The Effect of Hydrochloric Acid (HCL) Concentration on the Quality of Gourami Bone Gelatin (*Ospheronemus Gouramy* Lac)

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Keywords: Gelatin, Fish Bone, Halal Gelatin

Abstract: Gelatin is commonly made of the skin and bones of cattles or pigs. This causes problems in the society both in terms of the *halal* status and health. Fish bone (*Osphronemus gouramy* Lac) can be used for producing gelatin because it contains collagen protein. The purpose of this study was to produce good gelatin with different concentrations and to identify the physical characteristics of gelatin from the bones of gouramy fish at several concentrations. The treatment performed in this study used HCl was used with 48 hours immersion period and concentration of 1% - 5%. The value of rendement in this study ranged from 8.05% - 12.44%, the viscosity value ranged from 4.7 to 6.1 cP, the gel strength ranged from 71.90 to 90.61 bloom, the water content ranged from 9% - 12%, the grain ranged from 2.18% - 3.78%, pH levels ranged from 4.64% - 4.85% and the protein content ranged from 59.68% - 70.24%. Based on these results, it can be concluded that the different concentrations of acids significantly affect the value of gelatin produced. The best concentration is at 5%. At this concentration, the gelatin produced has the best physical and chemical properties compared to those produced with other concentrations.

1 INTRODUCTION

Gelatin is derived proteins from collagen that in denaturing because of termo-hidrolisis and to transform termo-reversible between sol and gel (Cho, 2004). Gelatin is a protein conversion is soluble in water (Sobral, 2001).

Besides food industries, gelatin is also used in non-food industries such as pharmaceutical industry, photography, cosmetics, and paper industries. Gelatin can be used in the forms of capsules, tablets and pastilles, gelatin sponge, surgical powder, medical research, plasma expander, and microencapsulation in the pharmaceutical field.

Gelatin available in the market is mostly made from the bones of pigs or other mammals such as cows. In the teaching of Islam, gelatin from pigs is considered *haram* (forbidden to consume). This relates to the Islamic Shari'a law, which requires its followers to consume only *halal* food. On the other hand, the consumption of beef gelatin is often worrying as cattles are often infected by diseases such as anthrax and mad cow. In addition, often, in the process of slaughtering, the name of Allah was not mentioned and the cut was not made on the jugular vein of cows, making the beef unlawful. The Quran describes the prohibition of eating unclean foods, as follows: *Prohibited to you are dead animals, blood, the flesh of swine, and that which has been dedicated to other than Allah, and [those animals] killed by strangling or by a violent blow or by a head-long fall or by the goring of horns, and those from which a wild animal has eaten, except what you [are able to] slaughter [before its death], and those which are sacrificed on stone altars, and [prohibited is] that you seek decision through divining arrows. That is grave disobedience*. (Al-Maidah verse: 3).The importance of *halal* foods is regulated in Islam as stated in the Qur'an: "*O mankind, eat from whatever is on earth [that is] lawful and good and do not follow the footsteps of Satan. Indeed, he is to you a clear enemy.*" (Al-Baqarah 168). Besides, *Rasulullah* PBUH also said: "*Whoever takes a bite of forbidden goods, then the prayer will not be accepted for forty days*" (HR. Abu Daud).

Rahmi Hafsari, A., Rosmiati, D. and Jamaludin, D.
The Effect of Hydrochloric Acid (HCL) Concentration on the Quality of Gourami Bone Gelatin (Ospheronemus Gouramy Lac).
DOI: 10.5220/0009947329832989
In *Proceedings of the 1st International Conference on Recent Innovations (ICRI 2018)*, pages 2983-2989
ISBN: 978-989-758-458-9

Therefore, obtaining alternative raw materials for producing *halal* gelatin is necessary. Gourami bones are one of the materials which can be used to produce *halal* gelatin. Further, gelatin from fish bones is safer in terms of health because it is not made from infected materials that can affect the health of consumers. So, it is expected that the gelatin has a high quality and meets the standards of commercial gelatin.

The purpose of this study was to determine the highest concentration of HCl to generate the best gelatin with different concentrations and to determine the effect of HCl concentration on the physical and chemical characteristics of Gourami bone gelatin.

2 MATERIALS AND METHODS

2.1 Equipments

The equipment used in this study is divided into two: the equipment used in the manufacture of gelatin is an analytical balance, beaker, small spoons, measuring cups, glasses, water bath, an evaporator, a pH meter, viscometer, Kjeldhal pumpkin.

