



RESEARCH ARTICLE

DETERMINATION OF QUALITY AND IDENTIFICATION OF GELATIN ON THE EEL (*ANGUILLA MARMORATA* (Q.) GAIMARD) FISH SKIN WITH VARIED CONCENTRATIONS OF ACETIC ACID

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ABSTRACT

Gelatin is one type of protein derived from natural collagen found within the skin, bones, and connective tissue of animals. This study, used eel (*Anguilla marmorata* (Q) Gaimard) fish skin as the material for gelatin which was extracted using acetic acid solution of 3%, 6% and 9% for 48 hours. This study aims at determining the concentration of acetic acid towards the yield value and the quality (organoleptic, water content, ash content, and arsenic content) in accordance with Indonesian National Standards. The method used to determine the water content is oven method, to determine ash content is muffle furnace method, to determine arsenic metal is Atomic Absorption Spectrophotometry (SSA) and to find out the gelatin functional groups is FTIR (Fourier Transform Infrared) spectroscopy. The result shows that the gelatin extracted using 3% acetic acid has the highest yield value of 18.2% and for the quality of gelatin concentration of 9% acetic acid is the most optimal concentration with yellowish gelatin color, odorless and flavorless, and has water content of 7.099%, ash content of 0.96% and arsenic content of <0.01mg / kg. The result of characterization using FTIR shows that sample obtained by characterization of functional group similar to gelatin.

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INTRODUCTION

Gelatin is widely used as food ingredients, such as gel-forming agents, thickeners, emulsifiers, and edible film. In terms of pharmaceutical, gelatin is commonly used in making capsule, be it soft capsules or hard capsules and is also used in the cosmetics industry in making shampoo and soap. In terms of engineering industry, gelatin is used as a material in making glue, paper, and paint (Park, 2007). Gelatin can be in the form of sheets, pieces, or powder in pale yellow or light brown. Dry gelatin can be stable in the air, but it is easily decomposed by microbes if it is moist or in solution form. Two types of gelatin are type A and type B gelatin, in which for type A, it comes from acid process with isoelectric point of pH 7 – pH 9; on the other hand, gelatin type B is derived from base process with isoelectric point of pH 4,7 – 5,2 (Depkes, 1995). The chemical properties of gelatin type A is that it has gel strength of 50-300 bloom, viscosity of 15-75 cPs, pH 3,8-5,5 and ash content of 0,3-2%, while gelatin type B has gel strength of 50-300 bloom, viscosity of 20-75 cPs, pH of 4.7-5.4 and ash content of 0.5-2% (GMIA, 2012).

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The production of gelatin worldwide reaches 326,000 tons/year; gelatin from pig skin occupies the highest production (46%), followed by cow skin (29.4%), cattle bone (23.1%), and other sources (1.5%). Statistically, import activity conducted by the Indonesian government from January to December 2013 amounted to 3,124,255 kg with a value of US\$ 16,741,918. The countries supplying gelatin to Indonesia are, among others, China, Japan, France, Australia, India, and New Zealand (Suhenny, et al., 2015). Based on the data regarding the highest gelatin production which is made out of pig skin, this can lead to a number of problems, especially in countries that are predominantly Muslim like Indonesia. Related to this matter, to overcome the problem while reducing dependency of gelatin import; hence, it is considered to be essential to develop gelatin product derived from raw material more acceptable by the society. One of the solutions is to produce gelatin from fish (Trilaksani, et al, 2012). Fish skin contains a cross-linking collagen structure; it means that the solution of hydrochloric acid, sulfuric acid, acetic acid, and phosphoric acid is more suitable to be used on fish skin samples (Karim and Bhat, 2009). According to Ulfa (2011), it is reported that acetic acid solution has the best potential for hydrolysis of collagen which can facilitate its solubility in hot water during gelatin extraction, so that the collagen structure is exposed due to the release of several bonds in the protein molecule and the fact that the gelatin gets extracted over and over.

Based on the aforementioned description, it is necessary to conduct study on the potential of gelatin production from giant mottled eel *Anguilla marmorata* (Q.) Gaimard as well as gelatin quality analysis including water content, ash content and arsenic content according to Indonesian National Standard, so that it can be recommended as raw material in making gelatin which is safe and acceptable by the society.

