Effect of Surfactants on Enzyme and Skin in Bating Process

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Abstract: Sodium dodecylsulfate(anionic surfactant), dodecyl trimethylammonium chloride (cationic surfactant), Peregal O(nonionic surfactant) of various dosages were added in pancreatin and AS1.398 enzyme liquor respectively to study the effect of these three types of surfactants on enzyme activity. Results indicated that the nonionic surfactant played a role of activating in comparison with ionic surfactants. In the second stage of the experiment, to verify the results are consistent with the application in leather making, these three surfactants were added in bating. After bated, the protein content in the bated liquor was measured and the histology sections of the bated skins were observed to study the hydrolysis degree of elastic fibrils by an optical microscope. The bated skins were then tanned with chromium. Also the shrinkage temperature of the wet blues, the tensile strength and elongation of the chrome-tanned leather were tested. Results indicated that with the three types of surfactants, the hydrolysis degree of elastic fibrils of the bated skins were as follows: nonionic surfactants>cationic surfactants> anionic surfactants. For physical properties of leathers, the nonionic surfactants in bating gave better results with respect to shrinkage temperature, tensile strength and elongation at break.

Key words: leather; bating; surfactant; enzyme activity; histology sections

1 Introduction

In early times enzyme was mainly used in the dehairing and bating process in the leather industry. However, with the development of enzyme preparation technology, it has been gradually widely used in many important leather processes[1-2], and become a sort of indispensable material in the leather making. Among these processes, enzyme is not simply replaced only by chemicals in bating, because this process plays an important role on leather softness, elasticity and smooth, etc. Now in the practical leather making, some surfactants are used to promote the enzyme osmosis during the bating process. But the relevant researches about the effect of surfactants on enzyme and skin in bating process have not been found.

In comparison with nonenzymatic catalysts, enzymes have the characteristics of specificity and efficiency. But they are sensitive to environmental conditions. For instance, enzymes are affected by substrate concentration, enzyme concentration, temperature, pH, activator concentration, inhibitor concentration, etc. Thus, the environmental conditions must be controlled to develop the enzyme catalytic function in the application of enzyme.

In this work, the relationship between different surfactants and enzymes has been studied by testing enzymes activity. Then the effect of different surfactants on skin had been investigated by testing the protein content of bating liquid, the hydrolysis degree of elastic fibrils of bated skins and the physical properties of chrome leather. The aim of this work was to acquire the rule of the effect of different surfactants on enzyme and skin in bating process, then offer the reference about the application of surfactants in bating and other enzyme treatment processes.

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2 Experimental

2.1 Materials

The goat skin was soaked and unhaired as usual. Industrial pancreatin(activity was 14 times, the best pH was 8.0) and AS1.398 protease(activity was 30000(u/g), the best pH was7.5) were from Chongzhou enzyme plant, China. The critical micelle concentration(CMC) of three types of surfactants as follow: Peregal O 0.05%(w/w), Sodium dodecylsulfate(SDSB) 0.10%(w/w) and Dodecyl trimethylammonium chloride(DTAC) 0.25%(w/w).

2.2 Determination of the Effect on Enzyme Activity[3]

3%w/w pancreatin and 0.2%w/w AS1.398 enzyme were respectively added in the pH 8.0 and 7.5 phosphate buffer and then mixed, the mixture was divided into 13 portions. According to CMC, the surfactants were added by 0.05% increment in the enzyme liquor. This reaction was performed for 45min at 37℃ in a water bath. The enzyme activity was tested by Folin method.

2.3 Bating and Tanning Process

The sampling of the goat skin was shows in Fig.1, part "a" was used to test the physical properties of leathers, part "b" was used to for histology. Bating, pickling and chrome tanning process was shown in Tab.1

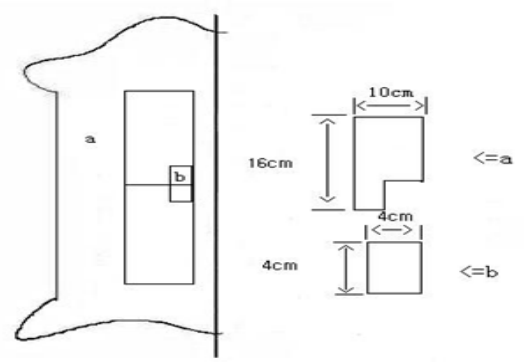

Fig.1 The sampling of the raw skin

Tab.1 The process of bating, pickling and chrome tanning

Process	Material	Temperature/℃	Time/min
Bating	100% Water	37	
	X% Surfactant		
	0.4% Ammonium sulphate		
	Y% Enzyme	45	
Bathing	300% Water	30	30
Pickling	60% Water	20	
	8% salt		5
	0.8% Methanoic acid（1:10）		15
	1.2% Sulfuric acid（1:20）		80 overnight pH 2.8
Chrome tanning	8% Chrome		120
	0.5% Sodium formate		120
	1.2% Sodium bicarbonate（1:20）		120 pH 3.8
	100% Water	40	120 overnight pH3.8-4.0
Bating		20	15

2.4 Analysis of Bated Liquid

After bated, the protein content in the bated liquor was measured by the Lowry method[4].

