THE Journal OF THE AMERICAN LEATHER CHEMISTS ASSOCIATION

January 2020 Vol. CXV, No.1 *JALCA* 115(1), 1–36, 2020

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THE JOURNAL OF THE AMERICAN LEATHER CHEMISTS ASSOCIATION (USPS #019-334) is published monthly by The American Leather Chemists Association, 1314 50th Street, Suite 103, Lubbock, Texas 79412. Telephone (806)744-1798 Fax (806)744-1785. Single copy price: $8.50 members, $17.00 non-member. Subscriptions: $185 for hard copy plus postage and handling of $60 for domestic subscribers and $70 for foreign subscribers; $185 for ezine only; and $205 for hard copy and ezine plus postage and handling of $60 for domestic subscribers and $70 for foreign subscribers.

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Investigation on Reducing Chromium Quantity in Chromium Containing Wastes from Leather Industry Using Oyster Mushroom (Pleurotus ostreatus)

by
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Abstract

In the leather industry, the wastes after the wet blue phase, which are created by the shaving process, are one of the substances that cause environmental pollution. Most of the time, these wastes are buried and may, under some circumstanses, cause serious environmental pollution problems. In this study, the chromium in wet blue shaved waste is to be minimized by using oyster mushrooms (*Pleurotus ostreatus*). Wet blue shaved wastes were mixed with 0.5%, 1%, 1.5% and 2% doses into the growth medium. After the oyster mushroom growth, the consumption of chromium from the growth media and uptaken by the mushrooms were investigated with Ege University Argefar Lab.'s House Method using a ICP-MS.

Introduction

Currently, pollution from different steps of production in many industrial branches cause serious irremediable problems.[1] Industrial production can be considered as the main reason of global warming, water pollution, air pollution and soil pollution.[2]

There are many chemicals used in leather industry that can cause pollution. Chromium is one of them and it is the most preferred tanning agent in leather industry. 80-90% of leather tanning is performed by Cr (III) salt.[3] At the end of the chromium tanning, leathers are held for the chemical reaction to continue. The leathers that become wet blue are shaved to adjust thickness. Chromium tanned shaving waste is one of the biggest factors that can cause pollution in leather industry. Commercially, biological and chemical treatments are endeavored; however, they are not fully succeeded. High amounts of chromium is present in shavings. In many countries, these shavings are buried in the ground and ignored but they might cause severe health issues in the future. Due to toxic Cr(VI) and toxic gas outlet, removal of the shavings by burning is not recommended.[4]

Chromate and chromate compounds can be used by plants and they can infiltrate through the deep soil layers causing pollution in water resources. Chromates can be absorbed to the positively charged soil particles in less amount and they are prevented from convection to the atmosphere. However, unregulated storage of solid wastes containing chromium can cause an increase in the chromium concentration in soil and cause pollution in water resources.[5]

The researchers reported that the application of trivalent as well as hexavalent chromium compounds had a noticeable negative effect on soil dehydrogenase activity. Soil dehydrogenase activity can be considered effective indicators of soil quality changes resulting from environmental stress or management practices.[6]

Even many researchers tried to use chromium tanned shaving waste for different aims [7,8,9,10,11,12] but still the problem is not solved. The researchers reported that the most common way to manage these solid wastes is by disposing of them at landfill sites.[12] The waste which contains chromium is buried and if the burying area is not insulated enough, the filtration, transportation or mixing of these wastes to the soil is inevitable.

The researchers reported that although Cr(III) is the most expected form in the tannery effluents, an increase in the hexavalent form can occur as a result of redox reactions occurring in the sludge, for instance, in water by manganese oxides and in soils by mobile ligands such as citric acid, diethylene triamine pentaacetic acid (DTPA), and fulvic acid mediated oxidation.[13]

It has been reported that Cr(III) is generally has no risk in leather processing.[14] Cr (VI) salt is not used in leather processing however Cr (VI) can be observed on finished leathers and this is not an intended feature.[15] For instance, pH increase during neutralization causes the oxidation of Cr(III) to Cr(VI) or the drying the leather may provide a mechanism for the formation of Cr(VI).[3]

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Manuscript received May 27, 2019, accepted for publication August 20, 2019.

Table I

Evaluation of Cr(III) and Cr(IV) from the point of human health[3] (IULTCS, IUR-1, August 2013)

Cr (III)	Sensitivity	Does not create sensitivity (no sensitization)
	Acute Toxicity	No harmful or toxic related to impact amount and compound
	Carcinogen	Is not included on CMR list
Cr (VI)	Sensitivity	Does create sensitivity
	Acute Toxicity	Toxic
	Carcinogen	Carcinogen and Mutagen

Discharge of heavy metals to the earth and water without disintegration is very important. It cannot be divided to non-toxic forms and leaves therefore a lasting effect on the ecosystem. Most of them are toxic even in very low concentrations. Arsenic, cadmium, chromium, copper, lead, mercury, selenium, silver, zinc, etc. are not only cytotoxic but also carcinogenic and mutagenic in the nature. This fact is clearly observed on several reports which show harmful effects of heavy metals on human health.[16]

We have to find effective, cheap and practical solution proposals for the removal of waste chromium without harming the environment if we want to use chromium as tanning material in leather production. It is required that chromium should be reduced before disposal so that Cr(VI) potential in the environment is decreased. Bioremediation, in this context, is an innovative and promising methodology for the removal of heavy metals. Microorganisms, since they have developed strategies to remain alive in environments including heavy metals, have adapted themselves to various detoxification mechanisms such as biosorption, bioaccumulation, biotransformation and biomineralization. They can thus make ex situ or in situ bioremediation. It is known that some fungi can easily grow on wet blue and affect the structure.[17,18]

Organisms which are mostly used for biological treatments in the scope of waste and environment technology are white rot fungi.[19] Disintegration feature of white rot fungi is determined through various studies. They occupy an important and privileged place due to their characteristics. It is known that white rot fungi included in Basidiomycetes group, play a role in the elimination of environmental pollution shown up due to dense industrial activity, and the oxidation of organic compounds possessing very different molecular structures, together with various enzymes they synthesize, ie: lignin peroxidase (LIP), peroxidase related to manganese (MnP), to begin with laccase (lak) enzyme.[20,21]

Wood destroying *Pleurotus ostreatus* are saprophytic fungi. Easy development and yield on organic materials including lignin and cellulose without requiring any fermentation due to their strong mycelium structure allow the use of different industrial and agricultural wastes in the cultivation of *Pleurotus ostreatus.*[22]

Pleurotus ostreatus is resistant to toxic chemicals existing in nature. It possesses a very strong oxidative biodecomposition potential.

We have tried in this research to show that oyster mushroom (*Pleurotus ostreatus*) will mineralize chromium wastes within its body and make them harmless and that the chromium can be decomposed without being oxidized to Cr(VI). Chromium on elementary level does not have any risk for human health is shown on Table I.[3] Leather wastes with chromium are mixed to the specially prepared compost in the research and chromium quantity existing in the compost before fungus inoculating and after fungus development and chromium quantity transferred to the fungus fruiting body are observed. Besides, impacts of chromium on the development and yield of fungus are also examined.

Experimental

Materials

Wheat straw and wheat bran to be utilized in the research are provided from local suppliers and wet blue shaving wastes from “Lider Leather Tannery” located in Menemen Leather Free Zone (Figure 1).

Figure 1. Shaving wastes containing chromium

Sypra PL 28 (*Pleurotus ostreatus*) type mycelium is used in the test and is provided from the importer company “OPE Agriculture”.

Methods

Nitrogen content of shaving wastes containing chromium is determined through Kjeldhal Method.[23]

Assembly of Tests

Preparation of Growing Environments

Wheat straw (WS) is used as basic material in the research, and wheat bran (WB) and chromium tanned shavings wastes (Cr) are used as additives. Mixture rates and codes of growing environments used in the research are given on Table II.

Figure 2. Preparation of Compost used on Tests

Substrate prepared from the mixture of wheat straw and wheat bran (WS80+WB20) is used as the admixture (C) in the research. Chromium tanned shavings wastes mixed in 4 different rates (0.5%, 1%, 1.5% and 2%) with wheat straw and wheat bran are used as control compost.

Table II

Mixture substrate contents

Compost Mixture Rates	Code
Wheat Straw (80%) + Wheat Bran (20%) (Mixture)	Control (WS80+WB20)
0.5% Cr + 99.5% Mixture (80% wheat straw + 20% wheat bran)	0.5% Cr
1% Cr + 99% Mixture (80% wheat straw + 20% wheat bran)	1% Cr
1.5% Cr + 98.5% Mixture (80% wheat straw + 20% wheat bran)	1.5% Cr
2% Cr + 98% Mixture (80% wheat straw + 20% wheat bran)	2% Cr

Shaving wastes containing chromium were ground and sterilized. Every substrate mixture having different chromium dosage was separated into 4 groups and study was conducted with 4 repetitions. Convenient substrate mixture for the growing of *Pleurotus ostreatus* was prepared in Bergama Technicial and Business Collage, Ege University. Materials were weighed with predetermined weights by taking percentage of dry substance of their mixture substrate as basis, for the preparation of substrate. Then, mixtures were dampened and humidity rate accessed to 70%.

Sterilization and Mycelium Grafting

Mixture substrate are pasteurized, after damping, by boiling in water (70°C) for 2 hours. Substrate are left for cooling after pasteurization and 1% plaster over the basis of weight is added to all mixtures to adjust pH level.

Temperature is rapidly decreased to nearly 25°C, by draining excess water on the table possessing perforated grill with ventilation from the bottom. Growing mixtures with decreased temperatures are put into bags of 40x50 cm dimensions, 2 kg of mixtures substrate being in each bag and inoculation is done, by homogenously mixing 2% of the weight of spawn suitable for inoculation. Bags which are inoculated with spawn are compressed and their openings are covered by fastening.

Figure 3. Fungus Growing Room and Pre-tests

Incubation and Harvest

Inoculated bags were incubated at 25°C±2°C temperature and 70-80% humidity during incubation period (15 days) until mycelium colonized. Bags are perforated after full colonization, for the induce fructification. Temperature is adjusted to 15°C±2°C in the production room to promote fungus formation, and the humidity is increased up to 85-90%.

12 hours of illumination with fluorescent lamps of 200 lux intensity is provided per day. Fresh air is supplied to the production room to promote primordium formation, and CO$_2$ level is gradually decreased. Mushrooms are harvested by cutting with a knife after having reached a giving size.

Chromium contents existing in the substrate and mushroom fruiting body are determined in "Argefar Lab., Ege University" through ICP-MS In House Method.[24]

Statistical Evaluation

Tests related to mushroom development and yields are structured with 4 repetitions, 4 bags existing on each repetition, according to random plots test design.[25] Variance analyses of data obtained are done through SPSS (ver. 17.0 for Windows) statistical program and Duncan Test of Multiple Comparisons is used for groupings.

Wilcoxon Signed Ranks Test is applied to prove whether there is any difference or not on statistical meaning in chromium quantity within the mushroom and compost samples collected before the spawning of *Pleurotus ostreatus* and after the harvest.[26]

Kruskal-Wallis Test is applied to prove whether there is any meaningful difference or not on determined Cr rates (0.5%, 1%, 1.5% and 2%).[26]

Results and Discussion

Nitrogen content in shaving wastes containing chromium is determined as 14.18%. Fungi of Pleurotus genus are cellulosic.[27] Even if nitrogen content may show a development on substrates which have a nitrogen content from 0.03% to 1.0%,[28] the best development is observed on 1.0% rate.

Addition to growing media of leather waste with chromium content is kept on rather low level due high N content. Correspondingly, N percentage content obtained from admixture substrate is given on Table III.

Table III

Composts possessing different chromium dosages

Substrate	N (%)
Control (C)	0.84
0.5 % (Cr)	1.02
1.0 % (Cr)	1.17
1.5 % (Cr)	1.27
2.0 % (Cr)	1.44

Mushroom development is determined during the research, on tests conducted on 5 different groups. It is observed that the mushroom has incorporated the chromium to its body and that chromium quantity is decreased in the environment when chromium quantity is examined before spawning after the preparation of compost and after the harvest. Arithmetic means of these values are seen on Table IV whereas arithmetic means of chromium left in the compost before and after spawning are seen on Figure 5. Chromium quantity existing in shaving waste containing chromium is also examined during tests and 42330 mg/kg Cr content is determined in shaving waste through ICP-MS In House Method.[24]

Kruskal-Wallis test is applied to examine whether Cr rate affects or not the capacity of *Pleurotus ostreatus* to incorporate into its body the Cr existing in the compost. H_0 is rejected according to test result obtained (Asymp. Sig. < 0.05). This fact proves that Cr rates create an important difference, from the statistical point of view, on the capacity of *Pleurotus ostreatus* to incorporate Cr into its body.

Statistical diagram graph of the chromium content in the media before mycelium inoculation and after the harvest and arithmetic means diagram of Cr quantity left in the compost before mycelium inoculation and after the harvest are similar each other (Figure 4 and Figure 5).

