The effect of different maceration solutions towards characteristic gelatin from bone of common carp (Cyprinus carpio)

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Abstract. Gelatin is a derivative of collagen which is found in connective tissue, skin and bones. Gelatin can be obtained from cow, pig or fish. Teleost fish bones have collagen protein content ranging from 15-17%. Common carp belong to the teleost fish group and research on gelatin from carp bones using different maceration solutions has never been done before. The purpose of this research was to determine the effect of different maceration solutions on the characteristics of gelatin from carp bones and to determine their conformity with the standard. This research is a laboratory experimental with Completely Randomized (CRD) consisting of 3 different designs of maceration solutions (NaOH; C₆H₈O₇ and a mixture of NaOH and C₆H₈O₇). The results showed that there was an effect in different maceration solutions on the characteristics of carp bone gelatin which was tested with the best treatment using a mixture of NaOH and C₆H₈O₇ solutions. Some of the characteristics of gelatin in this research are accordance with standards including water content, ash content, viscosity, gel strength, as well as color and pH in several treatments.

1. Introduction

Freshwaters have various kinds of biota that can be utilized and processed into various products, one of them is gelatin. Gelatin is a derivative of collagen which found in connective tissue, skin and bones. Gelatin can be obtained from cow, pig or fish [1]. Teleost fish bones have collagen protein content ranging from 15-17% [2]. Common carp (Cyprinus carpio) is one of freshwater teleost fish that is widely consumed by the public and has a collagen content of 16% [3,4]. The high content of collagen allows it to be further processed into a product in the form of gelatin. In general, the produce of gelatin can use an acid solution, a alkaline solution and a combination of an acid and a alkaline solution. The acid solution will produce more gelatin when using skin as raw material and it will take a dozen hours. Alkaline solutions can produce more gelatin if using raw materials such as bone but it takes longer to tens of hours [5].

The maceration process using a mixed solution takes a shorter time with the better quality of the gelatin produced than using acid or alkaline solutions only [6]. The different application of solutions can affect the characteristics of the gelatin produced because each solution has its own working principle. NaOH alkaline
solution will break the protein secondary bonds and close the empty space around the protein so there is a distribution of peptide molecules that affect the viscosity value [8]. C_6H_8O_7 acid solution will cause accelerated swelling of the collagen structure [9]. Research on gelatin using common carp bone material has never been done by any method. The effect of different maceration solutions on the characteristics of gelatin from bone of common carp (Cyprinus carpio) needs to be done to find out some information. The information includes whether differences in maceration solutions affect the characteristics of gelatin from carp bone (Cyprinus carpio) and whether the gelatin has characteristics according to the standard.

2. Material and methods
2.1. Material
The equipment used in the produce and characterization gelatin from bone of common carp (Cyprinus carpio) were cutting boards, knives, basins, 1000 ml and 2000 ml beaker glass, 1000 ml measuring cups, petri disks, stirring rods, sieves, analytical balance, 1000 ml erlenmeyer, funnel, spatula, tweezers, orbital shaker, water bath, thermometer, refrigerator, rotary evaporator, oven, pH meter, viscometer, texture analyzer, desiccator, furnace, porcelain cup and tangkrus. The materials used in this study were the spine (vertebrae) from the base of the operculum to the anus and the fins of common carp (Cyprinus carpio), aluminum foil, aquades, plastic, label paper, 1.5% NaCl, 0.2% NaOH, and C_6H_8O_7 0.2%.

2.2. Preparation of Common carp’s bone
Common carp samples were obtained from common carp farming ponds, Kepudibener Village, Turi District, Lamongan using a styrofoam box equipped with ice. The fish are packaged one by one using plastic and then frozen before being brought to the laboratory to maintain their quality. The frozen common carp were brought to the laboratory, thawed and then carried out for organoleptic tests. Common carp are filleted for the spine and fins only. Carp bones are washed using distilled water until clean. Bone samples were weighed as much as 150 g for each treatment and then cut into several parts. Common carp bones were cleaned by maceration using a 1.5% NaCl solution with a composition of 1:4 (w/v) for 1 hour with a change of solution every 30 minutes to avoid saturation of the solution. Bone was macerated with 1.5% NaCl solution using an orbital shaker at room temperature. 1.5% NaCl solution is intended to remove mucus and blood adhering to the bones [6].

