GELATIN EXTRACTION THROUGH ACETIC ACID USING TANNERY WASTE AS RAW MATERIAL

EXTRACCIÓN DE GELATINA CON ÁCIDO ACÉTICO UTILIZANDO DESECHOS DE CURTIEMBRE COMO MATERIA PRIMA

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Abstract

The present study proposes an alternative gelatin extraction method from waste materials generated by tanneries, including trimmings, offcuts, and leather scraps. These industrial byproducts yield substantial monthly volumes that are managed by an environmental entity and disposed of in a sanitary landfill. To harness the potential of these waste materials, experimental techniques were employed, involving protein analysis, assessment of response to varying concentrations of acetic acid, and the application of various treatments to individual samples. The objective was to maximize yield and minimize ecological impact. The most effective treatment was found to be T16 (1 mole of acid per liter) for trimmings, T12 (0.8 moles of acid per liter) for offcuts, and T8 (0.7 moles of acid per liter) for leather scraps. Approximately 90% of the obtained protein values correspond to collagen, with a more pronounced presence in the trimmings samples.

Keywords: *gelatin; extraction; tannery; acetic acid*

Resumen

El presente estudio propone una alternativa de extracción de gelatina a partir de desechos de curtidurías, incluyendo colas, retal y carnaza. Estos subproductos industriales generan volúmenes significativos mensuales que son gestionados por una entidad ambiental y dispuestos en un relleno sanitario. Con el propósito de aprovechar estos residuos, se emplearon técnicas experimentales como análisis proteico, evaluación de respuesta ante concentraciones de ácido acético y aplicación de diversos tratamientos a las muestras individuales. El objetivo era maximizar el rendimiento y minimizar el impacto ecológico. El tratamiento más eficaz resultó ser el T16 (1 mol de ácido por litro) para colas, el T12 (0,8 mol de ácido por litro) para retal y el T8 (0,7 mol de ácido por litro) para carnaza. Alrededor del 90% de los valores proteicos obtenidos corresponden al colágeno, con mayor presencia en las muestras de colas.

Palabras clave: gelatina; extracción; curtiembre; ácido acético

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Introduction

Gelatin is extracted from the connective tissue of animal skins, including bovine, porcine, fish, and bones. It is a derivative of collagen obtained through controlled partial thermal hydrolysis under specific conditions of temperature, solvent, and pH (Ahmad & Benjakul, 2011). Gelatin, derived from collagen with limited denaturation, is a high molecular weight biopolymer that exhibits various functional properties, such as film-forming capacity, water retention capacity, foam formation, and emulsifying properties. These characteristics make it an important component in the food industry, medicine, pharmacy, photography, and cosmetics (Ahmad et al., 2018).

Proteins are chains of amino acids (polymers) that play a fundamental role in living organisms (Prockop & Guzmán, 1981). There are globular and fibrous proteins, with collagen being an example of the latter. Collagen is a fiber abundant in most living organisms and is known for its high strength, capable of withstanding loads between 10 and 40 kg. It is composed of naturally occurring amino acids linked together in a specific sequence (Eluk, 2006). Collagen is constituted by approximately 20 amino acids, varying depending on the type of animal and its youth, with non-polar, polar, acidic, and basic side chains. Acid treatment removes the non-collagenous material from the raw material, causing the fibrous collagen particles to swell. This weakens the protein chain unions through topochemical hydrolysis, leading to the final rupture of covalent bonds present in collagen monomers while preserving a high molecular weight (Serna, Pineda, & Ayala, 2007).

Collagen, being a structural protein, contributes to the formation of bones and tendons (López, 2014). There are different types of collagens depending on their location in the body, including type I found in bones, type

II found in cartilage, and type III found in the skin (Bernales et al., 2004). Bovine tails contain type I collagen, while type II collagen is present in cartilaginous joints. These values would therefore be added to the collagen fibers found in the corium and subcutaneous tissue (León, 2009). Trimings refers to leftover cuts from tanned skin, mostly composed of exocrine glands. Fleshings corresponds to the inner side of the skin that was in contact with muscle and animal fat (Micheau, Hoa, & Borofka, 2020).

