The effect of differences extraction solutions on the gelatin characteristic fishbone of bader bang (*Barbonymus balteoides*)

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Abstract. Gelatin is a polypeptide derived from collagen in the skin and bones of mammals or fish. The utilization of bader bang especially bader bang fish bones as raw material for gelatin is still not widely found. The purpose of this study was to determine the effect of different extraction solutions on the characteristics of bader bang fish bone gelatin and to compare the characteristics of the bader bang fish bone gelatin produced with the standard. The study was carried out using a completely randomized design (CRD). The best results from the three treatments were found in the extraction of gelatin in the treatment NaOH 0.2% + H₂SO₄ 0.2% The characteristics of water content, ash content, viscosity and gel strength complied with SNI 06-3735 and British Standard 757.

1. Introduction

Gelatin comes from the Latin word gelatos which means freezing. Gelatin is a polypeptide derived from collagen in the skin and bones of mammals or fish. In the food processing industry, gelatin is used as a composition to increase the elasticity, consistency, and stability of food [1]. Gelatin has a variety of functional properties, such as water-binding capacity, film-forming properties, making it versatile in the food, pharmaceutical, and cosmetic industries [2].

Gelatin is mostly produced from pigskin and cowhide. This is due to its relatively lower price and substantial presence. The production of gelatin from pigskin often does not meet the requirements for halal food [3] and may contain swine flu. In addition, gelatin from cowhide is feared to be contaminated with bovine spongiform encephalopathy (BSE) or mad cow disease. Bader bang fish bones can be an alternative raw material for making gelatin. Fishbones are generally one of the fish wastes that contain abundant collagen in addition to the skin. The more collagen content, the more gelatin content. The amount of collagen in fish bones varies greatly between species, the collagen content in bony fish is about 3% and collagen in cartilaginous fish is about 10% of the total protein [4].

Bader bang fish is a freshwater fish and is an endemic fish species from Indonesia and is widely distributed in Kalimantan, Java, Cambodia, Malaysia, Thailand, and Vietnam [5]. Bader bang fish is a consumption fish whose existence is quite abundant and is a close relative of Tawes fish which has been
widely cultivated by the community. In contrast to tawes fish, bader bang fish are still not widely cultivated and are indicated to experience overexploitation and overfishing in several rivers on the island of Java [6].

The use of bader bang especially bader bang fish bones into products that have high selling value is still not widely found. Therefore, research on the effect of different acids on the mixed method is one of the methods of making gelatin that needs to be done to determine the differences in the characteristics of the gelatin produced. According to [7], the produce of gelatin by the mixed method with sulfuric acid can increase the yield of gelatin.

2. Material and methods

2.1 Material

The equipment used in this research were 250 ml and 500 ml beakers, trays, measuring cups 250 ml and 1000 ml measuring cups, 10 ml volume pipettes, cutting board, filter, knife, basin, plastic, fume hood, stopwatch, orbital shaker, magnetic stirrer, refrigerator, analytical balance, petri dish, hot air oven, water bath shaker, and rotary evaporator. Tools for testing include pH meter, viscometer, texture analyzer, desiccator, porcelain cup, and basin. The materials used in this research were 150 grams of fishbones, aluminum foil, aqua dest, 1.5% NaCl, 0.2% NaOH, 0.2% H2SO4.

2.2 Sample preparation

The sample used for the extraction of gelatin came from the bones of bader bang fish obtained from collectors of bader bang fish in the Brantas river, Kediri, East Java, which was brought using an icebox with ice cubes. The sample is then placed in a plastic container before being put in an icebox and taken to the laboratory to maintain the freshness of the sample.

The initial process carried out is thawing. Thawing is the process of thawing a frozen sample until the sample can be used [8]. The thawing process is placed in plastic with running water until the bones of the bader bang fish can be separated from each other or until the sample is soft. The sample was then washed with running water in a basin to remove dirt on the sample and the remaining meat attached to the bones and skin using a spoon. The sample was then cut into smaller sizes of about 3x3 cm using a knife and a cutting board as a base. This is done to simplify the extraction process, with the smaller the particle size, the easier it will be to get the extraction results and make the extraction process run well. The smaller the size of the material to be extracted, the wider the contact area between the material and the solvent and the greater the speed at which the system reaches equilibrium [8]. Bader bang fish bones were then weighed 150 grams per replication. Bader bang fish bones were then washed using NaCl solution with a concentration of 1.5% with a ratio of 1/4 (w/v) for 1 hour. Washing bader bang fish bones using NaCl serves to remove mucus and blood [9]. The next process is the preparation of a solution of H2SO4, NaOH, and H2SO4 + NaOH. Samples and solutions are ready to be used for the maceration process.

