

PRODUCTION AND CHARACTERISATION OF GELATIN FROM RABBIT BONE AS BIOPLASTICS MATERIAL BY ACID PRE-TREATMENT

Dwi Wulandari¹*, Indri Hermiyati¹†, Iswahyuni Iswahyuni¹†, Armila Zahra Tawarniate*

*Department of Leather Processing Technology, Polytechnic ATK Yogyakarta, Jl. South Ring Road, Sewon, BANTUL, Yogyakarta 55188 Indonesia.

†Department of Plastic Rubber Processing Technology, Polytechnic ATK Yogyakarta, Jl. South Ring Road, Sewon, BANTUL, Yogyakarta 55188 Indonesia.

Abstract: This study aims to find out the effect of hydrochloric acid curing concentration on the characteristics of rabbit bone gelatin and determine the level of hydrochloric acid concentration for the soaking process to produce the best characteristics of rabbit bone gelatin. The material used was 50 kg of Rex rabbit bones obtained from rabbit farms, HCl 4, 5 and 6% and distilled water. The rabbit skin was soaked in hydrochloric acid (4, 5 and 6%) for 4 d as treatment and replicated three times. Gelatin extraction was performed three times at temperatures of 65, 75 and 85°C for 4 h each time and the results obtained were filtered through filter paper. The filtrate was concentrated at 50°C for 5 h. The concentrated filtrate was then poured into a tray before drying in an oven at 50°C until dry. Milling was carried out until it became gelatin powder. This study used a completely randomised design with a unidirectional pattern, and if there was a significant difference, continued with Duncan's multiple range test. The results showed that the rabbit bone gelatin yield was between 6.18-8.52%, moisture 8.08-8.45%, ash content 8.15-10.93%, pH 3.85-4, protein content 57.09-62.84%, fat content 0.04-0.27%, gel strength 74.47-129.09 bloom, viscosity 3.06-4.26 cP, thick point 10-12°C, melting point 33-35°C and the molecular weights were 85, 120, and 212.5 kDa. The characteristics of rabbit bone gelatin still meet the Standar Nasional Indonesia gelatin range. Curing treatment with 6% HCl gave the best gelatin characteristics.

Key Words: rabbit, bioplastic, gelatin, rabbit bone.

INTRODUCTION

Currently, the processing industry is increasingly pushing for the introduction of more attractive products such as ready-to-eat and ready-to-cook, among others, including rabbit meat, which is mostly sold as whole carcasses or pieces (Petracci and Cavani, 2013). Therefore, the implementation of management planning and strategy as well as the continuous improvement of production and efficient use of by-products has also become a major task for the rabbit meat industry. It is further explained that for this implementation it is necessary to analyse the strengths and weaknesses of the main factors regarding the use of rabbit meat for the manufacture of further processed products. It is desirable to process all by-products into valuable products for human food, pet food, animal feed, medicine or fertiliser, and more recently for biodiesel manufacture (Mohan and Long, 2021).

Correspondence: D. Wulandari, dwiw2313@gmail.com. Received November 2021 - Accepted December 2021.
<https://doi.org/10.4995/wrs.2022.16639>

Cite as: Wulandari D., Hermiyati I., Iswahyuni, Tawarniate A.Z. 2022. Production and characterisation of gelatin from rabbit bone as bioplastics material by acid pre-treatment. *World Rabbit Sci.*, 30: 83-93. <https://doi.org/10.4995/wrs.2022.16639>

The amount of rabbit bone waste can be seen from the estimation of meat consumption from supermarkets, shops and home consumption to be calculated (Petracci *et al.*, 2018). If the amount of rabbit meat consumed increases, the number of bones will also increase, which will pose a new problem for the rabbit meat-producing industry.

Rabbit bone as waste is a potential source of gelatin that can increase its value, but has not been optimally explored. Bone waste is usually crushed and used as an additional ingredient in animal food.

Gelatin is a type of protein obtained from natural collagen found in skin, bones and connective tissue. Gelatin is produced by extracting and hydrolysing collagen. The extraction and hydrolysis processes lead to denaturation of the triple helix collagen arrangement protein into a single chain by combining with three peptide bonds to produce gelatin compounds (Panjaitan, 2016). Indonesia has a high demand for gelatin, especially as an ingredient for both food and non-food products. Gelatin is commonly used in the pharmaceutical, cosmetic and food industries as a foaming agent, binder, stabiliser, gelling agent and emulsifier (Huda *et al.*, 2013). Gelatin is also widely used as a wrapper for food that can be eaten (*edible film*), bioplastic or biodegradable plastic.

Bioplastics are plastics that can be used like conventional plastic, but are biodegradable by the activity of microorganisms into water and carbon dioxide gas after being used up and discharged into the environment. Bioplastics are made from nature and can return to nature, including plastic materials that are environmentally friendly (Sinaga *et al.*, 2014).

One type of biodegradable plastic is made from starch as raw material. Starch-based bioplastics are brittle and easily damaged when subjected to a load. One way to overcome this problem is by adding a plasticiser that reduces the stiffness of the polymer material. One of the natural ingredients that can function as a plasticiser is gelatin.

Pork and beef are the common sources of gelatin that are continuously explored. Other sources of gelatin are poultry, marine animals and insects. Gelatin extracted from bones and feet of poultry and fish have similar characteristics to pork gelatin (Miskah *et al.*, 2010; Jannah *et al.*, 2013). The functional group of catfish bone gelatin has the same structure and quality as commercial gelatin (Permata *et al.*, 2016). The same study was conducted for iridescent shark fish bone gelatin (Atma *et al.*, 2018) and with its duck bone gelatin (Khirzin *et al.*, 2019).