One of the initial parameters to consider is the moisture content, which serves as an indicator of product stability. Free water is the predominant form, which can be easily removed through various drying methods, while bound water is specifically combined with protein structures, which also possess water retention capacity. The water retention capacity is influenced by factors such as pH, ionic strength, salt types, and temperature (Fennema, 2000). Other considerations in this study include protein determination, gelatin extraction, and the statistical design of treatments to evaluate pH at different concentrations of acetic acid. Therefore, the objective of this study was to evaluate the extraction of gelatin using waste materials from tanneries (trimmings, tails, and fleshing) and identify the optimal extraction method using acid.

Materials and methods

An experimental laboratory analysis was conducted using raw materials obtained from a tannery in the Tungurahua province, Ambato, Ecuador. The waste generated by the company, including bovine fleshing, tails, and trimmings was utilized. Following the washing of the raw hides (based on the company's established formulation), the waste materials were used for the characterization of the raw material through proximate analysis.

Moisture Determination

The moisture content was determined according to the NTE INEN 565 standard (1983). Three grams of ground sample were weighed in a tared empty capsule and placed in an oven, dried at a temperature of 105 °C ± 1 °C for 24 hours until a constant weight was achieved. Finally, the sample was cooled in a desiccator and weighed. The moisture content was calculated using Equation 1 and Equation 2:

$$\%Dried\ matter = \frac{m_3 - m_2}{m_2 - m_1} \quad \text{(Eq 1)}$$

Where,
m_1: Weight of the crucible
m_2: Weight of the sample + crucible
m_3: Weight of the dried sample + crucible

$$\%Moisture = 100 - \%Dried\ Matter \quad \text{(Eq 2)}$$

Protein Determination

The protein content was determined following the AOAC (2016) standard for meat and meat products. Three grams of the samples were weighed and two Kjeldahl tablets and 20 mL of sulfuric acid (H2SO4) were added. The prepared sample was boiled for one hour until a color change occurred from opaque red to green.

Subsequently, the sample was cooled, and 70 mL of distilled water were added. After further cooling, 50 mL of 40% (w/v) sodium hydroxide (NaOH) solution were added. This solution was introduced into a Vapodest Gerhardt protein distillation apparatus (TT625, Spain) together with 30 mL of 1N boric acid (H3BO3) contained in a flask for filtration. The filtered solution was titrated with 0.1 mol/L hydrochloric acid (HCl) until a light pink color was reached. Finally, the raw protein content was calculated utilizing Equations 3 and 4.

$$\%Kjeldahl\ nitrogen = \frac{(Vs*VB)*M*14.01}{W*10} \quad \text{(Eq 3)}$$

$$\%Raw\ Protein = \%Kjeldahl * F \quad \text{(Eq 4)}$$

Where,
Vs = Volume (ml) of standardized acid employed for sample titration.
VB = Volume (mL) of standardized acid utilized for reagent blank titration.
M = Molarity of standard HCl.
14.01 = Atomic weight of nitrogen (N).
W = Weight (g) of the test standard.
10 = Conversion factor for mg/g to percentage.

Gelatin extraction

The skins received at all tanneries must comply with the standard NTE INEN 1809 (1991), which establishes the requirements for fresh or salted bovine skins from normal slaughter, emergency slaughter, or salvage of accidentally dead cattle, among other specifications. Subsequently, the raw skins are washed with water and salted to reach a moisture content of 60 - 65%. Then, the fleshing or depilation process is carried out to remove the hair, following the guidelines stated in NTE INEN 1870 (1994), which require the use of formulations containing calcium hydroxide, sodium sulfide, sodium hydrosulfide, amines, and sodium hydroxide to loosen the fibrous structure of the skin (Ortiz and Lumbí, 2016).

After the fleshing process, the skins go through a fleshing machine to remove any remaining flesh and fat. Additionally, manual cutting is performed to remove parts such as the neck, tail, and extremities. At this point, the trimmings, scraps, and tails are separated and collected in waste containers as raw materials. The samples are washed with distilled water for 30 minutes to remove any physical residues (dirt and hair). Due to the chemical products used during the fleshing stage, the skins tend to have a basic pH, so they are neutralized with running water until a pH of 6 - 7 is achieved. They are then cut into small pieces measuring approximately 1 to 2 cm² (Ortiz and Lumbí, 2016).

