Impact of Curing and Extraction Time on Yield and Quality of Base Gelatin from Goat Skin

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Abstract. The impact of curing and extraction time on yield and quality (water and ash content, gel and organolaptic properties) of the base gelatin from goat skin was investigated. The yield, water and ash content also gel strength of goat gelatin (GG) increased with increasing curing (10-50 days) and extraction time (4-5 hours). Higher water and ash content was observed with 30 days curing and 5 hours of extraction compared with the others conditions. In line with these, the higher gel strength was observed under the same conditions. The ash content of GG with 30 days curing and 5 hours extraction time increased by 0.26% compared to the GG with lower curing and extraction time. The Gel strength of all gelatins increased as the curing and extraction time of gelatin increased. To know the effect of curing and extraction time on gelatin organoleptic properties, ie color, has been analysed using Visible UV spectrophotometer. The results showed that there has been a detectable increase in color become darker along with increased curing and extraction time. Nevertheless, at higher extraction times (6 hours), the yield and quality of gelatin can not be analysed because the goat skin material has been destroyed. According to Indonesian Industrial Standard based on Indonesian National Standard (SNI) 06-3735-1995, it can be said that the best quality gelatin from goat skin was obtained from the manufacturing process using 30 days curing and 5 hours extraction time

1. Introduction

Gelatin is a protein derived from collagen by thermal denaturation or partial hydrolysis of collagen, the main protein in skin, hides, white connective tissues, and bones of the animal body [1]-[3]. It has the rheological properties of a thermos-reversible transformation between solution (sol) and gel form. Gelatin has many applications in food, pharmaceutical, medical and technical applications due to its surface-active properties [4]-[5].

Despite of wide applications, alternative gelatins have gained increasing attention in recent years as the demand for non-bovine and non-porcine gelatins has increased due to the BSE (bovine spongiform encephalopathy) crisis as well as religious and social reasons [1]. Porcine gelatin is not acceptable in Kosher and Halal foods, while bovine gelatin is prohibited for Hindus [1]. Halal law for Muslims has obliged the use of food and non-food products free of pork and its derivatives [3],[6]. Poultry gelatin has been also concerned, due to avian influenza.

As a consequence, gelatin from alternatively land animals, especially by-products from goat slaughtering, e.g. skin or bone, has been of increasing interest as a potential alternative. When goat are slaughtering, skin generated as by-product accounts for 6.4-11.6 g/100 g (based on the body weight) [7].
Recently, gelatin has been extracted from goat skin after the appropriate alkaline pre-treatment is implemented [8].

The quality of gelatin depends on its physical, chemical and structural characteristics. The most important physical properties of gelatin are gel strength. It is measured as bloom value, which can be classified as low (<150), medium (150-220) and high Bloom (220-300) [1], [9]. Generally, functional properties of gelatin are governed by several factors, such as raw material, pre-treatment and extraction conditions [5], [8-12]. Curing and extraction times are two of essential processes for gelatin manufacturing. The application of these two factors varies depending on product needs, type of equipment employed, timing of operations, and economics. Extraction procedures are controlled in the manufacture of both Type A and Type B gelatin since they influence both quality and quantity. Although continuous extraction is used by some processors, most methods still employ discrete batch fractions. Extraction is normally carried out in stainless steel vessels equipped with provisions for heating and temperature control. The number of extractions varies, 3-6 is typical. The first extraction generally takes place at 50-60 °C, subsequent extractions being made with successive increases in temperature of 5-10 °C. The final extraction is carried out close to the boiling point. Extracts are kept separate, analysed, and subsequently blended to meet various customer specifications [14]. However, the little information regarding the impact of curing and extraction times on yield and quality of base gelatin from goat skin exists. The objective of the present study was to determine yield and the quality of base gelatin from goat skin as affected by different curing and extraction time.

2. Materials and Methods

2.1. Materials
All chemicals were of analytical grade. Calcium oxide (CaO) and hydrochloride acid (HCl) were purchased from Sigma-Aldrich (Singapore).

2.2. Collection and Preparation of Goat Skins
Goats skins with the random age were collected from a local slaughter house in Surabaya, Jawa Timur, Indonesia. Ten kilograms of goat skins were randomly taken from twenty goats, pooled and used as the sample. The skins were packed in polyethylene bag, embedded in the insulated box containing ice and transported to the Department of Chemistry, Universitas Negeri Surabaya, within 2 h. Upon arrival, the skins were cleaned and washed with running water (28-30 ºC). Prepared skins were then cut into small pieces (1 x 3 cm) using knives, placed in polyethylene bags and stored at -20 ºC until use. The storage time was not longer than 2 months. Before use, the frozen skins were thawed using a tap water (28-30 ºC) for 15 min.

