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The Effect of Acid Concentration and Duration of Submersion toward the Characteristics of Gelatin of Eel Fish Bone (*Anguilla bicolor*) Produced through Acid Process

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Abstract. Eel fish (*Anguilla* sp.*)* is one kind of freshwater aquaculture that has high selling value. Efforts to reduce fish bone waste as well as an alternative way to overcome, its halal problem is by producing it into gelatin. Gelatin is a water-soluble conversion protein that can either be a gelling agent (gel-making ingredients) or a non-gelling agent. This study aims to determine the effect of acid concentration and the duration of submersion to gelatin of eel fish produced through the acid process. The used treatment was in a different concentration of HCl recpectively 4%, 5%, and 6% and 24 hours and 48 hours of submersion time. The ANOVA significance of 0.5 indicates that the acid concentration and the duration of submersion have an effect on the gelatin characteristics formed on the submersion, gel strength, viscosity, and pH. Based on the results of the submersion test, pH, viscosity, and gel strength, the best treatment was obtained at 4% HCl concentration with the submersion duration of 2 days consecutive period of 4.29%; 5.70; 5.90 cP; and 200.15 bloom. The proximate content of the best treatments including moisture content, ash content, and protein content were respectively 7.43%; 1.71%; and 87.84%.

1. Introduction

Eel fish (*Anguilla* sp.) is one type of freshwater fish that has high selling value and has been cultivated through intensive or extensive system especially in Asia [1]. The market demand for eel fish is very high, in 2010 world eel production is estimated at 8,440 tons worth 36 million US $ [2]. Seafood processing produces more than 60% by products, consisting of offal, head, bone, and skin. It is one of raw material for gelatin. The raw material of gelatin in Indonesia is still imported goods, especially from Europe, America, and China that are not guaranteed halal [3]. According to Gelatine Manufacturers of Europe, in the 2002 world gelatin production of pig skin was 41% while cow leather was 28.6%. The use of leather and pig bones as a raw material of gelatin is not appropriate when applied in a predominantly Muslim country like Indonesia [4]. One alternative way to overcome the above problems is to innovate by making gelatin from eel fish waste. In principle, the process of making gelatin can be divided into two types, namely Type A and Type B. In the process of making gelatin type A through the acid process, the raw materials are subjected to submersion in acidic solutions such as hydrochloric acid, sulfuric acid, sulfite or phosphoric acid, production of gelatin type B through alkaline processes, the treatment given is a soaking in lime water, this process is often known as the alkaline process [5]. Gelatin from fish bone including gelatin type A, so it is necessary to do the treatment process with an acid solvent that is hydrochloric acid to produce gelatin in accordance with standard and gelatin which halal. Therefore, it is necessary to research the use of solvent concentration and duration of submersion in order to obtain gelatin from eel bone fish extract (*Anguilla bicolor*) according to market standard and guaranteed halal.

2. Research Method

2.1. Materials

The raw materials used in the manufacture of gelatin from eel fish bone is *Anguilla bicolor* bone obtained from Unagi UNS Purwosari Surakarta. HCl support materials (4%, 5%, and 6%) and aquadest were obtained from chemicals store Agung Jaya in Surakarta. While the tool used is one-axis gas stove, medium-size pot, thermometer, aluminum stirrer, waste plastic, toothbrush, basin container, cabinet dryer (Tew Electric Heating Equipment IL-80EN), Analytical Scales (And GF -200), label paper, 10 ml measuring pipette, 1 ml pipette, propeller, erlenmeyer 1000 ml, 500 ml beaker glass, 1000 ml beaker glass, 500 ml measuring cup, 250 ml measuring cup, 500 ml measuring flask, aluminum foil , sieve, 100 ml beaker glass, pH meter (Hanna), glass stirrer, water bath (memmert), glass funnel, filter paper whatman no.9, edible tray, blender, plastic clip medium size, incubator, Universal Testing Machine (Zwick Z 0.5), Viscometer (Brookfield DV II + Pro), Porcelain Grille, Desiccator (Iwaki Asahi Techno Glass), kiln (Neycraft).

2.2. Degreasing Process

The process of degreasing is the process of preparation of raw materials, in this study using raw materials of eel and gelatin bones in the market as a control. In the preparation stage of the process of washing or cleaning the bones by removing dirt, meat residue, and fat on the bone. To facilitate the cleaning process, eel bone fish boiled at a temperature of 80^{0}C for 30 minutes, then bone cleaned and dried. The dried eel fish bone is cut to 2-3 cm [6].

