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Comparison between Bovine Hide and Pigskin Gelatins and Preparation of Gelatin Hydrolysates

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Abstract

Bovine hide and pigskin gelatins were prepared and their molecular weight profiles were examined by SDS-PAGE. The major molecular weights of bovine hide gelatin were 220 kDa, 140 kDa, and 130 kDa and the weights of pigskin gelatin were 210 kDa, 135 kDa and 120 kDa. Also, as a typical parameter of rheological property of the gelatin, viscosities of gelatin were measured under various conditions. Gelatin hydrolysates were prepared using typical commercial proteases and their angiotensin converting enzyme inhibitory activities were examined.

Key words: gelatin, ACE inhibitor, protein hydrolysate

INTRODUCTION

Gelatin is obtained by the partial hydrolysis of collagen, which is the major protein component in skins, bones, and connective tissues of the animal body and it is composed of α-, β-, and γ-chains (1-3). The conversion of collagen to gelatin involves the break-down of the collagen backbone chain by acid or base and the rupture of a considerable amount of cross-links that hold the chains together, resulting in the formation of a random coil structure. Gelatin derived from an acid-treatment is known as Type A, and gelatin derived from an alkali-treatment is known as Type B. Most commercial gelatin contains molecular species from 15 kDa to above 250 kDa (2). Although many studies on pigskin gelatin have been reported (4-8), there have not been any systematic studies on comparison of molecular weight profiles of pigskin and bovine hide gelatins.

In this study, to further elucidate the functional properties of gelatin for food application, gelatins were prepared from bovine hide and pigskin and their molecular weight profiles were determined and viscosity values were measured under various conditions. Also, to find out useful functional biomaterials, gelatin samples were enzymatically hydrolyzed and antihypertensive properties of the hydrolysates were determined by measuring angiotensin converting enzyme (ACE) inhibitory activity. ACE (peptidyldipeptide hydrolase, EC3.4.15.1) converts angiotensin I into angiotensin II by cleaving the C-terminal dipeptide (His-Leu) of angiotensin I and also inactivates bradykinin which depresses blood pressure. Thus, ACE inhibitor acts on the inhibition of ACE and results in a decrease of blood pressure and was screened from protein hydrolysates of various food sources (9-13). Therefore, in this study, after preparation of gelatin hydrolysates, it was examined for ACE inbitory activity.

MATERIALS AND METHODS

Preparation of gelatin

Bovine hide was dehaired and soaked in 15% $Ca(OH)_2$ solution for 4 weeks. After washing in running water for 24 h, the pH of the solution was adjusted to 4-5 and extracted at 55°C for 6 h. Extracted gelatin was dried to less than 10% of water content and crushed and passed through a 20 mesh sieve. Also, pigskin was washed in running water for 12 h and soaked in 2% of HCl solution at 25°C. After washing in running water for 24 h, the pH of the solution was adjusted to 4-5 and extracted at 55°C. Extracted pigskin gelatin was treated by the same method as that of bovine hide.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

SDS-PAGE was performed according to the method of Laemmli (14).

Viscosity measurement

Viscosity of gelatin was measured using a Brookfield viscometer (Brookfield Engineering Lab Inc., Model DV-1, USA).

Preparation of gelatin hydrolysates

Gelatin was hydrolyzed with 1% of Flavorzyme (Novo Co., Switzerland), Protamex (Novo Co., Switzerland), and Protease N.P. (Pacific Co., Korea) at 50°C for 2, 8, and 16 h, respectively and the hydrolysates were filtered using a PM-10 membrane (Amicon Co., USA).

Trinitrobenzenesulfonic acid (TNBS) assay

Concentration of peptide was determined according to the modified TNBS method (15).

Assay for ACE inhibitory activity

ACE inhibitory activity was measured by the modified meth-

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od of Cushman and Cheung (16). The reaction mixture contained 150 μl of 5 mM Hip-His-Leu as a substrate, 50 μl of rabbit lung acetone powder (5 m unit) in a 50 mM sodium borate buffer (pH 8.3), and 50 μl of sample solution. The reaction was carried out at 37°C for 30 min, and terminated by addition of 250 μl of 1 N HCl and 1 ml of ethylacetate. After centrifugation, the supernatants were dried, dissolved in a sodium borate buffer (pH 8.3), and the absorbances were measured at 228 nm.

