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Chemical Composition of Acid Soluble Collagen (ASC) Isolated from Indonesia Local "Kacang" Goat Skin *(Capra aegagrus hocus)*

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ABSTRACT

Collagen from the local "Kacang" goat skin is a natural raw material in the halal food industry in Indonesia. This study aims to isolate acid-soluble collagen (ASC) from "Kacang" goat skin and characterize its chemical properties. The collagen was derived from one-year-old goat skin and cured in acid condition for 48 hours at 4°C to eliminate meats, fat, and hair. The cleaned skins were treated at 1:10 (w/v) of 0.1 M NaOH for 0, 24, and 48 h at 4°C. It was then neutralized in distilled water and extracted with 0.5 M acetic acid at a 1:10 (w/v) ratio for 24, 48, and 72 h at 4°C. The yield of ASC was 21%, characterized by chemical composition, soluble protein, differential scanning calorimeter (DSC), and protein molecular weight. The chemical composition of ASC was 11.15% (moisture), 9.04% (protein), 0.98% (fat), and 0.052% (ash). ASC has the highest collagen solubility in NaCl 5% at pH 2. ASC also has thermal stability with a low profile pattern of molecular weight. In conclusion, "Kacang" goat skin from Indonesia might be used to make a value-added product because it has a high moisture content and low fat level.

Keywords: Acid soluble collagen (ASC), chemical composition, DSC, Indonesia local "kacang" goat skin, molecular weight, soluble protein

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INTRODUCTION

The need for collagen as raw material in food and non-food industries, such as medicine and cosmetics, is increasing yearly (Veeruraj et al., 2015). Due to the huge demand for collagen, humans explored and isolated collagen from the extracellular matrix of multicellular animals, from vertebrates to echinoderms. According to Chen et al. (2018), collagen makes up about 30% of the total protein in a vertebrate animal's body. Fibril collagen is organized into a network that creates a stable body frame (Holmes et al., 2018). There are currently 29 types of collagen molecules known from types I-XXIX from various animal tissues, with each type having a unique molecular structure, amino acid sequence, functional characteristics, and biophysical properties (Luo et al., 2018; Zhao et al., 2018). The collagen from cow and pig skins is used for functional foods, tissue engineering, and cosmetics because it has unique properties, physically and functionally (Luo et al., 2018; Subhan et al., 2015; Venkatesan et al., 2017).

The arrangement of peptides, structures, and functions varies among all these collagen molecules (Yousefi et al., 2017). Even though Indonesia has a huge potential for raw materials, the country currently depends on imported goods for its collagen supply. Wang et al. (2014) reported that the use of raw materials from pigs' skin and bones as a source of collagen is an issue for Muslims, even though the use of local materials other than pork is an alternative source for collagen production. Some industries make halal collagen due to Muslim consumers' growing awareness of halal food. Furthermore, collagen, the primary component of capsule shells used in the pharmaceutical business, is an imported good (Hidaka & Liu, 2003), mostly from the United States and Europe. These countries often use pig skin (46%), cow bones (23.1%), and other sources (1.5%) to isolate collagen. It has occurred because of the low cost of raw materials (Karim & Bhat, 2009). Additionally, some countries have high cases of foot and mouth disease (FMD) (Huang et al., 2011).

The "Kacang" goat skin holds great promise as a source of collagen in Indonesia. There are various local livestock species spread over Indonesia. Goat skin is typically a by-product used to make jackets and gloves. For best usage and to maximize its worth and reduce disposal costs, this by-product should be converted into a source of collagen. A chemical substance and a skin protein react during the collagen isolation process. Pepsin and materials derived from acids are still used as pre-extraction agents in collagen isolation. Animal collagen can be isolated through salting out, alkaline, acid, and enzymes (Yang & Shu, 2014). The acid and enzyme procedures are the ones that scientists like to use. Collagen can be extracted using the acid method using organic acids like citric, lactic, or chloroacetic acid (Skierka & Sadowska, 2007).

The results of isolating collagen from a material are then physically and chemically characterized to determine the properties of the collagen obtained. Protein concentration, moisture, ash, soluble protein, and fat content are examples of chemical properties. Physical characterizations include viscoelasticity, thermal stability, functional group, and solubility

(Matmaroh et al., 2011; Singh et al., 2011; Wang et al., 2014). As a result, this study's objectives are to isolate acid-soluble collagen (ASC) from "Kacang" goat skin and define its chemical composition.

MATERIALS AND METHODS

Materials

Collagen was prepared from goatskin at the age of one year. It was carefully removed by hand, cleaned with distilled water, and removed manually. We purchased acetic acid, sodium hydroxide, sodium chloride, HCl, methanol, BSA, and commasie blue from Merck in Darmstadt, Germany. İn this study, only analytical-grade reagents were employed.

