Leather Retanning Performance of Carboxylated Collagen Fibres Containing Adsorbed Cr(III)

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Abstract

Carboxylated collagen fibre (CCF) was prepared using glyoxylic acid modified collagen fibre which was extracted from fresh pigskin, the prepared CCF was used as an adsorbent to treat trivalent chromium ions (Cr(III)) in wastewater (Cr-CCF). Alkali-enzyme hydrolysis was used to extract collagen protein from the CCF after the adsorption of Cr(III) to obtain chromium-containing collagen hydrolyzate (Cr-CCH). Fourier transform infrared spectroscopy (FTIR) and gel permeation chromatography (GPC) were employed to analyse the structure and relative molecular mass distribution of the collagen in Cr-CCH. The Cr(III) content was determined by diphenylcarbazide spectrophotometry. The optimal hydrolysis conditions were optimised by single factor experiments with the collagen extraction rate as the index. Finally, sheep and sturgeon wet-blue was chosen to evaluate the retanning efficiency of Cr-CCH. The satisfactory sensory and mechanical properties of the finished leather demonstrated that Cr-CCH could become a good candidate as a retanning agent, which not only satisfied performance requirements for finished leather, but also achieved recycling of resources.

摘要：利用醋酸松弛法从猪皮中提取胶原纤维作为原料，选用乙醛酸为改性试剂对提取的胶原纤维进行羧基化改性，制备出羧基化胶原纤维，并用于吸附水中的三价铬离子（Cr(III)）。对吸附后的羧基化胶原纤维（Cr-CCF）利用酶—碱结合法进行水解，利用FT-IR、GPC等现代仪器分析方法对所得到的含铬胶原蛋白水解液（Cr-CCH）中的胶原蛋白结构、相对分子质量分布情况进行表征，通过二苯碳酰二肼分光光度法对其Cr(Ⅲ)含量进行了测定；以胶原蛋白提取率为考察指标通过单因素实验优化得到最佳水解条件。最后，以绵羊皮与鲟鱼皮蓝湿革为处理对象，利用所得到的Cr-CCH对其进行复鞣，以皮革的感官性能和机械性能作为评价指标，证明了Cr-CCH具有良好的复鞣性能,不仅可以满足人们对成革的性能要求，同时可以实现资源的循环利用

INTRODUCTION

The development of the fur and leather industry has provided economic benefits for satisfying the requirements of human beings.[1] However, the tanning process has brought about environmental pollution due to bad smells, organic wastes and high water consumption.[2] Particularly, chrome tanning has been widely applied due to its low cost, simple operation and good leather performance but, which possesses potential toxicity and carcinogenicity[3] during the oxidation of Cr(III) to Cr(VI). Therefore, numerous technologies including alkali precipitation, electrolysis, ion exchange, chemical flocculation and adsorption,[4,5] have been studied to treat chromium-containing wastewater. The adsorption method has received much attention because of its low-cost, simple operation and high efficiency.[6]

Collagen fibre is a white and transparent straight-chain structural protein which is abundant in the skin tissue, hooves and bone tissue of mammals, accounting for 25%-33% of the total protein in animals.[7] Collagen fibres appear amphipathic depending on the existence of carboxyl and amino groups at both ends of the peptide chain. In addition, hydroxyl, acylamino and other active groups[8] exist in collagen molecules, which can be easily chemical modified. What's more, the interaction forces between peptide chains containing electrovalent bonds, hydrogen bonds, Van der Waals forces and hydrophobic bonds provide desirable mechanical strength and biocompatibility[9] which appeal to many researchers concentrating their research on collagen fibres.

Various solid wastes such as meat residues, hair and leather trims will be produced during tanning, these will be discharged into soil or be incinerated in the atmosphere most of the time, generating other possibly more noxious forms of residual pollutants.[10] However, as is known, leather solid waste is composed of collagen fibres and other protein substances,[11] in which more than 80% consists of collagen apart from a little non-protein substance.[12] Therefore, we attempted to extract collagen fibres from leather solid waste and to modify them with glyoxylic acid to obtain CCF, which is used as an absorbent to purify wastewater containing Cr(III) and re-used as a retanning agent for leather after alkali-enzyme hydrolysis (Scheme 1). This study can provide an efficient method to treat Cr(III) and realize recycling of resources.