2.3. Demineralization Process

The next process is demineralization, which is the process of removing calcium and mineral salts contained in the bone so that the resulting bone becomes soft. Demineralization process is done by soaking the bone in HCl solution with 4%, 5%, and 6% concentration for 24 hours and 48 hours [7]. The ratio of bone and HCl solution is 1: 4. In the process of submersion with HCl solution for 48 hours, HCl solution is changed every 24 hours. The eels of ossein eels are then washed with distilled water until the pH is neutral (6-7) [8].

2.5. Extraction, Drying and Physical and Chemical Analysis

Then extraction process using hot water, wherein this process denaturation occurs, increase of hydrolysis, and solubility of gelatin. Neutral pH Ossein is inserted into a beaker glass and aquadest is added, the ossein and the aquadest ratio is 1: 3 (w / w). After that, the ossein solution was extracted from the water bath at 85 ± 2.5^{0}C for 6 hours [7]. The filtrate extracted then filtered with Whatman paper.

Filtrate that has been filtered with a paper of Whatman is then poured into an edible tray that has been cleaned for drying in a cabinet dryer at 50^{0}C for 24 hours to obtain gelatin sheet. The gelatin sheet obtained was crushed using a blender to make gelatin powder [8].

Eel bone gelatin analysis method includes Yield [9], viscosity test [10], gel strength test [11][12][13], acidity test [10], water content test [9], ash content test [9], and protein content test [9].

2.6. Data Analysis

The data obtained were then analyzed statistically with One Way Analyze of Variance (ANOVA) using SPSS 23 application. If the result of the analysis showed a real difference between treatments then continued by using Duncan Multiple Range Test (DMRT) with significance level $\alpha = 0{,}05$.

3. Results and discussion

3.1 Characteristics of Raw Materials

Table 1 Result of Proximate Fish Size Analysis

Parameters (% db/db)	Eel Fish Bone	Tilapia Fish Bone (*)	Red Snapper Fish Bone (**)	Catfish Bone (***)
Water content	7.94	7.92	8.06	11.35
Ash Content	37.11	62.56	59.21	42.54
Protein levels	29.36	20.85	26.91	14.55
Fat level	22.4	6.86	4.12	31.35

* [14], ** [7], *** [15]

Raw materials in this study were obtained from Unagi UNS Purwosari, Surakarta. Eel fish maintenance in Unagi UNS is done starting elver stadia (small ell fish) to adult eel fish ready to be sold. At a glance, eel fish looks similar to the eel, but the eel fish body is more elongated and has a triangular head and has four pectoral fins which are often called ear, rectum, back, and tail. Has a very fine scales and the body is covered with mucus. Eel fish body covered with mucus, so the water condition must be kept clean and pH to remain neutral. In this study using eel fish 6 months old with a weight of 400-600 grams, length reaches 90-150 cm with a body diameter ± 7.5 cm. Eel fish waste generated from Unagi UNS in the form of head, bone, scales, and viscera. The raw materials used are bone waste obtained from the production of eel fillet (Tabel 1). Eel fish bone raw materials used in gelatin making need to be considered freshness level. Fish bones in fresh condition can affect the quality of ossein and gelatin produced. [16], states that the fressher the fish bone is used then the quality of gelatin produced is also better.

3.2 Process Optimization

Tabel 2. Gelatin Characteristic of the eel fish bone (*Anguilla bicolor*) produced through an acidic process

Formula	Parameter			
	Yield (%)	pH Value	Viscosity (cP)	Strength Gel (bloom)
$F1_{24}$	9.31^{e}	5.33^{c}	4.87^{a}	199.75^{c}
$F1_{48}$	4.29^{b}	5.70^{a}	5.90^{d}	200.15^{c}
$F2_{24}$	7.80^{d}	5.11^{b}	5.30^{b}	165.78^{b}
$F2_{48}$	3.90^{b}	5.53^{d}	5.70^{cd}	160.37^{b}
$F3_{24}$	5.60^{c}	4.97^{a}	5.63^{c}	133.84^{a}
$F3_{48}$	3.04^{a}	5.31^{c}	5.17^{b}	175.54^{bc}

Different letter notation in the same column shows a significant difference at $\alpha = 0.05$. Gelatin Formula = $F1_{24}$ (HCl 4%, 24 hours); $F1_{48}$ (HCl 4%, 48 hours); $F2_{24}$ (HCl 5%, 24 hours); $F2_{48}$ (HCl 5%, 48 hours); $F3_{24}$ (HCl 6%, 24 hours); $F3_{48}$ (HCl 6%, 48 hours).

