of chitosan as a natural preservative of cilok fish. The tools and materials used are presented in the following table.

**Table 1.** The Tools and Materials for Making Chitosan and Fish *Cilok*

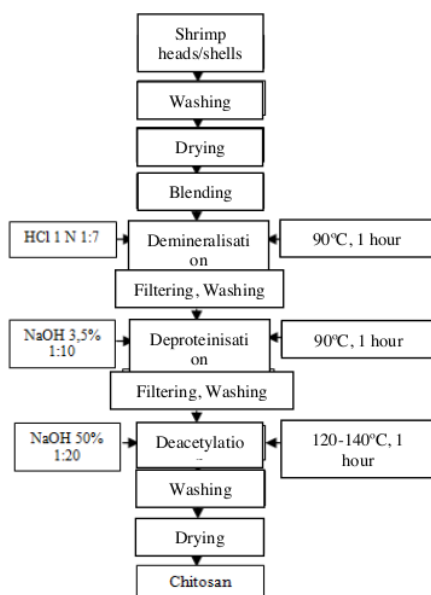
Name	Tools	Materials
Chitosan	Bunsen burner, tripod, clamps, beaker glass, measuring cup, volumetric flask, stirrer, thermometer, and litmus paper.	Fish heads and shells, acetate acid, HCl 1N, NaOH 3.5%, aquadest, NaOH 50%, and methanol.
Fish <i>Cilok</i>	Knife, cutting board, basin, blender, spoon, mortar, pestle, pan dan stove	Fish fillets, tapioca flour, starch, wheat, sugar, salt, pepper, onion dan water.

## A Procedure for Producing Chitosan

Chitosan is produced from a chitin isolation process. Chitin can be gained from a chitin isolation process from shrimp shells, consisting of two steps, demineralisation and deproteinisation. Demineralisation is conducted to eliminate organic compounds contained in the shrimp waste. Shrimp shell generally contains 30-50% minerals (dry weight). Dominant minerals found in shrimp are calcium in form of  $\text{CaCO}_3$  and a small amount of  $\text{Ca}_3(\text{PO}_4)_3$ . A scheme of chitosan extraction is presented in Figure 1.

Demineralisation can be conducted by adding HCl 1 N with the 1:7 ratio of the weight of materials and the volume of extractor for one hour in 90°C temperature. Deproteinisation aims to eliminate proteins from shrimp waste. Its effectiveness depends on the power of the alkaline solution and the degree of temperature used. A 3.5% NaOH solution heated in 90°C temperature for one hour can be used as an alternative to deproteinisation with a

1:10 ratio between dry shrimp waste and solution (Suptijah et. al.,1992).



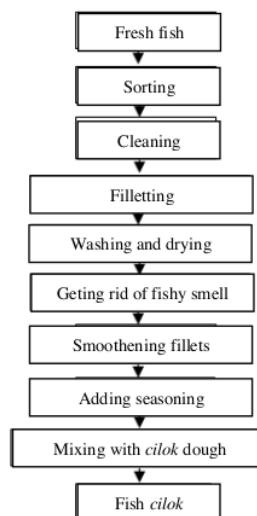
**Figure 1.** A Procedure for Producing Chitosan (Source: Suptijah, et. al., 1992)

Chitosan is produced by eliminating acetyl group ( $-\text{COCH}_3$ ) from chitin by using a NaOH concentrated solution (50%) with a 1:20 ratio for one hour in 120-140°C temperature (Suptijah et. al., 1992). The more acetyl groups are eliminated from the chitin polymer, the stronger the interaction among ions and hydrogen bonds from chitosan will be (Suptijah et. al., 1992).

#### A Procedure for Making Fish *Cilok*

According to Suprapti (2003), a general overview of making fish *cilok* is presented in Figure 2.

Fish fillets, after 5 minutes untouched, are blended until smooth and put them into a bowl. Mix them with ingredients, whipped egg whites and tapioca flour until a homogeneous, shapeable dough is formed. Make small balls (*cilok*) from the dough. Then, boil *cilok* until they are floating. After that, drain them.



