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Gelatin Preparation from Cow's Bone by Heat-Pressure Method

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The main sources of gelatin are pig skin, cow hide and fish bone or skin. The extraction of gelatin could be done by three different processes *i.e.*, acid process, alkali process and heat-pressure process. The heat-pressure method to extract gelatin may produce a gelatin with a lower quality but the processing time is also low. In this study gelatin was extracted from cow's bone by heat-pressure process and some properties of the product was investigated. The cow's bone cured in a high-pressure tank in boiling water for 5 h. Then the gelatin solution was filtered, defatted and the remaining solution was concentrated. This solution was dried at room temperature. The colour of the gelatin obtained was dark yellow with colorimetric indexes of L, a, b* of 57.5, 5.1, 24.7, respectively. The moisture content of gelatin was 9.6 % and the gelatin ash content is determined by pyrolysis at 900 °C that it was 2.4 %. The pH of solution (1 % w/v) was about 7. The viscosity of a 6.67 % solution at 25 °C was zero. The gelatin doesn't have good gel strength. The amino acid analysis of the produced gelatin exhibited that the high amounts of amino acids belong to glycine, glutamic acid and aspartic acid, respectively. Atomic absorption analysis also showed that elements such as Cu, Pb, Al, Ca and Zn are existed in the product.

Key Words: Gelatin, Cow's bone, Heat-pressure method.

INTRODUCTION

Gelatin is a natural protein that is obtained by thermal denaturation or physical and chemical degradation of collagen[1-4]. The wide interest in gelatin is now mainly due to its biodegradability, biocompatibility and non-immunogenic. Food, pharmaceutical and photographic industries are the main users of gelatin. Its most frequent uses in the biomedical field include hard and soft capsules, microspheres, sealants for vascular prostheses, wound dressing, adsorbent pad for surgical use and implantable devices. With respect to collagen, gelatin is much cheaper and easier to obtain in concentrated solutions[1,2,5,6].

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The most common and important sources of collagen and gelatin are bone, pig skin, cow hide, fish skin and fish bone[3,6,7]. For producing of gelatin there are four processes: a) the acid process (for type A gelatin) is mainly used with pig skin and fish skin. The skin is treated with acid at pH = 4[3,6,7], b) The alkaline process (for type B gelatin)is mainly used with bovine bones and hide. At this process bone is demineralized with 4 % HCl, then ossein (demineralized bone) is treated with lime[6-10], c) Enzymatic process which are often used to increase the efficiency and reduce processing time for type A gelatin[8], d) The heat-pressure process which is used for bone. The degreased bone is treted by heat-pressure (133 °C/3bar) process. The gelatin obtained is of limited quality and use[6]. Among all of these process, acid and alkaline process are the best and the most widely used[9,10]. The method reported by Cole. C.G.B[11] for producing gelatin of bovine hide, it takes about 5 weeks and requires chemicals and a large amount of water for washing.

Gelatin extraction of fish skin or bone[12] both need acidification, which for skin it lasts for 16 h and for bone for 9-12 d.

According to method reported by Coradin *et al.*[13], gelatin extraction of marine skin includes acidification and the extraction continues in water over the night.

In production type a gelatin from pig skin[8], acidification lasts for 8 to 30 h and for type B gelatin from bone[8], demineralization with acid lasts for 5 to 7 d and the process continues with liming for 35 to 70 d. In all the mentioned methods, a large amount of time is necessary for processing.

In present work, the heat-pressure method for producing gelatin was considered to obtain a gelatin with specific properties. The main reasons for choosing this method are saving the time and elimination of chemicals.

EXPERIMENTAL

Gelatin preparation: The cow's bone was crushed and washed with water. The crushed bones were treated in a high-pressure tank along with water at ratio of 1:3 (bone:water) at 104°C and 1.2 atmosphere pressure for 5-6 h. The gelatin solution was then filtered with a multi-layer of gauze (cloths filter) to remove all solid materials. This solution was first defatted by phase separation method then the remaining gelatin solution was boiled to achieve a concentrate gelatin solution. The solution was dried at room temperature for 48 h and milled. The produced powder by the name of cow's bone gelatin (CBG) is utilized[8].

Determination of colour: The colour of the CBG powder was determined with a colourimeter (colour eye 7000A-Gretagmacbeth), working with D65 (day light) at 10 visual angle, being used CIELab colour parameters (L*, a*, b*). The colour parameters were measured and calculated by computer and colour of gelatin was expressed as values of L*, a*, b* and also colour difference (DE*) expressed as compared with standard sample (Merck gelatin powder).

Determination of moisture content: Ceramic beaker was dried at 104 °C for 24 h. The beaker was cooled down in a vacuum incubator and precise weight was recorded. Approximately 1 g of CBG was put in ceramic beaker and dried at 104°C for 24 h, cooled down in a vacuum incubator and the exact weight was recorded and the moisture content in bone gelatin was calculated.

