Microbial Transglutaminases as Pre-tanning Agents in the Leather Industry

by

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Abstract

Microbial transglutaminases(MTG) was used to pre-tan hide to investigate its effect on the hydrothermal stability of hide and the improvement for exhaustion of chrome tanning agent. This study tested the shrinkage temperatures of samples treated with MTG only and tanned MTG followed by the chrome tanning agent separately. The Cr_2O_3 content in effluents was surveyed after chrome tanning. It was shown that the shrinkage temperature of hide sample treated with 0.6% MTG at pH 6.0 for 2h was above 80°C, much higher than 54.8°C, which is the Ts of control. While the Ts of leather sample, which was pre-treated with 0.6% of MTG first, tanned with only 4% chrome tanning agent were higher than 100°C, but initial pH is 2.0. Moreover, the exhaustion of chrome tanning agent in leather was improved significantly when the dosage of chrome salt was less than 4%.

Introduction

Tanning is the most important process for converting skins or hides to leather. Due to the excellent technical properties and hydrothermal stability of chrome tanned leather, for more than one hundred years, chrome tanning is in a dominant position for leather making.[1] Although novel and cleaner tanning processes have been developed world wide, the chrome-free tanning agents developed in the past decades wound not replace chrome-based tanning agent completely in next decades. In fact, the development of cleaner process for improvement the exhaustion of chromium is more interesting and important to some extent, which not only remains the excellent properties of chrome tanned leather but also decreases the chromium salt content in effluents and wastes.

Transglutaminase (TGase; protein-glutamineγ-glutamyltransferase, EC 2.3.2.13) are enzymes that catalyze an acyl transfer reaction in which the γ--carboxyamide groups of peptide-bound glutamine residues act as the acyl donors. The most common acyl acceptors of TGase are the ε-amino groups of lysine residues within peptides or the primary amino groups of some naturally occurring polyamines.[2,3] When lysine residues in proteins serve as acyl acceptors, intermolecular or intramolecular ε(γ-glutamil)lysine bonds are formed, resulting in the polymerization of proteins.[4,5] TGases are distributed in many kinds of organisms, therein microbial TGase (MTG) with a cysteine protease-like catalytic triad that has been isolated from the culture medium of Streptoverticillium sp. and Bacillus spp., which are widely applied in food industry[6] and leather.[7] Generally, the catalytic residue, Cys64, exists at the bottom of the cleft. Asp^{255} resides at the position nearest to Cys64 and is also adjacent to His^{274}. Interestingly, Cys^{64}, Asp^{255}, and His^{274} superimpose well on the catalytic triad "Cys-His-Asp". Moreover, due to higher reaction rate, broader substrate specificity, and lower deamidation activity, the microbial transglutaminase has developed a novel catalytic mechanism specialized for the cross-linking reaction.[8,9]

The transglutaminases (TGs) were capable of covalently crosslinking native collagen molecules together. The thermal stability of the bovine hide collagen treated by TGase (40 Unit /g collagen) at 40°C for 2h in water significantly improved to 91.4°C, described by WU.[11] In contrast, Collighan presented that transglutaminase-mediated crosslinking did not affect the denaturation temperature of either native bovine hide or soluble rat tail collagens, even that on average every collagen molecule contained at least one ε(γ-glutamyl)lysine crosslink.[10] Furthermore, the shrinkage temperature of leather samples treated with TGase increased little compared with the controls according to the research of LIU, which arising from inadequate catalysis by TGase and less cross-linking reaction among active glutamine residues and lysine residues located in the zone of collagen triple helix. In addition, the Ts of leather sample treated with TGase firstly and then with Chromosal B decreased 9°C compared with sample treated with Chromosal B only, as the prevention of TGase on the penetration of chrome salt.[12,13] Thus it is crucial to build an appropriate tanning process for the using of both MTG and chrome tanning agent.[14,15] Luckily, chromium salt in the hide did not affect the activity of MTG, found by Taylor.[16]

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In this paper, pickled hide was pre-tanned with MTG before chrome tanning in order to investigate the effect of MTG on the exhaustion of chrome-tanning agent. The Ts of leather samples and the Cr_2O_3 content in effluent after tanning were measured to optimize reaction conditions, such as dosage of MTG and chromium salt.