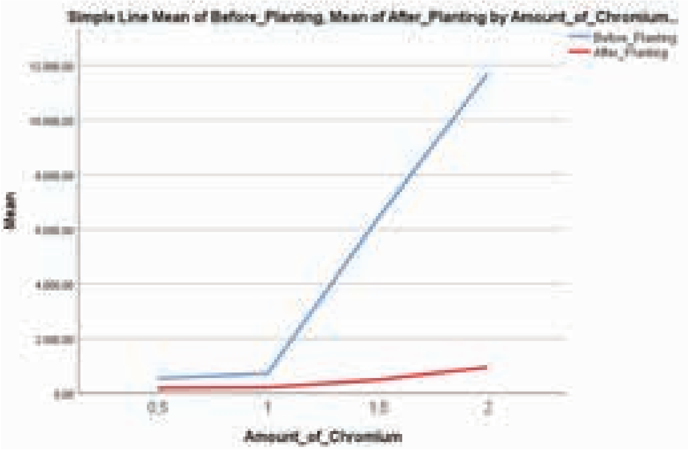

Figure 4. Statistical diagram graph of the chromium content in the media- before mycelium inoculation and after the harvest

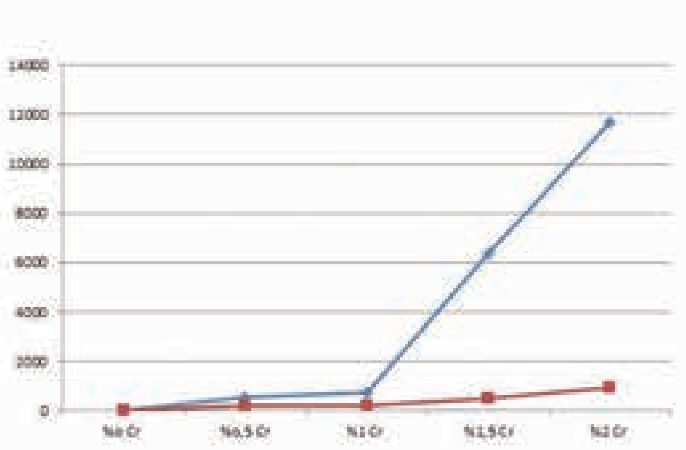

Figure 5. Arithmetic means diagram of Cr quantity left in the compost before mycelium inoculation and after the harvest

Table IV

Arithmetic means of Cr quantity left in the compost before and after mycelium inoculation and the one incorporated into the fungus fruiting body.

Compost (wet blue shaving dust)	Before *Pleurotus ostreatus* spawn inoculation (mean) (media) (Cr)	After *Pleurotus ostreatus* harvest (mean) (media) (Cr)	(Cr) within *Pleurotus ostreatus* fruiting body
0% (Control)	4.33 mg/kg	–	0.122 mg/kg
0,5% (Cr)	530 mg/kg	177.8 mg/kg	0.372 mg/kg
1% (Cr)	724 mg/kg	205.6 mg/kg	0.361 mg/kg
1,5% (Cr)	6374 mg/kg	482.8 mg/kg	0.568 mg/kg
2% (Cr)	11690 mg/kg	951.1 mg/kg	0.422 mg/kg

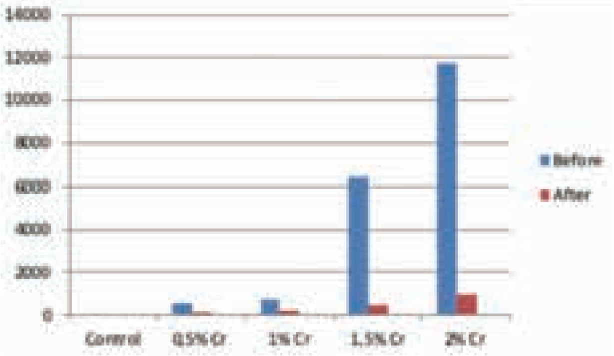

Figure 6. Chromium quantity left in the compost before the mycelium inoculation and after the harvest

Under analyses done, it has been determined that *Pleurotus ostreatus*, while uptake into its body the chromium from convenient growing media containing shaving waste with different chromium rates, achieves the highest rate when 1.5 % is added to the compost (Figure 7).

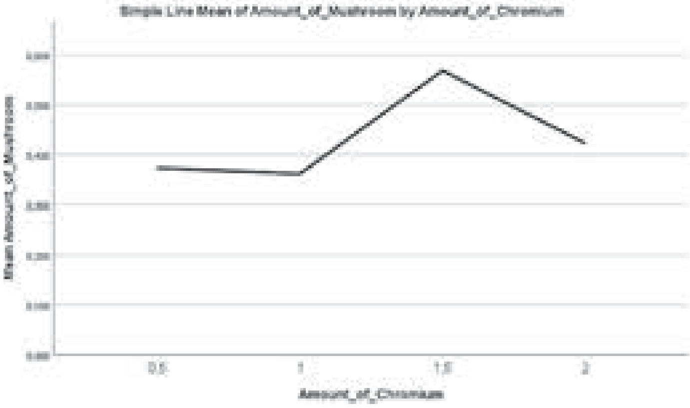

Figure 7. Uptake rate of chromium into the mushroom fruiting body

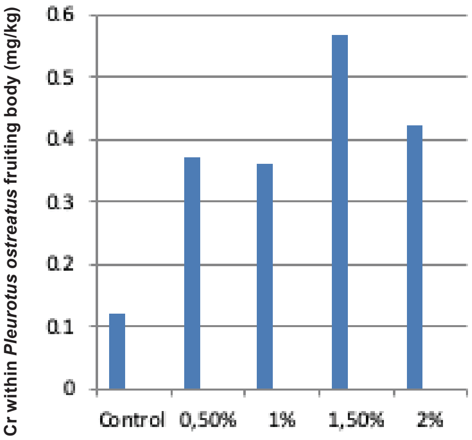

Figure 8. Chromium quantity the mushroom uptakes into its body in different compost contents

The difference between mushroom yield wherein compost that mixed with chromium or without chrome (control group) is determined as important statistically speaking.

According to results of the study seen on Table V, whereas the highest yield is obtained from the compost to which 1.5% shaving waste containing chromium is added (214.81 g/kg- total mushroom weight), 35% yield increase is observed when compared to the mixture (WS80+WB20) environment (159.00 g/kg). The lowest yield is obtained from control application. Substrate where 0.5%, 1.0% and 2.0% shaving waste containing chromium are included in the same group and a yield increase of 14-26% is obtained when compared to the control. A study obtained a yield of 214.6 g/kg from the substrate prepared with WS80 (wheat straw) + WB20 (wheat bran).[29] In another study, the yield of *P. ostreatus* in wheat straw growing media is indicated as 175 g/kg compost[30] and 246.5 g/kg compost.[31] Indicated through the study conducted, the highest yield obtained from 2 WS (wheat straw) + WB (wheat bran) media (300.24 g/kg).[32] It can be observed that yield values obtained in this study are coherent with data of these researchers.

Asterisks indicate significance at $*P < 0.05$, ^{ns} not significant; values within the same column followed by the same letter are not significantly different according to Duncan test.

In another study, it is indicated that high N quantity caused a yield decrease on *Pleurotus* type.[28] Concordantly, yield value obtained in the study from 2.0 shaving containing chromium, where N content is higher indicated a decrease when compared to yield values obtained from substrates prepared with other shavings containing chromium.

The difference between the quantity of caps obtained from compost media mixed with different rates of shaving waste containing chromium and the test conducted with the control media is determined as important statistically speaking. When Table V is examined and concerning the quantity of caps obtained during the test from different compost media, the highest quantity of caps (33,44 pieces) is obtained from the substrate where 1,5% shaving containing chromium is added (Figure 8). Then we have substrates where 1% (33,20 pieces), 2% (30,50 pieces) and 0,5% (30,25 pieces) wet blue shaving is added respectively. All substrates where wet blue shaving is added are included in the same group. The lowest quantity of caps is obtained from the control media with 20,69 pieces.

Figure 9. Mushroom which are grown in mushroom production room, developed in compost media with 1,5% wet blue shaving and which are available for harvest

Table V

Physical measurements of mushroom grown in different compost contents

Substrate	Yield (g/kg)	Quantity of mushroom (pcs/kg)	Cap Diameter (mm)	Stalk Diameter (mm)	Stalk Length (mm)
Control (Mixture) (WS80+WB20) (0%)	$^{*}159^{b}$	$^{*}20.69^{b}$	69.43^{ns}	9.71^{ns}	24.08^{ns}
0,5% Cr+99,5% Mixture	195.69^{b}	30.25^{a}	68.26	9.54	21.13
1,0% Cr+99% Mixture	200.06^{ab}	33.20^{a}	75.06	10.00	19.38
1,5% Cr+98,5% Mixture	214.81^{a}	33.44^{a}	74.17	9.92	24.29
2,0% Cr+98% Mixture	180.81^{ab}	30.50^{a}	69.12	10.46	24.42

Differences among values concerning cap diameter, stalk diameter and stalk length are not considered as important.

Conclusion

It has been understood through the study that *Pleurotus ostreatus* can easily develop and grow on leather shaving waste containing chromium and uptake the chromium into its body, when optimum conditions are provided. An important difference is determined in the quantity of chromium in the compost concerning composts prepared by adding different doses shaving waste containing chromium. The existence of chromium in the compost did not create any question in *Pleurotus ostreatus* growing from the stand point of mushroom development. On the contrary, a yield increase is observed due to nitrogen existing in leather shaving waste containing chromium. However, though nitrogen rate is the highest in the compost having 2% rate of shaving waste containing chromium, the yield and chromium absorption are not at the maximum rate. As a reason we may say that high rate of nitrogen creates a negative impact for the development of *Pleurotus ostreatus,* and this point is coherent with the study of Desrumeaux et al., 2003[33].

When we compare both from the stand point of uptake of chromium to the body and high yield, most successful results are observed on fungi developed on the compost where 1.5% shaving containing chromium is added.

It has clearly been determined as the result of our study that chromium does not have any negative impact for the development of *Pleurotus ostreatus*. Moreover, *Pleurotus ostreatus,* mineralizes the chromium by uptaking it to its body and decomposes it from the environment. We showed with this study that *Pleurotus ostreatus* can be used as mycoremediation in leather wastes containing chromium. Edibility of fungi obtained is the subject of another research project. On the other hand, whether these mushroom can be used as fertilizer or animal feed should also be researched. The mushroom obtained can also be used for several different purposes as chromium source. Cr (III) serves as an essential nutrient in plants[6] and exhibits a significant number of health benefits in animals and humans.[34] This study conducted is a basic research which can light the way for many scientists.

Acknowledgement

The authors would like to thank to Ege University Scientific Research Project Department Directorate for the financial support they provided (Project No: 16-MUH-102). The authors also acknowledge the project of "Industrial Doctorate Program of Textile and Leather–2007 DPT 001" supported by T.R. Ministry of Development.

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Analyzing the Mechanism and Effect of Acid Protease in Wet blue Bating Process for Leather Production

by
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Abstract

In recent years, in order to reduce the pollution produced in beamhouse and tanning sections, more and more tanneries purchase wet blue from other factories in other regions directly used as raw materials for finished leather production thereby those polluted preliminary steps can be eliminated. Therefore, the wet blue bating process is an essential step to minimize the differences of wet blue which are purchased from different regions. In this study, the properties of different acid protease are analyzed for selecting suitable protease used for wet blue bating. The analysis of chromium tolerance of different acid proteases reveals that, L1 and L4 produced from *Aspergillus* have higher chromium resistance than that of produced from Bacillus. The effect of L1 and L4 on wet blue and collagen shows that the L1 has more excellent performance, in which the molecular weight of functional protein is 48 KD. By SEM and MCT analysis, L1 can successfully disperse the collagen fibers of wet blue. Furthermore, the biodegradation rates of collagen and elastin were 0.006‰ and 0.5‰, respectively. It indicates that the acid protease mainly degraded elastin but not collagen in bating process thereby ensuring production safety. This paper provides the importance references for the application and the basis for the development of mechanism of acid protease in bating process.

Introduction

Protease has been applied in leather industry for a long-time because of its high efficiency. It is an irreplaceable biomaterial for improving leather quality and realizing cleaner production especially during the bating process.[1] According to the suitable pH in application, the industrial proteases are usually classified as acid protease,[2] neutral protease[3] or alkaline protease. Meantime, it should be noted that protease are produced by different strains such as *Aspergillus Niger,*[4-5] *Aspergillus Oryzae*[6-7] and *Bacillus*[8] etc. thereby resulting in their characteristic differences.

As known, chrome-tanned leather has excellent physical and chemical properties such as high shrinkage temperature, softness, fullness and good hygienic properties, thus chrome tanning is the most widely used tanning method in leather industry. Due to chrome is usually considered as the polluted heavy metal, a lot of tanneries choose to use wet blue (Semi-finished leather after Cr^{3+} tanning) as original material to produce finished leather, thus huge amounts of producing pollution in pre-tanning process and tanning process can be avoided. However, there are two problems needing to be solved when purchasing wet blue for production. After long-time transportation and preservation, the collagen fibers of wet blue are further cross-linked due to the existence of residual chrome in wet blue. The tightly arranged fiber bundles have a negative influence on the penetration of chemicals in subsequent process, which may reduce the quality of finished leather. Meantime, the wet blue is purchased from different regions, and the regional differences may result that it's difficult to maintain consistency of finished leather.

In order to solve these problems mentioned, a pretreatment should be performed to homogenize the wet blue properties from different regions by dispersing the collagen fibers of wet blue. As known, pH value of wet blue is approximately 4.0, thus acid protease should be selected for wet blue bating. Besides, in the wet blue bating process, the chromium tolerance of acid protease must be considered because heavy metal ions may result in enzyme deactivation. The suitable acid protease used in wet blue bating process should have high chrome tolerance, thus it can maintain activity in bating process.

In this paper, several acid proteases from different strains are screened. The acid proteases including L1 and L4, which are produced from *Aspergillus* strains, display good chromium resistance. Afterwards, the structure changes of collagen and the physical properties of crust leather influenced by L1 and L4 acid protease are evaluated. Thus, the reaction mechanism between acid protease and the main structural protein in wet blue is summarized. This study has important reference value for the selection and application of acid protease in the bating process of wet blue.

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Manuscript received July 07, 2019, accepted for publication August 19, 2019.

Experimental

Materials

The wet blue were purchased from Shandong Dexin Leather Co.,Ltd. Collagen and Hydroxyproline (Hyp) were purchased from Shanghai yuanye Bio-Technology Co., Ltd. Desmosine (DES) ELISA Kit (48T) were purchased from Jiangsu Kete Biological Technology Co.,Ltd. AL acid protease (EC.NO.3.4.21.62), ABG acid protease (EC.NO.232.752.2), L1 acid protease (EC.NO.3.4.23.18) and L4 acid protease (EC.NO.3.4.23.19) were purchased from Sichuan Dawei Technology Co., Ltd. (Chengdu, China), Novozymes (Denmark) and Longda Biotechnology (Shandong, China), respectively. Rainbow predyed wide molecular weight protein marker (10-260KD) were purchased from HeFei BoMei Biotechnology Co., Ltd. The chemicals used for analytical techniques were of analytical grade, and other chemicals used for leather processing were of commercial grade.