2.3. Pre-treatment of goat skins
Prepared skins were pre-treated with CaO solution 10% (w/v) at a ratio of 1:1 (w/v) at room temperature (28-30 ºC). The mixture was stirred manually for one time every two days. Alkaline solution was not removed and replaced by the same volume of freshly prepared solution for totally curing time. To determine the effect of curing time on yield and gelatin quality, at this stage curing time is varied for 10, 30, and 50 days. Alkali-pretreated skins were then washed with running water until the pH of wash water became neutral or slightly alkaline. The obtained skins were used for gelatin extraction.

2.4. Extraction of Gelatins
Pretreated skins were firstly placed in distilled water (60 ºC) with a skin/water ratio of 1:1 (w/v) in a temperature-controlled water bath. The mixture was continuously heated for 4 h. The mixture was then filtered using two layers of cloth. The resulting filtrate was heated at a temperature of 50 ºC until coagulated, followed by a drying process using an oven on the same temperature. To determine the effect of extraction time on yield and gelatin quality, at this stage the extraction time was varied for 4, 5, and 6 hours.
2.5. Analysis

2.5.1. Yield

The yield of the gelatin was calculated based on wet weight of fresh skin.

2.5.2. Determination of Gel Strength:

Gel strength of gelatin was determined according to the method of Ref. [15] using 6.67 g/100 ml gels prepared by mixing the dry gelatin in distilled water at 60 °C for 30 min and cooling down the solution in a refrigerator at 7 °C (maturation temperature) for 16-18 h. The gel strength of gelatin was determined at 7 ºC using a Model TA-TX2 texture analyzer with a 5 kN load cell equipped with a 1.27 cm diameter flat-faced cylindrical Teflon plunger. The dimensions of the sample were 2.7 cm in diameter and 3.7 cm in height. Gel strength was expressed as maximum force (in grams), required for the plunger to press the gel 4 mm depression at a rate of 0.5 mm/s [15]. The measurement was performed in triplicate [16].

2.5.3. Determination of Viscosity:

The viscosity of a 6.67% gelatin solution is determined at 60 ºC by measuring the flow time of 100 mL of the solution through a standard viscosity pipette. The viscosity (to the nearest millipoise) at 60 ºC of any sample with efflux time t (in second) may be calculated from the following equation:

\[ V = \frac{A \cdot t}{d} \]

where, V is a viscosity (mP); A, B are A and B pipette constants (refer to Annex I, calibration of viscosity pipette, to obtain A and B constants, if not available); t is an efflux time (seconds); and d is a solution density (for a 6.67% gelatin solution at 60 ºC, d = 1.001).

2.5.4. Determination of pH:

The pH of a 1.5% gelatin solution is determined by potentiometry at a temperature of 35 ± 1 ºC using a bench meter (ECION51041S; Eutech Instruments, Malaysia) [17].

2.5.5. Determination of Water and Ash Content:

The water and ash content of the gelatin product were determined according to the AOAC methods number 927.05 and 942.05, respectively [18]. All measurements were performed in triplicate [16].

2.5.6. Determination of Organoleptic Characteristics:

The CieLab coordinates (L*, a*, b*) of the goat gelatin (6.67 g/100 ml dissolved at 60 ºC) were directly measured using a spectrophotocolorimeter (Tintometre, Lovibond PFX 195 V 3.2, Amesbury, UK). In this coordinat system, the L* value is a measure of lightness, ranging from 0 (black) to 100 (white); the a* value ranges from -100 (greenness) to +100 (redness) and the b* value ranges from -100 (blueness) to +100 (yellowness) [16].