Determination of pH: The pH of 1 and 2 % gelatin solutions was determined by a pH-meter (Horiba - N12) at 25.6°C.

Determination of viscosity: The viscosity of a 6.67 % gelatin solution[12-14] at 25 °C was determined with rotary viscometer (Especialidades, Medieas, MYR S.L., Type V2-R, Spain) at speed of 10 and 30 rpm.

Determination of Ash: The ash content of CBG was determined by pyrolysis of 1g gelatin at 900 °C to reach a constant weight.

Amino acid analysis: Amino acid analysis was conducted using Pico Tag method. This method involves three steps: 1) Hydrolysis of the gelatin to yield free amino acids, 2) Pre-column derivatization of the sample that at this step gelatin is first hydrolyzed with HCl, then derivatized with phenylisothiocyanate to produce phenylthiocarbamyl amino acids. 3) Analysis by reverse phase HPLC (Waters 1525). Analyzed amino acids were detected by dual λ absorbance detector (Model: Waters 2487).

Atomic absorption analysis: The amount of the Cu, Ca, Zn, Al, Pb, Cr in CBG were quantified on a atomic absorption spectrophotometer (Varian-Spectr AA-200).

RESULTS AND DISCUSSION

The results of the determination of gelatin were expressed as the colour parameters L*, a*, b* and the difference of colour (ΔE *) in relation to the standard gelatin powder. The amount of the colour parameters of cows bone gelatin and standard gelatin are shown in Table-1.

The results of the moisture content, ash content, pH solution and viscosity of CBG are shown in Table-1. The amino acid composition of CBG is listed in Table-2. The results of atomic absorption analysis of CBG are shown in Table-3.

In order to produce gelatin from cow's bone by heat-pressure method the required time for treatment and concentrate steps is less than 10 h and drying at room temperature takes 48 h that can be decreased by using warm air. At the earlier methods mentioned for gelatin production[8,11-13], the time

TABLE-1
CHARACTERISTICS OF COW'S BONE GELATIN

Characteristics	Cow's bone gelatin	Merck gelatin
L*	57.50	71.7
a*	5.10	0.5
b*	27.70	19.6
ΔE*	15.70	–
Moisture content (%)	9.60	–
pH (solution 1 %)	7.01	–
pH (solution 2 %)	6.77	–
Ash (%)	2.40	–
Viscosity (mPa.s.)	0.00	–

TABLE-2
AMINO ACID COMPOSITION OF COW'S BONE AND
BOVINE HIDE GELATINS

Amino acids (AA)	gAA/100 g gelatin	
	Cow's bone gelatin	Bovine hide gelatin[15]
Aspartic acid	11.47	7.46
Threonine	3.15	2.11
Serine	2.94	3.62
Glutamic acid	15.56	11.28
Proline	9.40	12.52
Glycine	17.24	32.63
Alanine	6.67	10.88
Valine	2.09	2.18
Methionine	0.78	0.42
Isoleucine	1.15	1.44
Leucine	2.27	3.00
Tyrosine	0.66	0.40
Phenylanine	3.15	1.99
Lysine	3.78	3.46
Histidine	0.67	0.77
Arginine	2.38	9.90

TABLE-3
ELEMENTS (E) IN 1 g COW'S BONE GELATIN

E	Ca	Cu	Zn	Al	Pb	Cr
mg	0.340	0.003	0.033	0.280	0.320	< 0.004

is necessary for producing gelatin of bone is too long. Most of them also need chemicals during the processing. The method used in this research for producing gelatin of cow's bone, was done with short time and no chemicals, but in the severe conditions.

CBG obtained, was dark yellow in colour and its solution was turbid. The colourimetric results of CBG compared to standard gelatin showed that the CBG has a lower lightness because the L* for CBG is 57.5 and for standard gelatin and Nile Tilapia film is 71.7 and 90.02[16], respectively. The CBG with higher a*(5.1) than standard gelatin a*(0.5) looks more reddish. This difference can be observed visually and its yellowness b* (24.7) is higher than standard gelatin b*(19.6) and fish gelatin b* (11.34)[16].

Colour difference (ΔE*) of CBG and standard gelatin was mainly due to the variation of L*, that it means the CBG has the lower lightness. This is probably resulted of either existence of metal ions in gelatin or more hydrolysis of peptide bonds due to heat and pressure treatment.

Moisture content of CBG is 9.6 %. However, bovine hide gelatin's moisture content[15] is 10.3 %. The difference could be the result of variations of amino acids between two gelatins.

CBG contains 2.4 % ash which is allowed to use in food industry[3,8] and it is much less than the amount of ash reported[12] for fish bone (% 11.2).

Viscosity is affected by raw material and production process. It was observed that the viscosity of gelatin solutions with concentration less than 20 % was approximately zero[7]. The viscosity of 6.67 % CBG solution was zero. Obviously as gelatin concentration increases, the viscosity increases. The gelatin with zero viscosity was dissolved easily in cold water[17].