2.2 Materials

The raw materials used are Gourami bon dry waste as much as 2 kg, 6 M HC1 distilled water, filter paper and aluminum foil

2.3 Experimental Design

This study is divided into two phases. The first phase of the preliminary test, which is the process of making gelatin from the bones of Gourami (*Ospheronemus Gourami*) with a concentration of 2%, 4%, 6%, 8%, 10% and analyzing physical properties that yield. The second stage of research the manufacture of gelatin, namely the manufacture of gelatin concentrations that are the 1%, 2%, 3%, 4%, after the gelatin was chosen to analyze physical and chemical properties of gelatin that is yield, viscosity, gel strength, analysis of the degree of acidity (pH), moisture content, ash content, and protein.

2.4 Research Stages

2.4.1 Gelatin Extraction

Bone degreasing or boiled for 20 minutes at 70 ° C with bone and distilled water ratio 1: 2 (b: v) and then dried in the sun until bone dry. Degreasing the bones that have been soaked in a solution of HCI at a concentration of 1%, 2%, 3%, 4% and 5% for 24 hours (treatment A). The bones that have been soaked in an acid solution is called ossein and separated and then filtered. Ossein then neutralized with water. Ossein the neutral buffer is inserted into the glass beaker and add distilled water, ossein comparison with distilled water is 1: 2 (w / w). After it is extracted with a water bath at a temperature of 90 ° C for 7 hours. Then filtered through Whatman filter paper No. 40. Distillate concentrated by evaporator. Gelatin solution obtained was still in a state of liquid. The gelatin solution should be concentrated. Concentration is done with the evaporator, until a concentration to 25-30%, the concentration temperature is 800C, while the time it takes ± 5 hours (Handoko et al, 2011).

2.5 Research Analysis

2.5.1 Rendemen

Randemen obtained from the weight ratio of dry flour weight gelatin produced with fresh ingredients (the bone that has been washed). by AOAC, 1995:

$$\text{Rendemen} = \frac{gelatin\ results}{bone\ weight\ carp} \times 100\%$$

2.5.2 Gel Strength

Gel strength is done objectively by using the tool Rheoner RE3305. The level of gel strength expressed in units gf/cm2, which means the amount of compressive force to break up the deformation of the product. (British Standard 757, 1975).

2.5.3 Viscosity

The water content of the gelatin is determined by providing the chemicals as much as 75-100 ml in the samples estimated to contain as much as 2-5 ml water, then heated to boiling for 1 hour. Water vapor and chemicals are condensed and collected in the reservoir tube. Tools used as a container for include Strak tube-Dean and Sterling-Bidwell (Nancy, 2013).

$$\%\text{water content} = \frac{volume\ distillation}{sample\ weight}\ x\ 100$$

2.5.4 Ash Content

The ash content is determined by weighing the sample ± 2 grams ago, then put into porcelain dish until it is heated in a charcoal bath. Samples were evaporated water is put into the furnace temperature to 600 0C. The evaporation process until all material

changes color to gray, then the sample is weighed (AOAC, 1995).

$$\% \text{ Ash Content} = \frac{Ash\ Content}{Sample\ Weight} \text{ x } 100\%$$

2.5.5 Acidity (pH)

A total of 0.2 gram sample is weighed and dispersed into 20 ml of distilled water at a temperature of 80 ° C. The samples were homogenized with a magnetic stirrer, and then measured the degree of acidity at room temperature with a pH meter (British Standard 757, 1975)

2.5.6 Protein Level

A total of ± 0.25 gram dry sample, placed in a 100 ml Kjeldahl flask and added 0.25 grams of selenium and 3 ml of concentrated H2SO4. Then proceed with the process for 1 hour until a clear solution. Once cool add 50 ml of distilled water and 20 ml of 40% NaOH, and then distilled. Distilled accommodated in a Erlenmeyer flask containing a mixture of 10 ml of H3BO3 2% and 2 drops of indicator Brom Crsol Green Methyl Red pink. Once the volume of reservoirs (distillate) to 10 ml and the bluish-green, distillation was stopped and distilled titrated with 0.0235 N HCl until pink. The same treatment was also carried out on the blank. With this method obtained the total nitrogen content calculated using the formula (AOAC, 1995):

$$\% \text{ Protein Level} = \frac{(ml\ HCl - blank\ ml) x\ N\ HCl\ x\ 14.007\ x\ 100\ x\ 6.25}{ml\ sample}$$

2.6 Data Analysis

Data were analyzed with SPSS 20.0 by ANOVA. If the results of the analysis significantly different then tested further using Duncan (Gaspersz 1994).