2.3 Gelatin produce

Samples that are ready for use are then macerated. According to Tohmadlae et al. (2018) treatment was carried out using a solution of NaOH, H2SO4, and NaOH + H2SO4. Bader bang fish bones in the first treatment (P1) were soaked using 0.2% H2SO4 acid for 2 hours with changes every 1 hour to avoid saturation. Bader bang fish bones in the second treatment (P2) were soaked using 0.2% NaOH for 2 hours with changes every 1 hour to avoid saturation. Bader bang fish bones in the third treatment (P3) were immersed using 0.2% NaOH and H2SO4 for 2 hours each with changes every 1 hour to avoid saturation. The results of the treatment were filtered to take the bone which was then extracted using a water bath shaker at a temperature of 50°C for 3 hours. The extraction solution was then evaporated using a rotary evaporator at a temperature of 50°C until the remaining solution was about 50%. Drying was carried out using a hot air
oven for 16 hours at 50°C until the gelatin solution was dry. Drying is done to get the result in the form of the net weight of gelatin.

The solvents used in the maceration process were 0.2% NaOH for 2 hours, 0.2% H₂SO₄ for 2 hours, and 0.2% NaOH + 0.2% H₂SO₄ for 2 hours each [10]. The results of the maceration will be filtered and the sample is taken for extraction.

2.4 Characterization process
Gelatin characterization is needed to ensure that the extract obtained is gelatin and to guarantee the quality of the gelatin [11]. The gelatin characterization process was carried out in the form of calculating yield, gel strength, viscosity, water content, ash content, and pH.

2.4.1 Yield
Yield is the ratio of the dry weight of the extract to the amount of raw material. Bader bang fish bone gelatin which has been dried in a hot air oven for 16 hours at a temperature of 50°C is compared with the weight of the bader bang fishbone sample to get the yield value. Gelatin yield was calculated using the formula [12]:

\[
\text{Yield} \, (\%) = \frac{\text{Final weight of gelatin}}{\text{Initial weight of sample}} \times 100\%
\]

2.4.2 Gel strength
Gel strength analysis was carried out using a texture analyzer [13]. Liquid gelatin that has gone through the extraction process is put in a refrigerator at ± 4°C for 24 hours. Gel strength was determined using a Texture Analyzer (CT3 Brookfield, USA) set with a load cell of ±5 kg, a cross-head speed of 1 mm/s, and a diameter of 5 mm. The bloom bottle containing the gelatin solution is then placed in the middle and then the probe penetration is allowed to a depth of 4 mm.

2.4.3 Viscosity
Viscosity analysis was performed using a Brookfield viscometer [11]. Gelatin solution with a concentration of 6.67% (w/w) was prepared with distilled water (7 g gelatin plus 105 ml aqua dest) then the viscosity of the solution was measured using a Brookfield Viscometer. Measurements were carried out at a temperature of 60°C with a shear rate of 60 rpm [14].

2.4.4 Water content
Analysis of moisture content using the oven method [15]. The aluminum crucible was dried in an oven for 15 minutes at a temperature of 100°C. Then cooled in a desiccator for 10 minutes and weighed. A sample of 1-2 g was put into a cup of known weight, then dried in an oven at a temperature of 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. Calculation of water content is carried out by comparing the weight before and after oven in percent.

2.4.5 Ash content
Ash content analysis was carried out using the kiln method [15]. The porcelain dish was dried in an oven at 100°C, then cooled in a desiccator and weighed. One gram of the sample was weighed and put into a porcelain dish. Subsequently, the samples were ashed in an electric furnace at a temperature of 550°C for 5–6 hours or until ash was formed. The sample is then cooled in a desiccator and weighed. Calculation of ash content is done by calculating the ratio of weight before and after the kiln process.
2.4.6 pH value
The pH value (potential hydrogen) is the degree of acidity that arises due to the use of a solution during the extraction process [16]. The pH value test was carried out using a pH meter. The pH test was first carried out by rinsing the electrode with mineral-free water and dried with soft tissue. Electrodes dipped in wet gelatin were extracted using a water bath until the pH meter showed a stable reading.