The amino acid composition of CBG was found to be approximately similar to bovine hide gelatin (type B gelatin) (Table-2). However, there is a drastic difference between the amount of glycine, arginine and alanine in two gelatins. This is resulted from raw material sources[12]. It means that the amino acid composition may not be affected by the production method. The proline content of CBG (9.49 g/100 g gelatin) is higher than 3 and 8.9 g/100 g reported for Tilapia (cold water fish) and Myofibrilar proteins[16], respectively, but lower than 12.5 (g/100 g gelatin) reported for Nile perch fish[12] and bovine hide gelatins[15]. The CBG is found to contain proline at level between those of mammalian gelatins and those of coldwater fish species. However, the researcher reported that the proline content of gelatins has a strong influence on their functional properties[12,13,18]. The results of atomic absorption showed the existence of Zn, Cu, Al, Pb, Ca and a little Cr in CBG (Table-3). The amount of calcium (0.34 mg/g) is higher than 0.29 mg/g reported for fish gelatin[18-20] that it is resulted from mineral part of bone and used tap water(hard water) for gelatin production. The amount of lead is higher than 1.5 mg/kg reported by Food Chemical Codex[8,21-23]. This is probably as a result of high air-pollution.

Conclusion

In this research, gelatin was obtained from cow's bone by heat-pressure method. The advantages of this method compared to other conventional methods, are short processing time and no need chemicals. The obtained gelatin is approximately the same as other gelatins in the amount of ash, moisture content, pH and the composition and amounts of amino acids. The colour of cow's bone gelatin in comparison with other common gelatins is a little darker and the solution is more turbid, which is less important. The viscosity of diluted solutions is zero. However, as the concentration increases the viscosity increases so that the 10 % gel solution can produce suitable films. It was observed that the gelatin was approximately free from chrome, but the amount of lead was about 0.03 % which is higher than the reported amounts[22,23] (in conventional processes). Therefore, it is necessary to eliminate lead for using in food and medical applications.

REFERENCES

1. A. Bigi, G. Cojazzi, S. Panzavolta, N. Roveri and K. Rubini, *Biomaterials*, **23**, 4827 (2002).
2. A. Bigi, G. Cojazzi, S. Panzavolta, K. Rubini and N. Roveri, *Biomaterials*, **22**, 763 (2001).
3. R. Langer and J. Vacanti, *Science*, **260**, 920 (1993).
4. L. Ren, K. Tsuru, S. Hayakawa and A. Osaka, *J. Sol-Gel Sci. Tech.*, **21**, 115 (2001).
5. K.S. Tenhuisen and D.W. Brown, *J. Biomed. Mater. Res.*, **28**, 27 (1994).
6. H.W. Kang, Y. Tabata and Y. Ikada, *Biomaterials*, **20**, 1339 (1999).
7. L.L. Hench, *J. Am. Ceram. Soc.*, **74**, 1487 (1991).
8. T. Kokubo, *J. Ceram. Soc. Japan*, **99**, 965 (1991).
9. L. Lu, C.A. Garcia and A.G. Mikos, *J. Biomed. Mater. Res.*, **46**, 236 (1999).
10. C. Ohtsuki, T. Kokubo and T. Yamamuro, *J. Non-Cryst. Solids*, **143**, 84 (1992).
11. K. Tsuru, C. Ohtsuki, A. Osaka, T. Iwamoto and J.D. Mackenzie, *J. Mater. Sci.*, **8**, (1997).
12. S.V. Madihally and H.W.T. Matthew, *Biomaterials*, **20**, 1133 (1999).
13. T. Coradin, S. Bah and J. Livage, *Colloids Surf. B: Biointerfaces*, **35**, 53 (2004).
14. L. Ren, K. Tsuru, S. Hayakawa and A. Osaka, *Biomaterials*, **23**, 4765 (2002).
15. I. Brasack, H. Bottcher and U. Hempel, *J. Sol-Gel Sci. Technol.*, **19**, 479 (2000).
16. L. Ren, K. Tsuru, S. Hayakawa and A. Osaka, *J. Sol-Gel Sci. Technol.*, **21**, 115 (2001).
17. L. Ren, K. Tsuru, S. Hayakawa and A. Osaka, *J. Non-Cryst. Solids*, **285**, 116 (2001).
18. 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).
19. M. Schuleit and P.L. Luisi, *Biotechnol. Bioeng.*, **72**, 249 (2001).
20. D.M. Liu and I.W. Chen, *Acta Mater.*, **47**, 4535 (1999).
21. R. Cortesi, C. Nastruzzi and S.S. Davis, *Biomaterials*, **19**, 1641 (1998).
22. Q. Chen, F. Miyaji, T. Kokubo and T. Nakamura, *Biomaterials*, **20**, 1127 (1999).
23. V. Matejec, K. Rose, M. Hayer, M. Pospisllova and M. Chomat, *Sens. Actuators B: Chem.*, **39**, 438 (1997).

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