Indonesia, especially in Magetan District, has abundant potential for rabbit bones every day. At least 400 rabbits are slaughtered for consumption purposes. The value of rabbit bones as a basic ingredient for gelatin is unknown in the Magetan District due to the lack of information distributed on the subject. The 30% protein content of goatskin in their research is a source of collagen protein that has the potential to produce protein derivative products, including bioactive peptides (Hakim *et al.*, 2021). The protein content in bones is 33% (Herniawati, 2008), meaning that rabbit bones are a source of collagen protein which can be made into derivative products such as gelatin. When collagen is treated with acid or base followed by heat, the fibrous structure of collagen is broken down irreversibly to produce gelatin (Zhou and Regenstein, 2005). Acids can convert triple-helical collagen fibres into single chains, whereas alkaline solutions are only able to produce double chains (Ward and Court, 2009).

The use of hydrochloric acid in different concentrations as a solvent to produce gelatin from rabbit bone has not been reported. Therefore, research into the production and characterisation of gelatin produced from rabbit bone using a hydrochloric acid soaking solution needs to be conducted. The aims of this study are to find out the effect of the different concentrations of hydrochloric acid immersion solution (4, 5 and 6%) on the characteristics of rabbit bone gelatin.

MATERIALS AND METHODS

Materials

The material used in the study was the bones of Rex rabbit isolated from 50 kg of rabbit obtained from the Republic AE farm, in Magetan District, East Java Province, Indonesia. The chemicals used for curing were hydrochloric acid (HCl) at 4, 5 and 6% and distilled water. The tools used in this work included knives, analytical scales, water baths, buckets, glassware, thermometers, ovens, filter paper, Viscosimeter, Universal Testing Machine, Lovibond grinder and cooler.

Experimental design

The study used a completely randomised design with a unidirectional pattern, applying three different concentrations of hydrochloric acid curing agents as treatment, at 4, 5 and 6%, and was replicated three times.

Rabbit bone preparation

Collagen extraction was started by boiling the raw materials to remove the remaining meat. Rabbit bones were then cut into 2-3 cm, weighed and then washed with running water at pH 7. The bone was then soaked in 4, 5 and 6% hydrochloric acid for 4 d and neutralised with water at pH 7 at stratified temperature of 65, 75, and 85°C, respectively, for 4 h. The extracts were filtered through filter paper. The filtrate was then concentrated at 50°C for 5 h. The filtrate was then poured into a tray and dried in an oven at 50°C. The dry material was then milled. The process sequence is shown in Figure 1.

Gelatin yield weight determination

The weight of gelatin yield was calculated according to the method of (Giménez *et al.*, 2005) by dividing by the weight of extracted rabbit bones multiplied by 100%.

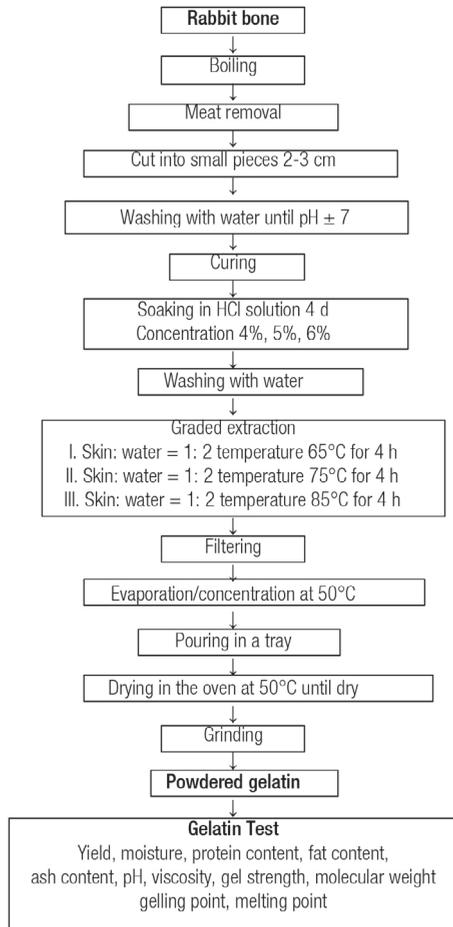


Figure 1: Rabbit bone gelatin preparation flowchart.

Chemical composition analyses

Chemical compositions gelatin extracted from rabbit bones were analysed for its moisture, fat, protein, and ash content using the method of AOAC (2012).

Gelatin pH measurement

The gelatin solution with a concentration of 6.67% (w/w) was prepared with distilled water before measuring the acidity of gelatin. The gelatin solution was heated to 70°C and homogenised with a magnetic stirrer, then the degree of acidity was measured at room temperature with a pH meter (British Standard 757, 1975).

Viscosity analysis

Gelatin solution at 6.67% concentration was boiled in a water bath and continuously stirred up to 60°C. Viscosity was measured using a Brookfield viscometer (Zhengzhou Nanbei Instrument Equipment Co., Henan, China). A spindle was previously heated to 60°C and then installed in the Brookfield viscometer. The spindle position in the hot solution was set accurately, then the viscometer was turned on and the temperature of the solution was measured. When the solution temperature reached 60°C, the viscosity value was read through the viscometer at a scale of 1-100. The reading was done after 1 min of full rotation 2 times for spindle n° 1.

