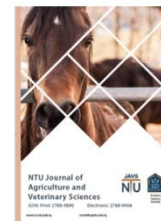




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## Studying of qualitative properties of gelatin extracted from bovine hides as by-product and marshmallow made from it

1<sup>st</sup> Mohamed Saab Adel, 2<sup>nd</sup> Basmaa Saaduldeen Sheet 

1,2. Dept. of Food Science, College of Agriculture and Forestry, University of Mosul,

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#### Corresponding author:

Mohamed Saab Adel  
Dept. of Food Science, College  
of Agriculture and Forestry,  
University of Mosul, Mosul,Iraq  
Email:[mohammed.22agp54@student.uomosul.edu.iq](mailto:mohammed.22agp54@student.uomosul.edu.iq)

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### ABSTRACT

Leaving animal tissue waste in the form of waste after slaughter pollutes the environment, so one of the goals of this study is to try to use it to make gelatin. Other goals include examining the functional, physical, and chemical properties of gelatin, comparing them to commercial gelatin, which has practical and economic value and researching some of the characteristics of marshmallows made from it. The findings indicated that the viscosity, in comparison to commercial gelatin, which reached 4.1°Cp, hide gelatin's gel strength, elasticity, cohesiveness, melting point, and gelation dropped to 3.5 cP, 271.2 g, 5.00 mm, 0.90, 29.00, and 14.57°C, respectively. cP, 446.6 g, 5.07 mm, 0.99, 32.67, and 17.67 °C, the pH values of skin gelatin were higher, at 5.60 and 4.50, respectively. After 30 and 60 minutes, the gelatin's hide expanded and stabilized the foam more than the commercial gelatin (75.25, 90.96, and 84.75%, respectively) (63.37, 90.91, and 87.88%, respectively). This study discovered 18 distinct types of amino acids, including threonine, tyrosine, asparagine, aspartic acid, leucine, phenylalanine, serine, glycine, histidine, lysine, alanine, arginine, cysteine, valine, isoleucine, methionine, proline and hydroxyproline. The gelatin's hide had lower levels of these amino acids than the commercial gelatin. In contrast to the commercial marshmallows, which achieved the same qualities of 589.5 g, 4.7 mm, and 0.87, the factory-made marshmallows' gel strength, elasticity, and cohesiveness were poor, measuring 465.8 g, 4.4 mm, and 0.87, respectively. The marshmallow sample prepared from commercial gelatin T0 received the highest marks for all qualities in the sensory evaluation, followed by the marshmallow sample made from gelatin from cow hides (T1). This means that it obtained great pleasure scores that were almost similar to the marshmallows made from commercial gelatin; this is due to the properties of the hide gelatin that are similar to those of commercial gelatin. Therefore, gelatin's hide, as an inexpensive by-product that can be used as a stabilizer, thickener and foaming component in many foods, as well as other applications.



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## Introduction

About 8% of the meat industry's waste, which amounts to over 100 million tons per year, is wasted, and if it is not correctly used, it can lead to a number of environmental issues. Collagen, which is abundant in this waste, can be utilized to create useful substances like gelatin [1], one of the most significant and versatile animal biopolymers ever employed. Numerous food applications as well as medicinal, cosmetic, and photographic products have made extensive use of it as a gel-forming, stabilizer, emulsifier, thickening and forming agent [2]. The kind of treatment, the kind and concentration of the soaking solvent, the length of time, the extraction temperature and duration, and mixing all affect the qualities of the extracted gelatin [3][4]. In general, depending on the pre-collagen processing, two distinct forms of gelatin can be created. Acid-treated Type A has an isoelectric point between pH 6 and 9. Collagen 6–9 is extracted from low-cross linked pig hide using this technique. Type B has an isoelectric point of pH 5 and has undergone basic treatment. Bovine hide is used to extract the more complicated collagen, which is less biodegradable than type A due to its more cross linked particles.[5]. The 18 amino acids that comprise gelatin are mostly composed of glycine, proline, and hydroxyproline (57%). The smaller portion (43%) is composed of aspartic acid, glutamic acid, arginine and alanine. Gelatin lacks the amino acid tryptophan, which is required for the creation of single ( $\alpha$ ), double ( $\beta$ ), and triple ( $\gamma$ ) strings that are covalently bound to molecules that are hydrophobic [6]. After an animal is killed, its leftover by products particularly its hides and bones pollute the environment.