MATERIALS AND METHODS

Apparatus

Experimental stainless steel drum with heat pump cycle control temperature (GS type, manufactured by Wuxi Xinda Light Industry Machinery Co. Ltd., China), experimental drum with 5-piece of drums (diameter 350mm), and detection instrument of leather shrinking temperature (MSW-YD4, manufactured by Shaanxi University of Science and Technology.)

Materials

Pickled bovine hide, chrome tanning agent (Cr_2O_3 content=24%±1.0%) and microbial transglutaminases (MTG, 100units/g)

Methods

Sampling

Symmetric pieces of pickled hide (300mm×150mm) were sampled on the left or right side 50mm far from the backbone.

Experimental Process with MTG as Pre-tanning Agent

In order to investigate the pre-tanned effect of MTG, different amounts of MTG were applied to treat pickled hide samples, then their shrinkage temperatures were tested. The experimental scheme was shown in Table I.

Experimental Process of pH Value in the Preliminary of Chrome Tannage

The pH plays a decisive role in the permeation of chrome-tanning agent. The samples pre-tanned with 0.8% MTG were pickled at pH 1.5, 2.0, 2.5 and 3.0 respectively, and then were tanned using 7.0% chrome-tanning agent. The shrinkage temperatures of leather samples were detected to investigate the effect of pH value on the penetration and combination of chrome tanning agent after pre-treating leather with MTG. The experimental scheme was shown in Table II.

Experimental Process of Chrome Tanning

The samples pre-treated with 0, 0.4%, 0.6% and 0.8% of MTG were tanned by 0, 2.0%, 3.0%, 4.0%, 5.0%, 6.0% and 7.0% of chromium-tanning agent at pH 2.0, respectively. The shrinkage temperatures of leather sample and Cr_2O_3 contents in effluents after tanning were tested. The experimental scheme was shown in Table III.

Testing Methods

(1) Shrinkage temperature of leather: complied with the ISO 3380-2003 Leather - Physical and mechanical tests - Determination of shrinkage temperature up to 100°C.

(2) Chromic oxide content in waste chrome tanning solution: referred to ISO 5398-2:2009 Leather - Chemical determination of chromic oxide content-Part 2: Quantification by colorimetric determination.

TABLE I
Processing of hides pre-treated with MTG.

Process	Operated scheme	Remark
Weighing	According to the twice of pickled hide weight, the amount of subsequent material was calculated.	
Softening	Water: 100%, room temperature; Salt: 8%, rotating 20min.	
Basifying	Add sodium Bicarbonate to the pH value of 6.5, temperature 40°C, rotating 30min.	Check hide cut with a drop of bromothymol blue indicator, the cut color is green.
Pre-tanning	Microbial transglutaminases(MTG), the amount: 0, 0.2%, 0.4%, 0.6%, 0.8% and 1%; Time: rotating 2h, drum stop overnight.	(5/30) (2h, every piece of hide was sampled a small piece, test shrinkage temperature.)
Washing	Rotating 1h, drain away water;	Test shrinkage temperature.

TABLE II
Processing of hides pre-treated with MTG and tanned with chorme-tanning agent at different pH.

Process	Operated scheme	Remark
Weighing	According to the twice of pickled hide weight, the amount of Subsequent material was calculated.	
Softening	Water: 100%, room temperature; Salt: 8%, rotating 20min.	
Basifying	Add sodium Bicarbonate to the pH value of 6.5, temperature 40°C, rotating 30min.	Check hide cut with a drop of Bromothymol blue indicator, the cut color is green.
Pre-tanning	Microbial transglutaminases(MTG): 0.8%; rotating 2h	(5/30)
Draining	Draining off the water.	
Pickling	Water: 80%, room temperature; salt: 4%, add sulphuric acid(10% concentration) to adjust pH value, the value: 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0, rotating 30min.	pH value was gradually decreased, adjusted to responding value in two hours.
Chrome-tanning	Chrome-tanning agent: 7.0%; Rotating2h-3h.	
Basifying	Add sodium Bicarbonate to the pH value of 3.8.	Add with several times
Heating	Add hot water(60°C): 60%, temperature of the solution in the drum arise to 35°C, rotating 1h.	
Aging	Stop drum, overnight; rotating 30min in the next morning, drain away water; washing, out of the drum.	Test shrinkage temperature.