3. Results and Discussion

3.1. Yield

The yield of gelatin is defined as the amount of dry gelatin produced from the amount of raw material in the net through the extraction process [20]. The results of yield of goat gelatin samples obtained using different curing and extraction conditions are reported in Table 1. It is evident that the curing and extraction times have a strong effect on the goat gelatin yield, where the yield significantly increases when the the curing and extraction times are higher, in other word the highest yield was obtained for the goat gelatin cured for 30 days and extracted for 5 hours (27.74%). The lowest yield was obtained for the goat gelatin cured for 10 days and extracted for 4 hours (24.20%). These results are in agreement with the study carried out by Said et al. (2014) who reported a higher yield when the gelatin from goat skin was performed with a longer curing and extraction time. The higher percentage of yield resulted from the production process of base goat gelatin with higher curing time is due to the longer time available for the alkaline curing solution for destruction of triple helix structures in collagen. The more the structure of triple helix collagen in goat skin decomposes, the more the quantity of collagen is extracted and then converted to gelatin.
The preparation of goat skin gelatin with 50 days curing time and 5 and 6 hours extraction time is not recommended because too long treatment in base and overheating have damaged all the bonding and interaction between the compound material and yielded a pulp-like texture.

### Table 1. Goat Gelatin Yield from Varying Curing and Extraction Time

| No | Goat Skin Sample | Curing Time (day) | Extraction Time (hour) | Yield (%) |
|----|------------------|-------------------|------------------------|-----------|
| 1  | GG-1             | 10                | 4                      | 20.87     |
| 2  | GG-2             | 30                | 4                      | 23.26     |
| 3  | GG-3             | 50                | 4                      | 25.79     |
| 4  | GG-4             | 30                | 5                      | 24.41     |
| 5  | GG-5             | 30                | 6                      | 26.17     |

3.2. Gel Strength

One of the functional properties of gelatin is gel strength [21]. Gel strength is one of the parameters to determine the physical quality of a gelatin product. Physical properties are influenced by the concentration of materials and curing time [10]. The formation of thermoreversible gels in water is one of gelatin’s most important properties. When an aqueous solution of gelatin with a concentration greater than approximately 0.5% is cooled to approximately 35-40°C it first increases in viscosity, and then later forms a gel. The rigidity or strength of the gel depends upon gelatin concentration, the intrinsic strength of the gelatin, pH, temperature, and the presence of any additives. The intrinsic strength of gelatin is a function of both structure and molecular mass.

The first step in gelation is the formation of locally ordered regions caused by the partial random return (renaturation) of gelatin to collagen-like helices (collagen fold). Next, a continuous fibriller three-dimensional network of fringed micelles forms throughout the system probably due to non-specific bond formation between the more ordered segments of the chains. Hydrophobic, hydrogen, and electrostatic bonds may be involved in the crossbonding. Since these bonds are disrupted on heating, the gel is thermoreversible. Formation of the crossbonds is the slowest part of the process, so that under ideal conditions the strength of the gel increases with time as more crossbonds are formed. The total effect is a time-dependent increase in average molecular mass and in order.

The gel forming quality of gelatin is a significant physical quality parameter. The measurement of this property is very important from both a control standpoint and as an indication of the amount of gelatin required by a particular application.

Gel strength of a goat gelatin at different curing (10 – 50 days) and extraction times (4 – 6 hours) are shown in Fig. 1. Gel strength of all gelatins increased as the curing and extraction time of gelatin increased. The increase in gel strength value with increasing base curing time occurs because the curing material works in breaking the amino acid polymer chain at the right and optimum limits, thus ultimately giving an effect of improvement in the gel formation process. Accordingly, increasing the extraction time will provide an extended opportunity for extracting the collagen protein from the goat skin material, which will then be converted to gelatin by hydrolysis reaction in the water solvent.

In water, the amino acid monomer chains subsequently combine with each other to form a continuous three-dimensional structure and bind water to form a compact gel structure. Gel strength is highly dependent on hydrogen bonds between water molecules with free hydroxyl groups of amino acid groups, protein chain size, concentration and distribution of collagen molecular weight [22]-[23]. It was noted that GG-4 showed higher gel strength than GG-1, GG-2, GG-3 and GG-5. The result suggested that GG-4 developed with 30 days of curing and 5 hours of extraction time has best physical properties. It can be inferred that curing and extraction times played a role in gel strength of base gelatin from goat skin.
Figure 1. Gel strength of base gelatin from goat skin at various curing and extraction times

Gelatin which has high gel strength is generally preferred because its application is easier. The gelatin gel strength generated from both types of processes (GG-2, GG-3, GG-4 and GG-5) still meets the standards required by GMIA ie 50-300 Bloom [13]. The use of high curing time in the production of B-type (base) gelatin can lead to an increase in gel strength value [24], whereas the quality of gelatin produced is highly depending on the extraction process performed on collagen proteins [25].