2.3. Gelatin produce
Extraction of gelatin from common carp’s bone (Cyprinus carpio) was carried out based on the research of Tohmadlae et al [6]. The initial process of making gelatin begins with the maceration process using an orbital shaker at room temperature. The common carp’s bones in the first treatment were macerated using a 0.2% NaOH solution with a composition of 1:4 (w/v) for 2 hours with changes every 1 hour and then rinsed with distilled water. The second treatment carp bones were macerated using a 0.2% C_6H_8O_7 solution of 1:4 (w/v) composition for 2 hours with changes every 1 hour and then rinsed with distilled water. The third treatment carp bones were macerated using a 0.2% NaOH solution with a composition of 1:4 (w/v) for 2 hours with a change every 1 hour and then rinsed using distilled water, then followed by a second maceration using a 0.2% C_6H_8O_7 solution with a composition of 1:4 (w/v) for 2 hours with changes every hour and then rinsed again with distilled water.

The common carp’s bones in the first, second and third treatments then went through an extraction process using a water bath for 3 hours at 50℃ using distilled water until the bone samples were completely submerged. The extraction result in the form of a gelatin solution is stored in the refrigerator. The gelatin solution was evaporated using a rotary evaporator at 80℃ with a speed of 70 rpm until the remaining 50% of the original solution. The evaporated sample was stored in the refrigerator for 24 hours to maintain its quality.
Liquid gelatin samples are required for testing pH, gel strength and molecular weight. Dry gelatin are required for testing water content, ash content, yield, viscosity and organoleptic so that the gelatin samples are dried using an oven at 50°C for 24 hours to obtain the net weight of carp’s bone gelatin.

2.4 Gelatin characterization process

Gelatin characterization process carried out in the form of quality and quantity testing. Gelatin quality testing includes organoleptic, gel strength, viscosity, pH, moisture content, ash content and molecular weight. While testing the quantity of gelatin carried out in the form of calculating the yield content.

a. Organoleptic

Organoleptic tests are divided into hedonic tests based on the panelist’s preference level and scoring tests based on certain specifications [9]. In this study, organoleptic tests of fresh fish and gelatin were conducted using a scoring test with the respondents of the college students of the Faculty of Fisheries and Marine Affairs, Airlangga University. According to SNI 01-2346, the number of untrained panelists consists were 30 peoples [10]. Panelists will provide quality values in the form of numbers on the organoleptic test sheet of fresh fish with specifications in the form of eye appearance, gills, mucus, odor, flesh and texture. According to organoleptic test of gelatin in the form of color and odor, each has 5 scales. Color parameters include 1 (brown), 2 (yellow-brown), 3 (yellow), 4 (yellowish white) and 5 (white). The odor parameters included 1 (very smelly), 2 (smelly), 3 (slightly odorless), 4 (no smell) and 5 (very no smell) [11].

b. Yield

Yield is a comparison between the final weight and the initial weight of the product multiplied by 100% [12]. Bone samples were weighed for each treatment to determine the initial weight of the sample and then the dry gelatin was weighed to determine the net weight of the gelatin.

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\text{Yield} \% = \frac{\text{Dry Gelatin}}{\text{Sample}} \times 100\%
\]

c. pH

pH is the degree of acidity that arises due to the use of a solution during the extraction process. The pH value test is carried out using a pH meter by dipping the tool into liquid wet gelatin for a while and then the pH meter screen will show the pH value of the gelatin [5].

d. Gel strength

According to the Gelatin Manufactures Institute of America on 2013, gel strength testing was carried out using a Brookfield CT-3 Texture Analyzer. The extracted liquid gelatin was poured into a 50 ml glass beaker, covered with aluminum foil and then stored in a refrigerator at 10°C for 16 hours until the liquid gelatin turned into a gel. Gel strength was measured using a load cell of ±5 kg, a cross-head speed of 1 mm/s and a diameter of 5 mm. A beaker glass containing gelatin that has formed into a gel is placed in the middle and then pressed with a probe and left to a depth of 4 mm. Gel strength is expressed in gram bloom units on the device screen [13].

e. Viscosity

According to the Gelatin Manufactures Institute of America on 2012, the viscosity value test is carried out using a viscometer. Gelatin solution with a concentration of 6.67% (w/w) was prepared with distilled water which had been heated at 60°C (7 g gelatin plus 105 ml aquadest). The gelatin solution is placed in a 250 ml beaker glass, the rotor is installed and the speed is set to 60 rpm, the surface of the rotor must be submerged in the solution. The viscometer is then turned on and the rotor will rotate for a few minutes until it shows the viscosity value in cP units [14].
f. Water content
Moisture content is one of the important parameters that determine the quality of gelatin. According to the Association of Official Analytical Chemist on 1995, analysis of moisture content was carried out using the oven method. The porcelain cup was dried in an oven for 15 minutes at 100°C then the cup was cooled in a desiccator for 10 minutes and weighed. 1 g of sample was put into a cup of known weight, and then dried in an oven at 105°C for 5 hours until a constant weight was reached. Then the cup and its contents were cooled in a desiccator for 10 minutes and weighed. The calculation of the water content is done by comparing the weight before and after the oven in percent units [15].

