Collagen extraction from bovine hoof by the acid-base method

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Summary

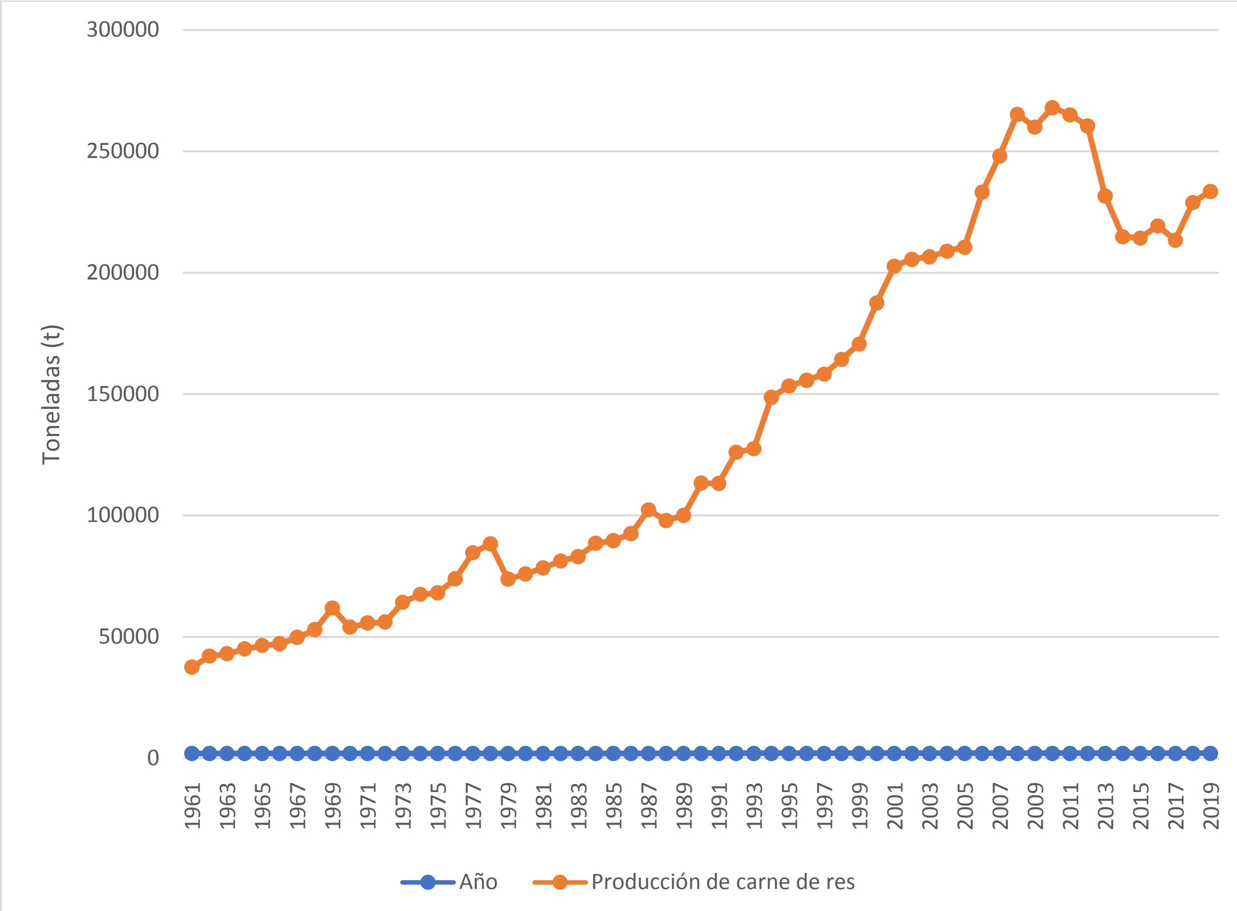
This article aims to evaluate the extraction of collagen from the hooves of cattle slaughter produced in the municipal bed of Riobamba-Ecuador. It is hypothesized that these residues may be an alternative and sustainable source of collagen for various industrial and biomedical applications. An optimized acid hydrolysis process is used to extract high-quality, high-yield collagen from the hooves of slaughtered cattle. The physicochemical and functional characterization of the extracted collagen was performed. The results confirm that it is possible to take full advantage of animal waste to obtain collagen. In addition, the positive environmental, economic and social implications of this study for the country are discussed. Further research with other types of animals and other extraction techniques is suggested to compare the collagen obtained. Likewise, it is recommended to evaluate the physicochemical and biological properties of the extracted collagen to determine its possible applications in different sectors.

