Extraction and characterization of gelatin from skin trimming pickled waste of tannery

D Rahmawati*, N M Setyadewi, Sugiharnton
1Center for Leather, Rubber and Plastics, Jl. Sokonandi No. 9 Yogyakarta 55166, Indonesia
* Corresponding author. Telp.:+62 274 512929, 563939, Fax.: +62 274 563655
e-mail: donna_rahma@kemenperin.go.id

Abstract. Hides and skins, the raw material used in the leather industry are a by-product of the meat industry. The tanning process results in a lot of waste, including solid and liquid waste. Pickled skin trimming is one of the solid waste derived from the tanning process and has a high protein and acid content. Suspected acid on the pickled skin trimming can be used to produce type A gelatine without the addition of acid from the outside. Pickled skin trimming was washed using limited water intended to remove the salt and to maintain the acid content so that the collagen can be partially hydrolyzed in-situ into gelatin. The purpose of this research was to determine the characteristics of pickled skin trimming's gelatin was produced from in-situ acid hydrolysis. The variations of washing were 1:10; 1:15; 1:20; 1:25; 1:30 pickled skin parts: parts of water (w/v). Hydrolysis for 48 hours by adding water as much as 5 parts by weight of the skin. Extraction with water as much as 4 parts by weight of the skin at a temperature of 70-80o C for 3 hours. The highest yield of 49.73% was obtained from the salt separation with pickled skin/water ratio of 1:20(w/v). Proximate composition, pH, functional group profiles, molecular weight distribution, and viscosity of gelatin extracted from pickled skin trimming produced similar characteristics (P>0.05). Gelatin had the characteristic that met GMIA standard for moisture content and viscosity, also had identical functional properties with commercial gelatin. Therefore, gelatin from pickled skin trimming waste can be utilized for pharmaceutical, biomaterial, tissue engineering and cosmetic industries with relatively cheap price.

Keywords: characteristics; gelatin; hydrolysis; in-situ acid; pickled skin trimming

1. Introduction

The leather industry is one of the industries that produce high quantities of wastes including solid waste, liquid waste, gaseous emission and unpleasant odours. Processing of 1000 kg of rawhide only produces 200 kg of tanned leather and the rest is the solid waste [1]. Solid waste is an important part of the total waste. A part of this waste are trimmings, splits, shavings, fleshing, buffing dust [2], [3].

Solid waste in the form of trimming such as pickled skin trimming. The process of pickling is a very important initial process at the leather processing. The pickling process has several functions as
one a way of preserving the skin that is not directly processed. The pickling process changes the skin condition to acid, usually using sulfuric acid and formic acid [4]. Skins or hide trimming wastes contain proteins and are also less contaminated by chemicals compared to the trimming of tanned and finished leathers. Currently, the waste of pickled skin trimming has not been utilized optimally, pickled skin trimming is rich in protein compounds, especially collagen protein. [1] state if this protein is not utilized properly they could pose serious environmental problems threatening the sustainability of leather making. Strict regulations on environmental safety have encouraged industrialists and scientists to re-look at waste decontamination and processing solid waste into valuable products. The products that can be produced from trimming waste such as collagen peptides, glue, industrial gelatin, feed and fertilizers [5].

Gelatin is an important biopolymer obtained from partial denaturation of collagen [6]. It has a wide range of applications in food, pharmaceutical, photographic and cosmetic industries [7]. Gelatin can be extracted from solid waste skin using alkaline or acid pretreatment or a combination of both followed by thermal hydrolysis. Hydrolysis aims to weaken the collagen structure, demineralization, dissolve the non-collagen protein, partially hydrolyze the peptide bond by still maintaining the consistency of collagen fibres, and kill the bacteria [8].

The research on utilization of gelatin from pickled skin trimming waste has been done by [9] and [10]. Both studies used KOH and NaOH as hydrolysis partial of collagen, nevertheless there has a drawback because it requires additional material of the base that will increase the cost of the process. Acid on the pickled skin trimming can be used to produce gelatin without the addition of acid from the outside. Therefore, this aims of this study were to extract and characterise gelatin from pickled skin trimming waste was produced from in-situ acid hydrolysis.

2. Materials and Methods

2.1. Materials

The research materials consist of skin trimming pickled of goatskin, water, materials for gelatin characteristic analysis. Skin trimming pickled tannery waste was obtained from PT Budi Makmur Jayamurni, Yogyakarta. The equipment used includes digital scales (Mettler Toledo AB 204-S), rotating drums, water baths (Memmert W 760 R), ovens (Memmert U 30 780682), pH meters (Mettler Toledo Seven Easy N 315), a set of Kjeldahl test kits, glassware, filters, and plastic trays.