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\% \text{ Water Content} = \frac{B - C}{B - A} \times 100\%
\]

Description :
A : Weight of empty porcelain cup (g)
B : Weight of porcelain cup and sample (g)
C : Weight of porcelain cup and dried sample (g)

g. Ash content
The ash content determines the amount of minerals in gelatin. According to the Association of Official Analytical Chemist on 1995 analysis of ash content can be carried out using the kiln method. The porcelain cup was dried in an oven at 100°C then cooled in a desiccator and weighed. A total of 3 g of the samples was weighed and put into a porcelain cup. Subsequently, the samples were ashed in a furnace at 550°C for 5 hours or until ash was formed. Then cooled samples in a desiccator and weighed. Calculation of ash content is done by calculating the ratio of weight before and after the kiln process [15].

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\% \text{ Ash Content} = \frac{W_1 - W_2}{W}
\]

Description :
W : Weight of sample (g)
W1 : Weight of porcelain cup and ashed samples (g)
W2 : Weight of empty porcelain cup (g)

h. Molecular weight
In testing the molecular weight of gelatin using SDS-Page, 10 μl of gelatin sample was inserted into the micro tube. The sample was denatured by dissolving the sample with 10 μl of loading protein made of 0.5% Sodium Dodecyl Sulphate (SDS) and 10% Dithiothreitol (DTT) for 5 minutes at 100°C. The sample is then put into the refrigerator before being placed into the gel electrophoresis. There are 2 parts of the gel, separating gel and stacking gel. The composition of separating and stacking gel for SDS-PAGE is 4%. Separating gel is made first and located at the bottom, while the stacking gel is at the top. Separating gel is made of 4.1 ml H2O, 3.3 ml acrylamide 30%, 2.5 ml tris-HCL 1.5, Sodium Dodecyl Sulphate (SDS) 10% 100 μl, tetramethylethylenediamine 10 μl and 1 of 10% ammonium persulfate. The stacking gel was made of 6.1 ml of H2O, 1.3 ml of 30% acrylamide, 100 μl of tris-HCL 0.5 M and 100 μl of 10% ammonium persulfate. The gel is allowed to dry but previously wells in the gel have been made. The gel was mounted on the gel electrophoresis device in a standing position and immersed in electrophoresis buffer made of fixing buffer until the gel was completely submerged. Then, 7 μl of marker and sample were added to the gel well. Running electrophoresis using a voltage of 110 V was carried out for ±40 minutes. The gel was removed and immersed in a commasie blue solution for 30 minutes then placed on a rocker. The next step is to rinse the gel with distilled water and then soak it again in the detaining solution.
for one night. Protein bands with different molecular weights will separate. The results obtained are documented through computer and scanning tools [16].

3. Results and discussion

The results of the study of the effect of differences maceration solutions towards characteristics of gelatin from bone of common carp (Cyprinus carpio) included the results of the organoleptic test of fish and the results of the gelatin characteristics test which included yield, pH, water content, ash content, viscosity, gel strength, organoleptic gelatin and molecular weight.

3.1. Fish organoleptic

Organoleptic testing of fish showed the level of freshness of the samples used as ingredients for making carp bone gelatin. The average results of organoleptic testing of fish are in Table 1.

| Parameter       | Mean ± SD |
|-----------------|-----------|
| Eyes            | 8.17 ± 0.53 |
| Gills           | 8.30 ± 0.46 |
| Mucus           | 8.23 ± 0.62 |
| Odor            | 7.93 ± 0.74 |
| Meat Appearance | 8.03 ± 0.76 |
| Texture         | 8.10 ± 0.54 |

The criteria for freshness of fish based on organoleptic testing using SNI 01-2346 on 2006, a scale of 1-3 are classified as not fresh, a scale of 4-6 is classified as kind of fresh and 7-9 is classified as fresh [10]. The results of organoleptic testing of fresh fish conducted by 30 panelists from college students of the Faculty of Fisheries and Marine, Airlangga University, showed numbers ranging from 7.93 to 8.30. This indicates that the fish used as samples for making gelatin in this study is classified as fresh fish.