**Figure 2.** A procedure for Making Fish *Cilok* (Source: Suprapti, 2003)

#### A Procedure for Testing Chitosan as a Food Preservative

In this research, a test on chitosan as the fish *cilok* preservative was conducted by giving three treatments as presented in Table 2.

**Table 2.** A Procedure of Chitosan Test

Treatment	Notes	Observation
1	Fish <i>cilok</i> were boiled, dried and dipped into 1,5% chitosan solution for two minutes. After that, they were dried and preserved in a room temperature.	Day: 1, 2, 3, 4, 5
2	Before processed, fish <i>cilok</i> were mixed with a 15 cc of 1,5% chitosan solution. After that, they were boiled, dried and preserved in a room temperature.	Day: 1, 2, 3, 4, 5
3	Fish <i>cilok</i> were boiled, dried and preserved in a room temperature without chitosan.	Day: 1, 2, 3, 4, 5



### Data Collection and Analysis Techniques

Research data were collected via documentation and organoleptic tests (Harjanti, 2014). Documentation was done by taking photos of fish *cilok* during the experiment. The organoleptic tests which were performed consisted of specifications on the whole appearance such as taste, colour, odor and texture. The tests were conducted towards the three treatments: 1) the fish *cilok* with 15% chitosan coating; the fish *cilok* mixed with 1,5% chitosan and 3) the fish *cilok* without chitosan. Observations on each treatment had been done for 5 days consecutively.

## RESULTS AND DISCUSSION

### Chitosan

In this research, shrimp heads and shells were used to produce chitosan. Dried shrimp heads and shells were blended, resulting in shrimp shell powder. The amount of the shrimp heads and shells was 300 gram while the amount of HCl 1N used in the demineralisation was 2,1 liters.

The demineralisation resulted in pink shrimp head and shell powder. The powder then was used in the next phase, deproteinisation. The deproteination phase is carried out by reacting the result of demineralization with strong base (NaOH), so that the protein will dissolve in NaOH solution. This deproteination reaction aims to break the bonds between protein and chitin by adding sodium hydroxide. In this process, the powder was mixed with 3 litres of 3,5% NaOH.

The deproteinisation resulted in white shrimp head and shell powder. The powder was then used in the next phase, deacetylation. According to Dompeipen, et.al (2016), Isolation of chitosan compounds was obtained by performing a deacetylation reaction on chitin. Deacetylation is the process of converting an acetyl group (-NHCOCH<sub>3</sub>) to chitin

into an amine group (-NH<sub>2</sub>) with the addition of strong bases such as NaOH.

The deacetylation reaction of chitin is basically an amide hydrolysis reaction of  $\alpha$ - (1-4) -2-acetamide-2-deoxy-D-glucose. The OH-ion concentration is very influential on the process of releasing the acetyl group from the chitin acetamide group. In this process, the powder was mixed with 6 litres of 50% concentrated NaOH. During the chitosan creation process, the amount of chitin produced was 50 gram while the amount of chitosan produced was 30 gram. Therefore, the rendement in the chitosan creation was 10%.

### Fish *Cilok*

The type of fish used for making *cilok* was Treadfin Bream (*Nemipterus nematophorus*). In this research, the dough was mixed with 1 kg fish fillets. Next, the dough was divided into three smaller portions. *Cilok* which is made of three parts has the same color, smell, texture and taste, namely white, chewy texture, and fishy taste.

Small balls (*cilok*) from the three smaller portions with 3 cm diameter were shaped. The first portion was used to create *cilok* with 1,5% chitosan coating. The second portion was used to create *cilok* mixed with 1,5% chitosan and the last portion was used to create *cilok* without chitosan.