Method

Chemical Composition of Raw "Kacang" Goat Skin

The chemical compositions of "Kacang" goat skin, such as moisture, protein, fat, ash content and isolated collagen, were analyzed and determined according to AOAC (2000).

Condition for the Optimum Process of Isolation of Collagen

In the extraction process, 0.1 M NaOH was used to isolate and optimize collagen and acetic acid for 24, 48, and 72 h. Each treatment in the study was carried out five times. The experimental design extracted collagen using a slightly modified technique (Wahyuningsih et al., 2018). The local slaughterhouse provided "Kacang" goat skin. Figure 1 shows a flow diagram of the collagen extraction procedure. The cleaned skins were trimmed to a length of around 10 cm. The treated skins were washed and placed in 0.1 M NaOH at a ratio of 1:10 (w/v) for 0, 24, and 48 h at 4°C. After the alkaline treatment, the skin was cleansed and neutralized with distilled water to a pH of 7. For 24, 48, and 72 h at 4°C, the skin was extracted with 0.5 M acetic acid at 1:10 (w/v). The extract was filtrated with filter paper, and then it was precipitated with 2.6 M NaCl. At 4°C and 10,000 g, the sample solution was centrifuged for 30 min. Total precipitates were collected, dialyzed with 0.1 M acetic acid, and then mixed with 0.5 M acetic acid a ratio of 1:5 w/v). The highest yields of the freeze-dried collagen that had been isolated were employed in the tests. Figure 2 displays the collagen from "Kacang" goat skin.

Characterization of "Kacang" Goat Skin Extracted Collagen

Protein Concentration

Lowry's method is used to calculate the protein concentration of ASC (Ledward, 2000). As a protein standard, 10 ml of distilled water was mixed with about 100 mg of bovine

serum albumin (BSA). The biuret reagent was added and allowed at room temperature for 30 min, and with a spectrophotometer (Shimadzu, United States), absorbance at 570 nm was determined. The absorbance at 570 nm was graphed against the volume of the protein standard solution. The collagen powder was then diluted 1:5 with distilled water. As a result, the final protein concentrations in the calibration curve's range were established.