Gel-strength measurement

The strength of the gel was determined according to Liu *et al.* (2009), using a Universal Testing Machine (Zwick, Ulm, Germany). Gelatin samples at a concentration of 6.67% w/v were dissolved in distilled water at 60°C. The solution was stirred until the gelatin was solubilised completely. The solution, in dimensions of 5 cm in diameter and 6 cm in height, was stored at 5°C for 16-18 h. The gelatin sample container was placed right at the bottom of the plunger (with a diameter of 13 mm) for the testing process. The measurement was conducted at a temperature of 10°C with a plunger speed of 10 mm/min and a depth of 4 mm. The gel strength value calculation was expressed in bloom= F_{max} (g/mm²) \times 12.7 (a surface area of the needle).

Gelling point measurement

Gelatin solution at a concentration of 6.67% (w/w) was prepared with distilled water in a 15 mL volume test tube connected to a Hanna digital thermometer sensor. The sample was lowered in temperature slowly by placing it in a container with crushed ice. The gelling point was determined when the sensor could lift the gel in the test tube (Suryaningrum and Utomo, 2002).

Melting point measurement

Gelatin solution at a concentration of 6.67% (w/w) was prepared with distilled water. Samples were incubated at 100°C for 17 \pm 2 h. Melting point measurements were carried out by heating the gelatin gel in a water bath. On the top of the gelatin gel, the buckwheat is placed, and when the buckwheat reaches the bottom of the gelatin gel, the temperature is determined as the melting point of the gelatin. (Suryaningrum and Utomo, 2002).

Molecular weight distribution determination

The distribution of molecular weight was determined according to the method of Laemmli (1970) with sodium sulphate dodecyl polyacrylamide gel electrophoresis (SDS-PAGE) using Atto Pugeran AE 6531 (Atto, Tokyo, Japan). The gelatin sample was diluted with sterile distilled water and mixed. The mixtures were centrifuged at 3000 rpm for 10 min at room temperature. Samples and supernatants were mixed at the ratio of 1:1, and denaturation was conducted at 100°C for 5 min before cooling at room temperature. The gelatin samples at the volume of 10 μ L were loaded into 12% resolving gel and a 5% stacking gel. The gel was then stained with Coomassie Blue.

Data analysis

If there was a significant difference, analysis was continued with Duncan's multiple range test (DMRT) according to Steel and Torrie (1993). The statistical significance level was set at $P < 0.05$. The statistical software package SPSS 15.0 (SPSS, Inc., Chicago, IL, USA) was used for these data analyses, while the molecular weight data were analysed descriptively.

RESULTS AND DISCUSSION

The physical and chemical properties of gelatin produced from rabbit bones are presented in Table 1. The higher the percentage of HCl, the higher the yield of gelatin. The average gelatin yield was $6.25 \pm 0.11\%$ to $8.42 \pm 0.43\%$. Statistical analysis showed that the gelatin yield was significantly different between treatments of 4% (6.25 ± 0.11), 5% (7.49 ± 0.16) and 6% (8.42 ± 0.43).

Type A gelatin can be extracted using acids such as HCl, H_2SO_4 . Acidic extraction is considered more effective than base extraction and produces higher yields. This is a standard method that produces halal gelatin (Yudhistira *et al.*, 2019). Variations in acid concentration also affect the ash content and moisture of chicken leg bone gelatin, while the immersion time affects the viscosity value (Huda *et al.*, 2013). Moreover, the acid concentration used in the demineralisation process greatly determines the quantity and quality of gelatin (See *et al.*, 2015).

The table shows that the higher the HCl concentration, the more gelatin was obtained. This is because the higher the HCl concentration, the easier it is to convert the collagen into gelatin. The yield of gelatin extracted from catfish bone hydrochloric acid increased along with the increasing HCl concentration until it reached the peak and decreased again, even though the HCl concentration was increased (Permata *et al.*, 2016).

Statistical analysis showed a significant difference in gelatin yield among treatments. This indicated that increasing the concentration of HCl affected the gelatin yield. This result was in line with the research conducted by (Huda *et al.*, 2013) in that the greater the concentration in the HCl solvent, the higher the yield of gelatin produced. The increase in gelatin yield was because the HCL reacted with calcium in the bones and caused the calcium salt in the bones to dissolve. HCl is a strong inorganic acid that produces more hydrogen ions, which causes the dissolution of intra and intermolecular cross-linked collagen (Mulyani *et al.*, 2017). The triple-helical structure of collagen turns into random coils, resulting in higher gelatin yields.

The treatment of HCl acid concentration in the production of rabbit bone gelatin resulted in a significant effect on moisture, ash content and gelatin fat content ($P < 0.05$). However, there was no significant effect on protein content and gelatin pH. The average moisture of gelatin was 8.42 ± 0.06 - $8.08 \pm 0.03\%$, ash content was 8.23 ± 0.07 - $10.87 \pm 0.06\%$, and the average ash content decreased with increasing HCl percentage; fat content was 0.03 ± 0.01 - $0.24 \pm 0.04\%$, while the average protein content was 57.63 ± 0.01 - $62.81 \pm 0.025\%$ and the pH of gelatin was 3.88 ± 0.01 - $3.95 \pm 0.01\%$.

