The Effect of Addition Different HCl Concentrations on The Physico-Chemical Properties of Cork Fish (*Ophiocephalus striatus*) Skin Gelatin

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***Abstract*-** Gelatin is a collagen derivative compound found in the skin, bones, and connective tissue of animals that are hydrolyzed using acidic or basic solutions. The industry that uses gelatin the most is the food industry. Cork fish skin has a very high protein and is rich in albumin so that it can be used by people with hypalbumin (low albumin) and accelerate wound healing. The process of making gelatin has 2 extraction methods, namely the acid and base method. The extraction process using the acid method is faster than using the base method, because the acid solution hydrolyzes collagen to gelatin more be faster. For this reason, HCl solutions are used in research to determine the effect of adding concentration on the process of making gelatin. Giving different concentrations during the manufacturing process is expected to produce the best quality gelatin concentration in chemical and physics. The HCl concentration used in the extraction process is 0.03 mol; 0.04 mol; 0.05 mol; 0.06 mol and 0.07 mol with 5 replications. Analysis of the results of the research used were physico analysis (viscosity and gel strength test), chemical analysis (protein content, fat content, ash content, moisture content, yield and pH) and overall acceptance based on the De Garmo method. The research data were analyzed using ANOVA (Analysis of Variance) to determine the effect of treatment on the parameter response performed, with the F test at the level of 5% and if the results were significantly different then the Tukey test was carried out at the level of 5%.

***Index Terms*-** Gelatin, Cork Fish Skin (*Ophiocephalus striatus*), HCl Solutions, Proximate analysis, Viscocity, Gel Strength.

I. INTRODUCTION

Fish cork (*Ophiocephalus striatus*) is fish army, which is quite big can grow up to long 1m, large headed somewhat flattened resemble the heads of a serpent with scales large on his head. Elongated rounded body, like a bullet control. A long dorsal fin and caudal fin globase at the tip. The upper side of the body from head to tail of dark colored, brownish, black or greenish. The under side of the body white, from the chin to the back (Suprayitno, 2014). Cork fish is usually used for public consumption because it has very high protein and is rich in albumin sources. According to Setiawan (2013), albumin is useful for helping the process of healing postoperative wounds and hypoalbumin sufferers. Inside the cork fish skin also contains protein and albumin.

Gelatin is a protein obtained from animal collagen tissue found in the skin, bones and connective tissue (Karim and Bhat, 2008). Gelatin from pig skin is 46%, cow skin 29.4%, beef bone 23.1%, and other sources 1.5% (Harianto et al., 2008). The use of pig skin is less acceptable because of halal status, while the quality of cow skin is of doubtful quality due to the widespread issue of mad cow disease. So that gelatin extracted from cork fish is used as an additive alternative that can be guaranteed of halal status and accepted by the whole community.

Quality gelatin production depends on the use of the right extraction method such as the acid and base method. The difference between the two methods lies in the immersion process. Acids are able to convert triple helix collagen fibers into single strands, while base immersion solutions are only able to produce double strands (Ward and Courts, 1977). This causes at the same time the amount of collagen hydrolyzed by more acidic solutions than base solutions (Tazwir et al., 2008). HCl has advantages over other types of acids because HCl is able to decompose collagen fibers more and more quickly without affecting the quality of the gelatin produced (Kurniadi, 2009).

II. MATERIALS AND METHODS

2.1 Materials

The raw material used in the study was dead cork fish skin obtained from fishermen in Jember Regency. Other materials used are: aquades, pro analysis acetic acid, sodium hydroxide pro analysis, hydrochloric acid pro analysis obtained from the Makmur store, and materials used for testing include: Na2CO3, NaOH, Na2S2O3, HCl, K2SO4, HgO , H2SO4, HClO4, HNO3, distilled water, acetone, and H3BO3, petroleum ether, sodium acetate and 41 whatman filter paper from the Panadia store.

2.2 Method of Research

The method used was experimental method. The analysis of data used in this research was through Completely Randomized Design (RAL) with five replications. There are five concentratios employed 0,03 mol; 0,04 mol; 0,05 mol; 0,06 mol and 0,07 mol. The proximate analysis of gelatin product was also conducted, yield, water content, ash content, protein content, fatty content, gel strength, viscosity and pH.