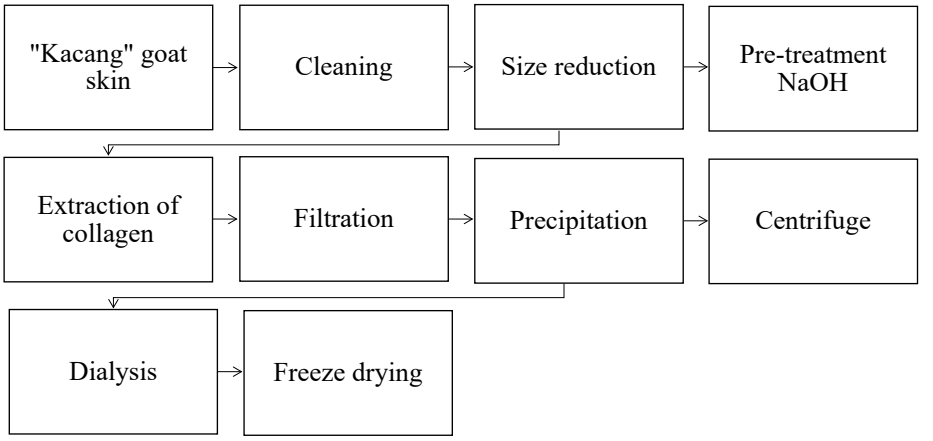

Figure 1. The process flow diagram for collagen extraction

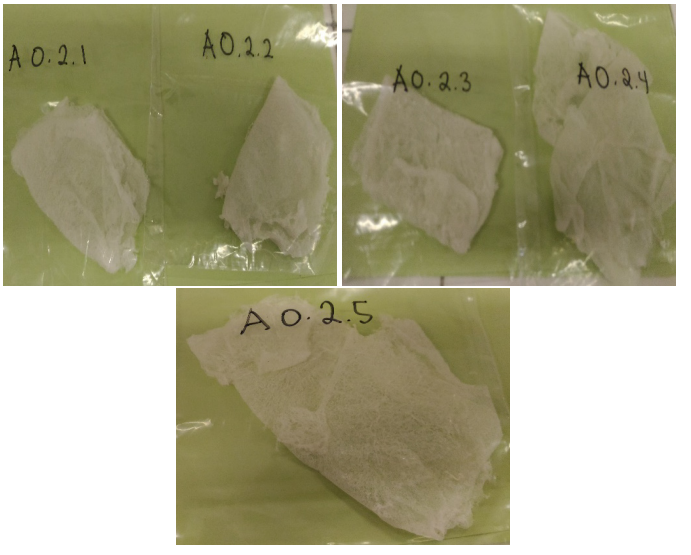

Figure 2. The collagen freeze-dried from "Kacang" goat skin

Solubility determination

With a few minor modifications, Jongjareonrak et al. (2005a) and Corre-Bordes et al. (2018) used varied pH levels to test the solubility of isolated collagens. The lyophilized collagen was dissolved in 0.5 M acetic acid with moderate stirring for 24 h to achieve a final 3 mg/ml concentration. The sample was transferred to a centrifuge tube with a volume of around 8 ml with 6 N NaOH or 6 N HCl; the pH values were changed from 1 to 10 across the pH scale. Distilled water was used to increase the volume to 10 ml. The solutions were agitated for 30 min at 4ºC, followed by a 30 min centrifugation at 4ºC. Lowry's method determined protein concentrations in the supernatant (Lowry et al., 1951). Calculations of protein solubility were made using data from the pH level with the highest protein content. Equation 1 was used to determine the relative solubility of collagen.

$$Relative\ solubility\ (\%) = \frac{Protein\ concentration\ of\ supernatant}{The\ highest\ protein\ concentration} x\ 100 \qquad [1]$$

Differential Scanning Calorimeter (DSC)

Liang et al. (2014) and Jeevithan et al. (2014) employed the differential scanning calorimeter with minor adjustments. The samples were sealed after being weighed on an aluminum pan. Temperature calibrations were done using an indium standard. Denaturation and melting temperatures are determined using DSC heating patterns. The first endothermic peak (Td), or thermal denaturation of collagen and the second peak (Tm), or melting temperature, were measured.

Protein Molecular Weight by SDS-Page Electrophoresis

SDS-PAGE electrophoresis was used to determine the proteins' molecular weight. Following the Laemmli (1970) method, SDS-PAGE was carried out using 5% stacking gels and 7.5% separating gels. The gels were separated and connected to the power source at 10 mA to track the dye introduced into the bromophenol blue. Gel staining was done using Coomassie blue and a 2:1 methanol to the acetic acid solution. The separated protein bands were recognized using a typical Sigma molecular mixture marker (protein marker).

Statistical Analysis

The condition optimization of collagen was analyzed using SPSS 16.0 with the General Linear Model (GLM) factorial pattern with each of the three replicates. The chemical and solubility collagen of the ASC "Kacang" goat collagen was analyzed using SPSS 16.0 with a one-way analysis of variance (ANOVA) design for each of the three replicates. Each analysis result was presented as a mean (SD). ANOVA and mean were compared statistically using SPSS to determine whether any differences were statistically significant ($p < 0.05$).

RESULTS AND DISCUSSION

Chemical Composition

The analysis objectives in this study are to determine the chemical composition of raw material and compare it with raw material extracted into ASC. Table 1 presents the chemical compositions of raw skin and ASC. The result showed raw skin has moisture (29.81 ± 2.31%), protein (31.05 ± 0.21%), and ash (0.36% ± 0.03), whereas the chemical composition of ASC is moisture (11.15 ± 0.08%), protein (9.04 ± 0.32%), and ash (0.052 ± 0.09%), the raw material has it higher than ASC. Collagen isolated by acid treatment contained higher fat (0.98%) than raw material (0.52%). The separate collagen's high-fat content demonstrated an effective defatting process. Silver catfish collagen only included 0.81 ± 0.32% ASC and 0.92 ± 0.08% fat, according to research by Sarbon et al. (2013). Ash content indicating low value also provides well on the collagen produced. The protein content of ASC was 9.04% lower than the raw skin material (31.05%). The extraction process may have failed, so the extracted protein was not optimal, and there was still much left in the raw material. Therefore, further study is needed to obtain optimal results so that the extracted protein can be optimal.

The moisture content of the raw skin of the "Kacang" goat and extracted collagen differs significantly ($p < 0.05$). Li et al. (2020) reported that collagen from the carapace tissue of a Chinese soft-shelled turtle (*Pelodiscus sinensis*) contains moisture (14.21%), protein (32.02%), fat (0.40%), and ash (48.6%). The extracted collagen of ASC showed low moisture, protein, and ash but high fat. High-fat content in the raw material can interfere with collagen extraction, so the yield cannot be optimal. It was removed before collagen extraction with NaOH, and this also indicates that the NaOH curing process before extraction in this study was not optimal, or the pre-treatment of NaOH was not long enough because there was still much fat involved during extraction.