2.2. Methods

2.2.1. Preparation of collagen from pickled skin trimming.

At first, weighed a certain amount of pickled skin trimming waste tannery, then washed with clean water using pickled skin/water ratio of 1:10; 1:15; 1:20; 1:25; and 1:30 (w/v). The skin was cut into the size of 1.5 x 1.5 cm, then dried in the sun. The dried skin was weighed, then placed in a 1000 ml beaker glass. The skin was hydrolyzed by adding water. The amount of water added as much as 5 parts by weight of dry skin. Hydrolysis performed for 48 hours. The remaining hydrolysis water was removed, then the skin was washed in order to remove the remaining acid. The gelatin from the hydrolyzed skin was then extracted using a water solvent at 70-80 ° C for 3 hours. Water used as much as 4 parts by weight of the skin and performed on a water bath. The extracted results were filtered using a filter cloth and the gelatin filtrate was dried in the sun to dry. This treatment produced dry gelatin. Dried gelatin was reduced in size using a blender, then filtered and packed in a plastic bag. Gelatin was ready for testing.
2.2.2. Characterization of pickled skin trimming gelatin

2.2.2.1. Yield determination [11]. Weight the pickled skin trimming waste tannery that has been dry and clean, then extracted according to the procedure. Then the gelatin weight was calculated. The gelatin yield was calculated based on the weight ratio of the dry gelatin obtained from the extraction with the dry weight of the processed skin. The gelatin recovery can be calculated by using the following equation:

\[
\text{Yield (\%)} = \frac{w_1}{w_0} \times 100
\]

where \(w_1\) is the weight of dry gelatin, \(w_0\) is the weight of initial dry pickled skin trimming

2.2.2.2. Proximate Analysis. The moisture, ash, protein, fat and carbohydrate contents of pickled skin trimming gelatin were determined using proximate analysis following AOAC guideline (2005).

2.2.2.3. Determination of Acidity (pH). Weighed gelatin as much as 0.3 grams, then dissolved in 30 ml of distilled water. Then heated to a temperature of \(70^\circ\) C, stirred until dissolved and homogenized using a magnetic stirrer, then cooled at room temperature to measure its acidity by using pH meter.

2.2.2.4. Molecular weight profile. The molecular weight distribution of pickled skin trimming gelatin was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS PAGE) was conducted based on a modified method [12]. The electrophoresis process begins with the preparation of loading buffers, sample buffers, gel dyes (Commasie Brilliant Blue), distillation solutions, 12% gel Separating and 3% Stacking gel. After the solution was ready, the electrophoresis device (AE-6530 page apparatus, ATTO) was strung together. Then ± 7.0 ml of separating gel solution was added APS 10% 58.5 μl and TEMED of 5.85 μl, then stirred until blended and then filled on a plate and coated with 1 ml of butanol, allowed to stand for 15 minutes to solidify. After solidifying the butanol layer was removed and added Stacking gel solution above Separating gel. Stacking gel of ± 2.0 ml added 58.5 μl APS 10% and 5.85 μl TEMED, then stirred until blended and then loaded on a plate and coated with 1 ml of butanol, allowed to stand for 15 minutes to solidify. After solidifying the butanol layer was removed and added Stacking gel solution above Separating gel. Stacking gel of ± 2.0 ml added 58.5 μl APS 10% and 5.85 μl TEMED, then stirred until blended and then loaded on a plate. A comb forming well was inserted between the plate and allowed to stand for 10 minutes after solid, comb was taken. The solid gel was ready to be assembled in electrophoresis and filled with loading buffer. Samples were inserted into columns with a volume of 5-10μl each well. The electrophoresis device was connected to a 120 V electric conductor for 2 - 4 hours. Electrophoresis was stopped until the blue colour touches the bottom of the gel. The gel was removed and coloured (Staining) with Coomassie Brilliant Blue (CBB) dye. Further rocked at 60 rpm for 24 hours. The solution was discarded and washed with a distillation solution for 15 minutes and repeated 3 times, then finally with 10% acetic acid solution for 30. The gel was then stored in 10% acetic acid solution

2.2.2.5. Functional Group Profile. A Thermo Scientific Nicolet iS 10 was used to the analyzed functional group of gelatin. FTIR spectra were 650-4000 cm\(^{-1}\) range and collected in 32 scans with automatic signal gain control at a resolution of 4 per cm against a background spectrum recorded from a clean, empty cell. FTIR was conducted with a mid-IR deuterated TriGlycine Sulfate (DTGS) detector and KBr/Ge mid-infrared beam splitter.