Determination of Chromium Tolerance of Acid Proteases

The circular samples of wet blue with a diameter of 5 cm (the thickness of the samples was 1.54 mm) were taken from wet blue and placed in 250 mL flask without acid protease. The water (v=200mL) was used as solution. and the pH of the original water is 7.0, because of the positive charge of wet blue, the pH value of the solution was about 4.0 during the experiment.10 ml of solution was collected at 0 h, 0.5 h, 1 h, 2 h, 4 h, 12 h, 24 h, 60 h and 90 h, respectively and filtered. The concentration of chromium was determined with 1,5-Diphenylcarbohydrazide Spectrophotometric Method.[9] Afterwards, the chromium tolerance of acid protease was measured with corresponding concentration of chromium sulfate solution. The acid protease activity was determined by Folin method using casein as substrate.[10-11] Under the condition of 40°C and pH=3, one unit (U/g) of protease activity is defined as the amount of enzyme required to catalyze the hydrolysis of casein to produce 1 μg of tyrosine in 1 min.[12-13]

Pretreatment of Wet blue

The square samples (20 cm × 30 cm) and circular samples with a diameter of 5 cm were taken symmetrically along the backbone of wet blue and treated with L1 and L4 acid protease, respectively. The samples were treated with different dosage of acid protease (w/w, 0%, 0.4%, 0.6%, 0.8%, 1%) with 100% water (w/w) at 40 °C for 4 h. The activity of L1 and L4 acid protease were 51200U and 48000U, respectively. After bating process, these samples were retanned, fatliquored, dryed and softened. Afterwards, the square samples were used to measure the softness while the circular samples were used to measure the air permeability.

Physical Properties Evaluation

The softness and air permeability of samples were measured to evaluate the sensory properties, and their change rates calculated. Before testing, the samples were firstly placed in a Humidity Chamber (T=20°C, Rh=65%) for 24 h. The softness of the samples were measured by Leather Softness tester (GT-303, Gotech Testing CO.,LTD. China). The air permeability of sample was measured by Leather Air Permeability Tester (GT-7007-Q, Gotech Testing CO.,LTD.China). Each sample was tested three times. In the end, the change rates of softness and air permeability of crust leather were calculated according to the following formula (1).

$$R = \frac{|R_1 - R_0|}{R_0} \times 100\% \qquad (1)$$

R — the change rate (%);
R_0 — the data before bating process;
R_1 — the data after bating process.

Circular Dichroism Spectrometer Analysis

Cowhide collagen (0.2 mg/mL) was reacted with L1 and L4 acid protease (200 U/g) at 40 °C for 4 h, and the spectra data were measured at 25 °C by Circular Dichroism Spectrometer (Chirascan, Applied Photophysics Ltd, UK). The CD spectra of samples were obtained after subtracting the reference spectrum respectively and expressed in terms of molar ellipticity.[14-15] Spectral data was collected at a rectangular quartz cell with an optical path of 1 mm and a time constant of 1s under nitrogen atmosphere. The scan speed was 20 nm/min and the measured far UV region was in the range of 190-260 nm. By CD spectra analysis, the Rpn value of collagen was calculated to characterize the degradation degree of acid protease to collagen.[16]

Micro-structure Observation

The porosity of collagen fibers (bundle) was observed by Micro-CT (Skyscan2211, Bruker, Germany) method.[17] After treating with L1 (200 U) at 40 °C for 4 h in Constant Temperature Incubator Shaker, the sample was observed while the untreated rectangular sample (15 mm 5 mm) was used as control sample. Besides, these samples were observed by SEM (Gemini 500, Zeiss, Germany) to evaluate the dispersion of collagen fibers.[18-19]

SDS-PAGE analysis

The acid protease activity was determined by Folin method with casein as substrate in the pH range of 2-11.[10, 20] The molecular weight of L1 was analyzed by SDS-PAGE methods at the optimal pH.[21-24] Separation gel at 12% and a 5% stacking gel were separately prepared. The loading quantity of marker and acid protease was 20 μL. The stacking gel voltage was 80 V and the separation gel voltage was 120 V. The thickness of the gel was 1 mm.

Preparation of Enzymatic Hydrolysate

Firstly, the molecular weight below 50 KD in L1 was separated by Tangential Flow Filtration (TFF) system (Minimate™ TFF Capsule with Omega™ 50K Membrane, Pall Corporation, USA). Several circular samples with diameter of 5 cm were treated by the effective components (33600U/mL) at 40°C for 4 h in Constant Temperature Incubator Shaker, and the hydrolysate was obtained. Then, the hydrolysate was filtered and stored at 4°C.

Determination of Hydroxyproline by HPLC Method

The hydrolysate was mixed (v:v=1:1) with HCl (6 mol/L) and digested at 150°C for 25 min by Microwave Digestion system (Ethos.UP, Milestone, Italy). The Hyp content was determined by HPLC method with pre-column derivation with 2, 4-dinitrofluorobenzene.[25-27] The mobile phase was A: ammonium acetate (30 mmol/L), B: acetonitrile. Gradient elution (0-7 min, 80%A; 7-10min, 60%A;10-30 min, 60%A) with a flow rate of 1 mL/min. Aiglent TC-C18 (5 µm, 4.6×250 mm), the detection wavelength was 360 nm, loading quantity of sample was 20 µL and the temperature of chromatographic column was 27°C.

Determination of Desmosine in the Hydrolysate by ELISA Method

The Des concentration was determined by using the method of double-antibody sandwich enzyme-linked immunosorbent assay (ELISA).[28-30] A solid-phase antibody was prepared by coating the microporous plate with purified Des antibody. Des was added to the micropore in turn, and then combined with HRP-labeled Des antibody to form an antibody-antigen-enzyme labeled antibody complex. After thorough washing, TMB was added to the substrate to develop the antibody. By HRP enzyme catalyzing, the color of TMB was firstly converted to blue, and finally turned to yellow under acid condition. The OD value was measured at 450 nm by Enzyme Labeling Instrument (Labsystems, Multiskan, MS-352, Finland), and the concentration of Des was calculated. The other test process was carried out according to the requirements of the instructions of the Des Element Kit.

Calculating the Biodegradation Rate of Collagen and Elastin

Collagen accounts for three-quarters of the dry weight of skin,[31] and Hyp is the typical amino acid of collagen. Each 100 g of collagen contains 12.8 g of hydroxyproline,[32] thus the constant for calculating collagen degradation rate is designed as 96. Meantime, Elastin content in the skin is relatively small, constituting about 2%-5% of the dry weight of skin.[33] The Des constant is designed as because 1 g elastin contains 17 µmol Des.[34] The untreated wet blue samples were dried to constant weight in an oven at 50 °C, and the total dry weight of samples was obtained. The biodegradation rate can be calculated according to the following formulas (2) and (3):

$$D_{Collagen} = \left[\frac{C_{Hyp} \times V}{96m}\right] \times 1000‰ \tag{2}$$

$$D_{Elastin} = \left[\frac{2\times10^4 \times C_{Des} \times V}{17 \times M_{Des} \times m_0}\right] \times 1000‰ \tag{3}$$

C — The concentration of Hyp and Des in the Hydrolysate, mg/L;
D — The biodegradation rates of Collagen and Elastin, ‰.
M— The molar mass of Des, 526.6031 g/mol;
m — The total dry weight of the samples, g;
V — The volume of Hydrolysate, L.

Results and Discussion

Chromium Tolerance Evaluation

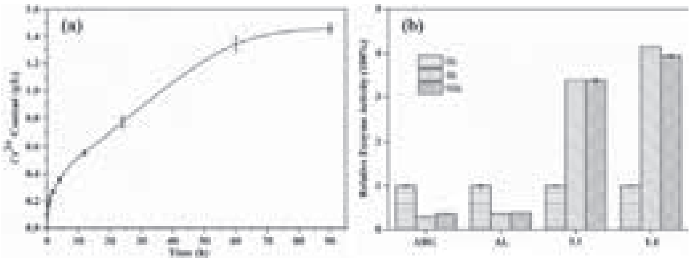

Figure 1. (a) Cr^{3+} leaching content of wet blue; (b) The relative enzymatic activities of the acid proteases with different Cr^{3+} concentrations.

The bating time is usually controlled at approximately 4 h. From Fig.1 (a), it shows that the Cr^{3+} concentration in solution increases rapidly within the first 4 hours. At 4 h, the chromium content reaches 0.36 g/L. The chromium content changes slightly with the time range of 60-90 h, and the chromium content reaches 1.5 g/L. Comparing the activities of different proteases as shown in Fig.1 (b), the protease activities of ABG and AL are inhibited by chromium at 4 h and the measured pH is 3, and their relative enzymatic activities was only 29% and 35%, respectively. At 90 h, the relative enzymatic activities are 36% and 38%, respectively. Meantime, the protease activities of L1 and L4 are obviously activated by Cr^{3+} ions. The relative enzymatic activities of L1 and L4 are 338% and 340% at 4 h, whilst their activities are improved to 414% and 393% at 90 h. Therefore, it concludes that the acid protease produced by *Aspergillus* has better tolerance to chromium than that of protease from *Bacillus*, thus it is more suitable for the bating process of wet blue.

Physical Properties Analysis

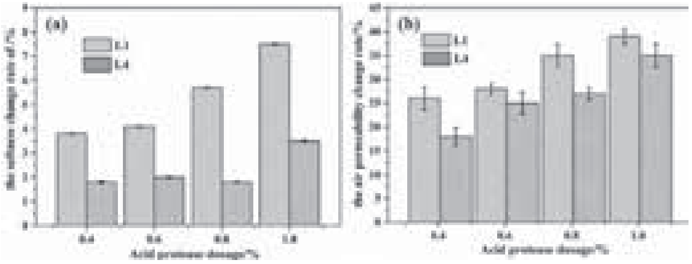

Figure 2. The changes of physical properties of wet blue treated with different acid proteases. (a) The softness change rate; (b) The air permeability rate.

As shown in Fig.2(a) and Fig.2(b), the softness and air permeability of wet blue bating with L1 and L4 increase obviously, and the wet blue treated with L1 is softer and has better air permeability than treated with L4. It is seen that the softness and air permeability of wet blue increase with the increasing of enzyme dosage. Therefore, using acid protease in bating process can improve both the softness and air permeability of wet blue successfully, and L1 acid protease has better application effect.

Effect of L1 and L4 Acid Protease act on Collagen

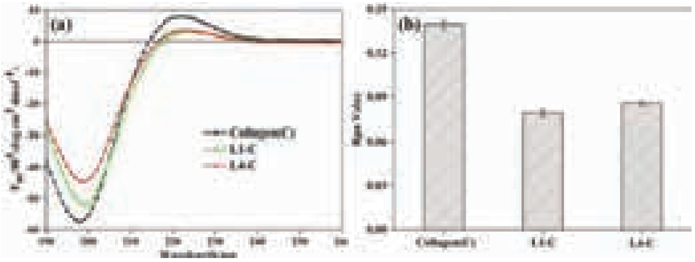

Figure 3. The Circular Dichroism (CD) spectra and Rpn of samples; (a) the CD spectra (b) The Rpn analysis.

Collagen has circular dichroism because of its stable triple helix structure. As shown in Fig.3(a), the CD spectra of all the three samples display the strong negative absorption peaks due to π–π* transition of amides at about 197 nm, while the strong positive absorption peaks due to n–π* transition of amides at 220 nm.[35-36] Because the ratio of the positive peak to the negative peak (Rpn) is unique to the structural conformation,[37] it can be used to identify the triple helical conformation. As shown in Fig.3(b), the Rpn value of original collagen is 0.139, and the collagen treated with protease L1 and L4 was 0.079 and 0.086, respectively, which indicates that the collagen can still maintain the triple helix structure after treated by acid protease. In addition, the Rpn of collagen is reduced in varying degrees by L1 and L4 acid protease. By the comparison, we can make the conclusion that L1 acid protease has a greater effect on collagen than L4 when using them for bating.

Enzymatic Properties of L1 acid protease

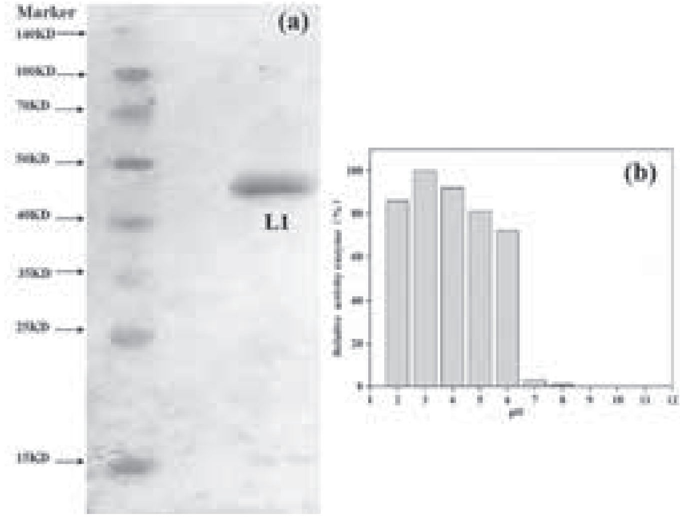

Figure 4. The enzymatic properties of L1 acid protease. (a) The SDS-PAGE of L1;(b) The optimum pH of L1.

From Fig.4(a), it is seen that the molecular weight of the functional protein is 48 KD, which display significantly effect in bating process. Fig.4(b) shows that the enzyme activity of L1 is stable under acid conditions. But under neutral or alkaline conditions, the enzyme is inactivated. The optimal pH of L1 is 3.0, and it is corresponding with the bating condition which the pH is in the range of 2.5-3.0.

