Recovery of Collagen from Phosphonium Tanned Leather Shavings and Application as Formaldehyde Scavenger

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Abstract

Samples of collagen extracted from phosphonium tanned leather shavings by three different methods (acid, alkali, magnesium oxide dissolution and alkaline protease hydrolysis treatments) were characterized with respect to their components and morphology of fibril network. The ratio of collagen gained was 90% by the process of alkali dissolution and enzyme hydrolysis, which was much higher than that of acid and alkali methods. The results from amino acid analysis showed that the components of extracted collagen were similar with that of commercial gelatin. The Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) results indicated that the extracted substance was the mixture of polypeptide and amino acid. The polypeptide and amino acid exposed to hydrophilic functional groups including carboxyl, hydroxyl, amino group etc. These functional groups especially amino group can react with formaldehyde which leads to scavenge formaldehyde. The morphological change of leather shavings treated by magnesium oxide and alkaline protease was clearly presented with scanning electron microscope. The percentage of formaldehyde removal and thickening rate of collagen were 51.8% and 10.4%, respectively. Therefore, the collagen from the leather shavings and used as the formaldehyde scavenger has a good potential in achieving the innocent treatment and reuses of the solid leather wastes.

Resumen

Muestras de colágeno extraídas de rebajaduras de cuero curtido al fosfonio por tres métodos diferentes (ácido, álcali, disolución por óxido de magnesio y tratamiento por hidrólisis alcalina con proteasa) fueron caracterizadas con respecto a sus componentes y su morfología de reticulación fibrilar. La proporción de colágeno recuperado fue 90% por el proceso de disolución por álcali e hidrólisis enzimática, la cual fue mucho más alta que las obtenidas de los procesos por ácido y [simplemente] álcali. Los resultados del análisis de los amino ácidos demostraron que los componentes del colágeno extraído fueron similares a los obtenidos de gelatina comercial. Los resultados de gel electroforesis por dodecil sulfato de sodio-poliacrilamida (SDS-PAGE) indicaron que las sustancias extraídas fueron una mezcla de polipéptidos y amino ácidos. Los polipéptidos y amino ácidos exponen grupos funcionales hidrofílicos tal cómo carboxilo, hidróxido, amino, etc. Estos grupos funcionales, en especial el grupo amino, potencialmente son reactivos al formaldehído, lo que los conducen a capturar formaldehído. Los cambios morfológicos en rebajaduras de cuero tratadas con óxido de magnesio y proteasa [de acción] alcalina son claramente discernibles por un microscopio por barrido electrónico. El porcentaje de formaldehído removido y la tasa del incremento en la viscosidad fueron 51,8% y 10,4%, respectivamente. Tal siendo el caso, que el colágeno obtenible de las rebajaduras y utilizable como capturador de formaldehído, tiene un buen uso potencial en el en logramiento de un tratamiento libre de toda crítica ecológica para el reciclaje de los desperdicios sólidos del cuero.

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INTRODUCTION

The leather industry is known to be associated with the generation of liquid, solid, and gaseous wastes. Solid wastes from the tanning industry are unavoidable.[1] The solid waste accounts for almost 25% of the weight of raw hide. During the manufacture process of high-grade leather, even 50% of the weight of raw hide became wastes.[2] Use of chromium in leather industry is being questioned owing to reports emerging on the toxicity and disposal problems associated with it.[3-5] Meantime, the leather industry will be restricted by more and more environmental protection policies. It is hence obvious that worldwide research is being focused on chrome-free tanning systems. Many studies based on less chrome and chrome-free technologies have emerged in the recent past.[6-9] In order to overcome the chrome related problems it is preferable to have a completely chrome free tanning system. A new Tetrakis hydroxymethyl phosphonium sulfate (THPS) tanning system is developed. The phosphonium tanning reduces the chrome pollution; while abundant phosphonium tanned leather shavings will cause another contamination. The recovery of phosphonium tanned leather shavings will become one of problems that need urgent solution for the development of chrome-free tanning technology.

