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EXTRACTION OF COLLAGEN FROM RAW HIDE (COW) & ITS APPLICATION IN FOOD PRODUCTS

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ABSTRACT

Background: Collagen is the predominant structural protein in animals, constituting roughly 30% of total protein in mammals. It serves an essential function in the extracellular matrix, offering structural stability to skin, tendons, cartilage, and connective tissues. (Shoulders & Raines, 2009). In the food sector, collagen and its derivatives especially gelatin and collagen hydrolysates—are valued for their functional properties, including gelling, emulsification, water retention, and nutritional benefits. (Silva et al., 2014). Cattle skins are a significant by-product of the leather and meat industries. Significant quantities of raw hide and trimmings are frequently discarded or underutilized, leading to waste management and environmental issues. Utilizing bovine hide for collagen extraction not only enhances its economic value but also promotes sustainability by minimizing waste. (Schmidt et al., 2016). The study aimed to produce cowhide collagen and apply it to food product development. This study employed two different extraction methods for cowhide: an acid and alkali combined synthesis method and an alkaline synthesis method. The observed parameters consisted of yield, moisture, ash, pH, protein, emulsifying, and gel strength tests. The result showed the difference in the pre-extraction process. Collagen has unique characteristics that make it widely applicable in food products. Methodology: All experiments were performed in the Process and Analytical Lab Department of Food Engineering and Technology at the State University of Bangladesh. Protein tests were performed in the WAFFEN Research Lab. In my experiments, the chemicals used without further purification were calcium hydroxide and acetic acid. Cow hides were collected from the local market. The procedure involved measuring the unprocessed material (cowhide). Additionally, the hide was soaked in water at 70 °C for 15 minutes to prepare it for the unhairing process. The hairs on the hide were then taken off using a manual method. The initial process involved weighing raw material (cowhide). The sample, in fresh condition and clean (without hair), was cut into a rectangle measuring 2 cm x 3 cm. A total of 4 beakers were prepared for the treatment containers of T1, T2, T3, and T4. Ca(OH)2 and CH3COOH solutions were each inserted into the beaker glass. 100 g of cowhide as raw material was divided into four pieces of beaker glass and soaked for 4 days at room temperature. The hide sample was then removed from the beaker and washed with running water. These samples were further extracted in a water bath at 70 °C for 24 hours. The extraction results were then filtered to produce a filtrate of liquid collagen extract. The result was dried in an oven at 60 °C for 24 hours to produce a dry collagen extract, and then packed in airtight glass jars. (Said, 2018). Result: A study was conducted on the extraction of collagen from cow hide. Collagen extract from cow hide was obtained by using the extraction and characterization of extracted collagen, yielding samples (1, 2, 3, 4). Yield is (11.20, 11.60, 6.70, 5.50) %. After analyzing the collagen, I found the moisture content to be (S1 = 7.8%, S2 = 7.2%, S3 = 6.8%, S4 = 6.7%). The ash content which is (S1= 1.4%, S2=1.55%, S3=1.75%. S4= 1.9%). The protein content was 77.51% and 41.02% in the collagen. Sample (1, 2, 3, 4) moisture content is (7.8%, 7.2%, 6.8%, 6.7%). Ash is (1.4, 1.55, 1.75, 1.90) %. PH is (8.90, 8.10, 7.50, 6.64), Protein is (77.51, Nil, Nil, 41.02) %. TDS (PPM) is (109, 117, 120, Nil), Stability (Hours) is (14, 12, 09, Nil). This Collagen shows strong gelatinizing properties. This Collagen shows strong emulsifying strength.

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Conclusion: A promising resource for protein can be found in collagen derived from animal byproducts. Their special characteristics, as well as biodegradability, edibility, and bioavailability, make them widely applicable in the food industry. Collagen is reviewed in this test for its biochemical and physical properties. However, a lack of standardized preparation procedures and a limited number of species sources limit the availability of collagen.

KEYWORDS: Collagen, rawhide (cow), extraction, application.

