Effect of acid, base and mixed treatment towards characteristics gelatin of bader bang (*Barbonymus balleroides*) skin

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Abstract. Gelatin is a type of protein obtained from natural collagen found in skin and bones. One of the fishery products that is thought to contain collagen. This research was conducted using laboratory experimental method in this study used a completely randomized design (CRD). The data obtained were then analyzed by ANOVA method and further test using Duncan Multiple Range Test (DMRT) at a significance level of 5%. The color and aroma data were analyzed using the Kruskal Wallis method and further testing using the Mann-Whitney method. The results of the treatment of 0.2% NaOH base (P1), 0.2% CH₃COOH acid (P2) and 0.2% NaOH mixture and 0.2% CH₃COOH (P3) have the main characteristic results as follows: yield 13.5%; 12.3%; 15.8%, pH 10.6; 5.6; 6.7, gel strength 241 bloom; 251 blooms; 273 bloom, viscosity 3.6 cP; 4.5 cP; 6.8 cP, 8.6% moisture content; 5.3%; 4.8%, ash content 2.6%; 1.8%; 0.8%, the color is brownish yellow (alkaline); yellow (sour); yellowish white (mixed); aroma i.e. smell (alkaline and sour); neutral (mixed). The best treatment was found in the mixed treatment (P3) NaOH 0.2% and CH₃COOH 0.2%.

1. Introduction

Proteins are long chains of amino acids linked together by many bonds called peptide bonds. Proteins are made up of 20 kinds of amino acids. Proteins are found in the living systems of all organisms, both those at low levels and high levels of organisms. Proteins have a complex main function in all biological processes [1]. Gelatin is a type of protein obtained from natural collagen found in skin and bones [2]. Utilization of fish skin is one of the ideas to use fishery products as raw material for making gelatin. One type of structural protein composed of various amino acids in the form of amino proline, hydroxyproline, alanine and glycine is collagen. Collagen that has gone through a partial hydrolysis process becomes a biopolymer product in the form of gelatin [3].

Bader bang fish are freshwater fish, the optimum acidity level is at pH 6.5 to 7, the optimum temperature suitable for bader bang fish is a temperature of 20°C to 26°C, lives in tropical waters, can be used as aquarium fish or commercial use [4]. The methods used in the manufacture of gelatin are acid, base and mixed methods. The extraction process using the mixed method will produce a pH that is close to neutral and form gelatin with a higher gel strength than extraction using only acid or base alone. The use of mixed methods needs to be done because the manufacture of gelatin with a mixture...
of acids and bases can break the collagen polypeptide chain bonds into dipeptide or monopeptide bonds, so that it can increase the gel strength value which is higher than extraction using acid or base alone [5].

The use of NaOH base solution during maceration will break the protein secondary bonds and close the empty space around the protein space so that the distribution of peptide molecules occurs which affects the viscosity value [6]. The use of CH3COOH acid solution (acetic acid) during maceration serves to hydrolyze collagen so that at the time of extraction, the gelatin structure in the skin is more easily decomposed [7]. The use of bader bang fish on the skin is a product that has high selling value. Research on the use of acid, alkaline and mixed treatments is one way to find out which gelatin product is the best treatment for man and has the most optimal quality and quantity. Therefore, there is a need for research on the differences in the treatment of acids, bases and mixtures on the characteristics of gelatin from bader bang fish.

2. Material and methods
This research was conducted from April 2021 to June 2021. The gelatin extraction process was carried out at the Analytical Chemistry Laboratory, Faculty of Fisheries and Marine, Universitas Airlangga. Tests for pH, water content, ash content and gel strength were carried out at the Analytical Chemistry Laboratory, Faculty of Fisheries and Marine, Airlangga University. The SDS-Page test was conducted at the Institute for Tropical Disease, Airlangga University. Viscosity testing was carried out at the Seaweed Capsule Shell Teaching Industry, Airlangga University. Equipment used in the manufacture and characterization of bader bang fish skin gelatin (Barbonymus balleroides) in the form of a cutting board, knife, basin, 1000 ml and 2000 ml beaker glass, 1000 ml measuring cup, petri disk, stirring rod, sieve, 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 backbone (vertebrae) from the base of the operculum to the anus and the fins of the bader bang fish (Barbonymus balleroides), aluminum foil, aquades, plastic, label paper, 1.5% NaCl, 0.2% NaOH, and 0.2% CH₃COOH.