MATERIALS AND METHODS

Materials: The material used is the eel (*Anguilla marmorata* (Q) Gaimard) fish skin. Other materials used are 10% formalin, acetic acid (CH_3COOH) of 3%, 6% and 9%, aquadest (H_2O), concentrated Nitric Acid (HNO_3), Hydrochloric acid (HCl) 6 N, potassium bromide powder (KBr).

Methods: The method used in making gelatin is steaming method: the first stage being degreasing (the process of removing fat) that the sample of skin fish of 2 kg that have been cleaned, soaked in boiling water for \pm 30 minutes and stirred, then being washed using running water and drained. Then the skin weighed for 50 grams each, then soaked using acetic acid (CH_3COOH) solution with each concentration of 3%, 6% and 9% in 250 ml erlenmeyer for 48 hours. After the immersion is finished, it is then filtered and washed with aquadest until the pH of the water becomes neutral as measured by pH measuring device. The neutralized skin sample was put in the erlenmeyer and aquadest was added with a 1 : 3 ratio at 700°C for 5 hours in the waterbath and then was filtered with a filter cloth to separate the filtrate and residue. After that, the filtrate was taken and inserted into the refrigerator to form a gel, then was dried in an oven at 50°C for \pm 48 hours. Upon drying, the size was reduced and the samples were ready for analysis. Gelatin obtained was then calculated in terms of yield value, organoleptic analysis including water content by using oven method, ash content by using muffle furnace method and arsenic content by using SSA and functional group characterization by using FTIR.

Data Analysis: The experimental design conducted for this study was Completely Randomized Design using 2 levels of acetic acid concentration treatment and demineralization time of 48 hours 3%, 48 hours 6% and 48 hours 9%. The data obtained were tested for their normality by Shapiro Wilk test. It is considered to be normally distributed if $p > 0.05$. It was then followed by homogeneity test (Levene test), $p > 0,05$ means that the data obtained were homogeneous. Furthermore, the statistical test of analysis of variant (ANOVA) one way at 95% confidence level and as the average value comparison test, SPSS (Statistical Product and Service Solution) program was employed and followed by Mann-Whitney test to find the most optimal concentration.

RESULTS AND DISCUSSION

The study aims to obtain gelatin derived from eel *Anguilla marmorata* (Q.) Gaimard from Poso lake and to analyze and to determine the quality content of the gelatin produced. Gelatin is a conversion protein of collagen; thus, the skin was used due to the fact that the number of collagen contained in animals is mostly within its skin for about 89%. Fish skin is a raw material which contains a cross-linking collagen structure, so it is suitable to be extracted using acid solution (Karim and Bhat, 2009).

The gelatin obtained from the acid solution is considered to be better than the one obtained from base process. It is because the acid solution is capable of converting the triple helical collagen fibers into single helices, while the base solution can only convert the triple helix collagen fibers into double helices, so that the gelatin will be more extracted when using acid solutions. Moreover, the advantage of using acid solution is that it is relatively cheaper, and time of extraction is much faster compared to using alkaline solution (Ulfah, 2011). The use of acid helps the increase of H^+ ion which makes it easier for water to penetrate into collagen fibers by electrostatic force between polar groups or hydrogen bonds and between non-polar and atomic groups (Jazwir, 2011). In this study, the gelatin produced is type A gelatin due to the use of acid solution, while gelatin derived from base process is type B gelatin (Yenti, 2015).