Table 1
Chemical composition and soluble protein concentration of ASC

Samples	Chemical composition (%)			
	Moisture	Protein	Fat	Ash
Raw skin	29.81 ± 2.31[a]	31.05 ± 0.21[c]	0.52 ± 0.07[a]	0.36 ± 0.03[d]
ASC	11.15 ± 0.08[b]	9.04 ± 0.23[b]	0.98 ± 0.32[b]	0.052 ± 0.09[a]

*Different superscripts ([a-d]) in the same column of collagen are the significant differences ($p < 0.05$)

Condition for Optimum Process of Isolating Collagen

Optimization of collagen isolation was analyzed using the General Linear Model (GLM) factorial pattern (Table 2). Pre-treatment with NaOH is followed by collagen extraction in

two phases (Pal & Suresh, 2016). The optimization of collagen isolation is based on the highest yields. Yield is the final result obtained from collagen extraction in the method is the more effective or not used (Alhana et al., 2015). Yield shows the effectiveness of raw materials in converting into products (Wulandari & Suptijah, 2015). Wet yield data of acid extraction was used to analyze the optimum method for isolating collagen from Indonesia's local goat skin. The optimum process of isolating collagen in this study was at 0 h pre-treatment of NaOH and extraction 48 h at 4°C with yields of 21%. Treatment extraction collagen without NaOH has the highest yield and was significant to the pre-treatment of NaOH in 24 and 48 h. Long pre-treatment NaOH and acid extraction time is known to impact the yield of acid-soluble collagen. Decreasing collagen yield can impact the pre-treatment time of NaOH and the extraction process. Based on the data analysis results in this study, pre-treatment time with NaOH and acid extraction can impact the yield produced.

Table 2
Yields of acid soluble collagen from "Kacang" goat skin

Pre-treatment of NaOH (h)	Yield (%)		
	Time to extraction (h)		
	24	48	72
0	11.40 ± 4.39^{a}	21.00 ± 8.34^{a}	17.8 ± 2.78^{a}
24	18.20 ± 4.15^{b}	8.89 ± 2.34^{b}	6.45 ± 3.29^{b}
48	3.60 ± 1.14^{c}	3.20 ± 1.64^{c}	4.80 ± 1.64^{c}

[a,b,c] Different Superscript differences in some letters indicated significant differences ($p < 0.05$)

The optimum conditions in this study were different from previous studies by Woo et al. (2008), who explained that the optimum condition for collagen extraction from yellow tuna skin was 24 h NaOH pre-treatment time and collagen extraction time was 23.5 h with a yield of 27.1% using NaOH concentrations of 0.2 and 0.5 M, but if NaOH is used with a concentration of 0.05 and 0.1 M is only a small amount of protein dissolves. So, in this study, a concentration of 0.1 M NaOH only caused slight swelling of the goat skin, but the longer the soaking, the greater the yield produced. Yoshimura et al. (2000) stated that soaking using NaOH in collagen extraction can affect swelling on the skin of *C. striata* fish. The telopeptide region of the collagen molecule can be damaged by alkaline treatment with NaOH during the pre-treatment method, which results in the soluble OH group that binds to the protein (Jaswir et al., 2011). The process can be caused by the migration of non-protein collagen and other components in the collagen matrix so that it is easily detached (Cho et al., 2005). Time, temperature, concentration of NaOH, and the type of raw material utilized impacted how effective the NaOH pre-treatment time was (Liu et al., 2015)—the period of hydrolysis impacted the extraction method because the mass transfer

rate was regulated by the duration of the diffusion process, which in turn impacted the total yield. The type of solvent, solvent concentration, temperature, and the raw material utilized can all impact the variations in ideal circumstances in the extraction process. The most often used organic solvent for collagen extraction was acetic acid. pH is linked to changes in protein density, which can affect electrostatic interactions and protein structure. At the same time, the solubility of collagen in acetic acid can impact the pH value during the extraction process (Verheul et al., 1998). When the pH is lower or higher than the isoelectric point (pI) of protein, the solubility of protein will increase due to the repulsive force between charges (Chi et al., 2014). The pI value of collagen is usually between pH 6-9 (Li et al., 2013; Wu et al., 2015). İn addition, collagen solubility was affected by the number and sequence of amino acids, molecular weight and conformation. Environmental factors also play an important role, such as pH, type of solvent, ionic strength, temperature and extraction process (Li et al., 2018; Zayas, 1997).

Characterization of Extracted "Kacang" Goat Skin Collagen

Soluble Protein Concentration

NaCl Effect on the Solubility of Collagen. The precipitation process greatly influences the total amount of soluble collagen. Collagen can be precipitated using NaCl. Adding salt in high concentrations causes protein aggregation through salting out, where the salt binds to water and precipitation occurs. The precipitation can happen because the ionic strength of the salt is higher than that of protein, so water is easily bound. The decrease in total protein bonding in water can cause the protein to precipitate easily (Winarno, 2008). Figure 3 shows ASC solubility with various concentrations of NaCl (1%, 2%, 3%, 4%, 5%, 6%). This study showed collagen solubility of ASC at 1% NaCl was significantly lower to 5% and 6% NaCl concentration treatment but similar with NaCl 2%, 3%, and 4% concentrations. The collagen solubility of ASC at 5% NaCl was significantly higher at 1% and 2% NaCl concentration treatment but showed similar results to NaCl 3%, 4%, and 6%. ASC solubility decreased and gradually declined from 1 to 5% NaCl concentration. ASC was more soluble in treatment NaCl 5% (580 ± 0,11 ppm).