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Scheme 1. Schematic illustration of the main experimental process

1 EXPERIMENTAL PROCEDURES

1.1 Material and apparatus

The fresh pigskin was supplied by Xianyang, Shaanxi. Collagen fibre was made in our laboratory. 40% aqueous glyoxylic acid was obtained from Hubei Zhonghecheng Chemical Co. Ltd. and chromium powder, 33% basic, was supplied by Sisecam Company (Turkey). Diphenylcarbazide, AR, was obtained from Tianjin Fuyu Fine Chemical Co. Ltd. 200,000μ/g alkaline protease was purchased from Nanning Pangbo Biological Engineering Co. Ltd. Magnesium oxide and sodium chloride, AR, were purchased from Tianjin Dengfeng Chemical Reagent Factory. Sheep and sturgeon wet-blue were supplied by Xianyang Changwu County Tannery and Wuhan Tianzhu Tianhe Investment Co. Ltd., respectively. Acetic acid, sulfuric acid and formic acid, AR, were obtained from Xi'an Miura Fine Chemical Factory. Sodium bicarbonate, AR, was provided by Shanghai Xinbao Fine Chemical Factory. Ammonium bicarbonate, AR, was purchased from Suzhou Boyuan Chemical Co. Ltd. Acid Black Dye (SDL), Polymer APS, Neutralizer (TJ-R819) and amino resin (RS) was obtained from Klein Chemical Co. Ltd, Shandong Lihou Light Industry New Material Co., Ltd, Sichuan Tingjiang New Materials Co. Ltd. and Fangyu Trading Co. Ltd., respectively. Fatliquor (Desopon QL), phospholipid (Desopon PF), lanolin (Desopon LB) were both provided by Desaier Chemical Industry Co. Ltd.

The THZ-82A constant-temperature bath oscillator was supplied by the Pudong Physical Optical Instrument Factory (China) and the Fourier transform infrared spectroscope (FT-IR) was purchased from Nicolet Instrument (USA). G025159l l Mgel permeation chromatograph (GPC) was obtained by the company of Waters (USA). The drum (GB) was provided by Huihong Leather Machinery Co. Ltd., GT-313A1 Thickness Gauge was supplied by Taiwan High Speed Rail Testing Instrument Co. Ltd., KJ-1068 tensile machine was purchased from Dongguan Kejian Testing Instrument Co. Ltd. The XK-3055 Cracking Strength Tester was obtained from Kunshan Xiangke Testing Instrument Co. Ltd. and the HZ-3708 shrinkage temperature tester was provided by Dongguan Lixian Instrument Technology Co. Ltd.

1.2 Preparation of CCF

A fresh pigskin was used as the raw material for the extraction of collagen fibres by the acetic acid relaxation method. The specific preparation process was described in the reference 13. Afterwards, 2.5g CF was soaked in 30mL distilled water, and then transferred into a flask after the fibres were fully