Because many chemical materials are used in leather manufacturing process, some hazardous substances are left in leather.[10] Formaldehyde is one of hazardous substances, so its content in leather must be lower than limited values. The DIN standard for maximum tolerance limit for formaldehyde in finished leather articles for adults is 150ppm and for children is 75ppm.[11] At present, phosphonium tanning and oxazolidine tanning became a hot topic. In acid, alkaline or water medium, oxazolidine is unstable. It is easy to open the ring and produce aldehyde. So the leather tanned by oxazolidine will release free formaldehyde. The structural formula of oxazolidine is shown as follows. Therefore, it is essential to study formaldehyde scavenger. At present, the substances which were used to scavenge formaldehyde included amide derivatives, strong oxidative materials, porous inorganic filler, and so on.

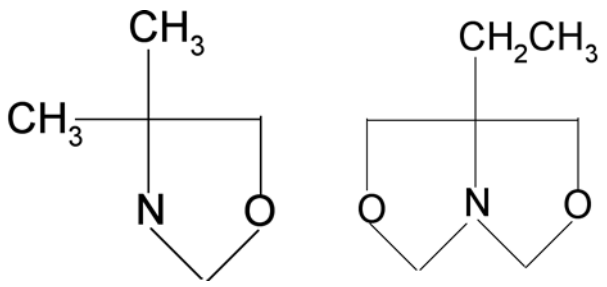


Figure 1. – The structural formula of oxazolidine.

In recent years, collagen is widely used in various fields, including foods, [12] cosmetics, [13] medical material and cell culture technology.[14-15] As a kind of protein, collagen has a specific amino acid sequence, size and structure. Collagen is generally produced for industrial use from animal pelts. In the study, the phosphonium tanned leather shavings were cut into small pieces, and then treated by acid, alkali or enzyme. Moreover, the chemical properties of collagen extracted from organic phosphonium tanned leather shavings was examined.[16] The aim was to employ a suitable extraction method for organic phosphonium tanned leather shavings.

EXPERIMENTAL

Materials and apparatus

Phosphonium tanned leather shavings were provided by Baoen Company in Shandong; PB05-1 alkaline protease (200000μ/g) was supplied by Pangbo biological engineering Co., Ltd in Nanning; Hyperbranched polymer with terminal amidogen(HPTA) was self-prepared with diethylene triamine and succinic anhydride; Anhydrous citric acid(purity: 99%), magnesium oxide(purity: 99%), sodium hydroxide(purity: 99%) and sodium dodecyl sulfate(purity: ≥95%) were all offered by Hua Bo-zhan chemical factory in Xi'an. TDL-40C centrifuge was provided by Anting Science apparatus factory in Shanghai; Beckman 121 amino acid analyzer was offered by Beckman Company of USA; DYY-7C electrophoresis instrument was provided by Bio-rad Company of USA; KYKY1000B scanning electron microscope was supplied by Chinese academic instrument factory.

Preparation of solubilized collagen

Acid-solubilized collagen: 25g phosphonium tanned leather shavings and 100g water were homogenized in 250mL three-neck flask, and then 20% citric acid (based on the weight of leather shavings) was added to hydrolyze the shavings at 40ºC for 7h. Alkali-isolated collagen: 25g phosphonium tanned leather shavings and 100g water were homogenized in 250mL three-neck flask, and then 20% sodium hydroxide (based on the weight of leather shavings) was added to isolate collagen at 40ºC for 7h. Alkali and enzyme-digested collagen: 50g phosphonium tanned leather shavings were immersed in 250g water in a three-neck round bottom flask, and then 0.1% sodium dodecyl sulfate and 6% (based on the weight of leather shavings) magnesium oxide were added to hydrolyze the shavings at 70°C for 2.5h. The optimal pH and temperature of alkaline protease were 8.0~9.0 and 45~55°C. The pH of reaction system was 8.0. Then lowered the temperature to 55°C, 0.6% alkaline protease (based on the weight of leather shavings) was incorporated into the three-neck flask, and keep the reaction for another 2h at a pH of 8.0. The temperature was raised to 90°C, so as to make alkaline protease lose activity. The resulting solutions were

all centrifuged at 4000rpm for 25min to remove insoluble substances. The residue was dried in oven and its weight was used to calculate the degree of extraction.

$$\text{The degree of extraction/\%} = \frac{M_0 - M_1}{M_0} \times 100\% \qquad \text{(Eq.1)}$$

In the formula, M_1 and M_0 were the weight of dry residue and leather shavings, respectively (g).

