

Improving Quality of Halal Milkfish Gelatin by Cross-Link with Sucrose and Glutaradehid

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Abstract. One of the newest sources of halal gelatin is from fish scales, but it has low quality for pharmaceutical dosage form. The purpose of this study is to improve the quality of milkfish gelatin by cross-link and halal analysis. Quality parameters are viscosity and swelling index. Gelatin samples were extracted by alkaline and acid method. The samples were divided into two large groups cross-link: gelatin and chitosan-gelatin. The sample was crosslinked with glutaraldehyde and oxidized sucrose. All samples were tested for identity using IR spectrophotometer, Oswald method for viscosity, and swelling index. Oxidized sucrose is made by reacting sucrose with sodium periodate. The halal study uses the treasurer method where the materials that used and the manufacturing process are identified the possibility the existence of haram ingredients. The results showed creating cross link gelatin was successful as shown in the IR spectrum. The first group gelatin and the second group chitosan-gelatin had improved viscosity and swelling index quality. The second group has a better swelling index. Samples that cross-linked with glutaraldehyde had better viscosity and swelling index compared to samples with cross-linked oxidized sucrose. This shows that cross links have good polymeric properties. For the study of halal crosslink gelatin, samples materials have no critical point or possibility contaminated by haram material. Similarly, the manufacturing process also does not find a critical point that requires supervision. Based on this study, cross-linked gelatin has better quality than gelatin. The gelatin materials is also halal.

Keywords: Cross-link, Gelatin, Halal, Fish

1 Introduction

All products in Indonesia must be halal certified. Government through Law No. 33 at 2014 about Halal Products Assurances, states that all products that enter, circulate, and trade within the territory of Indonesia must be halal-certified. The implementation of this law must be in 2019, five years after it was decided. In addition to being a demand of the law, the increasing understanding of Islam, especially in the use of halal products, has made several industries that initially refused

to have no choice except to accept the demands of Muslims as the biggest consumers in Indonesia. The emergence of controversy over the use of non-halal vaccines and the recent cases of pork contaminated noodles, shows an increasing awareness of the Muslim community about the use of halal products. In fact, several industries from other countries such as South Korea and Japan have developed their products to be halal certified. The aim of the developed halal product is being accepted as export commodities in Muslim countries.

The challenge of developing halal products of gelatin raw material is the source of material and quality improvement. Gelatin is one material that very popular in large products of food and pharmaceutical. Gelatin is the most widely used in candy products (soft candy) and capsule shells worldwide [1]. Gelatin is a mixture of polypeptides sourced from partial hydrolysis of collagen [2]. In the gelatin production, the main raw material that used is collagen which can be found in cattle and pigs [1]. The form of commercial gelatin is sheets, granules, or powder, mainly sourced from bones and skin of cattle, skin of pig, and, recently using pig bones [3, 4]. Other sources such as fish and poultry are very new and mostly processed to meet certain religious consumer groups [5, 6]. However, due to the development of science and technology, food products rapid diversification, food products can use non-halal components to reduce production costs. In the market, pork gelatin is cheaper than cattle gelatin or other gelatin produced from halal sources [7].

Gelatin is a mixture of polypeptides that are made through collagen hydrolysis. Gelatin can be extracted from the skin, bones and skin of mammals such as pigs and cattle [6]. In addition, gelatin can also be made from fish [8, 9, 10]. Commercial gelatin is obtained from cows and pigs, where around 90% of the gelatin comes from pigs [11]. Gelatin has gelling properties such as gel strength and gelling time, melting temperature and viscosity which are suitable for use in food products such as soft candy. In addition, the surface behavior of gelatin (for example, the formation and stabilization of foams and emulsions, the nature of adhesion and destruction of dosage forms) plays an important role in food and pharmaceutical products [1, 12]. One deficiency of fish gelatin is the low of gel strength. In the pharmaceutical field, the high gel strength required for the manufacture of hard capsule shells, above 200 brooms. Fish and chicken gelatin, as well as some gelatin from cattle, do not meet this requirement [4].

An alternative source for gelatin from waste products such as gelatin from fish scales will be a profitable alternative that can be considered. Indonesia is the second-largest country for fisheries production in 2016, with total production is over six million tons in 2014, making the potential is also large [13]. The highest production of fish is snapper, yellowfin tuna and skipjack for sea fish and milkfish and carp for cultured fish [13]. Gelatin from fish can be accepted by the Islamic community, and is also accepted with very little risk of being [14].