3. Results and discussion
The results of this study include the results of organoleptic tests of fish and test results of gelatin characteristics, namely yield, pH, gel strength, viscosity, water content, ash content, color, aroma and SDS-Page.

3.1. Fresh fish organoleptic
The results of organoleptic testing of fresh fish are shown in Table 1.

| Parameter | Average ± SD |
|-----------|--------------|
| Eye       | 7.5 ± 0.82   |
| Gill      | 7.57 ± 1.04  |
| Mucus     | 7.33 ± 0.92  |
| Meat      | 7.53 ± 1.16  |
| Smell     | 6.47 ± 1.47  |
| Texture   | 7.21 ± 0.97  |

Based on organoleptic and statistical tests, it is known that the organoleptic test results of bader bang fish on the appearance of the eyes, gills, and mucus each have an average value of 7.5; 7.57 and 7.33; while for meat, smell, and texture each has an average of 7.53; 6.47 and 7.21. According to the organoleptic standard of fresh fish, namely SNI 01-2346-2006 samples of bader bang fish have an average value of 7 which means the fish are still in a fresh state even though they experience a slight deterioration in quality due to the journey from the fish directly caught to the laboratory. According to
[8] in handling fresh fish, the temperature is kept low close to 0°C. Handling of fish must be done as quickly as possible to avoid deterioration of the quality of fish so that materials and cooling media are needed very quickly in lowering the temperature of the fish in the fish thermal center. Freshness of fish is closely related to the quality of gelatin [9]. The freshness of a raw material affects the continuity of the molecular weight distribution which is directly related to the high or low strength of a gel.

3.2. Characteristics of gelatin

The results of testing the characteristics of bader bang fish skin gelatin consist of yield, pH, gel strength, viscosity, water content, ash content, color and aroma of gelatin are shown in Table 2.

| Component           | P1 (Alkali) | P2 (Acid) | P3 (Mix) | SNI 06-3735 | British Standard 757 Gelatin |
|---------------------|-------------|-----------|----------|-------------|-----------------------------|
| Yield (%)           | 13.59±0.39  | 12.32±0.50| 15.84±0.50| -           | -                           |
| pH                  | 10.62±0.06  | 5.65±0.03 | 6.72±0.06| Netral (7)  | 4.5-6.5                     |
| Gel Power (Bloom)   | 241.83±1.04 | 251.66±1.75| 273.00±5.56| -           | 50-300 bloom                |
| Viscosity (cP)      | 3.66±0.07   | 4.55±0.11 | 6.83±0.11| -           | 1.5-7 cP                    |
| Water content (%)   | 8.62±0.08   | 5.35±0.10 | 4.84±0.12| ≤16%        | -                           |
| Ash Level (%)       | 2.64±0.09   | 1.83±0.07 | 0.85±0.09| ≤3.25%      | -                           |
| Color               | 2.40± 1.133 | 4.17± 0.592| 3.07± 0.980| Colorless  | Pale yellow                |
| Aroma               | 2.87± 0.819 | 3.27± 0.521| 3.52± 0.571| Normal      | -                           |

Description: different letter notations indicate a significant difference in the DMRT test level, which has a 95% confidence level. P1 (NaOH 0.2%), P2 (CH₃COOH 0.2%), P3 (NaOH 0.2% and CH₃COOH 0.2%)

Figure 1. Bader bang fish skin dry gelatin
Description: alkali (NaOH 0.2%); acid (CH₃COOH 0.2%); mix (NaOH 0.2% and CH₃COOH 0.2%)

