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Physical and Chemical Properties of Buffalo Skin Gelatin Extracted Using Crude Acid Protease from Goat Abomasum

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Abstract. The present study reports extraction buffalo skin gelatin (BSG) using crude acid protease from abomasum of goat (CAPAG). CAPAG was used for aiding gelatin extraction of buffalo skin using different levels of 0; 2.5; 5; 7.5 U/ g at different levels of hydrolysis temperature i.e., 28; 37; 40˚C. CAPAG aided process increased the yield of BSG, which reached 26.15 % using 7.5 U/g. The BSG was characterized based on its chemical and physical properties. BSG had high protein content (87.96 – 96.18 %), low moisture (6.99 – 7.51 %), low ash (0,51 – 0,57 %), and low fat (0,47 – 0,55 %) contents. The pH ranged 3.90 – 4.05 and had 204.88 – 212.33 g bloom of gel strength while the viscosity was 7.34-7.69 cP and showed 90-110 KDa on SDS Page output.

INTRODUCTION

Gelatin is a substantially pure protein food ingredient, obtained by the thermal denaturation of collagen, which is the structural mainstay and most common protein in the animal kingdom [1]. Gelatin specifically by the partial hydrolysis of collagen derived from the skin, white connective tissue and bones of animals [1]. In the food field, gelatin is employed as a gel former, texture former, thickener, and excellent water binder [2]. Harianto and Peranginangin [3] argue that gelatin is extracted from pigskin with the percentage of 44.5% (136,000 tons), cowhide with the percentage of 27.6% (84,000 tons), and other sources with the percentage of 1.3% (4,000 tons). Recent research produces gelatin from fish skin [4][5][6][7][8]. However, there is still limited research discussing gelatin utilization from mammals.

Collagen is a rigid, inextensible fibrous protein that is composed of three intertwined polypeptide chains and is a principal constituent of connective tissue in animals, including tendons, cartilage, bones, teeth, skin, and blood vessels. Collagen is the most abundant protein in vertebrates [9]. Buffalo is one of the mammals in Indonesia. The thick skin of buffalo, contains of 30 % protein [10], indicates an existence of fibrous protein that acts as a collagen fiber former.

Several types of collagens have a stable character toward alkali-acid treatments and heat extractions which causes difficulties in hydrolyzing them to be gelatin and makes relatively low products [11]. As an alternative process, protease categories can be added in such gelatin extraction. Acid protease can dissolve collagen by cutting more peptide bonds and produce more gelatin with less molecular weight than alkali-acid processes [5][7]. There is no research concerning the crude acid protease (CAP) extraction process from local goat abomasum and its characteristics has not been identified yet. These facts indicate that research on how to extract and characterize crude acid protease derived from goats is indeed required to be conducted.

Gelatin qualities are influenced by its physic-chemistry characters and extraction methods [8]. Gelatin produced by alkali-acid processes high gel strength values that are 248-297 g bloom [10] and 350.25 g bloom. By these gel strength values, when the gelatin is applied to jelly products, it gives a harder texture [12]. Enzymatically extracted gelatin has a lower gel strength that is 120-198 g bloom [8] than gelatin produced with an alkali-acid submersion.

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Adding acid protease to collagen extractions to be gelatin affects gelatin yields [8][5][4]. Using several enzyme levels when the extraction is being processed can decrease gelatin pH values and water content as well as increase its viscosity [13]. Increasing the crude acid protease concentration is reported being able to increase gelatin protein content [4] and brightness as well as decrease yellow color intensity in gelatin [5].

Moreover, the acid protease enzyme's work during gelatin extraction processes is influenced by its working condition temperature. It has to be noted that each acid protease enzyme that comes from different sources owns a different temperature optimization as well.

This research aims on observing characteristics of buffalo skin gelatin extracted by assistance of crude acid protease (CAP) from goat abomasum with some concentration levels that are 0, 2.5, 5, and 7.5 U/g as well as different temperatures that are 28, 37, and 40ºC.

MATERIALS AND METHODS

This research was conducted in buffalo skin supplied by CV Panji Jaya Kecamatan Segoroyoso Kabupaten Bantul Yogyakarta. Goat abomasum extracted was supplied by Jagal Kambing H. Bambang Hariyanto Pasarkliwon Solo.

The researcher used equipment such as blender (Philip), centrifuge (Sigma Sartorius 3-30K), pH meter (Mettler Toledo, Hanna Instrument HI 2210), dialysis tube (D6066-25 EA), MWCO 12000 Da (Sigma Aldrich), spectrophotometer UV-Vis (Shimadzu), electrophoresis equipment SDS-PAGE (Thermo Fisher Scientific), analytic scale (Fujitsu FS-AR210), oven (Memmert WNB-45 type, Germany), furnace, Chromameter (Konika Minolta Sensing, INC, Japan), universal texture machine (UTM Zwich Z 0.5) rheometer viscosity (Viscometer Brookfield LV), water bath (Sibata ES-240, Japan), and glass equipment for analysis necessities. This research consisted of extraction of buffalo skin collagen, and gelatin character testing.