Table 2 shows that gelatin content was obtained which ranged from 3.04% -9.31%. The highest value of gelatin yield was obtained by treatment of 4% concentration with 24 hours of submersion time which was 9,31%. From the results of the research, it was seen the tendency that the higher the hydrochloric acid concentration, the lower yield result. It is assumed that the amount of yield value is influenced by the concentration of HCl used in the submersion. High concentrations and long submersion times are assumed that it is able to reduce the amount of gelatin yield produced. The reason of this case is that the produced ossein in the treatment becomes very soft and crushed, so it causes many osseins are lost during the neutralization process.

Measurement of the gelatin pH value is important to do, because the pH of gelatin solvent affects other properties such as viscosity and gel strength, and will affect gelatin application in the product. Gelatin with a neutral pH will be stable and its use will become wider [17]. The obtained value of gelatin pH ranged from 4.97 to 5.70. This value still meets the standard type A gelatin required by GMIA[18] and referred to Amiruldin [19] which was ranged from 3.8 to 6.0. The pH value range of produced eel fish gelatin was higher than commercial gelatin based on the test result by Nurilmala [15] that was 5.00. The best pH value is close to neutral condition (pH 7) owned by treatment of 4% HCl concentration with 48 hours submersion time that is equal to 5,70. While the lowest pH value is in the treatment of 6% HCl concentration with 24 hours of submersion time that is equal to 4.97.

Gelatin with neutral pH will be very well used for meat products, pharmacy, photography, paint, and so on. While gelatin with low pH will be very good used in juice product, mayonnaise, sour flavor syrup, and so on [15]. Gelatin from eel fish bone has a pH value ranging from 4.97 to 5.70, making it suitable for acid products. Gelatin with a neutral pH value of 5.70, suitable for food and pharmaceutical products because it meets the standard of food and pharmaceutical gelatin issued by Norland [20] that is 5.5-7.0.

Viscosity is one of the important physical properties of gelatin. Based on the result of the research, it can be seen that the viscosity value of obtained gelatin eel fish ranged from 4.87 to 5.90 cP. The value still meets the standard type A of gelatin required by GMIA [18] that is referred to Amiruldin [19] ranging from 1.5-7.5 cP. From the analysis results, it can be seen that the highest viscosity value is in the treatment of 4% HCl concentration with 48 hours submersion time that is 5.90 cP. While the lowest viscosity value is found in 4% HCl concentration treatment with 24 hours submersion time that is 4,87 cP.

Based on the results of the research, it is able to know the tendency that the longer the submersion time, the more increase value of produced gelatin viscosity. This study shows that longer submersion time can optimize the process of amino acid chain formation, so the gelatin viscosity is greater [6]. The duration of submersion will affect the viscosity of gelatin as it will inhibit the collagen triple helix fibers into a single chain. In the case of Increasing the length of submersion, it effects that the more collagen fibers are broken into single chains (α) or single chains connected by covalent crosslinks (β and γ) result more collagen being converted. The viscosity of the gelatin solvent will increase as the gelatin concentration increases and the temperature decreases [21]. The more amount of water bound by gelatin, the thicker gel produced, which directly affects the higher measured viscosity value. The viscosity of gelatin will affect the final properties of a product [22].

Gel strength is one of the parameters to determine the physical quality of a gelatin product. Jones [22] states that gel strength is essential in determining the best treatment of gelatin extraction process, because one of the important gelatin properties is able to change the liquid into solid or to change the sol form into a reversible gel. This ability that causes gelatin very widely in its use, both in the field of food and non-food. Based on the results of the research, it can be seen that the obtained value of gel gelatin strength from eel fish bone is ranged 133.84 - 200.15 bloom. The gel strength value range is lower than commercial gelatin based on Nurilmala [15] test result that is 328.57 bloom. However, the value of gel eel fish bone strength resulted from this study meets the standard type A of gelatin required by GMIA [18] referred to Amiruldin [19] ranging from 50-300 bloom. This means that gelatin results of this study can be used for gel-shaped products. The results of gel strength analysis showed that the best gel strength value, when viewed from the highest value produced, is in the treatment of 4% HCl concentration with 48 hours submersion time, which is 200.15 bloom. However, when viewed from the efficiency of the process and the resulted yield value, the best treatment is 4% HCl concentration and 24 hours of submersion time, which is 199.75 bloom. While the lowest value of gel strength is in the treatment of 6% HCl concentration with 24 hours of submersion time, which is 133.84 bloom. Higher acid concentrations and increased temperatures led to continued hydrolysis of collagen that has been converted to gelatin which caused shorter amino acid chains so the gel strength became lower [24]. While the duration of submersion did not give a significant effect, so the result showed an unstable gelatin value.