One deficiency of fish gelatin is the low rheological properties, gel strength and viscosity [15, 6]. In the pharmaceutical field, the gel strength properties for the manufacture of hard capsule shells is above 200 brooms [11, 1]. Fish gelatin does not meet this requirement. One method to increase gel strength is to make cross-link gelatin [14, 15]. Gelatin can be cross-linked using certain ingredients, such as oxidized sucrose and glutaraldehyde, or combine with chitosan [16, 17, 18, 19]. Those substances will make gelatin bind to each other tightly through complex manufacturing. For this reason, we decide to make cross-link gelatin to improve the gelatin properties of fish, like to improve its viscosity and swelling index properties. Source of gelatin raw material is from industrial waste that can still be recycled.

2 Method

Materials

The sample that used for this study was milkfish scales. The standard gelatin used is commercial gelatin (Brataco). Chitosan was purchased from Kawasan Industri Makassar (KIMA). Other ingredients such as NaOH, HCl, Glutaraldehyde, Sucrose and Sodium Periodate are purchased from Merck.

Gelatin Extraction

Procedures for extracting gelatin from fish scales through several stages, namely [20]:

a. Sorting

Fresh fish scales are cleaned by removing waste, meat, or fat. The sample is heated by hot water (85-90°C) for 5 minutes to help washing fat from the sample and make it easier to remove scales from the skin [1]. Then, clean fish scales are dried.

b. Base and acid pre-treatment

Sample 50,0 g was soaked with base solution (NaOH 1,0 N) 350 ml for 45 minutes at room temperature. After alkaline soaking, the sample is filtered and washed with distilled water 3 replicates. Then, the sample was soaked with acid solution (HCl 1,0 N) 350 ml for 45 minutes at room temperature. After alkaline soaking, the sample is filtered and washed with distilled water 3 replicates. Pre-extraction with alkaline and acid aims to dissolve fat, proteins or other contaminants that are soluble in alkaline and acidic. Gelatin itself is difficult to dissolve in alkaline and acidic, but easily dissolves in neutral pH. In addition, base and acid also help soften the sample, making it easier to extract the gelatin [1].

c. Extraction

The soft sample was put into the extraction container and added distilled water 350 ml. The mixture is then neutralized with acid or base to neutralize pH. Then, the mixture is heated at 90°C for 8 hours [11].

d. Drying

The concentrated liquid gelatin obtained from extraction was dried using heating in oven at 80°C, generally for 72 hours. Then, dry gelatin is powdered. The effectiveness of extraction is determined by yield parameter of dry scales.

Manufacturing cross-link gelatin

a. Manufacturing oxidized sucrose

Sucrose 5.13 gram and Sodium Periodate 6.39 gram, dissolved in distilled water 50 ml and let stand for 6 hours. The mixture then dried in oven at 70°C.

b. Composing gelatin + chitosan + cross-linker.

Gelatin 10 gram was dissolved into 100 ml of Chitosan solution 1% in acetic acid solution 1%. Cross-linker material (glutaraldehyde or oxidized sucrose) with weights according to table 1 are dissolved in distilled water 10 ml. Compose the two solutions and stir until homogeneous. The mixture is allowed to stand for 24 hours. The mixture then dried at 70°C.

c. Composing gelatin and cross-linker

Gelatin 10 gram is dissolved in 100 ml of distilled water. Cross-linker material (glutaraldehyde or oxidized sucrose) with weights according to table 1. Compose the two solutions and stir until homogeneous. The mixture is allowed to stand for 24 hours. The mixture then dried at 70°C.