2.4.7 Organoleptic and hedonic
According to [17] organoleptic/sensory is a test method using the human senses as the main tool to assess product quality using an organoleptic score sheet table. Assessment using this sense tool includes the quality specifications of appearance, smell, taste, and texture needed to assess the freshness of bader bang fish.

According to [17], hedonic testing is a test method used to measure the level of preference for bader bang fish bone gelatin using a scoring test with a preference level of 1-5. The hedonic test assessments this time included color (1: brown, 2: yellowish-brown, 3: whitish-yellow, 4: yellowish-white, 5: white) and odor (1: very smelly, 2: smelly, 3: slightly smelly, 4: no smell, very no smell) on dry gelatin of bader fish bones, bang.

2.4.8 Molecular weight
The molecular weight of gelatin was determined by Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) with 7.5% separating gel and 4% stacking gel. A 50% (w/v) gelatin solution was mixed with buffer (0.5 M Tris-HCl, pH 6.8 containing 10% SDS (v/v), glycerol, 0.5% bromophenol blue, 2-mercaptoethanol) with a ratio of 1:1 (v/v). The mixture was incubated at 90 °C for 30 min and centrifuged at 6.000 rpm to remove insoluble debris. The volume of each sample is 10 liters per well. Electrophoresis was carried out at a constant voltage of 180 V. After electrophoresis, the gel was stained with 0.1% (w/v) Coomassie blue R-250 in 40% (v/v) methanol, 10% (v/v) acetic acid and 50% (v/v) water, and then stained with 40% (v/v) methanol, 10% (v/v) acetic acid and 50% (v/v) water [12].

3. Results and discussion
The results from the research of the effect of different extraction solutions on the characteristics of bone gelatin of bader bang fish (*Barbonymus balleroides*) include the results of color, odor, yield, pH value, water content, ash content, viscosity, gel strength, molecular weight and fish organoleptic.

3.1 Fish organoleptic
Organoleptic testing is a way to determine the freshness of fish [18]. The average results of organoleptic testing of fish are shown in Table 1. Based on organoleptic and statistical tests, it is known that the organoleptic test results of bader bang fish on the parameters of eyes, gills, and mucus each have an average value of 7.43 ± 0.93; 7.53 ± 0.77 and 7.50 ± 0.73, while for meat, odor, and texture, each has an average of 7.20 ± 1.06; 7.37 ± 1.03; 7.57 ± 0.77.
Table 1. Fish organoleptic

| Parameter | Mean ± SD |
|-----------|-----------|
| Eyes      | 7.43 ± 0.93 |
| Gills     | 7.53 ± 0.77 |
| Mucus     | 7.50 ± 0.73 |
| Odor      | 7.20 ± 1.06 |
| Meat      | 7.37 ± 1.03 |
| Texture   | 7.57 ± 0.77 |

Based on SNI 01-2346-2006 samples of bader bang fish have an average value ranging from 7.20-7.57 which indicates that the fish is still in a fresh state. Fish freshness cannot be increased but maintained [19]. A way to maintain the quality of fish is by applying a cold chain that can inhibit the freezing of enzymes and microbes [20]. The freshness of fish is closely related to the quality of gelatin, one of which is color. According to [21] raw materials play an important role in the brightness of the resulting gelatin color.

3.2 Gelatin characteristics

The characteristics of bader bang fish bone gelatin in this study include yield, pH value, water content, ash content, viscosity, gel strength, color, and odor which are shown in Table 2.

Table 2. Gelatin characteristics

| Parameter          | Mean ± SD   | SNI 06-3735 | British Standard 757 |
|--------------------|-------------|-------------|-----------------------|
|                    | P1          | P2          | P3                      |
| Yield (%)          | 7.62a ± 0.17 | 8.61b ± 0.24 | 9.24c ± 0.28            | -          | -          |
| pH                 | 6.51a ± 0.12 | 9.19c ± 0.36 | 7.26b ± 0.15            | Close to neutral | 4.5-6.5 |
| Water Content (%)  | 6.54b ± 0.24 | 7.11c ± 0.24 | 4.43a ± 0.24            | ≦ 16%      | -          |
| Ash Content (%)    | 0.43b ± 0.03 | 0.56c ± 0.03 | 0.33a ± 0.02            | ≦ 3.25%    | -          |
| Viscosity (cPs)    | 7.37 ± 0.08c | 6.42 ± 0.10b | 3.75 ± 0.13a            | ≦ 16       | -          |
| Gel Strength (bloom) | 94.16b ± 3.68 | 72.66a ± 1.04 | 120.66a ± 17.00         | -          | 50-300 bloom |
| Color              | 4.43b ± 0.50 | 2.33a ± 0.88 | 4.37b ± 0.76            | Colorless to yellowish | Pale yellow |
| Odor               | 2.33c ± 0.75 | 1.67a ± 0.60 | 2.27b ± 0.52            | No smell    | -          |

