Paper B40

Detection of the changes of biochemical parameters during beamhouse leather-processing

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Abstract

Utilization of lime-free processing to replace conventional liming during beamhouse processing is global trend. It not only reduces the yield of sludge and environmental loads, but also reduces costs. The releasing of inter-fibrillar components facilitate collagen fiber opening. But the retain of collagen fiber in hide is desirable. The objective of the present study was using biochemical examination to monitor the changes of pH value, Baume, protease activity, collagen concentration, protein and glycosaminoglycan concentration during beamhouse processing to improve leather quality. On conventional leather processing, the pH value were increased by lime addition. Due to addition of 0.3% enzyme, the protease activity increased in time dependent. This phenomenon should be correlated with endogenous enzymes of raw hides. The release amount of inter-fibrillar components was correlated with pH value. The protease activity of lime-free method reached 169.4±1.8 U and the releasing amount of collagen reached maximum at 350 minutes. Compared to traditional methods, the relationship among protease, inter-fibrillar components and collagen of lime-free method have shown a range of high correlation coefficients (0.400~0.550). The utilization of lime-free method can reduce environmental pollution and costs. Nevertheless, the releasing amount of collagen of lime-free method was four fold higher than traditional method. Previous dilemma will be remained to solve by optimization processing.

Key words：Leather processing, Lime-free method, Collagen, Glycosaminoglycan

1. Introduction

Leather processing involves a series of unit of operations that can be classified into three groups: (1) beamhouse operation, which clean the hides or skins; (2) tanning, which permanently stabilizes the hides or skin matrix; and (3) post-tanning and finishing operations, where aesthetic value added[1]. The removal of the interfibrillar components viz., proteins, proteoglycan and glycosaminoglycans during liming and re-liming processes are goal conventional beamhouse operation[2]. Furthermore, the liming processing can induce the "fiber opening effect", where loosens the fibers making it easier for tanning agents, dyes, fat liquors and other substances to diffuse into skin. Enzymatic dehairing via proteolytic enzymes combines small amount of sodium sulfide causes loosening hair follicle are global trends. However, the excessive hydrolysis may induce the collagen fiber degradation and influence the leather quality. The objective of present study was observed the change of interfibrillar components and collagen during beamhouse processing and provide information facilitate to develop of cleaner techniques and green processing.

2. Materials and method

2.1. Specimen collection

Two wet salted bovine skins were used as raw materials for present study. The skin was cut into adjacent quarter parts according backbone and belly, one part of skin were approximate 5 kg (Fig.1 and Fig.2). The processing procedure was derived to "conventional beamhouse processing" and "lime-free beamhouse processing" the All chemicals used in the present study were commercial grade or analytical grade.

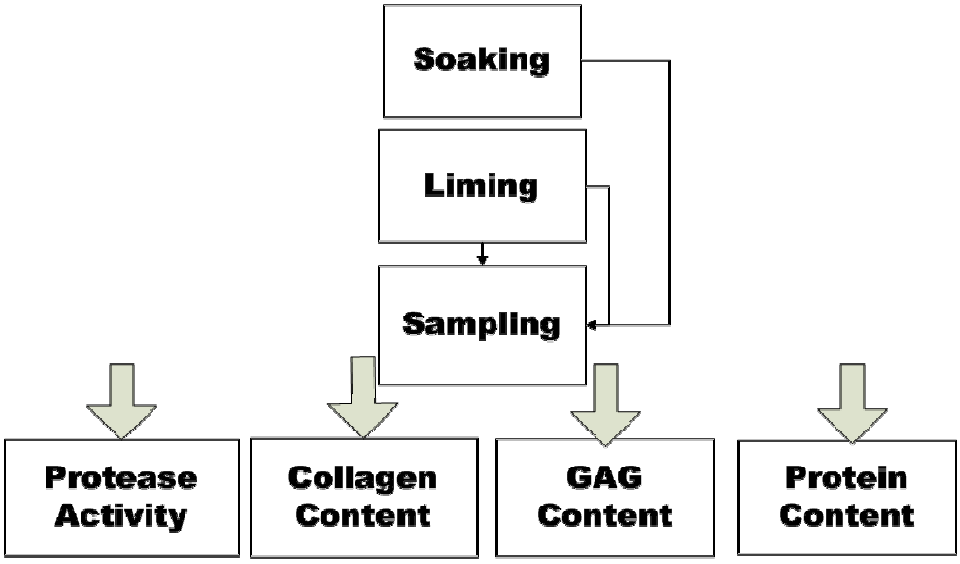

Fig. 1. Analysis scheme of present study.