2.3 Yield Analyze

The yield was obtained from a comparison between the weight of dry gelatin flour produced with the weight of fresh ingredients (skin that has been washed clean).

2.4 Water Content Analyze

The procedure for determining water content is done by weighing 5 grams of gelatin cork fish skin and placed in an empty cup that has been weighed heavily, the cup and lid have been dried in the oven and cooled in the desiccator. The cup containing the sample was then closed and put into an oven with a temperature of 100-102 ºC for 6 hours. The cup is then cooled in a desiccator and after the cold the cup is weighed.

2.5 Ash Content Analyze

The procedure for determining the ash content is carried out by weighing gelatin cork fish skin as much as 5 grams of sample and put into an ignition dish that has been weighed and burned in a furnace at a temperature of 600 ºC and cooled in a desiccator. The cup containing the sample is inserted into the ignition furnace and burned to obtain grayish ash. This ignition is carried out in two stages, namely first at a temperature of around 400ºC for 1 hour and second at a temperature of 550ºC for 5 hours. The cup containing the ash was cooled in a desiccator and then weighed.

2.6 Protein Content Analyze

Determination of protein content was carried out by the micro-kjeldahl method. The sample was weighed as much as 0.2 grams and put in a 30 ml kjeldahl flask. Then add 2 g K2SO4, 50 mg HgO and 2.5 ml H2SO4. The samples are destroyed for 1-1.5 hours until the liquid is clear green and then cooled and added with distilled water slowly. The contents of the flask are transferred into a distillation device, plus 10 ml of concentrated NaOH until it is blackish brown and then distilled. The distillation results are stored in 125 ml erlenmeyer containing 5 ml of H3BO3 and titrated with 0.02N HCl until the color changes to pink.

2.7 Fatty Content Analyze

The gelatin cork fish skin of 2 grams is weighed and wrapped in filter paper and then covered with fat-free cotton and put in a fat flask. After that the sample placed into a Soxhlet extraction tool, with the condenser tool above and the fat pumpkin underneath. Petroleum benzene is added to the fat flask then extracted for ± 6 hours at a temperature of 40 ° C until the solvent that drops back to the fat flask becomes clear. The solvent in the fat flask is distilled so that all the fat solvents evaporate. The extracted fat flask is then dried in an oven at 105 ° C. After that the flask is cooled in a desiccator and weighed.

2.8 Gel Strength Analyze

Gelatin solution with a concentration of 6.67% (b / b) was prepared with distilled water (7.5 grams of gelatin plus 105 ml of distilled water). The solution is stirred using a magnetic stirrer until it is homogeneous then heated to a temperature of 80 oC for 15 minutes. The solution was poured in Standard Bloom Jars (bottles with a diameter of 58–60 mm, height 85 mm), closed and left to stand for 2 minutes. Then incubated at 10 ° C for 17 ± 2 hours. Gel strength was measured using the STEVEN-LFRA brand Texture Analyzer. This tool uses a probe with an area of 0.1923 cm2. The sample was placed under the probe and pressed with a load of 97 grams. The curve height is then measured by using the calipers.

2.9 Viscosity Analyze

Gelatin solution with a concentration of 6.67% (b / b) was prepared with distilled water (7 grams gelatin plus 105 ml of aquades) then the solution was measured for viscosity using a Brookfield Syncro-Visric Viscometer. Measurements were carried out at 60ºC with a shear rate of 60 rpm using a spindle. The measurement results are multiplied by the conversion factor. This test uses spindle no.1 with the conversion factor being 1, the viscosity value is expressed in units of centipoise (cP).

2.10 pH

An example of 0.2 gram is dispersed in 20 ml of distilled water at 80ºC. The example is homogenized with a magnetic stirrer. Then measured the degree of acidity (pH) at room temperature with a pH meter.

III. RESULTS AND DISCUSSION

Based on the proximate results of the physico-chemical properties of gelatin in cork fish skin with HCl concentrations of 0.03 mol; 0.04 mol; 0.05 mol; 0.06 mol and 0.07 mol, commercial gelatin, standard gelatin based on SNI and standard gelatin based on GMIA can be seen in Table 1.