Description: Different superscript letter notation mean there is a significant difference in the DMRT test level with a 95%. P1 (H₂SO₄ 0.2%); P2 (NaOH 0.2%); P3 (NaOH 0.2% + H₂SO₄ 0.2%).
The results of the gelatin yield test are shown in Table 2. Based on the Analysis of Variance (ANOVA) statistical analysis of the yield, it showed that there was an effect of different extraction solutions on the yield of bader bang fish bone gelatin (<0.05). The results of the Duncan Multiple Range Test (DMRT) further test showed that the average yield of gelatin in the 0.2% H₂SO₄ treatment (P1) was significantly different from 0.2% NaOH (P2). 0.2% NaOH (P2) was significantly different from NaOH 0.2% + H₂SO₄ 0.2% (P3) and H₂SO₄ 0.2% (P1) were significantly different from NaOH 0.2% + H₂SO₄ 0.2% (P3). H₂SO₄ 0.2% (P1) yielded a yield value of 7.62 ± 0.17%. NaOH 0.2% (P2) resulted in a yield value of 8.61 ± 0.24%. and NaOH 0.2% + H₂SO₄ 0.2% (P3) resulted in a yield value of 9.24 ± 0.28%. Treatment with 0.2% NaOH + 0.2% H₂SO₄ (P3) had the highest average yield of gelatin with 9.24 ± 0.28% and treatment with 0.2% H₂SO₄ (P1) with an average yield of gelatin. lowest with 7.62 ± 0.17%.

The average yield of bader bang fish bone gelatin in this study was significantly different for each treatment ranging from 7.62-9.24%. The difference in yield of bader bang fish bone gelatin was caused by differences in the extraction method. The concentration of the solution used to remove non-collagenous protein. and the type of material used [22]. According to [23] that proteins are not only denatured by heat but can also be denatured by pH. Acid concentrations and temperatures that are too high cause hydrolysis so that some of the gelatin is also degraded and causes a decrease in gelatin yield. The conversion of collagen to gelatin is influenced by temperature, heating time, and pH [23].

One of the chemical properties that is important to know is the pH value because the pH value is closely related to the viscosity and strength of the gel [24]. The results of testing the pH value of bader bang fish bone gelatin are shown in Table 2. The pH characteristic is one of the important things to do because pH affects other characteristics such as viscosity and gel strength. The results of the Analysis of Variance (ANOVA) test stated that there was an effect of differences in the extraction solution on the pH value of bader bang fish bone gelatin (<0.05). The results of the Duncan Multiple Range Test (DMRT) further test also showed that the average pH value of gelatin in the 0.2% H₂SO₄ treatment (P1) was significantly different from 0.2% NaOH (P2). 0.2% NaOH (P2) was different. significantly different with 0.2% NaOH + 0.2% H₂SO₄ (P3) and 0.2% H₂SO₄ (P1) significantly different from 0.2% NaOH + 0.2% H₂SO₄ (P3). 0.2% H₂SO₄ (P1) produces a pH value of 6.51 ± 0.12%. 0.2% NaOH (P2) produces a pH value of 9.19 ± 0.36%. and 0.2% NaOH + H₂SO₄ 0.2% (P3) resulted in a pH value of 7.26 ± 0.15%. Treatment with 0.2% NaOH (P2) had the highest average gelatin pH value with 9.19 ± 0.36% and treatment with 0.2% H₂SO₄ (P1) with the lowest average gelatin pH value with 6.51 ± 0.12%.

The results of the average pH value treatment of 0.2% H₂SO₄ (P1) and 0.2% NaOH + 0.2% H₂SO₄ (P3) can be said to meet SNI 06-3735 with a pH standard close to neutral and British Standard 757 with a pH standard of 4.5 - 6.5. This shows that the use of the type of solution in the treatment affects the pH [25].