The higher solubility of ASC with treatment NaCl 5% is 580 ± 0,11 ppm, which was significant with 1% NaCl. The collagen solubility of ASC in this study generally decreases at a 6% increase in concentration of NaCl. According to the research by Li et al. (2020), the collagen of the skin of the Nile tilapia by chemical and fermentation has a solubility of collagen at 1% and 2%. Other studies have also been reported by Li et al. (2013) on collagen solubility of ASC from bone and skin of Spanish mackerel (*Scomberomorus phonics*) constant of NaCl up to 2% (w/v) and decreasing of NaCl concentration is 3% (w/v). According to research by Woo et al. (2008), collagen solubility of ASC from yellowfin tuna dorsal skin collagen decreases when the concentration of NaCl increases at 2% (w/v).

In Song et al. (2021), FASC and CASC from Nile tilapia skin have high solubility at 3% and decrease at 4% (w/v) NaCl. It is probably caused by the structure of ASC from the Indonesian local Kacang" goatskin, which is different from the structure of collagen from fish. The protein solubility of ASC in this study was relatively stable, increasing at over a concentration of NaOH 2% and slowly decreasing at a concentration of 6% NaCl. The collagen structure of ASC in this study was relatively stronger and resistant to NaCl at above 2%. The salting-out phenomenon was affected by collagen solubility when NaCl concentration was increased. The collagen solubility decreases because of increasing ionic strength, so salt ions compete with water and protein precipitates (Vojdani, 1996). Collagen solubility declines when an increase in NaCl concentration causes the protein to precipitate by combinations of chains and the competition with salts ionic water and enhancing hydrophobic-hydrophobic interactions (Bae et al., 2008; Jongjareonrak et al., 2005b). NaCl with high concentrations causes the hydration layer on the collagen surface to be destroyed and hydrophobic sites to be exposed, thereby causing increased interactions and hydrophobicity collagen precipitation and aggregation (Woo et al., 2008; Yu et al., 2014).

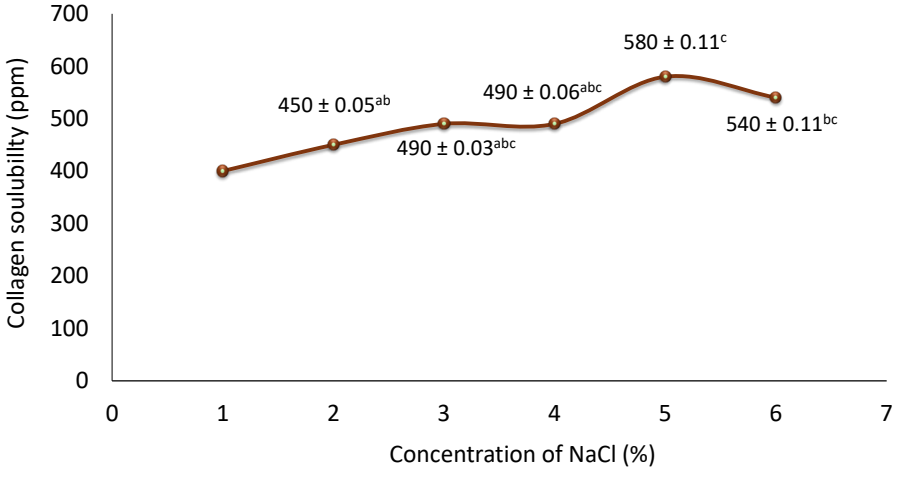

Figure 3. ASC solubility with variation concentration of NaCl

pH Effect on the Solubility of Collagen. pH conditions highly influence the collagen solubility of the solution. ASC samples were observed at pH 1–10. Figure 4 illustrates how pH affects ASC collagen solubility. In this study, collagen solubility was pH neutral, and the level of solubility of collagen in each sample experienced a decrease. However, the solubility of collagen increased once again when the pH was increased to an alkaline. Dissolved collagen levels in this study showed that the solubility of collagen in the treatment