2.2.2.6. Determination of Viscosity. A 6.67% (w/v) gelatin solution was obtained by dissolving dried gelatin in deionized water at 60°C. The viscosity of this gelatin solution was determined with a Brookfield DV-II+Pro viscometer equipped with an ultra-low viscosity adapter (16 ml solution, 20 rpm).

2.2.2.7. Statistical Analysis. This study was designed using a completely randomized design with various amounts of water for salt separation of the pickled skin trimming as treatments with three replicates. Data were analyzed using
analysis of variance (ANOVA) and the differences between treatments were considered statistically significant at P<0.05. Descriptive analysis was used for functional group and molecular weight profile.

3. Results

3.1. Yield.

Yield in gelatin production is often related to the efficiency of the extraction. The indicative of production process efficiency is increased of yield [13]; [14]. The yield of gelatin extracted in situ acids are given in Figure 1. The values were in the range of 40.79±9.26% to 49.73±0.14%. The highest yield of 49.73±0.14% was obtained from the salt separation with pickled skin/water ratio of 1:20(w/v).

This yield was higher than those described by [10] for pickled skin trimming gelatin which hydrolyzed with NaOH 2% (46.47%) and pickled skin trimming gelatin which hydrolyzed with KOH 1-4% (16.50-28.60 %), NaOH 1-4% (23.68-34.42%) [9].

The use of water as much as 20 parts by weight of pickled skin trimming has been able to remove salt as a hydrolysis inhibitor of collagen fibres in the skin. The acid content in the pickled skin trimming was still high, pH at hydrolysis water 2.43 or lower when compared with the use of more washing water.

[15] states that in the absence of salt, water absorption or skin swelling occurs most at pH 2 or 13, thus collagen would be readily hydrolyzed to be converted into gelatin.

In other words that pickled skin/water ratio of 1:20(w/v) is the best treatment because the salt that inhibits the hydrolysis of collagen has been eliminated but the remaining acid to hydrolyze the collagen was still quite high.

![Figure 1](image_url)  
**Figure 1.** Extraction yields (%) of gelatin from pickled skin trimming salt separation using the different amount of water

3.2. Proximate composition

The proximate composition and pH of gelatin from pickled skin trimming salt separation using the different amount of water are given in Table 1.

| Code (pickled skin: water) | pH gelatin | Moisture content (% w/wb) | Ash content (% w/wb) | Protein (% w/wb) | Fat content (% w/wb) | Carbohydrate (%) | Energy Kal/100g |
|-----------------------------|------------|---------------------------|----------------------|------------------|---------------------|-------------------|-----------------|
| A (1:10)                    | 2.97±0.01a | 8.16±0.15a                | 2.50±0.34a           | 80.96±0.18ab     | 0.38±0.17a          | 2.34±0.40cd       | 358.20±1.42b    |
| B (1:15)                    | 2.90±0.01b | 9.13±0.66b                | 3.18±0.51b           | 81.16±0.24a      | 0.55±0.37a          | 0.40±0.10a        | 353.12±3.97a    |
| C (1:20)                    | 2.97±0.01a | 8.57±0.39ab               | 2.68±0.24ab          | 80.74±0.67a      | 0.29±0.18a          | 2.92±0.92d        | 358.67±1.30b    |
| D (1:25)                    | 2.97±0.01a | 8.76±0.22ab               | 3.09±0.25ab          | 81.40±0.16b      | 0.48±0.13a          | 0.87±0.70ab       | 355.27±1.56ab   |
| E (1:30)                    | 3.00±0.01a | 8.45±0.40ab               | 2.61±0.03ab          | 80.45±0.35a      | 0.65±0.04a          | 1.75±0.46bc       | 356.05±0.65ab   |
Note: The same letter notation in the same column shows no significant difference (P> 0.05)

The moisture content of gelatin was in the range of 8.16±0.15 to 9.13±0.66%. The highest moisture content of 9.13±0.66% was found in gelatin by pickled skin /water ratio of 1:1 (w/v) and lowest moisture content 8.16±0.15% in gelatin by pickled skin/water ratio of 1:10 (w/v). These values were higher than the results of [16] examined the gelatin from goat skin with CH₃COOH as a curing material, were in the range of 5.78–6.16%. [10] reported that moisture content of gelatin from pickled skin trimming with NaOH and KOH as hydrolysis agent were in the range of 6.68–8.70%. The moisture content of these results met the SNI 06-3735-1995 standard, that is the maximum gelatin moisture content of 16% [17]