The water content of a material is determined by the nature and ability of a material to attract water [26]. The results of testing the water content of the gelatin in the bones of bader bang fish are shown in Table 2. Based on the results of the Analysis of Variance (ANOVA) analysis. It is stated that there is an effect of differences in the extraction solution on the water content of gelatin in the bones of bader bang fish (<0.05). The results of the Duncan Multiple Range Test (DMRT) further tests also showed that the average pH value of gelatin in the 0.2%H₂SO₄ treatment (P1) was significantly different from 0.2% NaOH (P2). 0.2% NaOH (P2) was different. significantly different with 0.2% NaOH + 0.2% H₂SO₄ (P3) and 0.2% H₂SO₄ (P1) significantly different from 0.2% NaOH + 0.2% H₂SO₄ (P3). H₂SO₄ 0.2% (P1) produces water content of 6.51 ± 0.12%. NaOH 0.2% (P2) produces water content of 7.11 ± 0.24%. and NaOH 0.2% + H₂SO₄ 0.2% (P3) produces a water content of 4.43 ± 0.24%.

The highest water content of gelatin was found in 0.2% NaOH (P2) with 7.11 ± 0.24 and the lowest water content was in 0.2% NaOH + 0.2% H₂SO₄ (P3) treatment with 4.43 ± 0.24. The results of the water
content test of bader bang fish bone gelatin in this study ranged from 4.43 to 7.11%. The average value of water content in all treatments can meet SNI 06-3735 with a standard water content of 16%. The use of a strong concentration and type of acid can affect the lower water content. This is because the use of a strong concentration and type of acid can cause the reaction to run faster so that more peptide bonds are broken and more free water is available [27]. According to [28] alkaline solvents can also break down the amino acid structure to become weak and eventually denaturation occurs. The denaturation process causes molecular changes and decreases the amount of bound water, resulting in lower water content of gelatin [29].

The ash content of gelatin shows the number of minerals contained in the gelatin [30]. The results of the test for the gelatin ash content of bader bang fish bones are shown in Table 2. The results of the Analysis of Variance (ANOVA) analysis state that there is an effect of different extraction solutions on the ash content of bader bang fish bone gelatin (<0.05). Duncan Multiple Range Test (DMRT) further test showed that the average pH value of gelatin in the 0.2% H2SO4 treatment (P1) was significantly different from 0.2% NaOH (P2). 0.2% NaOH (P2) was significantly different from NaOH 0.2% + H2SO4 0.2% (P3) and H2SO4 0.2% (P1) were significantly different from NaOH 0.2% + H2SO4 0.2% (P3). H2SO4 0.2% (P1) produces an ash content of 0.43 ± 0.03%. NaOH 0.2% (P2) produces an ash content of 0.56 ± 0.03%. and NaOH 0.2% + H2SO4 0.2% (P3) produces an ash content of 0.33 ± 0.02%. The highest value of gelatin ash content was found in 0.2% NaOH (P2) with 0.56 ± 0.03% and the lowest ash content was in the 0.2% NaOH + 0.2% H2SO4 (P3) treatment with 0.33 ± 0.02%.

The ash content of the bader bang fish bone gelatin this time ranged from 0.33-0.56%. All treatments in this study met SNI 06-3735 with a standard of 3.25%. The difference in ash content can be caused by the type of solvent used. Acid solvents tend to produce gelatin with lower ash content than alkaline solvents. This is because the acid solvent dissolves calcium in the bones into Ca+ ions which will dissolve in the solvent so that the number of minerals is reduced [31]. Alkaline solvents can also affect the value of ash content because hydrolysis of non-protein bonds including minerals can be carried out by immersing NaOH [32].

Viscosity is one of the important physical properties of gelatin. Viscosity is the degree of viscosity of a solution. Viscosity testing is carried out to determine the level of gelatin viscosity [33]. The results of testing the viscosity of bader bang fish bone gelatin are shown in Table 2. Viscosity is the resistance of a liquid to flow [28]. Based on the results of statistical tests showed that there was an effect of different extraction solutions on the viscosity of bader bang fish bone gelatin (<0.05). The Duncan Multiple Range Test (DMRT) further test also showed that the average viscosity of gelatin in the 0.2% H2SO4 treatment (P1) was significantly different from 0.2% NaOH (P2). 0.2% NaOH (P2) was significantly different from NaOH 0.2% + H2SO4 0.2% (P3) and H2SO4 0.2% (P1) were significantly different from NaOH 0.2% + H2SO4 0.2% (P3). H2SO4 0.2% (P1) produces a viscosity of 3.24 ± 0.17 cPs. 0.2% NaOH (P2) produces a viscosity of 2.35 ± 0.18 cPs. and 0.2% NaOH + H2SO4 0.2% (P3) resulted in a viscosity of 5.06 ± 0.10 cPs. The highest
average viscosity value was found in 0.2% NaOH + 0.2% H₂SO₄ (P3) with 5.06 ± 0.10 cPs and the lowest average viscosity value was found in 0.2% NaOH (P2) with 2.35 ± 0.18 cPs.