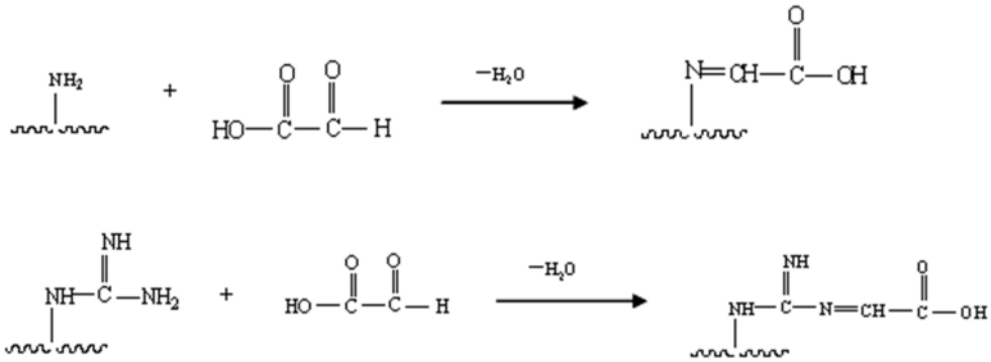

Figure 1.The modification reaction of collagen fibre

swollen. 2.0g glyoxylic acid (40%) was diluted by 40mL distilled water and adjusted by NaOH solution (0.1M) to pH6.5. Thereafter, the solution obtained was added into the flask dropwise in a water bath at 35°C over 5 hours. Finally, the CCF was prepared by filtering with nylon cloth and washing with distilled water.

1.3 Preparation of Cr-CCF

On the basis of our previous research, Cr-CCF was prepared by adding 0.1g CCF into an iodine flask with 100mL chromium solution (100mg/L), in which the pH was adjusted to 4.5. The iodine flask was shaken in a thermostat oscillator at 25°C for 3 hours (Fig 1).

1.4 Alkali-enzyme hydrolysis of Cr-CCF

Alkali-enzyme hydrolysis was used to extract collagen protein from CCF after the adsorption of Cr(III) as in the following process: MgO was employed as basic treatment to relax the Cr-CCF. Cr-CCF was fully soaked in water with a particular solid-liquid ratio (m(Cr-CCF):m(H_2O)), in which the system reacted under the specified conditions (pH, temperature, time). Subsequently, the cooling system above was reacted with the addition of alkaline protease (wt%, absolute dry weight) under constant temperature for a certain period, then the pH was adjusted by H_2SO_4 (0.1M). Finally the Cr-CCH and unhydrolyzed Cr-CCF residue was obtained by centrifugation and filtration. The extraction ratio of collagen (R, %) was calculated as below:

$$R = \frac{m_1 - m_2}{m_1} \times 100\%$$

Where: m_1, m_2 is the mass of drying Cr-CCF and Cr-CCF residue respectively. In addition, the single factor of solid-liquid ratio, the dosage of MgO and Alkali-enzyme, reacting temperature and time should be considered to confirm the optimal conditions of fibre hydrolysis.

1.5 Characterization of Cr-CCH

FTIR was employed to measure the molecular structure and functional group of Cr-CCH after vacuum drying. The Cr-CCH was characterized by GPC based on the principle of volume exclusion to investigate the distribution of collagen molecular weight in the hydrolysate. In order to facilitate the subsequent retanning test, the GB7466-87 diphenylcarbazide spectrophotometric method was used to determine the Cr(III) content of the materials.

1.6 Retanning performance of Cr-CCH

Sheep and sturgeon wet-blue was chosen to evaluate the retanning efficiency of Cr-CCH. The retanning process is listed in Tables I and II below.

2 RESULTS AND DISCUSSION

2.1 The single factor effect of Cr-CCH

The results of single factor experiments are shown in Figure 2. With the increase of the solid-liquid ratio, the extract efficiency rapidly increased and tended towards a steady value after the ratio reached 1:7. Cr-CCF was infiltrated incompletely with only a little water and the viscosity of the system would obviously reduce under opposite conditions, which had a significant effect on the enzymatic reaction rate. Figure 2 (b, c, d) shows the results of MgO dosage and alkali treatment temperature and time. MgO with its weak basicity gave good dispersion of the collagen peptide chains and which provided optimal pH conditions when the dosage reached 4%. The solubility of MgO in water was

TABLE I
Retanning process of sheep leather

Technology	Materials	Dosage %	Temperature	pH	Explanation
Weight					
Washing	Water	200	40°C		90 min
Retanning	Water	150	40°C		
	Standard chrome tanning powders	4			120 min
	Cr-CCH/chromium tanning agents	X			90 min
	$NaHCO_3$	0.5-0.7		4.2-4.5	3 × 20 min + 60 min
	Leave overnight, run for 30 min the next day and drain away				
Neutralizing	Water	200	40°C	5.5-6.0	60 min
	NH_3HCO_3	1.5			
	Drain, wash, drain				
Dyeing, stuffing	Water	150	55°C		30 min
	Acid black dye (SDL)	2			
	Fatliquor (Desopon QL)	15			60 min
	Formic acid	1.0			3 × 20 min+30 min
	pH3.5-4.0, drain, wash and out of drum				

