

**Comparative Quality of Proteins and Morphological Structures of Gelatin From Sheepskin With Acid and Alkaline Treatment****Muhamad Hasdar<sup>1</sup>, Mohamamad Jusuf Randi<sup>1</sup>**Food Science and Technology, Faculty of Science and Technology,  
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Email: hasdarmuhammad@umus.ac.id**ABSTRACT**

*Sheepskin is one alternative to raw materials for producing gelatin. This study aims to compare the quality of gelatin from sheepskin based on different types of pretreatment solutions used, namely HCl 1,5% solution and NaOH 1,5% solution. Data analysis used t-test to compare two treatment methods. Yield and protein gelatin there is a difference where the treatment of HCl 1,5% solution is higher than the treatment of NaOH 1,5% solution. The brighter visual quality of gelatin colour was produced by HCl 1,5% treatment compared to NaOH 1,5% and commercial gelatin treatments. The results of the microstructure using Scanning Electron Microscopy (SEM) showed that there were still lots of clumps of protein and cavities in the gelatin NaOH 1,5% and commercial gelatin treatments. Gelatin with HCl 1,5% treatment showed the best results.*

**Keywords:** *gelatin, sheepskin, HCl, NaOH, SEM*

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**INTRODUCTION**

Gelatin is a type of protein derived from the collagen tissue of the animal skin which has a high economic value for applications in the food or non-food industry. The gelatin in various food industries can be utilized as emulsifier, binder, stabiliser, control elasticity (Karim and Bhat, 2009), and can form a transparent and strong film (Djagny et al., 2001). The gelatin in the pharmaceutical industry is used as a capsule manufacturer and a tablet binder (Wangtueai and Noomhorm, 2009).

Gelatin is a multifunctional natural material with a very unique nature that is melting at body temperature and can form a thermoreversible gel. These two properties are making gelatin difficult to be replaced by other products. The utilization of gelatin in various industries is highly determined by the quality of the gelatin, where the quality of gelatin can be determined based on its physicochemical properties (Mad-Ali et al., 2016). One of the properties of gelatin is reversible where gelatin can form the soles when heated and when cooled can form the gel again. Gels are formed due to the hydrogen bonding between the gelatin.

Generally, gelatin is divided into two types, type A and type B, this division is based on the pretreatment method. Type A usually uses pretreatment of acid solutions, while Type B uses pretreatment of alkaline solutions. Gelatin type A and type B will produce gelatin quality which is also different (Zhou and Regenstein, 2006). The use of acidic and alkaline solutions can help break the collagen helical triple bonds (Amertaning et al., 2019) and produce gelatin products that have different characteristics (Gomez-guillen and Montero, 2001). Gelatin is a material that can adapt to the environment wherever gelatin is placed because it does not harm the body and does not cause toxic (Yue et al., 2015).

Gelatin derived from the skin is usually the result of collagen expression with repetitive structural motifs, namely Gly-X-Y, where X and Y are usually occupied by proline and hydroxyproline (Mariod and Adam, 2013). Gelatin which has high-quality proline and hydroxyproline will reflect the high quality of gel strength, making it easier to be processed into advanced products for pharmaceutical, food and photography products.

Gelatin produced from the skin is usually made from cow's skin (Said et al., 2018), Pork (Sompie et al., 2012), Goat (Mad-Ali et al., 2017), fish (Yang et al., 2019), and Chicken feet (Mrázek et al., 2019). Sheepskin is one of the alternatives of raw material to producing gelatin. Sheepskins are found in Indonesia, especially in small animal houses. Usually, sheepskin is used for the raw material of jackets and skin crackers (Nasr et al., 2013). Sheepskin conversion to gelatin is a form of diversification of products that must be developed to support the needs of gelatin as the raw material of gelatin-based on raw material sources. This research compares the quality of the gelatin skin based on the different types of pretreatment solution used is the solution of HCl and NaOH solution.