Viscosity is one of the important physical properties of gelatin. Viscosity is the degree of viscosity of a solution. Viscosity testing is carried out to determine the level of gelatin viscosity [33]. The results of testing the viscosity of bader bang fish bone gelatin are shown in Table 2. Viscosity is the resistance of a liquid to flow (Said et al., 2011). Based on the results of statistical tests showed that there was an effect of different extraction solutions on the viscosity of bader bang fish bone gelatin (<0.05). The Duncan Multiple Range Test (DMRT) further test also showed that the average viscosity of gelatin in the 0.2% H₂SO₄ treatment (P1) was significantly different from 0.2% NaOH (P2). 0.2% NaOH (P2) was significantly different from NaOH 0.2% + H₂SO₄ 0.2% (P3) and H₂SO₄ 0.2% (P1) were significantly different from NaOH 0.2% + H₂SO₄ 0.2% (P3). 0.2% H₂SO₄ (P1) produces a viscosity of 3.24 ± 0.17 cPs. 0.2% NaOH (P2) produces a viscosity of 2.35 ± 0.18 cPs. and 0.2% NaOH + H₂SO₄ 0.2% (P3) resulted in a viscosity of 5.06 ± 0.10 cPs. The highest average viscosity value was found in 0.2% NaOH + 0.2% H₂SO₄ (P3) with 5.06 ± 0.10 cPs and the lowest average viscosity value was found in 0.2% NaOH (P2) with 2.35 ± 0.18 cPs.

The results of testing the water content of carp bone gelatin are in Table 2 and the results of the statistical calculation of the water content are attached in Appendix 3. 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 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 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 (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. 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. 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.

The results of testing the ash content of carp bone gelatin are in Table 2 and the results of statistical calculations of ash content are attached in Appendix 4. 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 (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, namely 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. NaOH solution that is too high will cause the demineralization process to be incomplete. The incomplete demineralization process then causes excessive hydrolysis so that unneeded minerals will precipitate [2]. Ash content shows the amount of minerals contained in food [20]. The longer immersion time causes more calcium and other minerals to dissolve during the demineralization process. The lower the ash content in gelatin, the better the quality of gelatin because the less minerals and impurities contained in gelatin.

Viscosity is one of the important physical properties of gelatin. Viscosity is the degree of viscosity of a solution. Viscosity testing is carried out to determine the level of gelatin viscosity [33]. The results of testing the viscosity of bader bang fish bone gelatin are shown in Table 2. Viscosity is the resistance of a liquid to flow [28]. Based on the results of statistical tests showed that there was an effect of different extraction solutions on the viscosity of bader bang fish bone gelatin (<0.05). The Duncan Multiple Range Test (DMRT) further test also showed that the average viscosity of gelatin in the 0.2% H₂SO₄ treatment (P1) was significantly different from 0.2% NaOH (P2). 0.2% NaOH (P2) was significantly different from NaOH 0.2% + H₂SO₄ 0.2% (P3) and H₂SO₄ 0.2% (P1) were significantly different from NaOH 0.2% + H₂SO₄ 0.2% (P3). 0.2% H₂SO₄ (P1) produces a viscosity of 3.24 ± 0.17 cPs. 0.2% NaOH (P2) produces a viscosity of 2.35 ± 0.18 cPs. and 0.2% NaOH + H₂SO₄ 0. 2% (P3) resulted in a viscosity of 5.06 ± 0.10 cPs. The highest average viscosity value was found in 0.2% NaOH + 0.2% H₂SO₄ (P3) with 5.06 ± 0.10 cPs and the lowest average viscosity value was found in 0.2% NaOH (P2) with 2.35 ± 0.18 cPs

Viscosity values were significantly different for each treatment and had a range between 2.35-5.06 cPs. The average value of the viscosity of all treatments can be said to meet the standards set by British Standard 757 with 1.5-7 cPs. Gelatin viscosity can be affected by temperature, pH, and concentration. Viscosity is also strongly related to the length of the amino acid chain. The higher the temperature and the concentration of the solution can break the peptide bonds of amino acids into very short so that it can reduce the viscosity [28].