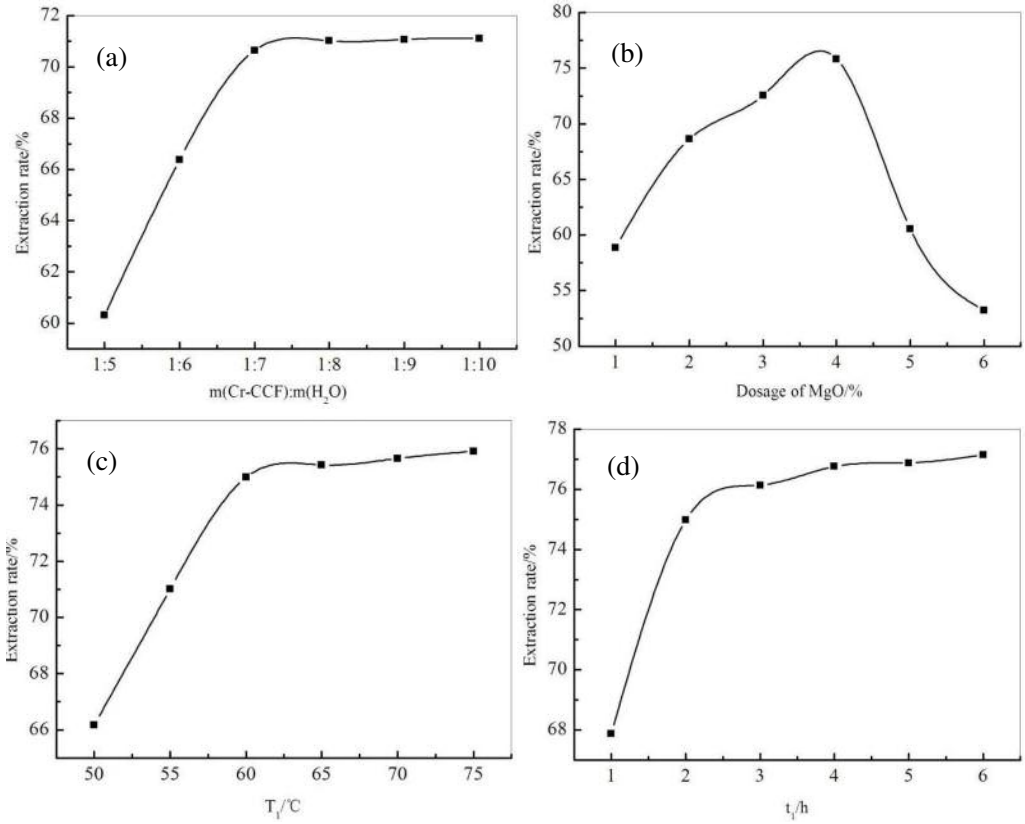

Figure 2. Effect of (**a**) m(Cr-CCF):m(H_2O) (**b**) MgO dosage (**c**) Alkali treatment temperature (T_1) (**d**) Alkali treatment time (t_1) on the hydrolysis process.

