

## **CHARACTERIZATION OF KACANG GOAT SKIN PEP SIN SOLUBLE COLLAGEN (Psc) AND THEIR POTENCY AS AN ANTIOXIDANT**

Rina Wahyuningsih<sup>1)</sup>, Rusman<sup>2)</sup>, Nurliyani<sup>2)</sup>, Abdul Rohman<sup>3), 4)</sup>, Nanung Agus Fitriyanto<sup>2)</sup>, Yuny Erwanto<sup>\*2), 4)</sup>

<sup>1)</sup> Research Division for Natural Product Technology-Indonesian Institute of Sciences (LIPI) Yogyakarta, Indonesia

<sup>2)</sup> Department of Animal Products Technology, Faculty of Animal Science, Univesitas Gadjah Mada, Bulaksumur, Yogyakarta 55281

<sup>3)</sup> Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Univesitas Gadjah Mada, Bulaksumur, Yogyakarta 55281

<sup>4)</sup> Institute for Halal Industry & System, Universitas Gadjah Mada, Jl. Kaliurang Km. 4,5 Yogyakarta 55281  
\*Corresponding Email: yunyer@ugm.ac.id

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### **ABSTRACT**

Collagen have been interesting material for many utilization such as food, pharmaceutical and cosmetic in various products and target administration, consequently collagen should be prepared as well as type of application. The objective of this research is to prepare collagen from goat skin and investigate the character and their potency as an antioxidant. Kacang goat skin aged 2 years was used for collagen production. Small slice skin was extracted by curing with 0.1% (w/v) pepsin in acetic acid 0.5 M, for 24, 48, dan 72 h at 4°C. The variables observed were molecular weight by Sodium dodecyl sulfat polyacrylamide gel electrophoresis (SDS PAGE), microstructure using scanning electron microscope, thermal stability by differential scanning calorimetric, and the antioxidant potency through 2,2-diphenyl-1-picrylhydrazyl (DPPH) inhibition analysis. The result showed the molecular weight range from 25 kDa to 180 kDa, microstructure showed the collagen fibril crosslink, collagen start to denature at 62,28°C, highest dissolved with 1% NaCl concentration and has highest DPPH inhibition at 60 min after hydrolysis. In conclusion, pepsin soluble collagen derived from kacang goat skin has antioxidant capacity and could be used in some applications.

**Keywords:** Antioxidant activity; characteristic; collagen; goat skin.

## INTRODUCTION

Collagen is one of the most important abundant single protein in the body of vertebrate animals because constituting 30% of total proteins was represented collagen (Bhatnagar et al. 1999; Grover, Cameron, and Best 2012). In the body cells, collagen is used for structural integrity, improving strength and resistance in tissues (Woo et al., 2008). Collagen has 28 variants different types (types I-XXVIII). Type I collagen has many functions in the body, such as to biomaterial in wound dressing systems, in human used to implants of tissue engineering, and to drug delivery systems (Lee, Singla, and Lee 2001; Friess, 1998). One of the type I collagen was many found in skins from animal tissues. Study of collagen the most used skin from fish, bovine, and pig skin.

Using collagen from bovine and pig skin has many carries potential risks such as some disease bovine spongiform encephalopathy (BSE) and religious problems (Jongjareonrak et al. 2005; Klompong et al. 2007). Alternative of a raw material to production of collagen must be to find different sources. So far study of collagen from goat skin is very limited. Object this study of collagen is using Indonesian local kacang goat skin which has a high population in Indonesian.