The purpose of this study is to use these products to produce gelatin and use it to make a variety of sweets, like marshmallows. It also aims to investigate some of the physicochemical characteristics of gelatin that is extracted from cowhide and compare it with commercial gelatin to determine which is the best. During a 30-day storage period at room temperature, the rheological and sensory characteristics of marshmallows made with this gelatin will also be examined and compared to those of commercial gelatin.

## Materials and methods

The one-year-old Friesian cow hides were bought from the Gogjali slaughterhouse in Mosul, while commercial bovine gelatin powder (made by Gelken Gelatin Co. Ltd., China) was bought from the Mosul-Nineveh-Iraq market. The trials were conducted in the Baghdad laboratories of the Ministry of Science and Technology, the postgraduate laboratories of the College of Food Sciences at Al-Qasim Green University in Babylon, the Central Laboratory of the College of

Agriculture and Forestry at the University of Mosul, and the Microbiology and Food Research Laboratory.

### Gelatin extraction from bovine hide

The method described by [7] was used to extract gelatin from the bovine hide, with adjustments made to the temperature, extraction duration, and choice of acid concentration. The salted hide was split into pieces using a knife to make cleaning easier once it was received and cleaned to get rid of the salt. After that, the parts were cleaned under running water to get rid of any contaminants, including blood. After that, they were scraped with a knife to get rid of any remaining meat or fat. To get rid of non-collagenous material, the hair was shaved off and then immersed in a 0.5 M NaOH solution (1:4 w/v) for 24 hours. To get rid of dead skin and base residues, the pieces were warmed up a little before being thoroughly cleaned with distilled water till the pH reached 6-7. After being patted dry with a towel, the skin was immersed in a 0.3 M acetic acid solution (1:4 w/v) for four hours. Then a pH wash with distilled water until it was between 5 -6, the hides were dried with a cloth and weighed, then put in a standard pressure cooker with 1.5 liters of distilled water per kilogram of hide. After around five minutes of heating to 120°C, the cooker was lowered to 55–65°C for fifty-five minutes.

The final solution was cooled for 24 hours at 4°C after being filtered through a fine cloth to exclude contaminants. A knife was used to cut any remaining fat, which was scraped off the surface. After that, the gelatin solution was put in securely packed nylon bags and freeze-dried at -18°C.

### Freeze drying

According to the device instructions, the extracted gelatin was dried by freeze-drying. - A 50 ml volumetric flask containing samples of the gelatin solution was frozen in a laboratory refrigerator. After that, place it in the apparatus that was its pressure to 103 Torr (mmHg) and set at -30°C. After drying, the samples were ground in an electric grinder and stored in tightly sealed plastic containers at room temperature until testing.

### The determination of pH

The pH was measured for three replicates in accordance with [8] after a 1% from solution of gelatin was prepared by Weight 1 gram of gelatin powder sample, adding distilled water until the specified mark and dissolving it in a water bath at 60°C for 30 minutes before allowing it to cool to room temperature (20–25°C), three replicates' pH values were taken.

### Determination of viscosity

In the postgraduate laboratory of the College of Food Sciences at Al-Qasim Green University in Babylon, the viscosity of gelatin was measured using the Brookfield device, which was based on the method of [9] with adjustments of temperature and spindle number. To create 6.67% of gelatin

solution, 6.67 g of gelatin powder was weighed, put in a 100 ml volumetric flask, filled to the mark with distilled water and dissolved in a water bath at 30°C. Afterward, it was let to cool to ambient temperature (20–25 °C), then the viscosity measuring vessel was filled with the solution and the device was set to 20°C. The spindle which number L4, was attached to the device and submerged in the sample, and the device was run at 10 revolutions per minute. The reading was taken in centipoise at 30 seconds and for three repetitions.

#### Amino acid analyzer

The amino acids from gelatin powder were identified using an amino acid analyzer based on the methodology [10]. The extraction conditions were determined to be 0.1 M sodium acetate as the mobile phase, pH 7.2: methanol 1:9, and a flow rate of 1 mL/min. To separate amino acids, a 250 mm × 4.6 mm reversed-phase C18-NH<sub>2</sub> column was employed. For detection, a fluorescence detector was employed at two wavelengths: 360 and 455 nm, respectively. To a 25 ml volumetric flask, 3 grams of weighed gelatin powder were added 4 mL of 6 M hydrochloric acid is then added, and the flask is sealed.