Table 1. Cross-link trial groups

No	Group (Composition in gram)	Code
1	Milkfish Gelatin	Mi
2	Commercial Gelatin	Co
3	Chitosan	Ci
4	Milkfish Gelatin + Chitosan (10:1)	MiCi
5	Commercial Gelatin + Chitosan (10:1)	CoCi
6	Milkfish Gelatin+Chitosan+Oxidized Sucrose (10:1:0,5)	MiCiSu 5
7	Milkfish Gelatin+Chitosan+Oxidized Sucrose (10:1:0,25)	MiCiSu 2,5
8	Milkfish Gelatin+Chitosan+Oxidized Sucrose (10:1:0,1)	MiCiSu 1
9	Milkfish Gelatin+Chitosan+Oxidized Sucrose (10:1:0,05)	MiCiSu 0,5
10	Milkfish Gelatin+Chitosan + Glutaraldehyde (10:1:0,25)	MiCiGu 2,5
11	Milkfish Gelatin+Chitosan + Glutaraldehyde (10:1:0,1)	MiCiGu 1
12	Milkfish Gelatin+Chitosan + Glutaraldehyde (10:1:0,05)	MiCiGu 0,5
13	Milkfish Gelatin+Chitosan + Glutaraldehyde (10:1:0,01)	MiCiGu 0,1
14	Milkfish Gelatin+Chitosan + Glutaraldehyde (10:1:0,005)	MiCiGu 0,05
15	Milkfish Gelatin+Oxidized Sucrose (10:0,5)	MiSu 5
16	Milkfish Gelatin+Oxidized Sucrose (10:0,25)	MiSu 2,5
17	Milkfish Gelatin+Oxidized Sucrose (10:0,1)	MiSu 1
18	Milkfish Gelatin+Oxidized Sucrose (10:0,05)	MiSu 0,5
19	Milkfish Gelatin+Oxidized Sucrose (10:0,01)	MiSu 0,1
20	Milkfish Gelatin+Glutaraldehyde(10:0,25)	MiGu 2,5
21	Milkfish Gelatin+Glutaraldehyde(10:0,1)	MiGu 1
22	Milkfish Gelatin+Glutaraldehyde (10:0,05)	MiGu 0,5
23	Milkfish Gelatin+Glutaraldehyde(10:0,01)	MiGu 0,1
24	Milkfish Gelatin+Glutaraldehyde(10:0,005)	MiGu 0,05
25	Commercial Gelatin+Chitosan+Oxidized Sucrose(10:1:0,1)	CoCiSu 1
26	Commercial Gelatin+Chitosan+Glutaraldehyde(10:1:0,05)	CoCiGu 0,5
27	Commercial Gelatin +Oxidized Sucrose(10:0,1)	CoSu 1
28	Commercial Gelatin+Glutaraldehyde(10:0,05)	CoGu 0,5

Quality Testing

- a. Organoleptic
The sample is observed in color on white paper.
- b. Identification with IR Spectroscopy

Samples were dried at 105°C for 5 hours. Dry samples were directly measured by IR spectral transmittance. Measurements were made at wavelengths of 400-4000 cm⁻¹. Crosslink identification is confirmed by the changes in the IR spectrum of the sample.

c. Swelling index

Observed weights before (dry) and after incubation (swelling). Dry weight is the weight of the sample at 20°C to a constant weight. Swelling ratio is calculated using the formula

$$= \frac{B_s - B_d}{B_d} \times 100$$

Where B_s and B_d are gelatin weights at swelling and dry.

d. Viscosity

The sample weighed 6.67 g and then dissolved in 100 ml distilled water at 70°C. The gelatin solution cooled at 40°C, then was measured for viscosity using calibrated-canon Fenske routine viscometer [21].

3 Result and Discussion

Halal Studies of Cross-link fish gelatin

Gelatin and its derivatives that produced in this research are halal. The evaluation of halal is based on the principle *inushul fiqh*, that state “all things are halal unless there is an argument for haramness of them.” Materials that used in this study, such as water, sodium hydroxide, hydrochloric acid, chitosan, sucrose, sodium periodate, glutaraldehyde and commercial gelatin, are halal. The equipment is also certain to be free from *najs*, such as heavy, medium and light *najs*. Materials that are possibly to be haram are commercial gelatin and glutaraldehyde. Even though chitosan is a processed product, the process does not use haram substances, such as the shells of marine animals, hydrochloric acid and sodium hydroxide [22]. The commercial gelatin that used has a halal certificate by LP-POM MUI. Glutaraldehyde synthesis from acrolein and vinyl ethers, by Diels-Alder reaction or cyclopentene oxidation [23]. Acrolein is possibly sourced from burning glycerol, and it possibly sourced from lard. However, this chemical process has changed the character and chemical structure of the material so it meets the requirements of *istihalah*. Thus, glutaraldehyde is a halal material. In addition, if the synthesis pathway is through cyclopentene oxidation, the glutaraldehyde product is made from halal raw materials.