Determination of free formaldehyde content

The determination of formaldehyde content in leather was shown in references 17 and 18.

$$\text{The percentage of formaldehyde removal/\%} = \frac{W_1 - W_2}{W_1} \times 100\% \qquad \text{(Eq. 2)}$$

In the formula, W_1 was the content of formaldehyde in blank leather sample and W_2 was the content of formaldehyde in leather treated by collagen-derived formaldehyde scavenger (mg/kg).

SDS-PAGE measurement

According to the reference 19, the relative molecular weight of collagen polypeptide was determined.

Amino acid analysis

100mg collagen was hydrolyzed by 6mol/L hydrochloric acid of 10mL at 110°C for 24h. After filtrating and concentrating, the solution was dissolved in 50mL volume flask. 1mL solution, being dried and diluted by citric acid buffer solution whose pH was 2.2, was tested using amino acid analyzer. The analysis conditions: time was 60min, chromatogram column was 3μm×4.6mm×60mm, the flow speed of buffer solution and ninhydrin was 0.4mL/min and 0.3mL/min, respectively.

Scanning electron microscope (SEM) study

The morphological characteristics of leather shavings hydrolyzed by alkali-enzyme method were studied by SEM. The dried specimens were mounted on metal stubs with surface adhesive tape and then coated with gold using coater. The samples were then introduced into the specimen chamber of a SEM with a voltage of 25KV, and the surface morphology of leather shavings was examined.

Application test

The reaction of amino group and formaldehyde is greatly influenced by pH. In strong acid medium the amino group is protonized and no lone electron pair attacks aldehyde group, as a result, the reaction was restrained. But if in an even more alkaline medium, alkali will combine with proton and influence the transfer and dehydration of proton. So after neutralization the extracted collagen solution was used to scavenger formaldehyde. Moreover, it was compared with other formaldehyde scavengers. The functional groups especially amidogen, in chitosan, HPTA and collagen polypeptides, can react with formaldehyde, so their reaction mechanism of chitosan, hyperbranched polymer with terminal amidogen and formaldehyde were the same as that of collagen peptides.[20-22] For hydrogen peroxide,[23] the effect of pH on the reaction with formaldehyde was minimal, so it can be used in the same procedure as well. The tanning procedure was shown as follows.

TABLE I

The formaldehyde removal procedure of leather

Procedure	Chemical	dosage/%	T/°C	t/min	Remarks
	water	150	30		Drain
	salt	8		30	
Tanning	water	200	25		
	formaldehyde	3			
	Salt	8		120	Check cut
Neutralization	sodium bicarbonate	X	25		pH=8.0
Capturing formaldehyde	Formaldehyde scavenger	3		120	
Raising temperature			40	60	Over night, the next day running 30 min, washing and drying

RESULTS AND DISSCUSSIONS

The degree of extraction

The degree of extraction by using different methods was shown in TABLE II.

TABLE II

The degree of extraction of collagen by different methods

Extraction method	Extraction time/h	degree of extraction/%
Acid	7	22.3
Alkali	7	16.5
Alkali-enzyme	4.5	90.0

As shown in TABLE II, relatively little collagen was extracted from phosphonium tanned leather shavings by acid and alkali, while most of collagen was digested by alkali-enzyme treatment. However, since the time required for alkali-enzyme method was much less than that required for acid and alkali methods, alkali-alkaline protease method was a better method. Because the tanning mechanisms of chrome and organic phosphonium were different and it was necessary to dechrome in extracting collagen from chrome tanned leather shavings, acid method and alkali method were suitable for extracting collagen from chrome tanned leather shavings, but not for phosphonium tanned leather shavings.

SDS-PAGE analysis

SDS-PAGE is a commonly used method to detect relative molecular weight of protein. The relative molecular weight of collagen was shown in Figure 2.

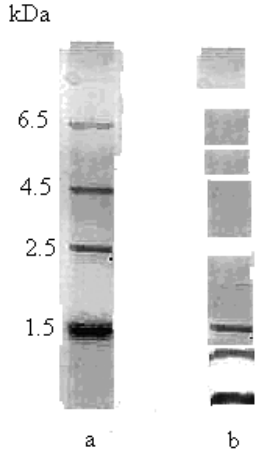


Figure 1. – The SDS-PAGE results of extracted collagen.