Method of isolation collagen from skin was extraction with using chemical methods and enzymatic methods. Extraction of collagen using chemical methods with acetic acid has low produce yield so to maximise produce of collagen yield was using enzymatic methods. One of the enzyme used to extraction of collagen is pepsin. Pepsin is enzyme can hydrolysis the non collagenous proteins and reduce of the antigenicity (Benjakul, Nalinanon, and

Shahidi, 2012; Regenstein and Zhou, 2007). The objective of this study was to isolation of collagen from new source with using Indonesian local kacang goat skin and extracted with enzymatic process. Enzyme used to extraction collagen is pepsin. Collagen have been interesting material for many utilization such as food, pharmaceutical and cosmetic in various products and target administration, consequently collagen should be prepared as well as type of application. The objective of this research is to prepare collagen from goat skin and investigate the character and their potency as an antioxidant. The variables observed were molecular weight by SDS PAGE, microstructure using scanning electron microscope, thermal stability by differential scanning calorimetric, and the antioxidant potency through DPPH inhibition analysis.

## MATERIALS AND METHODS

### Materials

Indonesia Local kacang goat skin, sodium hydroxide and sodium chloride were purchased from Merck KgaA (Germany), acetic acid was purchased from Sigma-Aldrich (Germany), pepsin from porcine gastric mucosa was purchased from Merck (Germany), DPPH (1,1-diphenyl-2picrylhydrazyl), methanol, sodium hydroxide was purchased from Merck Kga (Germany), and trichloroacetic acid (TCA).

### Methods

#### *Extraction of collagen*

One hundred grams of skin was precisely weighed. Small slice skin was extracted by curing with 0.1% (w/v) pepsin in acetic acid 0.5 M, for 24, 48, dan 72 h at 4°C. The extract was filtered with Whatman No.1 paper. The collagen was precipitated

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\*Corresponding author:

Yuny Erwanto

Email: yunyer@ugm.ac.id

Department of Animal Products Technology,  
Faculty of Animal Science, Univesitas Gadjah  
Mada, Bulaksumur, Yogyakarta 55281

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by adding NaCl at final concentration of 2.6 M. The pellet was collected by centrifuging at 7000 g for 30 min at 4°C and then re-dissolved in 0.5 M acetic acid. The resulting solutions were dialyzed against 0.1 M acetic acid for 24 h with a change of solution once per 3 h, and finally, a change used distilled water sequentially. The collagen was obtained by freeze-drying.

### **SDS-polyacrylamide gel electrophoresis (PAGE)**

Electrophoresis patterns were measured by using method of Laemmli (1970). The lyophilized of collagen was dissolved with 0,5 M Tris HCl buffer pH 6.8 to make a 3 mg/ml. The sample was added loading dye at ratio 1:1 and heated for 2 min. Polyacrylamide gel was prepared with 7.5% gradient gel and 4% stacking gel. The sample solutions were loaded into each gel and electrophoresed for about 2 h at a constant voltage of 110 V. Gel after electrophoresis was stained (50% methanol, 40% H<sub>2</sub>O, 10% acetic acid, 1% brilliant blue) destained with 50% methanol, 40% H<sub>2</sub>O, and 10% acetic acid. Finally gel was solution change with 10% acetic acid.

### **Scanning electron microscopy (SEM)**

The samples were coated with palladium for about 8 min with the sputtering system (JEOL. JEC-3000PC). The image was taken by using a scanning electron microscopy from JEOL (JSM-6510LA) under vacuum at 20 kV with a magnification of 100x and 1000x was applied.

### **Differential scanning calorimetry (DSC)**

DSC studies were done using the DSC-60 Plus (Shimadzu) which was calibrated for temperature and enthalpy using indium as the standard. Sampel PSC was accurately weighed into aluminum

pan, hermetically sealed and scanned from 0 to 300°C. An empty sealed aluminium pan was used as the reference. The maximum transaction temperature ( $T_{max}$ ) was recorded as the peak temperature.

