Biodegradation raw trimming waste from goat’s skin tannery by enzymatic method

A Pratiwi¹, T R Hakim¹, N A Fitriyanto¹, M Z Abidin¹, Y Erwanto¹

¹Department of Animal Science, Universitas Gadjah Mada, Jl. Fauna No.3, Bulaksumur, Yogyakarta, Indonesia

*Corresponding author: yunyer@ugm.ac.id

Abstract. The tannery industry produces large amount of waste from many parts that cannot be used in making leather products. Process of minimizing the waste can be done by trimming the goat skin. The treatment of trimmed goat skin waste from tanneries can potentially produce value-added products. A study on enzymatic treatment of leather processing waste from local goat skin was conducted. This method utilized pepsin which belong to alkaline protease. Pepsin hydrolysed the skin for 48 hours at 4 °C. Hydrolysed raw skin was used to produce collagen. The size measurement of hydrolysed collagen can be analysed and after electrophoresis using SDS-polyacrylamide gel electrophoresis (SDS-PAGE) technique. Hydrolysed of collagen resulted in bioactive peptide identification. Amino acid composition in the hydrolysate obtained by the enzyme hydrolysis was determined. This relative simple biological treatment of leather processing waste may provide a practical solution. The treatment method can be used to produce value-added collagen as alternative source of functional food, medical and pharmaceutical applications.

Keywords: Leather processing waste; Biodegradation; Pepsin; Collagen

1. Introduction

Indonesia is currently the sixth biggest exporter of leather products, footwear, and finished leather goods in the world. Therefore, this sector is selected as a priority sector, made globally competitive and contribute significantly to the national economy. The Ministry of Industry Indonesia reported the export value of leather products, footwear and finished leather goods was valued at USD 4.16 Billion in January-September 2018, an increase of 6.28 % from the same period in 2017. There are 500 tanneries producing about 70,000 tons of raw livestock skins [1]. A lot of useless materials from tanneries industry, only 25 % and 55 % being products. Useless material become pollutant at environment surrounding [2]. Useless material contain protein can be hydrolysed with fermentative and organic chemical [3]. The result of hydrolysed one of them as bioactive peptide. Bioactive peptide produced also has potential use in cosmetics, as food ingredient and pharmaceutical products.

Chemical component on goatskins are water content (60-70 %), proteins (25-30 %), fat (2 %), other materials (1 %) [4]. Protein collagen obtained from solid waste. The amount of collagen in animal protein is 25-30 % [5]. Identified collagen from solid waste/hydrolysed collagen gave benefit for industry [6]. Purpose of research to degrade biological of the collagen to reduce the potency of waste production in leather processing.
2. Materials and Methods

2.1. Materials
Trimmed raw skin of the local Kacang goat was sliced into 1cm² size. The average weight of the sliced skin was 200g. All skins were packed in a polyethylene bag and stored at -20 °C for further use. All chemicals (Sigma Aldrich) used were of analytical grade.

2.2. Collagen analysis.
The goat skin and isolated collagen have chemical compositions were analysed. The chemical compositions, such as fat, peptide, humidity and ash contents were determined according to the methods of AOAC [7]. The amount of hydrolysed collagen can be analysed and quantified after electrophoresis using molecular masses (SDS-PAGE) [8].

2.3. Collagen extraction
Extraction collagen from 200 g (slice 1 cm²) raw skin was made using 0.5 M acetic acid 2 L containing 0.1 % (w/v) pepsin for 48 h at 4°C. Filtrations was made using Whatman paper (No1). Precipitation process was made by adding 2.6 M NaCl, stirred overnight at room temperature and then made centrifugation at 4500 rpm for 20 minutes at 4 °C. The precipitated was dissolved into 0.05 M acetic acid solution before dialysis with 20 mM phosphate buffer. Finally it was freeze dried to collect the collagen. The collagen yields were calculated as follows:

\[
\text{Collagen Yield} \text{ (\%}) = \frac{\text{dried collagen}}{\text{wet weight of raw skin}} \times 100
\]

2.4. Enzymatic treatment
Pepsin (from MERCK) was used for collagen extraction. Pepsin was weighted and diluted on acetic acid 0.5 M.