The type A (acid) method (Table 1) was used for gelatin extraction. In this method, the designated skin pieces from each sample are immersed in a concentration of acetic acid (0.5-1 mol/L) for a period of 24 hours (Table 2). This process removes the non-collagenous material from the sample and causes the fibrous collagen particles to swell, resulting in the final breakdown of covalent bonds present in the collagen monomers while maintaining the protein's high molecular weight. The samples are then neutralized a second time with distilled

water until a pH in the range of 5 - 6 is reached (Serna, Pineda, and Ayala, 2007). The samples are dried in an oven, following a three-stage process with different temperatures (70, 75, and 80°C) for 5 hours each (Wulandari et al., 2016).

Table 1. Samples and acid concentrations.

Items	Acetic acid concentrations and samples
a: concentration of acetic acid	a0 = 0.5 M
	a1 = 0,6 M
	a2 = 0,7 M
	a3= 0,8 M
	a4 = 0,9 M
	a5 = 1M
b: sample	b0 = tales
	b1 = fleshing
	b2 = trimmings

Table 2. Treatments and combinations (samples and concentration of acetic acid)

Combination	Treatment
a0b0	T1
a0b1	T2
a0b2	T3
a1b0	T4
a1b1	T5
a1b2	T6
a2b0	T7
a2b1	T8
a2b2	T9
a3b0	T10
a3b1	T11
a3b2	T12
a4b0	T13
a4b1	T14
a4b2	T15
a5b0	T16
a5b1	T17
a5b2	T18

Finally, an ANOVA analysis was carried out to validate the obtained data.

Results and discussion

Physicochemical Analysis

The collected samples after fleshing were analyzed for moisture content and protein percentage. The moisture content was determined to be 56.8% for all three samples, which falls within the range of 50 to 65% (Hidalgo, 2013).

Table 3 also shows the protein content for each sample, which is in line with Morera's findings (2007) stating that bovine skin contains up to 33% protein. The sample of tails exhibits the highest protein content, followed by scraps and trimmings (influenced by the chemical structure of each waste component). It is evident that the trimmings have the lowest protein content at 9.42% since they mainly consist of the endodermis, which has thin collagen fibers, compared to scraps and tails that contain both dermis and endodermis layers, resulting in a higher concentration of collagen proteins. Scraps are composed of exocrine glands, which contribute to a more complex chemical structure compared to trimmings. Tails have a protein content of 30.4% due to the presence of bones and tendons, which are primarily composed of this structural protein (López, 2014).

Table 3. Protein and Moisture content.

Sample	Moisture (%)	Proteín (%)
Fleshing	56.8	9.42
Tails	56.8	30.40
Trimmings	56.8	22.60

Gelatin extraction

Table 4 displays the mass and pH values after 24-hour extraction with acetic acid and subsequent neutralization with distilled water to achieve a pH of 5-6. In the case of scraps, due to their simple composition, low concentrations of acid easily penetrate the intermolecular spaces. However, for trimmings with a more complex composition, intermediate acid concentrations are required.

Table 4. Mass of the gelatin obtained and pH for each acid treatment.

Treatment CH_3COOH (mol/l)	Tails		Fleshing		Trimmings	
	Peso (g)	pH	Peso (g)	pH	Peso (g)	pH
-	100.789	7.90	100.977	7.80	100.295	7.40
0.5	105.122	7.65	108.282	6.85	106.382	7.00
0.6	109.134	7.35	112.456	6.70	107.322	6.75
0.7	108.123	7.45	120.343	5.40	110.522	6.50
0.8	109.604	7.15	**122.429**	5.10	**111.529**	6.10
0.9	110.311	6.30	115.633	3.55	110.464	4.55
1.0	**115.249**	6.00	105.532	3.70	109.524	4.45

High concentrations of acetic acid are needed to penetrate tails due to the presence of thicker tissues and coccygeal vertebrae, which contain collagen-rich material. At low acid concentrations, collagen does not swell. For all samples, reaching a pH level of 5-6 results in maximum weight due to acid-induced swelling of the collagen protein and increased absorption of acetic acid.