INTRODUCTION

The need for collagen to meet human use is increasing every year. Thus, humans are expected to be part of the extracellular matrix of multicellular animals, from echinoderms to vertebrates. Fibrillar collagen is a network that forms a stable body frame (Holmes et al., 2018). Collagen is the primary structural component of vertebrate animals, comprising approximately 30% of the total body protein (Chen et al., 2016). Collagen is utilized as an industrial raw material in both food and non-food industries, and its demand is increasing annually (Veeruraj et al., 2015). Currently, 29 types of collagen molecules have been identified. All these collagen molecules have differences in the order of peptides, structures, and functions (Yousefi et al., 2017). The supply of collagen remains dependent on imported products, while the potential of raw materials in the country is substantial. The use of raw materials from the skin and pig bones is a problem for Islamic countries. One of the current problems is related to the aspect of collagen halality. Halal collagen marketed by several countries in Europe and America is still in doubt. This issue is entirely groundless because collagen, as the base material of capsule shells (pharmaceutical industry), is an imported product (Liu et al., 2007). Collagen used in many Muslim countries is mainly imported from the United States and European countries. These countries generally produce collagen from pig skin (46%), cowhides (29%), cow bones (23.1%), and other sources (1.5%). This is done because the raw material price is relatively cheap (Karim & Bhat, 2008). Additionally, these countries were identified as having a high number of cases of foot-and-mouth disease (FMD) (Said, 2018). In Indonesia, the hide of Bali cattle has a vast potential to be developed as a raw material for collagen synthesis. Beef cattle are one of the livestock species with the largest population after poultry and goats. These figures pertain to the poultry population (2,175,613,000 heads), goats (18,410,000 heads), and beef cattle (16,599,000 heads) (Ministry of Agriculture of the Republic of Indonesia, 2017). Hide of Bali cattle is a halal product that the Muslim community can accept, which could potentially be developed as a raw material for collagen synthesis. The process of collagen synthesis involves a reaction between a chemical compound and a skin protein. In the process of collagen synthesis, the use of materials from acids and alkali as pre-extraction materials is still

being explored and studied. Collagen is the most abundant protein in mammals, making up to 30% of whole-body protein content (Chung et al., 2001). The collagen molecule is a triple helix of three distinct alpha chains of repeating units of (Gly-X-Y)N amino acids, where X and Y are any amino acids. However, X is often proline, and Y is often hydroxyproline (Mbg et al., 2015); proline (Shoulders & Raines, 2009). Purified collagen can be used in regenerative medicine and cosmetics, such as collagen injections for improving appearance, in body lotions, and mascaras (Peng et al., 2004). Collagen is also used in casings (Simelane & Ustunol, 2005), supplements, films (O'Sullivan et al., 2006), pharmaceuticals(Meena et al., 1999), as a precursor to biodegradable materials, for tissue engineering, and more recently in 3D printing (Pereira & Bártolo, 2015) (Lee et al., 2001) (Poland & Kaufman, 1988). The demand for collagen is rising at approximately 20% annually, and the global collagen and hyaluronic acid (HA)- based biomaterials market is predicted to reach US\$4.6 billion by 2020 (Analysts, 2015). Hides, a byproduct of meat production, are mainly used for leather production (Cui et al., 2012). The bovine hide is approximately 30% protein, and the inner corium layer of the hide is rich in collagen. This collagen has a higher denaturation temperature compared to collagen from marine sources. Collagen can be extracted from fish and porcine sources, but presents limitations. The applications of fish collagen are limited due to its lower hydroxyproline content (Aberoumand, 2012), which results in a collagen with a low denaturation temperature. In contrast, porcine products are prohibited by Muslim and Jewish communities (Schmidt et al., 2016). Off-cuts, which are not used in the leather industry, mostly end up in landfills or, at most, as animal feed. Converting this waste material into a high-value end-product, such as collagen, will benefit both the environment and the leather processor. The structure of bovine hide is influenced by factors such as age, sex, diet, and environment (Noorzai et al., 2020), which may impact collagen structure and product yield. The research examines the effect of the extraction method on collagen yield.

MATERIALS AND METHODS

All experiments were conducted in the Process and Analytical Laboratory, Department of Food Engineering and Technology, at the **State University of Bangladesh**. Protein tests were performed in the **WAFFEN Research Lab**.

Chemicals: In my experiments, the chemicals used without further purification were calcium hydroxide and acetic acid.

Preparation of Cow Hide: Raw hide (Cow) was gathered from the nearby market. The process involved weighing raw material (cowhide). Furthermore, the hide was immersed in water at 70 °C for 15 minutes to prepare for the unhairing process. Hairs of the hide were then removed through a manual process. The hide was

washed with running water for 10 min. The hide was cut into a small size, then it was ready for use as a raw material for further collagen preparation.

Extraction Method: The sequence of the operations performed for the extraction of collagen from raw hide(Cow hide) is presented in Figure 1.