3.3. Viscosity
Viscosity is the holding ability of a liquid to flow. The flow process of a liquid is influenced by viscosity due to adsorbs and colloidal development [21].

Table 2. Viscosity of Goat Skin Gelatin From Varying Curing and Extraction Time

| No | Goat Skin Gelatin Sample | Curing Time (day) | Extraction Time (hour) | Viscosity (cP) |
|----|----------------------------|-------------------|------------------------|---------------|
| 1  | GG-1                      | 10                | 4                      | 23.30         |
| 2  | GG-2                      | 30                | 4                      | 22.10         |
| 3  | GG-3                      | 50                | 4                      | 20.80         |
| 4  | GG-4                      | 30                | 5                      | 27.60         |
| 5  | GG-5                      | 30                | 6                      | 26.80         |

The result of gelatin viscosity analysis, as shown in Table 2 shows the effect of curing and extraction time on gelatin viscosity value. Increased viscosity values are influenced by the structure of amino acid molecules that make up gelatin proteins. An increasingly long chain of amino acids will increase the viscosity value of gelatin. Increased curing time in the gelatin production process can decrease the viscosity value. This is because the optimum decomposition process of amino acid peptide bond into shorter molecular chains by curing particle so its viscosity is reduced. The higher the curing times applied, the higher the quantity of short molecular chains in the gelatin compound and the lower the viscosity of the resulting gelatin product. Molecular weight distribution appears to play a more important role in the effect on viscosity than it does on gel strength. Some gelatins of higher gel strength may have lower viscosities than gelatins of lower gel strength.

Increased extraction time has increased the collagen compound obtained, which will hydrolyze into gelatin in water solvent. The increase in particle content of the gelatin compound has increased the particle density level in the gelatin solution and induced an increase in the viscosity of the gelatin solution. However, the application of relatively high temperatures in long-term extraction processes, which in this case for 6 hours has resulted in a decrease in the viscosity value of goat skin gelatin products produced. Based on the data in Table 2, it is seen that the viscosity value of goat skin gelatin
produced by increasing curing time and extraction is not much different from the value required by GMIA for the acidic and bases process, each of which is in the range of 15-75 mps and 20-75 mps. The highest viscosity values were obtained from base processes with curing and extraction time respectively for 30 days and 5 hours.

3.4. pH
One of the important chemical properties of gelatin is the degree of acidity (pH) as it relates to other properties such as the ability to bind with water, viscosity and emulsion capacity.

| No | Goat Skin Gelatin Sample | pH  |
|----|-------------------------|-----|
| 1  | GG-1                    | 8.63|
| 2  | GG-2                    | 8.68|
| 3  | GG-3                    | 8.70|
| 4  | GG-4                    | 8.70|
| 5  | GG-5                    | 8.75|

The results of gelatin pH analysis of goat skin in Table 3 showed that the contact time of goat skin material with curing solution will not only affect the percentage of the obtained gelatin yield, but also affect the maximum adsorption of the curing solution into the goat skin material. The condition affects: (1) the longer drying time required to obtain the dry gelatin product; (2) the increased moisture content of the goat skin gelatin product obtained. In addition, the maximum adsorption of the curing solution into the goat skin material has maximized the deposition of the curing solution, which in this case is the alkaline compound, which is further extracted together with collagen which is subsequently hydrolyzed into gelatin. The condition triggers the formation of gelatin products with higher pH value of the gelatin.

In line with this result, the increasing time of extraction has maximized the ingress of curing solution into goat skin material and has an effect on the water and ash content of the goat gelatin product. The high content of the alkaline curing solution that goes into the goat skin material has made it difficult to dry the gelatin product obtained and leave the gelatin product with a higher moisture content. The cured solution deposited on the goat skin material will increase as the time of extraction is applied. The curing solution deposited on the goat skin material will also be extracted together with gelatin compound. This condition will lead to the production of goat skin gelatin product with higher pH. The goat skin gelatin product produced by alkaline process with increased curing and extraction time in this research did not exceed the commercial pH value of the GMIA standard, ie in the pH range of 5-7.5.