The waste materials used in this study contain collagen, which was subsequently hydrolyzed to produce gelatin (Hastutiningrum, 2009). The use of acetic acid causes collagen to break down into smaller peptides. The product obtained through acidic extraction is superior to basic extraction because acids can easily penetrate the triple helix structure of collagen fibers. Additionally, the extraction time is shorter, and the cost is lower (Hasdar, 2011).

Working with high concentrations of acid is not recommended. Although it may increase the amount of gelatin obtained by enhancing collagen hydrolysis, it does not guarantee a high-quality product. The described process can adversely affect the chemical bonds within the collagen molecules, resulting in reduced quality of the resulting gelatin (Wang et al., 2008). For the scraps and trimmings samples, it is not advisable to use high concentrations of acetic acid because a pH range of 0.5 to 0.7 mol/L can be achieved, eliminating the need for excessive reagent consumption. Finally, as it is observed in Table 2, the best acid treatments for each raw material resulted at 1.0 mol/L of acetic acid for Tails and, 0.8 mol/L of acetic acid for Fleshing and Trimmings.

Finally, a statistic analysis was carried out obtaining Table 5. It is concluded that the concentration factor is statistically significant, indicating that the variability among the means of the concentrations is greater than the variability within these observations. It is observed that the calculated F-value is higher than the critical F-value. Therefore, we infer that the mean weight varies at different concentrations. Additionally, the obtained p-value is 0.0026, suggesting that with a 95% confidence level, we can assume the significance of the concentration factor.

Table 5. Analysis of Variance for the Concentration Factor.

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	392.598347	6	65.4330579	3.46407661	2.60E-02	2.847726
Within Groups	264.446464	14	18.8890331			
Total	657.044812	20				

In Figure 1, the behavior of the means for the samples (1 Tails, 2 Fleshing, and 3 Trimmings) is observed. Likewise, the behavior of the means for the different concentrations being tested is shown. It can be observed that the mean weight of Carnaza at a concentration of 0.8 exhibits the most favorable average.

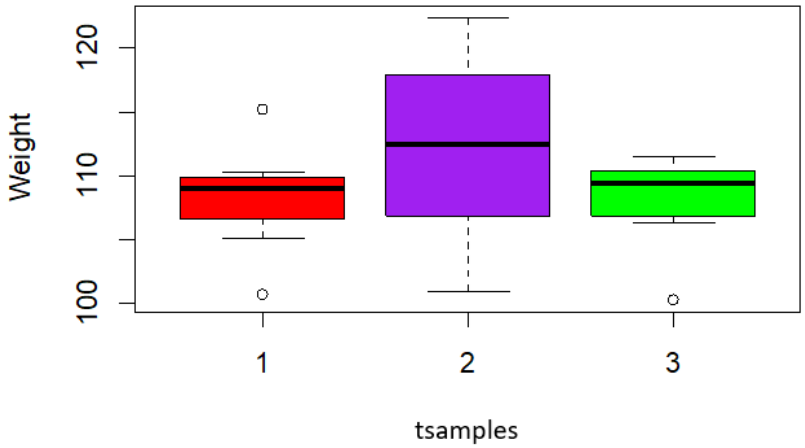

Figure 1. Weight against sample types.

In Figure 2, the progression of the average weight concerning concentration is depicted, with the observation values conforming to the assumptions of the model. The trend of the average weight demonstrates its optimal value at concentrations ranging between 0.7 and 0.8. Additionally, the factor of rejection type also exhibits notable values, as illustrated in Figure 2(a). Outliers can be observed in the 'Tail' and 'Retail' categories, while in the 'Carnaza' category, although broader variability is evident, outliers are not observed.