The sample was heated for 6 hours at 150°C in a preheated oven. After hydrolysis, the sample was dried, and the acid was extracted using a rotary evaporator. One milliliter of a sodium citrate solution with a pH of 2.2 was added. Following that, 7.5 mmol of orthophthaldehyde was added to the sample containing the citrate solution, which was then filtered and stored until analysis. 18 standard amino acids (0.05 µmol/ mL<sup>-1</sup> for each amino acid) were first injected to determine the retention times for each amino acid and α-amino-butyric acid (0.05 µmol/mL<sup>-1</sup>) in order to measure and identify the amino acid content of each sample. After the samples were injected, the database stored on the computer that was connected to the amino acid analyzer equipment was used to create the curves of the recognized amino acids.

#### Gel strength (bloom) determination

In the graduate laboratory of the College of Food Sciences at Al-Qasim Green University in Babylon, the gelatin gel strength was measured. A 6.67% gelatin solution was made from gelatin powder using the procedure described in [11].

#### Melting point determination

A 6.67% (w/v) gelatin solution derived from bovine hide and commercial gelatin was prepared in accordance with the method of [12].

#### Gelling point determination

The method of [13] was used to establish the gelling point, which was adjusted by the cooling temperature. Twenty milliliters of gelatin solution 6.67% (w/v) extracted from bovine hide and commercial gelatin both separately were put into test tubes for comparing them. A thermometer was then used, and the tubes were put in a container with water at 40°C. A few ice cubes were then

added gradually, and the tubes were left until the gel formation started. The temperature was then recorded..

#### Evaluation of foam stability and expansion

With a modification of the homogenization procedure, the foam properties (expansion and stability) of gelatin solutions were assessed using the methodology described by [14]. A 1% (w/v) gelatin solution was made and homogenized by a magnetic stirrer for one minute at room temperature (25°C). After immediately pouring the whipped solution into a 250 ml glass cylinder, the total volume was measured at 0 minutes. After that, it was whipped for 30 minutes, during which the volume was also measured. The proportion of foam expansion was computed. The whipped gelatin solution was then allowed to sit at room temperature for half an hour and an hour. Using the following formulas, the percentage of foam stability was determined for three replicates:

$$FE\% = VT/V_0 \times 100. (1)$$

$$FS\% = V_t(\text{min})/V_{\text{initial}} \times 100. (2)$$

FS = foam stability, FE = foam expansion. Where: Total volume (ml) following 30 minutes of whipping and 1 minute of homogenization = VT.

Initial volume after homogenization (ml) = V<sub>0</sub>.

Total volume (ml) at room temperature after 30 and 60 minutes = V<sub>t</sub>.

For foam stability, the volume of foam that remained after 30 and 60 minutes was measured.

#### Marshmallow candy

Marshmallows were made by mixing 20g of gelatin powder with 125ml of distilled water and leaving it to soak in the water. Then, 400g of sugar was mixed with the same amount of water used previously and the mixture was heated with stirring to 70°C for 15 minutes. Then, 45g of honey was added as a substitute for glucose syrup. The temperature was gradually raised until it reached 115°C, which is the end point. Then, the gelatin soaked in water was whipped with an electric whisk and the hot syrup, a little salt and vanilla were gradually added to it while continuing to whip until the mixture reached a suitable viscosity. Then, it was poured into a mold sprinkled with a mixture of powdered sugar and starch. It was left for 30 minutes, cut and stored in a tightly sealed plastic container for the purpose of sensory evaluation. The product itself was also manufactured without cutting and a test of gel strength, elasticity and cohesion was conducted, which are important in determining the quality of the product.

#### Evaluation of the marshmallow's gel strength, flexibility and cohesion

In the graduate studies laboratory at the College of Food Science at Al-Qasim Green University in Babylon, the strength of marshmallow gel was calculated by measuring the marshmallow after it was manufactured, using the Brookfield texture analyzer, and taking readings in grams for three replicates.

### Sensory evaluation of marshmallow

Use a 9-point hedonic scale (strongly like, very much like, moderately like, slightly like, neither like nor dislike, slightly like, moderately like, strongly dislike, very strongly dislike) as stated in [15] when stored at room temperature (20-25°C) for 0, 15 and 30 days.

### Statistical analysis

A completely randomized design (C.R.D. Factorial Complex Random Design) was used to analyze the data statistically using Duncan's test to compare means according to [16], and statistical analysis was done at 0.05 probability level using SAS 2001.

