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Effect of Glucose, Sucrose, Cellulose, Glycerol, Chitosan Addition to Improve Gelatin Quality of Fish, Chicken, and Cow

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Abstract : Gelatin potency from fish and chicken is very profound because reuse of the skin/shell and the bone is still limited. However, utilization in pharmacy is very limited because gel strength and viscosity from fish and chicken are poor.
To ameliorate quality of fish and chicken gelatin, it can be combined with other substances that are expected to form cross-linking between gelatin and the substances. This research intend is to improve quality of gelatin viscosity by cross-linking approach. Objective of this research is to determine whether glucose, sucrose, cellulose, glycerol, or chitosan is the best substance to improve quality of gelatin viscosity as well as to analyze effect of the best substance to gelatin viscosity made from fish, chicken, and cow.
This research was performed by sample demineralization of cow skin, cow cartilage, fish shell, fish bone, chicken skin, and chicken bone with acid until ossein formed. The ossein was extracted with distilled water producing gelatin. The best substance to improve gelatin viscosity was gained by cross linked glucose, sucrose, cellulose, glycerol, and chitosan with standard gelatin then comparing their viscosity. This the best substance was used as cross-linking component combined with gelatin of fish, chicken, and cow by heating method at temperature 70^{o}C for 1 hour. Afterward, the viscosity of these gelatins were measured.
The result is that chitosan has the highest viscosity which is used to form gelatin cross-linking of fish, chicken, and cow. Adding of 1% chitosan significantly affects increasing of fish, chicken, and cow gelatin, therefore it is expected can improve gelatin quality of fish and chicken.
Key words : gelatin, cross-linking, chitosan, viscosity.

Introduction

Nowadays, gelatin is a very popular substance for many food and pharmacy products. Gelatin is most used for candy products (soft candy) and capsule shells all over the world[1]. Gelatin is a polypeptide compound that is derived from partial hydrolysis of collagen[2]. In producing big scale gelatin, the main substance is collagen which can be isolated from cow and pig[1]. Commercial shape of gelatin is thin plate, granules, or powder particularly that is made from bone and skin of cow, pig skin, and the latest is pig bone[3, 4]. Other gelatin sources are fish and fowl as the latest this time and generally are produced to fulfill consumer group from

certain religion[4,5,6]. But, because of knowledge and technology development, rapid food product diversification, food products can be compiled by non-halal compositions in order to reduce production cost. In market, pig gelatin is cheaper than cow gelatin or other gelatins that are produced from halal source[7].

In Islam, the criteria of recommended food for consumption shall be Halal and 'good' (tayyib), as stated in the Al Qur'an Surah al-Baqarah/2:168. Halal means any food that is allowed by God for consumption and obtained not in any way unlawful/Haram. While the 'good' means the sacred food substances, not something bad or harmful to the body and mind[8,9,10,11]. Therefore, consuming products that are not considered , such as those containing pig gelatin, is forbidden (Haram)[7, 12]. Some Islamic countries have established strict regulation for producer and importer by stamping their products with halal certificate to differentiate their products with non-halal products[3]. In some countries, halal certification is still limited for food products, but for pharmaceutical products are not regulated yet. Pharmaceutical products is only be part of halal debate when society realize with halal concept in all aspects of life. Narrow understanding about halal is that food/ drug has to be free from forbidden compound or substance which contains pork or alcohol, meanwhile the genuine concept about halal is that all processes started from product preparation until finished product have conformed sharia provisions. Halal promotes not only what we consume has to be halal (obey approved sharia law) but also product has to be 'Tayyib' (good quality)[13].

Gelatin is mixture of polypeptide which is made by collagen hydrolysis. Gelatin can be extracted from animal skin, bone, and skin of mammals like pig and cow[6]. Besides that, gelatin can be produced from fish as well[14,15,16]. According to GMIA, commercial gelatin is obtained from cow and pig in which approximately 90% of gelatin made from pig[17]. Gelatin has gelling characteristics such as gel strength and gelling time, adjustment and melting point, as well as viscosity that are suitable to be used in food product like soft candy. Except that, gelatin surface behavior (for instance formation and stabilization of foam and emulsion, gluing property and disintegration from dosage forms) has been agreed the application for food and pharmaceutical products [1,18]. One of weaknesses of fish, chicken, and cow gelatin is poor gel strength. In pharmacy, gel strength which is required in producing hard capsule shells and microencapsulation is more than 200 broom. Fish and chicken gelatin, and also cow gelatin do not meet this requirements[18]. One of ways to improve gel strength is by making cross-linking gelatin. Gelatin can be formed by cross-linking with assistance of certain substances for example glucose, sucrose, cellulose, glycerol, and chitosan. This gelatin will be modified so that molecules will bond tightly one to another by formation of inter molecules bonding complex. In case of that, we intended to make raw material of cross-linking gelatin in order to improve gel strength of fish, chicken, and cow gelatin and also examined their quality as raw material for hard capsule shells and microencapsulation (drug delivery system). Purpose of this research is to determine whether glucose, sucrose, cellulose, glycerol, or chitosan is the best substance to improve quality of gelatin viscosity. Furthermore the research is to analyze effect of the best substance toward viscosity of fish, chicken, and cow gelatin. Source of raw material that we used is from industrial waste that still can be recycled.