Steps during the process of gelatin extraction which is degreasing or fat removal process is an immersion process of fish skin sample in boiling water to separate the fat or other dirt which remains attached to the skin (Yuliani, 2014), after degreasing, the step proceeds to the immersion using the solution acetic acid of 3%, 6% and 9% for 48 hours. Ulfah (2011) states that acetic acid solution is used since it can hydrolyze collagen to facilitate its solubility in hot water during gelatin extraction, so that the collagen structure is exposed due to the release of several bonds in the protein molecule. According to Pelu (1998), acetic acid is a good organic acid to be used to produce good quality gelatin compared to other acid solutions as it is unable to damage the color of the resulting gelatin. In addition, acetic acid can convert the collagen fibers of triple helices into single helix which facilitate its solubility (Ward and Court, 1977). A study by Sompie (2015) asserts that the use of acetic acid of 3%, 6% and 9% in the immersion process for 48 hours can produce gelatin in accordance with Indonesian National Standard and immersion process for 48 hours can result in high yield value. The immersion process aims to convert collagen into an appropriate form for extracting by the interaction of H^+ ions from acidic solutions with collagen. Some of the hydrogen bonds in the tropocollagen as well as the cross-linking bonds which connect the tropocollagen are hydrolyzed and result in tropocollagen chains which begin to lose the triple helical structure, the immersion process also results in swelling that can remove unwanted materials, such as fat and non-collagen proteins in the skin with minimal collagen loss. When the skin containing collagen is soaked with acid solution and followed by heating in water, the collagen fibril structure is broken irreversibly (Martianingsih, 2010). The samples which have been soaked are, then, washed with running water until it reaches a neutral pH (6-7), because, commonly, such pH is the isoelectric point of the non-collagen protein components of the skin which makes it easy to be coagulated and removed (Hinterwaldner, 1977).

The next step is the extraction by using a waterbath at 700°C . Wulandari (2013) in her study on the effect of temperature during extraction process states that the suitable temperature is at 700°C . In addition, according to Pelu (1998), gelatin extraction temperature ideally starts from 490°C ; the higher the temperature used the amount of gelatin produced increases. However, Wulandari (2013) states that if the extraction temperature is above 700°C , the gelatin produced may decrease because such temperature can cause denaturation of the gelatin and the decrease of the yield value.

Below is the yield percentage of the gelatin from the eel (*Anguilla marmorata* (Q) Gaimard) skin.

Sample	Yield percentage
Gelatin (3% acetic acid)	18,2%
Gelatin (6% acetic acid)	17,26%
Gelatin (9% acetic acid)	16,4%

Below is the result of organoleptic testing of the gelatin from the eel (*Anguilla marmorata* (Q) Gaimard) skin

Sample	Organoleptic Parameter			SNI 06-3735(1995)		
	Color	Odor	Taste	Color	Odor	Taste
Gelatin (3% acetic acid)	Yellowish	Odorless	Tasteless	Yellowish – colorless	Odorless	Tasteless
Gelatin (6% acetic acid)	Yellowish	Odorless	Tasteless			
Gelatin (9% acetic acid)	Yellowish	Odorless	Tasteless			

Below is the result of testing regarding water, ash, and arsenic content of gelatin from the eel (*Anguilla marmorata* (Q) Gaimard) skin

Parameter	Result			SNI 06-3735 (1995)
	Gelatin (3% acetic acid)	Gelatin (6% acetic acid)	Gelatin (9% acetic acid)	
Water content	8.266 %	7.3 %	7.066 %	Maks 16%
Ash content	1.16 %	1.06%	0.96%	Maks 3,25%
Arsenic content	0.08mg/kg	0.07mg/kg	< 0.01 mg/kg	Maks 2 mg/kg

Extraction using hot water continues the destruction of the cross-links as well as to destruct the hydrogen bonds that are the collagen-stabilizing factors of the structure. Damaged hydrogen bonds and broken covalent bonds destabilize the water-soluble gelatin conversion (Djabourov, 1993). The extracted tropocollagen (collagen molecule) undergoes a hydrolysis reaction which occurs when immersion in an acid solution. In the hydrolysis reaction, the hydrogen bonds and covalent cross-linking of the tropocollagen chains are disconnected and result in a triple helix tropocollagen transformed into a water-soluble single helix called gelatin (Martianingsih, 2010). The gelatin obtained from the extraction was filtered with filter cloth to separate between the filtrate and the residue. Then, the obtained filtrate was cooled in the refrigerator to solidify the gelatin. The cooling process results in the transition of a random roll structure into a new helical structure and strengthens the gelatin gel produced (Martianingsih, 2010), afterwards, the gelatin was dried at an oven at 50°C for 48 hours. The purpose of drying is to reduce the risk of gelatin damage by microorganisms (Yenti, 2015).