Observation of the Porosity or Dispersion of Collagen Fiber (Bundle)

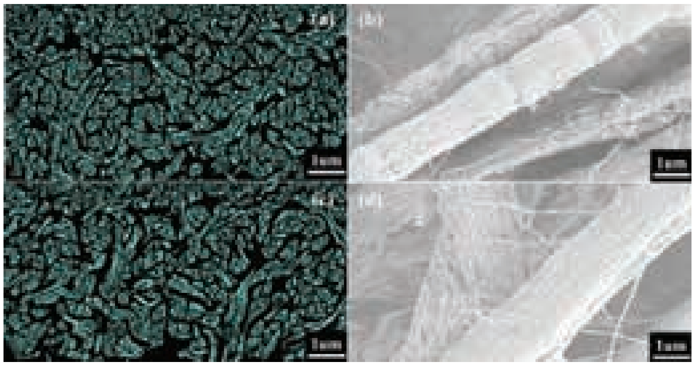

Figure 5. (a) The Micro-CT image of untreated sample (b) The SEM image of untreated sample. (c) The Micro-CT image of sample treated with L1. (d) The SEM image of sample treated with L1.

Comparing with the Fig.5 (a) and Fig.5(c), it can be seen that the porosity of collagen fiber bundles in Fig.5(c) is larger than that in Fig.5 (a), which results in the excellent air permeability of crust leather. From Fig.5 (b) and Fig.5 (d), the collagen fibers of sample untreated are tightly woven. Therefore, this structure results in the less than ideal properties of wet blue including low softness and weak hygienic properties. After treated with acid protease, the tightly woven structure of collagen fibers is dispersed. It explains the increase of air permeability and softness after acid protease treating.

Analyze the Biodegradation Rate of L1 to structural protein in Wet blue

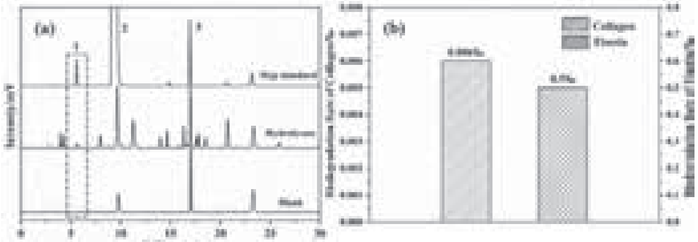

Figure 6. (a) The HPLC chromatograms. (b) The biodegradation rate of Collagen and Elast*in. 1: Hyp; Peak 2: 2, 4-Dinitrophenol; 3: 2, 4-Dinitrofluorobenzene*

In Fig.6(a), when elution time approached 6 minutes, the elution peak of Hyp appeared. According to the standard curve equation of Hyp and Des, the concentrations of Hyp and Des in enzymatic hydrolysate were 0.17 mg/L and 0.071 mg/L, respectively. According to formulas (2) and (3), Fig.6(b) shows that the biodegradation rates of collagen and elastin were 0.006‰ and 0.5‰, respectively. As known, the chromium in wet blue mainly reacts with carboxyl groups in hide and the carboxyl groups in elastin is less than that in collagen. Therefore, the degradation of elastin by acid protease is effective to disperse the collagen fibers by removing interstitial protease, while the application of acid protease has no adverse effect on chrome-tanned leather. In brief, the result illustrates that the acid protease is more target to elastin and does not lower the quality of finished leather.

Conclusions

The results show that the acid protease produced from *Aspergillus Niger and Aspergillus Oryza* has better chromium resistance than that from *Bacillus subtilis*, thus the acid protease from *Aspergillus* is more suitable for wet blue bating process. In the acid bating process, acid protease can degrade elastin in wet blue and disperse collagen fibers thereby improving the properties of crust leather. Furthermore, the degradation degree of elastin in wet blue by acid protease is directly corresponding with the physical and chemical properties of crust leather. In conclusion, we successfully screened the suitable acid proteases for acid bating process and investigated the degradation mechanism of wet blue. This research has great significance for the application of acid protease on wet blue bating.

Acknowledgements

This study was financially supported by the National Key Research and Development Program of China under contract No. 2017YFB0308402. And the national natural science foundation of china (No.21808170).

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A Novel Non-Pickling Combination Tanning for Chrome-free Leather Based on Reactive Benzenesulphonate and Tannic acid

by
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Abstract

A non-pickling combination tanning based on reactive benzenesulphonate (rBS) and Tannic acid (TA) has been developed for the chrome-free leather manufacturing. By optimizing the tanning process, the two-bath combination tanning with 4% rBS and 10% TA at the final pH 3.0-3.5 can raise the shrinkage temperature (T_s) of goatskins to ~86°C. Morphological analysis results reveal that the chrome-free leather exhibits a clear even grain surface and isolated collagen fiber network structure. The novel combination tanning approach not only improves light fastness, but also confers high physical and mechanical properties to the chrome-free leather which can meet the Chinese standard requirements for furniture leather and shoe upper leather. The non-pickling tanning without use of salt or acid is also benefit to clean leather-making production.

Introduction

Chrome tanning remains the most widely used tannage in world leather industry due to excellent comprehensive properties of the resultant leather.[1] However, the utilization of chromium in conventional chrome tanning procedure is only 60-70%, which not only causes serious waste of chromium resources, but also brings about negative effects on the environment because of large quantities of chromium-containing sludge[2] and leather waste.[3] Trivalent chromium salts used in tanning systems are potentially oxidized to hexavalent chromium in the presence of alkali, UV radiation and high temperature, as well as post-tanning materials such as fatliquoring agents containing unsaturated bonds.[4] Since Cr(VI) is considered as highly allergenic,[5] carcinogenic[6] and genotoxic[7] to animals and humans, the chrome-tanning solid waste has been listed in National Hazardous Waste list (HW21) in China since 2008. Moreover, increasingly strict restrictions on the Cr(VI) level in leather articles have been issued in succession.[5] In addition, high concentration of salt contamination is introduced in conventional pickling-chrome tanning process.[8] The Cl^- content in effluents from leather plants must be below 3000 mg/L before discharge to the environment as required by Mandatory National Standard GB 30486-2013, China.[9] Therefore, exploring of eco-friendly chrome-free tanning agent and green tanning process is becoming highly desirable for the sustainable development of the leather industry.[10]

At present, chrome-free tanning agents mainly include non-chrome mineral tanning agents (aluminum, titanium and zirconium) and organic tanning agents such as vegetables, aldehydes and syntan etc.[11] And the combination tanning[12] is always applied due to the relatively poor tanning property of those agents by solo tanning. Among them, the chrome-free combination tanning based on vegetable tannins has drawn great attention because of its natural origin, suitable molecular weight and appropriate tanning properties.[13] So far, combination tannages by tannic acid-zinc sulfate or sodium silicate,[14] tannic acid-aluminum-silica[15] and tannic acid-organophosphorus[16] have been reported. However, the potential environmental and health risks from metal salts (Al^{3+}) and free formaldehyde in some syntans cannot be neglected. The newly developed combination tannage based on TA and nano-clay shows that the improvement in leather physical performances and environmental impact can be achieved by the appropriate combination of the two tanning agents with cooperation effect.[17,18]

In addition, some novel chrome-free tanning materials have been developed and commercialized. We noticed a novel amphoteric organic compound syntan, reactive benzenesulphonate (Figure 1). The reactivity of the two chlorine groups ($-Cl^-$) in the benzenesulphonate molecule are temperature-dependent. The first one (marked in red in the structure, Figure 1) can react with amino group of collagen at 30-50°C; while the reaction temperature for the second one needs to be above 80°C. And its sulfonate groups ($-SO_3^-$) and secondary amine can also interact with $-NH_3^+$ and $-COO^-$ on collagen under certain conditions.[19] Its tanning effect can be simply performed at initial pH 7 and produce light color leather. Nevertheless, the tanned leather has some shortcomings such as low shrinkage temperature and poor storage resistance.

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Manuscript received August 8, 2019, accepted for publication September 24, 2019.

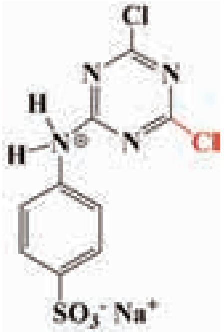

Figure 1. The structure representation of reactive benzenesulphonate

In the present work, we have developed a combination tannage based on reactive benzenesulphonate and TA in order to overcome the negative aspects of solo tanning and avoid potential metal and neutral salt pollution. The parameters of the tanning process have been optimized and the characteristics of the obtained leather have been investigated. Our aim is to develop a novel non-pickling combination tanning technology for chrome-free leather.

Experiments

Materials

The limed goatskin pelts with an average area of 4~5 square meters were taken as raw materials for leather processing. The dosages of the chemicals were all based on the weight of limed pelts. Reactive benzenesulphonate (denoted as rBS hereafter), sodium p-[(4,6-dichloro-1,3,5-triazin-2-yl)amino]benzenesulphonate (CAS: 4156-21-2), is from Granofin® Easy F-90 liq with 20~25 wt% active component, courtesy of Stahl Company. Tannic acid (TA, tannins content: 81 wt%) was purchased from Institute of Chemical Industry of Nanjing Forest Products, China. All the chemicals used in leather processing were of commercial grade.

Particle size determination of TA and rBS

0.1 g/L TA and rBS aqueous dispersions were prepared respectively before measurement and then measured at 25°C using a nanoparticle size and Zeta potentiometer (Zetasizer NanoZS, Malvern, England). Each value reported is an average of three parallel test data.

Tanning process

Table I gives the combination tanning process recipe for chrome-free leather. The limed goat pelts were delimed, bated and washed before tanning. For two-bath combination tanning, the pelts were first tanned with 1~10 wt% rBS for 8 h without pickling,[20] then rinsed and tanned by TA with varied amounts from 5~15 wt% for another 3 h. The final pH of tanning floats was adjusted to 3.0~5.0 with an interval of 0.5 by $NaHCO_3$. The obtained chrome-free leathers were piled overnight. In order to compare the tanning effect, first adding TA and then rBS (TA-rBS) in separate baths was also studied. In addition, rBS-TA tanning in the same float, namely one-bath combination tanning was carried out as well to explore the tanning mechanism. Solo rBS tanning was performed under the same conditions (Table I) as control. The dosages of tanning materials were optimized by the measurement of the shrinkage temperature (T_s) of the crust leathers and the uptake of TA in the tanned float.

Determination of shrinkage temperature

The shrinkage temperature of crust leather was determined by a shrinkage tester using the ASTM method.[20] A 10 mm 60 mm sample was cut out from the tanned leather and hung vertically in water. The rate of heating was kept at 2°C per minute. The temperature at the first definite sign of shrinking of the samples was taken as T_s. Each experimental result was obtained from average of three samples.

Measurement of the uptake of TA

The standard curve of TA concentration against the maximum absorbance value was measured and plotted (R^2 = 0.9990) by using UV-visible spectrophotometer (UV3600, Shimadzu, Japan) according to previously reported method.[21] Then, 10 mL spent liquor from solo TA tanning and experimental tanning processes were collected and centrifuged at 2500 rpm for 5 min to remove suspended solids. The TA concentration in the spent liquor was analyzed by UV-visible spectrophotometer and the uptake of TA was calculated by the formulation as follow.

$$\text{Uptake of TA (\%)} = [(C_O - C_S) / C_O] \times 100 \qquad (1)$$

Where C_O is the concentration of TA offered and C_S is the concentration of TA in the spent liquor.

Table I

rBS-TA combination tanning Process

Process	Material	Dosage (%)	Time (h)	Temperature	pH
Limed goats were weighed, delimed, bated and washed as conventional process.					
Tanning	Water	40		35 °C	~7.8
	rBS	1~10	2		~6.0
	Water (55-65°C)	30	2	40 °C	~5.6
	Water (55-65°C)	30	4	45 °C	~5.0
	Draining, and washing				
	Water	100		25 °C	5.0
	TA	5~15	3		
	HCOOH	0~0.5	1	35 °C	3.0~5.0
Check the final pH, stay overnight and measure T_s.					

Zeta potential measurements

10 g chrome-free leather samples were pulverized into powder particles and dispersed in 400 mL deionized water to form suspension. The pH of the suspension was adjusted to 2.5~6.0 with an appropriate amount of 0.1 mol/L HCl or NaOH solution, respectively, and then stirred at 150 rpm, 30°C for 30 min. The Zeta potential of the suspension was measured using a flow potential analyzer (MütekTM SZP-10, BTG, Germany). Each experimental result is obtained from average of three parallel test data. The zero point indicates the isoelectric point (IEP) of leather.[22]

SEM analysis

After lyophilized at -50°C in a freeze dryer for 24 h, experimental crust leather samples were cut into specimens with uniform thickness (~1 cm × 1 cm) by a microtome (CM1900, Leica, Germany), respectively. The cross-section images of samples at low and high magnification levels were obtained by a scanning electron microscope (JSM-7500F, JEOL, Japan). The accelerating voltage was set as 15 KV.

Determination of color difference

The control and experimental crust leather were irradiated by 15 W ultraviolet lamp at room temperature for different times before measurement.[23] Then the color parameters of the irradiated crust leather were measured by Chromaticity analyzer at the spectral detection wavelength of 400~700 nm. Color measurement of lightness-darkness (L^*), redness-greenness (a^*) and yellowness-blueness (b^*) were recorded, and the color difference (ΔE) was calculated using the following equations:

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \tag{2}$$

Where ΔL^*, Δa^* and Δb^* represent the lightness, redness-greenness and yellowness-blueness differences between irradiated and unirradiated crust leather, respectively, and ΔE represents the total color difference.

Physical and mechanical properties measurements

The control and experimental leather specimens of standard dimensions for various physical tests were obtained according to IULTCS methods.[24] The specimens were first conditioned at 20 ± 2°C in a suitable environment (RH = 65±2 %) for 48 h. Then physical and mechanical properties, such as tensile strength, elongation at break,[25] tear strength[26] and bursting strength[27] were measured as per standard procedures. Each value is an average of three samples.

