The Properties of the Hydrolyzate of Collagen Fiber Treated within Alkali-Neutral Salt Systems

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Abstract: Skin collagen fiber was treated within alkali neutral salt systems. The unsolved fiber and the soluble hydrolyzed protein were analyzed respectively. The hydrolyzate of collagen fiber in alkali and neutral salt systems had both non fibrous components and fibrous components, which were ruptured by alkali solutions. The fiber axial changed to flat which distinguished with the circle axial of natural collagen fiber. The pI values of the solutions were at acidic region. There was a trend that increasing the concentration of neutral salt would shift the value of the hydrolyzate's pI to more acidic region. The characteristic triple helix structure of collagen was denatured completely when hydrolyzated in alkali neutral salt solution. Alkali took charge of the hydrolysis of collagen fiber.

Key words: collagen fiber; alkali; neutral salt

1 Introduction

Type I collagen is the main protein constituent of skin and hide with an insoluble fibrillar form structure. Collagen is characterized with triple helical conformation which is formed by three interwoven α chains. This assembly is the direct consequence of the primary structure characterized by the repetition of Gly-X-Y triplet sequences [1]. Intra-molecular and inter-molecular hydrogen bonds are responsible for the stability of the triple helix in collagen [2]. The trimers of collagen will be formed to fibrils and fibers by the supramolecular assembly. A feature that distinguishes them from other fibrillar forms of macromolecules is that they are most easily recognized by their axial 67 nm periodicity, which can be seen in atomic force microscopy, electron microscopy, and can also be inferred from X-ray diffraction data [3].

Collagen fiber of the skin and hide would be swelled by absorbing water in alkali solutions, and would be hydrolyzed, thus the hydrolyzate could be solved in the solution. During the swelling process, the ion linkages and hydrogen bonds among collagen molecules will be open up. The swelled skin and hide expressed with weight increasing, fibril diameter enlarging, and the hardness of the skin increasing. The isoelectric point of collagen fiber would be shifted toward lower pH value after treated with alkali solution, because the amide groups of the side chain were hydrolyzed to carboxylic groups by the reaction of alkali [4, 5].

In leather making process, skin would be treated with lime, enzyme and acid to open up the collagen fiber, in order to give the finished leather a suitable softness, fullness and handle. Although technicians recognized that collagen fiber could be "open-up" in the lime solution, but the aggregation state of the collagen fiber treated in the alkali solution was seldom researched. Maxwell et al [6] used small angle and wide angle X-ray diffraction to measure collagen fiber changes before and after lime treatment.

The fiber structure of animal skin and hide exist section difference, which will influence the

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evaluation of the experiments. To avoid this problem, we used acid relaxed collagen which was prepared from pigskin, to study the physical and chemical behaviors of collagen fiber in alkali solutions.

2 Materials and Methods

2.1 Materials

Acid relaxed collagen fiber was extracted from pigskin [7, 8]. The fresh pigskin was fleshed and degreased, and unhaired by coated protease on the flesh side. Then it was sliced and swelled in 0.5 M acetic acid at 4°C, with 200 u/g A.S.1398 protease at 35°C, in 1% ammonium nitrate for 3 hour, treated with 0.5 M sodium borohydride, with a pH about 10.0. The slices were acid relaxed in 0.05 M acetic acid at about 4°C. Then they were dispersed by tissue disperser. During disperse, cold diluted acetic acid was added to decrease the viscosity. The dispersed liquid was filtered twice, and neutralized to pH 6.5-6.8 with 5% sodium bicarbonate. The neutralized collagen fiber aggregates were then floated to the surface and collected to be ice dried.

2.2 Hydrolysis of collagen fiber in the solution of alkalis

The alkalis used in this experiment were sodium hydroxide, potassium hydroxide and calcium hydroxide. The concentration of them was 0.05 M, 0.1 M, 0.5 M, to calcium hydroxide the content is saturated solution. The temperatures of the experiment were 4°C and 25°C. 50 ml certain concentration of alkali solutions, 50 mg acid relaxed collagen fiber were put into 100 ml conical flask, which was shaken under certain temperature for 48 h. The hydrolyzed solution was determined with circular dichroism spectrum, SDS-PAGE electrophoresis and Zeta potential titration respectively. The un-hydrolyzed fiber was ice dried and investigated with SEM and TEM.