3 RESULTS AND DISCUSSIONS

3.1 Analysis of Physical Properties of Gelatin

3.1.1 Rendemen

Based on Figure.1 The yield values obtained in gelatin by treatment with HCl 5%, ie 12.44%, while the value of the smallest yield generated 1% HCl concentration of 8.05%. According to Mulyani et al (2013), HCl has a bigger and stronger ability to degrade CaCO3 minerals of calcium is in the bones of Gourami compared with a base. Conversion of collagen into gelatin can be affected by temperature, heating time, and pH. In attachment.5 the values ranged on average from 8.16 to 12.44%. The size of the yield value is influenced by the concentration of HCl is different. The yield shows the effectiveness of the methods used in a study, especially about optimalitasnya in producing a product. The higher the yield value indicates the treatment is applied to the research more effective (Miwada and Simpen, 2007)

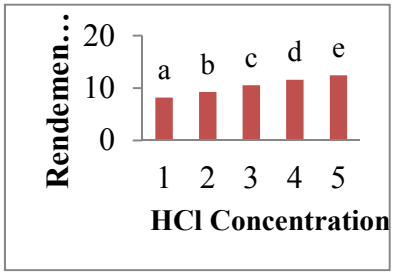

Figure 1 The value of bone Gourami rendemen gelatin. Description: Different letters indicate a significant difference at a significant level α = 0,05

3.1.2 Viscosity

Viscosity is the power flow of molecules in a solution either in water, a simple organic liquid and an aqueous suspension (Fever, 1989). The average value of Gourami viscosity bone gelatin is 4.7 to 6.1 cP (Figure.2). This research viscosity grades according to standards set by GMIA in 2012 is between 1.5 to 7.5 cP. According to Setiawati (2009), viscosity or viscosity of the gelatin solution is closely related to the water content of dry gelatin. The lower the water content of dry gelatin then its ability to bind water (to form a gel) will be higher. The more the amount of water bound by the gelatin will become increasingly viscous gel, which directly affect the higher the viscosity measured value.

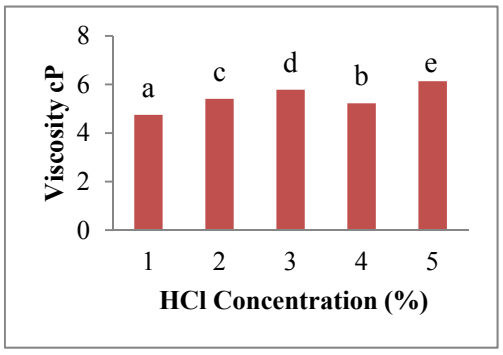

Figure 2 Viscosity Values Gourami Bone Gelatin. Description: Different letters indicate a significant difference at a significant level α = 0.05

3.1.3 Gel Strength

Gel strength is important in knowing the best determination, because one of the important properties of gelatin is able to transform the liquid into a gel which is reversible. Ability is what causes the gelatin very wide use, both in the field of food and non-food (Harris, 2008).

Based on advanced test Duncan showed that the treatment with using a 5% concentration showed the highest gel strength, which amounted to 90.61 bloom, while for the results of the gel strength smallest one is at a concentration of 1% at 71.90 bloom (Figure.3). The use of different concentrations significant effect, this is caused by collagen with acid hydrolysis process went quite well with increasing concentrations and the heating process that would damage the gelatin to form a gel structure. According Astawan (2002), high gel strength associated with long-chain amino acids, which long chains of amino acids that will produce a large gel strength. Optimal hydrolysis process will result in a long chain of amino acids, and at the time of conversion of collagen into gelatin will produce a high gel strength.

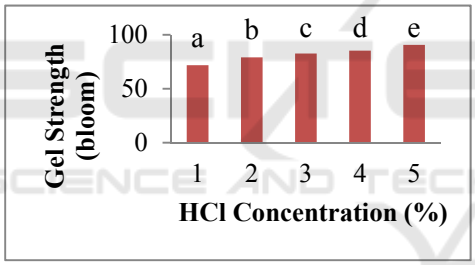

Figure 3: Value of Gelatin Gourami Bone Strengt. Description: different letters indicate a significant difference at a significant level α = 0.05

3.2 Gelatin Chemical Properties Analysis

3.2.1 Water Content

Water is an essential ingredients in a food. Water can be either intracellular or extracellular components of a product (deMan1989). Water in food ingredients will determine the acceptability, freshness and durability of the material. Water can also affect the appearance, texture, taste, and quality of foodstuffs (Winarno, 1992).