Fish eye appearance based on organoleptic testing using SNI 01-2346 on 2006 by panelists showed bright fish eye appearance, flat eyeball and clear cornea with an average of 8.17. This is confirmed that the eyeball will look bright fresh fish and the fish is not fresh will look cloudy [17]. Common carp gills in this research had an average of 8.30 based on organoleptic testing by panelists. The organoleptic value of the gills indicates that the red gills are less bright and not slimy. Fish gills are the part that contains the most blood and is a medium for microbial development [18]. Fish that are not quickly treated using low temperatures after being caught will experience a decline in quality more quickly [19].

The mucus on the surface of the fish body based on organoleptic testing using SNI 01-2346 on 2006 by the panelists showed that the mucus on the surface of the fish body was clear, transparent, bright and there was no color change with an average of 8.23. The hyperaemia stage or the release of clear mucus around the fish's body is a natural reaction of fish, the lower the mucus value, the lower the quality of fish freshness [18]. The appearance of the color and flesh of the fish based on organoleptic testing using SNI 01-2346 on 2006 by the panelists showed that the cut looks brilliant, there is no milking along the spine and has an intact abdominal wall with an average of 8.03. Fish that have experienced a decrease in quality will have soft meat conditions due to the autolysis process and cause the meat to be easily separated from the bones [20].

The odor of carp based on organoleptic testing using SNI 01-2346 on 2006 by the panelists showed a neutral odor with an average of 7.93. Odor is influenced by the activity of spoilage bacteria that develop after the fish is dead. Bacterial activity will decompose fats and proteins to produce ammonia, indole and...
H₂S compounds that cause unpleasant odors in fish [18]. The texture of carp based on organoleptic testing using SNI 01-2346 on 2006 by the panelists showed that the texture of the fish was rather dense, elastic when pressed using the fingers and difficult to tear the meat from the spine with an average of 8.10. Handling fish at low temperatures will suppress the activity of spoilage bacteria so that the quality of fish can be maintained [21].

3.2. Characteristic of gelatin
The results of testing the characteristics of carp’s bone gelatin which include yield, pH, water content, ash content, viscosity, gel strength, color and odor of gelatin are shown in Table 2. Pictures of the results of dried carp’s bone gelatin are shown in Figure 1.

Table 2. Characteristics of Gelatin

| Parameter          | Mean ± SD          | SNI 06-3735 | British Standard 757 |
|--------------------|--------------------|-------------|-----------------------|
|                    | P1                 | P2          | P3                    |
| Color              | 1.57 ± 0.56a       | 4.77 ± 0.43c | 3.37 ± 0.85b          | White until yellowish |
| Odor               | 2.27 ± 0.64a       | 3.23 ± 0.62b | 3.17 ± 0.59b          | No odor               |
| Yield (%)          | 8.88 ± 0.15b       | 7.71 ± 0.17a | 10.01 ± 0.14c         | -                     |
| pH                 | 9.23 ± 0.06a       | 5.21 ± 0.07a | 6.43 ± 0.09b          | - 4.5-6.5            |
| Water Content (%)  | 7.37 ± 0.08c       | 6.42 ± 0.10b | 3.75 ± 0.13a          | ≤ 16                  |
| Ash Content (%)    | 2.22 ± 0.08c       | 1.68 ± 0.05b | 0.91 ± 0.07a          | ≤ 3.25                |
| Viscosity (cP)     | 2.55 ± 0.10a       | 4.35 ± 0.11b | 5.24 ± 0.11c          | - 1.5-7              |
| Gel Strength (bloom) | 87.66 ± 1.75a    | 126.16 ± 2.25b | 138.33 ± 2.36c        | - 50-300             |

Description: different superscript letter notation means there is a difference significantly at the DMRT test level with a 95% confidence level. P1 (NaOH); P2 (C₆H₈O₇); P3 (NaOH and C₆H₈O₇).

The results of the color appearance of dry gelatin are shown in Figure 4 and the organoleptic results of the color and odor of carp bone gelatin are shown in Table 2. The results of the Kruskall Wallis test show that the statistical data of the color and odor parameters of treatment 1 are different from treatment 2 and 3 (p<0.05), treatment 2 was different from treatment 1 and 3 (p<0.05) and treatment 3 was different from...
treatment 1 and 2 (p<0.05). Mann Whitney further test results on the gelatin color parameter showed that treatment 1 was significantly different from treatment 2 and 3 (p<0.05), treatment 2 was significantly different from treatment 1 and 3 (p<0.05) and treatment 3 was significantly different from treatment 1 and 2 (p<0.05).