## **MATERIALS AND METHODS**

### *Raw Material*

The material used in this research is the sheepskin obtained from the animal slaughterhouse in Brebes District, commercial gelatin obtained from a pastry supplies shop in Tegal District, Aquades, a solution NaOH 1.5% (v/v), and a solution of HCl 1.5% (v/v). Materials used in a glass beaker, measuring glass, pipette, cutter knife, filter cloth, digital scale, blender, and plastic tray.

### *Gelatin Preparation*

The sheepskin has been cleared of wool and the rest of the flesh in the wash with running water. Then cut the skin with a size of  $\pm 2$  cm and weighed by 200 gr and then moved. Furthermore, each treatment is performed immersion with a solution of HCl 1,5% (v/v) and a solution of NaOH 1,5% concentration (v/v) for 4 hours. In the next process is the extraction of gelatin for 2 hours at 60° C. The extraction process is done by modifying the Immersion method performed by Hasdar et al., (2019) to soak the skin using aquades with a comparison of 1:3 (skin pretreatment: aquades) placed in the glass beaker then for 2 hours in the hot plate. Filtering gelatin extract with a filter cloth to separate the skin solids from the solution gelatin and deodorization. Drying of gelatin solution using an oven with a temperature of 50-60 °c for  $\pm 24$  hours (Atma and Ramdhani, 2018). After the drying process is done smoothing sheet gelatin using a blender.

### *Total Yield*

The yield was obtained from the comparison of the weight of gelatin produced with the weight of raw materials for wet skin (Kaleli et al., 2017). The yield can be obtained using the formula :

$$\text{Yield (\%)} = \frac{\text{Dry weight of sheepskin gelatin}}{\text{Wet weight of sheepskin}} \times 100 \%$$

### *Total Protein*

Protein analysis using sheepskin gelatin samples are taken based on the sequence of replication. Crude protein analysis refers to the method of Semimikro-Kjeldahl (AOAC, 1995). Samples of gelatin were taken as much as 0.5 g weighed and then inserted into the pumpkin Kjeldahl 30 ml. Then added 0.9 g K<sub>2</sub>SO<sub>4</sub>, 40 mg HgO, and 2 ml H<sub>2</sub>SO<sub>4</sub>. If the sample weight of the gelatin is more than 15 mg, it is added 0.1 ml H<sub>2</sub>SO<sub>4</sub> for every 10 mg of organic matter above 15 mg. Sample of gelatin simmer for 1-1.5 hours until the liquid becomes clear. Then the solution is inserted into the distillation device and added 10 ml of the solution NaOH 30%.

The distillation device produces  $\text{NH}_3$  gas which is then captured by the  $\text{H}_3\text{BO}_3$  in the Erlenmeyer which has been added 3 drops of the indicator (mixture 2 parts red methyl 0.2% in alcohol and 1 part methylene blue 0.2% in alcohol). The gelatin condensate fluid is then assigned to the HCl 0.1 N which has been standardized until the condensate changes to grey colour. Setting blanko using the same method as the sample assignment. The protein value is derived from the calculation result between the difference in the titration volume and the values multiplied by the normality of HCl and 0.014, the conversion factor gelatin (i.e. 5.55), the dilution factor divided by the sample weight multiplied by 100%.

#### Data Analysis

Data analysis using T test (Kim, 2015) to compare the results of the treatment of HCl 1,5% and NaOH 1,5% solution

#### Analysis Mikrostruktur

Gelatin powder with a variation of HCl 1,5%, NaOH 1,5%, and commercial, weighed each as much as 2 mg. The gelatin sample is analyzed by using the SEM tool (electron Scanning Microscopies) to determine the morphology of the surface of the gelatin with a size of 1-2  $\mu$  m. The analysis is done by magnification up to 5,000 times.