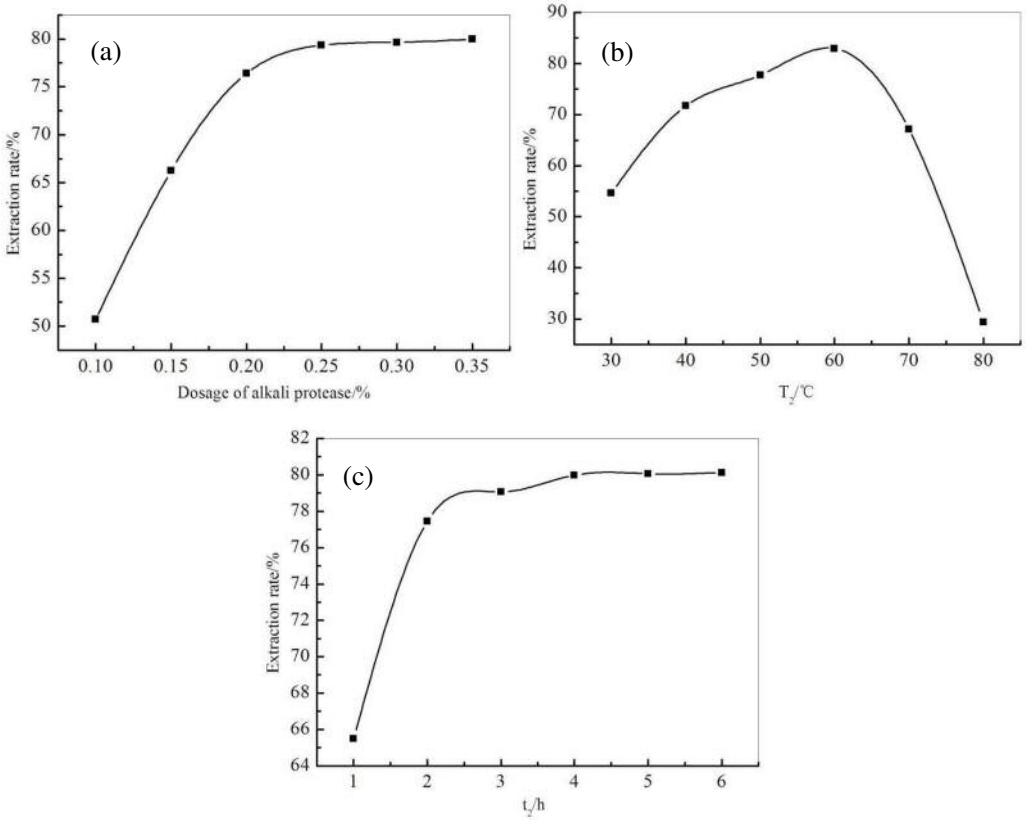

Figure 3. Effect of (**a**) alkaline protease dosage (**b**) alkaline protease treatment temperature (T_1) (**c**) alkaline protease treatment time (t_1) on the hydrolysis process.

TABLE II
Retanning process of sturgeon leather

Technology	Materials	Dosage %	Temperature	pH	Explanation
Weight					
Bleaching	Formic acid	0.4			
	Water	400	40°C		2 h
Neutralizing	Water	300	40°C		
	Neutralizer (TJ-R819)	1			
	Sodium formate	1.2	38	5.5±	
Retanning	Water 300 45 °C				
	Amino resin (RS)	4			60 min
	Cr-CCH	8			2×60 min + 30 min
	Polymer APS	4			60 min, overnight
	Run for 1hour, drain and wash				
Stuffing	Water	300	55°C		
	Fatliquor (Desopon QL)	7			90 min
	Phospholipid (Desopon PF)	7			
	Lanolin (Desopon LB)	10			3 × 60 min
Dyeing	Water	400	45°C		
	Acid black dye (SDL)	0.8			90 min
		1.5			60 min
Fixating	Formic acid	1.5		4.0	3 × 20 min + 30 min
	Drain, wash, house drying				

determined by the alkali treatment temperature, which obtained a saturation state around 65°C. In this case, $Mg(OH)_2$ (MgO was dissolved in water) could penetrate into the fibres better and enhance the loosening ability on the fibres, therefore, the extraction rate of collagen was obviously increased. The collagen extraction rate increased sharply when the time continued to 3 hours, and there was no prominent change after 3 hours. In view of the energy consumption and financial cost, 3 hours was considered as the optimal duration.

The effect of alkaline protease dosage, reaction temperature and time was shown in Figure 3. The alkaline protease at the dosage of 0.25% provided the appropriate concentration of enzyme which was beneficial to the hydrolysis of the collagen fibres, and the optimum temperature for the enzymatic reaction was 60°C. In accordance with the result of Figure 3(c), the preferential action duration was 3 hours.