Gel strength is an important physical property of gelatin because gel strength indicates the ability of gelatin in gel formation so that the use of gelatin is very wide in the food and non-food fields [24]. The results of testing the gel strength of the bader bang fish bone gelatin are shown in Table 2. The results of the statistical analysis test indicate that there is an effect of different extraction solutions on the strength of the bader bang fish bone gelatin gel (<0.05). Duncan Multiple Range Test (DMRT) further test showed that the average gel strength of gelatin in the 0.2% H₂SO₄ treatment (P1) was significantly different from 0.2% NaOH (P2). 0.2% NaOH (P2) was significantly different from NaOH 0.2% + H₂SO₄ 0.2% (P3) and H₂SO₄ 0.2% (P1) were significantly different from NaOH 0.2% + H₂SO₄ 0.2% (P3), 0.2% H₂SO₄ (P1) produced a gel strength of 94.16 ± 3.68 blooms, 0.2% NaOH (P2) produced a gel strength of 72.66a ± 1.04 blooms, and 0.2% NaOH + H₂SO₄ 0.2% (P3) resulted in a gel strength of 120.66c ± 17.00 bloom, NaOH 0.2% + H₂SO₄ 0.2% (P3) had the highest average gel strength value with 120.66 ± 17.00 blooms. The lowest average gel strength was found in 0.2% NaOH (P2) with 72.66 ± 1.04 blooms.
The average value of gel strength in all treatments showed significant differences with values ranging from 72.66 to 120.66 blooms. The average value of gel strength in all treatments can meet the standards set by British Standard 757 (50-300 bloom) [3] stated that a good extraction pH is a pH that is close to neutral. This is because extraction using a solution with a pH close to neutral can keep the amino acid chain bonds from being damaged so that it can produce a higher gel strength value. The value of gel strength is directly proportional to the value of viscosity, which is the longer the amino acid chain. The greater the value of gel strength. The length of this amino acid chain is due to the optimum breakdown of collagen. Excessive denaturation will cause the amino acid chain to become shorter so that it can reduce the value of gel strength and viscosity.

![Figure 1. Bader bang fishbone dried gelatin
Description: P1 (H₂SO₄ 0.2%) P2 (NaOH 0.2%); P3 (NaOH 0.2% + H₂SO₄ 0.2%).](image)

Good gelatin is generally colorless or white. The results of the Kruskal Wallis test showed that there was an effect of different extraction solutions on the color of the bader bang fish bone gelatin (<0.05). Mann-Whitney further test showed that the average color value of gelatin in the 0.2% H₂SO₄ treatment (P1) was significantly different from 0.2% NaOH (P2), 0.2% NaOH (P2) was significantly different from 0.2% NaOH + H₂SO₄ 0.2% (P3) but H₂SO₄ 0.2% (P1) was not significantly different from NaOH 0.2% + H₂SO₄ 0.2% (P3), H₂SO₄ 0.2% (P1) produces a color value of 4.43 ± 0.50 (yellowish-white), 0.2% NaOH (P2) produces a color value of 2.33 ± 0.88 (yellowish-brown), and NaOH 0.2% + H₂SO₄ 0.2% (P3) produces a color value of 4.37 ± 0.76 (yellowish-white). The results showed that the highest color was found in 0.2% H₂SO₄ (P1), namely 4.43 ± 0.50 (yellowish-white), and the lowest color was found in 0.2% NaOH (P2), namely 2.33 ± 0.88 (yellowish-brown).

The average value of 0.2% NaOH color (P2) does not meet SNI 06-3735 because it has a value of 2.33 or yellowish-brown but 0.2% H₂SO₄ (P1) and 0.2% NaOH + H₂SO₄ 0.2% (P3) shows values of 4.43 and 4.37 respectively or yellowish-white which can meet SNI 06-3735 with a color standard from colorless to yellowish. The color of gelatin usually depends on the pretreatment given. The use of alkaline solutions in gelatin extraction produces gelatin with a darker yellowish color [15]. The concentration and type of solvent can also affect the color of the gelatin because the stronger the solvent used. The darker the color of the gelatin produced [27]. Another factor is the treatment with 0.2% NaOH (P2) has a higher ash content than the others. According to [32]. The high ash content in gelatin can also produce gelatin with a darker color.