Keywords: collagen, hooves , cattle, sustainable, extraction

Introduction

According to FAO, in 2019, Latin America produced 31.8 million tons of beef, representing 25% of world production. For Ecuador, according to this same organization, it was estimated at 233425 t of beef production for 2020(1) (Figure 1). If it is assumed that each slaughtered animal produces 40% of its live weight in waste. Cattle (male or female) are ruminant mammals, robust and their mass can reach more than 700 kg. Beef accounts for 25% of meat consumption worldwide (FAO, 2009). They are bred for meat, milk and skin consumption, which is usually transformed into leather. The slaughtering process must be carried out following technical and sanitary standards, but there is no official Good Practice Guide (GM) in the country. Cattle slaughter in Ecuador faces some challenges, such as climate, disease, cattle rustling and clandestine slaughter(2).

Figure 1. Beef production in Ecuador from 1961-2020.



Source: 1K6F, Crystal Structure of the Collagen Triple Helix Model [(Pro-Pro-Gly)10]3. PDB DOI: 10.2210/pdb1K6F/pdb.

Waste from slaughterhouses in Ecuador is an environmental and health problem that requires comprehensive and sustainable management. These residues include rumen contents, sewage, viscera, hides, blood, fats and other inedible organic remains of slaughtered animals that are discarded for municipal solid waste collection. This implies a low efficiency in the use of the waste generated.

Collagen is one of the most abundant structural proteins in animals. It is present is the component of the extracellular matrix of various connective tissues such as skin, bones, cartilage and tendons. Collagen molecules consist of 3 long helical chains of amino acid residues with non-helical terminals on the 2 sides. Collagen chains commonly consist of Gly-X-Y, where Gly is the amino acid glycine while X and Y are generally amino acids like proline and 4-hydroxyproline respectively.

Graph 2. Three-dimensional structure of the triple helix of collagen.

Fuente: 1K6F, Crystal Structure of the Collagen Triple Helix Model [(Pro-Pro-Gly)10]3. PDB DOI: 10.2210/pdb1K6F/pdb.

One of the alternatives for the use of bovine hull waste is the extraction of collagen and keratin, which are proteins with useful properties for the pharmaceutical, cosmetic, food and textile industries. In this way, a product of high commercial value could be obtained from a waste that is normally discarded or incinerated. However, bovine hooves contain both collagen (in the chorion layer) and keratin (in the horn layer) that can be extracted using mild enzymatic, chemical or physical methods. Collagen and keratin are two important structural proteins that have many applications in the biomedical, cosmetic, food and textile industries. In addition, the market value of collagen is much higher than that of leather, ranging from $37 per gram to $1000 per gram for lab-grade native collagen.

Extracted collagen and keratin can have many uses in industry(3). Collagen is commonly used as a food supplement and in cosmetics for its skin-enhancing properties as a moisturizer and for its anti-aging properties and improve skin health and appearance.(4). It is also used in the production of medical devices, such as artificial skin and bone replacements. Keratin is commonly used in the cosmetic industry as a hair and nail strengthener. It is also used in the production of industrial adhesives, as it has strong binding properties. It is also used in the food industry as a thickening and gelling agent in a variety of products.(5).

In the city of Riobamba, cattle slaughter is carried out in the municipal Camal and in slaughterhouses located in different areas of the city. These slaughterhouses are regulated by the Ministry of Agriculture and Livestock and comply with hygiene and health regulations to guarantee the quality of the meat, however others are not.(6).

The slaughter process consists of the capture and slaughter of the animal, followed by the butchering and classification of the meat into different cuts. The meat undergoes quality inspections and disease testing before being marketed in city markets and elsewhere in the country.

In this research, an acid-base method is used for the extraction of collagen from bovine hooves. Collagen extracted through a solution of citric acid and sodium hydroxide is an efficient method. The extracted proteins have many uses in various industries, including cosmetics, food supplements, medical devices, and industrial adhesives. Keratin, on the other hand, is used in various hair and skin care products for its strengthening and moisturizing properties. It is also used in the food industry as a source of protein and in the production of feed additives for livestock.

Materials and methods.

Collection, cleaning and demineralization of samples.

The samples were collected at the Municipal Chamber of Riobamba. The helmets were washed with municipal drinking water to remove dirt and other impurities that attach to the helmets. The addition of a solution of 30 ppm of NaClO, to eliminate the microorganisms present on the inner and outer surface for a time of 30 hours; this treatment allowed the dissolution of NaClO to penetrate by osmosis to the surface cells and eliminate the microorganisms. The elimination of NaClO residues was carried out through whitening with plenty of water.

Acid pretreatment.