3.5. Water and Ash Content
Water content in a product is closely related to the shelf life of the product, especially in terms of metabolic activity that occurs. The role of water in food is one of the factors that influence metabolism activity such as enzyme, microbial, chemical, enzymatic and non-enzymatic reaction activity so that it can cause changes in nutritional value and its organoleptic properties. While, determination of ash content is one way to know the purity of a material. Ash is an inorganic residue from the burning of organic materials.

Water and ash content of goat gelatin with higher curing and extraction time in comparison with its lower is shown in Table 4. Among all goat gelatin, GG-1 showed the lowest water content compared with GG-2; GG-3; GG-4 and GG-5. Higher curing time had resulted in maximum absorption of curing solution by goat skin material during the process. This is evidenced by the increasingly shrinking volume of curing solution and the expanding of goat skin material along with the increased curing time. The condition was due to the high water swelling of goat skin.

The maximum absorption of curing solution by goat skin material has increased the mineral deposition potential. This then leads to the production of gelatin products with the highest ash content.
Among all goat gelatin, GG-1 showed the lowest ash content (4.16%) compared with the others (4.35% for GG-2; 5.23% for GG-3; 4.61% for GG-4; and 5.95% for GG-5).

### Table 4. Water And Ash Content From Varying Curing and Extraction Time

| No | Goat Skin Gelatin Sample | Content (%) | Water | Ash |
|----|--------------------------|-------------|-------|-----|
| 1  | GG-1                     | 5.00        | 4.16  |
| 2  | GG-2                     | 10.00       | 4.35  |
| 3  | GG-3                     | 10.00       | 5.23  |
| 4  | GG-4                     | 10.00       | 4.61  |
| 5  | GG-5                     | 10.00       | 5.95  |

In line with this result, the increase in extraction time had significantly affected the water content of the GG product. Increased extraction time from 4 hours on GG-2 production to 5 and 6 hours, respectively on GG-4 and GG-5 production did not result in an increase in the water content of the gelatin product. The extraction accompanied by 4-6 hours of heating process has caused optimum damage to the molecular structure of the material to allow for high gelatin yields to be obtained without the increase in water content.

In addition, increased extraction time also has a negative effect where the mineral content of gelatin products produced becomes higher. The high levels of minerals deposited in goat skin material along with the increased curing time applied have increased the potential increase of ash content of gelatin products as a result of washed out curing material along with extracted collagen from the goat skin which will then hydrolyze into gelatin. Different raw materials and processes used might result in varying water and ash contents in gelatins. It was noted that all gelatins had the lower water and ash content than the recommended maximum value based on SNI 06-3735-1995 (i.e 16% water content and 3.25% ash content).

### 3.6. Chemical Characteristics

To find out the differences in the quality of GG with commercial gelatin on the market, functional groups analysis using FTIR were carried out. FTIR analysis of commercial gelatin showed the emergence of a number of peaks, including the wave number 3629.97 cm\(^{-1}\) and 3568.41 cm\(^{-1}\) (O-H free); 3271.33 cm\(^{-1}\) and 2938.64 cm\(^{-1}\) (O-H overlap with C-H); 1628.62 cm\(^{-1}\) and 1522.72 cm\(^{-1}\) (N-H); 1438.29 cm\(^{-1}\) and 1399.74 cm\(^{-1}\) (C-O-H bending); 1332.15 cm\(^{-1}\) (O-H bending); 1235.65 cm\(^{-1}\) (O-C overlap with C-N); 1079.58 cm\(^{-1}\) and 1032.74 cm\(^{-1}\) (C-O overlap with C-N).

With a very high level of similarity, the FTIR results of the GG in this study also showed the appearance of peaks in wave numbers 3268.84 cm\(^{-1}\) and 2930.52 cm\(^{-1}\) (O-H overlap with C-H); 1628.24 cm\(^{-1}\) and 1527.12 cm\(^{-1}\) (N-H); 1438.45 cm\(^{-1}\) and 1400.16 cm\(^{-1}\) (C-O-H bending); 1333.29 cm\(^{-1}\) (O-H bending); 1239.22 cm\(^{-1}\) (O-C overlap with C-N); 1080.06 cm\(^{-1}\) (C-O overlap with C-N). In addition, GG shows the appearance of the characteristic peaks at wave number 871.47 cm\(^{-1}\) which is an indication of the existence of C-H bending and ring puckering.