3.3 Gelatin Characteristic

Tabel 3. The proximate content of Gelatin Eel Fish Bone (*Anguilla bicolor*) Produced through Acid Process

Formula	Parameter		
	Water Content (%)	Ash Content (%)	Protein Content (%)
$F1_{24}$	$7,87^{c}$	$1,91^{c}$	$81,17^{a}$
$F1_{48}$	$7,43^{b}$	$1,71^{a}$	$87,84^{c}$
$F2_{24}$	$7,55^{bc}$	$1,88^{c}$	$83,09^{b}$
$F2_{48}$	$7,25^{ab}$	$1,68^{a}$	$86,50^{d}$
$F3_{24}$	$6,93^{a}$	$1,79^{b}$	$84,35^{c}$
$F2_{48}$	$7,27^{ab}$	$1,62^{a}$	$84,93^{c}$

Different letter notation in the same column shows a significant difference at $\alpha = 0.05$. Gelatin Formula = $F1_{24}$ (HCl 4%, 24 hours); $F1_{48}$ (HCl 4%, 48 hours); $F2_{24}$ (HCl 5%, 24 hours); $F2_{48}$ (HCl 5%, 48 hours); $F3_{24}$ (HCl 6%, 24 hours); $F3_{48}$ (HCl 6%, 48 hours).

The testing of water content in gelatin is intended to determine how much water content in gelatin. The role of water in food is one of the factors that influence metabolism activity such as enzyme activity, microbial activity, and chemical activity that is the occurrence of rancidity and non-enzymatic reactions, causing changes in organoleptic properties and nutritional value [25]. The water content of eel fish bone gelatin is between 6.93% -7.87%. The content still fulfills the gelatin quality standard by [26] which is maximum 16% and [27] that is not more than 18%. The longer the submersion time, the more decrease water content. The decrease in the gelatin water content is due to the longer submersion, the more diffused acids in the eel fish bone tissue, so the collagen structure becomes more exposed and produces a weakly bonded gelatin structure [28]. Differences in the obtained moisture value are also suspected because the drying time for each sample is different. This means that for each sample takes different time to become dry sample [29].

Ash content, is important to know the mineral content of the material and to know the purity of a food. In this study it can be seen that gelatin contains mineral salts. Most of the mineral salt contained in eel fish bone gelatin is calcium, it is known from the results of a gelatin solvent that is yellow. Calcium is the most abundant mineral that causes a gelatinous solvent of turbid yellow [22]. Eel fish bone gelatin content of ash content ranged between 1.62% -1.91%. The content still fulfills gelatin quality standard by SNI [26] which is maximum of 3.25% and JECFA [27] is not more than 2%. The amount of produced gelatin ash content of eel fish bone is also not much different from commercial gelatin based on Nurilmala [15] test result that is equal to 1.66%. The result of analysis shows that there is a decrease of ash content along with increasing of acid solvent concentration. In this case, the higher the acid solvent concentration, the more calcium dissolved in the solvent, so the amount of calcium in the ossein will decrease. Reduced calcium in ossein causes a decrease in the value of ash content, because the extracted calcium in those process becomes less. The influence of the use of acid concentration is very significant. It is characterized by a decreasing rate of ash content of gelatin as the concentration of used acid solvent [6].

The protein content of eel fish bone gelatin ranged between 81.17% -87.84%. The range of protein content in produced gelatin from it is bigger than commercial gelatin based on Nurilmala [15] test result that is 85.99%. Higher levels of protein bone gelatin from eel fish bone happen, because the raw materials used have a higher protein content. The submersion process results bond termination reaction of the hydrogen and opens the collagen coil structure that occurs optimum so that the amount of extracted protein becomes numerous [29].

4. Conclusion

Characteristics of eel fish bone gelatin (*Anguilla bicolor*) obtained from this study include yield, pH value, viscosity, and gel strength respectively ranging from 3.04% -9.31%; 4,97-5,70; 4.87-5.90 cP; and 133.84-200.15 bloom. The best treatment in this research is 4% HCl concentration with 2 days submersion period, has a value of Yield, pH, viscosity, and gel strength respectively 4.29%; 5.70; 5.90 cP; and 200.15 bloom, and the proximate content includes moisture content, ash content, and protein content respectively of 7.43%; 1.71%; and 87.84%.

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