## RESULT AND DISCUSSIONS

#### Colour Visuals

Visually, the appearance of the colour of sheepskin gelatin is a brighter, HCl 1,5% than the treatment of NaOH 1,5% and commercial gelatin. The colour of the lamb gelatin treatment NaOH brighter than the commercial gelatin. Colour differences of gelatin are caused by differences in pretreatment methods (Rammaya et al., 2012), drying methods (Kim et al., 2020), and raw materials (Kamatchi and Leela, 2016). The colour difference of gelatin must indicate the difference of quality both physically and chemically, but still can be used as food raw material or non-food depending on the needs of advanced products to be formed (Ratnasari et al., 2013). The colour apparition of sheepskin and commercial gelatin powder can be seen in figure 1.



Figure 1. The appearance and colour of sheep skin gelatin

#### Gelatin Yield

Yield is the percentage of the weight of the gelatin obtained from the yield of skin collagen denaturation (Jaswir et al., 2017). The greater the yield gained, the more efficient the treatment is applied (Shyni et al., 2014). The skin's gelatin yield is based on the difference in treatment of pretreatment material can be seen in Table 1 below.

Tabel 1. Yield sheepskin gelatin treatment HCl 1.5% and NaOH 1,5% treatment

Replication	Treatment	
	HCl 1,5%	NaOH 1,5%
1	23,44 %	20,56 %
2	23,35 %	21,07 %
3	24,16 %	20,58 %
4	23,37 %	20,79 %
5	25,21 %	20,09 %
Average	23,91% ± 0,80	20,62 % ± 0,36

$t_{\text{count}} > t_{\text{table}}$  : a difference  
 $t_{\text{count}} < t_{\text{table}}$  : not difference

The average yield of gelatin yield of HCl 1,5% treatment was  $23.91 \pm 0.80$  higher compared to the average gelatin yield of NaOH 1,5% treatment was  $20.62 \pm 0.36$ . The difference in the average yield of sheepskin gelatin between treatments is also directly proportional to the results of the t-test analysis in which the results obtained  $t_{\text{count}}$  of 8.357 and  $t_{\text{table}}$  of 1.860, which means there is a difference in yield between the sheepskin gelatin HCl 1,5% treatment and NaOH 1,5% treatment. This difference in gelatin yield shows that the ability of HCl 1,5% solution is better than the NaOH 1,5% solution in producing sheepskin gelatin yield. A high percentage of gelatin yield shows that HCl 1,5% treatment is more efficient. The ability of HCl 1,5% solution can change the stability of sheepskin collagen fibres namely triple-helical molecules to become single strand, while the NaOH 1,5% solution soaking is only able to produce double chains (Bigi et al., 2004), thus causing the amount of collagen produced during the hydrolysis process to be more by pretreatment with HCl 1,5% solution rather than NaOH 1,5% pretreatment. The conversion of skin collagen to gelatin is strongly influenced by chemical pretreatment, temperature, heating time and pH (Abdullah et al., 2018).

The yield of type B gelatin from sheepskin in this study is 20.09% -21.07%, slightly lower than the type B gelatin from goatskin produced by Mad-Ali et al., (2017), 22.1% - 23.1%, and higher than the research of Bahar et al., (2020) which resulted in a yield of 5.17-10.41%. This difference in yield is due to differences in the old method of NaOH immersion and concentration. The percentage of gelatin yield is influenced by several factors such as the type of raw material, the extraction process, and the pretreatment process (Kittiphattanabawon et al., 2010). The average yield of type A gelatin from sheepskin produced in this study was  $23.91\% \pm 0.80$  which is much higher than the average yield of type A gelatin from goatskin produced in the study of Zilhadia et al., (2018) namely  $10.26\% \pm 1.07$ , the difference in average yield is due to differences in method and immersion time at the time of pretreatment. HCl solution is very effective in breaking down triple-helical molecules into single strands in a short period (Monsur et al., 2014). The length of the immersion process in HCL solution will make a lot of acid solution into the skin collagen fibrils tissue which eventually collagen fibrils disappear during the process of extraction with water or hydrolyzed completely to produce less yield (Kołodziejaska et al., 2008).

### Protein Content

Gelatin as one type of high-protein biopolymer (Roy et al., 2017), obtained from the hydrolysis of collagen in the skin (Mariod and Adam, 2013). Gelatin, which has a high protein level, indicates good quality (Derkach et al., 2019). High protein levels in gelatin are expected to provide additional nutrients to the next processed food products (Baziwane and He, 2003).