### 3.7. Organoleptic Characteristics

The results of general identification (color, physical form, odor and granular texture) on the resulting gelatin product showed that descriptively gelatin product produced by alkaline process with difference of curing time and extraction did not show any significant difference. Based on the data in Table 5 it appears that the appearance of the color of the GG-5 looks darker than the others. Differences in the appearance of color can be caused by the influence of the type of production processes [26]. The results of this identification is also not much different from quality required by SNI that is varied from not colored until yellowish and weak yellow or light brown.
The color change continued along with the increase in extraction time used. This is due to the higher structural damage to gelatin compounds caused by the use of high temperature with long periods of time in the extraction process. This condition will then also damage the structure of chromophores and maximize the change of chromophore groups in gelatin products. This then causes the GG-1 product to look brighter than GG-2, GG-3, GG-4, and GG-5 product.

**Figure 2.** Infra red spectra: (a) commercial gelatin; and (b) GG

| No | Identification Result | Product Characteristics | Odor | Granular texture |
|----|-----------------------|-------------------------|------|-----------------|
| 1  | GG-1                  | Yellowish, Sheets - powder | Weak like broth | Smooth & homogeneity |
| 2  | GG-2                  | Weak yellow, Sheets - powder | Weak like broth | Smooth & homogeneity |
| 3  | GG-3                  | Weak yellow, Sheets - powder | Weak like broth | Smooth & homogeneity |
| 4  | GG-4                  | Weak yellow, Light brown, Sheets - powder | Weak like broth | Smooth & homogeneity |
| 5  | GG-5                  | Light brown, Sheets - powder | Weak like broth | Smooth & homogeneity |
The results of gelatin color identification with chroma meter based on the color notation "L" are presented in Tables 6. The "L" (light) notation expresses a brightness level with a range of values 0 (black) - 100 (white). This value is depicted as a reflective light that produces a white, gray and black chromatic color.

The results of the color test in Table 6 showed that the application of different curing and extraction times has an effect on the change of gelatin product "L" color notation value. The increase of the "L" color notation value along with the increase in curing time indicated a change of molecular structure especially in the chromophores group of gelatin product which produced, as a result of the increasing destruction in amino acid polymer rings by curing solution.

| No | Goat Skin Gelatin Sample | Color Notation “L” |
|----|--------------------------|---------------------|
| 1  | GG-1                     | 91.23/89.00         |
| 2  | GG-2                     | 90.28               |
| 3  | GG-3                     | 85.56               |
| 4  | GG-4                     | 88.33               |
| 5  | GG-5                     | 84.72               |

4. Conclusions
Curing and extraction time have the same impacts on yield, gel strength, viscosity, pH, water and ash content, and also organoleptic characteristics. Increased curing and extraction times have led to an increase in yield, gel strength, water content and color of goat skin gelatin. At the same time, increased curing and extraction time has decreased the viscosity and ash content of base gelatin from goat skin. Particularly for pH, no significant effect was observed on curing and extraction time of gelatin pH. Goat gelatin has a very high resemblance to commercial gelatin on the market.

References
[1] P. Kaewruang, S. Benjakul, T. Prodpran, A.B. Encarnacion, S. Nalinanon, “Impact of divalent salts and bovine gelatin on gel properties of phosphorylated gelatin from the skin of unicorn leatherjacket,” LWT Food Science and Technology, vol. 55, pp. 477–482, 2014.
[2] T. Tasara, S. Schumacher, R. Stephan, “Conventional and real-time PCR411 based approaches for molecular detection and quantitation of bovine species 412 material in edible gelatin,” Journal of Food Protection, vol. 68, pp. 2420–2426, 2005.
[3] H.I.A. Amqizal, H.A. Al-Kahtani, E.A. Ismail, K. Hayat, I. Jaswir, “Identification and verification of porcine DNA in commercial gelatin and gelatin containing processed foods,” Food Control, vol. 78, pp. 297–303, 2017.
[4] R. Balti,M. Jridi, A. Sila, N. Souissi, N. Nedjar-Aroume, D. Guillochon, “Extraction and functional properties of gelatin from the skin of cuttlefish (Sepia officinalis) using smooth hound crude acid protease-aided process, Food Hydrocolloids, vol. 25, pp. 943-950, 2011.
[5] S. Mad-Ali, S. Benjakul, T. Prodpran, S. Maqsood, “Characteristics and gel properties of gelatin from goat skin as affected by pretreatments using sodium sulfate and hydrogen peroxide,” Journal of the Science of Food and Agriculture, vol. 96, pp. 2193-2203, 2016.
[6] A.A. Karim, R. Bhat, “Fish gelatin: properties, challenges, and prospects as alternative to man gelatin,” Food Hydrocolloids, vol. 23, pp. 563-576, 2009.
[7] B.G. Warmington, A.H. Kirton, “Genetic and non-genetic influences on growth and carcass traits of goats,” Small Ruminant Research, vol. 3, pp. 147-165, 1990.
[8] F.A. Johnston-Banks, Gelatin. In P. Harris (Ed.), Food gels (pp. 233-289), London: Elsevier Applied Science Publ., 1990.
[9] S. Benjakul, P. Kittiphattanabawon, J.M. Regenstein, Fish gelatin. In B.K. Simpson, L.M.L. Nollet, F. Toldrae (Eds.), Food Biochemistry and Food Processing (pp. 388-405), Ames: John Wiley & Sons Inc., 2012.