Results and Discussion

Optimization of rBS and TA combination tanning

Combination tanning methods

Figure 2 shows the T_s of the crust leathers obtained by different tanning methods. It is clear that the adding order of tanning agents has significant effect on the T_s. First tanning with rBS and then TA (rBS-TA) results in higher hydrothermal stability of crust leathers than that of TA-rBS. This is reasonable because the underlying tanning mechanisms of TA and rBS are different. Vegetable tannin like TA reacts with collagen primarily via hydrogen bonding and has a strong filling effect within the collagen fiber structure.[28] Whereas rBS can form covalent bonds with the amino groups of collagen and exhibit tanning effect. In the case of TA-rBS tanning, the smaller TA particles (Figure 3) initially penetrate into collagen fibers, then fix to polar side chains of collagen with available carboxyl, amino or hydroxyl groups (depending on pH). Especially, the possible interaction of the polyphenols with amino sidechains is unfavorable to the covalent bonding between rBS and the amino groups of collagen. Thus, adding of TA before rBS may block the diffusion paths and hinder the penetration and bonding of the larger-sized rBS molecules.

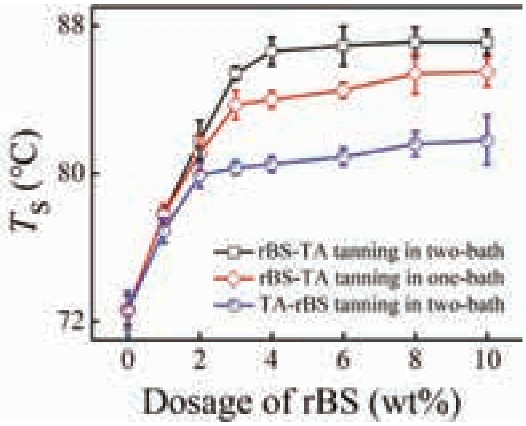

Figure 2. T_s of crust leathers obtained by varied tanning methods. The dosage of TA was kept at 10% based on the weight of limed pelts.

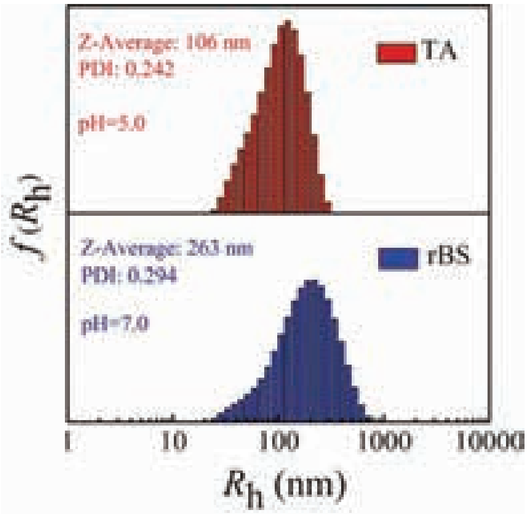

Figure 3. Hydrodynamic radius distributions $f(R_h)$ of TA and rBS at 25 °C.

Furthermore, Figure 2 also shows that for rBS-TA combination tanning, two-bath method is better than one-bath, as reflected in higher T_s. In other words, it is necessary to rinse the rBS-tanned leather before shifting to TA tanning float, because the higher temperature (~45°C) and residual benzenesulphonate in rBS pre-tanned float may facilitate the formation of TA-based aggregates in the outer layer of the pelt. Such insufficient combination tanning can only cause limited increase of T_s to the leather with the increasing of rBS. Therefore, rBS-TA two-bath combination tanning is the optimal tanning system, and it is used for further experiments.

The dosage of rBS and TA

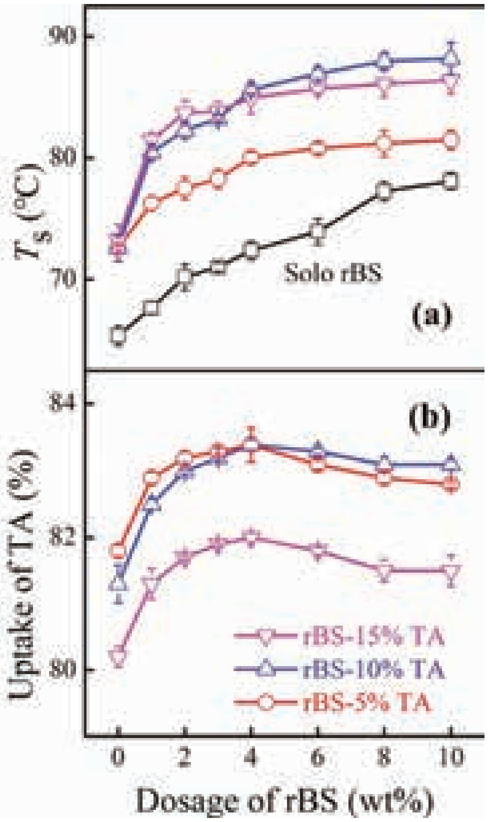

Figure 4. T_s of crust leather (a) and the uptake of TA (b) as a function of rBS dosage

Figure 4a shows the T_s of rBS-TA combination tanned crust leather. Note that the solo TA tanning with 5~15% dosage can raise the T_s of bated goatskins by 6~8°C. The solo rBS tanning with 1~10% dosage gives an increase in T_s of 3~12°C. In contrast, the T_s of rBS-TA combination tanned leather reaches ~85°C. Namely, the rise in T_s is much higher than that of solo TA and rBS tanning at the same dosage, showing the synergistic tanning effect of rBS and TA. Moreover, the uptake of TA significantly increases in comparison to solo TA tanning, indicating that rBS can facilitate the fixation of TA in crust leather (Figure 4b). When rBS is above 4%, the T_s values plateaus, whereas the corresponding uptakes of TA decrease slightly. It can be related to the blockage of available bonding sites of collagen fibers by rBS and TA at certain concentrations. On the whole, the use of 4% rBS and 10% TA for the combination tanning can be both efficient and economical compared with the reported 10% benzenesulphonate solo tanning.[19]

Final pH

It is known that for both collagen and tanning agents, the availability of reactive groups depend on the float pH, which in turn has a substantial influence on the tanning reactivity.[29] Figure 5 shows the effect of final pH in the tanning float on the T_s of crust leather. When the pH is adjusted to 3.3~3.5, the T_s reaches the peak. The reason can be that at the pH close to the precipitation point of TA (pH = 2.5), TA particles tend to aggregate and deposit onto collagen fibers,[18] promoting the synergistic interaction between rBS and TA. However, further lowering pH, the efficient binding of tanning materials and collagen fibers may decrease instead in view of the T_s. Therefore, the final pH in the combination tannage is optimized as 3.3~3.5.

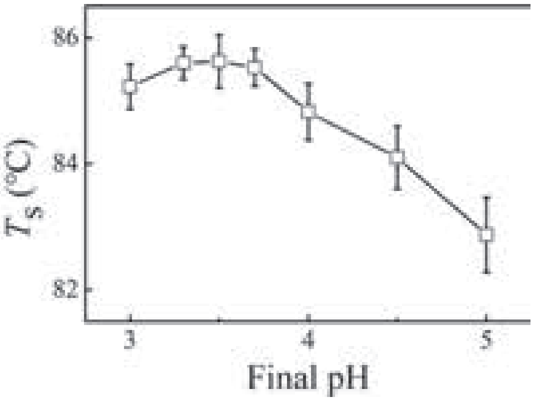

Figure 5. T_s of crust leather as a function of final pH.

Zeta potential analysis

The isoelectric point (IEP) of collagen and its changes during leather making can indicate the state of hide substance and help to understand the bonding between tanning agents and the groups on the collagen molecule, revealing the tanning mechanism.[30] As given in Figure 6, IEP of solo rBS tanned leather is at pH ~4.81, lower than that of bated goatskins (~5.2). It is because the surface positive charge of leather (NH_3^+) is blocked by rBS. After the introduction of TA, IEP of the chrome-free leather decreased to pH ~3.66, showing some kind of characteristics of vegetable tanned leather (IEP 3.2~4.0).[29] Moreover, the float pH 4.5~5.0 in the later rBS tanning is basically consistent with the initial pH of TA tanning, which is beneficial to the penetration of TA into collagen fibers. Therefore, non-pickling tanning is reasonably feasible to the present combination tanning system, so that the salt pollution from conventional chrome tannage can be eliminated.

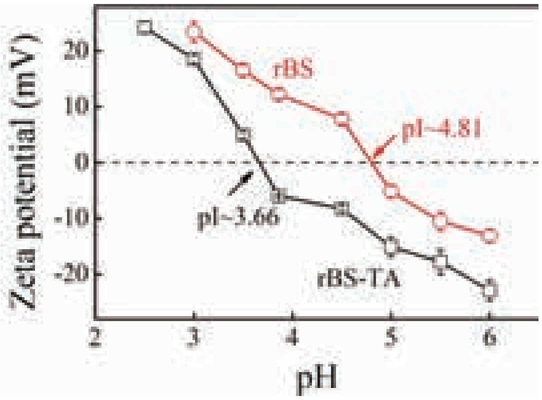

Figure 6. Zeta potentials of solo rBS and rBS-TA combination tanned leathers at different pH.

3.3 Morphological analysis

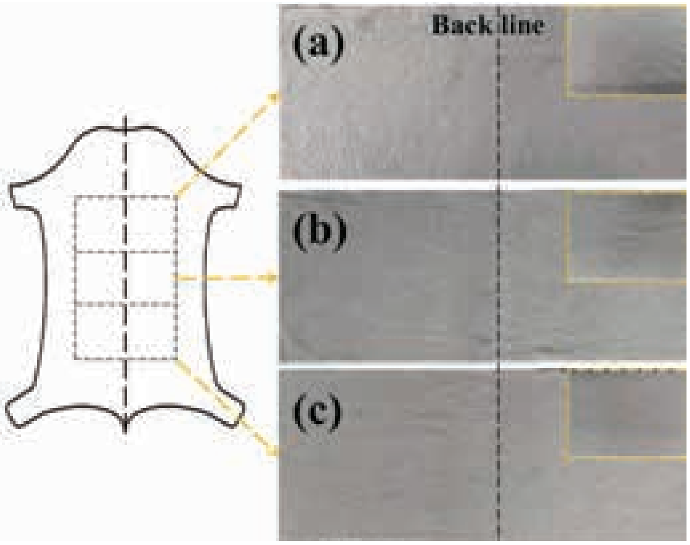

Figure 7. The photos of solo TA tanned (**a**), solo rBS tanned (**b**) and rBS-TA combination tanned (**c**) crust leathers.

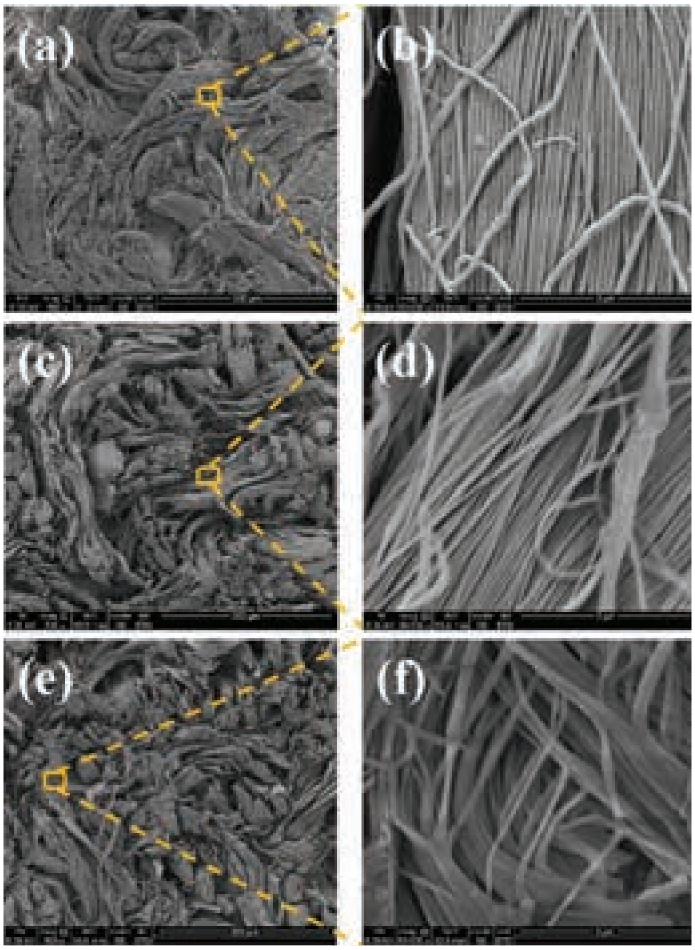

Figure 8. SEM morphologies of cross sections of solo TA tanned leather (**a** and **b**) , solo rBS tanned leather (**c** and **d**) and rBS-TA combination tanned leather (**e** and **f**).

Figure 7 shows the photos of solo TA, solo rBS and rBS-TA combination tanned crust leathers. The presence of TA confers the crusts (Figure 7a and c) tight and clear grain surface, mainly attributed to the filling and astringent nature of TA. In contrast, the solo rBS tanned leather (Figure 7b) exhibits fine and even grain, and the rBS-TA tanned leather (Figure 7c) does combine the features of both, showing clear and fine grain surface with fullness, elasticity and foam hand-feel.

SEM observations (Figure 8) further indicate that solo TA tanned leather exhibits tight and thick collagen fiber bundles (Figure 8a and b). Whereas, the collagen fibers of solo rBS tanned and the combination tanned leathers seem loosened and isolated to some degree (Figure 8c-8f). The results suggest the advantages of rBS-TA combination tannage.