The average value-king of protein levels of sheepskin gelatin with the different treatment of pretreatment ingredients obtained in this study is presented in table 2 below.

Tabel 2. Protein content (%) sheepskin gelatin HCl 1,5% treatment and NaOH 1,5% treatment

Replication	Treatment	
	HCl 1,5%	NaOH 1,5%
1	90,17 %	85,15 %
2	89,55 %	86,02 %
3	90,38 %	85,36 %
4	90,15%	85,58 %
5	90,91 %	84,79 %
Average	90,29 % ± 0,39	85,38 % ± 0,46

$t_{\text{count}} > t_{\text{table}}$  : a difference  
 $t_{\text{count}} < t_{\text{table}}$  : not difference

Based on the results of the average total protein in table 2 it is known that the average gelatin protein with HCl 1,5% treatment is  $90.29\% \pm 0.39$  higher than the average NaOH 1,5% treatment that is  $85.38 \pm 0.46$ . The difference in sheep gelatin protein content between HCl 1,5% treatment and NaOH 1,5% treatment turned out to be in line with the results of the t-test test analysis where the results obtained  $t_{\text{count}}$  of 18.112 and  $t_{\text{table}}$  of 1.860 so it can be concluded there are different levels of protein between treatments, where the average HCl 1,5% treatment is higher compared to the NaOH 1,5% solution treatment. The use of NaOH 1,5% solution as a pretreatment is not able to produce high protein levels when compared with the use of HCl 1,5% solution. Strong acid solutions usually have a better effect on producing gelatin protein (Ahmad and Benjakul, 2011). NaOH 1,5% solution can only hydrolyze triple helix from sheepskin collagen to double helix, while HCl 1,5% solution can convert triple helix from sheepskin collagen into a single strand. The change in skin collagen to gelatin is largely determined by the ability of pretreatment chemicals (Zhang et al., 2006).

Protein gelatin of sheepskin type A and type B produced in this study is better when compared with the results of the study of Zarei et al., (2019) which is  $18.90\% \pm 0.27$  using protease enzymes as pretreatment material. The average level of gelatin protein in sheepskin type A in this study is higher when compared with the results of research Zilhadia et al., (2018) which produces an average level of goatskin protein that is 86.58%, this is caused by differences in raw materials, methods pretreatment and duration of soaking in HCl solution. Long immersion time and pretreatment material concentration cause collagen fibres to shrink and break into irregular structures so that they undergo a perfect dissolution process during the process of hydrolysis of skin collagen (Mulyani et al., 2017).

The average protein content of type B sheep skin protein in this study was  $85.38\% \pm 0.46$  smaller than the research of Said et al., (2011) which produced type B goat skin gelatin using a solution of  $\text{Ca}(\text{OH})_2$  as a pretreatment material and performs three stages of extraction to produce 91.63% -93.60% protein. Three stages of extraction are carried out to re-hydrolyze the collagen bonds that are still trapped in the skin due to not being completely loosened by an alkaline solution. The small amount of protein in this study was caused by 1.5% NaOH solution which was only able to change the triple helix bond of sheepskin collagen to double helix. Protein levels can be higher by increasing the time of immersion and replacing the solution with new ones periodically during the immersion. So that NaOH 1,5% solution can change the triple helix bond of sheepskin collagen into a single strand.

### *Gelatin Microstructure Analysis*

SEM (Scanning Electron Microscopy) analysis was used to look at the morphology of the surface of sheepskin gelatin. The morphological forms of the three sheepskin gelatin samples were each seen in the same magnification of 5000 x. In Figure 2 below.