The percentage of HCl that binds to 6% resulted in a decrease in moisture, as strong acids have a greater ability to hydrolyse collagen into shorter gelatin peptides, so that during drying, the surface area becomes wider, causing more water evaporation. The moisture of gelatine produced in this study was between 8.06-8.47%, which still met the SNI 1995 (National Indonesian Standard 1995), which required a maximum of 16%.

Table 1 shows the ash content of rabbit bone gelatin, which is somewhat higher than that of SNI 1995, where the maximum is 3.25%. This result indicated that the mineral content of rabbit bone gelatin was quite high. The results of analysis for Ca content of rabbit bone gelatin was low (29.41%), because the rabbit bone as a source of gelatin is rich in calcium content. The more the HCl concentration was increased, the lower the ash content. This is due to the reaction between HCl and calcium phosphate of bone produced calcium salts that dissolved during this reaction and softened the bones. The higher the concentration of HCl, the more dissolved calcium salts caused the mineral content of rabbit bones to decrease during extraction. The decrease in ash content indicates the absence of inorganic salt content in the gelatin produced during pre-treatment with acid (Ahmad and Benjakul, 2011). In line with that, Wardhani *et al.* (2017) also reported that the ash content was affected by the demineralisation process during gelatin extraction from fish scales, and this content process was reduced by using NaCl and NaOH.

Table 1: Physical and chemical properties of rabbit bone gelatin with curing percentage of HCl.

Parameters	Replay	Hydrochloric acid percentage		
		4%	5%	6%
Yield	1	6.18	7.28	7.95
	2	6.38	7.58	8.79
	3	6.20	7.25	8.52
Total		18.76	22.21	25.26
Average		6.25±0.11 ^a	7.40±0.16 ^b	8.42±0.43 ^c
Moisture (%)	1	8.47	8.43	8.08
	2	8.35	8.45	8.12
	3	8.44	8.42	8.06
Total		25.26	25.30	24.26
Average		8.42±0.06 ^a	8.43±0.02 ^a	8.08±0.03 ^b
Ash (%)	1	10.93	9.14	8.15
	2	10.81	9.19	8.26
	3	10.88	9.15	8.28
Total		30.62	27.48	24.69
Average		10.87±0.06 ^a	9.16±0.03 ^b	8.23±0.07 ^c
Fat (%)	1	0.03	0.06	0.20
	2	0.04	0.05	0.24
	3	0.03	0.05	0.27
Total		0.10	0.16	0.71
Average		0.03±0.01 ^a	0.05±0.01 ^a	0.24±0.04 ^b
Protein (%)	1	57.82	59.70	63.04
	2	58.09	60.66	62.84
	3	57.97	60.51	62.55
Total		172.88	180.87	188.43
Average		57.63±0.14 ^a	60.29±0.52 ^a	62.81±0.25 ^a
pH	1	3.88	3.90	3.94
	2	3.90	4.00	3.95
	3	3.88	3.80	3.95
Total		11.66	11.79	11.84
Average		3.89±0.01 ^a	3.90±0.10 ^a	3.95±0.01 ^a
Viscosity (cP)	1	3.06	3.42	4.20
	2	3.08	3.47	4.24
	3	3.06	3.44	4.26
Total		9.23	10.33	12.27
Average		3.07±0.01 ^a	3.44±0.03 ^b	4.23±0.03 ^c
Gel Strength (Bloom)	1	75.00	99.17	128.23
	2	74.47	97.30	129.09
	3	74.91	98.59	127.02
Total		224.38	295.06	384.34
Average		74.79±0.29 ^a	98.35±0.96 ^b	128.11±1.04 ^c
Gelling Point (°C)	1	10	11	12
	2	10	10	11
	3	11	11	12
Total		31	32	35
Average		10.33±0.58 ^a	10.67±0.58 ^a	11.67±0.58 ^b
Melting Point (°C)	1	33	33.5	35
	2	33	34	34
	3	33	33	34
Total		99	100.5	103
Average		33.00±0.00 ^a	33.50±0.50 ^b	34.33±0.58 ^b

Different superscripts in the same row and column indicate significant differences ($P < 0.05$).

The fat content of rabbit bone gelatin in this current study ranged from 0.03 to 0.1%, which was much lower than that stipulated by Standar Nasional Indonesia (SNI) 06-3735-1995, which is a maximum of 5%. The HCl concentration gave a significant effect on the fat with ($P < 0.05$). The good quality of gelatin is indicated by the low-fat content (Said *et al.*, 2012). The low-fat content is because the bone that is used as a gelatin source contains 66.4% of minerals.

It was also influenced by the time and temperature used during extraction. The extraction temperature in this study used a stratified temperature of 65, 75, and 85°C for 4 h, respectively. The longer the heating time, the lower the fat content, as unsaturated fatty acids will be oxidised by heat and decomposed into shorter carbon chains that make them more soluble. The duration of boiling and high temperatures are thought to be able to optimally separate the fat content in the bones, so the resulting gelatin contains low fat.

The protein content of rabbit bone gelatin in this study ranged from 57.82 to 62.84%. The greater concentration of HCl treatment resulted in higher protein content. The greater the concentration of HCl, the stronger it is in breaking hydrogen bonds and opening the collagen fabric, so more collagen is converted into gelatin. Protein content in gelatin can also be influenced by the quality of ossein produced during the demineralisation process (Jannah *et al.*, 2013).