Experimental

1. Gelatin Extraction from sample

Six groups sample are cow skin, cow bone, chicken skin, chicken bone, snapper fish shell, and snapper fish bone. Every sample was gained in fresh condition, then washed or cleaned. Cleaning process was performed by expel dirt, remain meat, fat in the skin. To ease the cleaning process, it was performed by heating in boiled water for 30 minutes. After that, sample was cut size about 3 cm to enlarge surface area.

The next step was demineralization. Demineralization is refining process of calcium and mineral salts which are contained in bone or skin so that bone and skin become tender, called ossein, in which contains collagen inside. Demineralization process was performed by using 5% acidic acid and 5% citric acid for chicken skin and cow bone, 5% nitric acid solution and 5% chloride acid for shell and bone fish, 5% sulfate acid and 5% citric acid for chicken bone and cow bone soaked for 72 hours. Acetic acid is chosen because generally it is not toxic than other acid substances, citric acid can eliminate putrid smell of the sample. Sulfate acid and chloride acid is strong acid that can soften fish bone. Ossein was washed with flowing water until its pH is neutral.

Gelatin was extracted from neutral ossein in a beaker glass using distilled water. Comparison between ossein and distilled water is 1: 1 (w/v). After that gelatin was extracted in water bath at temperature 70°C for 24 hours. Next step it was filtered with filter fabric. Gelatin thick liquid that was obtained was poured into petri dish to dry in freeze dryer for 48 hours. Then it was refined when dry [19,20].

2. **Determination of the Best Cross-linking Substance**

Experiment groups at this step were divided to six groups based on cross-linking substances that were used. Gelatin will be cross-linked with glucose, sucrose, cellulose, glycerol, and chitosan [21,22,23,24,25].

Making of cross-linking gelatin was begun by making gelatin solution that was made by adding boiled water and was stirred until gel dough was resulted. After that, gelatin solution was mixed with cross-linking substances in the same quantity at temperature 70°C while stirring slowly. Mixture concentrations are presented below.

Table 1. Gelatin solution concentrations and cross-linking substances

Mixture	**Concentration**	
A	20% gelatin	5% sucrose
B	20% gelatin	5% glucose
C	20% gelatin	5% CMC
D	20% gelatin	5% glycerol
E	20% gelatin	5% NaH_2PO_4
F	8% gelatin	2% chitosanin1% acetic acid

Gelatin and CMC solution were made by adding boiled water and stirring until gel dough was formed. Then, those mixtures were dried in oven at temperature 75°C until dry. Dry mixtures were made to be 2% solution and their viscosity was examined by using Oswald/ Brookfield viscometer. Cross-linking substance that has the highest viscosity value was used as cross-linking substance for samples of cow, chicken, and fish gelatin.

3. **Making of Cross-linking Gelatin from Skin and Bone of Cow, Chicken, and Fish**

Examined samples were divided to six experiment groups based on raw material sources, soleskin and nose cartilage of cow, skin and feet bone of chicken, shell and bone of snapper fish. Making of cross-linking gelatin was firstly performed by making gelatin solution from each gelatin source by adding boiled water and stirring until gel dough was formed. Then, gelatin solution was combined in the same quantity with cross-linking substance at temperature 70°C by stirring slowly. Mixture concentrations are suitable to experiment result in point 2.

The cross-linking gelatin was dried in oven at temperature 75°C until dry. The dry cross-linking gelatin was made to be 2% solution and performed viscosity test by using Oswald viscometer. Gelatin viscosity resulted from cross-linking method was compared with viscosity before cross-linking treatment to analyze effect of crosslinking process.