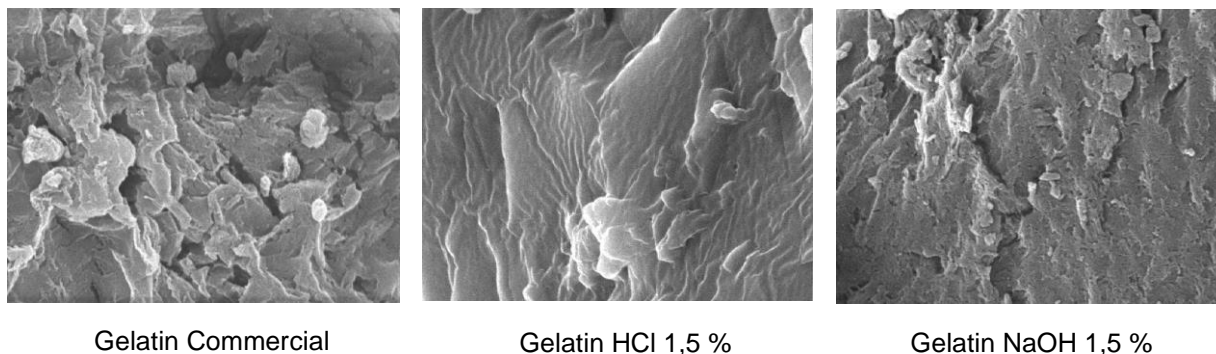


Figure 2. Microstructure sheepskin gelatin of HCl 1,5% treatment, NaOH treatment 1,5%, and commercial gelatin

In figure 2. Shows the surface of the sheepskin gelatin layer with HCl treatment is smoother and flat compared to gelatin treated with NaOH and commercial gelatin, but there is still a lump of protein. Commercial gelatin also has cavities on most surfaces as well as on sheepskin gelatin NaOH treatment. When linked between gelatin microstructure with protein quality and yield, it can be concluded that high yield will produce a high protein and have better microstructure quality. The quality of gelatin morphology is closely related to the quality of viscosity (Nuamsrinuan et al., 2015) The quality of gelatin morphology is also influenced by cross-linking that occurs in gelatin (Gaspar-Pintilieșcu et al., 2019).

### **CONCLUSION**

Yield and protein gelatin of sheepskin with HCl 1,5% pretreatment treatment have higher quality than NaOH 1,5% pretreatment treatment. Brighter visual colour of gelatin was produced by HCl 1,5% pretreatment treatment. The flat and dense microstructure is also produced by HCl 1,5% pretreatment treatment.

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### **REFERENCES**

- Abdullah, M.S.P., Noordin, M.I., Mohd Ismail, S.I., Mustapha, N.M., Jasamai, M., Danik, M.F., Wan Ismail, W.A., Shamsuddin, A.F., 2018. Recent advances in the use of animal-sourced gelatine as natural polymers for food, cosmetics and pharmaceutical applications. *Sains Malaysiana* 47, 323–336.
- 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.
- Amertaning, D., Bachrudin, Z., . J., Chin, K.B., Erwanto, Y., 2019. Characteristics of gelatin extracted from indonesian local cattle hides using acid and base curing. *Pakistan J. Nutr.* 18, 443–454.