The extracted collagen was the mixture of polypeptide with different relative molecular weight. Moreover, there was a little amino acid in collagen. So the appropriate gelatin concentration in SDS electrophoresis should be strictly controlled. Figure 1 showed the electrophoresis patterns of the standard substance(a) and extracted collagen(b) from phosphonium tanned leather shavings. The collagen samples displayed three bands and their position all were below 1500. The collagen with higher molecular weight was few. Its relative molecular weight respectively was 1632, 659 and 83, which proved the extracted collagen was the mixture of polypeptide and amino acid. Because the activity of alkali protease was very strong, the relative molecular weight of extracted collagen was small. The formaldehyde removal mechanism of collagen was the reaction of amino group and aldehyde group, so the smaller of the relative molecular weight, the more of the amino group and the better of the formaldehyde removal effect. It could be used for formaldehyde scavenger.

Amino acid analysis

The amino acid components of extracted collagen, gelatin and acid-solubilized collagen were compared. The results were shown in Figure 2 and TABLE III.

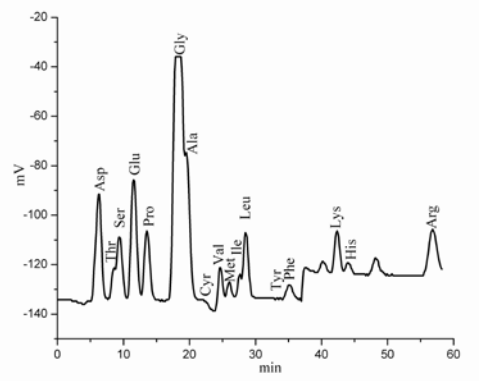


Figure 2. – The amino acid components of extracted collagen.

As shown in Figure 2 and TABLE III, the percentage of glycin in total amino acids was 22.18% which was less than that of acid-solubilized collagen and gelatin. The determination methods and conditions had influence on the content of amino acid; especially the effect of the control to hydrolysis degree was obvious. According to the reference, the content of proline in protein was few, but in collagen it was the most.[18] Take the consideration of the results from GPC and amino acid analysis, the extracted substance from phosphonium tanned leather shavings was the mixture of polypeptide and amino acid.

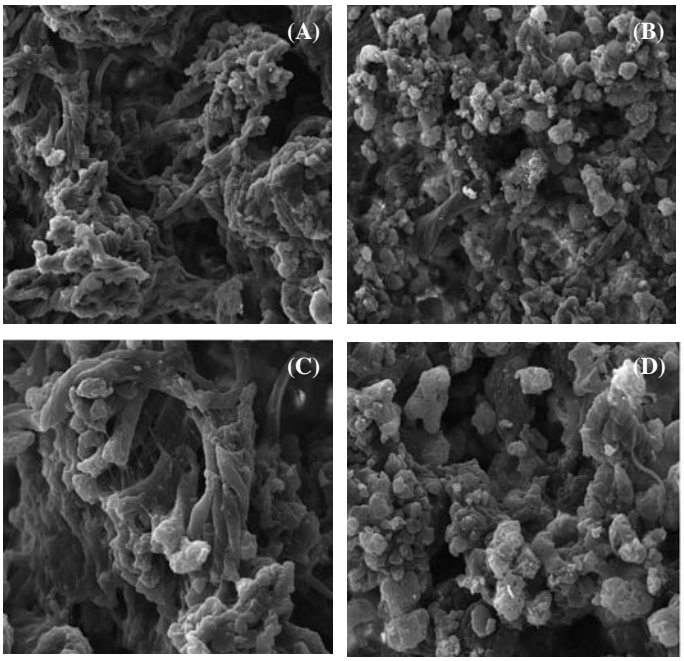
SEM analysis

Observation of the appearance and network structure of the collagen isolated from phosphonium tanned leather by two-step method of alkali and alkaline protease was carried out by SEM, and SEM images were shown in Figure 3.

TABLE III

The contrast of amino acid components of different collagen

Components	Different collagen		
	Extracted collagen	**Acid-solubilized collagen[24]**	**Commercial gelatin[24]**
Asp	7.08	4.46	4.68
Thr	1.56	1.82	1.71
Ser	2.80	3.97	3.65
Glu	8.76	7.37	7.20
Pro	10.94	11.33	13.04
Gly	22.18	34.10	32.60
Ala	6.085	11.51	11.08
Cys	0.121	—	—
Val	1.635	1.90	2.19
Met	1.156	0.51	0.54
Ile	1.526	1.04	0.96
Leu	2.586	2.40	2.37
Tyr	0.142	0.28	0.32
Phe	1.506	1.18	1.44
Lys	2.18	2.40	2.62
His	0.595	0.19	0.60
Arg	5.707	4.71	4.82



Remarks: the magnification times of A and B figures were 200, the magnification times of C and D figures were 400.