## RESULTS AND DISCUSSION

### PH

The pH values of the commercial gelatin and bovine hide varieties are displayed in the same table. It is observed that the pH of gelatin derived from bovine skin was considerably higher at the level ( $P < 0.05$ ) and reached 5.60, but the pH of commercial gelatin was significantly lower and reached 4.50. It's possible that the concentration of acid, the method, and the length of time it is soaked in it may have an impact on the gelatin extracted from hide, which is why its pH is higher than that of commercial gelatin. During research on the physical, chemical, and functional characteristics of buffalo bone gelatin, the difference may be due to the type of connective tissue and the type of treatment [17] was mentioned and the variation in the type and technique of treatment before extraction could be the cause of the pH discrepancy. The range of values found in this study, which ranges from -4.50 to 60.5, is within the gelatin quality criteria [18], despite the variation in pH levels.

**Table 1.** Gelatin's pH and viscosity

| Gelatin type | pH     | Viscosity (cP) |
|--------------|--------|----------------|
| Commercial   | 4.50 b | 4.1a           |
| Hide         | 5.60 a | 3.5 b          |

\*Differing letters in the same column denote significant differences at the 0.05 probability level.

\*The figures represent the mean of three repetitions.

### Viscosity

A viscosity test on gelatin is used to determine the viscosity level of gelatin solution at a certain concentration. High viscosity indicates good-quality gelatin. It is noted from Table (1) that the viscosity of the gelatin solution extracted from the hide was significantly lower at the level ( $P < 0.05$ ) which reached 3.5 cP, the sodium hydroxide solution employed for the treatment (soaking) may be the cause of the low viscosity in the gelatin solution recovered from the hide when compared to the commercial gelatin solution's viscosity of 4.1 cP, as soaking with the base only leads to a decrease in the molecular weight and thus a

decrease in viscosity. This was confirmed by [10], who indicated that the type of solutions used in the treatment and the extraction period may affect the viscosity of the resulting gelatin, as they found that using an acid with a base such as hydrochloric acid with sodium hydroxide, gave a better viscosity than using the base alone, and they also found that an extraction period of 4 hours gave a better viscosity than 6 hours.

### The different kinds of amino acids that compose gelatin

Upon detecting the amino acids found in the commercial and hide derived gelatin types listed in Table (2), it was discovered that the composition of gelatin from these acids differed in type and quantity in the extract from bovine hide than the commercial one when compared with it, especially the amino acids responsible for the composition of the gel and determining its properties, such as the amino acids cysteine, glycine, proline and hydroxyproline, which obtained the highest percentages in the two types of gelatin compared to the percentages of the rest of the amino acids. They reached 9.82, 16.19, 13.45 and 8.77% in commercial gelatin, respectively, followed by gelatin extracted from hide, as their percentages reached 9.35, 13.47, 12.68 and 8.75%, respectively. The same table also shows that, in comparison to non-essential amino acids, the essential amino acids threonine, Histidine, lysine, Valine, isoleucine, methionine, Leucine and phenylalanine were low in these two types and the essential amino acid Tryptophan was not detected in both types; therefore, gelatin is considered a protein with low nutritional value, and the non-essential glutamic acid was not detected. A difference in the kind of connective tissue, the manufacturing process for gelatin, and the pretreatment could be the cause of the variation in the amino acid ratio between the two types of gelatin [19, 20].

The same table shows that the percentage of total amino acids in gelatin extracted from bovine hide was low, reaching 91.23%, whereas commercial gelatin had a greater percentage, reaching 92.13%. This was demonstrated by [21], who clarified that, in addition to the previously mentioned factors, the proportion of protein in the gelatin determines the cause of the variation in the percentage of total amino acids between gelatin samples.

When comparing the physical, chemical, and functional characteristics of duck feet and commercial bovine gelatin, some results were in agreement [22], they discovered that the percentage of certain amino acids, like lysine (3.31%), was similar to the percentage of gelatin extracted from skin and commercial gelatin, which was 3.37 and 3.41%, respectively, and that the percentage of phenylalanine in commercial gelatin was 2.47%, which was the same as the percentage found in this study. The remaining amino acid percentages,

however, varied from this study. Because of these differences in animal and breed type, connective tissue type, pretreatment type, and extraction method and conditions, the rest of the researches agreed with the percentages of some amino acids but disagreed with others.