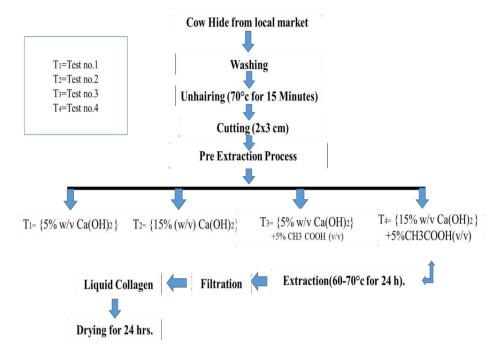


Fig.: flow sheet for the extraction of collagen from cow hide.

The initial process involved weighing raw material (cowhide). The sample, in fresh condition and clean (without hair), was cut into a rectangle measuring 2x3 cm. A total of 4 beakers were prepared for the treatment containers of T1, T2, T3, and T4. Ca(OH)2 and CH3COOH solutions were each inserted into the beaker glass. 100 g of cowhide as raw material was divided into four pieces of beaker glass and soaked for 4 days at room

temperature. The hide sample was then removed from the beaker and washed with running water. These samples were further extracted in a water bath at 70 °C for 24 hours. The extraction results were then filtered to produce a filtrate of liquid collagen extract. The result was dried in an oven at 60 °C for 24 hours to produce a dry collagen extract, and then packed in airtight glass iars.

Formula for the calculation of yield

Yield%=

Final weight x100

Initial weight

Yield%(S₁) =

$$\frac{12.2 \times 100}{100}$$
=12.2%

TESTING PROCEDURE FOR COLLAGEN FROM RAW HIDE (COW)

Moisture Content: Collagen was weighed and placed in a hot air oven for drying (60°C for 7-9 hours). After

drying was completed, the sample was placed in a desiccator for cooling. The dry weight of the collagen was then determined, and its moisture content was calculated.

Moisture
$$\% = \frac{\text{(Wt. of sample before drying } - \text{Wt. of sample after drying)} \times 100}{\text{Wt. of sample before drying}}$$

Ash Content: Ash is an index of the mineral content of the collagen powder. A 2-gm sample was weighed on a pre-weighed 3-crucible. It was incinerated over a hot plate till charring was complete. Then, the crucibles were transferred to a muffle furnace maintained at 550-600°C until a light grey ash formed or until they reached constant weight. They were then cooled in a desiccator, and the weight was noted. This determines ash content.

$$Ash \% = \frac{Weight of ash \times 100}{Wt. of sample}$$

pH: Take 0.5 g of the sample and 100 mL of distilled water in a beaker, then stir and measure the pH at room temperature. Heat the beaker to 50 °C.°C then measured pH using a digital pH meter.

TDS: Total Dissolved Solids. Take a 0.5 g sample and add 100 mL of distilled water to a beaker. Stir and measure the TDS at room temperature. Heat the beaker to 50 °C.°C Then, the measured TDS was used with a digital TDS meter.

Gel strength test: Gel strength is a measure of the gel's firmness and resistance to deformation. It is crucial for ensuring product stability, texture, and overall performance.

Emulsifying strength test: Place 50 mL of crude oil and 50 mL of laboratory-grade distilled water in a 150ml beaker. Add 5%, 10%, and 15% collagen, respectively, to the beaker. Then, centrifuge at 2500rpm for 5 min to create a homogeneous emulsion. Place the emulsion at room temperature. Note down how long it takes for the emulsion to separate.

Protein test: To determine protein content in a 2-gram sample using the Kjeldahl method, first, digest the sample with concentrated sulfuric acid and a catalyst (such as selenium or copper) to convert nitrogen in proteins to ammonium sulfate. After digestion, neutralize the solution with sodium hydroxide, then distill the released ammonia into a known volume of boric acid. Finally, the ammonia content is titrated with a standard acid, and the protein content is calculated by multiplying the nitrogen content by a factor of 6.25. (N. J. Thiex et al., 2002)

Kjeldahl nitrogen % =
$$\frac{(\text{Vs Vb}) \times \text{M} \times 14.01}{\text{W} \times 10}$$

RESULTS Yield%

A study was conducted on the extraction of collagen from cowhide. Collagen extract from cow hide was obtained through the extraction and characterization of the extracted collagen.

Table 02: Yield (%) from raw hide.