2.5 Histological Analysis of Bated Skin [5]

By fixing in 10% w/w neutral formaldehyde solution for 24h, the bated skin was cut by a frozen slicer and dyed according to Verhoeff method, the elastic fiber dispersion was observed by an optical microscope and photographed.

2.6 Analysis of Physical Properties of Leather[5]

(1) Determination of the shrinkage temperature

HG shrinkage temperature detector was used to measure the shrinkage temperature of the wet-blue leather in the glycerin and water solution (ratio 3:1).

(2) Determination of the tensile strength

After the wet-blue leather naturally dried, the tensile strength was tested in 100 mm / min tensile speed using standard methods.

(3) Determination of the elongation at break

The ratio of the original length and the break length was measured as standard methods.

3 Results and discussion

3.1 Effect of Surfactants on Enzyme

The effect of surfactants on pancreatin activity in pH8.0 is presented in Fig.2. It shows that for DTAC or SDBS, pancretin activity decreases significantly, particularly, the activity is 30% of the original only using SDBS. But for Peregal O the activity increases 10%. The effect of surfactants on AS1.398 enzyme activity in pH7.5 is presented in Fig.3. It also shows a similar rule, the AS1.398 enzyme activity inactivates completely with SDBS. However, the activity increases almost 100% using Peregal O. This indicated that Peregal O could activate the two enzymes only. On account of DTAC and SDBS are composed by surfactant ion and inorganic ions Na^+ or Cl^-, so NaCl was used as control sample to research whether the enzyme inactivation is mainly due to surfactant ion. NaCl does not affect the activity as presented in Fig.2 and Fig.3. The result was considered that the enzyme inactivation was mainly due to surfactant ion. That because when the surfactant concentration reached to CMC, the liquid surface tension decreased rapidly, and made solute dispersed in solution very well. But when the concentration exceeded a certain level, they formed the reverse micelles and reacted with enzyme protein hydrophobic district. Finally the protein was dissolved to lose its activity as presented in Fig.4. This also accords with the conclusion that Nicholas[6] and Marilena[7] proposed. Comparatively, the reverse micelles character of nonionic surfactant Peregal O was not obvious, so Peregal O mainly played the role of solubilization.