RESULTS AND DISCUSSION

The Amount of MTG and Pre-tanning Time

The shrinkage temperatures of samples tanned with 0, 0.2%, 0.4%, 0.6%, 0.8% and 1.0% of MTG in 40°C at pH 6.5 for 2h or overnight respectively were shown in Figure 1.

Generally, MTG enzyme activity reaches peak in 40-45°C at pH 6.0-7.0. Thus MTG was adopted in the process of pre-tannage according to the practical pH and temperature in leather making. Additionally, MTG was easy to catalyze collagen on the surface of hide to covalently cross-link, resulting that it was difficult for MTG to penetrate into the inner of hide. Even the partial enzyme penetrated to the inner, its catalyze site could not access to residues act. Through changing reaction condition or adding appropriate auxiliary agent, the penetration of enzyme into inner of hide would be improved, and covalently cross-link would increase. Thus it is very important to confirm the penetration and catalysis of MTG on the cross-section of hide samples. A simple and effective indicator i.e. bromothymol blue was used in this paper. As shown in Figure1, the shrinkage temperature of

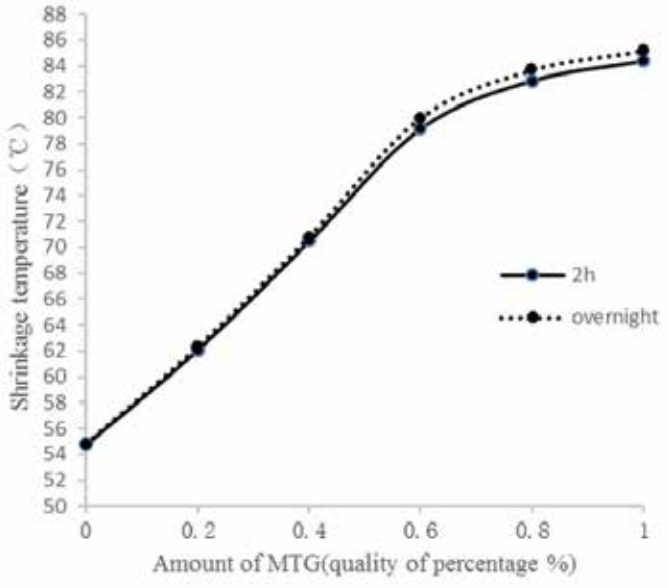

Figure 1. The effect of dosage of MTG and pre-tanning time on the shrinkage temperatures of samples.

TABLE III
Processing of hides tanned with different amount of MTG and chrome-tanning agent.

Process	Operated scheme	Remark
Weighing	According to the twice of pickled hide weight, the amount of Subsequent material was calculated.	
Softening	Water: 100%, room temperature; Salt: 8%, rotating 20min.	
Basifying	Add sodium Bicarbonate to the pH value of 6.5, temperature 40°C, rotating 30min.	Check hide cut with a drop of Bromothymol blue indicator, the cut color is green.
Pre-tanning	Microbial transglutaminases(MTG), the amount: 0, 0.4%, 0.6%and 0.8%; rotating 2h.	(5/30)
Draining	Draining off the water.	
Pickling	Water: 80%, room temperature; salt: 4%; add sulphuric acid(10% concentration) to adjust pH value 2.0, rotating 30min.	pH value was gradually decreased, adjusted to responding value in two hours.
Chrome-tanning	Chrome-tanning agent, the amount: 0, 2.0%, 3.0%, 4.0%, 5.0%, 6.0% and 7.0%; rotating 2h-3h.	
Basifying	Add sodium Bicarbonate to the pH value of 3.8.	Add with several times
Heating	Add hot water (60°C): 60%, temperature of the solution in the drum arise to 35°C, rotating 1h.	
Aging	Stop drum, overnight; rotating 30min in the next morning, drain away water; washing, out of the drum.	Test shrinkage temperature and Cr_2O_3 content in waste chrome tanning solution

pickled hide was 54.8°C, while the shrinkage temperatures of samples treated with MTG increased obviously with the increase of MTG dosage. The Ts of hide treated with 1% of MTG for 2h was above 84°C, whilst the Ts of hide treated with 1% of MTG for 24h was almost equal to the former. It was concluded that MTG penetrated into the hide well and catalyzed the crosslinking reaction among collagen molecules in 2h. It was ascribed to the increasing of active groups in pickled hides under the activation of acid, alkaline, salt and enzyme in the processes of soaking, degreasing, unhairing, liming, deliming, and bating, sequentially. Exposed γ--carboxyamide groups of peptide-bound glutamine and ε-amino groups of lysine on collagen matrix were easy to be crosslinked under the catalysis of enzyme.