2.2. Protease activity

Proteolytic activity was determined according to Kembhavi, Kulkarni, and Panti [3] with some modifications. The 0.6 % sodium caseinate/0.1 M sodium phosphate buffer solution (pH 8.0) used as substrate. One unit of enzyme activity (U) was arbitrarily defined as the amount of enzyme required to cause an increase of 0.001 in absorbance at 280 nm, under the assay conditions. Enzymatic activity was calculated as follows: U/ml = (ΔAbs280 nm × 10 × dilution factor)/0.2. The specific activity was expressed as units of enzyme activity per mg of protein.

2.3. Collagen quantification

Collagen quantification was carried out according to a previous study [4]. The higher amount of released 4-hydroxyproline (4-Hyp) detected in collagen presence in liquor.

Fig.2. Appearance of experimental drum in the present study

2.4. Glycosaminoglycan quantification

The amount of glucuronic acid present in the liquor was determined by carbazole reaction [5].

2.5. Protein quantification

Protein and uronic acid concentration were determined by the biuret method [6] and using the 0~10 mg/mL bovine serum albumin (BSA) to calibration.

2.6 Statistical analysis

The entire protocol was repeated three times for each liquor. All data in this experiment were analyzed by GLM program and compared with Duncan's new multiple ranges test contained in the Sigma Stat system (version 3.2), p-value < 0.05 was considered significant.

3. Results and discussion

Beamhouse operations employ complex principles of biochemistry and inorganic chemistry and are the most difficult areas of leather manufacture for understanding[7]. The beamhouse operations are tremendous importance in ultimate quality of the leather. However the absence of monitoring technique to determine the changes of biochemical parameters during beamhouse processing.

We determined the changes of pH value, Baume, protease activity, protein concentration, collagen content and glycosaminoglycan concentration of liquors from conventional or lime-free beamhouse processing, respectively.

The first experiment was carried out according conventional beamhouse processing (Table. 1) which using lime to remove hair and facilitate "fiber opening". The pH and Baume were remarkable increased in liquor 4 due to the lime addition (Table. 3). Protease activity is an important index during beamhouse processing. The protease can be detected in liquor 1 means the hide still contained small amount of endogenous protease which may affect leather quality. The protease activity was increased in liquor 2 due to the addition of 0.3% exogenous enzyme and retained activity during conventional beamhouse processing (Table. 3). The protease activity reach maximum in liquor 9, this phenomenon may correlate with the overnight drum process between liquor 8 and liquor 9. Protein, collagen and glycosaminoglycan were indicators of interfibrillar, fibillar and extra cellular matrix (ECM), respectively. The results shown the

protein, collagen and glycosaminoglycan concentration in liquor were increased in liquor 4 and almost reach maximum in liquor. Previous result should be relate to the addition of lime between liquor 3 and liquor 4. Furthermore, the protein, collagen and glycosaminoglycan concentration in liquors were decreased in liquor 7 due to drain water between liquor 6 and liquor 7. Moreover the protein, collagen and glycosaminoglycan concentration peaked in liquor 9 means the overnight drum process would be enhanced the accumulation of protein, collagen and glycosaminoglycan.