The focus of halal analysis for halal certification is to examine the possible halalness of each ingredient by detecting the critical point. Materials categorized as critical points are meat, animal derivatives, processed vegetable ingredients, industrial side-products of yeast, microbial products, and food additives (BTM) such as dyes, flavors, seasonings, aspartame sweeteners, vitamin premixes, coatings, emulsifying agents, antifoaming or others [24]. All materials are included in the critical point because it is possibly from haram material, or it is contaminated by *najs* and not holiness yet (*thaharah* process). Halal materials must be guaranteed not contain or contaminated with haram and *najs* materials, not just conjecture.

In accordance with the previous *ushl fiqh* principle, materials that included in the category of haram are pigs, *khmar*, carcasses (animals not slaughtered according to Shari'a), blood, animals with spurs or fanged animals, animals that live on land and water, and parts of the human body with different levels of *najs* [25]. Products, raw materials or processed raw materials that sourced from these materials are categorized as haram. In addition, if the material is solid or the equipment

that used is contaminated with *najis*, the material or equipment must be purified with *thaharah* process. However, if the material is made from pigs, even though it has been *thaharah* processed, it is still categorized as haram. Some of haram ingredients are not *najis*, such as whole or parts of the human body and some animals that have spurs, but they are still haram to eat.

Gelatin extraction

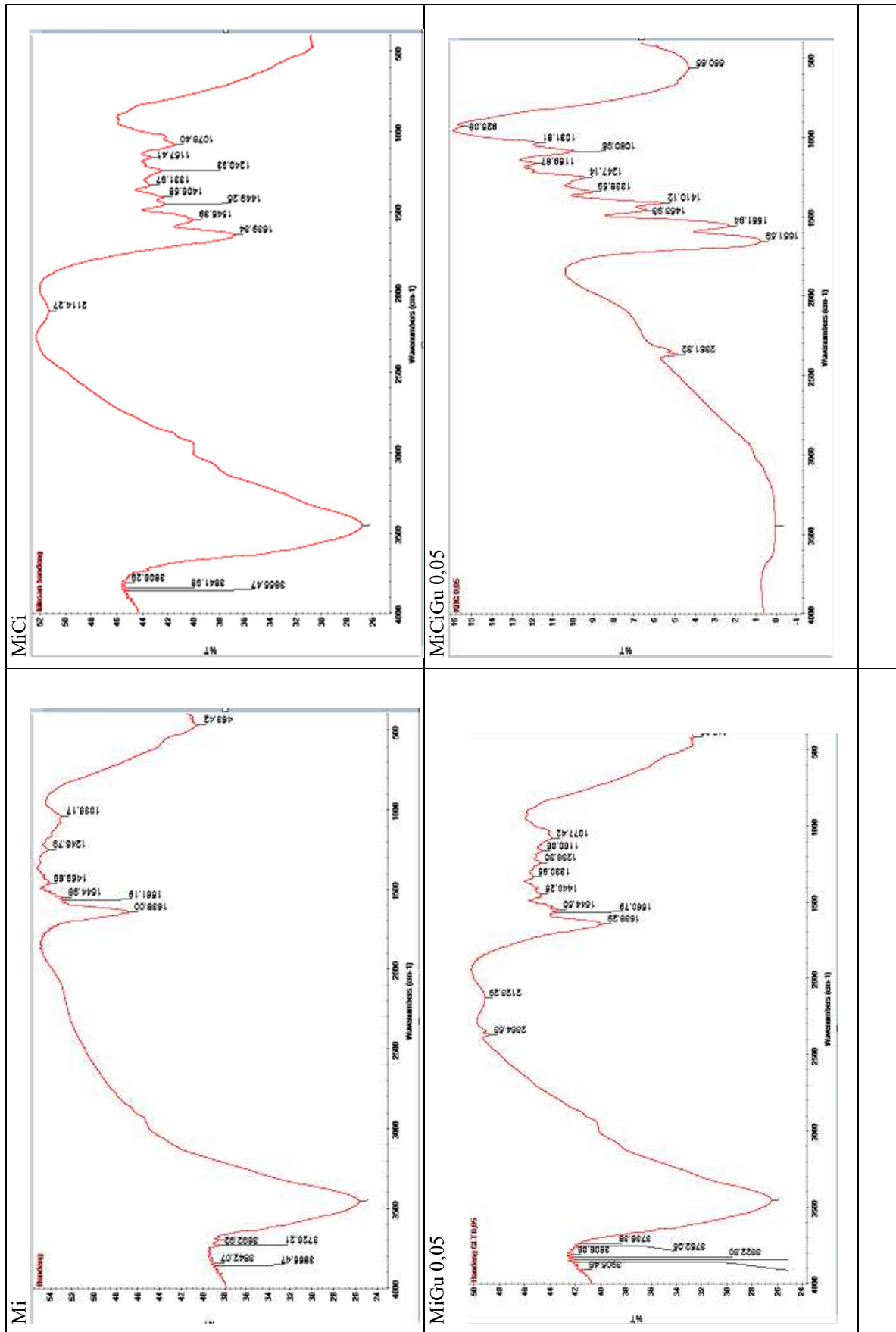
Extraction method with alkaline and acid pre-treatment is effective to extracting gelatin from milkfish scales. The yield of extraction is 38.8%, calculated against the weight of the scales that used. The extraction technique that used in this study refers to the research of Boran and Regenstein [20] with some modifications. This yield is greater than others yield of extraction from scales such as lizardfish (10.7%), tilapia (12.3%), and sea bream (9.55%) [26, 27, 28]. The milkfish gelatin is sheet-shaped, transparent in color and the smell of the fish has disappeared.