The gelatin extract from pickled skin trimming had the ash content from 2.50±0.34-3.18±0.51%. Highest ash content value on gelatin by pickled skin/water ratio of 1:15 (w/v), while the lowest ash content in gelatin by pickled skin/water ratio of 1:10 (w/v). The maximum gelatin ash content is 3.25% [17] and 0.3-2.0% [18]. Low ash content suggesting the efficient removal of minerals and fat from the skin material. The indicative of good quality gelatin extraction process is low ash content [6]. The high ash content in this result is probably caused by the process of drying gelatin by drying in the open space, allowing dust or mineral components to be blown by the wind into the gelatin

The crude protein content of gelatin from pickled skin trimming ranged from 80.45±0.35 to 81.40±0.16%. Highest levels protein content in gelatin by pickled skin/water ratio of 1:25 (w/v) and lowest protein content in gelatin by pickled skin/water ratio of 1:30 (w/v). The gelatin protein content of these result was higher when compared with the gelatin protein content of reported pickled skin trimming [10] that the gelatin protein content of pickled skin trimming resulted from hydrolysis with 1% NaOH base solution was 65.75%. [19] reported that gelatin from goatskin, the protein content were in the range of 89.37-90.74%. This result was also lower than commercial gelatin, were in the range of 83.36-89.74%. [19]. Differences in gelatin protein levels are due to differences in hydrolysis materials and time used [19].

The increase in acid concentration resulted from many proteins dissolved in the extraction process. The protein content of gelatin increased when a big number of dissolved protein [13]. At the pickled skin trimming/water ratio of 1:10 until 1:30 (w/v)the protein content did not further increase because the acid content is not significantly different.

The fat content of this gelatin equal to 0.29±0.18-0.65±0.04%. Overall the gelatin fat content is quite low and the value is almost the same. Fat animals may consist of various types of triglycerides, phospholipids, cholesterol, and wax. The fat on the skin has been removed in the degreasing process stages so that the fat content in the skin of pickle is very small, the fat that is involved in the process of gelatin extraction from the pickled skin is also very low.

The highest level of gelatin carbohydrate was obtained from the pickled skin trimming/water ratio of 1:20 was 2.92±0.92%, while the lowest was obtained in the pickled skin trimming/water ratio of 1:15 was 0.40±0.10%. Overall carbohydrate levels of gelatin results were not much different, and the value was quite low. Carbohydrates in the skin are slight, so the carbohydrate content in gelatin is also low. Glycosaminoglycans are long-branched polysaccharides of repeatable disaccharide units. The presence of glycosaminoglycans often attaches to the protein core to form proteoglycans. The proteoglycan association through the protein core with collagen fibrils and along with glycosaminoglycans plays the role of collagen fibrils and the key role in regulating the size of collagen fibrils during fibrillogenesis.

The energy content of gelatin was obtained from pickled skin trimming/water ratio of 1:20 (w/v) was 358.67±1.30 Kal / 100g. Then the treatment of 1:10 (w/v) was 358.20±1.42 Kal / 100g, in the 1:30 (w/v) was 356.05±0.65 Kal / 100g, the 1:25 (w/v) was 355.27±1.56 Kal / 100g, and the lowest was obtained in the 1:15 (w/v) that is 353.12±3.97 Kal / 100 g.

Proteins, fats and carbohydrates are energy-producing components, the energy of fat is about 2.25 to 2.50 times the energy of carbohydrates or proteins. In gelatin, protein is the largest component, and
so does its content in this gelatin from pickled skin trimming. On the other hand, the fat content is very low. These conditions make the gelatin energy content of the research also not significantly different.

3.3. pH
The degree of gelatin acidity from five treatments showed that the values were not significantly different (Table 1). The acid content of gelatin comes from the skin to be processed into gelatin, in this case, derived from the acid residing on the pickled skin. Because the degree of acidity of the skin at the time of hydrolysis is relatively the same, then the degree of acidity of gelatin is also not significantly different. However, the highest degree of acidity (pH) of gelatin is found in the treatment E of 3.00. From these circumstances, it can be argued that the increased use of water in pickled skin trimming washing causes more and more acid on the skin of the involved pure particles.

3.4. Molecular weight profile (SDS-PAGE)
The physical properties of gelatin depend not only on the amino acid composition but also on the relative content of α-chains, β-components and higher molecular weight aggregates, as well as on the presence of lower molecular weight protein fragments.

The molecular weight profile of pickled skin trimming gelatin was analyzed by SDS-PAGE (Figure 2). The acid content of each treatment is relatively the same, so the ability to break the collagen molecule is the same, and gelatin is produced with the same molecular weight. From Figure 2 it can be seen that the weight molecule of gelatin is 130kDa.