4. **Viscosity Test**

Sample was weighed 2 g, dissolved in distilled water until 100 ml. Then it was heated at temperature 70°C until gel was formed. The mixture was left at room temperature, then its viscosity was examined using Ostwald viscometer by putting 10 ml of mixture solution with cross-linking substance in Oswald viscometer, measuring flowing time of the mixture. Except that, viscosity of mixture solution was measured using 10 ml pycnometer before and after combined with mixture solution. Viscosity of each mixture was examined at temperature 27°C. The viscosity is calculated with formula $\rho =$ weight/ volume and the viscosity is calculated with formula:

$$\frac{\text{viscosity 1}}{\text{viscosity 2}} = \frac{\rho 1 \,.\, t1}{\rho 2 \,.\, t2}$$

Result and Discussion

The first stage of this research is gelatin extraction from sample. The samples are six, they are bone of red snapper fish (*Lutjanus bitaeniatus*), shell of red snapper fish, bone of broilers feet (*Gallus domesticus*), skin of broilers, nose cartilage of cow (*Bos sondaicus*), and sole skin of cow. The samples are processed in resulting gelatin through some steps:

1. Degreasing

Degreasing is preparation stage of raw material. In this stage, cleaning process or sample cleaning is performed. Cleaning process is performed by expelling dirt, remaining meat, and fat in the sample. To ease this cleaning process can be performed by heating bone or skin in boiled water for 30 minutes. Afterward, the bone or skin is drained and cut in order to widen sample surface so that it is easier to be extracted, and extraction process can undergo faster as well.

2. Demineralization

Demineralization is sample softening process to ease sample extortion. Using acid in low concentration is intended to dissolve calcium and mineral that compose bone and skin structure so that the structure becomes softer. Besides that soaking in acid condition in order to change collagen fiber helix triple to single chain. The soaking will generate ossein that is soft bone and shell. After that, ossein is washed using flowing water until it reaches normal pH

3. Extraction

Ossein is extracted on water bath at temperature 70^{o}C by using distilled water with comparison 1: 1. This comparison is used so that in drying process will be easy because of less water contain. Extraction is to convert collagen to gelatin. Neutral water, acid solution or base solution can be used to hydrolyze collagen as of breaking to gelatin. In this research, we use neutral water because it is inert so that relatively it is safe rather than use acid or base. Heating can fasten dissolved process of gelatin in case of that it is easy to be extracted. At cool or room temperature, gelatin are solid or semisolid as of complicating extraction process. Good temperature is at 70^{o}C because it is an optimum temperature to produce great quantity of product. At high temperature, it is worried that affect gelatin protein.

4. Drying

Drying is the last process of gelatin extraction. Drying is performed by using freeze dryer. Reason of choosing this equipment is because gelatin is not long lasting at high temperature, meanwhile freeze dryer is an equipment in which a sample undergoes freezing and drying, separates partial or almost all water contain by sublimation mechanism.

Yield or product is one of important parameter in gelatin production, yield is product which is gained by comparison of gelatin weight to sample weight. The sample weight is 1 kg. Yield value is significant parameter to know efficiency level of a production process. Yield value is affected the most by raw material as well as extraction temperature. The higher the extraction temperature, the greater the gelatin yield or product resulted. It is occurred because of temperature increasing so that gelatin extraction process will be more well-rounded and gelatin quantity will be greater. Based on experiment result, feet skin of cow is sample which contains the highest gelatin. This part contains much collagen that protects cow feet from injury. Feet skin of cow comparatively does not contain bone so that calcium content is lower inside.

Table 2. Yield of Extraction Process of Each Sample

Gelatin Source	Yield (%)
Gelatin of Fish Shell	2,50
Gelatin of Fish Bone	3,31
Gelatin of Chicken Skin	2,64
Gelatin of Chicken Bone	2,24
Gelatin of Cow Skin	5,69
Gelatin of Cow Cartilage	3,50

The second stage of this research is determining the best cross-linking substance. Generally, the substances are derived from polymer of polysaccharide or degradation result of polysaccharide, but glycerol belongs to poly-hydroxyl. The substances are glucose, sucrose, glycerol, cellulose, and chitosan. These substances have been investigated that they can create bonding between gelatin and the substances. Generally, the substances have hydroxyl group which can function as either acceptor or donor of hydrogen bonding, can form hydrogen bridge with hydroxyl group or amine group or side groups in amino acid that composes gelatin.

The best substance used as cross-linking substance is decided based on mixture viscosity. The mixture is made from comparison of 4 parts of gelatin and 1 part of cross-linking substance that is stirred gently at temperature 70^{o}C. Heating is useful in formation of cross-linking bonding. Energy generated from heating is expected to be source of activation energy that is needed by a molecule before two molecules are bonded each other. This energy can be obtained by radiation of electromagnetic wave.

Gelatin viscosity requirement appointed by GMIA is 15-75 milipoise, but this standard is gained if using high measurement of gelatin concentration. High viscosity is related to molecule weight while gelatin molecule weight is related directly to length of its amino acid chain. It means that the longer the chain, the higher the viscosity value. Concentration of different acid solution affects to gelatin molecule weight resulted. The bigger the molecule weight, the slower the solution flow rate as of improve viscosity value. Besides that, molecular viscosity exhibits intensity of hydrogen bonding or cross-linking formed inter molecules. The bigger the intensity of hydrogen bonding, so that the higher the hydrogen bonding. It can be one of criteria to analyze quality of cross-linking substance. In this research uses concentration of 2% gelatin cross-linking mixture to examine viscosity.