The protein content of gelatin extracted from rabbit bones in this study was lower than that extracted from chicken bones (Jannah *et al.*, 2013), i.e., 79.66-85% protein for free-range chicken bone gelatin and 78.58-86.27% for broiler chickens. The low protein content may be related to the protein content of bones as the raw material, which is 24%. Ward and Court (2009) stated that the protein content of gelatin varies depending on the species of animal used as raw material, the source of collagen and the type of collagen. Meanwhile, according to Choi and Regenstain (2000), the quality of gelatin depends on the source of the raw material, the species or connective tissue extracted and the method used to produce it. The low levels of this protein can also be because not all the collagen has been separated from bone tissue during the mineralisation.

The pH value of the rabbit bone gelatin in the study ranged from 3.80 to 4. That met the standard for type A gelatin, since the acidic gelatin was 3.8-5.5. The pH value of the extracted gelatin did not meet the SNI standard, namely 4.5-6.5. The low pH value was achieved due to the use of HCl as a strong acid. During demineralisation, the development of collagen (ossein) occurred, causing the high level of HCl to be absorbed into the expanding collagen and trapped in a network of collagen fibrils. When neutralised by washing, some HCl remained, thus affecting the acidity of the resulting gelatin.

The physical properties of gelatin produced from rabbit bone gelatin with HCl percentage treatment are also presented in Table 1. Statistical analysis of the physical properties of rabbit bone gelatin showed significant differences in gel strength, density point and melting point. The higher percentage of HCl resulted in the greater physical properties of gelatin. The average gelatin viscosity was 3.07 ± 0.01 - 4.23 ± 0.03 cP, gel strength was 74.79 ± 0.29 - 128.11 ± 1.04 Bloom, thickness point was 10.33 ± 0.05 - 11.67 ± 0.58 °C, and melting point was 33.00 ± 0.00 - 34.33 ± 0.58 °C.

The viscosity value of rabbit bone gelatin complies with the requirements of the 1995 SNI, namely 2.5-5 cP. The higher the concentration of HCl up to 6%, the higher the gelatin viscosity, as the higher acid concentration affects the collagen structure. The more swollen and more open amino acid chain structure caused the release of more amino acid chains, thus increasing the viscosity value. The viscosity value is directly proportional to the protein content. Santoso *et al.* (2015) explained that the protein in gelatin is highly influential on the formation of the gel. The higher the protein level, the greater its ability to form a gel and increase the viscosity.

The increase in viscosity value is influenced by the molecular structure of amino acids that make up the gelatin protein. The longer amino acid composition will increase the gelatin viscosity value (Bahar *et al.*, 2018). Further explained, the difference in curing and extraction time affected the viscosity of gelatin. The increased viscosity was influenced by the molecular structure of amino acids that make up the gelatin protein. The longer the amino acid chain, the more the gelatin viscosity increased.

The results of this study showed that the viscosity ranged from 3.06 to 4.26 cP. These results were similar to those of (Pertiwi *et al.*, 2018), who reported 3.83 cP for iridescent shark fish bones, and those of (Darwin *et al.*, 2018), who found 2.68-4.74 cP for tilapia fish bones.

The gel strength values obtained in this study ranged from 74.47-129.09 Bloom. The gel strength of rabbit bone gelatin still met the GMIA (2012) range, which is 50-300 Bloom and is classified as gelatin with low gel (<150 Bloom)

(Rahman and Jamalulail, 2012). The difference in the HCl concentration significantly affected the gel strength; this was because due to the increasing concentration of acid, the hydrolysis went well and the heating process damaged the gelatin, forming a gel structure (Hafsari *et al.*, 2018)

Gelatin gel formation is influenced by several factors, including pH, temperature and concentration of the curing agent (Tazwir *et al.*, 2014). The results showed that the greater the HCl concentration, the higher the value of the gel strength. The increased concentration of HCl increased the termination of the amino acid polymer chain which caused large amounts of collagen to be converted to gelatin, which also caused the gel strength to increase. According to Kharim and Bath (2009), the concentration of curing agents affects the strength of gelatin gel. High gel strength is related to the high molecular weight of the peptide. Sinthusamran *et al.* (2014) explained that gelatins with different molecular weight distributions and amino acid compositions have different gel strengths.

The gelling point of gelatin increased (T1=33.00°C, T2=33.50°C, and T3=34.33°C) along with the increase of HCl concentration, as well as the melting point. The increase in HCl levels caused more collagen to be converted into protein during extraction. High levels of protein determine the content of amino acids such as proline and hydroxyproline. Eysturskard *et al.* (2009) stated that the higher the proline and hydroxyproline, the higher the gelling point of gelatin, and it is proportional to the protein content of gelatin. Zulkifli *et al.* (2014) also explained that the gel point of tuna bone is higher due to the increased protein content with an increase in the volume of vinegar.

The melting point of gelatin in this study ranged from 33-35°C, lower than commercial gelatin, which was 37°C, but still in the Food Chemical Codex (1996) range, which was below 35°C. Gelatin can also melt in the mouth.

The analysis of the molecular weight of rabbit bone gelatin using SDS-PAGE is shown in Figure 2. The distribution of molecular weight increased along with the increase in HCl concentration. Figure 2 shows the molecular weight of T1 is 85 kDa, T2=120 kDa, T3=212 kDa.