### **Solubility of collagen with different NaCl concentrations**

The solubilities of collagen samples was determined by the method of Montero, Jiménez-Colmenero, and Borderías (1991) with a slight modification. The sample was dissolved in 0.5 M acetic acid to obtain a final concentration of 3 mg/ml and the mixtures was stirred at 4°C for 24 h. Thereafter, all the mixtures was centrifuged at 5000 g for 15 min 4°C and the supernatants was used for solubility study. Supernatants was mixed 5 ml of NaCl in 0.5 M acetic acid at various concentrations to give the final concentrations of 1%, 2%, 3%, 4%, 5%, and 6%. The mixture was stirred continuously at 4°C for 30 min, followed by centrifuging at 10,000 g for 60 min at 4°C. Protein content in the supernatant was measured and the relative solubility was calculated as previously described.

### **DPPH radical scavenging activity**

DPPH radical scavenging activity of PSC hydrolysate was determined using method based (Jeevithan et al., 2015) with slight modification. Sample solution 4.5 mg/ml was mixed with 500 µl of methanol and 125 µl 0.02% (w/v) of DPPH in 99.5% methanol. The mixture was shaken and incubated in the dark place at 60 min. Solution was measured of absorbance at 517 nm using spectrofotometer. The ascorbic acid as a positive control and all determinations were based on the means of three measurements. The calculation of DPPH radical scavenging activity was calculated as follows:

$$\text{Radical scavenging activity} = [(A_{\text{blank}} - A_{\text{sample}}) / A_{\text{blank}} \times 100]$$

Where  $A_{\text{blank}}$  = absorbance of the control,  $A_{\text{sample}}$  = absorbance of the sample

## RESULT AND DISCUSSION

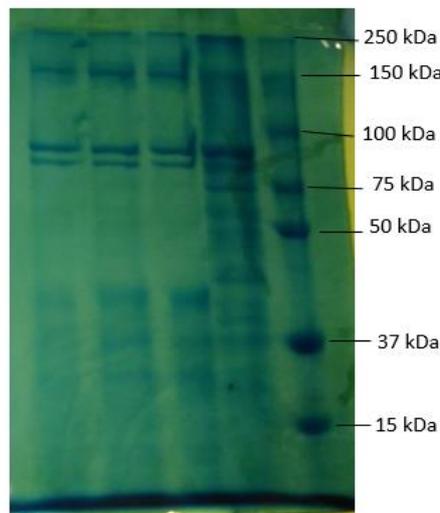
### Electrophoretic pattern of PSC from Indonesian local kacang goat skin by SDS-PAGE

Electrophoretic patterns of step by step processing PSC from Indonesian local kacang goat skin were measured by SDS-PAGE and compared in Fig. 1. The protein pattern each isolation process PSC had two  $\alpha$  chain ( $\alpha 1$  and  $\alpha 2$ ),  $\beta$ , and  $\gamma$ -component. The molecular weight range from 25 kDa to 180 kDa. PSC from Indonesian local kacang goat skin has molecular mass lower than that of PSC from raw material others. PSC from Northern pike (*Esox lucius*) had molecular mass about 118 kDa ( $\alpha 1$ ) and 108 kDa ( $\alpha 2$ ) (Kozłowska et al., 2015).

Beside it, other study of PSC from grass carp (*Ctenopharyngodon idella*) had molecular mass approximately 120 kDa for

$\alpha 1$  and 100 kDa for  $\alpha 2$  (Liu et al., 2015). PSC in this study had small molecular weight because the extraction process in 38°C higher than that extraction process of other raw materials. In low temperature, the collagen extract from goat skin become to a gel which difficult to filter process. PSC had significantly lower proportions of crosslinked component and smaller molecular weight (Liu et al., 2015).

Pepsin was remove of all the crosslinked region at the telopeptide of tropocollagen,  $\beta$ -, and  $\gamma$ -chain without the effecting structural integrity of super triple-helix chain (Kittiphattanabawon et al., 2010). Molecular weight is related to thermal stability. According to (Zhang et al., 2012). Duan et al. 2009 stated that collagen which has a high molecular weight will have a more stable heat resistance compared to collagen which has a low molecular weight.