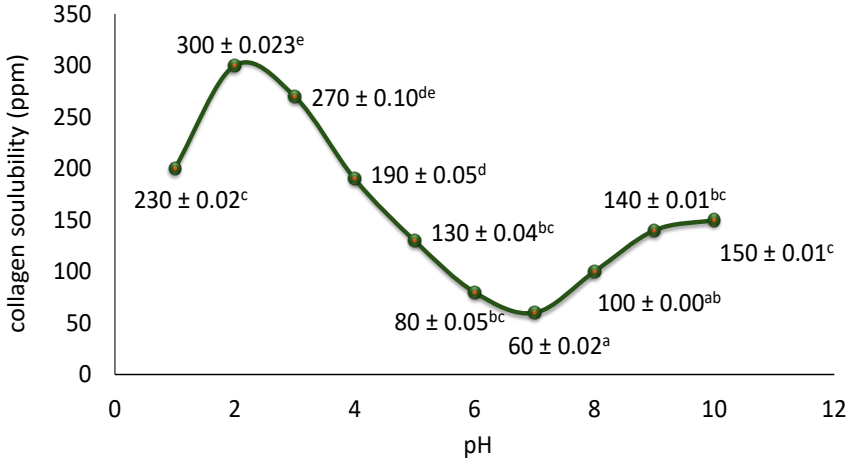

Figure 4. ASC solubility with variation pH

at pH 2 with a value of 300 ppm was significant to the treatment at pH 1, 4, 5, 6, 7, 8, 9, and 10 but not significant with treatment at pH 3 (Figure 4). The higher collagen solubility of ASC in this study was at pH 2 (300 ± 0.03 ppm). Collagen is easily dissolved in acid conditions. Protein can be positively or negatively charged if the pH value is above and below the isoelectric point, which causes protein solubility to increase due to repulsive forces between protein chains (Vojdani, 1996).

Based on research, the higher collagen ASC solubility in acidic conditions at pH 3 is 300 ± 0.03 ppm and decreases at pH 3 to 7. In a different study from Li et al. (2013), the collagen solubility of ASC from the skin of Spanish mackerel reduced at pH 7 and increased at a pH range between 1–4. Woo et al. (2008) reported that higher ASC solubility in the dorsal skin of yellowfin tuna at pH 4 slowly decreases at pH 5 to pH 6, then increases at pH 7 and becomes relatively stable to pH 9. In Kittiphattanabawon et al. (2005), collagen from Bigeye snapper fish (*P. Tayenus*) skin is pH 7–8 and has low solubility and higher collagen solubility in pH 2 and 5. In Song et al. (2021), FASC and CASC from Nile tilapia skin are highly soluble at pH 1–4 and increase at pH 7–11. Increased protein solubility can be caused by an increase in the negative charge of collagen molecules and repulsive forces between chains (Jongjareonrak et al., 2005b). ASC's higher average collagen solubility in acidic conditions decreases in alkaline conditions. The point of isoelectric (p*I*) value is affected by protein solubility. When p*I* is lower or higher than pH in a protein solution, it will cause protein solubility to increase due to an increase in the attraction between protein molecules net positive or negative charge residues. In contrast, p*I* induces aggregation, and precipitation causes the protein's overall charge to be almost zero because it increases as interactions between hydrophobic sites occur (Wong et al., 1989). Collagen has the p*I* range of pH 6–9 (Foegeding et al., 1996).

Differential Scanning Calorimeter (DSC)

Based on changes in physical and chemical properties, thermal analysis can characterize materials and determine their thermodynamic properties (Klančnik et al., 2010). The glass transition (Tg), melting point (Tm), and temperature in the polymer (Td) are three thermal properties that can be measured using DSC of the thermal analysis. The DSC thermograms of ASC from Indonesian local goatskin are shown in Figure 5. A sample of ASC from "Kacang" goatskin was used and rehydrated before DSC analysis using 0.05 M acetic acid. ASC has one endothermic peak (T_{max}) at 99.92°C, which means thermal denaturation of collagen and heat resistance at 99.92°C. ASC's temperature starts at 30.34°C, and the final melting temperature is 153.71°C. Thermal denaturation is related to the first endothermic peak, with maximum peak denaturation temperature (Td) of 30.3°C and 99.9°C. Td is indicated as an amino acid composition from the compact structure of collagen (Zhang et al., 2016). The number of amino acids has intramolecular and intermolecular cross-linking, correlated with the Td of collagen (Veeruraj et al., 2013; Zhang et al., 2016). The second peak is a melting temperature (Tm), which destroys material due to structural damage to the peptide crystal by high temperature (Liang et al., 2014). Kozlowska et al. (2015) reported in ASC that the fish skin of the Northern pike has an exothermal peak; T1 is 79.3°C, and T2 is 198.4°C. The first peak is related to the temperature at collagen denaturation, and water is bonding with protein molecules. The second exothermal peak describes the change in the structure of complex collagen parts into protein, which is destroyed. Based on this literature, ASC in this study at a temperature of 99.92°C and changes in collagen structure were denatured.