Gelatin obtained was then identified in terms of its gelatin functional group using FTIR spectroscopy. FTIR analysis is used to prove whether the compound obtained is gelatin. This study began with the preparation of gelatin samples. The obtained gelatin is minimized, so that it is powdered in order to be analyzed using FTIR (Suptijah, 2012). The typical gelatin-absorbent peak curve is divided into 4 parts: amide A, amide I, amide II, and amide III which is the absorption area of a typical gelatin functional group. The area of the absorption of amide A is shown in ν 3600-2300 cm^{-1} , amide I in ν 1661-1636 cm^{-1} , amide II in ν 1560-1335 cm^{-1} , and amide III at ν 1240-670 cm^{-1} (Suptijah, 2013). Below is the picture of the FTIR analysis of gelatin from the eel (*Anguilla marmorata* (Q) Gaimard) skin. The spectra obtained from the extracted sample using 3% acetic acid has absorption area of amide A as shown at ν 3421,72 cm^{-1} , 3086,11 cm^{-1} , 2951,09 cm^{-1} , 2360,87 cm^{-1} , and 2331,94 cm^{-1} . For samples extracted by using 6% acetic acid, it is shown at ν 3460,30 cm^{-1} , 2968,45 cm^{-1} , 2358,94 cm^{-1} , and 2330,01 cm^{-1} . For samples extracted using 9% acetic acid, it is shown at ν 3464,15 cm^{-1} , 2968.45 cm^{-1} , 2358,94 cm^{-1} , and 2330,01 cm^{-1} .

When compared with commercial gelatin, the absorbance of amide A is shown at 3303,27 cm^{-1} and 2960,89 cm^{-1} (Hermanto, 2014). The presence of the absorption peak is due to the presence of hydrogen bonds and the presence of OH group, the absorption area of amide A is shown at ν 3600-2300 cm^{-1} . The widespread form of the peak is evidence of an OH group of hydroxyproline (Prihatiningsih, 2014). The result obtained in the extraction sample using acetic acid of 3%, 6% and 9% respectively has a curve of amide A with widened-shape peak.

Amide I is a typical gelatin group present at the frequency indicated at ν 1661-1636 cm^{-1} known as the absorption area for the α -helix and β -sheet structure of the random coil structure of collagen. In amide I, it is denoted the presence of a double bond of the carbonyl group C=O, bending of NH bond, and CN strain. The absorption area of amide I shows the presence of a C=O strain and an OH group paired with a carboxyl group (Prihatiningsih, 2014). In the spectra obtained from the samples being extracted using 3% acetic acid, amide I absorption area is shown at ν 1658,78 cm^{-1} . For samples extracted using 6% acetic acid, it is shown at ν 1660.71 cm^{-1} , and 1629,85 cm^{-1} . For samples extracted using 9% acetic acid, it is shown at ν 1629.85. In the commercial gelatin, the absorption area of amide I is shown at ν 1649.76 cm^{-1} . The absorption area of amide II is caused by the deformation of the N-H bond in the protein. This absorption area is associated with the deformation of tropocollagen (collagen molecule) into an α -helix chain, the absorption area is shown at ν 1560-1335 cm^{-1} (Prihatiningsih, 2014). In the sample extracted using 3% acetic acid, the amide II absorption area is shown at 1560.41 cm^{-1} , 1448.54 cm^{-1} , and 1396.46 cm^{-1} . For samples extracted using 6% acetic acid, it is shown at ν 1544,98 cm^{-1} , 1440,83 cm^{-1} , and 1396,46 cm^{-1} . For samples extracted by using 9% acetic acid, it is shown at ν 1440.83 cm^{-1} , and 1396.46 cm^{-1} . In commercial gelatin, it is shown at ν 1543.88 cm^{-1} , 1453.48 cm^{-1} and 1333.75 cm^{-1} . Amide III is the last typical gelatin group and is an area associated with the triple helical structure of collagen and is shown at ν 1240-670 cm^{-1} (Prihatiningsih, 2014). Samples extracted with acetic acid 3% of the amide uptake area III are shown at ν 1240,23 cm^{-1} , 1078,21 cm^{-1} , 1031,92 cm^{-1} , and 929,69 cm^{-1} .