**Figure 1.** SDS-PAGE pattern of PSC from Indonesian local kacang goat skin on 7.5% gradient gel and 4% stacking gel.

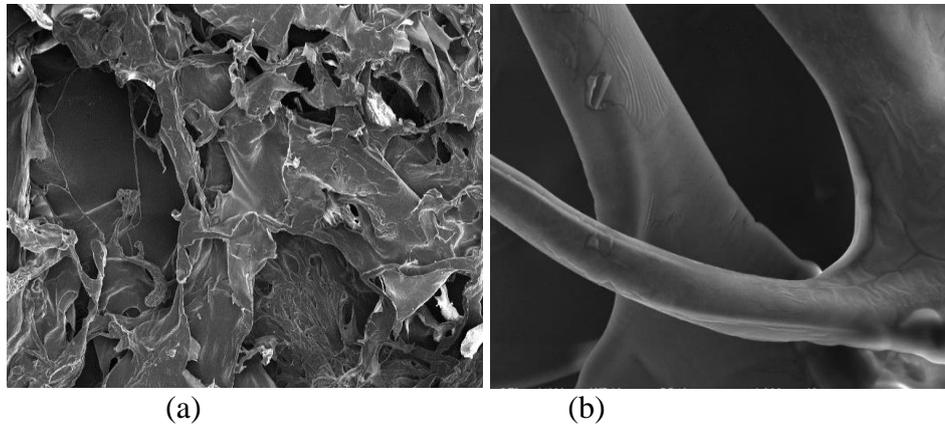
### Scanning electron microscopy

The lyophilized of PSC from Indonesian local kacang goat skin had a microstructure showed the collagen fibril crosslink. Structure of collagen from Indonesian local kacang goat skin can showed in this below Fig 2. According to (Jaswir et al., 2011), the use of acetic acid as a solution in extraction of collagen can be cause the skin to expand due to water penetration into the skin structure, so that in

condition  $H^+$  ions will be increase and water into the skin structure through electrostatic forces between polar groups or hydrogen bonds between polar groups and negative atoms. Swelling of the skin structure is very affected on the full structure of the fiber into the procolagen tropocollagen fiber. The increasing swelling of collagen was caused by increases solubility from hydrogel of collagen. The separation of collagen fibre structure was affected from swelling of the

skin structure and can be interfere the non-covalent bond of collagen. The breakdown of the reticular tissue and elastin tissue was causing collagen swelling at low pH increasing by breaking intermolecular

interactions and hydrogen bonds. The cohesion of protein molecules can be reduced breaking intermolecular interaction between collagen fibrils and acid solutions (Suparno & Prasetyo, 2019).