2.2 The characterisation of hydrolysis performance

2.2.1 FTIR

Figure 4 demonstrated the presence of collagen protein in the hydrolysate in comparison with collagen as marketed. As the FTIR spectra depicted, a strong and broad peak was exhibited at 3388cm^{-1} due to stretching vibration N-H of collagen. The characteristic adsorption peak of amido linkage (CO-NH) showed at 2983cm^{-1}. Other considerable peaks were observed at 1642cm^{-1} and 1400cm^{-1}, [14] which was attributed to the presence of -C=O (amide I) and -C-N- (amide II) vibrations, respectively.

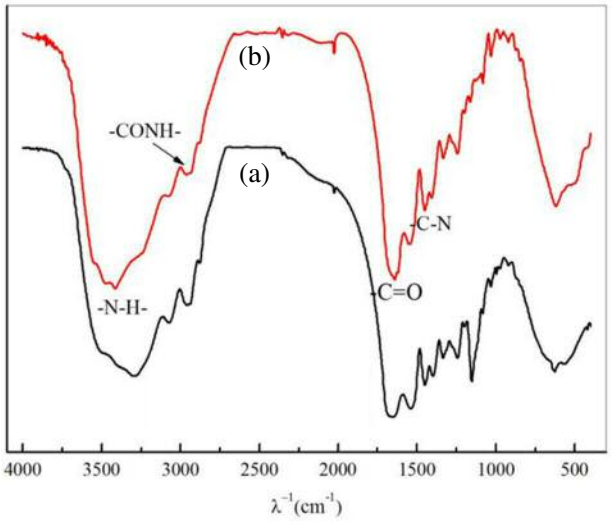

Figure 4. FTIR spectra of (**a**) hydrolyzate collagen and (**b**) market collagen.

2.2.2 GPC

Figure 5 showed the GPC spectra of Cr-CCH, and the collagen molecular mass distribution. The number average molecular weight (Mn), weight average molecular weight (Mw), statistical average molecular weight (Mp) and the maximum molecular weight of collagen (Mz) are listed in Table III. The data demonstrated the lower relative molecular weight of collagen obtained from alkali-enzyme hydrolysis Cr-CCF, which could promote the permeation of the molecule into the leather and improve leather retanning performance.

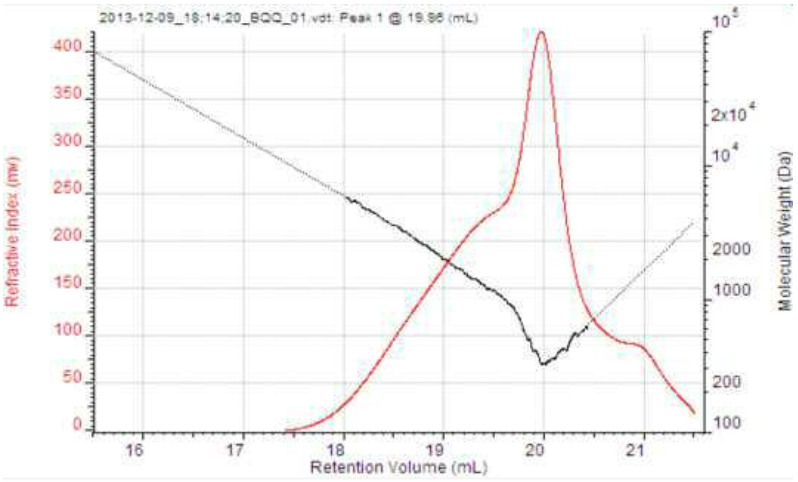

Figure 5. GPC spectra of chromium-containing collagen hydrolysate.