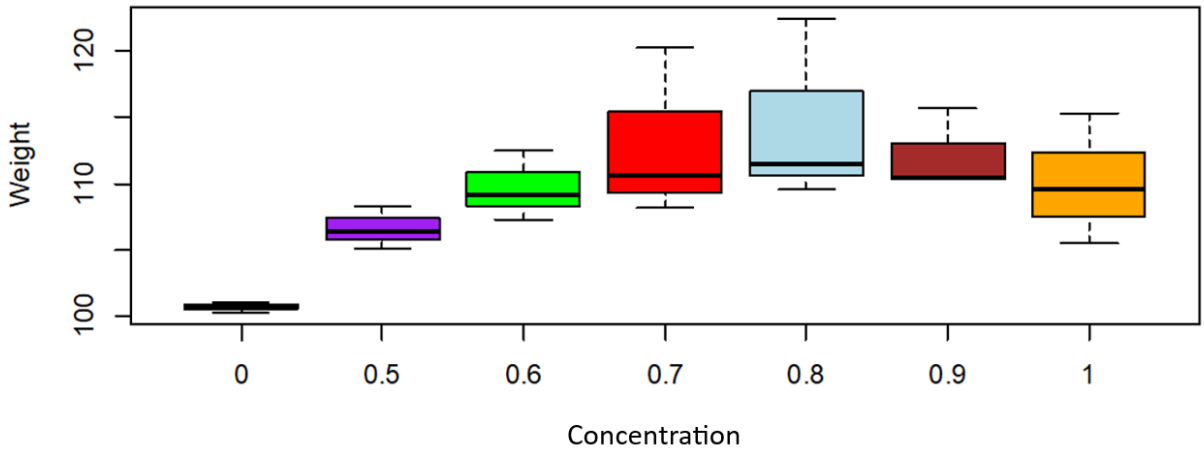

Figure 2. Weight against acetic acid concentration.

Figure 3 displays the dispersion of observations grouped by rejection type and labeled according to test concentration. It is evident that the observations obtained across the analyzed rejection types are not widely scattered between Tails and Trimmings. Conversely, in the Fleshig category, the trend of better average weight outcomes at concentrations of 0.8 and 0.7 is distinctly apparent.

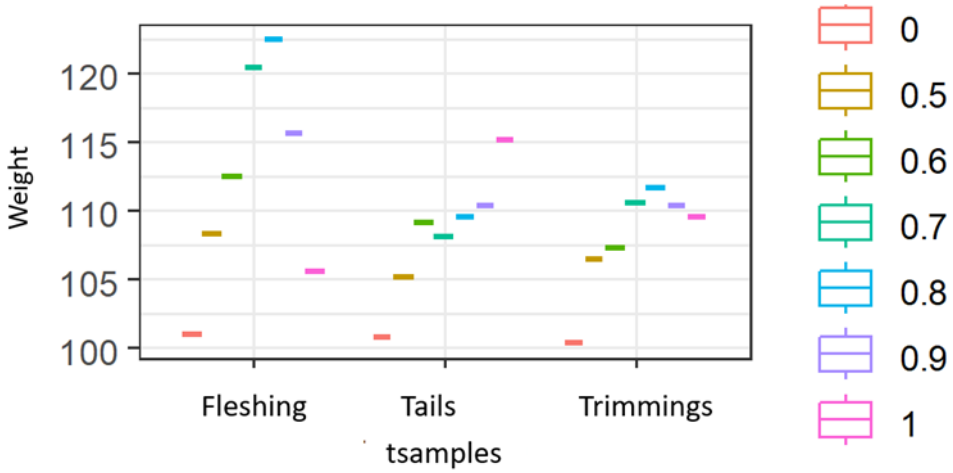

Figure 3. Weight against sample types and acetic acid concentration.

Conclusions

Based on the results of this study, its discussion, comparison of obtained values with other authors, and detailed analysis, the following three main conclusions can be drawn. Firstly, a moisture content of 56.8% was obtained for all three waste types. Protein extraction yielded 30.4% from tail samples, 22.6% from trimmings samples, and 9.42% from fleshing samples. Sencondly, 90% of the obtained protein values represent collagen, with the highest incidence in tail samples due to the significant presence of structural protein, tissues, and bones within this waste type. The second-highest value was extracted from trimmings samples, which contain thicker collagen fibers. Finally, the optimal extraction methods using acetic acid according to rejection type and reagent concentration were: T16 (1 mol/L acid concentration) for tail samples, T12 (0.8 mol/L acid concentration) for trimmings samples, and T8 (0.7 mol/L acid concentration) when working with fleshing samples. Higher acetic acid concentration is necessary for tail samples due to the presence of distinct collagen types in the sample. The methodology employed proves effective when dealing with bovine waste using acetic acid.

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