Parameter	Concentrations					Deviation Standart	Commercial Gelatin	Standart Gelatin (SNI, 1995)	Gelatin (GMIA, 2012)
	0,03 mol	0,04 mol	0,05 Mol	0,06 mol	0,07 mol				
Yield (%)	13,97	14,74	16,65	18,98	20,63	0,023	-	-	-
Water Content(%)	11,85	11,56	11,12	10,83	10,49	0,029	12,937	Maks.16	10,5-12
Ash Content (%)	3,61	3,53	3,44	3,32	3,26	0,034	1,633	Maks. 3,25	0,3
Protein Content(%)	68,54	70,11	76,84	84,12	82,01	0,062	87,707	-	-
Fatty Content (%)	0,96	0,83	0,77	0,65	0,57	0,029	0,23	Maks.5	-
Gel Strength (Bloom)	89,64	86,06	80,25	73,01	70,95	0,036	318,300	-	50-300
Viskosity (cP)	9,68	10,62	12,05	13,21	15,83	0,022	9,75	-	15 - 75
pH	3,54	3,49	3,41	3,37	3,32	0,025	4,00	-	3,8 -5,5

Based on Table 1, the results of the analysis proximate can be read thatthe quality of gelatin produced is not much different from the quality standards required by SNI, GMIA and commercial gelatin. The highest yield and fat content at HCl concentration was 0.07 mol. Water content, gel strength and highest pH at HCl concentration of 0.03 mol. The highest protein content at a concentration of 0.06 mol.

3.1 Yield

The results showed that the highest yield was obtained at 0.07 mol HCl concentration with a value of 20.66%, while the lowest yield was at 0.03 mol HCl concentration with a value of 13.94%.So as the higher concentration of HCl, the higher yield produced. This is because the higher the concentration of acid, the more the structure of collagen fibers is broken down and binds to water so that more gelatin produced. The tendency to increase yield with increasing acid concentration is possible because of the large number of H+ ions that interact with the structure of the trophagenagen. The triple helical collagen structure loses its triple helical structure because the hydrogen bonds in collagen and the bonds between the tropochagen are weakened, so that collagen is converted and becomes an ideal form for extraction (Martianingsih et al, 2009)

3.2 Water Content

The results showed that the highest water content was obtained at 0.03 mol HCl concentration with a value of 11.90%, while the lowest water content at HCl concentration was 0.07 mol with a value of 10.45%. So as the higher HCl concentration, the lower water content will be produced. This is due to the high concentration of HCl which causes the binding power of the water to become weaker in gelatin, so that the water evaporates easily when drying. Increasing the concentration in the acid solution causes a decrease in the gelatin water content. This is due to the more acid (H+ ions) in the soaking solution, causing the structure of collagen to be more open, thus the less water is physically trapped in the structure of the collagen matrix which causes the water content to be lower (BSN, 1995).

3.3 Ash Content

The results showed that the highest ash content was obtained at 0.03 mol HCl concentration with a value of 3.65%, while the lowest ash content was at 0.07 mol HCl concentration with a value of 3.22%.So as the higher HCl concentration, the lower ash content will be produced. This is due to the large amount of minerals that are dissolved during the washing process. The high ash content of the gelatin of cork fish skin is suspected because there are still many amounts of minerals that are not dissolved in the washing process. According to Yuliani (2014), the process of immersion in an acid solution in addition to aiming to convert collagen into collagen which is ready to be extracted in water, also to dissolve minerals such as calcium and other salts. Thus the higher the concentration of acid used causes more dissolved minerals. This causes the lower mineral content in ossein, which means the lower the mineral content in the gelatin produced.

3.4 Protein Content

The results of the study that the highest protein was obtained at 0.06 mol HCl concentration with a value of 84.15%, while the lowest protein content was 0.03 mol HCl with a value of 68.43%. So as the higher HCl concentration, the higher value of the protein to be produced. This is because during the extraction process more and more compounds are extracted, causing the resulting gelatin to become purer so that the protein produced is higher. In addition, it is also related to other proximate compositions such as ash content and fat content of gelatin from dry fish skin lower than fresh fish skin. The high level of gelatin protein is due to the low non-gelatin component such as ash content and other non-gelatin components (Peranginangin, 2005).