For samples extracted using 6% acetic acid, it is shown at v 1078.21 cm^{-1} , 1031.92 cm^{-1} , and 929.69 cm^{-1} . For the samples obtained using 9% acetic acid, it is shown at v 1240.23 cm^{-1} , 1078.21 cm^{-1} , 1031.92 cm^{-1} , and 929.69 cm^{-1} . For commercial gelatin, the absorption area of amide III is shown at v 1244.12 cm^{-1} . From the result obtained, the samples extracted using acetic acid 3%, 6% and 9% have absorption number of amide A, amide I, amide II and amide III. Differences in absorption areas lead to relatively different specific wavenumber. This is also due to the differences in gelatin-producing ingredients as well as the materials used for gelatin extraction. Gelatin as well as proteins contains a structure which consists of carbon, hydrogen, hydroxyl (OH), carbonyl (C=O), and amine groups (NH) (Suptijah, 2013). The hydroxyl (OH) functional group has an absorption area at 3300-3500 cm^{-1} , the carbonyl group (C=O) has an absorption area at the wave number of 1640-1670 cm^{-1} and the amine group (NH) has an absorption area at the wave number of 3310-3500 cm^{-1} (Martianingsih, 2010). Below is the table of absorption peak of FTIR analysis regarding gelatin from the eel (*Anguilla marmorata* (Q) Gaimard) skin.

The FTIR results obtained indicates that samples extracted using 3% acetic acid contains hydroxyl (OH) and amine (NH) functional groups at 3421.72 cm^{-1} and carbonyl (C=O) groups at wavenumber of 1658.78 cm^{-1} . In the sample extracted using 6% acetic acid, it contains hydroxyl (OH) and amine (NH) functional groups at 3460.30 cm^{-1} and carbonyl group (C=O) at 1660.71 cm^{-1} . Samples extracted using 9% acetic acid contain hydroxyl (OH) and amine (NH) functional groups at 3464.15 cm^{-1} and carbonyl groups (C=O) at 1668.71 cm^{-1} . When compared with commercial gelatin, OH and NH functional groups are shown at 3522.02 cm^{-1} , and functional group C=O is shown at 1650.71 cm^{-1} (Prihatiningsih, 2014 and Hermanto, 2014). The differences in the absorption area of the functional group can possibly be due to the differences in the gelatin-producing material and the solution used for gelatin extraction. The results extracted using acetic acid 3%, 6% and 9% contain the same functional group as commercial gelatin, thus it can be concluded that the extracted sample using 3%, 6% and 9% acetic acid is gelatin.

Gelatin obtained was, then, calculated its yield value in which the calculation of yield value is an indicator to determine the effective and efficient treatment or the method used (Syahraeni, 2017). It means that the higher the yield value the treatment applied to the study is more efficient (Miwanda and Simpen, 2008). The yield is calculated based on the ratio between the gelatin produced and the weight of the materials. The yield of gelatin ranges from 16.34% -18.2%, so that when compared to others, the yield value produced in this study is categorized as high yield value, therefore, it can be concluded that the method used for this study is effective and efficient. The gelatin produced was, then, analyzed according to Indonesian National Standard quality including organoleptic parameter (color, odor and taste), water content, ash content, quantitative analysis of arsenic metal. Organoleptic testing is an important factor to measure the level of consumer preferences or acceptance of a product. The organoleptic test is closely related to the quality of the product as it is directly related to consumer preference (Said, *et al*, 2011). Parameters in organoleptic testing include observation of color, odor and taste. The organoleptic test involves panelists of 10 respondents. Color is the main parameter which determines the level of consumer acceptance of a product. Subjective observation with the eyesight remains greatly decisive in