Extraction of Buffalo Skin Collagen

Before extracting gelatin, we prepared the CAPAG first. CAPAG was produced based on Balti *et al*.'s methods [14] that had been modified, and its characterization has been reported [15]. The extraction of buffalo skin collagen to be gelatin was based on Lassoued *et al*.'s methods [7] and Mulyani [10]. The size of buffalo skin was condensed (2 × 2 cm^2) and submerged in NaOH of 0.5 M (1:3 b/v) for two hours. After being condensed and submerged, the skin was washed with running water to pH of 7 and submerged in HCl of 0.9 M (1:3 b/v) for four hours. After submersion, the skin was washed with running water to pH of 7. The skin that had been submerged by bases and acids was enzymatically extracted using goat abomasum CAP (0; 2.5; 5.0; 7.5 U/g) at the temperature of 28, 37, and 40ºC for three hours. After that, CAP was inactivated using NaOH of 5 M until pH of 7. Then, a thermal extraction was done using 65-70ºC for four hours. The result was screened to gain gelatin supernatant. This supernatant was dried using a cabinet dryer at the temperature of 60ºC for 48 hours to produce dried gelatin. The gelatin was then to be powder gelatin.

RESULT AND DISCUSSION

Yield

Gelatin yield value is a comparison between a dried gelatin number produced and a total weight of buffalo skin used. The gelatin yield value on this research is 20.32-26.29% (Table 1). Acid protease utilization in the form of goat abomasum CAP can increase gelatin yields. The similar trend of the more the proportion of acid protease utilization is used, the more the yields will be produced is also applied by [14] [8] [5] [4]. Hydrolysis at the temperature of 37ºC is the most effective way to extract gelatin since this temperature is the natural temperature for digestion [16] in goat abomasum as the enzyme source.

Gelatin pH

The pH value of buffalo skin gelatin in this research is 3.90-4.05 (Table 1). The value obtained is in the standard range of gelatin pH based on GMIA 2007, that is 3.80-6.00. Gelatin with low pH values is perfectly applied in products like juice, jelly, and syrup.

Viscosity

The value of buffalo skin gelatin viscosity is 7.34-7.69 cP (Table 1). Moreover, in the research about an extraction of buffalo skin gelatin with alkali-acid processes by Mulyani *et al* [10], it concluded that the buffalo skin gelatin produced had the viscosity of 16.37-22.17 cP. CAP utilization in gelatin extraction can decrease gelatin viscosity to the standard value of gelatin viscosity based on GMIA [18] that is 1.5-7.5 cP.

TABLE 1. Gelatin Properties Based on Temperature

Parameters	Hydrolysis Temperature (°C)		
	28	37	40
Yield (%)	21.16^{a}	26.29^{b}	22.76^{a}
pH	3.96^{a}	3.98^{bc}	4.00^{c}
Viscosity(cP)	7.47^{a}	7.50^{a}	7.53^{a}
Gel Strength (g Bloom)	211.20^{a}	205.05^{a}	212.33^{a}
Water	7.42^{a}	7.30^{a}	7.16^{a}
Ash	0.54^{ab}	0.57^{b}	0.51^{a}
Protein	93.29^{a}	91.16^{a}	91.34^{a}
Color L	64.71^{a}	65.19^{a}	64.65^{a}
Color a	6.81^{a}	6.89^{a}	6.80^{a}
Color b	16.59^{a}	17.15^{a}	16.79^{a}

Note: The notation of different small letter superscript in the same column shows there is a real effect/difference ($p \geq 0.05$).

Gel Strength

To know the rigidity of gel formed by 6.67% solution of gelatin was measured using gel strength test. Bloom, as unit measurement, described the force (weight) that required to depress 4 mm surface of the sample. Gelatin in this research was obtained possesses values of gel strength between 204.88-212.33 g bloom (Table 1). This value of gel strength fulfils the GMIA standard of gelatin quality [18] that is around 50-300 g bloom. Gelatin whose value of gel strength is 200-260 can be applied in yogurt, sausage, broth, canned meat, and candy industries as a stabilizer, emulsifier, binding agent, and texture former [1].

Gelatin Color

The brightness of gelatin produced is 64.29-65.40, profile-a gelatin is 6.78-6.89, and profile-b gelatin is 16.43-17.15 (Table 1). Color profiles of extracted buffalo skin gelatin using alkali-acid have values of L, a, and b that are 69.92-70.97, 0.55-1.54, and 17.54-19.59 (orderly mentioned) [10]. Descriptively, gelatin in this research has darker, redder, and bluer colors compared to buffalo skin gelatin extracted from alkali-acid.