### Gelatin gel's Cohesion, flexibility, and gel strength (Bloom's value)

The rheological characteristics of commercial and bovine hide gelatin, such as cohesion, flexibility, and gel strength, are shown in Table (3). The cohesion property, which is a measure of the degree of difficulty in dismantling the internal structure of the gel, the same table indicates that the cohesion value of gelatin gel extracted from bovine hide did not differ significantly ( $P>0.05$ ) from the cohesion value of commercial gelatin, as their values reached 0.99 and 0.90, compared to commercial gelatin gel as well; the reason for the decrease in the cohesion value in gelatin may be due to the low protein percentage, which greatly affects the cohesion of gelatin gel. The results were close to the results of [28] when they studied the tissue analysis and functional properties of gelatin from the scales of three types of freshwater fish, which they compared with pig gelatin, as the cohesion value of pig gelatin gel reached 0.913, which is close to the cohesion value of bovine hide and commercial gelatin gel.

The same table shows that the flexibility value of gelatin gel did not differ significantly ( $P>0.05$ ) between commercial gelatin and gelatin extracted from bovine hide, reaching 5.07 and 5.00 mm, respectively. This is because the flexibility property simulates the action that the tongue and teeth exert on the gelatinous material. The nature of the network that was developed could be the cause of the great degree of flexibility.

In contrast to commercial gelatin, which had a greater gel strength value of 446.6 g, it was observed that the gel strength of gelatin extracted from hide reduced dramatically ( $P<0.05$ ) to 271.2 g. The gelatin gel strength is typically divided into three categories: low, which is defined as less than 150 g, medium, which is defined as 150–220 g, and high, which is defined as 220–300 g [23]. Since commercial gelatin is greater than the high classification, the gelatin gel value that was extracted from the hide is thought to fall into the medium category, which is ideal for many food applications. The type of connective tissue, the gelatin's source and the age of the cow are the main causes of the significant variations in gel strength amongst gelatin types. The concentration of residual amino acids in the gelatin, the animal's breed, and the concentration all have an impact on the gel's strength; the lower the concentration, the weaker the gel [24].

**Table 2.** lists the different kinds of amino acids that are present in bovine gelatin.

| Type of amino acids  | Commercial %Amino acids bovine gelatin of | %Amino acids of bovine hide gelatin |
|----------------------|---|-------------------------------------|
| Polar amino acids    |   |                                     |
| Asparagine           | 2.05                                      | 2.60                                |
| Aspartic acid        | 2.57                                      | 3.03                                |
| Serine               | 5.08                                      | 5.72                                |
| Threonine            | 3.66                                      | 4.13                                |
| Tyrosine             | 3.27                                      | 3.70                                |
| Arginine             | 4.53                                      | 4.86                                |
| Cysteine             | 9.82                                      | 9.35                                |
| Glycine              | 16.19                                     | 13.47                               |
| Histidine            | 2.78                                      | 2.80                                |
| Lysine               | 3.41                                      | 3.37                                |
| Nonpolar amino acids |   |                                     |
| Alanine              | 2.14                                      | 2.17                                |
| Valine               | 2.05                                      | 2.07                                |
| Isoleucine           | 2.47                                      | 2.59                                |
| Methionine           | 4.65                                      | 4.37                                |
| Proline              | 13.45                                     | 12.68                               |
| hydroxyproline       | 8.77                                      | 8.75                                |
| Leucine              | 3.04                                      | 3.11                                |
| Phenylalanine        | 2.47                                      | 2.49                                |
| The total            | 92.13                                     | 91.23                               |

**Table 3.** Cohesion, flexibility, and gel strength (Bloom's value)

| Cohesion | Flexibility (mm) | Bloom's value (g) | Type of gelatin |
|----------|------------------|-------------------|-----------------|
| 0.99 a   | 5.07 a           | 446.6 a           | commercial      |
| 0.90 a   | 5.00 a           | 271.2 b           | hide            |

\*Differing letters in the same column denote significant differences at the 0.05 probability level.

\*The figures represent the mean of three repetitions

gelatin gel, which in turn is connected to the extraction technique, the rise in molecular weight and its distribution, and the increase in amino acid content. In their investigation into the characteristics of gelatin derived from catfish head bones, [25] verified this, whereas [26] stated that high flexibility results from the disintegration of the gel structure into a few large pieces during the initial pressure in the device, while low flexibility results from the disintegration of the gel into many small pieces. [27] added that flexibility is related to the strength of the gel, as it increases with its height, and they explained that flexibility means the flexible return of the sample after deformation in terms of speed and strength.

### Gelatin's melting and gelation points

The melting and gelation points of the two varieties of commercial and bovine hide extracted gelatins are displayed in Table (4). The aforementioned table indicates that the melting and gelation points of commercial gelatin were significantly higher at the level ( $p<0.05$ ), at

32.67°C and 17.67°C, respectively, whereas they decreased in gelatin derived from bovine hide, reaching 29.00°C and 14.57°C, respectively. The basic differences in the protein composition and the major treatment type utilized in the manufacturing of gelatin may be the cause of the disparity in melting and gelation points [23] [28].