A pretreatment was performed in an acid medium with the purpose of breaking the covalent intermolecular crosslinks between the collagen molecules(7), for which it was done by adding 5% citric acid covering all the raw material and leaving at rest for 30 hours, observing a swelling of these and softening that allows cutting the material into strips of approximately 2 cm wide. Then water was added and brought to cooking, the liquid was decanted, an aliquot was used to determine the ash content; obtained from a dark ash.

Acid-base treatment.

For acid treatment, the raw material treated in the previous step was placed in a container and immersed in 5% citric acid. After this treatment the hooves were cut into strips of about 2 cm. After 24 hours, continuous washes were carried out to remove the acid from the raw material.

For basic treatment, the strip sample was treated with 5% NaOH for 36 hours; the fluid was removed and swelling and flexibility were observed in the samples; Successive washes removed all hydroxide residues. The samples in the gelatinous state were liquefied with water and taken to cooking for 2 hours, the temperature was lowered and filtered hot.

The product obtained from this process was collagen soluble in hot water. The filtering of this product was placed in trays that were left in an oven with air circulation until the liquid is removed, and from this process dry sheets were obtained, which were ground to obtain powder and quality control was carried out.

Product quality control.

Physicochemical tests:

- Humidity: by the traditional method according to AOAC 925.09-1925, Solids (total) and loss on drying (moisture)(8).
- Ash: by the gravimetric method according to AOAC 920.153-1920, Ash of meat(9).
- Fat: by the ethereal extraction method according to AOAC 920.39-1920, Fat (crude) or ether extract in animal feed(10).
- Protein: by the Kjeldahl method according to AOAC 928.08-1974, Nitrogen in meat. Kjeldahl method(11).

Results and discussion

Performance Determination

Two batches were processed from bovine hooves, using the treatment of medium acid-base, but varying the contact times with which it is obtained from 1814.37 grams of hooves was obtained 10.28 grams of the collagen product. The second batch of which is 1360.78 grams of hulls increased the treatment time with each of the reagents of demineralized, pretreatment and treatment, obtaining 26 grams. The yield was less than 1.00%.

$$\%\,\text{Yield} = \frac{g\ \text{Product}}{g\ \text{Raw material}} * 100 \qquad \text{Equation 1}$$

$$\%\,\text{Yield} = \frac{10\ g}{1814\ g} * 100 = 0.56\%$$

The yield of 0.56% can be considered very low with respect to the amount of matter processed and the product obtained however the process can be substantially improved by changing the pretreatment conditions with NaOH.

The pretreatment with NaOH and the pre-treatment time depends on the thickness of the treated material, the alkalis are particularly effective in the extraction of collagen from hard and thick materials, therefore the performance obtained could have been improved using a pretreatment with in basic medium(12). However, this alkali pretreatment can take anywhere from a few days to weeks to complete.

In this research work a simple method of collagen extraction using an acid-base method was used, however, this process can be improved using ultrasound or microwaves in conjunction with this chemical process. And even a combined process of enzymatic extraction using the physical processes mentioned above, can give better results. Additionally, it is worth mentioning that the temperature influenced the performance obtained because the temperature was greater than 4 ° C what is the recommended temperature for collagen extraction processes to minimize its degradation(13). However, the material obtained presented good properties in its characterization, such as its emulsification capacity, water retention, thermal stability and viscosity(14).

Physicochemical characterization.

Ash: The determination of ash was performed by the gravimetric method according to AOAC 920.153-1920(9). Producing a result of 5.95% ash (Table 1). However, typical values of ash content in collagen can range from less than 1% to around 5%, depending on the source of collagen and the production method used. For example, bovine collagen type I may have a typical ash content of 1-2%, while fish collagen may have a typical ash content of 1-5%.(15).

It is important to note that ash content values in collagen can be an indication of collagen quality, as elevated ash levels may indicate the presence of impurities or contaminants in collagen. Therefore, collagen manufacturers usually carry out quality tests to ensure that the ash content is within acceptable limits for its intended use.

Table 1. Results of physicochemical characterization

Parameter	Average value	Unit
Ashes	5,95	%
Humidity	62.74	%
Grease	2.81	%
Proteins	16.44	% PT
Emulsificación	10	%
Water holding capacity	0.2582	CRA

Humidity: The moisture determination was performed by the traditional method according to AOAC 925.09-1925, Solids (total) and loss on drying (moisture)(8). The average moisture determination of our samples was 62.74% (Table 1), a very high result considering that the moisture content can vary depending on various factors, such as the source of collagen, the method of extraction and processing, the way the sample is stored, among others. However, in general, it is considered that the moisture content in collagen can be in a range of around 10 to 12%.(16). Importantly, too high or too low moisture content can affect collagen quality and stability. Moisture determination should be performed on each collagen sample prior to use in specific applications, as this will ensure the quality and stability of collagen under the corresponding storage and use conditions.