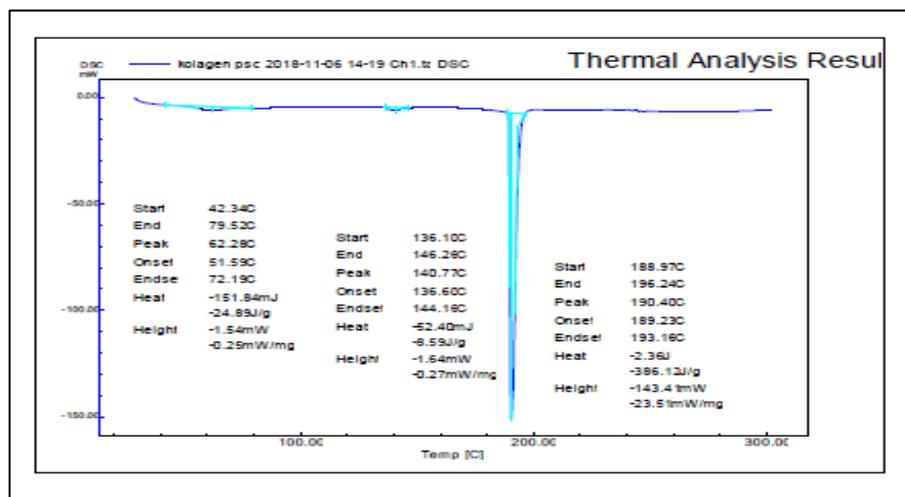


**Figure 2.** SEM of PSC from Indonesian local kacang goat skin. Scale bar = 100 μm x 100; (b) scale bar = 10 μm x 1000

**Differential scanning calorimetry**

Thermal stability of collagen in lyophilized collagen was examined using differential scanning calorimetry (DSC) analysis. DSC is measurement of the thermal denaturation of protein between sample and reference zone (Sionkowska et al., 2015). During the heating process of collagen in a nitrogen, DSC curve is formed. DSC curve of PSC from Indonesian local kacang goat skin is shown in Fig 3. Collagen in this study start to denature at 62.28°C. Thermal analysis functions to determine the thermodynamic properties and character of

the material as a function of temperature based on changes in physical and chemical properties (Petrovič et al., 2011). DSC is one of the thermal analyzes used to measure the difference in heat flow in the sample and the transition phases, namely glass transition (Tg), melting point (Tm), and temperature (Td) in the polymer. The first peak is related to temperature when denatured collagen and water are bound in protein molecules. The second exothermic peak illustrates the change in the structure of the complex part of collagen into a completely reconstructed protein kimia (Klančnik et al., 2010).

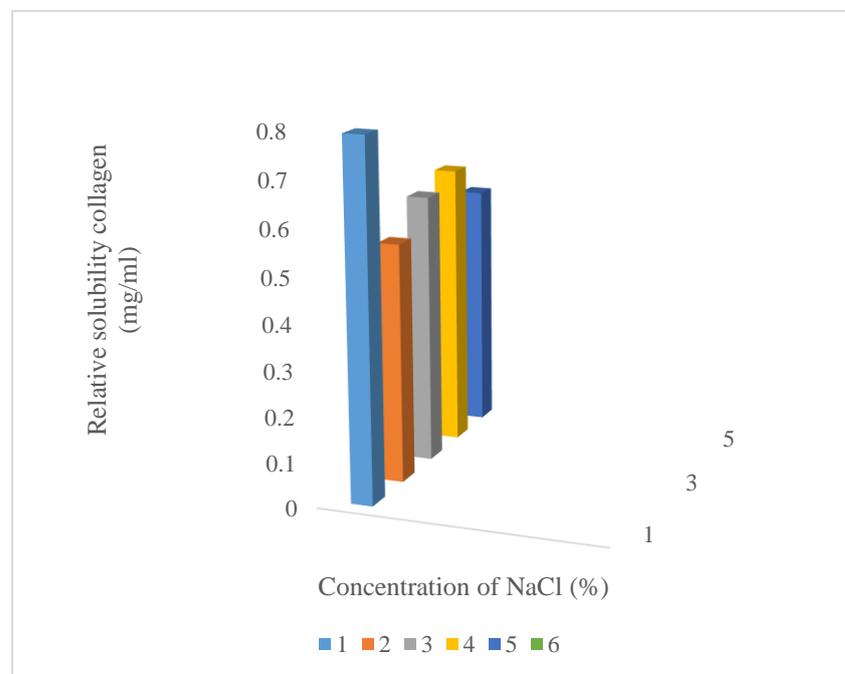


**Figure 3.** Differential scanning calorimetry collagen from Indonesian local kacang goat skin

### Solubility of collagen with different NaCl concentrations

The amount of dissolved collagen is also greatly influenced by the collagen deposition process. Collagen can be deposited using NaCl. The purpose of the addition of salt with high concentrations is the occurrence of salting out, which means that salt can bind water so that the aggregation of proteins occurs and the protein experiences precipitation. This can occur because the ionic strength of salt is higher than protein so water is easily bound. Decreasing the amount of water that is bound to proteins causes protein to easily settle (Winarno, 2008). Solubility of

collagen from Indonesian Local kacang goat skin with different NaCl concentration was shown Fig 4. Collagen in this study has highest dissolved with 1% NaCl concentration which is  $0.79 \pm 0.07$  mg / ml. Collagen solubility tends to decrease with increasing concentration of NaCl given. Increased NaCl concentration causes protein solubility in the solution to decrease, this can occur because of an increase in hydrophobic-hydrophobic interactions in the solution that causes water molecules and salt ions to compete with each other and aggregation occurs so that the protein precipitates (settles) (Bae et al. 2008 and Jongjareonrak et al. 2005).