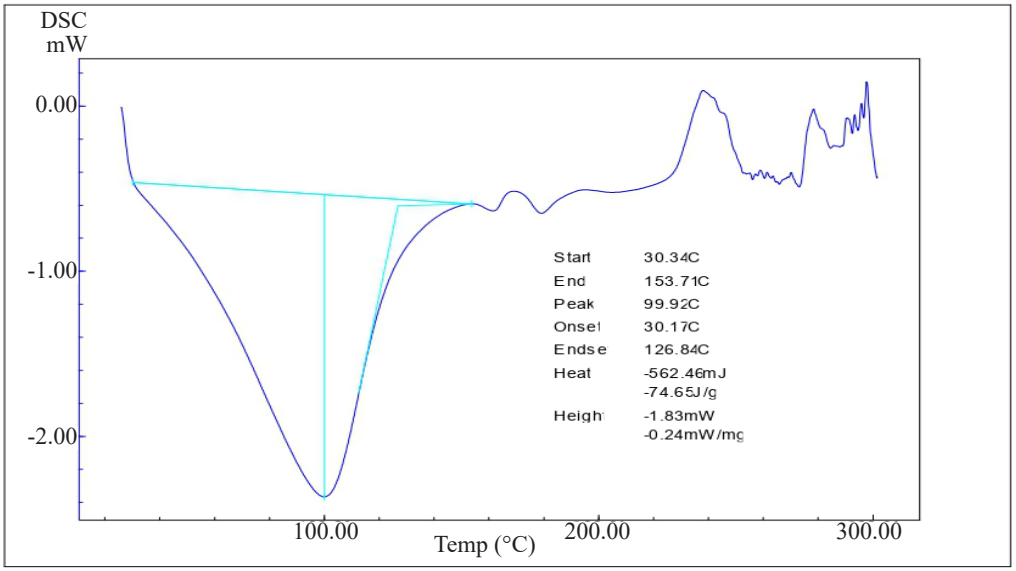

Figure 5. The DSC thermograms of ASC from Indonesian local goatskin

ASC from Indonesian local racing goatskin has thermal stability (Td and Tm) was lower than ASC from turtle carapace (40°C and 125°C) (Li et al., 2020), Chinese soft-shell turtle calipash collagen (between 35°C and 105.14°C) (Zhang et al., 2016), and CASC and FASC from Nile tilapia skin has Td values was 36.8 and 36.5 (Song et al., 2021). Different tissues in collagen feed and the animal's environment suggest the difference in thermal stability. Bae et al. (2008) said collagen with a high amino acid (proline and hydroxyproline) would be more heat-resistant. Besides that, the amino acid composition of collagen varies between species because it depends on the habitat, especially the temperature of the initial habitat. The collagen derived from a habitat with high temperatures will have a higher amino acid compared to the collagen derived from species in a habitat with low temperatures, so it has a higher melting temperature and thermal stability. Td of collagen is related to amino acid composition and non-helical regions with more regular regions and compact collagen structure (Ali et al., 2017; Zhang et al., 2016). In addition, collagen Td also directly correlates with the number of amino acids related to intra-molecular and intermolecular cross-link, and collagen stability is greatly influenced by the number of amino acids (Song et al., 2021; Veeruraj et al., 2013; Zhang et al., 2016). Besides that, collagen molecular weight also influences the thermal stability of collagen (Pal et al., 2015).

Protein Molecular Weight

SDS-PAGE profiles ASC's protein patterns and assesses collagen's type and composition (Figure 6). In this study, the protein pattern in ASC for each step of the collagen extraction process (Step 1), the initial screening after each sample extraction did not look clean, and other bands were still visible. It may be due to the initial step of extraction being contaminated with other materials besides collagen. However, after the precipitation step, until freeze-drying, the bands formed were clear, and no other material was contaminated. ASC has α1-chain, α2-chain, and β-chain. The molecular weight of ASC was between 57.82 and 162.06 kDa, with the α1-chain being 57.8 kDa and 71.83 kDa for α2-chain.

There are cross-linked collagen molecules because of the collagen structure's high molecular weight component. The high amount of cross-linked collagen is indicated by the thickness of structural protein band intensity in β structure (Singh et al., 2011). In general, the structure of ASC from Indonesian Local "Kacang" Goat Skin is indicated as Type 1 collagen. Damodaran (2017) stated that Type 1 collagen has a triple helix structure with a molecular weight of around 100 kDa on the α1 and α2 chains. Giraud-Guille et al. (2000) said the collagen derived from bovine and fish skin consists of 2 α-chains (α1 and α2 chains). Woo et al. (2008) added collagen derived from yellowfin tuna dorsal skin also consists of 2 α-chains (α1 and α2 chain). Saito et al. (2002) reported that Type 1 collagen consists of heterologous α1 and α2 chains forming a triple helix (α1)2α2, where the dimers and trimers of the chain are high molecular weight components.