2.3 Gel electrophoresis [9]

The hydrolyzate solutions were determined by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). The gel concentration of electrophoresis was 8%. The voltage was keeping at 200 V and the electrophoresis was running for 2.5 hours (Powerpac© Basic Power Supply; Bio-Rad Company). The gel was then stained with Coomassie blue R-250, and destained with 10% methanol and acetic acid mixture.

2.4 Isoelectric Point (pI) Determination

Zeta potentials of the hydrolyzate solutions were determined with Zetasize Nano series (Malvern) [10]. It was autotitrated with 0.25 M hydrochloric acid, and the titration pH range was from their current pH to 3.5.

2.5 Scanning electron microscopy (SEM) of un-hydrolyzed collagen fiber

Samples of collagen fiber were attached to alum SEM stubs using carbon tabs. Specimens were sputter coated with gold prior to examination using a JSM-5900LV SEM (Japan Electron Optical Laboratory Co., LTD).

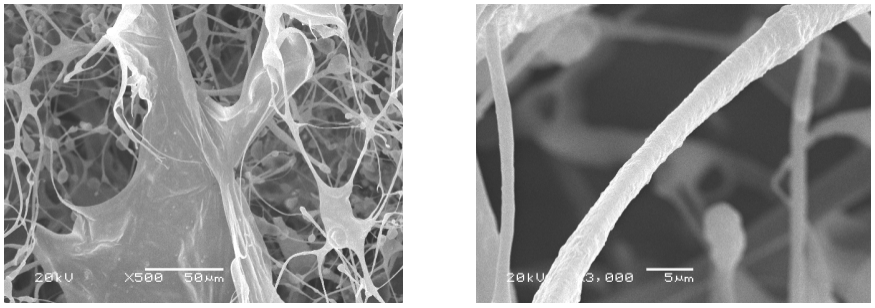
2.6 FT-IR spectrum

FT-IR studies of the relaxed collagen fiber, unsolved collagen fiber and protein in the solution were recorded using a FT-IR instrument (Nicolet MAGNA. IR506, PE).

3 Results and discussion

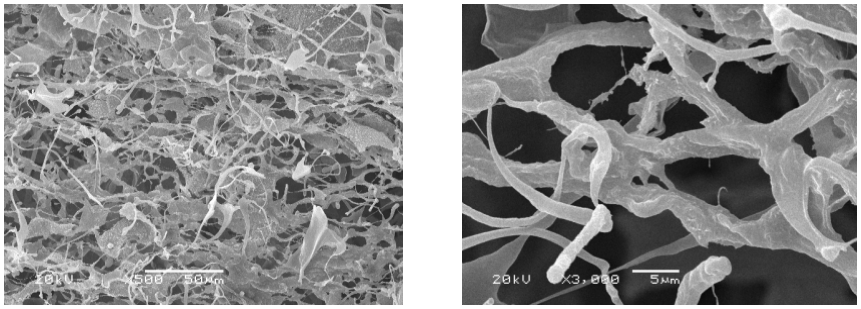
3.1SEM investigate of the hydrolyzate of collagen fiber under alkali-neutral salt system

The hydrolyzates of collagen fiber under alkali-neutral salt system were dialyzed and ice dried, the dried samples were investigated with SEM, and the photos were showed in Fig. 1.