In this paper, the main purpose was to investigate whether the amount of chrome-tanning agent was reduced with MTG as pre-tanning agent. As long as the shrinkage temperature can basically meet the request of subsequent process, the MTG usage was 0.4%-0.8% in following experiments to study the effect of MTG on the exhaustion of chrome tanning agents at different pH.

The Effect of Initial pH on Ts of Leather Samples

The shrinkage temperatures of leather samples pre-treated with 0.8% of MTG and tanned by 7% of chrome-tanning agent at pH 1.5, 2.0, 2.5, and 3.0 respectively, were shown in Figure. 2. It should be note that chrome tanning agent penetrated into hide well at pH less than 3.

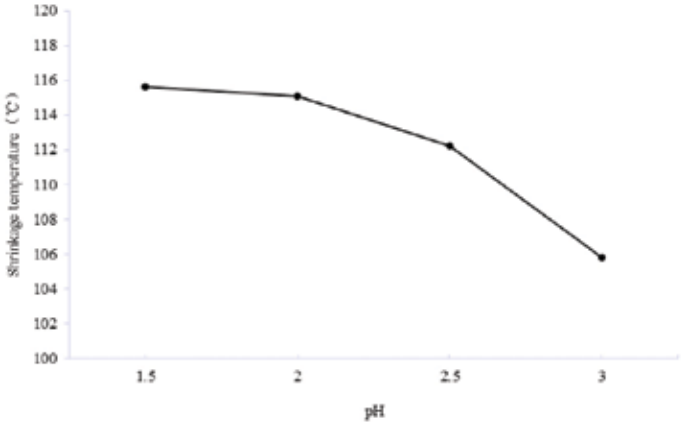

Figure 2. The effect of initial pH value on shrinkage temperatures of leather samples pre-treated with MTG and then tanned with chrome tanning agent.

As shown in Figure 2, Ts leather sample pre-treated with 0.8% of MTG and tanned by 7% of chrome-tanning agent at initial pH 1.5 was about 115°C, while it decreased to about 105°C at initial pH 3. This was because of the change of penetration of chrome tanning agent, which was due to the increase of isoelectric points of hide samples pre-tanned with MTG. The γ--carboxyamide groups of peptide-bound glutamine residues on collagen molecules as the acyl donors cross-linked with ε-amino groups of lysine residues (acyl acceptors) within other collagen molecules under the catalysis of MTG (as shown in equation 1). In other word, alkaline groups containing nitrogen on collagen molecules decreased while carboxyl groups increased comparatively. Therefore, lower pH was necessary to inactivate carboxyl groups, which benefited for the penetration of chrome tanning agents. However, it was harmful, in some extent, to practical producing and properties of final leather products when the pH was lower than 2. And thus it was appropriate for the tanning of hide pre-treated with MTG at pH 2.

Equation (1)

The Amount of Chrome Tanning Agent

The synergy between MTG and chromium salts was important. Thus the hide samples pre-tanned firstly with 0, 0.4%, 0.6% and 0.8% of MTG were tanned by using 0, 2.0%, 3.0%, 4.0%, 5.0%, 6.0% and 7.0% of chrome tanning agent, respectively. The shrinkage temperatures of leather samples and Cr_2O_3 content in effluents were tested, which were shown in Figure 3 and Figure 4 respectively.

Generally, the shrinkage temperature of leather tanned with at least 6% of chrome tanning agent would be above 100°C. As shown in Figure 3, the Ts of leather sample, which was pre-treated with 0.6% of MTG first, tanned with only 4% chrome tanning agent were higher than 100°C. It could be concluded that satisfied tanning properties would be achieved by using moderate amount of MTG firstly and less of chrome tanning agent in practical leather making. It was probably because that only carboxyl groups were crosslinked through chromium salt in traditional leather making, whilst amino groups on collagen molecules were also crosslinked under the catalysis of MTG in our research. The similar hydrothermal stability was achieved by using less chrome tanning agent. However, the amount of MTG should not be too much for consideration of cost effectiveness.