The molecular weight of gelatin from rabbit bone that was determined by the SDS-PAGE method is presented in Figure 2. The gelatin protein band seemed to be thin and faint. The T1, T2, and T3 treatments were using HCl at concentrations of 4, 5 and 6% respectively, the extraction temperature was 65°C, and was then increased to 75°C and 85°C. After 4 h of extraction of T1, T2 and T2 treatments, proteins were generated with molecular weights of 85, 120 and 212.5 kDa, respectively, while that of commercial gelatin was 225 kDa. These results are different from those of Mahmoodani *et al.* (2014), who found that the gelatin extracted from catfish bone using hydrochloric acid resulted in a protein with a molecular weight of >97-120 kDa and indicated as α bonds protein, while β and γ bonds were between 200-300 kDa.



Figure 2: Results of gel electrophoresis of rabbit bone gelatin. M=Marker, T1=immersion with 4% HCl, T2=immersion with 5% HCl, T3=immersion with 6% HCl, GK=commercial gelatin.

The protein pattern generated from the T3 treatment showed a high molecular weight of 212.5 kDa. This gelatin protein is a β sheet protein where the molecular weight is between 160-250 kDa, while the T1 and T2 treatments generated protein with a molecular weight of 97-120 kDa as α sheet group protein.

Figure 2 shows that the higher the concentration of HCl, the greater the molecular weight, because of the greater hydrolysis of collagen, and the longer cleaved polypeptide. This is seen through the increase in melting point, thickness point, viscosity and gel strength, along with the increasing of HCl concentration and decreasing of moisture. A large molecular weight in this result indicated there was more cross-linking and less water binding.

The results of the study were similar to those of (Hardikawati *et al.*, 2016), where a similar molecular weight (85-212.5 kDa) of gelatin proteins was obtained from the skin of broiler chicken using citric acid and NaOH as curing treatment. Different sources of gelatin and different curing treatment resulted in different molecular weights of gelatin proteins. The iridescent shark fishbone treated with HCl and NaOH resulted in 116-200 kDa gelatin proteins (Mahmoodani *et al.*, 2014), while the iridescent shark fishbone with citric acid pre-treatment resulted in 38-162 kDa gelatin proteins (Pertiwi *et al.*, 2018), iridescent shark fishbone with pineapple waste treatment resulted in 75-225 kDa gelatin proteins (Atma *et al.*, 2018), and iridescent shark fishbone with 0.1 M NaOH immersion resulted in 25-200 kDa gelatin proteins (Nuryanto *et al.*, 2018).

CONCLUSION

The results of the production of rabbit bone gelatin pre-treated with HCl concentrations at 4, 5 and 6% for 4 d and extracted at a temperature of 65, 75, and 85°C, respectively, for 4 h each showed the gelatin with the characteristics that still met the requirements of SNI 06-3735-1995. The gelatin with the best characteristics is gelatin produced using a 6% HCl treatment with a yield of 8.42%, moisture of 8.08%, ash content of 8.23%, fat content of 0.24%, pH of 3.95, protein content of 62.81%, gel strength of 128.11 Bloom, viscosity of 4.23 cP, gelling point of 12°C, melting point of 35°C and a molecular weight of 212.5 kDa.

This characteristic of rabbit bone gelatin has potential as a raw material for the manufacture of bioplastics or biodegradable plastics which will be destroyed by microorganism activity. Bioplastics from rabbit bone gelatin are environmentally friendly plastics.

Acknowledgements: The author would like to thank the ATK polytechnic for funding, Rabbit Farms of Republic AE from Magetan Regency for their support in obtaining some raw material, PAU UGM for carrying out chemical tests, Engineering Laboratory, Faculty of Agricultural Technology UGM for carrying out physio test and UGM Faculty of Fisheries Microbiology Laboratory for carrying out determination of distribution of molecular weight.

REFERENCES

- Ahmad M., Benjakul S. 2011. Characteristics of gelatin from the skin of unicorn leatherjacket (*Aluterus monoceros*) as influenced by acid pretreatment and extraction time. *Food Hydrocoll.*, 25: 381-388. <https://doi.org/10.1016/j.foodhyd.2010.07.004>
- AOAC. 2012. Official Methods of Analysis of AOAC International. In Association of Official Analysis Chemists International. Arlington: Association of Official Analytical Chemists Inc.
- Atma Y., Ramdhani H., Mustopa A.Z., Pertiwi M., Maisarah R. 2018. Physicochemical characteristics of catfish bone gelatin (*Pangasius sutchi*) extracted using pineapple waste (*Ananas comosus*). *Agritech.*, 38: 56-63. <https://doi.org/10.22146/agritech.29821>
- Bahar A., Kusumawati N., Anggrani M.A., Muslim S. 2018. Optimization of curing and extraction time on the production of base gelatin from bovine skin. *Material*, 112: 58-62. (*Iconhomecs 2017*), <https://doi.org/10.2991/iconhomecs-17.2018.14>
- British Standard 757. 1975. Sampling and Testing of Gelatin. In Imeson: Thickening and Gelling Agents for Food. 1992. Academic Press, New York.
- Choi S.S., Regenstein J.M. 2000. Physicochemical and sensory characteristics of fish gelatin. *J. Food Sci.*, 65: 194-199. <https://doi.org/10.1111/j.1365-2621.2000.tb15978.x>
- Darwin D., Ridhay A., Hardi J. 2018. Study of extracting gelatin from tilapia fish bones (*Oreochromis mossambicus*). *Kovalen: J. Ris. Kim.*, 4: 1-15. <https://doi.org/10.22487/kovalen.2018.v4.i1.10177>
- Eysturskard J., Haug I.J., Elharfaoui N., Djabourov M., Draget K. I. 2009. Structural and mechanical properties of fish gelatin as a function of extraction conditions. *Food Hydrocoll.*, 23: 1702-1711. <https://doi.org/10.1016/j.foodhyd.2009.01.008>
- Food Chemicals Codex. 1996. Food and Nutrition Board, National Academy of Sciences. 4th ed. Washington DC: National Academy Press.