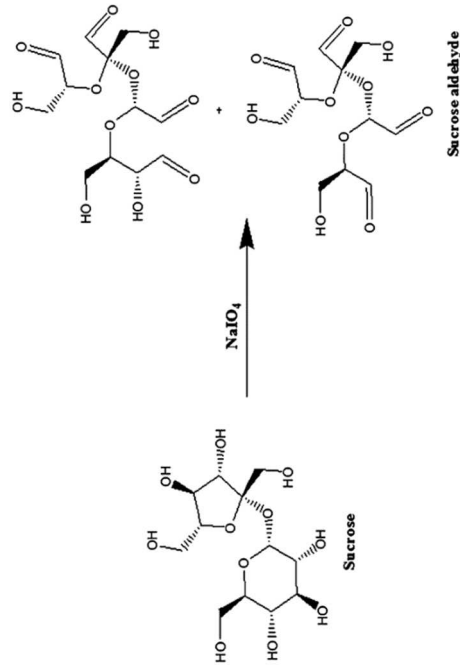
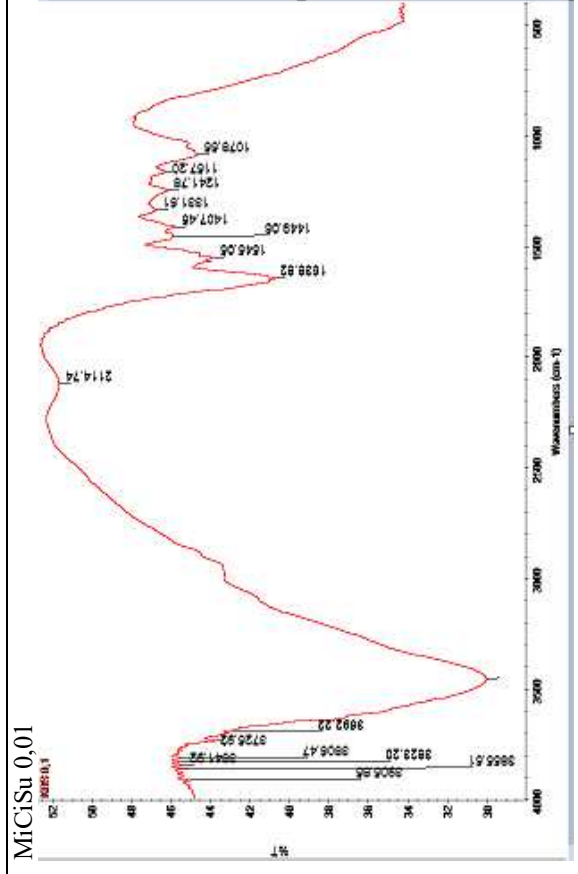
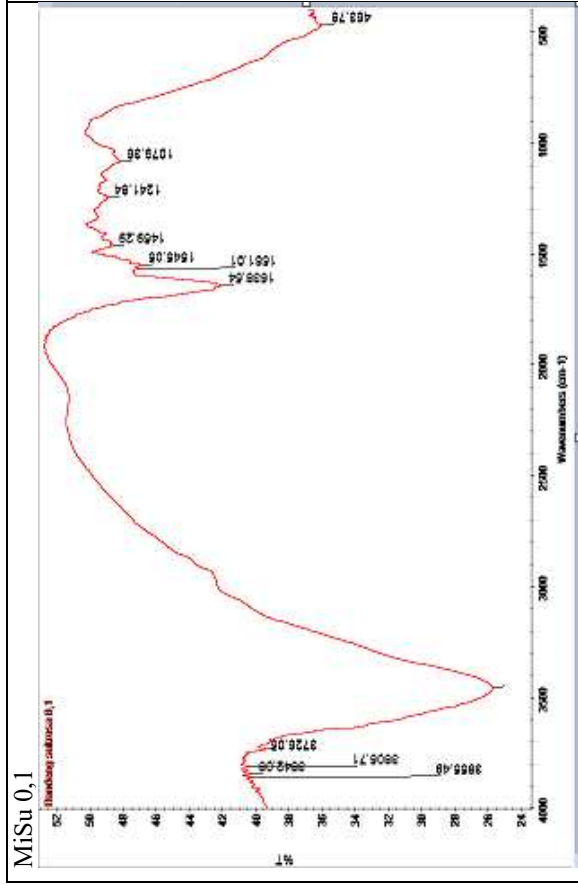
Gelatin cross-link