- [AOAC]. 1995 "Official methods of analysis of the association of official analytical chemist". Washington, DC, 1995
- Atma, Y., Ramdhani, H., 2018. Gelatin extraction from the indigenous *Pangasius catfish* bone using pineapple liquid waste. *Indones. J. Biotechnol.* 22, 86–91.
- Bahar, A., Kusumawati, N., Muslim, S., 2020. Preparation and characterization of goatskin gelatin as halal alternative to bovine gelatin. *Rasayan J. Chem.* 13, 85–98.
- Baziwane, D., He, Q., 2003. Gelatin: the paramount food additive. *Food Rev. Int.* 19, 423–435.
- Bigi, A., Panzavolta, S., Rubini, K., 2004. Relationship between triple-helix content and mechanical properties of gelatin films. *Biomaterials* 25, 5675–5680.
- Derkach, S.R., Kuchina, Y.A., Baryshnikov, A. V., Kolotova, D.S., Voron'ko, N.G., 2019. Tailoring cod gelatin structure and physical properties with acid and alkaline extraction. *Polymers (Basel)*. 11, 1–17.
- Djagny, K.B., Wang, Z., Xu, S., 2001. Gelatin: a valuable protein for food and pharmaceutical industries: Review. *Crit. Rev. Food Sci. Nutr.* 41, 481–492.
- Gaspar-Pintilieșcu, A., Stefan, L.M., Anton, E.D., Berger, D., Matei, C., Negreanu-Pirjol, T., Moldovan, L., 2019. Physicochemical and biological properties of gelatin extracted from marine snail *rapana venosa*. *Mar. Drugs* 17, 1–14.
- Gomez-guillœn, M.C., Montero, P., 2001. Extraction of gelatin from megrim (*lepidorhombus bosci*) skins with several organic acids. *J. Food Sci.* 66, 213–216.
- Hasdar, M., Rahmawati, Y.D., Erwanto, Y., Rusman, 2019. Quality protein, viscosity, gel strength and structural morphology of sheepskin gelatin catalyzed hcl with different concentrations. In: *IOP Conference Series: Earth and Environmental Science*.
- Jaswir, I., Yusof, N., Jamal, P., Jami, M.S., 2017. Novel method for gelatin extraction of various local fish using high pressure processing (hpp). *Int. Food Res. J.* 24, 533–539.
- Kaleli, A., Kulikovskiy, M.S., Solak, C.N., 2017. Some new records for marine diatom flora of turkey from akliman, sinop (black sea). *Turkish J. Fish. Aquat. Sci.* 17, 1387–1395.
- Kamatchi, P., Leela, K., 2016. Extraction , characterization and application of gelatin from *carcharhinus amblyrhyncho* and *sphyraena barracuda*. *J. Biotechnol. Biochem.* 2, 40–49.
- Karim, A.A., Bhat, R., 2009. Fish gelatin: properties, challenges, and prospects as an alternative to mammalian gelatins. *Food Hydrocoll.* 23, 563–576.
- Kim, T.K., 2015. T test as a parametric statistic. *Korean J. Anesthesiol.* 68, 540–546.
- Kim, T.K., Ham, Y.K., Shin, D.M., Kim, H.W., Jang, H.W., Kim, Y.B., Choi, Y.S., 2020. Extraction of crude gelatin from duck skin: effects of heating methods on gelatin yield. *Poult. Sci.* 99, 590–596.
- Kittiphattanabawon, P., Benjakul, S., Visessanguan, W., Shahidi, F., 2010. Comparative study on characteristics of gelatin from the skins of brownbanded bamboo shark and blacktip shark as affected by extraction conditions. *Food Hydrocoll.* 24, 164–171.
- Kołodziejska, I., Skierka, E., Sadowska, M., Kołodziejski, W., Niecikowska, C., 2008. Effect of extracting time and temperature on yield of gelatin from different fish offal. *Food Chem.* 107, 700–706.
- Mad-Ali, S., Benjakul, S., Prodpran, T., Maqsood, S., 2016. Characteristics and gel properties of gelatin from goat skin as influenced by alkaline-pretreatment conditions. *Asian-Australasian J. Anim. Sci.* 29, 845–854.
- Mad-Ali, S., Benjakul, S., Prodpran, T., Maqsood, S., 2017. Characteristics and gel properties of gelatin from goat skin as affected by extraction conditions. *J. Food Process. Preserv.* 41, 1–10.
- Mariod, A.A., Adam, H.F., 2013. Review: Gelatin, source, extraction and industrial applications. *Acta Sci. Pol. Technol. Aliment.* 12, 135–147.
- Monsur, H.A., Jaswir, I., Salleh, H.M., Alkahtani, H.A., 2014. Effects of pretreatment on properties of gelatin from perch (*Lates niloticus*) skin. *Int. J. Food Prop.* 17, 1224–1236.
- Mrázek, P., Mokrejš, P., Gál, R., Orsavová, J., 2019. Chicken skin gelatine as an alternative to pork and beef gelatines. *Potravin. Slovak J. Food Sci.* 13, 224–233.
- Mulyani, S., Setyabudi, F.M.C.S., Pranoto, Y., Santoso, U., 2017. The effect of pretreatment using hydrochloric acid on the characteristics of buffalo hide gelatin. *J. Indones. Trop.*