Gelatin color in this study has 5 levels of preference test. The color has the standard requirements of SNI-06 3735 which is colorless to yellowish and in British Standard 757 it is pale yellow. The colors obtained by alkaline treatment were 2 (brown yellow), acid treatment 4 (yellowish white) and
mixed treatment 3 (yellow). The results that show the best treatment are in the acid treatment (CH3COOH), which is yellowish white. It is known, the base treatment is not according to the standard because it is brownish yellow. However, in the treatment of acids and mixtures according to standard requirements, namely yellowish white for acids and whitish yellow for mixtures. According to [10] stated that the difference in color was caused by the extraction and solvent conditions indicated by their respective pH. In addition, the color itself depends on the type of raw material and in general, color does not affect the functional properties of gelatin [11].

In this research, gelatin aroma parameter has 5 levels. Aroma has British Standard 757 requirements, which is normal (acceptable to consumers). Aroma obtained by alkaline treatment was 2 (odor), acid treatment 3 (neutral) and mixed treatment 4 (no odor). The results that show the best treatment are in the mixed treatment (NaOH and CH3COOH), which is odorless. However, the base and acid treatments cannot meet the standards because they have a fishy smell which, according to the panelists, is unacceptable to consumers. In contrast to the mixed treatment, there was a slight fishy smell which resulted in meeting the standards that according to the panelists were acceptable to consumers. This can be due to the presence of volatile compounds derived from raw materials. Volatile compounds will interact with proteins contained in fish, causing a specific or distinctive fish aroma found in gelatin [12].

The yield value of bader bang fish skin gelatin was significantly different for each treatment. The average yield obtained by alkaline treatment is 13.59%, acid treatment is 12.32%, and mixed treatment is 15.84%. The results that showed the best treatment were found in the mixed treatment (NaOH and CH3COOH) which was 15.84%. The difference in yield results could be due to the nature of the solution affecting the possibility of being used to remove non-collagenous proteins and the type of material used [13]. Low yields occur due to incomplete hydrolysis of collagen or loss of collagen during washing [14]. The yield value may vary depending on the protein composition of the fish skin of each species and the extraction method used [15].

The pH produced by bader bang fish skin gelatin has a significant difference. The average pH obtained by alkaline treatment was 10.62, acid treatment was 5.65 and mixed treatment was 6.72. The results that show the best treatment are in the mixed treatment (NaOH and CH3COOH) which is 6.72. pH is one of the chemical properties that is known to be closely related to gel strength and viscosity [16]. The pH value of gelatin is influenced by the type of extraction material. Making gelatin with a mixture of acids and bases will produce a neutral pH. Gelatin extraction using a mixed solution also produces a higher gel strength value than acid-only or alkaline-only extraction [17].

The gel strength value of bader bang fish skin gelatin was produced in the range of 241-273 bloom. The gel strength obtained by alkaline treatment was 241.83 bloom, acid treatment was 251.66 bloom and mixed treatment was 273 bloom. The results that show the best treatment are in the mixed treatment (NaOH and CH3COOH) which is 273 blooms. Gel strength is an aspect to determine the quality of gelatin. According to [18] stated that the quality of gelatin can be assessed from various aspects, including del strength and viscosity, the higher the gel strength value, the higher the gelatin quality. It is known that alkaline treated gelatin has the lowest value, the cause is the possibility of the collagen hydrolysis process that occurs resulting in short and weak polypeptide chains and has very little intermolecular branching so that the gel strength is also low [19].