- Gelatin Manufacturers Institut of America. 2012. Gelatin. <http://www.gelatin-gmia.com>.
- Giménez B., Gómez-Guillén M. C., Montero P. 2005. Storage of dried fish skins on quality characteristics of extracted gelatin. *Food Hydrocoll.*, 19: 958-963. <https://doi.org/10.1016/j.foodhyd.2004.12.012>
- Hafsari A.R., Rosmiati D., Jamaludin D. 2018. The effect of hydrochloric acid (HCl) concentration on the quality of gourami bone gelatin (*Ospheronemus Gouramy* Lac). In *Proc.: 1st International Conference on Recent Innovations (ICRI): 2983-2989*. <https://doi.org/10.5220/0009947329832989>
- Hakim T.R., Pratiwi A., Jamhari., Fitriyanto N.A., Rusman, Abidin M.Z., Matulesy D.N., Erwanto Y. 2021. Extraction of collagen from the skin of kacang goat and production of its hydrolysate as an inhibitor of angiotensin-converting enzyme. *Trop. Anim. Sci. J.*, 44: 222-228. <https://doi.org/10.5398/tasj.2021.44.2.222>
- Hardikawati T., Puspawati N.M., Ratnayani K. 2016. Study of the effect of variations in the concentration of citric acid on the gel strength of broiler skin gelatin products associated with the protein pattern. *J. Kim.*, 10: 115-124. <https://doi.org/10.24843/jchem.2016.v10.i01.p16>
- Herniawati. 2008. Minerals and Homeostasis. *Biology Education Department, Faculty of Mathematics and Natural Sciences. Indonesian Education University. Bandung*. Available at http://file.upi.edu/Direktori/FPMIPA/JUR._PEND._BIOLOGI/197003311997022-HERNAWATI/FILE_7.pdf Accessed March 2022.
- Huda W., Atmaka W., Nurhartadi E. 2013. Study of physical and chemical characteristics of chicken leg bone extract gelatin (*Gallus gallus bankiva*) with variations in soaking time and acid concentration. *J. Teknosains Pangan.*, 2: 70-75.
- Jannah A., Maunatin A., Windayanti A., Findianti Y., Mufidah Z. 2013. Isolation and characterization of gelatin from chicken bones using the acid method. *Alchemy*, 2: 184-189. <https://doi.org/10.18860/al.v0i0.2904>
- Karim A.A., Bhat R. 2009. Review of Fish Gelatin: Properties, Challenges and prospects as an alternative to mammalian gelatins. *Trends Food Sci. Technol.*, 19: 644-656. <https://doi.org/10.1016/j.tifs.2008.08.001>
- Khirzin M. H., Ton S., Fatkhurrohmah F. 2019. Extraction and characterization of duck bone gelatin using the acid extraction method. *J. Sain Peternak Indones.*, 14: 119-127. <https://doi.org/10.31186/jspi.id.14.2.119-127>
- Laemmli U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227: 680-685. <https://doi.org/10.1038/227680a0>
- Liu F., Chiou B. Sen, Avena-Bustillos R.J., Zhang Y., Li Y., McHugh T.H., Zhong F. 2017. Study of combined effects of glycerol and transglutaminase on properties of gelatin films. *Food Hydrocoll.*, 65: 1-9. <https://doi.org/10.1016/j.foodhyd.2016.10.004>
- Mahmoodani F., Ardekani V.S., See S.F., Yusop S.M., Babji A.S. 2014. Optimization and physical properties of gelatin extracted from pangasius catfish (*Pangasius sutchi*) bone. *J. Food Sci. Technol.*, 51: 3104-3113. <https://doi.org/10.1007/s13197-012-0816-7>
- Miskah S., Ramadianti I.M., Hanif A.F. 2010. Effect of concentration of H₂COOH & HCl as solvent and immersion time on the manufacture of gelatin made from chicken leg bone/skin. *J. Tek. Kim.*, 17: 1-6. Available at <http://jtk.unsri.ac.id/index.php/jtk/article/view/97/94> Accessed March 2022.
- Mulyani S., Setyabudi F.S.M.C.S., Pranoto Y., Santoso U. 2017. Physicochemical properties of gelatin extracted from buffalo hide pretreated with different acids. *Korean J. Food Sci. Anim. Resour.*, 37: 708-715. <https://doi.org/10.5851/kosfa.2017.37.5.708>
- Mohan A., Long J. M. 2021. Chapter 23 - Valorization of wastes and by-products from the meat industry. In: *Rajeev Bhat (Ed). Valorization of Agri-Food Wastes and By-Products*. Academic Press, pp. 457-474. <https://doi.org/10.1016/B978-0-12-824044-1.00010-6>
- Nuryanto R., Trisunaryanti W., Falah I.I., Triyono. 2018. Extraction of gelatin from catfish bone using NaOH and its utilization as a template on mesoporous silica-alumina. *IOP Conference Series: Materials Science and Engineering.*, 349: 1-7. <https://doi.org/10.1088/1757-899X/349/1/012051>
- Panjaitan T.F.C. 2016. Optimization of gelatin extraction from tuna fish bones (*Thunnus albacares*). *J. Wiyata.*, 3: 11-16. Available at <http://ojs.iik.ac.id/index.php/wiyata/article/view/65> Accessed March 2022.
- Permata Y., Widiastri F., Sudaryanto Y., Anteng A. 2016. Gelatin from catfish bones (*Clarias batrachus*): Preparation by acid method, characterization and application as thickener in syrup industry. *J. Ilm Widya Tek.*, 15: 146-152.
- Pertiwi M., Atma Y., Mustopa A., Maisarah R. 2018. Physical and chemical characteristics of gelatin from catfish bones with citric acid pre-treatment. *J. Apl. Teknol. Pangan.*, 7: 83-91. <https://doi.org/10.17728/jatp.2470>
- Petracci M., Cavani C. 2013. Rabbit meat processing: historical perspective to future directions. *World Rabbit Sci.*, 21: 217-226. <https://doi.org/10.4995/wrs.2013.1329>
- Petracci M., Soglia F., Baldi G., Balzani L., Mudalal S., Cavani C. 2018. Technical note: estimation of real rabbit meat consumption in Italy. *World Rabbit Sci.*, 26: 91-96. <https://doi.org/10.4995/wrs.2018.7802>
- Rahman M.N.A., Jamalulail S.A.S.K.A. 2012. Extractions, Physicochemical Characterizations and Sensory Quality of Chicken Feet Gelatin. *Borneo Sci.*, 30: 1-13. Available at <https://www.researchgate.net/publication/235338137> Accessed March 2022.
- Said M.I., Triatmojo S., Erwanto Y., Fudholi A. 2012. Characteristics of goat skin gelatin that produced through acid and alkali process. *Agritech: J. Fac. Agric. Technol. UGM.*, 31: 190-200. <https://doi.org/10.22146/agritech.9744>
- Santoso C., Surti T., Sumardianto S. 2015. Differences in the use of the concentration of citric acid solution in the manufacture of stingray cartilage gelatin (*Himantura gerrardi*). *J. Pengolah. dan Bioteknol. Has. Perikan.*, 4: 106-114. Available at <https://ejournal3.undip.ac.id/index.php/jpbhp/article/view/9200> Accessed March 2022.
- See S.F., Ghassem M., Mamot S., Babji A.S. 2015. Effect of different pretreatments on functional properties of African catfish (*Clarias gariepinus*) skin gelatin. *J. Food Sci. Technol.*, 52: 753-762. <https://doi.org/10.1007/s13197-013-1043-6>
- Sinaga R.F., Ginting G.M., Hasibuan R., Ginying M.H.S. 2014. The effect of additional glycerol on the properties of tensile strength and elongation at the break-up of bioplastics from taro tuber starch. *J. Tek. Kim. USU.*, 3: 19-24. Available at <http://repository.usu.ac.id/handle/123456789/70014> Accessed March 2022.
- Sinthusamran S., Benjakul S., Kishimura H. 2014. Characteristics and gel properties of gelatin from skin of seabass (*Lates calcarifer*) as influenced by extraction conditions. *Food Chem.*, 152: 276-284. <https://doi.org/10.1016/j.foodchem.2013.11.109>