RESULTS AND DISCUSSION

Comparison between bovine hide and pigskin gelatins

Gelatin samples were prepared from pigskin and bovine hide samples according to the method described in Fig. 1. Although there have been many reports on characterization of pigskin gelatin (4-8), there is no systematic study on the comparison of molecular weight profiles of pigskin and bovine hide gelatins. Especially, SDS-PAGE profile of gelatin is not easy to obtain due to technical difficulties such as solubilizing of gelatin sample. In this study, we used a sample buffer containing 8 M urea for SDS-PAGE. Molecular weight profiles of bovine hide and pigskin gelatins were shown in Fig. 2. Major molecular weights of bovine hide gelatin were 220 kDa, 140 kDa, and 130 kDa, while pigskin gelatin were 210 KDa, 135 kDa, and 120 kDa species, indicating that they were mostly of molecular species smaller than collagen α-chain (2). Also, pigskin gelatin had a slightly smaller molecular species than bovine hide gelatin, mainly due to the difference of the acid (type A) and base treatment (type B). Regarding the application of gelatin prepared from various raw materials such as bovine hide, pigskin, beef bone etc., it suggests that the molecular weight of gelatin should be an important criteria in terms of its industrial application. Since the molecular weight profile of gelatin significantly affects rheological properties of gelatin, gelatin samples were compared with regard to viscosity, which is one of the typical parameters. As

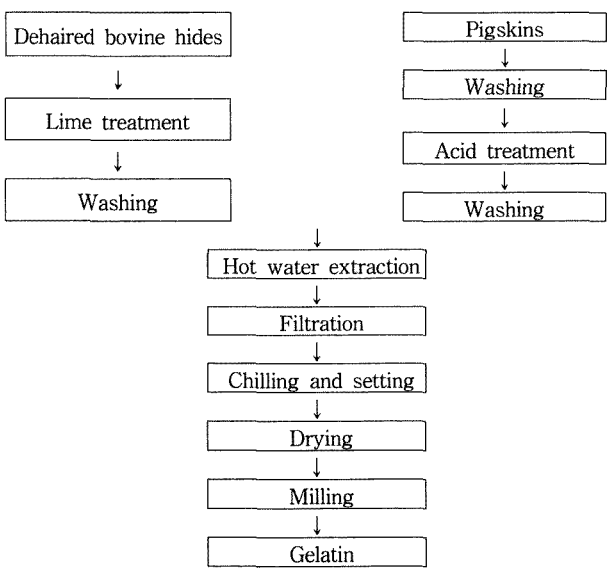

Fig. 1. Schematic diagram of preparation of gelatin.

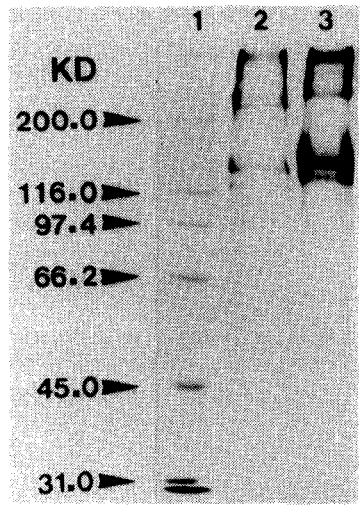

Fig. 2. SDS-PAGE profile of pigskin and bovine hide gelatin. Lane 1: molecular weight marker, myosin heavy chain (205 kDa), β-galactosidase (116 kDa), phosphorylase b (97.4 kDa), bovine serum albumin (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (29 kDa). 2: pigskin gelatin. 3: bovine hide gelatin.

expected, the viscosity of gelatin depends on gelatin concentration and temperature (Fig. 3). Viscosity increased rapidly

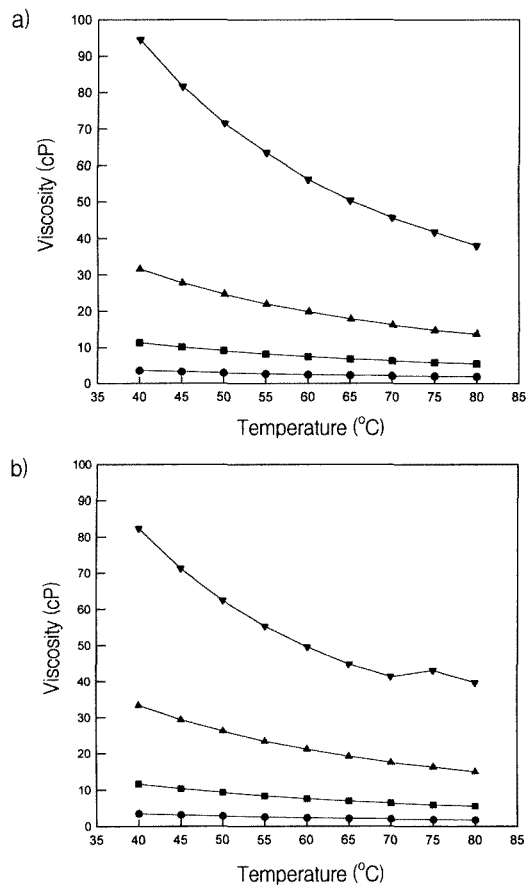

Fig. 3. Effect of gelatin concentration on the viscosity change of gelatin samples at various temperatures.
a) bovine hide gelatin, b) pigskin gelatin
●-●: 5% gelatin solution, ■-■: 10% gelatin solution, ▲-▲: 15% gelatin solution, ▼-▼: 20% gelatin solution

when temperature decreased below 40°C, especially in the case of 20% gelatin concentration. The increase in viscosity became more significant with increase of the concentration. Among the types of gelatins, at 20% gelatin concentration, the viscosity of bovine hide gelatin was 94.5 cP at 40°C, while the viscosity was 82.4 cP for pigskin gelatin. Although there was only a small difference in the viscosities of the gelatins, it is probably be due to the difference in the distribution of molecular weight size of the gelatins. However, the difference became smaller with the increase in temperature.