Figure 4. – The appearance of leather shavings in different reaction stages.

In the experiment, phosphonium tanned leather shavings were first treated by magnesium oxide, and then further hydrolyzed with alkaline protease. The morphological change of collagen fiber after treated by magnesium oxide and alkaline protease was observed. In Figure 3, SEM images of leather shavings treated only by magnesium oxide (Figure A and C) showed that some collagen fibers were broken. B and D SEM images were the morphological characteristics of leather shavings treated by alkaline protease. The figures showed that the collagen fiber had been cut into smaller parts, and in practical experiment the reaction system became liquor. By centrifuging, only a few residues were left. The comparison study of A and B, C and D showed that the hydrolyzation of magnesium oxide and alkaline protease to leather shavings was excellent. Alkaline protease is a nonspecific endopeptidase. Firstly, alkaline protease makes collagen fiber separate from leather shavings, and then the collagen fiber was further hydrolyzed to be polypeptide or amino acid with small relative molecular weight. The magnification times of figure C and D were 400, so the morphological change of collagen fiber could be clearly observed.

TABLE IV

Comparison of the percentage of formaldehyde removal and the effect on leather thickness

Formaldehyde scavenger	Formaldehyde content(mg/kg)	Percentage of formaldehyde removal (%)	Thickening rate (%)
Blank sample	236	—	—
HPTA	98.4	58.3	14.8
Chitosan	152.5	35.4	6.5
Hydrogen peroxide	175.8	25.5	2.8
Collagen polypeptide	113.8	51.8	10.4

Removal of formaldehyde

The percentage of formaldehyde removal and the effect on leather thickness were studied. The results were shown in TABLE IV.

As indicated in TABLE IV, the percentage of formaldehyde removal of HPTA reached to 58.3% which was higher than that of other formaldehyde scavengers. The reason was the amidogen content of HPTA was more than that of other formaldehyde scavengers. The structural formula was shown in Figure 4. The content of formaldehyde in leather, treated by hyperbranched polymer with terminal amidogen and collagen polypeptide, was less than 150mg/kg. Furthermore, the thickening rate of hyperbranched polymer with terminal amidogen and collagen were more obvious. The relative molecular weight of HPTA was more than 2000,[20] which was bigger than that of collagen, so it showed better filling property.

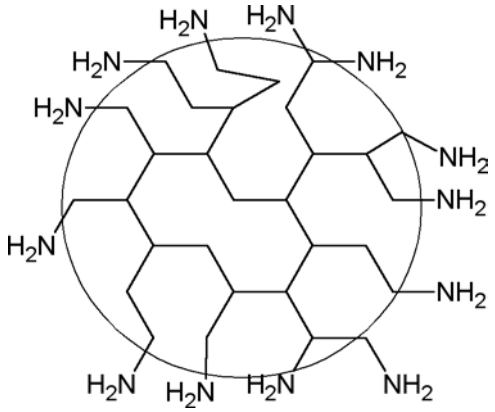


Figure 4. – The structural formula of HPTA.

CONCLUSIONS

The phosphonium tanned leather shavings were hydrolyzed by alkali, acid and alkali-enzyme methods. For two-step method of alkali and alkaline protease, the degree of extraction reached to 90% which was higher than that of alkali method and acid method. The obtained collagen was characterized by amino acid analyzer, SEM and SDS-PAGE. The results of amino acid analysis showed that extracted collagen had seventeen amino acids and was similar with that of commercial gelatin. The results from GPC showed that extracted substance by two-step method was the mixture of polypeptide and amino acid with small relative molecular weight.

The results of application experiment showed that the percentage of formaldehyde removal of extracted collagen reached to 51.8% which was less than that of hyperbranched polymer with terminal amidogen. The content of formaldehyde in leather, treated by hyperbranched polymer with terminal amidogen and collagen polypeptide, was less than 150mg/kg. Furthermore, the thickening rate of hyperbranched polymer with terminal amidogen and collagen were more obvious. In following study, increasing the content of amidogen in collagen was an important job.

ACKNOWLEDGMENTS

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