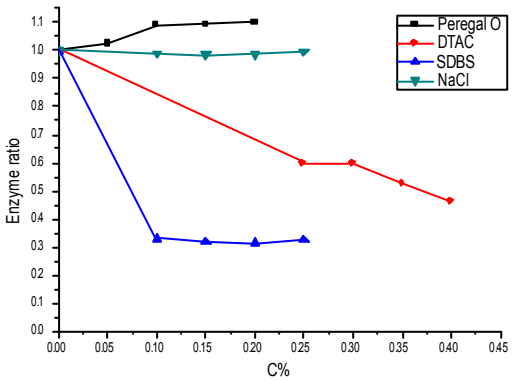

Fig.2 Effect of surfactants on pancreatin activity

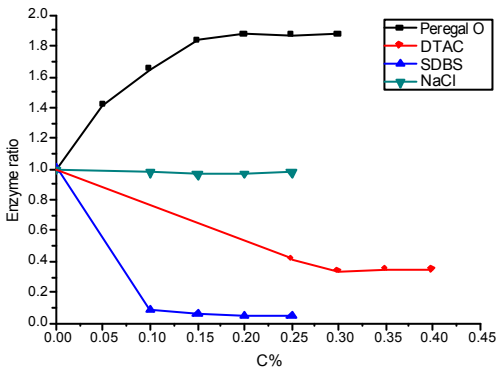

Fig.3 Effect of surfactants on AS1.398 activity

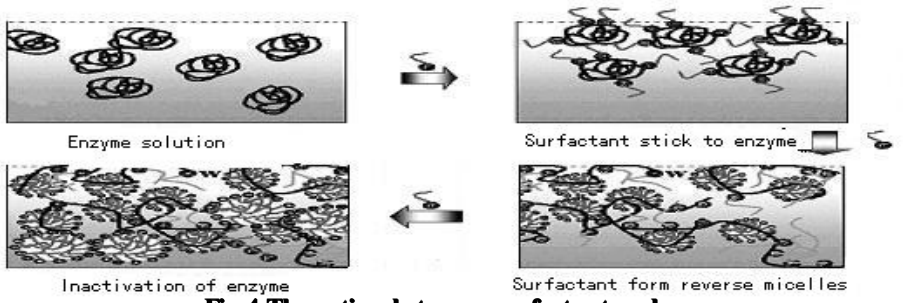

Fig.4 The action between surfactant and enzyme

3.3 Analysis of the Bated Liquid

Enzyme is used to remove dirt, eliminate the skin swelling, further disperse the collagen fibers and dissolve the elastic fibers, globular protein. So the relationship between enzymes and leather can be appraised by measuring the protein content of the bated liquor. Fig.5 and Fig.6 show that for SDBS or DTAC the protein content declines. However, using Peregal O, the protein concentration increases in the bathed liquid. This reason was that surfactant could accelerate the enzyme penetration which caused a lot of fibrous tissue hydrolysis. As the result of experiment 3.1, the two ionic surfactants caused enzyme inactivation, so its hydrolysis ability decreased. The two figures show that when the three types of surfactants amount reach a certain value respectively, the enzyme activity and protein hydrolysis are stable. Therefore, the dosages of three types of surfactant were as follows: Peregal O 0.15%, DTAC 0.35%, SDBS 0.20% in the following research.

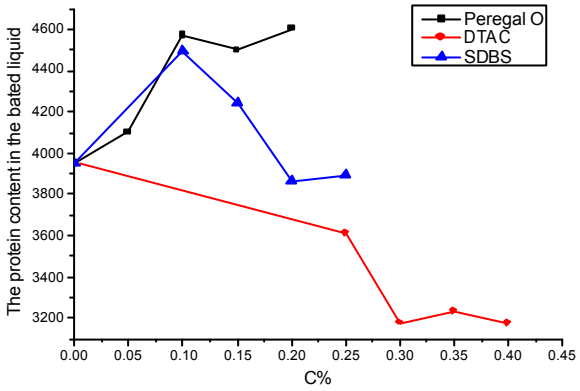

Fig.5 Effect of pancreatin on protein hydrolysis with surfactants

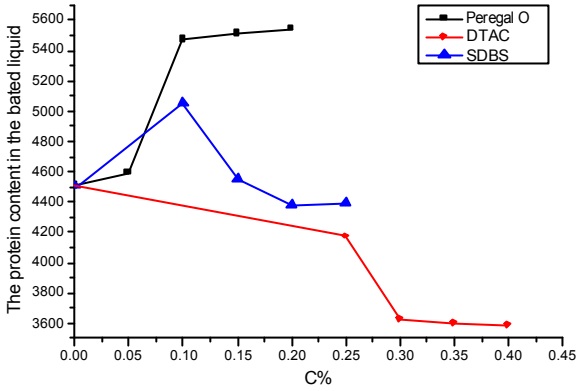

Fig.6 Effect of AS1.398 on protein hydrolysis with surfactants

3.4 Analysis of Bated Skin Histology

Fig. 7 and Fig.8 show that with Peregal O, the hydrolysis degree of elastic fibrils is distinguished, but it is not obvious with ionic surfactants. This phenomenon was due to surfactants could boost the enzyme hydrolysis ability. As the results of experiment 3.1, among the three surfactants, Peregal O could activate enzyme only. Therefore, using the three types of surfactant in bating, the hydrolysis degree of elastic fibers was as follows: Peregal O> DTAC> SDBS.

3.5 Physical Properties of Leather

For Peregal O, shrinkage temperature, tensile strength and elongation of the sample are obviously improved compared with these of the blank sample as presented in Tab.2. Moreover, using pancreatin in the bating process the temperature reaches 120 ℃, enhancing 6 ℃, the tensile strength enhances 2.738MPa, the elongation increases 29.205% .While using AS1.398, the temperature reaches 126 ℃,

enhancing 5 ℃, the tensile strength enhances 3.172MPa, the elongation increases 22.968%.For ionic surfactants these do not change significantly. This was due to the structural stability and the mechanical strength of the leather improved by tanning. Different types of surfactants in bating would impact the leather shrinkage temperature, tensile strength and the elongation at break. The cause was that enzyme could hydrolyze non-collagen protein and made the structure of collagen fibers looser, that caused the collagen reactivity group increasing, and thus the bond point between the chrome tanning agents and the skin collagen was more. According to as the result of experiment 3.4, using Peregal O in bating process the hydrolysis degree of elastic fibrils of the bated skins was distinguished. So using Peregal O in bating, the physical properties of leather were better compared with ionic surfactants.