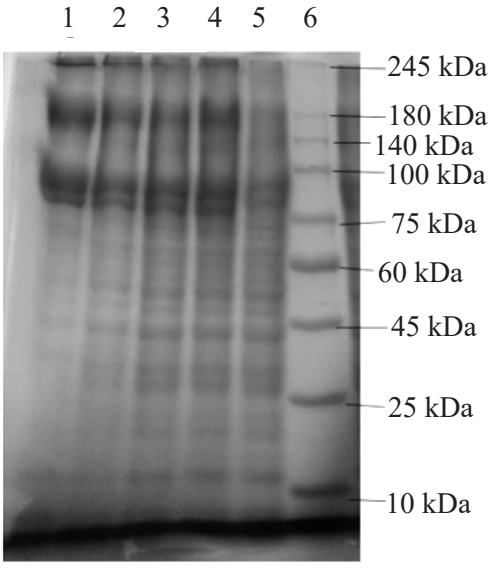

Figure 6. SDS-Page patterns of ASC from Indonesia's local "Kacang" goatskin. (1) step of freeze-drying, (2) step of dialysis, (3) step of centrifugation, (4) step of precipitation, (5) step of filtration, (6) marker of protein.

The ASC from Northern fish pike skin has a molecular weight of 118 kDa for the α1 and 108 kDa for the α2 chain (Kozlowska et al., 2015). In a study by Li et al. (2020), collagen ASC from the carapace tissue of a Chinese soft-shelled turtle (*Pelodiscus sinensis*) has a molecular weight between 100 kDa and 135 kDa and suggests Type 1 collagen. A comparable study from Martínez-Ortiz et al. (2015) used collagen derived from rabbit skin. The collagen has a molecular weight of 118 kDa for α1 and 102 kDa for the α2 chain, so ASC in this study was lower than the molecular weight from fish, turtle, and rabbit skin. The difference in molecular weight can occur due to several factors, such as the type of species, habitat, feed, and age of species. Collagen has two bands, α1 and α2 chains. Type 1 collagen found in bones, dermis, tendons, ligaments, and cornea has a molecular composition [α1(I) 2α 2(I)]. ASC-SK and ASC-SP from spines and skulls of skipjack tuna *(Katsuwonus pelamis)* are Type 1 collagen because it has heterotrimers containing two identical α1 chains and one α2 chain in the form of [α1(I)]2α2(I) molecules (Sato et al., 1989). In general, the Type I collagen consists of two α1 chains and one α2 chain as the main component ([α1]2α2). Hwang et al. (2007) stated that the intensity of the α2 chain band is lower due to the presence of components dimerized into β components and forming β12 dimer. Kimura et al. (1991) reported that the Type I collagen from lathyritic carp bones had two molecular forms: the first molecule [α1]2α2 is the main component. The second molecule α1α2α3 is minor, and Ogawa et al. (2004) reported that the Type I collagen heterotrimer (α1α2α3) was found as the main component of ASC from sheep head bone and black drum. The α3 chain has the same molecular weight as the α1 chain using electrophoresis (Di et al., 2014).

The study of Han et al. (2011) showed that collagen derived from bluefin tuna skin has a molecular weight of α1 chain in 120 kDa, α2 chain in 112 kDa, and β-chain in 205 kDa, whereas Type 1 collagen carp scales have α1 chain in 117.3 kDa and α2 chain in 107.4 kDa. In this study, ASC was known to have a higher molecular weight found in the structures and high cross-linking, as in Type 1 collagen. The research from Singh et al. (2011) showed that the structure in β and γ chains is a cross-linked collagen molecule

forming dimers and trimers. The higher the number of collagen molecules, the thicker the intensity in the protein structure band. Thermal stability also has a relationship with molecular weight. The result of the previous characterization stated that ASC had high thermal stability. According to Duan et al. (2009), high molecular-weight collagen has more stable heat resistance than low molecular-weight collagen. ASC in this study was higher in solubility at acidic pH conditions. When the pH is higher or lower than p*I*, it causes protein molecules to be greater, and the solubility of collagen increases as the repulsive force between the chains increases. Collagen stability decreases, possibly due to increased NaCl concentration, which is affected by protein precipitation and salting-out. The changes in collagen solubility in several types of fish can be caused by changes in structure, molecular weight, and number of amino acids (Li et al., 2020).

CONCLUSION

ASC from Indonesia's Local "Kacang" goat skin has a high moisture content but a low fat level. Additionally, collagen has an optimum process at 0 h pre-treatment of NaOH and extraction time of 48 h at 4°C with yields of 21%. Collagen has higher solubilization in NaCl 5% and at the acid pH ranges; besides, it has a temperature thermal stability with low molecular weight. Therefore, this study suggested that "Kacang" goat skin might be used to manufacture a product that has added value. Therefore, it is necessary to carry out further studies on the functional properties of this collagen.

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