The α chain molecular weight range is 90 to <200 kDa; β chains are 200 to <270 kD and γ chains are 270–300 kD [20], [21].

![Figure 2](image)

**Figure 2.** The SDS-PAGE pattern of gelatin from the pickled skin trimming wash treatment using the different amount of water

3.5. Functional Groups (FTIR)
Table 2 and Figure 3 show the functional group of gelatin extracted from pickled skin trimming/water ratio of 1:20 (w/v). The functional properties of gelatin extracted from pickled skin trimming were compared with commercial gelatin from the reference and have similar FTIR spectra but absorption peak intensities were different. This means that two types of gelatin have identical
functional properties. The functional group of commercial gelatin are relatively similar to gelatin extracted from pickled skin trimming. If the compound that has a similar functional group tend to have similar chemical reactivity, gelatin from pickled skin trimming could be the substitute for commercial gelatin [22]

FTIR spectra for gelatin extracted from pickled skin trimming formed six characteristics peaks corresponding to alkanes (3000-2850 cm\(^{-1}\), C-H group), alkene (1680-1600 cm\(^{-1}\), C=C group), aromatics (1600-1475 cm\(^{-1}\), C=C group), amide (1700-1640 cm\(^{-1}\), C=O group), carboxylic acid (3400-2400 cm\(^{-1}\), O-H group), esters (1300-1000 cm\(^{-1}\), C-O group).

Absorption at 3348 shows wide peak caused by the N-H strain bond of the amide group associated with the hydrogen bond, and the presence of the OH group. The wide peak is evidence of OH groups of hydroxyproline [8].

| Wave number at the peak absorption (cm\(^{-1}\)) | Estimate functional group | Name          |
|-----------------------------------------------|---------------------------|---------------|
| 3348.18                                       | O - H                     | Carboxylic acid|
| 2927.73                                       | C - H                     | Alkane        |
| 1658.66                                       | C = O                     | Amide         |
| 1616.23                                       | C = C                     | Alkene        |
| 1531.37                                       | C = C                     | Aromatic      |
| 1234.35                                       | C - O                     | Ester         |

Table 2. Analysis of functional groups on pickled skin trimming gelatin using FTIR

Gelatin commercial (Reference) [22]

| Wave number at the peak absorption (cm\(^{-1}\)) | Estimate functional group | Name          |
|-----------------------------------------------|---------------------------|---------------|
| 3274.92                                       | O - H                     | Carboxylic acid|
| 1628.26                                       | C = O                     | Amide         |
| 1524.12                                       | C = C                     | Aromatic      |
| 1230.27                                       | C - O                     | Ester         |

Figure 3. Fourier Transform Infrared (FTIR) of gelatin extracted from pickled skin trimming

3.6. Viscosity

The viscosity of gelatin from pickled skin trimming are given in Table 3 shows that various concentrations of water quantities in the washing treatment have no effect (p> 0.05) to the viscosity of gelatin. The viscosity value (cP) of gelatin ranges from 4.3-4.9 cP.
Various quantities of water for washing treatment has no significant effect on the degree of acidity (pH) of the solution. In situ acid hydrolysis means that hydrolysis is carried out by using an acid derived from the material itself without the addition of acid from the outside. Because the acid concentration for hydrolysis is almost the same causing similar viscosity.

Increasing the hydrolysis material in the gelatin production process can decrease the viscosity value because the curing material has broken the amino acid peptide bond into a very short molecular chain so its viscosity decreases. The curing material may also increase the viscosity value if it is capable of breaking the peptide bond at the proper bond with the longer molecule [23]

### Table 3. Viscosity of gelatin extracted from pickled skin trimming

| Pickled skin trimming/water ratio (w/v) | Viscosity (cP) |
|----------------------------------------|----------------|
| A(1:10)                                | 4.8            |
| B(1:15)                                | 4.3            |
| C(1:20)                                | 4.9            |
| D(1:25)                                | 4.6            |
| E(1:30)                                | 4.5            |

The values of gelatin viscosity from pickled skin trimming have met the GMIA standard [18] which were 1.5 – 7.5 cP.

### 4. Conclusion

Proximate composition, functional group profiles, molecular weight distribution, and viscosity of gelatin extracted from pickled skin trimming showed that salt separation treatment with a different amount of water or hydrolysis with in situ acid produced similar characteristics. The pickled skin trimming/water ratio of 1:20 (w/v) represented the highest yield. The characteristics of the pickled skin trimming gelatin with washing treatment of salt determination using water had the characteristic that met GMIA standard for moisture content and viscosity, also had the identical functional properties with commercial gelatin.

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