Materials commonly used as cross-linkers for gelatin are glutaraldehyde and oxidized sucrose. A cross-link reaction is formed between the aldehyde group (CHO) in glutaraldehyde and oxidized sucrose with the amine group (NH₂) in protein (lysine) and chitosan. The functional group that formed is imine (N=C). The bonds connect inter-macromolecular structures, such as inter-gelatin, inter-chitosan or gelatin-chitosan. This macromolecular structure is getting bigger so the viscosity is getting higher and the swelling index is getting lower.

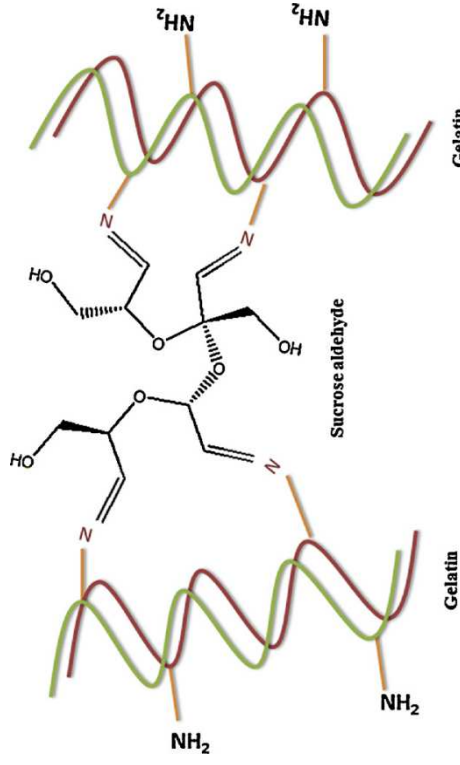
This research succeeded in forming gelatin cross-link. The sign that a cross-link bond has been formed can be observed in the infrared spectrum in Table 2. Based on the reaction in figure 2, an imine group (C=N) is formed between gelatin and oxidized sucrose. This imine group is marked with a peak at wave number 1690-1640. The spectrum of the imine group is overlap with keto spectrum of the amide, which is 1680-1630. Mi spectrum at the peak 1658 showed the presence of amide groups of proteins. While the spectrum at 1688 of MiGu 0.05 and MiSu 0.1 shows imine groups that overlap with amide groups. This peak intensity is higher than the spectrum of Mi, and the shift of spectrum to 1688 is an indication of the formation of imine groups at MiGu 0.05 and MiSu 0.1. Similarly, in a compose of gelatin and chitosan. The peak of 1639 with a slightly high intensity indicates the amide found in proteins and amines (1640-1500 → N-H bend). After cross-link with glutaraldehyde and oxidized sucrose, as seen in the spectrum of MiCiGu 0.05 (peak 1651) and MiCiSu 0.01 (peak 1658) also shows imine groups that overlap with amide groups, with higher intensity. This higher intensity is due to the formation of the imide group after cross-link, adding to the intensity of the previous peak.

Table 2. Infrared Spectrum of Gelatin and Derivates





Picture 1. Reaction formed oxidized sucrose [16].