Further testing of Mann Whitney on odor parameters showed that there was a significant difference between treatment 1 and treatment 2 and 3 (p<0.05) but treatment 2 was not significantly different from treatment 3 (p>0.05). Gelatin organoleptic results showed that the most influential gelatin color average value based on superscript notation sequentially was in treatment 2 of 4.77 (yellowish white); treatment 3 was 3.37 (yellow) and treatment 1 was 1.57 (brown). The most influential average gelatin odor based on superscript notation was found in treatment 2 of 3.23 (slightly odorless) followed by treatment 3 of 3.17 (slightly odorless) and treatment 1 of 2.27 (smelly).

The color of carp bone gelatin based on organoleptic testing using SNI 06-3735 on 1995 [22]. The gelatin’s organoleptic test by the panelists showed a brown to white color with an average of 1.57-4.77. The colors in this study were significantly different between treatments, which proved that the different maceration solutions affected the gelatin color. The color of gelatin from carp bone treatment 1 was macerated using NaOH solution produced a value of 1.57 which based on SNI 06-3735 on 1995 was brown. The color of gelatin from carp bones in treatment 2 was macerated using a solution of C6H8O7 resulted in a value of 4.77 which based on SNI 06-3735 on 1995 was yellowish white. The color of the common carp bone gelatin in treatment 3 was macerated with a mixture of NaOH and C6H8O7 solutions resulted in a value of 3.37 which based on SNI 06-3735 on 1995 was yellow. The color of gelatin macerated using NaOH solution does not match the standard [22]. While gelatin macerated using a solution of C6H8O7 and a mixture of NaOH and C6H8O7 solutions is in accordance with SNI 06-3735 on 1995 which is colorless to yellowish. The difference in the color of gelatin is due to the influence of the alkaline NaOH solution and the C6H8O7 acid solution which can cause the release of color pigments from the raw material so as to increase the color of the gelatin [23]. The brown color formed in gelatin is caused by a less than optimal demineralization process so that there are still a lot of mineral deposits that cause browning of the gelatin color. The color of gelatin is also influenced by the color of the raw materials used [23]. The raw material used for carp fins is orange and causing a brown to yellow color in gelatin.

The odor of gelatin produced in this research was based on organoleptic testing using SNI 06-3735 on 1995 by panelists ranging from 2.27 to 3.23. All treatments were different from each other which showed that different maceration solutions had an effect on the gelatin odor. But in treatment 2 and treatment 3 there was no significant difference. Based on SNI 06-3735 on 1995, the odor of gelatin in this research did not match the standard, which was odorless [22]. The odor of gelatin can come from the raw materials used which are then trapped during the gelatin manufacturing process. This happens because of the presence of volatile compounds that cause a pungent and distinctive fish odor such as ammonia [24]. Volatile compounds in fish can come from the environment and accumulate on the subsurface of the body [25]. C6H8O7 solution with the right concentration is known to be able to minimize the fishy odor by decomposing the volatile compounds present [26].

The results of the yield test are shown in Table 2. The results of the ANOVA test showed the difference between treatment 1 and treatment 2 and 3 (p<0.05). treatment 2 was different from treatment 1 and 3 (p<0.05) and treatment 3 were different from treatments 1 and 2 (p<0.05). After further testing of DMRT, it was found that treatment 1 was significantly different from treatment 2 and 3 (p<0.05). treatment 2 was significantly different from treatment 1 and 3 (p<0.05) and treatment 3 was significantly different from treatment 1 and 2 (p<0.05). The results of testing the yield of carp bone gelatin in this study showed the most influential average yield value based on superscript notation sequentially was treatment 3 of 10.01%; treatment 1 was 8.88% and treatment 2 was 7.71%.
The average yield of carp bone gelatin was significantly different between treatments and ranged from 7.71% to 10.01%. This proves that there is an effect of different maceration solutions on the yield of carp bone gelatin. Gelatin from carp bones macerated using a mixture of NaOH and C₆H₈O₇ solutions produced the highest yield value of 10.01%. The yield of common carp bone gelatin macerated using NaOH solution resulted in a yield of 8.88%. The yield of carp bone gelatin macerated using C₆H₈O₇ resulted in a yield of 7.71%. The yield of carp bone gelatin produced in this study was higher than the yield of catfish bone gelatin in the research of Pertiwi et al. [27] which was 6.04%. The high and low yields were caused by the fish species and the type of acid and base solution used. Acid solution C₆H₈O₇ will increase H⁺ ions and alkaline solution will increase OH⁻ ions so that the amount of conversion of collagen to gelatin increases which accelerates the process of gelatin formation and increases the resulting yield [28]. Too high acid concentrations cause further hydrolysis so that some of the gelatin will be degraded and cause low levels of gelatin yield [29]. The long soaking time also causes a reduction in the amount of yield because the bone will become very soft and dissolved during the neutralization process [27].