×500 **×3000**

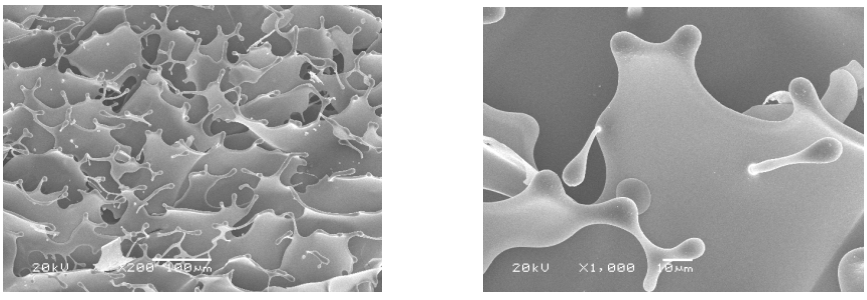
a 0.1 mol/L NaOH + 0.1 mol/L NaCl



×500 **×3000**

b 0.1 mol/L NaOH + 0.1 mol/L Na_2SO_4

Fig. 1 SEM photos of the hydrolyzate in 0.1 mol/L NaOH-neutral salt (30°C)



×200 **×1000**

Fig. 2 SEM photos of gelatin

The hydrolyzate of collagen fiber in alkali and neutral salt system had fibrous components and non fibrous components. However, the fibrous components were coruptured according to the photos amplified with 3000 times. The photos of gelatin were showed in Fig. 2, in which no fibrous components existed.

3.2 Results of FT-IR investigation

In order to investigate the structure of the collagen hydrolyzate in alkali neutral salt system, the hydrolyzates were dialyzed and ice dried, analyzed with FT-IR spectrum. Figure 3 showed the FT-IR

spectrums of the hydrolyzates in the solutions of 0.1mol/L NaOH-0.1mol/Lsodium sulfate, 0.1mol/L NaOH-1.0mol/Lsodium chloride, 0.1mol/L NaOH and unsolved collagen fiber.

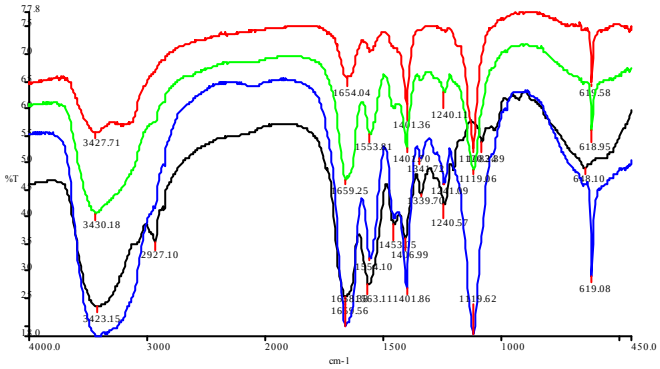


Fig. 3 FT-IR Spectrums of hydrolyzates in various alkali neutral salt system

The curves from up to bottom were: 0.1 mol/L NaOH; 0.1 mol/L NaOH -1.0 mol/L NaCl; 0.1 mol/L NaOH - 0.1 mol/L Na_2SO_4; unsolved collagen fiber

It could be seen that two strong peaks were appeared at 1660 cm^{-1} and 1552 cm^{-1} for the unsolved collagen fiber, however, the peaks were decreased at 1660 cm^{-1} and 1552 cm^{-1} for hydrolyzate in the solutions of alkali and neutral salt, but two new strong peaks were appeared at 1401cm^{-1} and 1118cm^{-1}. 1401cm^{-1} was the vibration peak of carboxyl group, 1118cm^{-1} was the character peak of sulfate, which was the remain of un dialyze of sulfate. The results of Payne and Veis [11] showed that during denaturation of the triple helix, the dominant 1660 cm^{-1} component in the native collagen spectrum diminished and the 1633 cm^{-1} peak became relatively intensified. So it could be concluded that the unsolved collagen fiber still maintained natural structure of collagen, but the hydrolyzate in alkali neutral salt solution was denatured completely.

3.3 Results of isoelectric point

Table 1 listed the isoelectric points of the hydrolyzate solutions of 0.1mol/L sodium hydroxide with different neutral salt treated collagen fiber for 48h under 20°C.