Picture 2. The reaction between oxidized sucrose with gelatin [16].

Table 3. Swelling index and viscosity of gelatin cross-link

No	Group Code	Organoleptic	Swelling index %	Viscosity cP
1	Mi	Yellowish	nd	2,314
2	Co	Yellowish	nd	1,128
3	Ci	White	470,74	nd
4	MiCi	Yellowish	2745,65	nd
5	CoCi	Brownish yellow	219,39	nd
6	MiCiSu 5	Brownish	268,06	nd
7	MiCiSu 2,5	Brownish	841,34	nd
8	MiCiSu 1	Brownish	1280,65	nd
9	MiCiSu 0,5	Yellowish	1602,745	nd
10	MiCiGu 2,5	Blackish brown	642,96	nd
11	MiCiGu 1	Brownish	699,68	nd
12	MiCiGu 0,5	Brownish	745,02	nd
13	MiCiGu 0,1	Brownish yellow	2173,63	nd
14	MiCiGu 0,05	Yellowish	2227,77	nd
15	MiSu 5	Brownish yellow	591,17	nd
16	MiSu 2,5	Brownish yellow	753,37	nd
17	MiSu 1	Yellowish	nd	4,954
18	MiSu 0,5	Yellowish	nd	3,137
19	MiSu 0,1	Yellowish	nd	2,991
20	MiGu 2,5	Blackish brown	879,41	nd
21	MiGu 1	Brownish	1160,03	nd
22	MiGu 0,5	Yellowish	nd	4,170
23	MiGu 0,1	Yellowish	nd	3,590
24	MiGu 0,05	Yellowish	nd	3,372
25	CoCiSu 1	Brownish	155,60	nd
26	CoCiGu 0,5	Brownish	388,26	nd
27	CoSu 1	Yellowish	nd	2,844
28	CoGu 0,5	Brownish	nd	2,475

nd = not determined

The cross-link gelatin showed improved viscosity and swelling index characters. Samples containing chitosan and cross-link gelatin with high concentration solutions are not soluble in water. Samples after added with water, only just swell. Because it is insoluble in water, this sample cannot be tested for its viscosity character. Conversely, in other samples, gelatin cross link is water soluble, and swelling index cannot be tested, but its viscosity is easily tested.

The higher the concentration of the cross-linker material, the smaller swelling index of the sample. Swelling index is a character that shows the ability of the sample to bind water. As a result of the reaction with the cross-linker, the area that initially can bind water becomes reduced. Some

have reacted with cross-linker material. In addition, cross-link bonds make gelatin little swell due to the large number of bonds that formed.

The cross-link gelatin with oxidized sucrose is stronger than with glutaraldehyde, and conversely, the gelatin-chitosan cross-link with glutaraldehyde is better than with oxidized sucrose. Swelling index of gelatin with oxidized sucrose 2.5% is 753%, while with glutaraldehyde 2.5% is 879%. When compared to the standard of commercial gelatin (CoSu and CoGu) and milkfish gelatin, the cross-link gelatin is harder. For other samples, the gelatin-chitosan swelling index with glutaraldehyde 2.5% was 643%, while with oxidized sucrose 2.5% was 841%. Gelatin-chitosan cross-link is stronger than gelatin cross-link.

Cross-link gelatin with glutaraldehyde has better viscosity than oxidized sucrose. The viscosity of gelatin with oxidized sucrose 0.5% was 3.137 cP, whereas with glutaraldehyde 2.5% was 4.170 cP. Viscosity of standard gelatin CoSu 1% is 2.844 and CoGu 0.5% is 2.475 cP. Viscosity of gelatin that has been cross-linked is also better than fish gelatin that is not modified by cross-link. Viscosity of fish gelatin is 2.314 cP and commercial gelatin is 1.128 cP.

This cross-link gelatin can be utilized in the pharmaceutical field. Cross-link gelatin that is not soluble in water can be used as a disintegrator for tablets and raw materials for hard capsule shells; whereas, water-soluble cross-link gelatin can be used as raw material for gel and jelly.

4 Conclusion

Gelatin from milkfish scales can be improved its viscosity and swelling index characteristics, thus increasing the quality of milkfish gelatin. The method is cross-linked with oxidized sucrose and glutaraldehyde, with or without combination with chitosan. All materials that used in the process of making gelatin and cross-link gelatin are halal.

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