The results of the pH test of carp bone gelatin are in Table 2. The results of the ANOVA test showed the difference between treatment 1 and treatment 2 and 3 (p<0.05). treatment 2 was different from treatment 1 and 3. (p<0.05) and treatment 3 were different from treatments 1 and 2 (p<0.05). After further testing using the DMRT test, it was found that treatment 1 was significantly different from treatment 2 and treatment 3 (p<0.05). treatment 2 was significantly different from treatment 1 and 3 (p<0.05) and treatment 3 was significantly different from treatments 1 and 2 (p<0.05). The results of testing the pH of carp bone gelatin showed that the most influential average pH value based on superscript notation sequentially was treatment 2 of 5.21; treatment 3 was 6.43 and treatment 1 was 9.23.

The average pH of carp bone gelatin between treatments was significantly different and ranged from 9.23 to 6.43. This proves that the difference in maceration solution affects the pH of carp bone gelatin. Maceration using NaOH solution produces an alkaline pH of 9.23. Maceration using a solution of C₆H₈O₇ produces an acidic pH of 5.21 and maceration using a mixture of NaOH and C₆H₈O₇ solutions will produce a pH that is close to neutral which is 6.43. Gelatin that was macerated using NaOH solution was not in accordance with the standard, while the pH value of gelatin produced using a solution of C₆H₈O₇ and a mixture of NaOH and C₆H₈O₇ solutions in this study was in accordance with British Standard 757 on 1975 gelatin with a pH range of 4.5-6.5 [30]. The pH value is influenced by the type of solution used. The process of bone maceration using a C₆H₈O₇ acid solution can cause the development of collagen fibers. The solution will be absorbed and trapped in a network of collagen fibrils so it is difficult to neutralize during the washing process and is carried away during the extraction process which then affects the pH of the gelatin [31]. Washing after the maceration process using an alkaline solution also cannot remove all the bases contained in the collagen network, causing the remaining solution to be extracted and affecting the pH value to become alkaline [32]. The pH value that is close to neutral is able to keep the helix chain from breaking easily, thereby increasing the quality of gelatin [5].

The results of testing the water content of carp’s bone gelatin are in Table 2. The results of the ANOVA test showed the difference between treatment 1 and treatment 2 and 3 (p<0.05). treatment 2 was different from treatment 1 and 3 (p<0.05) and treatment 3 was different from treatment 1 and 2 (p<0.05). After further testing of DMRT, it was found that treatment 1 was significantly different from treatment 2 and 3 (p<0.05). treatment 2 was significantly different from treatment 1 and 3 (p<0.05) and treatment 3 was significantly different from treatment 1 and 2 (p<0.05). The results of testing the water content of carp bone gelatin showed that the average value of the most influential water content based on superscript notation sequentially was in treatment 3 of 3.75%; treatment 2 was 6.42% and treatment 1 was 7.37%.

The water content of carp’s bone gelatin produced in this study was significantly different between treatments and had an average of 3.75%-7.37%. This proves that the difference in maceration solution affects the water content of carp’s bone gelatin. Common carp bone gelatin which was macerated using a
solution of C₆H₈O₇ produced a water content of 6.42%. The maceration process using NaOH solution produces a water content of 7.37%. Maceration using a mixture of NaOH and C₆H₈O₇ solutions produces a water content of 3.75%. These three treatments have a water content value that is in accordance with the standard of SNI 06-3735 on 1995 which is below 16%. The high and low water content in gelatin is caused by the opening of the collagen bond structure by an acid or alkaline solution. The more exposed collagen structure will increase the binding capacity of gelatin to adsorbed water and weaken the binding capacity of free water. This weak binding capacity in free water causes easy evaporation of water during the drying process so that a low water content is obtained [33]. The length of time of immersion also affects the number of exposed collagen bond structures. The longer the immersion, the more open the structure of the collagen bonds will be and the binding power of gelatin in free water will be weaker [34]. The water content shows the amount of water content in the product which then determines the shelf life of the product. The lower the water content, the higher the durability of the product [35].