TABLE III
Molecular mass distribution of collagen in chromium-containing collagen hydrolysate

Distribution	Mn	Mw	Mp	Mz	Coefficient (Mw/Mn)
	756	1382	327	2454	1.827

TABLE IV
Cr(III) contents of Cr-CCH and chromium tannins

	Cr-CCH	chrome tannins
Cr(III) content (% mass percentage)	0.9137	11.48

TABLE V
The specifications of Cr-CCH

Appearance	Light green liquid
Ionicity	cationic
pH	5.16
Collagen content	10.56%
Cr(III) content	0.9137%
Solid content	16.91%

2.2.3 Determination of Cr(III) content and specifications of Cr-CCH

The GB7466-87 diphenylcarbamide spectrophotometric method was employed to evaluate the Cr(III) content in Cr-CCH and chrome tannins, respectively. The results of the determinations are listed in Table IV. In addition, Table V showed the specifications of Cr-CCH.

2.4 The retanning performance of Cr-CCH

On the basis of sheep wet-blue as raw material, a contrast retanning test was carried out to evaluate Cr-CCH retanning performance (The dosage of retanning agent based on the Cr(III) content). Compared with chromium-containing tannins, Cr-CCH retanned leather had superiority in softness, flexibility and oiliness, while showing poor leather fullness due to the smaller molecular weight of collagen in Cr-CCH. In the leather retanning process, smaller molecules tended to penetrate into leather uniformly and fully, which promoted the softness and flexibility, and had a lower effect on fatliquoring. On the contrary, large molecules provided better fullness of leather. The sensory performance of the leather is listed in Table VI. Similarly, the comparison of the physical properties of the leather (Table VII) verified that the Cr-CCH retanning agent met the demands of finished leather and also realised resource recycling.

TABLE VI
Comparison of sensory performance of the leather

Property	Cr-CCH	Chrome tannins
Softness	9[a]	7
Fullness	8	9
Flexibility	9	8
Oiliness	7	6

[a] All the numbers are the score values for the leather property

TABLE VII
Comparison of the physical properties of the leather

Property	Cr-CCH	Chrome tannins
Ts	98°C	91°C
Thickening	21%	32%
Tensile strength	6.83MPa	6.71MPa
Tear strength	45.96N/mm	46.13N/mm
Elongation	77.12%	76.98%

TABLE VIII
The sensual evaluation results of sturgeon leather

Property	Softness	Fullness	Flexibility	Oiliness
Score value	8	9	8	7

TABLE IX
The physical properties of sturgeon leather

Property	Tear strength	Tensile strength	Flexing endurance	Burst height	Bursting strength	Ts
Shoe leather	30N/mm	10.0N/mm^2	≥20000	8mm	350N/mm	100°C
Garment leather	20N/mm	10.09N/mm^2	—	—	—	100°C
Sturgeon leather	140N/mm	19.6N/mm^2	≥36000	11mm	621N/mm	98°C

In order to further confirm the practical application value, sturgeon wet-blue from thicker hides and with higher fat content was used to study the retanning performance with Cr-CCH, which had more difficulty in tanning agent permeation. The sensual evaluation results in Table VIII show that the finished leather possessed satisfactory properties for softness, fullness, flexibility and oiliness. In addition, Table IX shows the physical properties of sturgeon leather obtained using the Cr-CCH retanning agent. On comparison with the standard of shoe upper leather QB/T 1873-2004 and the standard of clothing leather QB 1872-1993, the sturgeon finished leather possessed outstanding physical capabilities, which are expected to be applied in the leather industry.

3. CONCLUSIONS

In this study, an efficient, economical and environment-friendly absorbent (CCF) was prepared successfully by the glyoxylic acid method. The practical application value was evaluated by the adsorption of Cr(III), alkali-enzyme hydrolysis of Cr-CCF and retanning on sheep and sturgeon wet-blue, which demonstrated that the as-prepared Cr-CCF could adsorb Cr(III) in water andalso that Cr-CCH possessed satisfactory retanning performance with preferable sensory properties (softness, fullness, flexibility and oiliness) and physical properties (tear strength, tensile strength, folding fastness, burst height, bursting strength and Ts) . Therefore, this work provided a high potential application and wide application prospect.

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