**Table 4.** Gelatin's melting and gelation points

| Gelatin type | Point of melting (°C) | point of gelation (°C) |
|--------------|-----------------------|------------------------|
| commercial   | 32.67 a               | 17.67 a                |
| hide         | 29.00 b               | 14.57 b                |

\*Differing letters in the same column denote significant differences at the 0.05 probability level.

\*The figures represent the mean of three repetitions.

The high melting point and gelation of commercial gelatin may be due to the high viscosity values and thus the high gel strength, in addition to the fact that the source of gelatin, the strain and age of the animal, the molecular weight, the position of the peptide chain cleavage, and the concentration of amino acids resulting from the hydrolysis and remaining in the gelatin are the main factors contributing to its rheological properties [24] [29]. The reason for the low melting point of gelatin extracted from hide may be due to a slight breakage of both  $\alpha$  and  $\beta$  chains [30]. In general, it is noted that the high melting points of all types of gelatin were consistent with the high gelation point.

#### Gelatin's foam stability and foam expansion

The ability of gelatin to form foam and its resilience are essential properties, especially when it comes to its employment in certain confections like marshmallows. Foam stability and expansion were assessed after 30 and 60 minutes of whipping, stability was assessed to assess the varieties of gelatin utilized in the study, as may be seen the foam expansion and stability properties of the commercial foam are listed in Table (5).

and hide extract gelatin varieties used in this investigation, (commercial and hide-extracted), indicating the foam expansion rate in hide extracted gelatin was substantially greater at the level ( $p < 0.05$ ), with the foam expansion rate in bovine hide-extracted gelatin reaching 75.25% as opposed to 63.37% for commercial gelatin; even while a large proportion of protein and amino acids enter the composition of commercial gelatin, the high percentage of hydrophobic amino acids may be the cause of the significant foam expansion in the gelatin isolated from hide.

**Table 5.** The foam stability and foam expansion of gelatin

| Foam stability% |         | Type of gelatin | Foam expansion % | Foam stability % |
|-----------------|---------|-----------------|------------------|------------------|
| 30 min          | 60 min  |                 |                  |                  |
| commercial      | 63.37 a |                 | 90.91 a          | 87.88 a          |
| hide            | 75.25 b |                 | 90.96 a          | 84.75 b          |

\*Differing letters in the same column denote significant differences at the 0.05 probability level.

\*The figures represent the mean of three repetitions.

When they investigated [31] the chemical, physical, and biological characteristics of gelatin recovered from squid skin treated with pepsin and alkali, verified this. The foam expansion rate is increased by the high protein content, which causes it to spread swiftly to the gas-liquid phase's surface and create denser, more stable layers or films [32]. Even though the protein lacks tryptophan and has terminal peptide chains with hydrophobic amino acids such as alanine, valine, isoleucine, leucine, proline, methionine, phenylalanine, and tyrosine in trace amounts, it also speeds up the development of gelatin foam [23]. Verified by [33] who mentioned that the high levels of isoleucine, Leucine, and Valine in the bovine gelatin increased the amount of foam. Gelatin gels that have been freeze-dried exhibit increased foaming, expansion, and stability as a result of the protein-water interaction, which creates finer particles [34].

According to the same table, the gelatin extracted from the hide and commercial gelatin reached 90.96 and 90.91% foam stability, respectively, after being left in the gelatin foam for 30 minutes. This difference was not statistically significant ( $P > 0.05$ ). Nevertheless, it was observed that after 60 minutes, the foam stability of all commercial and hide-derived gelatin types dropped to 87.88% and 84.75 percent, respectively. The structure, molecular weight, and characteristics of the protein that makes up gelatin foam can all have an impact on its characteristics. Stronger films and more stable foam are produced by compounds with higher molecular weights and more hydrophobic amino acids [35]. Since gelatin with a high molecular weight generates a thick layer of the film, resulting in a foam with high stability, the protein's molecular weight is crucial in producing a nice foam with high stability [36].

The decrease in foam stability when the gelatin solution is left for 60 minutes may be due to the force of gravity that works to evaporate water and converge the air cells, which leads to a decrease in the foam [38]. All the results did not agree with [37] who mentioned in their research on the emulsification and foaming properties of buffalo skin gelatin extracted using acid and alkali compared to commercial gelatin, whose stability percentage reached 48.54%, this percentage is lower than this study.