The results of testing the ash content of carp bone gelatin are in Table 2. The results of the ANOVA test showed the difference between treatment 1 and treatment 2 and 3 (p<0.05). treatment 2 was different from treatment 1 and 3 (p<0.05) and treatment 3 was different from treatment 1 and 2 (p<0.05). After further testing of DMRT. it was found that treatment 1 was significantly different from treatment 2 and 3 (p<0.05). treatment 2 was significantly different from treatment 1 and 3 (p<0.05) and treatment 3 was significantly different from treatment 1 and 2 (p<0.05). The results of testing the ash content of carp bone gelatin showed that the average value of the most influential ash content based on superscript notation sequentially was in treatment 3 as much as 0.91%; treatment 2 as much as 1.68% and treatment 1 as much as 2.22%.

The ash content of carp bone gelatin produced in this study was significantly different between treatments and ranged from 2.22%-0.91%. This proves that the difference in maceration solution affects the ash content of gelatin. The three treatments in this study had an ash content value that was in accordance with the standard of SNI 06-3735 on 1995 which was below 3.25%. The results of different ash content between treatments indicated that differences in maceration solution affected the ash content of carp bone gelatin. The ash content of macerated carp bone gelatin using a mixture of NaOH and C₆H₈O₇ solutions had the lowest yield compared to the other two treatments which is 0.91%. Maceration using a solution of C₆H₈O₇ gave an ash content of 1.68%. Maceration using NaOH gives an ash content of 2.22%. The high and low ash content is due to the acid solution of C₆H₈O₇ and the alkaline solution of NaOH causing the demineralization process. Bone calcium will be bound in the form of calcium phosphate into calcium citrate so that the bones become soft and the minerals contained will be dissolved [23]. NaOH solution that is too high will cause the demineralization process to be incomplete [36]. The incomplete demineralization process then causes excessive hydrolysis so that unneeded minerals will precipitate [37]. Ash content shows the amount of minerals contained in food [27]. The longer immersion time causes more calcium and other minerals to dissolve during the demineralization process [38]. The lower the ash content in gelatin, the better the quality of gelatin because the less minerals and impurities contained in gelatin [39].

The results of the viscosity test of carp bone gelatin are shown in Table 2. The results of the ANOVA test showed the difference between treatment 1 and treatment 2 and 3 (p<0.05). treatment 2 was different from treatment 1 and 3 (p<0.05) and treatment 3 were different from treatments 1 and 2 (p<0.05). After further testing of DMRT. it was found that treatment 1 was significantly different from treatment 2 and 3 (p<0.05). treatment 2 was significantly different from treatment 1 and 3 (p<0.05) and treatment 3 was significantly different from treatment 1 and 2 (p<0.05). The results of testing the viscosity of carp bone gelatin showed that the average value of the most influential water content based on superscript notation sequentially was in treatment 3 of 5.24 cP; treatment 2 was 4.35 cP and treatment 1 was 2.55 cP.

The viscosity of carp bone gelatin in this study was significantly different between treatments and had a value range of 2.55-5.24 cP. This shows that the difference in maceration solution affects the viscosity of carp bone gelatin. The results of the gelatin viscosity of these three treatments were in accordance with
British Standard 757 on 1975 which was 1.5-7 cP. Gelatin viscosity from carp bone macerated using NaOH solution had the lowest yield. which was 2.55 cP. Gelatin from carp bones macerated using C₆H₈O₇ solution produced a viscosity value of 4.35 cP. Common carp bone gelatin which was macerated using a mixture of NaOH and C₆H₈O₇ solutions had the highest viscosity value of 5.24 cP. The acid solution will cause the decomposition of collagen and the density of protein bonds which then form a long chain of helix so that the viscosity will increase [40]. Gelatin viscosity affects the gel strength value. the higher the viscosity value. the higher the gel strength value [28]. Viscosity testing shows the viscosity of gelatin. the higher the viscosity value. the thicker the gelatin produced [14]. The low value of gelatin viscosity can be caused by the high water content contained in gelatin. thereby reducing the gel viscosity [42].