The synergistic tanning mechanism of rBS and TA

Figure 9 shows the synergistic tanning mechanism of rBS and TA. It is generally accepted that Type I collagen plays a key role in the manufacture of leather and in determining the properties of leather. The content of acidic (7.82%) and basic (8.61%) amino acids, amino acids containing hydroxyl groups (15.24%) and peptide groups is decisive for the reactivity of the collagen.[29] As to rBS tanning in an aqueous solution, reactive chlorine on benzenesulphonate molecule is hydrolyzed to hydroxyl first, then reacts with side-chain aminos of Lys, Hyl and Arg residues by C–N covalent bonding. The covalent crosslinking is regarded as the predominant reaction in rBS tanning. Meanwhile, the released H^+ can automatically reduce the pH of the tanning float, promoting the formation of $-NH_3^+$ on collagen and its subsequent bonding with sulfonate groups ($-SO_3^-$) of the benzenesulphonate by electrostatic salt linkage.[19] Phenolic hydroxyl groups of TA can not only form hydrogen bonding with hydroxyl groups of Hyp, Hyl, Ser and Tyr residues or peptide groups in the fundamental chain,[31] but also can interact with secondary amine of benzenesulphonate molecules by hydrogen bonding. Thus, the synergistic crosslinking within collagen fibers can be formed, which substantially contributes to the enhanced hydrothermal stability of the chrome-free leather.

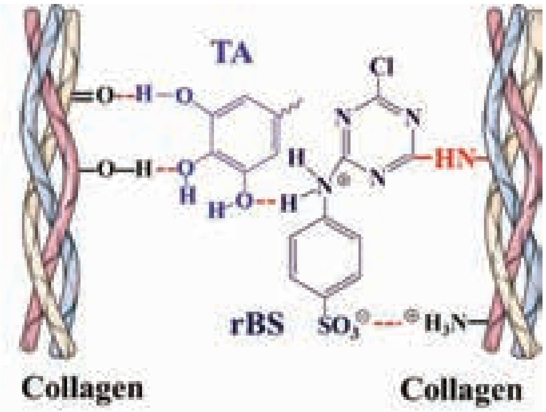

Figure 9. Schematic representation showing synergistic interactions of rBS and TA with collagen in the combination tannage

Color difference analysis

Light fastness is assessed by the exposure of the crust leathers in UV irradiation.[32] As shown in Figure 10a, the color difference (ΔE) of rBS-TA combination tanned leather is lower than that of solo rBS tanning under different irradiation time, illustrating that the introduction of TA improves the light fastness of crust leather. The enhanced resistance to UV irradiation can be due to the UV absorption capacity and antioxidant ability of TA.[33] In addition, the introduction of TA slightly reduces L^*value of the combination tanned leather (Figure 10b) due to the darkness effect of TA. Nevertheless, rBS-TA combination tanned leather still gives a light shade (Figure 7).

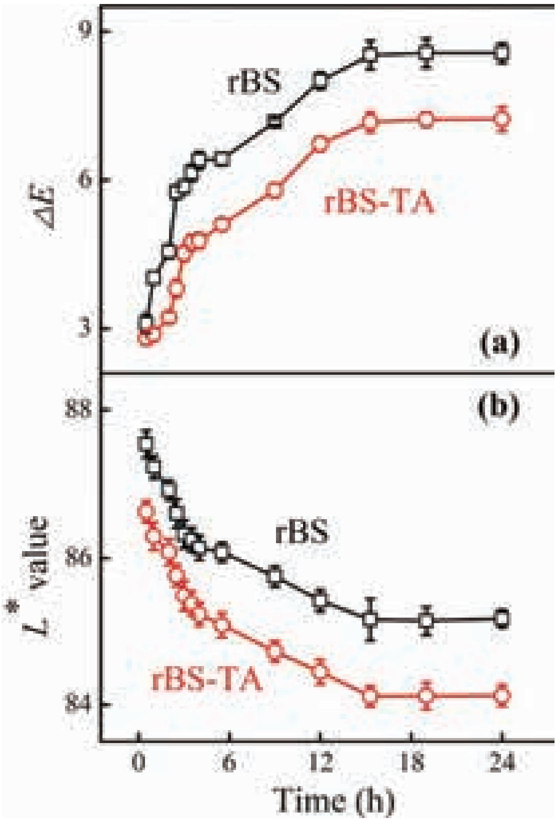

Figure 10. ΔE (a) and L^* value (b) of crust leathers under UV irradiation for 24 h.

Physical and mechanical properties measurements

The physical and mechanical properties of crust leathers including tensile strength, tear strength, elongation at 10N, elongation at break, bursting strength and T_s are shown in Table II. Note that all of the physical properties of rBS-TA combination tanned leather are better than that of solo rBS tanned leather. This is consistent with the increased hydrothermal stability owing to synergistic tanning effect of rBS and TA. Moreover, all of the physical properties can meet the Chinese standard requirements for furniture leather[34] and shoe upper leather[35]. Therefore, the rBS-TA non-pickling combination tannage is promising for the manufacture of furniture leather and shoe upper leather.

Table II

Physical and mechanical properties of crust leathers

Parameters	rBS	rBS-TA	furniture leather	shoe upper leather
T_s / °C	77.3±0.8	86.5±0.7	/	≥80
Tensile strength/ MPa	13.8±2.4	20.4±2.1	/	/
Tearing load / N	48.8±3.1	52.2±4.4	≥40	≥20
Tear strength / N·mm^{-1}	43.5±3.9	45.4±3.3		
Elongation at 10N / %	21.8±3.2	26.2±4.1	/	≤40
Elongation at break / %	44.3±2.1	58.3±2.4	35~60	/
Bursting strength / N·mm^{-1}	182.3±3.2	259.7±3.6	/	≥200

Conclusions

A novel non-pickling combination tanning for chrome-free leather based on reactive benzenesulphonate and tannic acid has been developed in this work. Compared with TA and rBS solo tanned leathers, the hydrothermal stability of the rBS-TA tanned leather increases significantly due to the presence of synergistic effect between rBS and TA. The optimized combination tanning, i.e 4% rBS and 10% tannic acid at the final pH 3.0-3.5 in two-bath process, can confer the leather with a T_s of above 86°C. And the obtained leather has fine isolated collagen fiber network structures. Moreover, the introduction of TA improves physical properties and light fastness of leather. The non-pickling combination tannage can not only completely eliminate the risks of chromium salts and free formaldehyde, but also reduce the neutral salts used in conventional pickling process, most beneficial to clean leather production.

Acknowledgements

This work was financially supported by National Natural Science Foundation (NNSF) of China (21978177), Innovation Team Program of Science & Technology Department of Sichuan Province (Grant 2017TD0010), and National Key R&D Program of China (2017YFB0308402).

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Insight into the Correlations Between Fiber Dispersion and Physical Properties of Chrome Tanned Leather

by

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Abstract

The correlations between fiber dispersion and physical properties of chrome tanned leather were investigated to provide new insights into improving leather quality and designing tanning agents and tannages. Wet blues were prepared by tanning pickled pelts with different amounts of chrome powder, and their pore structures in the range of 5.48 nm–120 µm were measured using a mercury intrusion porosimetry (MIP). The porosity and total pore area of wet blue increased with increasing amount of chrome powder from 2% to 8%. These MIP data combined with the images observed using a field emission scanning electron microscopy and a scanning transmission electron microscopy–energy dispersive spectroscopy indicated that chrome tanning agent opened up the microfibrils, fibrils, elementary fibers and fiber bundles of wet blue. The sufficient fiber dispersion of wet blue was attributed to the fact that chrome tanning agent penetrated into the microfibrils and fixed collagen fibers from microfibril level to fiber bundle level. The wet blues were then fatliquored to prepare crust leathers. The tensile strength, tear strength, elongation at break, softness, fullness and water vapor permeability of the leathers improved with increasing chrome powder. These results implied that there is a positive correlation between fiber dispersion and physical properties of chrome tanned leather.

Introduction

Leather products are popular goods due to their high mechanical strength, unique organoleptic properties, excellent water vapor permeability and so on.[1] Modern leather processing mainly involves beamhouse, tanning and post-tanning processes.[2] The beamhouse process removes unwanted components from raw hides/skins and opens up their collagen fibers to facilitate the penetration of tanning agents.[3–5] The tanning process converts putrescible raw hides/skins into durable leathers by crosslinking collagen fibers with tanning agents.[6–9] During this process, adhered fibers of pelts are turned into a stably dispersed state.[8] The post-tanning process improves the organoleptic and mechanical properties of leathers with various retanning agents, dyes and fatliquors.[10,11] The usage of these agents also changes the dispersion of collagen fibers.[2] Generally, the fiber dispersion of leather is affected by almost all processes. Tanners have believed that the properties of leather are closely related to its fiber dispersion. However, the correlation between fiber dispersion and physical properties of leather remains unclear.

Leather is a fibrous material with hierarchical structure, which from primary level to high level is woven by tropocollagens, microfibrils, fibrils, elementary fibers and fiber bundles.[12–15] The tanning process greatly influences the microstructure of collagen fibers, and the effect of tanning agents on the fiber dispersion at high level (elementary fibers and fiber bundles) has been observed by scanning electron microscopy (SEM) and biological microscopy. [3,6–8,15–18] However, minimal attention has been paid to the change in fiber dispersion at primary level (tropocollagens, microfibrils and fibrils), because it is difficult to observe the fiber dispersion at this level by common technologies.[3,4] Previous studies showed that the microstructure of fabrics affects their macroscopic properties. Niu et al. reported that the increased orientation of fiber molecular structure improves the tensile strength and elongation of wool fiber, compared with parent wool fiber.[19] Hence, the fiber dispersion of leather at primary level may significantly affect the macroscopic properties of leather. We previously verified that the mercury intrusion porosimetry (MIP) can measure the pore structure of leather in a wide range to quantify its fiber dispersion from primary level to high level with high accuracy.[20] Thus, we planned to investigate the change in fiber dispersion at primary level during tanning process by using MIP.

This study aimed to assess the correlation between fiber dispersion and physical properties of chrome tanned leather and to give some suggestions for improving leather quality and developing novel tanning agents and tannages. Wet blue with different fiber dispersion degrees were prepared by tanning pickled pelts with different amounts of chrome powder. Field emission scanning electron microscopy (FESEM), scanning transmission electron microscopy–energy dispersive X-ray spectroscopy (STEM-EDS) and MIP were used to analyze the fiber dispersion of these wet blue from primary

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Manuscript received August 23, 2019, accepted for publication September 30, 2019.

level to high level. After fatliquoring, the physical properties of the crust leathers, including tensile strength, tear strength, elongation at break, softness, fullness and water vapor permeability were determined, so that the correlation between fiber dispersion and physical properties of leather can be analyzed.

Experimental

Materials

Pickled cattle pelts were purchased from Ruixing Leather Co., Ltd. (Haining, China). Chrome powder (24% Cr_2O_3, 33% basicity) was supplied by Minfeng Chemical Co., Ltd. (Chongqing, China). The other reagents used for leather processing were of commercial grade. The chemicals utilized for analyses of leathers were of analytical grade.

Preparation of Wet Blue and Crust Leathers

To obtain wet blue, four pieces of pickled cattle pelts cut from back region were tanned by 2%, 4%, 6% and 8% of chrome powder, respectively, as shown in Table I. The wet blue were then rewetted, neutralized and fatliquored following the post-tanning processes shown in Table I to prepare crust leathers. The wet blue and crust leathers tanned by 2%, 4%, 6% and 8% of chrome power were recorded as Cr-2, Cr-4, Cr-6 and Cr-8, respectively.

Morphological Observation

The pickled pelt and wet blue samples were collected and lyophilized by freeze dryer (LGJ-30F, XinYi, China). Next, the cross sections of these samples were observed by FESEM (Nova Nanosem 450, FEI, USA). Besides, the lyophilized samples were embedded in epoxy resin and then sliced into 100 nm thickness using a freezing microtome (UC7, Leica, Germany). The thin slices were placed on copper fabricates for imaging at 200 kV by STEM (JEM 2100F, JEOL, Japan), and for surface elemental analysis by EDS (X-Max^N 80T, Oxford, UK). The cross sections of crust leather samples were observed by SEM (Phenom Pro, Phenom-world, Netherlands).

Measurement of Pore Structure

The pickled pelt and wet blue samples were lyophilized, and the crust leather samples were dried at 45 °C for 48 h. Then, the pore structures of the pickled pelt, wet blue and crust leather samples were measured using a mercury intrusion porosimetry (AutoPore IV 9500, Micromeritics, USA) as described by He et al.[20]

Determination of Chrome Content

A total of 0.1 g dried wet blue sample was digested with the mixture of nitric acid (10 mL) and H_2O_2 solution (5 mL, 30wt%) with a microwave digestion instrument (Multiwave PRO, Anton Paar,

Table I

Tanning and post-tanning processes

Process	Chemicals	Amount[a] (%)	Temperature (°C)	Time (min)	Remarks
Pickling	Water	100	22		
	Sodium chloride	7		10	
	Formic acid	0.3		30	pH=2.9
Tanning	Chrome powder	X	22	180	X=2, 4, 6, 8
	Sodium formate	1	22	30	
	Sodium bicarbonate	(0.1-0.3)×n		15×n+30	pH =3.8
	Water	100	40	180	
Next day, run 30 min. Horsing up and wringing. Wet blue sampling.					
Rewetting	Water	400	35		
	Degreasing agent	0.3		60	
Washing	Water	400	35	10	
Neutralizing	Water	200	35	30	
	Sodium formate	2			
	Sodium bicarbonate	1.2		10+60	pH=6.2
Washing	Water	400	40	10	
Fatliquoring	Water	200	50		
	Compound fatliquor	6			
	Surface fatliquor	3			
	Synthetic fatliqour	1		60	
	Formic acid	0.6		30	pH=3.8
Washing	Water	400	25	3×10 min	
Horsing up, drying and staking. Crust leather sampling.					

[a] The percentage of chemicals was based on the double weight of pickled pelt.

Austria). The digestion solution was properly diluted to 50 mL to determine the chrome content by inductively coupled plasma atomic emission spectroscopy (ICP-AES, Optima 8000DV, PerkinElmer, USA). The chrome content of wet blue was calculated as follows:

Where c is the chrome concentration measured by ICP-AES (mg/L), 0.05 is the volume of diluted digestion solution (L), and m is the mass of the dried sample (g).