Differences in water content varying allegedly due to the use of materials that are not evenly distributed between the hard bone and cartilage in each treatment thus affecting the results of the water content of the gelatin bones of Gourami, besides the difference in the water content of the gelatin is affected by the drying process each sempel for every sempel the concentration difference has a drying time is different.The statement was supported by Rev. et al (2013) that for every sample requires a different time to be a dry sample.

The average value of the water content of Gourami bone gelatin ranging between 9% - 12%. The highest water content is at 5% HCl concentration of 12%, while the smallest water content is at a concentration of 3% by 9% (Figure.4). Differences in water content was allegedly influenced by different raw materials in each sample. Water content of the fifth HCl concentration of gelatin still meet the quality standards of ISO No. 53 of 1995 which is up to 16% and the standard of JECFA in 2003 a maximum of 18%.

According to deMan (1997) gelatin moisture content affects the shelf life of a product, because it is closely related to metabolic activity that occurred during the gelatin is stored. The role of water in food is one of the factors that influence the metabolic activity such as enzyme activity, microbial activity and chemical activity, namely the occurrence of rancidity and non-enzymatic reactions giving rise to changes in organoleptic properties and quality values.

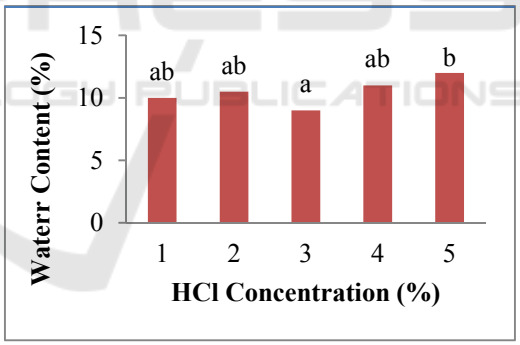

Figure 4: Water content Gelatin Gourami Bone. Description: Different letters indicate a significant difference at a significant level α = 0.05

3.2.2 Ash Content

Ash is an organic substance that is not burned in the combustion process of organic substances. These substances include sodium, chlorine, calcium, phosphorus, magnesium, and sulfur (Winarno 1992). Ash content value of foodstuffs showed a large amount of minerals contained in the food of a substance (Apriyantono 1989).

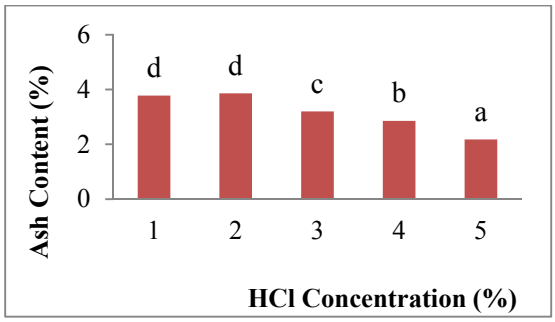

Figure 5: Levels of Ash Gelatin Gourami Bone . Description: Different letters indicate a significant difference at a significant lvel α = 0.05

Duncan advanced test showed that the concentration of HCl gives a significantly different effect ($P \leq 0.05$) with a concentration of 3%, 4% and 5%, but not significantly different ($P \geq 0.05$) with a concentration of 1% and 2% (Figure.5). Decreased calcium in the ossein is what causes the reduction of the value of its own ash content. Revelation et al (2013) states that the use of different acid concentrations have a significant influence on the ash content of gelatin. Marked with the rate of decline of the gelatin ash content along with the increasing concentration of acid used solvents. Allegedly higher acid concentrations can dissolve bone mineral in large quantities by the leaching process. According to Mulyani et al (2013), the use of HCl in the immersion process will react with the calcium phosphate bone. This will produce a soluble calcium salt and bones become soft, because HCl has bigger and stronger ability to degrade CaCO3 minerals of calcium is in the bones of Gourami compared with the use of bases

Value ash content contained in a product indicates the level of purity of the product.The level of purity is affected by the composition and mineral content. Abu contained in gelatin derived from salts or minerals in the bones of fish used (Ratri, 1998).

3.2.3 Acidity (pH)

The pH value of the degree of acidity gelatin is gelatin which is one of the important parameters in quality standards gelatin. pH gelatin based gelatin quality in general is expected to approach a neutral pH (Heidi, 2006).