The results of the gel strength test of carp bone gelatin are in Table 2. The results of the ANOVA test showed the difference between treatment 1 and treatment 2 and 3 (p<0.05). treatment 2 was different from treatment 1 and 3 (p<0.05) and treatment 3 was different from treatment 1 and 2 (p<0.05). After further testing of DMRT. it was found that treatment 1 was significantly different from treatment 2 and 3 (p<0.05). treatment 2 was significantly different from treatment 1 and 3 (p<0.05) and treatment 3 was significantly different from treatment 1 and 2 (p<0.05). The results of the gel strength test showed that the average value of the most influential gel strength based on superscript notation sequentially was in treatment 3 of 138.33 blooms. treatment 2 of 126.16 blooms and treatment 1 of 87.66 blooms.

The gel strength produced from carp bone gelatin in this study was significantly different between treatments and ranged from 87.66 to 138.33 blooms. This proves that the difference in maceration solution affects the strength of the gelatin gel of carp bones. The gel strength value of these three treatments was in accordance with British Standard 757 on 1975 which was 50-300 bloom. Maceration of gelatin from carp bones using a mixture of NaOH and C₆H₈O₇ solutions produced the highest gel strength of 138.33 blooms.

While maceration using C₆H₈O₇ solution of 126.16 blooms and maceration using NaOH solution of 87.66 blooms. The high and low gel strength is influenced by the length of the helix chain. water content and gelatin viscosity. the lower the water content and the higher the viscosity. the higher the gelatin gel strength [28]. The long immersion time can form a long helix chain. C₆H₈O₇ acid solution is able to form long -helix chains so that the gelatin network structure formed will be more rigid and resistant to high pressure [43]. Too high a concentration of NaOH can cause the destruction of the amino acid chain so that a short helix chain is formed and the gel strength value is low [44].

Testing the molecular weight of carp bone gelatin can be done using SDS-Page. Pictures of molecular weight test results are shown in Figure 2.
The results of the molecular weight testing of this study in treatment 1, 2 and 3 showed a smeared or faint protein band so that the molecular weight of all treatments could not be known which proved the extraction result was gelatin.

The marker used for molecular weight testing using SDS-Page gelatin is 10-180 kDa [45]. The results of the molecular weight test in this study showed protein bands that looked faint so it was not possible to calculate the molecular weight. The protein band in molecular weight testing using SDS-Page aims to ensure the presence of gelatin in the extraction results. This faint protein band indicates that the presence of impurities or other macromolecules such as ash and fat content has not completely disappeared [46]. Maceration using a mixture of NaOH and C₆H₈O₇ solutions showed a slightly clearer band than maceration using NaOH and C₆H₈O₇ solutions. This is related to the ash content in gelatin in this treatment is lower than other treatments. Maceration using NaOH solution and C₆H₈O₇ solution showed higher ash content and possibly other impurities. Another possibility is that there is still fat content from meat that does not completely disappear at the beginning of the gelatin making process because the fish used is still fresh and it is difficult to remove the meat that is attached to the bones. The result of gelatin which still contains a lot of impurities requires additional processing to obtain gelatin with better quality. One way is to add a process at the beginning of the manufacture of gelatin such as bone boiling to maximize the demineralization process and the removal of fat and meat that sticks to the bones [47].

The brown color of gelatin is caused by the raw materials used and the high mineral content or ash content of gelatin. The raw material used has a strong orange color pigment that causes the gelatin to brown to yellowish. Raw materials also affect the odor of gelatin which comes from volatile compounds found in carp fins and bones that are not decomposed by alkaline solutions so that the gelatin produced has a fishy odor. The high ash content is caused by the non-optimal demineralization process or mineral decomposition in gelatin which then causes a lot of impurity deposits in carp bone gelatin. The high deposition of impurities in carp bone gelatin causes browning of the gelatin color and the faintness of the protein bands on the molecular weight test results using SDS-Page so the molecular weight cannot be calculated which proves that the extraction result is gelatin. The low water content of gelatin is caused by the weakening of the binding capacity of free water by acid and alkaline solutions so that a lot of free water is lost during the drying process. Low water content causes high viscosity and gel strength values because there is less free water contained in gelatin and the helix chain formed is longer and denser. The length of the helix chain causes the gelatin molecule to become tightly packed which then results in thickening and solidification of the formed carp bone gelatin. The pH value that is close to neutral also affects the viscosity and gel strength because a neutral pH will keep the helix chain from shortening and damaging the helix chain.

4. Conclusions
Differences in maceration solution affect the characteristics of carp bone gelatin (Cyprinus carpio) and the best treatment uses a mixture of NaOH and C₆H₈O₇ solutions. The characteristics of carp bone gelatin produced in accordance with SNI 06-3735 and British Standard 757 include water content, ash content, viscosity and gel strength.

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