#### The marshmallow candy's cohesiveness, flexibility, and gel strength (Bloom value)

The rheological characteristics of marshmallow candy, including gel strength, cohesion, and flexibility, are displayed in Table (6). It was shown that marshmallow candy prepared with gelatin recovered from bovine hide T1 had a considerably lower gel strength or Bloom value ( $p < 0.05$ ) at 465.8 g as opposed to the strength of the same

candy gel made from commercial gelatin T<sub>0</sub>, which was significantly higher at 589.5 g. The high amount of the amino acid glycine in marshmallow gel—13.47% compared to 16.19% in commercial gelatin—may be the cause of its great strength when manufactured from gelatin extracted from hide.

**Table 6.** Marshmallow candy's rheological characteristics (cohesion, gel strength, and flexibility)

| Gelatin type   | Strength of gel (g) | Flexibility (mm) | Cohesion |
|----------------|---------------------|------------------|----------|
| T <sub>0</sub> | 589.5a              | 4.7 a            | 0.94 a   |
| T <sub>1</sub> | 465.8 b             | 4.4 b            | 0.87 b   |

\*T<sub>0</sub>=Marshmallow made from commercial gelatin

\*T<sub>1</sub>= Marshmallow made from gelatin extracted from bovine hide.

\*Differing letters in the same column denote significant differences at the 0.05 probability level.

\*The figures represent the mean of three repetitions.

This acid can bind water well so that the movement of its molecules is reduced and confined in the structure, which leads to the formation of a strong gel [38], in addition to the high percentage of proline and hydroxyproline, which contribute to increasing the strength of the gel [39]. Therefore, it is noted that the strength of marshmallow gel is directly proportional to the results of the gel strength and viscosity of the gelatin used in its manufacture [40].

As for the flexibility property, it is noted from the same table that the flexibility of marshmallows made from bovine hide gelatin was significantly lower ( $P < 0.05$ ) than that made from commercial gelatin reached 4.4 and 4.7mm, respectively; the reason for the high elasticity, may result from the type of network that forms during the gelatin gel formation process, which is linked to the rise in amino acid concentration, the increase in molecular weight, distribution as well as the extraction method used [25][41], in addition to the effect of flexibility on the type of gelatin, which gives it a rubbery nature [40] [42] attributed the differences in the flexibility of marshmallows to the differences in the strength of gelatin gel, as the higher the gel strength, the more flexible the marshmallows formed. The strength of its gel is affected by the water content and the percentage of the amino acid glycine in gelatin, which can bind water well So that the movement of its molecules is reduced and confined in the structure, which leads to the formation of an ideal gel [38]. [26]defined flexibility as the elastic return of the sample after deformation in terms of speed and force.

Table (6) shows that the cohesion of gelatin extracted from hide was lower than that of commercial gelatin, reaching 0.87 and 0.94 respectively and less cohesive than it; the reason for the low cohesion value in marshmallows made from gelatin extracted from bovine hide may be due to the low percentage of protein and the

percentage of amino acids entering into its composition, which play a major role in forming the gelatin network in it, especially the two amino acids proline and hydroxyproline, which greatly affect this property. From the results obtained from this table, it can be said that the three properties of marshmallows (gel strength, flexibility and cohesion) are related to each other. All of them are due to the same properties of the gelatin made from it, so when the gel strength is high, the flexibility and cohesion are also high for the reasons mentioned above.

#### Sensory evaluation of marshmallow

The purpose of the sensory evaluation is to determine the judges' degree of acceptance of marshmallows prepared with commercial gelatin as a comparison sample and marshmallows created from bovine hide gelatin. According to Table (7), samples T<sub>1</sub> and T<sub>0</sub>, which were manufactured immediately after zero days of storage and reached 8.5 and 8.7 degrees, respectively, show that the marshmallow manufactured from bovine hide gelatin had a substantial drop in the degrees of enjoyment for the flavor attribute at the level ( $p < 0.05$ ).

compared to the commercial one. However, the degrees of acceptance of this attribute decreased in the two samples during the storage periods of 15 and 30 days, but they were equal to each other significantly ( $p > 0.05$ ) in the degree of acceptance of the same attribute, reaching 8.3 and 8.2 degrees, respectively, and 8.2 and 8.1 degrees, respectively, also during the two mentioned storage periods. From these results, it is clear that the taste of marshmallows made from bovine hide gelatin was similar to the taste of those made from commercial gelatin and was accepted by the evaluators.