The Cr_2O_3 contents in effluents discharged after being tanned with MTG firstly and followed with chrome tanning agent were shown in Figure 4. The exhaustion of chrome tanning agent in leather was improved obviously when the dosage of chrome salt was less than 4%, which depended on the positive action of MTG. The Cr_2O_3 content in discharged effluent, in which the initial content of Cr_2O_3 was 4%, decreased from 0.9g/L to 0.15g/L as the dosage of MTG increasing from 0 to 0.6%. It is likely that MTG catalyzed a transamidation reaction between glutamyl and lysyl side chains of target proteins, exposed more carboxyl group, and increased the reaction group. Thus after improved the alkalinity, more carboxyl groups aroused the disassociation, entered the inner of complex to coordinate with chromium(III). Meanwhile the resulting ε(γ-glutamil)lysine crosslinked bonds enlarged the pore of collage fiber, boosted chromium(III) hydrolysis and polynuclear hydroxyl coordination, and formed $[Cr(H_2O)_6]^{3+}$. The multi-coordination of collagen was a great significance to the thermal stability, so the amount of chrome-tanning agent was effectively decreased with MTG as pre-tanning agent, and the bonding rate of chromium(III) with collagen was improved. However, it has less positive effect on the exhaustion of chrome tanning agent in case of the amount of chrome tanning agent being more than 5%.

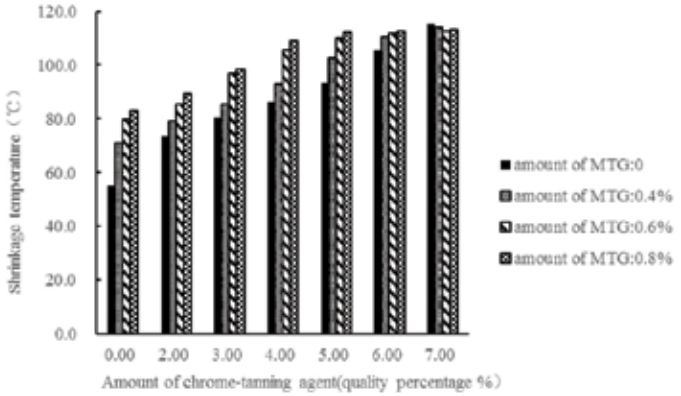

Figure 3. The shrinkage temperatures of leather samples pre-tanned with MTG firstly and tanned with chrome tanning agent.

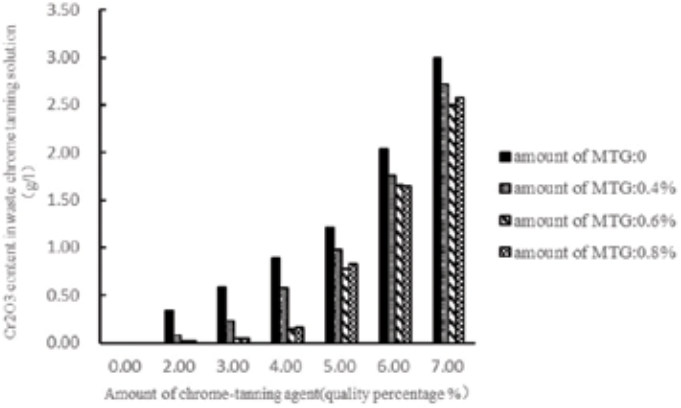

Figure 4. Cr_2O_3 content in waste chrome tanning solution.

CONCLUSIONS

1. The shrinkage temperature of hide sample treated with 0.6% MTG at pH 6.0 for 2h was above 80°C. It was proved that MTG could be used as pre-tanning agent in leather making.

2. The isoelectric point of hide pre-tanned with MTG would increase, and lower pH was necessary for chrome tanning in order to improve its penetration.

3. Compared with traditional chrome tanning, similar hydrothermal stability could be achieved by using moderate amount of MTG and less chromium salt, which was due to the crosslinking of both carboxyl groups with chromium salts and polymerization of amino groups.

4. The exhaustion of chrome salt in leather was improved significantly by using 0.6% MTG for pre-tanning when chrome tanning agent dosage was less than 4% and the content of Cr_2O_3 in effluents decreased effectively.

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