Determination of Physical Properties of Crust Leather

The dried crust leather samples were conditioned at 20 °C and 65% relative humidity for 48 h in accordance with IUP 3 method, and then sampled to determine physical properties. The tensile strength, tear strength and elongation at break of the samples were measured according to the IUP 6 and IUP 8 methods using a tensile tester (AI-7000SN, Gotech, China). The softness of the samples was measured using a standard leather softness tester (GT-303, Gotech, China) following the IUP 36 method. The fullness of sample was evaluated by compressed and resilient thicknesses using the method described by Penget al.[21] The water vapor permeability was measured using a GT-7005-E instrument (Gotech, China) following the IUP 15 method.

Results and Discussion

Fiber Dispersion of Wet Blue

As mentioned earlier, the aim of this study was to assess the correlation between fiber dispersion and physical properties of chrome tanned leather. Therefore, wet blue with different fiber dispersion were first prepared by tanning pickled pelts using different amounts of chrome powder varying from 2% to 8%. The fiber dispersion of pickled pelt and wet blue was observed by FESEM at different magnifications. The low magnification FESEM images in Figure 1 show that the fiber bundles (Φ 20–200 μm)[12] of the pickled pelt were adhesive, while those of the wet blue were highly dispersed. Moreover, the degrees of fiber dispersion of wet blue increased with increasing chrome content. Here, the chrome content of wet blue increased from 1.05% to 2.27% when the amount of chrome powder rose from 2% to 8%. This suggested that high chrome content of wet blue favored the crosslinking and fixation collagen fibers. It is worth noting that the degrees of fiber dispersion increased slightly when the amount of chrome powder exceeded 6%, because the carboxyl on collagen that can react with chrome is limited.

The high magnification FESEM images in Figure 2 show the fiber dispersion of the pickled pelt and wet blue at primary level. The chrome tanning process increased the dispersion of fibrils and the clearness of collagen D–period. This implied that the chrome tanning agent penetrated into the fibrils. The STEM images in Figures 3(a) and 3(d) show that alternately arranged light banding (~0.6 D gap) and dark banding (~0.4 D overlap) were observed in

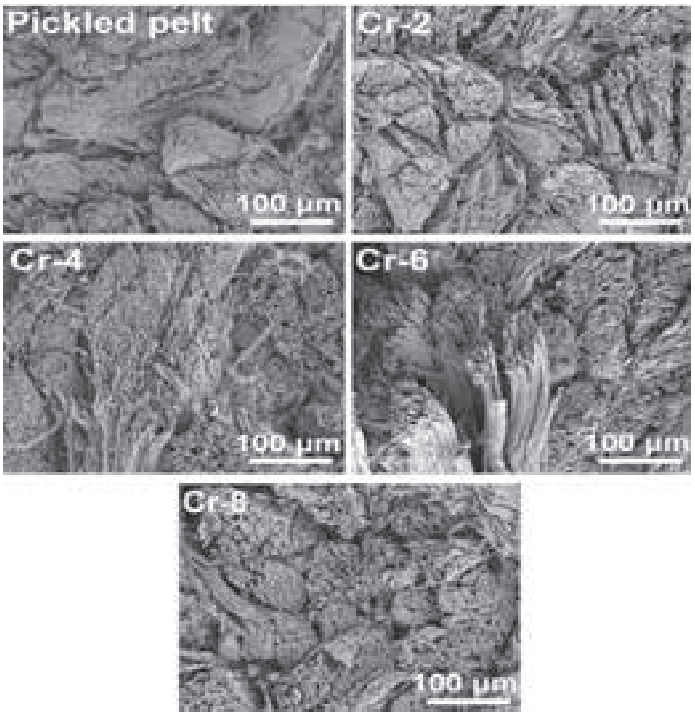

Figure 1. Low magnification FESEM images of cross sections of pickled pelt and wet blue.

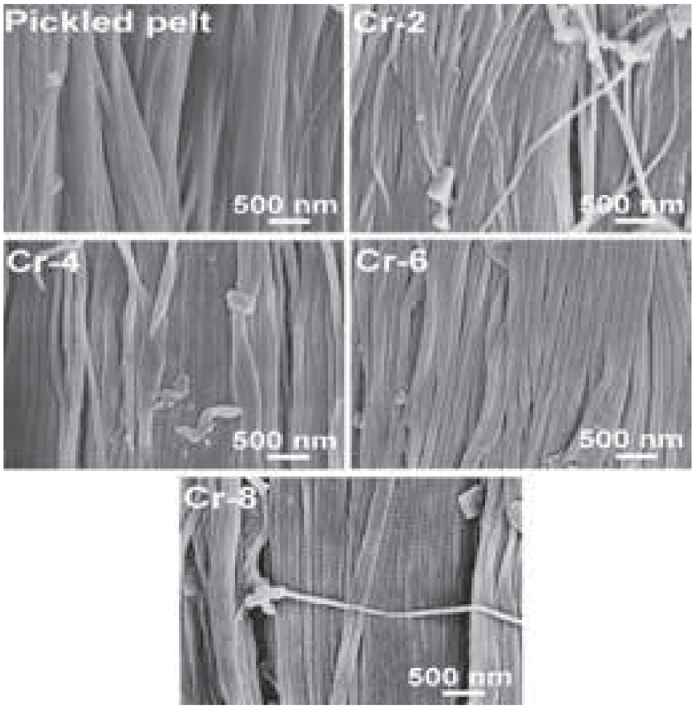

Figure 2. High magnification FESEM images of cross sections of pickled pelt and wet blue.

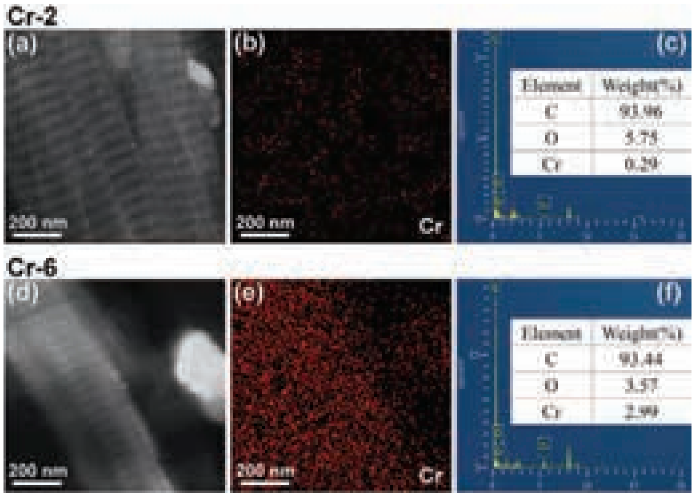

Figure 3. STEM and EDS mapping images of wet blue tanned by 2% chrome powder (a-c) and 6% chrome powder (d-f).

Table II

Effect of amount of chrome powder on pore structure of wet blue.

Wet blue	Average pore diameter (nm)	Porosity (%)	Total pore area (m^2/g)
Pickled pelt	1955.79	49.73	1.449
Cr-2	1691.38	50.25	2.212
Cr-4	1130.14	50.90	2.998
Cr-6	841.36	56.79	4.066
Cr-8	806.04	57.80	4.418

fibrils. The characteristic D-period structure was approximately 65 nm, which was close to the value reported in literature.[22] The wet blue had uniform distribution of chrome within the fibrils (Figures 3(b) and 3(e)), which also indicated that the chrome tanning agent could penetrate into the fibrils. By comparing Figures 3(c) and 3(f), it was found that higher content of chrome in the fibrils led to better fiber dispersion at fibril level.

In our previous work, we demonstrated that the dispersion of collagen fibers can be evaluated by its pore structure with MIP.[20] Table II lists the pore structure properties of pickled pelt and wet blue. The pickled pelt had the largest average pore diameter but the lowest porosity and total pore area. As for the wet blue, the average pore diameter decreased while the porosity and total pore area increased with increasing chrome powder. These results suggested that better fiber dispersion of wet blue was obtained with higher amount of chrome powder.

The pore size distribution was categorized using a theoretical model to further evaluate the fiber dispersion of pickled pelt and wet blue. The pore sizes of microfibrils, fibrils, elementary fibers and fiber bundles are smaller than 12 nm, 100 nm, 1000–3,000 nm and larger than 3000 nm, respectively.[23] Figure 4 shows that the pores in pickled pelt mainly ranged from 3000 nm to 50000 nm, whereas nearly none of them ranged from 5.48 nm to100 nm. These findings combined with the low magnification FESEM images (Figure 1) indicated that the fiber dispersion of pickled pelt was mainly concentrated at the fiber bundle level. After chrome tanning, the distribution of pore size in the range of 5.48–3000 nm increased from 26.91% to higher than 50%. Moreover, the pores in the range of 5.48–100 nm increased with increasing chrome powder. These results implied that a large number of pores at the microfibril, fibril and elementary fiber levels were formed by chrome tanning.

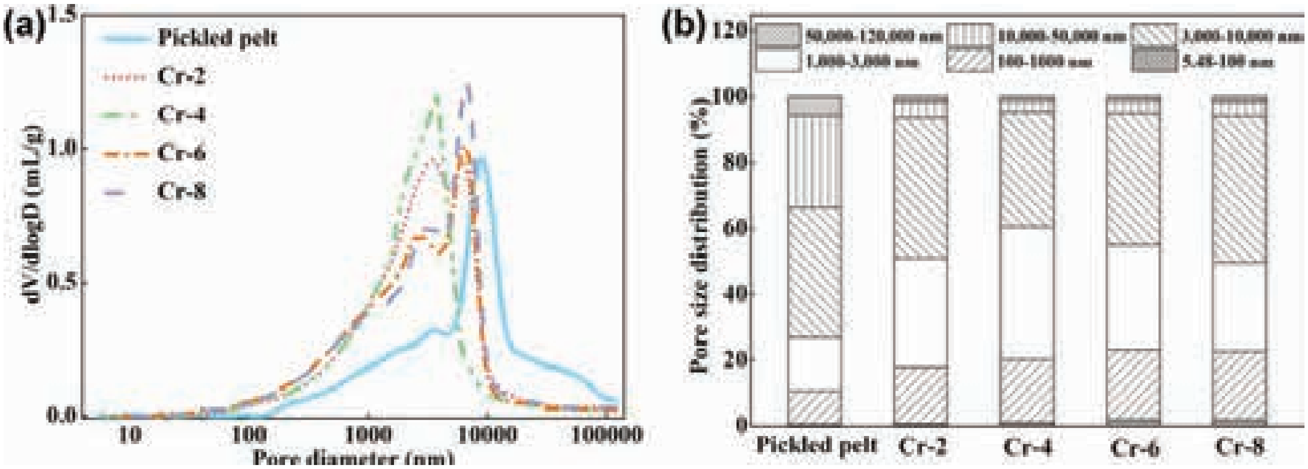

Figure 4. Effect of amount of chrome powder on pore size distribution of wet blue.

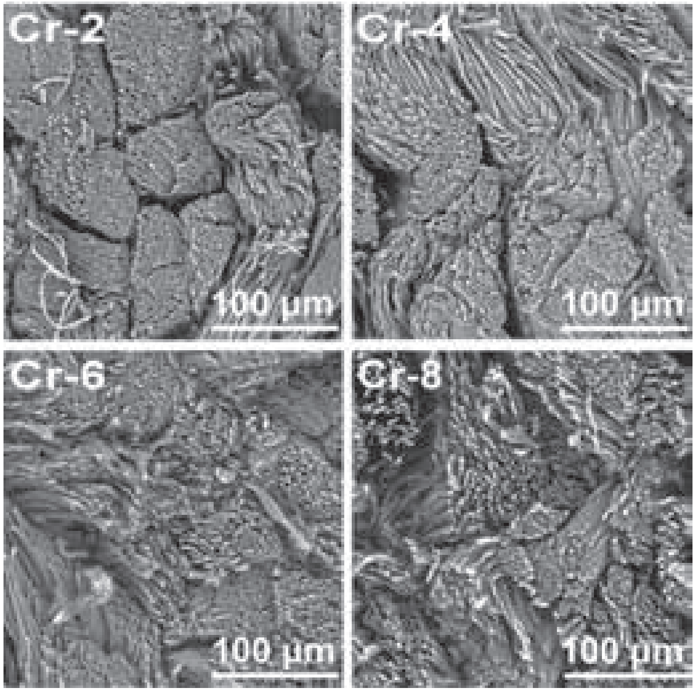

Figure 5. SEM images of cross sections of crust leathers.

Table III

Effect of amount of chrome powder on pore structure of crust leather.

Crust leather	Average pore diameter (nm)	Porosity (%)	Total pore area (m2/g)
Cr-2	1937.52	44.56	1.373
Cr-4	1442.02	44.81	1.744
Cr-6	1176.50	45.41	1.821
Cr-8	829.76	47.66	2.770

In summary, the results of FESEM, STEM–EDS and MIP confirmed that leathers with different fiber dispersion were obtained by tanning pickled pelts using different amounts of chrome tanning agent. Chrome tanning process plays an important role in the fiber dispersion of leather from primary level to high level because of deep penetration of chrome tanning agent.

Fiber Dispersion of Crust Leather

The wet blue samples were then fatliquored to prepare crust leathers, which can be determined for physical properties of leather. Because fatliquors would affect the dispersion of collagen fibers,[2] the fiber dispersion of crust leathers was analyzed by SEM and MIP. As shown in Figure 5, the degrees of fiber dispersion of crust leathers increased with increasing chrome powder, which was consistent with the trend of fiber dispersion of wet blue (Figure 1). The pore structure parameters and pore size distribution of crust leathers were listed in Table III and Figure 6, respectively. The average pore diameters of crust leathers were larger, and their porosities and total pore areas were lower than those of wet blue (Table II). Especially, the crust leathers had almost no pores in the range of 5.48-100 nm. These results implied that fatliquors penetrated crust leathers from primary level to high level, wrapping the collagen fibers. Nevertheless, the trend of crust leather pore structure with increasing chrome powder was consistent with that of wet blue. These results indicated that the fatliquoring process almost preserved the fiber dispersion of leather obtained from the chrome tanning process, although it resulted in a slight decrease in the porosity of leather. As a result, the crust leathers also had different fiber dispersion depending on the amount of chrome powder in tanning process.