[10] I. Kolodziejka, K. Kaczorowski, B. Piotrowska, M. Sadowska, “Modification of the properties of gelatin from skins of Baltic cod (Gadus morhua) with transglutaminase,” Food Chemistry, vol. 86, pp. 203-209, 2004.

[11] M. Nagarajan, S. Benjakul, T. Prodpran, P. Songtipya, H. Kishimura, “Characteristics and functional properties of gelatin from splendid squid (Loligo formasana) skin as affected by extraction temperatures, Food Hydrocolloids, vol. 29, pp. 389-397, 2012.

[12] J.M. Regenstein, P. Zhou, Collagen and gelatin from marine by-product, In F. Shahidi (Ed.), Maximizing the value of marine by-products (pp. 279-303), Cambridge: Woodhead Publishing Limited.

[13] GMIA, Gelatin. New York: Gelatin Manufacturers Institute of America, Inc, 2012.

[14] BSI (British Standards Institution), Methods for sampling and testing gelatin (physical and chemical methods). London: BSI.

[15] I. Lassoued, M. Jridi, R. Nasri, A. Dammak, M. Hajji, M. Nasri, A. Barkia, “Characteristics and functional properties of gelatin from thornback ray skin obtained by pepsin-aided process in comparison with commercial halal bovine gelatin,” Food Hydrocolloids, vol. 41, pp. 309-318, 2014.

[16] AOAC, Official methods of analysis (17th ed.). Washington, D.C: Association of Official Analytical Chemists, 2000.

[17] A. Bahar, M.A. Anggarani, N. Kusumawati, S. Muslim, “Optimization of curing and extraction time on the production of base gelatin skin material”, Advanced in Social Science, Education and Humanities Research, vol. 112, pp. 58-62, 2018.

[18] U.K. Laemmli, “Cleavage of structural proteins during the assembly of head of bacteriophage T4,” Nature, vol. 277, pp. 680-685, 1970.

[19] P. Diaz-Calderon, E. Flores, A. Gonzales-Munoz, M. Pepczynska, F. Quero, J. Enrione, “Influence of extraction variables on the structure and physical properties of salmon gelatin,” Food Hydrocolloids, vol. 71, pp. 118-128, 2017.

[20] Gimenez, “The role of salt washing of fish skins in chemical and rheological properties of gelatin extracted,’ Food Hydrocolloids, vol. 19, pp. 951-957, 2005.

[21] R. Schrieber and H. Gareis, Gelatin handbook, theory and industrial practice. WILEY-VCH, ISBN 978-3-527-31548-2, 2007.

[22] J.A. Arnesen and A. Gildberg, “Preparation and characterization of gelatin from the skin of harp seal (Phoca groendlandica),” Bioresource Technology, vol. 82, pp. 191-194, 2002.

[23] R. Bhat and A.A. Karim, “Ultraviolet irradiation improves gel strength of fish gelatin,” Food Chemistry, vol. 113, pp. 1160-1164, 2008.

[24] H.W. Ockerman and C.L. Hansen, Animal By Product Processing and Utilization. USA: CRC Press, 2000.

[25] L.M. Kasankala, Y. Xue, Y. Weilong, S.D. Hong, H. Q, "Optimization of gelatin extraction from grass carp (Ctenopharyngodon idella) fish skin by response surface methodology,” Bioresource Tech., vol. 98, pp. 3338-3343, 2007.

[26] Glicksman, Gum Technology in The Food Industry. New York: Academic Press, 1969.

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