The viscosity value of bader bang fish skin gelatin was produced in the range of 3-6 cP. The viscosity obtained by alkaline treatment was 3.66 cP, acid treatment was 4.55 cP and mixed treatment was 6.83 cP. The results that showed the best treatment were found in the mixed treatment (NaOH and CH3COOH) which was 6.83 cP. The resulting viscosity value meets the standard requirements set by British Standard 757, namely 1.5-7 cP. As well as gel strength, viscosity is an aspect to determine the quality of gelatin. The cause of viscosity in alkaline treatment produces a low value because NaOH (base) breaks the amino acid chain into smaller units so that the fluid flows more easily and does not form viscosity [20].
The water content in the research results of bader bang fish skin gelatin is in the range of 4.8-8.6%. The water content obtained by alkaline treatment was 8.62%, acid treatment was 5.35% and mixed treatment was 4.84%. The results that show the best treatment are in the mixed treatment (NaOH and CH₃COOH) which is 4.84%. The value of the water content of all treatments met the standard requirements of SNI 06-3735, namely 16%. It is known, the value of water content in the mixed treatment is low, but the lower the water content, the better the gelatin produced. In alkaline treatment, the water content decreased due to the more open collagen structure [21], weak bonds resulted in gelatin with a weak structure, so that the water holding capacity of gelatin was less strong. Weak binding capacity makes water volatile so that the water content of gelatin is lower [22].

The ash content in the research results of bader bang fish skin gelatin is in the range of 0.85-2.64%. The ash content obtained by alkaline treatment was 2.64%, acid treatment was 1.83% and mixed treatment was 0.85%. The results that show the best treatment are in the mixed treatment (NaOH and CH₃COOH) which is 0.85%. The ash content of all treatments met the standard requirements of SNI 06-3735, namely 3.25%. It is known, the value of ash content in the mixed treatment is low, but the lower the ash content, the better the gelatin produced. In alkaline treatment, it produces poor ash content because alkaline solvents dissolve calcium in bones so that the amount of minerals is reduced [23]. The value of ash content in alkaline treatment is influenced by hydrolysis of non-protein bonds that can be carried out during NaOH immersion [6].

3.3. Molecular weight

The results of molecular weight testing using SDS-Page are shown in Table 3. and Figure 2.

### Table 3. SDS-Page data results

| Treatment | SDS-PAGE (kDa) |  |
|-----------|----------------|---|
|           | B   | α1    | α2   |
| Alkali    | 222.203 | 120.956 | 107.444 |
| Acid      | 230.200 | 122.730 | 110.472 |
| Mix       | 250.203 | 130.906 | 121.000 |

![Figure 2. Result of SDS-Page gelatin](image)

Description: Marker; A (acid treatment CH₃COOH 0.2%); B (alkali treatment NaOH 0.2%); C (mic treatment NaOH 0.2% and CH₃COOH 0.2%)
The results of the SDS-Page test on the molecular weight of gelatin are visible. The base treatment gelatin was (222.203 kDa), 1 (120.956 kDa), 2 (107.444 kDa). Acid treated gelatin has a molecular weight of (230.200 kDa), 1 (122.730 kDa), 2 (110.472 kDa). Gelatin mixed treatment resulted in molecular weights (250.203 kDa), 1 (130.906 kDa), 2 (121.000 kDa). On SDS-Page the electroforesis pattern shows that gelatin contains two chains, namely 1 and 2 chains. The resulting gelatin also contains chemical components with high molecular weight, namely (chain dimers) components. The presence of the component indicates the presence of cross-linked in the molecule [5]. It is known that all gelatin samples contained gelatin protein molecular weights. Based on these results, the molecular weight decreases along with the concentration of the extracting material. It can break the peptide bonds of proteins into amino acids. Factors that affect peptide bonds include temperature, extraction time and concentration of extracting material [24].

4. Conclusion
Based on the results of the study it can be concluded that different treatment of acid, base and mixture had a significant effect on the characteristics of the skin gelatin of bader bang fish (Barbonymus balleroides) and it was known that the mixed treatment (NaOH and CH3COOH) had the best treatment. Characteristics of bader bang fish skin gelatin which refers to and complies with SNI 06-3735 and British Standard 757 Gelatin includes gel strength, viscosity, water content and ash content.

5. References
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