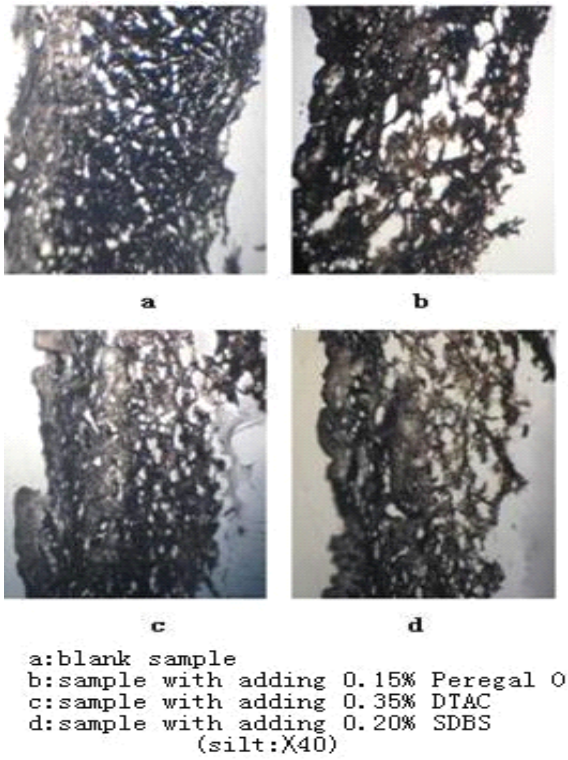

Fig.7 Pancreatin bated samples histology pictures

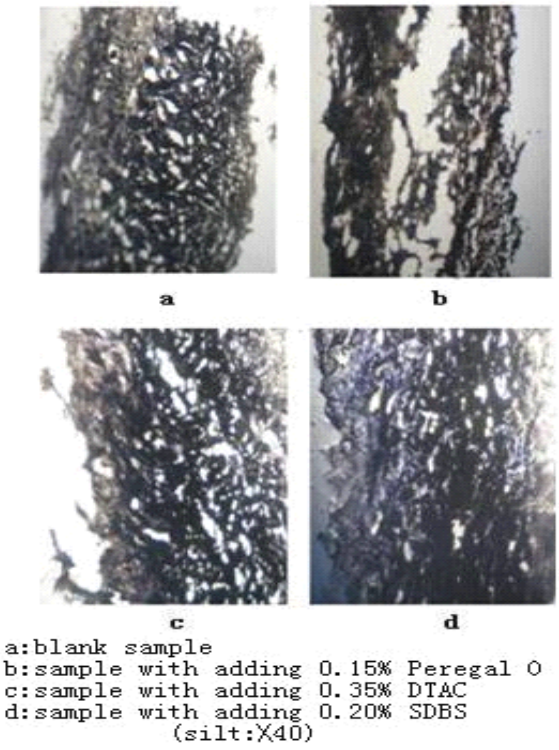

Fig.8 AS1.398 bated samples histology pictures

Tab.2 Tanning shrinkage temperature, tensile strength and elongation

Enzyme (%)	Surfactant &volume (%)		Temperature(℃)	Tensile strength(MPa)	Elongation at break
Pancreatin	*Blank*	*0*	*114*	*6.438*	*49.026*
	Peregal O	*0.15*	*120*	*9.176*	*78.231*
	DTAC	*0.35*	*112*	*7.719*	*50.026*
	SDBS	*0.20*	*114*	*7.274*	*46.840*
AS1.398	*Blank*	*0*	*121*	*6.507*	*58.988*
	Peregal O	*0.15*	*126*	*9.679*	*81.956*
	DTAC	*0.35*	*120*	*8.009*	*58.602*

SDBS	*0.20*	*118*	*7.853*	*46.731*

4 Conclusions

Anionic, cationic, and non-ionic surfactants have different effect on enzyme activity. And the surfactant concentration exceeded its CMC, the ionic surfactants made the activity declined, but the nonionic surfactant made enzyme activity raised.

When the three kinds of surfactants were used in bating, the hydrolysis degree of elastic fibrils of the bated skins was as follows: nonionic surfactants>cationic surfactants> anionic surfactants. Compared with the ionic surfactants, using non-ionic surfactants the physical properties of leather was better. Therefore the nonionic surfactant can be priorly chosen for bating process.

Acknowledgements

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