

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Industrial – Grade gelatin from Animal Bone Marrow through Free - Chemical Method.

M. A. Habib*.

Chemistry of Tanning Materials and Leather Technology Department , National Research Centre, P.O. 12622 Dokki, Giza, Egypt.

Chemistry Department, College of Science, Al-Imam Mohamed ibn Saud Islamic University (IMSIU), Riyadh, Kingdom of Saudi Arabia.

ABSTRACT

Gelatin is an important ingredient in different products. Its use in many applications makes the demand for it steadily increasing. It is mainly produced from cow and pig skins as well as bones, hooves and fish. The traditional methods involve acidic or alkaline hydrolysis of the animal protein using mineral acids or base under harsh condition. This work is devoted to obtain animal gelatin with a considerable quality, via low-cost and free chemical hydrolysis process, from animal bones that are a low-cost slaughterhouse by-product. The gelatin has been extracted from the crushed animal bone through simmering in stainless steel stock autoclave in aqueous solution (1: 1.75, crushed animal bone to water), at a temperature lower than the boiling point of water under 1.5atmospheric pressure for nine hours. The upper fatty material has been removed by skimming of and the gelatin layer was filtered through cheesecloth and the filtrate was concentrated to get the product. The results showed that the properties of extracted gelatin close to commercial gelatin and can be used in various industrial applications. It can be refined, sterilized and purified from some metal contaminants for use as food gelatin. Therefore, the work successes to add value to low- cost by-product via transferring it into of value product.

Keyword: Gelatin – animal bone – by-product - free chemical process

<https://doi.org/10.33887/rjpbcs/2019.10.6.10>

**Corresponding author*

INTRODUCTION

Cleaner production involves the greatest decline of the wastes generated during manufacture. It involves careful saving resources and re-use of by-products. Moreover, it reduces cost and saves raw resources. Slaughterhouses generate a huge amount of animal bones as by-products. They don't find the ideal utilization and getting a benefit, a great part of these by-product bones goes to waste, especially in the small local massacres. This research aimed to maximize the benefit of the by-product bones through the production of gelatin. Gelatin is considered as a biopolymer; it could be extracted or derived from the protein building fibers. Therefore, it is prepared through thermal disintegration of protein¹. Physically, it is colorless and tasteless solid substance. The importance of gelatin comes from being a raw material for many important products. Cosmetics, photographic, and food are the most common application of gelatin rather than medical and pharmaceutical applications. The unique properties of gelatin gain it great value². Therefore, it is biodegradable and biocompatible natural substance. It is used as medical adsorbent pads. The most important and widespread use of gelatin is that it is an essential substance for capsules for drug products and medicinal emulsion³. Pigskin and cowhides are the favorite sources of gelatin rather than fish and animal bones⁴. Gelatin manufacture involves use acidic and alkaline processes. The first one mainly depends on collagen dismantling in the acidic medium in pH near 4, and is suitable for type A gelatin (pig and fish), the next involves demineralization with mineral acids such as HCl followed by treatment with lime, this method is suitable for hides and bones⁵. Enzyme can be used to facilitate process and reduce the time especially for type A gelatin⁶. Acidic and basic hydrolysis is most favorite methods but it mainly depends on the use of chemicals and large amount of water as well as the time of processing⁷. In this regards, acidification of bones to extract gelatin need about 12 day⁸. Moreover, manufacture of gelatin from animal hide by Cole. C.G.B method needs large water quantity in addition to chemicals and about thirty days time duration⁹. Production of type B gelatine from animal bone requires 5-7 days for demineralization with mineral acid followed by liming for 35-70 day duration time. Other clear example is the extraction of gelatine from yellow fish tuna by Rahman et al, the frozen fish skin require to pre-treated with 0.5 M & 0.1 N of NaCl followed by 0.1 N acetic acid, rather than the washing with water for several times. And process is complete through heating at 50 °C for 18 hours¹⁰. Consequently, traditional chemical methods of gelatine manufacture require long time duration rather than chemicals and the need for huge water quantities. Animal bone marrow is renewable and valuable by-product; contains proteins and fatty acids. Therefore, can be transferred to value - added materials such as lubricant¹¹ and leather fatliquoring agents¹¹⁻¹³. This work aimed to produce an industrial product that is an essential ingredient for several applications (cosmetic photographic and pharmaceutical) that mainly depend in gel formation. The goal is achieved through hydrolyzing the protein of bone marrow at heat – pressure processing to obtain gelatin from by-product bones generated from slaughter- house, avoiding chemical usage and reducing the time and water.