*Cilok* fish in the first treatment, after cooking then draining and waiting until the temperature is not too hot. Furthermore, fish *cilok* is dipped in 1.5% chitosan solution for 2 minutes. The fish is then drained and stored at room temperature. In this first treatment, chitosan protects *cilok* fish from the outer surface of the *cilok*, so it can protect the *cilok* from oxidation and protect it from bacterial contamination.

*Cilok* fish in the second treatment, before *cilok* is cooked, *cilok* is mixed with a 1.5% chitosan solution of 55 cc. *Cilok* then cooked. After cooking, *cilok* is drained and stored at room temperature. In

this second treatment, the possibility of chitosan can also protect cilok from oxidation and bacterial contamination.

Cilok fish at the third treatment, cooked cilok then drained and stored at room temperature. Cilok fish is not given a chitosan coating or mixture. Based on the three types of treatment, later it can be observed which cilok is more durable in storage, so that it can be seen which chitosan treatment is more suitable to be used as cilok natural preservative.

### Test Result Data

#### 1. Cilok with 1,5% chitosan coating

Data from the observation towards fish cilok with 1,5% chitosan coating is presented in Table 3 and Figure 3 below.

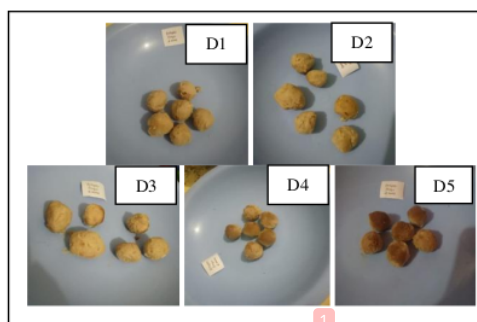
**Table 3.** The Result Data of the Observation on Fish Cilok with 1,5% Chitosan Coating

Day	Parameters	Observation results
(1)	(2)	(3)
D1	Taste	Fish flavoured
	Colour	White
	Odor/aroma	Fish flavoured
	Texture	Chewy
D2	Taste	Fish flavoured
	Colour	White
	Odor/aroma	Fish flavoured
	Texture	Chewy
D3	Taste	Fish flavoured
	Colour	White
	Odor/aroma	Fish flavoured
	Texture	Chewy
D4	Taste	Fish flavoured
	Colour	Brownish
	Odor/aroma	Fish flavoured
	Texture	Chewy
D5	Taste	Fish flavoured
	Colour	Brownish
	Odor/aroma	Fish flavoured
	Texture	Inelastic

Based on the observation on Day 1, there were no significant change in taste, colour, odor and texture since fungi and bacteria had not infected the fish cilok. The same situation were observed on Day 2 and 3 since the fish cilok with 1,5% chitosan coating in cold temperature for two minutes were able to delay oxidation

and minimize any contaminant caused by bacteria and fungi.

On Day 4 there was a significant change. The colour of the fish cilok changed from white to brownish. On Day 5, there were changes in the texture of the fish cilok, from chewy to inelastic. Such changes happened because the fish cilok started being oxidated.



**Figure 3.** The Condition of Cilok with 1,5% Chitosan Coating.

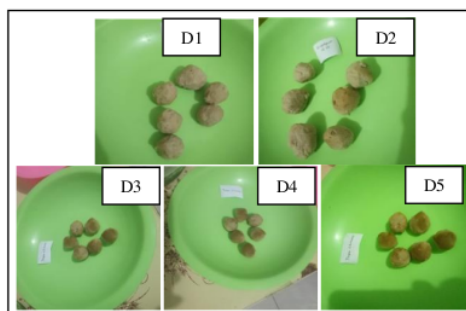
#### 2. The cilok mixed with 1,5% chitosan solution

The result data of the observation on the fish cilok mixed with a 15 cc of 1,5% chitosan solution is presented in Table 4 and Figure 4.

Based on the results of the observations on Day 1 and Day 2, there were no significant changes in taste, colour, odor and texture. On Day 3, change occurred in the texture, from chewy to inelastic. It was caused by the fact that the fish cilok started getting oxidated. On Day 4, the colour changed from white to brown, the odor went bad and the texture became inelastic. On Day 5, the cilok tasted sour and slightly bitter, had brownish colour, smelt bad and became inelastic. These changes were caused by the fact that the fish cilok had been oxidated and bacteria and fungi started growing in them.