3.5 Fatty Content

The results of the study that kadar lemak tertinggi didapatkan pada konsentrasi HCl 0,03 mol dengan nilai 1,01%, sedangkan kadar lemak terendah pada konsentrasi HCl 0,07 mol dengan nilai sebesar 0,53%. So as the higher concentration of HCl, the lower value of the fat to be produced. The fat content of gelatin in low cork fish skin makes it possible to store gelatin in a long time without causing significant changes in quality and odor. Fat content in gelatin is very dependent on the treatment during the gelatin making process. The manufacturing process starts from the skin cleansing stage until the filtration stage is extracted. Good treatment at each stage of the gelatin making process will reduce the fat content in the raw material (Trilaksani et al., 2012).

3.6 Gel Strength

The results showed that the highest gel strength was obtained at 0.03 mol HCl concentration with a value of 89.66 gr bloom, while the lowest gel strength was 0.07 mol HCl concentration with a value of 70.93 gr bloom. So as the higher concentration of HCl, the lower value of the gel strength produced. This is because the higher the concentration of acid, the amino acid chain produced is getting shorter so that the strength of the gel gets lower. Gel strength values decrease with increasing concentration and long duration. Increased acid concentration means an increase in the number of H+ ions in solution and will cause the collagen protein to undergo further hydrolysis so that the collagen protein polypeptide chain gets shorter. Shorter polypeptide chains not only can increase their solubility, but can also reduce the ability to thicken (Kusnandar, 2010).

3.7 Viscosity

The results showed that the highest viscosity was obtained at 0.07 mol HCl concentration with a value of 15.86 cP, while the lowest viscosity at 0.03 mol HCl concentration with a value of 9.65 cP. So as the higher concentration of HCl, the higher viscosity produced. the value of viscosity or thickness of gelatin solution is very closely related to the water content of dry gelatin. The smaller the gelatinous water content is dry, the higher ability to bind water (to form a gel). The more amount of water bound by the gelatin, make solution will become thicker, which directly affects the higher value of the measured viscosity.

Gelatin viscosity is influenced by gelatin pH, temperature, gelatin concentration and the addition of other electrolytes in the gelatin solution. The lower the temperature of the gelatin solution (maximum 40ºC) and the higher the concentration of gelatin, the higher the viscosity ($p < 0.05$). The viscosity value of this gelatin will affect the final product of a product (Karim 2009).

3.9 pH

The results showed that the highest pH was obtained at 0.03 mol HCl concentration with a value of 3.58, while the lowest pH was at 0.07 mol HCl concentration with a value of 3.28. So as the higher HCl concentration, the lower value of pH produced. This is because the higher concentration of acid during immersion, the higher content of H+ ions produced, this results in a lower pH value in the solution.

According to Stainsby, (1977) the concentration of acid is getting higher, causing more acidic cations trapped in the ossein, so that the measured pH is lower (acid) and the hydrolysis of collagen will continue in the decomposition process of collagen polymer. HCl during immersion (demineralization) acid cation is trapped in collagen protein so that the gelatin produced has a lower pH.

IV. CONCLUSION

Based on the results of this study, conclusions were obtained that physical based observations were not much different from the quality standards required by SNI. Different HCl concentrations (0.03 mol; 0.04 mol; 0.05 mol; 0.06 mol and 0.07 mol) significantly affected the physico-chemical properties (yield, water content, ash content, protein content, fat content , gel strength, viscosity, pH). The yield and viscosity of gelatin in cork fish skin using different HCL concentrations showed higher values with increasing concentration. Whereas the moisture content, ash content, fat content, gel strength, and gelatin pH of cork fish skin using different HCL concentrations showed lower values with increasing concentration. The best HCl concentration was located at a concentration of 0.07 moles. This result was obtained from water content, ash content, fat content, gel strength and viscosity which produce the best value of the other HCl concentrations (0.03 mol; 0.04 mol;
0.05 mol and 0.06 mol).

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