Table 3. Viscosity of Cross-linking Standard Gelatin Substance

Sample	**Viscosity (mP)**
Glucose + Gelatin	12.59
Sucrose + Gelatin	12.24
Glycerol + Gelatin	13.17
Sodium CMC + Gelatin	16.49
Chitosan + Gelatin	274.92*

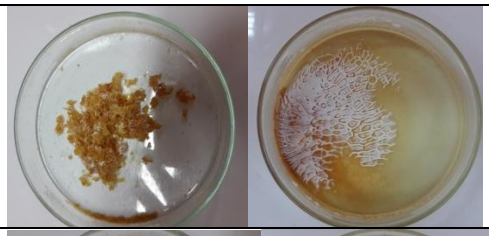
*mixture with the highest viscosity, used in the next stage as cross-linking substance

Before samples of cow, fish, and chicken are cross-linked to improve their quality, it is necessary to determine the best cross-linking substance. So that 5 substances are used as cross-linking substance, they are sucrose, glucose, cellulose (in form of sodium carboxyl methyl cellulose, Sodium CMC), chitosan, glycerol and phosphate salt. Those substances are chosen because relatively they are not toxic, and have hydroxyl group or oxygen atom which can arrange hydrogen bonding with either hydroxyl group or amine of protein.

Chosen substance as cross-linking substance is chitosan. This substance is selected because it increases gelatin viscosity. Its viscosity is too much different from other substances (275 milipoise, other substances are below 20 milipoise). However, this substance has limitation because it needs acid to dissolve it. In this research, acetic acid is used to assist solubility of 2 g chitosan in 100 ml. Heat is required to fasten chitosan solubility process.

The last stage is making gelatin mixture with cross-linking substance. Samples originating from skin and bone of cow, chicken, and fish are cross-linked with chitosan. Solution concentrations are 2% chitosan and 8% gelatin. Both solutions are combined in the same volume (100 ml) and the dough is swelled at temperature 70^{o}C while stirring gently for 60 minutes in order to gain homogeny mixture. This mixture is dried in oven at 70^{o}C until dry. The drying time is 48 hours. All products of cross-linking substance are yellow brown.

Table 4. Pictures of Cross-linking Gelatin with Chitosan

Sample	Picture	Appearance
Gelatin of Snapper Fish Shell		Powder, white yellow, spesific odor
Gelatin of Snapper Fish Bone		Powder, white yellow, spesific odor
Gelatin of Chicken Skin		Powder, yellow brown, specific odor
Gelatin of Chicken Feet Bone		Powder, white brown, specific odor
Gelatin of Cow Sole Skin		Powder, yellow brown, specific odor
Gelatin of Cow Nose Cartilage		Powder, yellow brown, specific odor

To examine effect of adding cross-linking substances toward gelatin quality, the viscosity is tested and compared with viscosity before and after adding cross-linking substance. The results are shown in the table below.

Table 5. Viscosity of Gelatin Substance by Source Before and After Cross-linking Treatment

Gelatin Source	Viscosity		Viscosity Increase (percent)
	Before Cross-linking Treatment (milipoise)	After Cross-linking with Chitosan (milipoise)	
Gelatin of Cow Nose	14.07	49.1	248.97
Gelatin of Cow Skin	13.4	42.95	220.52
Gelatin of Snapper Fish Shell	10.31	33.47	224.64
Gelatin of Snapper Fish Bone	11.62	13.15	13.17
Gelatin of Chicken Skin	45.83	76.23	66.33
Gelatin of Chicken Bone	11.02	31.93	189.75

According to statistic test in table 6 shows significant difference between viscosity of six gelatin substances and before and after cross-linking treatment. It is exhibited based on F value > F table (5.172 > 4.964) with accuracy 0.05. It indicates significant effect of cross-linking substance to gelatin viscosity increase. This increase is in comparison of gelatin: chitosan = 4: 1.

Table 6. Result of Anova Test Single Factor

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	1646.895	1	1646.895	5.172078	0.046238	4.964603
Within Groups	3184.203	10	318.420			
Total	4831.098	11				

Conclusion

1. The best substance as mixture with gelatin to improve its viscosity is chitosan with 1% concentration.

2. One percent chitosan affects to increase gelatinviscosity which is from fish shell, bone fish, chicken skin, chicken bone, cow skin, and cow bone.

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