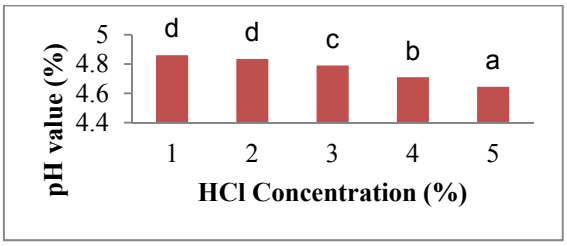

Figure 6 pH of Gelatin Bone Gourami . Description: Different letters indicate significant differences at the level of α = 0.05

In this study it was found that the higher the concentration of HCl pH value lower. The low pH value is due to the use of strong acid HCl. Allegedly in the event of collagen development time soaking with HCl, much residual unreacted HCl absorbed in the collagen which expands and caught in the collagen fibril network, making it difficult neutralized when washing that eventually brought current extraction processes that affect the level of acidity. In Appendix 5 the average pH value in Gourami bone gelatin ranged from 4.64 to 4.85%. The highest pH value is at a concentration of 1% by 4.85% (Figure.6). This is because the higher the concentration is used it will be increasingly concentrated acid and also the acid ossein which is bound to be more and more. While the lowest value contained at 5% concentration of 4.65%. Nurimala (2004), stating Low pH values in this study because it is still carrying over of residual HCl on fish bones and carried the time of extraction, thus affecting the value of acidity in the resulting gelatin. The pH value of Gourami bone gelatin in the amount of 4.64 to 4.85%. According Hinterwaldner (1977) in Nofri et al (2014) gelatin pH value relates to the process used. Acid process tends to produce low pH, whereas the alkaline process will have a tendency to produce a high pH. Gelatin with a neutral pH is more preferred that the neutralization process plays an important role in the manufacture of gelatin.

3.2.4 Protein Level

Proteins are polymers of about 21 different amino acids and peptides associated with the bond. Proteins in the gelatin included in the group Scleroprotein simple protein, because gelatin is obtained from the hydrolysis of collagen. (De Man, 1989).

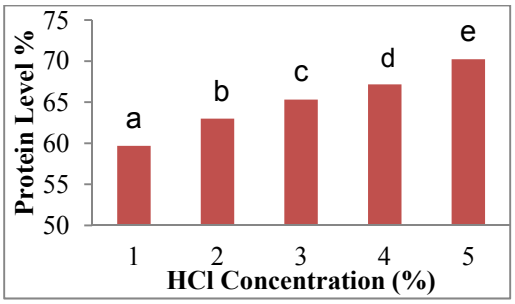

Figure 7 Levels of Protein Gelatin Bone Gourami. Description: notation different letters indicate a significant difference at a significant level α = 0.05

Based on Figure 4.7 the value of the highest levels of the protein present in a concentration of 5% amounting to 70.24%, while the value of the lowest levels of the protein present in a concentration of 1% at 59.68%. Suspected high acid capable hydrolyze collagen into gelatin. According to Rusli (2004), the protein content of the gelatin is affected by the immersion process of bone. Soaking process resulted in the termination reaction of hydrogen bonds and the opening of the coil structure of collagen occurring optimally so that the amount of protein extracted into many. Table 4.1 Based on the analysis of physical and chemical properties of gelatin best results are at concentrations of 5% with the result that much better with the other concentration. This proves the higher the concentration, the better the results of quality gelatin. The value of physical and chemical properties a concentration of 5% produces the highest protein content with other concentrations of 70.24%, with a yield of 12.44%, a value of 6.1 cP viscosity, gel strength 90.61 bloom, the water content of 12%, pH 4.64%, and ash content of 2.18%. According to Martianingsih and Lukman (2010), the immersion process using the acid solution will convert collagen into a form suitable at the time of extraction in the presence of H + ions from the acid solution with collagen.

4 CONCLUSION

Acid concentrations varying significantly affect the resulting gelatin. Based on this research the best concentration present in concentrations of 5%. At the concentration of gelatin values obtained with the physical and chemical properties compared with other concentrations. Treatment of acid concentrations varying influence on the physical and chemical properties of Gourami bone gelatin. The results of the analysis of physical and chemical properties showed significantly different results with commercial gelatin and gelatin laboratory standards. Based on the analysis of physical properties that yield value of 8.05 to 12.44%, ranging between 4.74 to 6.13 cP viscosity and gel strength of 71.90 to 90.61 bloom. As for the chemical analysis that the ash content ranged from 3.78 to 2.18%, water content ranging between 10-12%, the pH value ranging between 4.64 to 4.85% for protein content ranged from 59.68 to 70 , 24.

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