- Anim. Agric. 42, 14–22.
- Nasr, A.I., Abdelsalam, M.M., Azzam, A.H., 2013. Effect of tanning method and region on physical and chemical properties of barki sheep leather. *Egypt. J. Sheep Goat Sci.* 8, 123–130.
- Nuamsrinuan, N., Suttisiri, N., Hoonnivathana, E., Limsuwan, P., Naemchanthara, K., 2015. Characterization and gel properties of gelatin extracted from waste fish scales. *Appl. Mech. Mater.* 804, 187–190.
- Rammaya, K., Ying, V.Q., Babji, A.S., 2012. Physicochemical analysis of Gelatin extracted from mechanically deboned chicken meat ( mdcM ) residue. *Int. J. Food, Nutr. Public Heal.* 5, 147–168.
- Ratnasari, I., Yuwono, S.S., Nusyam, H., Widjanarko, S.B., 2013. Extraction and characterization of gelatin from different fresh water fishes as alternative sources of gelatin. *Int. Food Res. J.* 20, 3085–3091.
- Roy, B.C., Omana, D.A., Betti, M., Bruce, H.L., 2017. Extraction and characterization of gelatin from bovine lung. *Food Sci. Technol. Res.* 23, 255–266.
- Said, M.I., Burhan, T., Haerati, 2018. Synthesis of collagen from Bali cattle's hide using a combination of acid and alkali on the extracting process. *J. Indones. Trop. Anim. Agric.* 43, 247–256.
- Said, M.I., Triatmojo, S., Erwanto, Y., Fudholi, A., 2011. Gelatin properties of goat skin produced by calcium hydroxide as curing material. *Media Peternak.* 34, 184–189.
- Shyni, K., Hema, G.S., Ninan, G., Mathew, S., Joshy, C.G., Lakshmanan, P.T., 2014. Isolation and characterization of gelatin from the skins of skipjack tuna (*katsuwonus pelamis*), dog shark (*scoliodon sorrakowah*), and rohu (*labeo rohita*). *Food Hydrocoll.* 39, 68–76.
- Sompie, M., Triatmojo, S., Pertiwinigrum, A., Pranoto, Y., 2012. The effects of animal age and acetic acid concentration on pigskin gelatin characteristics. *J. Indones. Trop. Anim. Agric.* 37, 176–182.
- Yang, X.R., Zhao, Y.Q., Qiu, Y.T., Chi, C.F., Wang, B., 2019. Preparation and characterization of gelatin and antioxidant peptides from gelatin hydrolysate of skipjack tuna (*katsuwonus pelamis*) bone stimulated by in vitro gastrointestinal digestion. *mar. Drugs* 17.
- Yue, K., Trujillo-de Santiago, G., Alvarez, M.M., Tamayol, A., Annabi, N., Khademhosseini, A., 2015. Synthesis, properties, and biomedical applications of gelatin methacryloyl (GelMA) hydrogels. *Biomaterials*.
- Zarei, M., Mazaheri Tehrani, M., Rashidi, H., Najafi, M.F., 2019. Optimization of gelatin extraction process, from sheep skin waste using alcalase enzyme by response surface method 8.
- Zhang, Z., Li, G., Shi, B., 2006. Physicochemical properties of collagen, gelatin and collagen hydrolysate derived from bovine limed split wastes. *J. Soc. Leather Technol. Chem.* 90, 23–28.
- Zhou, P., Regenstein, J.M., 2006. Effects of alkaline and acid pretreatments on alaska pollock skin gelatin extraction. *J. Food Sci.* 70, c392–c396.
- Zilhada, Yahdiana, H., Irwandi, J., Effionora, A., 2018. Characterization and functional properties of gelatin extracted from goatskin. *Int. Food Res. J.* 25, 275–281.