Grease: The fat determination was performed by the ethereal extraction method according to AOAC 920.39-1920, Fat (crude) or ether extract in animal feed(10). Obtaining an average result of 2.81% (Table 1). The amount of fat in collagen samples from bovine hooves can vary depending on the animal's age, diet, health status, and other factors. In general, bovine hooves are expected to contain less fat than other tissues, such as skin or bone.

Based on the available literature, typical values of the amount of fat in collagen samples from bovine hooves can range from less than 1% to around 5% fat. For example, one study found that the amount of fat in bovine hooves ranged from 0.6% to 3.3% by dry weight. (17). Another study found that the amount of fat in bovine hooves was 4.4% by dry weight.(18).

It is important to note that these values may vary depending on the tissue source and sample conditions.

Protein: the determination of the total protein was carried out by the Kjeldahl method according to AOAC 928.08-1974, Nitrogen in meat. Kjeldahl method(11). Given the average result of 16.44% (Table 1), the amount of protein in collagen samples may vary depending on tissue type and animal source, as well as by the method used for protein extraction and determination.

The amount of protein in collagen samples obtained from bovine hooves may vary depending on the extraction and determination method used. According to the available literature, typical values of protein quantity by the Kjeldahl method in bovine hoof collagen samples can range from 20% to 70% of the total protein in the sample.

One study found that the amount of protein in bovine hoof collagen samples was 68% of the sample's dry weight. (19). Another study found that the amount of protein in bovine hoof collagen samples was 60-70% of the dry weight of the sample.

Emulsificación: the notification test yielded an average of 10%, well below the results found by other authors (35%) (20). Emulsification tests are used to evaluate collagen's ability to form stable emulsions, which are important in many applications. In these tests, collagen is mixed with oil and water and the emulsion is analyzed to determine stability and droplet size distribution. Several factors, such as pH, temperature and salt concentration, can affect the emulsifying properties of collagen. The results of these tests can help determine the optimal conditions for using collagen as an emulsifier and can aid in the development of new foods and cosmetic products.(19).

Water holding capacity: the average result found in our research (0.2582 CRA), Water holding capacity is a crucial property of collagen extracted from cattle, as it influences the texture and appearance of the final products. Water holding capacity refers to the ability of collagen to bind to and retain water molecules. This property is important in many food applications, such as meat products, where collagen acts as a binding agent, improving texture, juiciness and yield.

Collagen extracted from cattle has a high water retention capacity due to its unique molecular structure. Collagen is a triple helix protein consisting of long chains of amino acids. These chains clump together to form fibrils, which are then assembled into fibers. The spaces between the fibers act like a sponge, trapping water molecules and holding them within the collagen matrix. Several factors can affect the water-holding capacity of collagen, including pH, ionic strength, and temperature. High pH values and high ionic strength can increase water holding capacity by promoting protein-protein interactions, while high temperatures can cause denaturation of the protein, reducing its ability to retain water. Water retention tests are performed properly by measuring the conductivity of the collagen sample(3).

Determination of feasibility

$$\text{Viability} = \frac{\text{Product Cost}}{\text{Production Cost}} * 100 \qquad \text{Equation 2}$$

$$\text{Viability} = \frac{15.60}{15.72} = 0.99$$

The viability of this process has been calculated to determine if it is possible to carry it out satisfactorily and in order to obtain utility of the proposed process with respect to the economic investment that is made during the extraction process, and thus give an added value to this waste that until now has not been treated.

Conclusions.

This article has studied the production of collagen from the residues (hulls) of cattle slaughter in the municipal bed of Riobamba-Ecuador. The hypothesis put forward was that these residues may be an alternative and sustainable source of collagen for various industrial and biomedical applications. The results obtained showed that it is possible to extract high-quality collagen from the hooves of slaughtered cattle by an optimized acid hydrolysis process. Physicochemical and functional evaluations of the extracted collagen were performed. Although the yield was not as expected, these findings confirm the viability of the process and contribute to the knowledge about the integral use of animal waste. In addition, they have positive environmental, economic and social implications for the country, since they reduce the pollution generated by these wastes, generate added value from them and offer new opportunities for employment and innovation. However, this study has some limitations, such as the use of only one type of waste and a single extraction technique. Therefore, it is suggested to conduct further research with other types of animals (pigs, poultry) and other extraction techniques (enzymatic, physical) to compare the quality and performance of the collagen obtained. It is also recommended to extend the evaluation of the physicochemical and biological properties of the extracted collagen to determine its possible applications in different sectors.

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