The results of the aroma test of bader bang fish bone gelatin are shown in Table 2. The results of the Kruskal Wallis test show that there is an effect of different extraction solutions on the aroma of bader bang fish bone gelatin (<0.05). Mann-Whitney further test showed that the average aroma value of gelatin in the 0.2% H₂SO₄ treatment (P1) was significantly different from 0.2% NaOH (P2). 0.2% NaOH (P2) was significantly different from 0.2% NaOH + H₂SO₄ 0.2% (P3) and H₂SO₄ 0.2% (P1) were significantly different from NaOH 0.2% + H₂SO₄ 0.2% (P3). H₂SO₄ 0.2% (P1) produces an aroma value of 2.33 ± 0.75 (smell). 0.2% NaOH (P2) produces an aroma value of 1.67 ± 0.60 (very smelly), and NaOH 0.2% + H₂SO₄ 0.2% (P3) resulted in an aroma value of 2.27 ± 0.52 (odor). The highest average value of gelatin aroma was
found in the 0.2% H₂SO₄ treatment (P1) with 2.33 ± 0.75 (odor) while the average gelatin aroma value was found in 0.2% NaOH treatment (P2) with 1.67 ± 0.60 (very smelly).

All treatments in this study could not meet the standards set by SNI 06-3735 because they had a value of 1.67-2.33 or still had a fishy aroma or fish-specific odor and could not be accepted by the panelists. The low acceptance of the bader bang fish bone gelatin aroma is because there is still a specific or distinctive fish aroma in the bader bang fish bone gelatin. This is possible because there are volatile compounds derived from raw materials. These volatile compounds will interact with the proteins contained in fish during the processing process, causing a specific or distinctive aroma of fish found in gelatin. On the other hand, according to [34], the use of strong acids in the gelatin extraction process can reduce the fishy odor of gelatin.

The molecular weight test of bader bang fish bone gelatin can be carried out using SDS-PAGE. A picture of the results of testing the molecular weight of bader bang fish bone gelatin is shown in Figure 2.

![Figure 2. Molecular weight test results](image)

Description: M (Marker); P1 (H₂SO₄ 0.2%); P2 (NaOH 0.2%); P3 (NaOH 0.2% dan H₂SO₄ 0.2%)

The results of the molecular weight test using SDS-PAGE showed that the protein bands in all treatments (P1, P2, and P3) looked faint so it was not possible to know the molecular weight of the protein which indicated that the extraction result was gelatin.

The results from molecular weight testing using SDS-PAGE are in the form of separate protein bands based on differences in molecular weight [20]. The results of molecular weight testing using SDS-PAGE showed that the protein bands in all treatments (P1, P2, and P3) were faint, so it was not possible to know the molecular weight of the protein which indicated that the extraction result was gelatin. This can be caused by the extraction temperature using a minimum temperature of 50°C [35]. The content of gelatin which still contains impurities such as fat is also one of the causes of protein bands to look faint [36]. The average molecular weight of each gelatin ranges from 50-200 kDa [37].

Based on the results and discussion shows that the freshness of fish is closely related to the quality of gelatin, one of which is color. This is because the freshness of the raw materials plays an important role in the brightness of the gelatin color produced. The raw material also affects the aroma of the gelatin produced because the volatile compounds in the raw material cause the gelatin to have a fishy smell. The pH value is a value that greatly affects other characteristics such as yield, viscosity, and gel strength. Good extraction is to use a pH that is close to neutral. This is because the pH that is close to neutral can keep the amino acid chain bonds from being damaged so that it can produce a higher gel strength value. The value of gel strength is directly proportional to viscosity, where the longer the amino acid chain, the greater the value of gel.
strength and viscosity. The conversion of collagen to gelatin is also influenced by pH so that it affects the gelatin yield. The water content can affect the color and aroma of gelatin because the higher the water content, the higher the enzyme activity and the growth of spoilage bacteria. Water content can also affect the viscosity and strength of the gel because more water content in gelatin interferes with the peptide bonds of amino acids in gelatin. The less than optimal demineralization process causes the high value of ash content. The higher the value of ash content can cause the color of the gelatin to be darker due to the high mineral content in gelatin.

4. Conclusion
Based on the results of the study it can be concluded that the difference in the extraction solution on the characteristics of the bader bang fish bone gelatin has a significant effect on the characteristics of the bader bang fish bone gelatin and the best treatment is found in the 0.2% NaOH + 0.2% H2SO4 (P3) treatment. Characteristics of bader bang fish bone gelatin that can meet SNI 06-3735 and British Standard 757 include water content, ash content, viscosity, and gel strength.

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