- SNI 06-3735. 1995. Gelatin Quality and Test Method. *National Standardization Board. Jakarta*
- Steel R.G., Torrie J.H. 1993. Principle and Procedure of Statistic. *Mc. Graw Hill Book Company. Inc New York.*
- Suryaningrum T.D., Utomo B.S.B. 2002. Instructions for Analysis of Seaweed and Its Processed Products. *Fishery and Marine Product Processing and Socio-Economic Research Center, Jakarta.*
- Tazwir T., Ayudiarti D. L., Peranginangin R. 2014. Optimization of gelatin production from kaci-kaci fish bones (*Plectorhynchus chaetodonoides* Lac.) using various acid concentrations and extraction times. *J. Pascapanen dan Biotekno. Kelaut. dan Perikan., 2: 35-43.* <https://doi.org/10.15578/jbqp.v2i1.26>
- Ward A.G., Courts. 2009. The Science and Technology of Gelatin. *Digitized edition, Academy Press, New York.*
- Wardhani D.H., Rahmawati E., Arifin G.T., Cahyono H. 2017. Characteristics of demineralized gelatin from lizardfish (*Saurida* spp.) scales using NaOH-NaCl solution. *J. Bahan Alam Terbarukan., 6: 132-142.* <https://doi.org/10.15294/jbat.v6i2.9621>
- Yudhistira B., Palupi E., Atmaka W. 2019. The effect of acid concentration and duration of submersion toward the characteristics of gelatin of eel fishbone (*Anguilla bicolor*) produced through The acid process. *IOP Conference Series: Earth Environ. Sci. 246: 1-7.* <https://doi.org/10.1088/1755-1315/246/1/012046>
- Zhou P., Regenstein J.M. 2005. Effect of alkaline and acid pretreatments on Alaska pollock skin gelatin extraction. *J. Food Sci., 70: 992-396* <https://doi.org/10.1111/j.1365-2621.2005.tb11435.x>
- Zulkifli M., Naidu A.S., Yusuf N. 2014. Yield, gel point, and the melting point of tuna bone gelatin processed with palm vinegar. *J. Ilm. Perikan dan Kelaut., 2: 73-77.* <https://doi.org/10.37905/v2i2.1256>
-