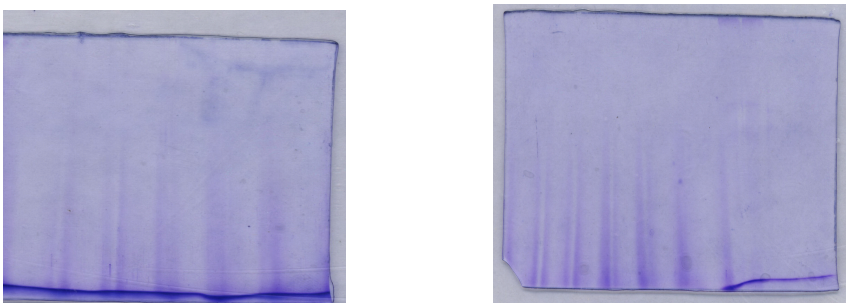
Table 1 Influence of salt concentration to PI of the hydrolyzates

NaCl /mol/L	pI	Na_2SO_4 /mol/L	pI
0	5.00	0	5.00
0.05	4.88	0.05	4.80
0.1	4.76	0.1	4.74
0.5	4.68	0.5	4.72
1.0	4.56	1.0	4.61

The pI values of the solutions were at acidic region, which was similar to the gelatin. We could found that there was a trend that the concentration of neutral salt was increased the pI value of the hydrolyzate would shift to more acidic region. The reason might be the influence of the ion strength to the determination with zeta potential method.

3.4 Results of SDS-PAGE

The molecular weight and its distribution of the hydrolyzate of collagen fiber in alkali and neutral salt were determined by SDS-PAGE electrophoresis.



a NaOH-neutral salt **b $Ca(OH)_2$ –neutral salt**

Fig. 4 SDS-PAGE spectrum of hydrolyzate of collagen fiber in alkali neutral salt

The hydrolyzate of collagen fiber treated with alkali and neutral salt system showed a continuous band but no special band was appeared in SDS-PAGE spectrum (Fig. 4), which was similar to the results of collagen fiber treated with alkali solutions only. It indicated that the addition of neutral salt would promote or decrease the hydrolysis rate of collagen fiber in alkali solutions, but could not change the hydrolysis location of collagen fiber. Alkali took charge of the hydrolysis of collagen fiber.

4 Conclusions

The hydrolyzate of collagen fiber in alkali and neutral salt systems had both non fibrous components and fibrous components, which were ruptured by alkali solutions. The fiber axial changed to flat which distinguished with the circle axial of natural collagen fiber. The pI values of the solutions were at acidic region. There was a trend that increasing the concentration of neutral salt would shift the value of the hydrolyzate's pI to more acidic region. The characteristic triple helix structure of collagen was denatured completely when hydrolyzated in alkali neutral salt solution. Neutral salt would promote or decrease the hydrolysis rate of collagen fiber in alkali solutions, but could not change the hydrolysis location of collagen fiber. Alkali took charge of the hydrolysis of collagen fiber.

Reference

[1] B. Brodsky; Shah N. The triple-helix motif in proteins. FASEB J, 1995, 9: 1537-1546.

[2] K. J. Bienkiewicz. Physical Chemistry of Leather Making. Rober E, Krieger Publishing Company, Inc. 1983.

[3] E. Heidemann. Fundamentals of Leather Manufacturing. Eduard Roether KB, 1993.

[4] K. H. Gustavson. The Chemistry and Reactivity of Collagen. Academic Press Inc. New York 10, 1956.

[5] I. Bergman; R. Loxley. Anal. Chem, 1963, 35(2): 1961-1965.

[6] C. A. Maxwell; T. J. Wess; C. J. Kennedy. Biomacromolecules, 2006, 7: 2321-2326.

[7] H. M. Cheng; R. Wang; Y. M. Wang; etal. Journal of Sichuan University (Engineering Science Edition), 2007, 39 (3): 78-82.

[8] I. Oneson; D. Fletcher; J. Olivo; etal. JALCA, 1970, 65(9): 440-450.

[9] Y. J. Guo. Protein electrophoresis experiment technology. Beijing: Science Press, 2001.

[10] H. M. Cheng; L. Wang; Z. Q. Li; etal. Leather Science and Technology, 2006, 16(6): 40-43.

[11] K. J. Payne; A. Veis. Biopolymers, 1988, 27: 1749-1760.