organoleptic test in terms of color (Syahraeni, 2017). The result of color organoleptic test on gelatin 3%, 6% and 9% obtains yellowish color as the preferred one, while in terms of taste and odor, it is obtained that the odorless one is acceptable by consumer. The result of organoleptic testing is in accordance with Indonesian National Standard (SNI). The next parameter measured is water content. Water content is an important parameter of a food product because water content is closely related to the shelf life of gelatin. The role of water in food is one of the factors which influence metabolism activity, such as enzyme, microbial, and chemical activities; for example, decay and non-enzymatic reactions that cause changes in organoleptic properties and nutritional value (Syahraeni, 2017). The average percentage of water content obtained from gelatin 3%, 6% and 9% are 8.2%, 7.3% and 7.0%, respectively. Based on the results, the data obtained were homogeneous, so that further test of one way Anova was conducted and shown in the appendix 3. It shows that there are significant differences in concentrations of 3% and 6% and concentrations of 3% and 9%, while for concentrations of 6% and 9%, no significant difference is found. Determining the best concentration can be conducted using Mann-Wheteny test; it is obtained that the best concentration is at 9%. The result of analysis of water content has met the Indonesian National Standard (SNI) that is 16% maximum. The level of water content of a material is determined by the nature and ability of the material to absorb water as well as the drying process carried out on the material (Syahraeni, 2017).

The next measured parameter is ash content. Ash content indicates the amount of minerals contained (Apriantono, *et al*, 1989). The average percentage of ash content of 3%, 6% and 9% gelatin produced is 1.18%, 1.06% and 0.96%, respectively. Based on the result, the data obtained were homogeneous, so that further test of one way Anova was conducted and shown in the appendix 3. It shows that there are significant differences in concentrations of 3% and 9% and concentrations of 6% and 9%, while for concentrations of 3% and 6%, no significant difference is found. To find out the best concentration, Mann-Wheteny test was conducted. It is obtained that the best concentration is 6%. The result of ash content analysis has met the Indonesian National Standard that is 3.25% maximum. The amount of ash content is determined during the soaking process; the higher the acid concentration is the more minerals dissolved during the soaking process (Syahraeni, 2017). The following test is the measurement of arsenic content in gelatin. Arsenic (As) is a naturally abundant element with atomic number of 33, atomic weight of 74.92 g/mol, has 2 solids form that is yellow and gray yellow. Arsenic is a natural compound as part of soil, water, and rock. Arsenic found in water comes from weather changes as well as industrial activities, ground washing, and urban population activity. In this study, the measurement of arsenic content was conducted on gelatin from the skin of giant mottled eel (*Anguilla marmorata*) in which the arsenic content within the fish is 4.64 mg/kg, while within the gelatin, the arsenic content is 2 mg/kg. Excessive levels of arsenic can cause skin abnormalities, increase the risk of liver, bile, kidney, and lung cancer, irritation and pain in the digestive system, nausea, vomitus, and diarrhea, exacerbating malnutrition, decrease the production of red blood cells and white blood cells, abnormalities of heart function, damage to blood vessels, liver and kidney damage and impaired neurological function (Widowati, 2008). The result of arsenic content test shows that gelatin 3% contains arsenic of 0.08 mg/kg, gelatin 6% contains

arsenic of 0.07 mg/kg and gelatin 9% contains arsenic of <0.01 mg/kg. It means that the arsenic content on gelatin is in accordance with Indonesian National Standard as it does not exceed the maximum limit of 3.25 mg/kg. Based on the result, the data obtained were homogeneous, so further test of one way Anova was conducted. The test shows that there is not any significant difference between the concentrations of 3%, 6% and 9%. However, Mann-Wheteny test shows that the best concentration is of 9%. Based on Mann-Wheteny statistic analysis, it can be seen from the parameter of water content, ash and arsenic content, the significant difference between 6% and 9% towards 3% is shown, but for the optimum concentration in determining quality including organoleptic parameter, water content, ash and arsenic content is gelatin extracted using 9% acetic acid.

Conclusion

Based on the study, the following conclusions are drawn: the optimum concentration of acetic acid towards gelatin quality from the skin of giant mottled eel *Anguilla marmorata* seen from organoleptic parameter, water content, ash content, arsenic content and functional group of gelatin is 9%. The analysis of gelatin from the skin of giant mottled eel *Anguilla marmorata* results in yield value ranging between 16.04% - 18.02%. Organoleptic, water content, ash content, arsenic content analyses have met the requirements of SNI 06-3735 (1995).

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