2.5. Molecular masses analyses by (SDS PAGE)
Molecular masses analyses of peptides was carried out according to the reported procedures with slightly modifications using polyacrylamide concentration 12 % at gradient gel and 4 % stacking gel. The molecular masses analysed was running time at 90 minutes for 110 V. The gel was staining for to 2 hour then distaining for 30 minutes and stored at fixed solution.

2.6. Statistical analysis
SPSS program was used in the analyses and data were expressed as a mean (±SD) for collagen yield. Data was analysed by analysis of variant (ANOVA).

3. Results and Discussion

3.1. Chemical structures of leather processing of raw skin and biodegradation enzyme
Chemical contents of leather processing waste are shown in Table 1. It contained approximately 30.5 % protein. The proteins content in raw skin reduced from 30.5 to 9.0 % after hydrolysis with the enzyme. Type I collagen was used to compare result of degradation collagen.

| Parameter  | Raw Skin | Hydrolysed Skin |
|------------|----------|-----------------|
| Moisture (%) | 69.65    | 88.20           |
| Ash (%)    | 0.36     | 0.04            |
| Protein (%)| 30.55    | 9.00            |
| Fat (%)    | 0.51     | 0.93            |
3.2. Yield of collagen
The yields obtained for enzyme collagen of Kacang goat skin were 4.169±0.06 %. It showed that the raw material can be extracted by pepsin enzyme and compared based on previous research that Bali cow skin had a yield of 11.07±3.50 % [5].

3.3. Molecular Masses analysis
Molecular masses of protein hydrolysed by pepsin enzyme from raw skin was analysed with SDS-PAGE indicated (Figure 1) the result showed the collagen composed of α1, α2 and chain which was classified as Type I collagen (Figure 1).

![Figure 1. Confirmation collagen hydrolysed raw materials by enzyme pepsin.](image)

4. Conclusion
This study indicated that pepsin are capable to degrade leather processing waste from tanneries, meaning that it can provide a solution to overcome the pollution problem at tanneries in Indonesia. In addition to the potential of using pepsin to treat tanning industry waste, the value-added collagen can be produce and utilities and alternative source for functional food, medical and pharmaceutical applications.

References
[1] Industry L 2018 E PORT One of the National Outstanding Sector Indonesia’s Leather Industry : 1–12
[2] Onukak I E, Mohammed-Dabo I A, Ameh A O, Okoduwa S I R and Fasanya O O 2017 Production and characterization of biomass briquettes from tannery solid waste Recycling 2
[3] Noorzai S, Verbeek C J R, Lay M C and Swan J 2019 Collagen Extraction from Various Waste Bovine Hide Sources Waste and Biomass Valorization
[4] Unango F J, Duraisamy R and Ramasamy K M 2019 Preparation and characterization of eco-friendly ash salts for Goat skins preservation Int. J. Innov. Technol. Explor. Eng. 8 184–90
[5] Said M I, Burhan T and Haerati 2018 Synthesis of collagen from Bali cattle’s hide using a combination of acid and alkali on the extracting process J. Indones. Trop. Anim. Agric. 43 247–56

[6] Schmidt M M, Dornelles R C P, Mello R O, Kubota E H, Mazutti M A, Kempka A P and Demiate I M 2016 Collagen extraction process Int. Food Res. J. 23 913–22

[7] Black J L 2004 Animal Feed Encycl. Grain Sci. 1 11–20

[8] ThermoFisher Scientific 2015 Protein gel electrophoresis technical handbook Comprehensive solutions designed to drive your success Thermo Fish. Sci. 63–9