**Figure 4.** Solubility of collagen from Indonesian Local kacang goat skin in 0.5 M acetic acid with different NaCl concentration

### Radical scavenging activity of collagen

Radical scavenging activity from Indonesian Local kacang goat skin at different time hydrolysis was shown in Fig 5. Statistical analysis to radical scavenging activity with DPPH using SPSS 16.0 factorial complete randomized design model with 3 replications.

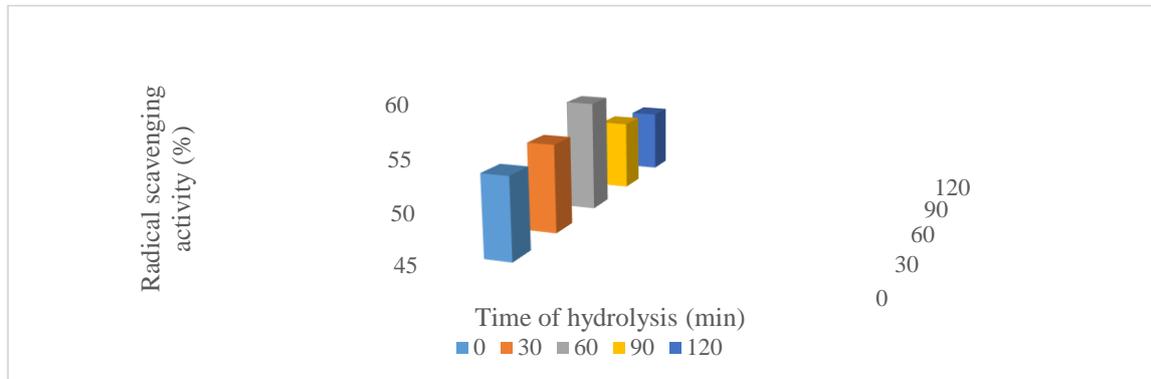
Collagen in this study also has highest DPPH inhibition at 60 min after hydrolysis with a value of 56.42% and IC50 value 4.64.

The antioxidant content in the sample can be measured by its antioxidant activity by looking at its ability to inhibit DPPH radical inhibitory activity which is a stable free radical in methanol and in the oxidized form has a strong absorption at a wavelength of 517 nm.

DPPH free radicals are able to form stable diamagnetic molecules by receiving electrons or hydrogen radicals from other compounds (Gutteridge & Halliwell, 2000).

According to Molyneux (2004), antioxidant properties based on IC<sub>50</sub> values are divided into 4, namely the IC<sub>50</sub> value <50 ppm has very strong antioxidant properties, IC<sub>50</sub> 50 to 100 ppm means it has strong antioxidant properties, IC<sub>50</sub> 100 to 150 ppm means it

has moderate antioxidant properties, and IC<sub>50</sub> 150 to 200 ppm means it has weak antioxidant properties. Based on these references it can be seen that ASC and PSC have very strong antioxidant properties (IC<sub>50</sub> values <50 ppm).



**Figure 5.** Radical scavenging activity from Indonesian Local kacang goat skin at different time hydrolysis

## CONCLUSIONS

This study investigated of characterizing collagen from kacang goat skin and the potency of antioxidant activity. Generally, kacang goat skin collagen prepared by pepsin in acetic acid has proper character in some application, example using as cosmetic and pharmaceutical industry, carriers in the meat processing, edible film and coatings of products. Therefore, there is a possibility of collagen from Kacang goat skin as an alternative source of other collagen for industrial purposes.

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