EXPERIMENTAL

Work-setup

Animal bone by-product parts were supplied from a local slaughterhouse; the bones were washed through draining in freshwater to remove wastes and dust. The bones were mechanically crushed to the appropriate size (about 8cm³).

Gelatin extraction

The crushed bone was then charged to stainless steel reactor. Appropriate amount of fresh water was added (1: 1.75 w/w, bone: water). Firstly, the bones were simmer in the tank for two hours, the collected or coagulated fats was skimmed off from the surface. The reactor was then subjected to 2 atmosphere pressure, and the bones were heated for five hours at temperature near the boiling point of water. The soup was left to cool down and any floating fat was skimmed off. The liquid was returned to the tank and heated for further 4 hours at the same temperature and pressure. The bone was removed from the tank and the soup was filtered by cheese cloths to remove impurities and solids. The soup was then concentrated by heating at the boiling point of water until the volume of the liquid reduced to quarter of its initial volume. The resulted solution was dried at room temperature for 24 hour; the solid product was milled and left to dry for another 24 at room temperature.

Evaluation of the extracted gelatin

The properties of extracted gelatin have been tested according to slandered test methods. Moisture and ash content were measured, the pH was measured at a concentration of 2% using pH meter. The viscosity of 7% gelatin solution was measured by rotary viscometer with a speed of 30 rpm and 25°C¹⁴. The amino acid of the extracted gelatin was evaluated by Pico Tag method¹⁵. The heavy metal analysis was conducted by atomic absorption spectrometer.

RESULT AND DISCUSSION

The data in table 1 illustrates the properties of the extracted gelatin, the data show that the moisture and ash content are in acceptable ratio. The ash content ratio is allowing for use in industries as technical gelatin¹⁶. However, the extracted sample has 9.89 % nitrogen content, although it is not high enough however it is unacceptable range¹⁷. The pH of the product was in acceptable value. Although viscosity of the product is relatively low, the resent process that was used to extract gelatin from animal bone by-product, the extraction methods have several reasons which justifier the advantage of the extracting process, therefore, it was free - chemical and low processing time compared with the traditional alkaline and acidic methods that require long-time and all of them takes place under harsh condition. Therefore, the recent method offers gelatin with considerable quality via low-cost and short time free - chemical processing. The solution of the obtained product is turbid and the gelatin itself is yellowish color. However, the properties of the product depend on the extraction method not only the extracted source¹⁸. Vice versa the amino acid composition of the product is independent on the processing methods therefore; it depends on the nature of the hydrolyzed source. Table 2 shows the amino acid composition of the extracted gelatin. The results show that the extracted gelatin has a similar amino acid composition for the commercial gelatin which extracted from hydrolyzed animal hide⁸, however, some slight difference can be observed. This deference comes from the nature of the hydrolyzed source. The heavy metal analysis of the extracted gelatin is shown in Table 3. It can be seen that the percentages of heavy metals are within acceptable limits as a technical - commercial gelatin sample. The increase in lead may be from friction with the containers and used tools. The data show a remarkable increase in the calcium ratio even this ratio compared with the fish gelatin¹⁹. The increase in calcium results from the high calcium content in raw material animal bones. High lead content (> 0.0015 g/mg) makes the extracted gelatin unsuitable for food processing and can only be used in industrial applications as technical gelatin²⁰. However, the product can be purified to reduce the heavy metal ratio converting the gelatin to into a food product. The work adds value to a low-cost by-product bio-mass via transferring it into of value product, which is considered an important and ingredient for many industrial applications.