As for the enjoyment scores for the odor characteristic, it is noted from the same table that the scores for this characteristic did not differ significantly ( $p > 0.05$ ) between samples T<sub>0</sub> and T<sub>1</sub> in all storage periods (zero, 15 and 30 days). They were 8.3 and 8.5 degrees, respectively, in zero days, and were 8.3 and 8.4 degrees, respectively, in the 15-day storage period, and 8.0 and 8.3 degrees, respectively, for samples T<sub>0</sub> and T<sub>1</sub> in the 30-day storage period. [43] stated that the smell is caused by the mixture of ingredients that make up the product.

Table (7) shows that samples T<sub>0</sub> and T<sub>1</sub> had significantly high ( $p < 0.05$ ) texture characteristic scores, reaching 8.6 and 8.5 degrees, respectively. According to the study's findings, the gelatin used to make marshmallows had high viscosity and gel strength, high foam expansion and stability, high flexibility, and high cohesion, all of which helped to give the marshmallows the consistency the evaluators wanted. At 15 and 30 days of storage, the texture trait scores for the two samples were equal.

**Table 7.** Sensory evaluation of marshmallow

| Storage Time | Characteristics    | Type of Marshmallow |                |
|--------------|--------------------|---------------------|----------------|
|              |                    | T <sub>0</sub>      | T <sub>1</sub> |
| 0 days       | Taste              | 8.7 a               | 8.5 a          |
|              | Aroma              | 8.3 a               | 8.5 a          |
|              | Texture            | 8.5 a               | 8.6 a          |
|              | Appearance         | 8.6 a               | 8.5 a          |
|              | General Acceptance | 8.5 a               | 8.5 a          |
| 15 days      | Taste              | 8.2 ab              | 8.3 ab         |
|              | Aroma              | 8.3 a               | 8.4 a          |
|              | Texture            | 8.4 ab              | 8.4 ab         |
|              | Appearance         | 8.4 a               | 8.4 a          |
|              | General Acceptance | 8.5 a               | 8.5 a          |
| 30 days      | Taste              | 8.1 ab              | 8.2 ab         |
|              | Aroma              | 8.0 a               | 8.3 a          |
|              | Texture            | 7.8 ab              | 8.2 ab         |
|              | Appearance         | 8.2 a               | 8.2 a          |
|              | General Acceptance | 8.4 a               | 8.4 a          |

\*T<sub>0</sub>=Marshmallow made from commercial gelatin

\*T<sub>1</sub>=Marshmallow made from gelatin extracted from bovine hide

\*Differing letters in the same column denote significant differences at the 0.05 probability level.

\*The figures represent the mean of three repetitions.

Regarding the appearance trait scores, the same table's findings demonstrated that there was no discernible difference between marshmallow samples T<sub>0</sub> and T<sub>1</sub> ( $p>0.05$ ) in this trait at zero, 15 and 30 days of storage, as they reached 8.6 and 8.5 degrees respectively at zero days of storage, and reached 8.4 degrees for both samples at 15 days of storage, and 8.2 degrees were given to both samples when stored for 30 days.

The results indicated in Table (7) also showed that the general acceptance scores were high in samples T<sub>0</sub> and T<sub>1</sub>. The evaluators stated that they liked these samples very much from the beginning of storage to the end and did not differ significantly between them. They gave them scores of 8.5 for both samples in the periods of zero and 15 days, and scores of 8.4 were given in the storage period of 30 days. This is consistent with the enjoyment scores discussed previously (taste, aroma, texture and appearance). It is noted from the same table that the highest score for all attributes was given to the marshmallow sample made from commercial gelatin T<sub>0</sub>, followed by the marshmallow sample made from bovine hide gelatin T<sub>1</sub>. This means that it received great enjoyment scores almost similar to the scores of marshmallows made from commercial gelatin; because hide gelatin's qualities are comparable to those of commercial gelatin.

## Conclusions

Bovine hide gelatin has similar qualities to commercial gelatin, with high pH, foam stability after 30 minutes, foam expansion and low viscosity, gel strength, cohesion, melting point, gelation, and foam stability at 60 minutes. The

proportion of amino acids in the hide-derived gelatin was somewhat lower than that of commercial gelatin. Even though the gelatin that is extracted from hide has slightly inferior qualities, it is still regarded as a cheap by-product that may be utilized to thicken and stabilize a variety of foods as well as in jelly desserts.

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