Sample	Yield%
Sample(01)	11.20%
Sample(02)	11.60%
Sample(03)	6.70%
Sample(04)	5.50%

Table 02 shows that the Sample (1, 2, 3, 4) Yield is (11.20, 11.60, 6.70, 5.50) %

Moisture Content

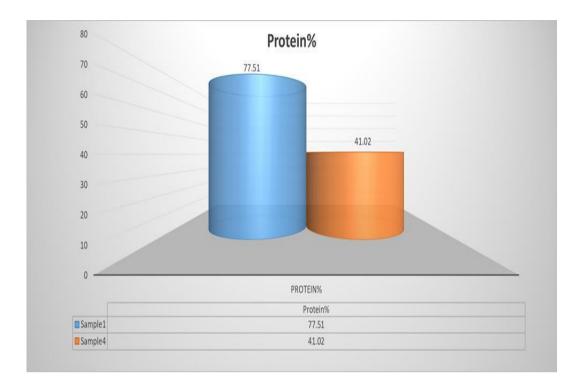
After analyzing the collagen, I found the moisture content to be (S1 = 7.8%, S2 = 7.2%, S3 = 6.8%, S4 = 6.7%). In my test, t89+*he moisture content of collagen from cow hide averaged 7.1%. The normal range of moisture content for collagen is 4-8%. A low moisture content is necessary for safe storage, as well as to inhibit the growth of microorganisms that can affect quality by producing collagen. (Muhmad Zadeh, 2010).

Ash Content

The ash content, which is 1.4% (S1), 1.55% (S2), 1.75% (S3), and 1.9% (S4) in collagen extracted from cow hide, indicates a moderate mineral residue that may contribute to its structural stability. This value aligns with reported ranges for natural collagen, highlighting its potential for use in food and beverage applications.

Protein

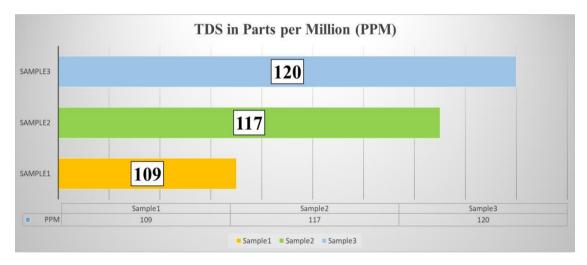
The protein content of 77.51% and 41.02% in the collagen extracted from the cow hide highlights its rich collagen composition, which is essential for its effectiveness as a gel-making agent.



TDS (Total dissolved solids)

A TDS meter is used to measure the total dissolved solids

(TDS) in water. It is used to measure the collagen in dissolved water at ambient temperature.



pН

The pH of the isinglass was measured by using a pH meter at an ambient temperature. An electronic pH meter is used to obtain accurate pH measurements. A pH meter is

an instrument used to measure hydrogen ion activity in solutions; in other words, it measures the acidity/alkalinity of a solution.

Table 01: Proximate analysis.

Sample	Moisture (%)	Ash (%)	pН	Protein (%)	TDS (PPM)	Stability(Hours)
Sample(01)	7.8	1.40	8.90	77.51	109	14
Sample(02)	7.2	1.55	8.10		117	12
Sample(03)	6.8	1.75	7.50		120	9
Sample(04)	6.7	1.90	6.64	41.02		

Table 01 shows that the Sample (1, 2, 3, 4) moisture content is (7.8, 7.2, 6.8, 6.7) %. Ash is (1.4,1.55, 1.75, 1.90) %. PH is (8.90, 8.10, 7.50, 6.64), Protein is (77.51,

Nil, Nil, 41.02) %. TDS (PPM) is (109, 117, 120, Nil), Stability (Hours) is (14, 12, 09, Nil).

Gel strength test

Indicates a firmer gel with greater resistance to

deformation. This is desirable for products like jellies that require a specific texture.





Jelly preparation.

Table 03: Gel strength test.

Sample	Result
Collagen	Exhibit strong jellying properties

Table 03 shows that this collagen exhibits **strong gelatinizing properties.**

Emulsifying strength test

Collagen-stabilized emulsions are stable over time, with minimal phase separation and droplet coalescence.



Table 04: Emulsifying strength test.

8				
Sample	Result			
Collagen	Collagen shows strong emulsifying strength.			

Table 04 shows that Collagen shows **strong emulsifying strength. Recommendations** It is recommended to use cowhide to produce Collagen.

CONCLUSION

A promising resource for protein can be found in collagen derived from animal byproducts. Their special characteristics, as well as biodegradability, edibility, and bioavailability, make them widely applicable in the food industry. Collagen is reviewed in this test for its

biochemical and physical properties. However, a lack of standardized preparation procedures and a limited number of species sources limit the availability of collagen.

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guidance and valuable support.

Conflicts of interest

The authors state no conflicting interests.

Limitation

Due to technical, logistical, and financial constraints, we were unable to conduct the study on a large scale.

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