Physical Properties of Crust Leather

Table IV and Figure 7 present the physical properties of crust leathers, including mechanical properties (tensile strength, tear strength and elongation at break), organoleptic properties (softness and fullness), and hygienic property (water vapor permeability). The data in Table IV show that the tensile strength, tear strength and elongation at

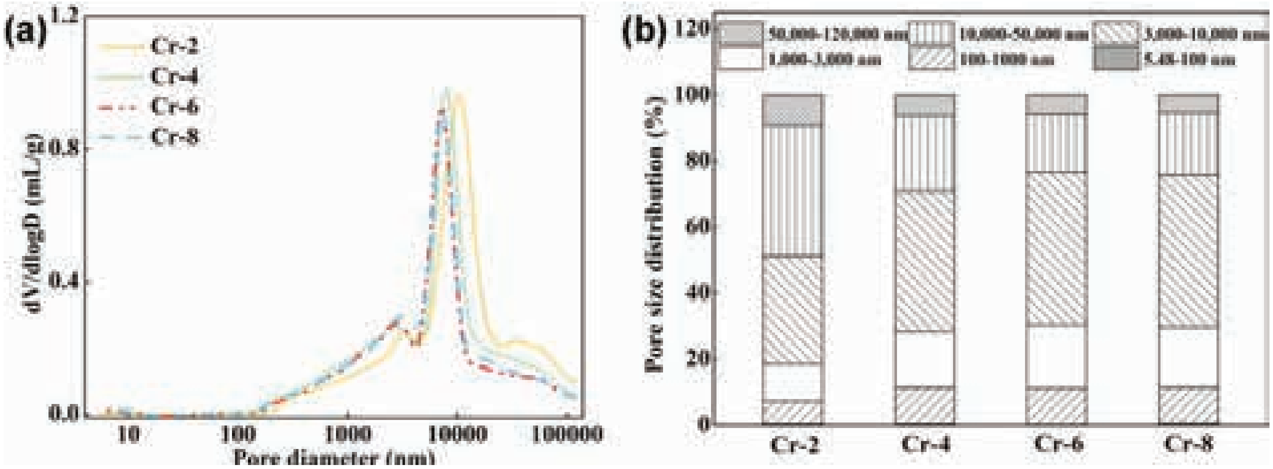

Figure 6. Effect of amount of chrome powder on pore size distribution of crust leather.

break of crust leathers increased with increasing chrome powder. However, these mechanical properties did not remarkably improve after the amount of chrome powder exceeded 6%. As mentioned previously, the fiber dispersion of wet blue and crust leather increased with increasing amount of chrome powder, particularly in the range of 2%-6%. Hence, the mechanical properties of leather were positively correlated with its fiber dispersion. This should be due to the fact that the crosslinking among collagen fibers caused by chrome tanning agent is helpful to improve both the mechanical properties and the porosity of leather. [6,24,25] The softness of crust leather increased from 7.14 mm to 8.21 mm (Figures 7(a)) when the amount of chrome powder rose from 2% to 8%. The compressed and resilient thicknesses of crust leather gradually increased with increasing chrome powder (Figures 7(b) and 7(c)), and the Cr-6 and Cr-8 crust leathers had the highest and similar compressed and resilient thicknesses. These data indicated that the fullness of crust leather was improved with increasing chrome powder. The above phenomena suggested that the organoleptic properties of leather were also positively correlated with its fiber dispersion because the pore structure of leather is the key factor to the compressibility of leather.[21,26] Figure 7(d) shows that the water vapor permeability increased from 8.88 mg/cm^2×h to 10.89 mg/cm^2×h when the amount of chrome powder rose from 2% to 8%. This is because more chrome powder resulted in higher porosity of leather, which could make more water molecules pass through the leather from high humidity environment to low humidity environment.[27,28] In summary, high physical properties of crust leather were accompanied by and even depended on its good fiber dispersion.

Table IV

Mechanical properties of crust leathers.

Crust leather	Tensile strength (N/mm2)	Tear strength (N/mm)	Elongation at break (%)
Cr-2	22.84±0.68	96.94±0.53	47.21±5.55
Cr-4	24.86±1.63	97.27±1.17	53.21±4.87
Cr-6	25.24±0.58	117.82±3.12	55.50±1.15
Cr-8	23.29±2.04	110.28±4.11	64.32±2.87

Conclusion

Chrome tanning process endowed leather with high fiber dispersion at different levels because chrome tanning agent could penetrate into elementary fibers, fibrils and microfibrils to fix collagen fibers. The crust leather with higher fiber dispersion presented better physical properties, indicating that the fiber dispersion is an important factor producing leathers with excellent physical properties. These results may provide a new insight into tanning performance and developing novel tanning agents and tannages.

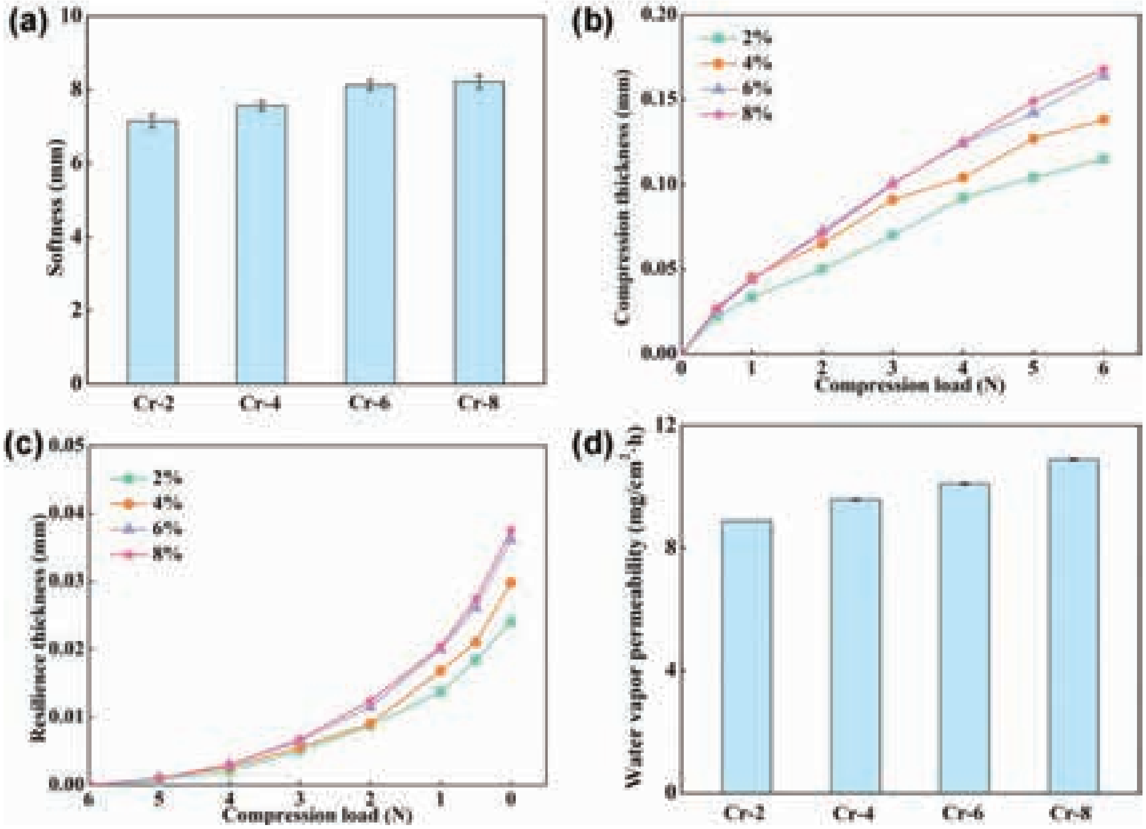

Figure 7.Effects of amount of chrome powder on properties of crust leathers: (a) softness, (b) compression performance, (c) resilience performance and (d) water vapor permeability.

Acknowledgement

This project is financially supported by the National Natural Science Foundation of China (21978176) and the National Key R&D Program (2017YFB0308500).

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Lifelines

Eser Eke Bayramoğlu is a full Professor at Ege University, Faculty of Engineering, Department of Leather Engineering. Currently teaches leather microbiology, hazardous fungi during leather production, leather production practice, microorganism control for the leather industry, finishing materials and techniques, leather handicrafts and marketing; has relevant skills and rich experience on the research of leather making technology and new product development from green chemicals. Nine national research projects have been undertaken, including 4 national upper graduate projects. Twenty-four awards have been won since 1993 including 17 publication awards. She created a microbiology laboratory in her department. She also worked with her graduate student to help establish a cosmetic company, Flamel Chemistry, which is producing keratin from waste hair and wool.

Anıl Özçelik has Undergraduate and a Masters degree from Ege University, Faculty of Engineering, Department of Leather Engineering. Worked on fungi for both of undergraduate thesis and master thesis. Won an award from intermediate school science competition as consultant teacher with research on fungi. Completed the TUBITAK Project for producing nano keratin as a researcher. Attended Waalwijk – Stahl Campus Event / Finishing Techniques and Applications training, completed internship with Erasmus+ at INCDTP / Bucharest on microbiology. Also worked in production at Flamel Chemistry which is a cosmetic company.

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Deyi Zhu, see *JALCA*, 114,131-137,2019

Jinzhi Song, see *JALCA*, 114,131-137,2019.

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Yanchun Li, see *JALCA*, 114,131-137,2019.

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Xiu He, see JALCA 114, 69, 2019.

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Yunhang Zeng, see JALCA 109, 207, 2014

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Jianfei Zhou, see JALCA 112, 258, 2017.

Bi Shi, see JALCA 99, 220, 2004.

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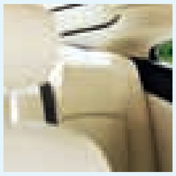

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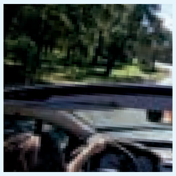

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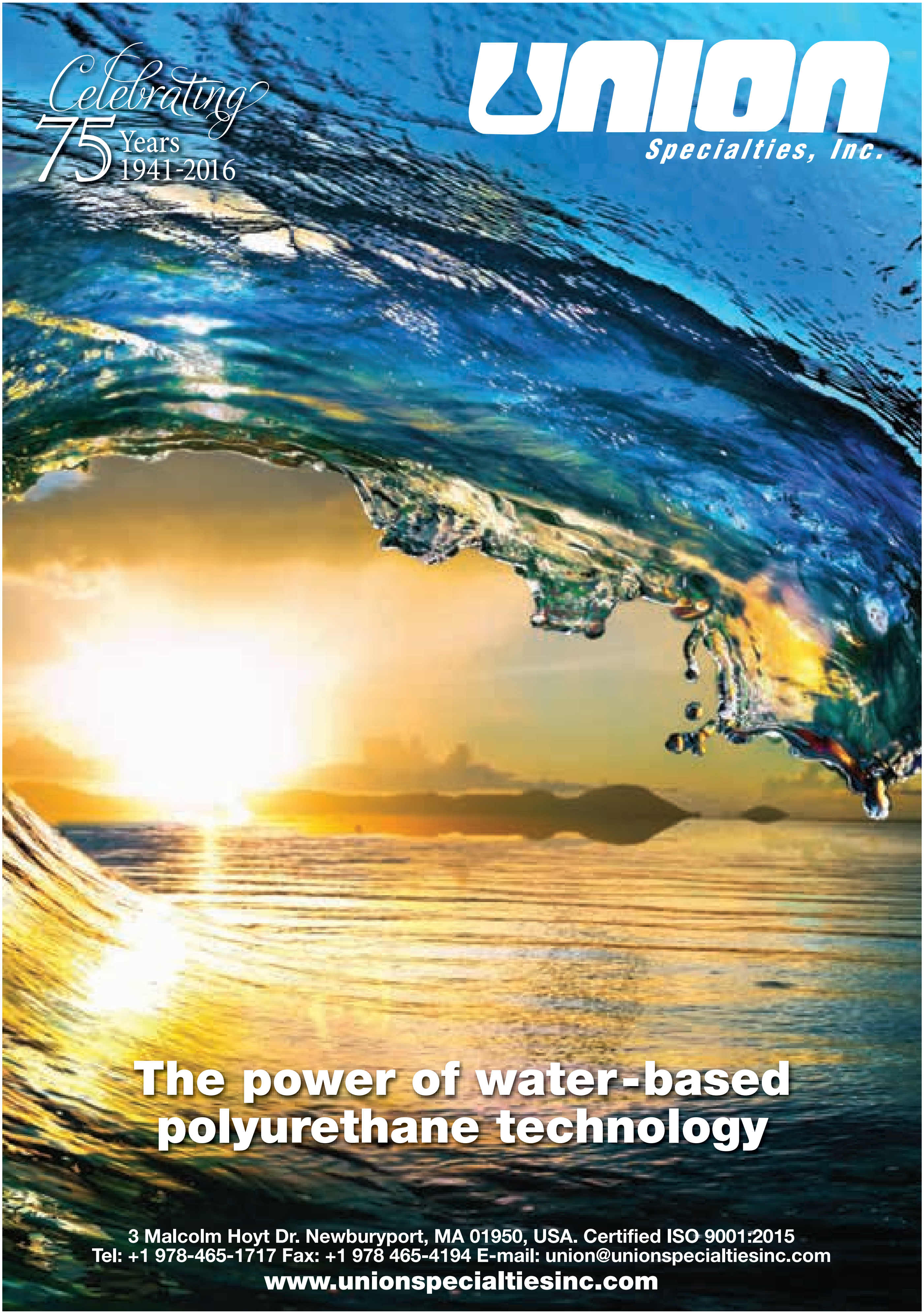
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