**Table 4.** The Result Data of The Observation on Fish *Cilok* mixed with 1,5% chitosan solution

Day	Parameters	Observation results
(1)	(2)	(3)
D1	Taste	Fish flavoured
	Colour	White
	Odor/aroma	Fish flavoured
	Texture	Chewy
D2	Taste	Fish flavoured
	Colour	White
	Odor/aroma	Fish flavoured
	Texture	Chewy
D3	Taste	Fish flavoured
	Colour	White
	Odor/aroma	Fish flavoured
	Texture	Inelastic
D4	Taste	Fish flavoured
	Colour	Brownish
	Odor/aroma	Started rotting
	Texture	Inelastic
D5	Taste	Sour to slightly bitter
	Colour	Brown
	Odor/aroma	Rotten
	Texture	Very inelastic



**Figure 4.** The Condition of *Cilok* Mixed with a 15 cc of 1,5% chitosan solution.

### 3. The fish *cilok* without chitosan

The result data of the observation on the fish *cilok* without chitosan is presented in Table 5 and Figure 5.

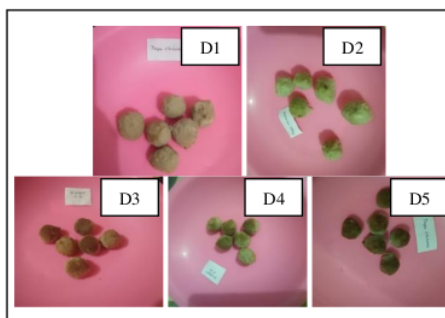
Based on the observation result on Day 1, the *cilok* had normal fishy taste, white colour, fishy odor and chewy texture. On Day 2, changes happened. The colour changed from white to brownish and the texture changed from chewy to slightly mushy. On Day 3, changes occurred in the taste. They tasted sour and slightly bitter, the odour smell bad, and the texture stayed

mushy. These changes were results of the oxidation and the growth of bacteria and fungi in the *cilok*.

On Day 4, the odor got worse, the taste became sour and slightly bitter, the colour turned to brownish and the texture became slimy and mushy. These were caused by the increasing contaminations by fungi and bacteria. On Day 5, the *cilok* tasted bitter, had blackish and brownish colour, smelt bad, and had mushy texture due to the growth of fungi and bacteria. This shows that the *cilok* without chitosan spoiled fast.

**Table 5.** The Result Data of The Observation on the Fish *Cilok* without Chitosan

Day	Parameters	Observation results
(1)	(2)	(3)
D1	Taste	Fish flavoured
	Colour	White
	Odor/aroma	Fish flavoured
	Texture	Chewy
D2	Taste	Fish flavoured
	Colour	Brownish
	Odor/aroma	Fish flavoured
	Texture	Mushy
D3	Taste	Sour to slightly bitter
	Colour	Brownish
	Odor/aroma	Started rotting
	Texture	Mushy
D4	Taste	Sour to slightly bitter
	Colour	Brownish
	Odor/aroma	Rotten
	Texture	Mushy
D5	Taste	Bitter
	Colour	Blackish brown
	Odor/aroma	Rotten
	Texture	Mushy



**Figure 5.** The Condition of *Cilok* without Chitosan



## CONCLUSION

The conclusions of this research are as follows: (1) Chitosan can be produced by processing shrimp shells and heads through demineralisation, deproteinization and deacetylation. (2) Fish *cilok* can be made from Treadfin Bream (*Nemipterus Nematophorus*). (3) Chitosan is a natural preservative for fish *cilok*. It is proven by the results of the experiment and observations that the fish *cilok* with 1,5% chitosan coating were able to last longer up to fourth day preservation (D+3) in a room temperature.

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