Preparation of gelatin hydrolysates

To find out whether there are functional materials such as ACE inhibitory peptides and to utilize the inexpensive gelatin, gelatin samples were enzymatically hydrolyzed using three different proteases. The degree of hydrolysis was measured by the modified TNBS assay (Fig. 4). The results indicated that the available amino group concentration as criteria for the degree of hydrolysis increased with the time of hydrolysis. However, in contrast to Flavorzyme, the degree of hydrolysis using Protamex and Protease NP did not change significantly with the time of hydrolysis, suggesting that two hours of hydrolysis was sufficient to prepare the gelatin hydrolysates. Especially, in the case of pigskin gelatin, after 8 h, the degree of hydrolysis decreased for Flavorzyme. Compared with the degree of hydrolysis for other proteins (17), this is favorable. Among the proteases, Flavorzyme was the best for the hydrolysis of bovine hide and pigskin gelatin. Based on TNBS data, ACE inhibitory activities of gelatin hydrolysates were measured and IC_{50} values were calculated (Table 1), assuming that the available amino group concentration was directly related to peptide concentration. For bovine hide gelatin, Protamex was the best although Flavorzyme was the best in terms of the degree of hydrolysis. This result strongly indicates that there is an appropriate degree of hydrolysis needed for preparation of ACE inhibitory molecules such as peptides. Also, in the case of pigskin gelatin, Protease NP was the best. Compared to IC_{50} values reported in the literature (10), these IC_{50} values are relatively high. Especially, Oshma et al. (18) reported that ACE inhibitory peptides purified from gelatin digested by bacterial collagenase was around 10 μg as IC_{50}. However, considering the crude fraction of ACE inhibitory materials, it is more promising since purification schemes should improve ACE inhibitory activity significantly. Thus, by using rather inexpensive raw materials of bovine hide and pigskin, crude preparation of enzymatic hydrolysates using commercial proteases may be a suitable way of reutilizing and isolating functional biomaterials such as ACE inhibitors.

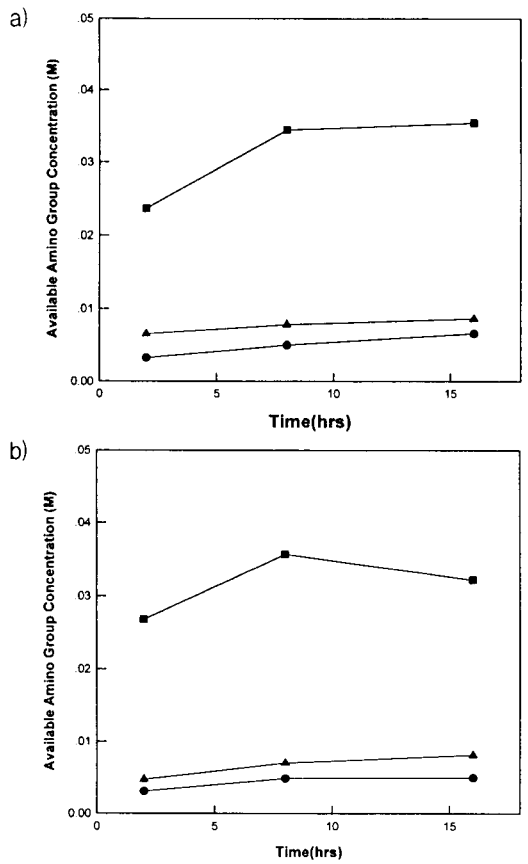

Fig. 4. Measurement of degree of hydrolysis of gelatin.
Degree of hydrolysis was measured by determining available amino group concentration according to the modified TNBS assay.
a) bovine hide gelatin, b) pigskin gelatin
■-■; Flavorzyme, ▲-▲; Protamex, ●-●; Protease NP

Table 1. ACE inhibition of pigskin and bovine hide gelatin hydrolysates

Sample	Enzyme	IC_{50} (mM)
Bovine hide gelatin	Protease NP	12
	Flavorzyme	27
	Protamex	6
Pigskin gelatin	Protease NP	3
	Flavorzyme	19
	Protamex	7

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