Table 1: properties of extracted gelatin

property	value
Ash content %	6.80
Moisture content %	5.90
pH (2% con.)	6.72
Viscosity (poise)	9.20
Nitrogen content %	9.89
Fat content %	0.36

Table 2 : amino acid composition of the extracted gelatin

The amino acid	% of amino acid
Aspartic acid	10.15
Serine	3.12
Threonine	2.69
Proline	8.91
Glutamic acid	14.95
Glycine	18.11
Valine	3.06
Alanine	5.77
Isoleucine	0.95
Methionine	1.10
Leucine	2.12
Phenylalanine	2.98
Tyrosine	0.79
Histidine	0.84
Arginine	2.44
Lysine	3.97

Table 3: heavy metal analysis of the extracted gelatin

Element	mg/g of gelatin
Pb	0.440
Al	0.890
Cu	0.013
Zn	0.040
Ca	0.59

CONCLUSION

Gelatin is an important natural bio-polymer, it required for several industrial and pharmaceutical application. Simple and low-cost free chemical method has been examined to extract gelatin based on temperature – pressure instead of traditional alkaline and acidic hydrolyzes methods. Gelatin has been extracted from the animal bone that generated as slaughterhouse by-product. The physical properties of the product are closed to the commercial industrial gelatin; the extracted product can be utilized in industrial activities as technical gelatin.

REFERENCES

- [1] H.W. Kang, Y. Tabata and Y. Ikada, *Biomaterials*, **20**, 1339 (1999).
- [2] L. Ren, K. Tsuru, S. Hayakawa and A. Osaka, *J. Sol-Gel Sci. Tech.*, **21**, 115 (2001).
- [3] Narang, A. S., Delmarre, D. and Gao, D. 2007. Stable drug encapsulation in micelles and microemulsions. *International Journal of Pharmaceutics* 345:9–25.
- [4] H.W. Kang, Y. Tabata and Y. Ikada, *Biomaterials*, **20**, 1339 (1999).
- [5] L. Lu, C.A. Garcia and A.G. Mikos, *J. Biomed. Mater. Res.*, **46**, 236 (1999).
- [6] T. Kokubo, *J. Ceram. Soc. Japan*, **99**, 965 (1991).
- [7] L. Lu, C.A. Garcia and A.G. Mikos, *J. Biomed. Mater. Res.*, **46**, 236 (1999).
- [8] S.V. Madhally and H.W.T. Matthew, *Biomaterials*, **20**, 1133 (1999).
- [9] K. Tsuru, C. Ohtsuki, A. Osaka, T. Iwamoto and J.D. Mackenzie, *J. Mater. Sci.*, **8**, (1997).
- [10] Rahman, M. S., Al-Saidi, G. S. and Guizani, M. 2008. Thermal characterisation of gelatin extracted from yellowfin tuna skin and commercial mammalian gelatin. *Food Chemistry* 108:472–481.
- [11] Mohamed, O.A., Habib, M.A. and El Sayed, N.H., Production of leather fat liquoring agents from local sources as alternative for imported liquors, *Egypt J. Chem.* **52**, 507 (2009).
- [12] Ola A M, Habib, M A and El Sayed N H. *J ILTA* 2012; LX : 456 – 468. *Afr. J. Environ. Sci. Technol.*

- [13] MA and Heba YM . Research Journal of Pharmaceutical, Biological and Chemical Sciences, 8(4), 2017 , 474.
- [14] T. Coradin, S. Bah and J. Livage, Colloids Surf. B: Biointerfaces, 35, 53 (2004).
- [15] R. Langer and J. Vacanti, Science, 260, 920 (1993).
- [16] j. A. White, R. J. Hart and J. C. Fry. Journal of Automatic Chemis. 8(4), 1986, 170-177.
- [17] I. Brasack, H. Bottcher and U. Hempel, J. Sol-Gel Sci. Technol., 19, 479 (2000).
- [18] Gómez-Guillén, M.C., Giménez, B., López- Caballero, M.E., Montero, M.P., Food Hydrocoll, 2011, **25**, 8, 1813-1827.
- [19] V.M. Gunko, I.V. Mikhailova, V.I. Zarko, I.I. Gerashchenko, N.V. Guzenko, W. Janusz, R. Leboda and S. Chibowski, J. Colloid Interface Sci., 260, 56 (2003).
- [20] Q. Chen, F. Miyaji, T. Kokubo and T. Nakamura, Biomaterials, 20, 1127 (1999).