Water Content

Water content in food also determines its acceptance, freshness, and durability. Water content of buffalo skin gelatin resulted in this research is 6.99-7.42% (Table 1). The data was in good value depend on water content values that suggested by GMIA (10.5%). Dry gelatin is hygroscopic, and storage under moist ambient can make gelatin absorbs atmospheric moisture and reveal low moisture percentage.

Ash Content

Ash content of certain material indicates quantities of mineral existence contained by the material. Ash content of buffalo skin gelatin obtained is 0.51-0.56% (Table 1). The ash content value in this research is lower than the ash content value of buffalo skin gelatin extracted by alkali-acid, that is 0.53-1.23% [10]. This fact proves that adding the goat abomasum CAP can alleviate ash content contained by buffalo skin gelatin more than extracting gelatin by alkali-acid.

Protein Content

Gelatins consist of peptides and proteins produced by partial hydrolysis of collagen extracted from skin, bones, and some connective tissue of animals. During hydrolysis, some of protein collagen are broken. By enzymatic extraction, buffalo skin collagen degraded into pieces of gelatin that has protein content about 87.93-96.18% (Table 1). Moreover, other research stated that buffalo skin gelatin that was extracted using alkali-acid extraction (without adding enzymatic extraction) only contained 83.38-91.11% of protein [10]. From this data we can conclude that using enzymatic extraction can degrade more protein content in gelatin.

Fat Content

Table 1 shows that the percentage of ash content of buffalo skin gelatin extracted using CAPAG ranged between 0.47-0.55%. This range of fat content was in good value and accordance with fat standard content required by SNI which is no more that 3.25% content of fat. Other result from different research of fish gelatin showed similar result such us 0.5% [19], 0.78-0.97% [5], and 0.48-1.75% [4] that fulfil the SNI requirement about maximally fat content.

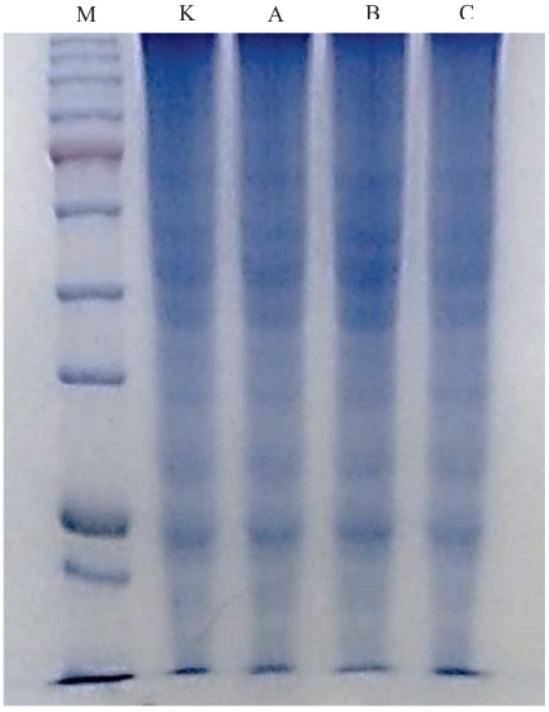

FIGURE 1. Distribution of Gelatin Molecular Weight (K = control, A: gelatin extracted using 7,5 U/g CAP at 28˚C, B: gelatin extracted using 7,5 U/g CAP at 37˚C, C: gelatin extracted using 7,5 U/g CAP at 40˚C)

Distribution of Gelatin Molecular Weight

Gelatin molecular weight distribution can be inferred from SDS-Page (sodium dodecyl sulphate–polyacrylamide gel electrophoresis) as commonly kit used as a method to separate proteins with molecular weight between 5 and 250 kDa (kilodalton). Gelatin's SDS-Page data performed in Figure 1 that stated that SDS-gel produced bands that were thicker than the range of gelatin α-helix chain band that was 90-110 (kDa) [1]. This result indicates that buffalo skin collagen has been degraded to be gelatin in proper α-helix chain band.

Collagen natives that are still in the form of triple helix have molecular weight of about 300 K; while gelatin that has been hydrolyzed, they don't have triple helix structures anymore and the chain is decomposed to be shorter. So that in SDS PAGE analysis results, which is produced several bands under 300 KDa, indicated that gelatin from buffalo skin extracted using CAPAG is achieved.

CONCLUSION

Goat abomasum CAP performs the highest activity when conditioned in pH of 3 and the temperature of 40ºC. Utilizing goat abomasum CAP in gelatin extraction is capable to increase gelatin yields. Buffalo skin gelatin has pH of about 3.90-4.05, gel strength value of 204.88-212.33g bloom, viscosity value of 7.34-7.69 cP, shows that there is a distribution of molecular weight in 90-110 KDa, and has proximate characteristics of protein content of 87.96-96.18%, water content of 6.99-7.51%, ash content 0.51-0.57, and fat content of 0.47-0.55%.

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