Table. 1 Changes of biochemical parameters during conventional beamhouse processing

Process	%	Weight	Chemical	Turn	Stop
Presoaking	300	15 kg	Water		
	0.2	10g	Sodium carbonate		
	0.1	5g	STANDOX F330 (degreasing agent)		
	0.1	5g	STARCIDE C (bactericide)	60	**Liquor1**
Drain water					
Main soaking	200	10 kg	Water		
	0.3	15 g	INVIGAL SC (Protease)		
	0.1	5 g	STANDOX F255 (degreasing agent)		
	0.1	5 g	STANDOX F330 (degreasing agent)		
	1.0	50 g	Sodium carbonate	210	**Liquor 2**
Drain water					
Liming	100	5 kg	Water		
	1	50 g	SIRO L330P (liming agent)	60	**Liquor 3**
	+				
	1.2	60 g	Lime	60	**Liquor 4**
	+				
	1.2	60g	Sodium sulfite		
	1.2	60g	Sodium hydrosulfide	60	**Liquor 5**
	+				
	1.0	50g	Lime		
	0.8	40g	Sodium sulfite	60	**Liquor 6**
	+				
Liquor	100	5kg	Water		
	0.5	25g	Lime	10 **Liquor 7**	**60 Liquor 8**
Overnight			O.N.	5	**60 Liquor 9**

The conventional processing uses high proportion of lime[1]. The most significant achievement of bioprocess methodology is to reduce toxic waste and total solids. Second experiment used enzyme to replace lime or sulfide. In present study we used 0.1% INVIGAL SC and 0.1% NO.2 (commercial high specificity of alkaline bacterial protease) in liquor 2 and liquor 5, respectively. Moreover, the sodium cabonate was used to modulate pH to enhance hair-remove (Table. 2). The pH and Baume were increased due to the chemicals addition (Table. 4). The protein, collagen and glycosaminoglycan concentration of liquor were significant increased in liquor 6. The release amount of protein and collagen reached maximum in liquor 13 and slight decreased between liquor 15 and liquor 16.

Table. 2 Changes of biochemical parameters during lime-free beamhouse processing

Process	%	Weight	Chemical	Turn	Stop	
Presoaking	300	18kg	Water			
	0.2	12g	Sodium carbonate			
	0.1	6g	STANDOX F330 (degreasing agent)			
	0.1	6g	STARCIDE C (bactericide)	60	**Liquor 1**	
Drain water						
Main soaking	200	12kg	Water			
	0.3	18g	INVIGAL SC (Protease)			
	0.1	6g	STANDOX F255 (degreasing agent)			
	0.1	6g	STANDOX F330 (degreasing agent)			
	1.0	60g	Sodium carbonate	210	**Liquor 2**	
Drain water						
Liming	70	4.2kg	Water			
	1	60g	SIRO L505 (liming agent)			
	0.3	18g	STANDOX F330 (degreasing agent)	20 **Liquor 3**	10	
	0.1	6g	SIRO NL		10	
	1.0	60g	Sodium hydroxide,	10 **Liquor 4**	10	
	1.2	84g	Sodium sulfite	10 **Liquor 6, 7, 8**	10	Repeat X3
	1	60g	Sodium hydroxide,	10 **Liquor 9, 10**	10	Repeat X2
	1	60g	Sodium hydroxide,	10 **Liquor 11, 12, 13**	10	X3
	1	60g	Sodium hydroxide,	10 **Liquor 14**	50 **Liquor 15**	
	80	4.8kg	Water	5		
			O.N.	1	10 **Liquor 16**	

On comparison between conventional and lime-free processing, the synergistic effect of high pH value and exogenous protease actually facilitate the release of inter-fibrillar matrix (protein and glycosaminoglycan). However, the release amounts of collagen also increased simultaneously and reached four folds than conventional processing. These results revealed the enzyme may be providing better efficiency on unhairing and fiber opening up, but may induce the breakage of dermis collagen fiber.

4. Conclusions

The enzyme-assisted processes can be reducing toxic waste and environmental loads, but the excessive hydrolysis may possible damage of leather-making substances. However previous disadvantage could be controlled through proper process optimization and regulated using other additives.

Table. 3 Changes of biochemical parameters during conventional beamhouse processing

	pH	Be°	Protease activity (U)	Protein concentration (mg/mL)	4-Hyp concentration (μg/mL)	Glycosaminoglycan content (μg/mL)
Liquor 1	9.08±0.06	-	26.42±15.63	221.87±90.98	2.72±0.01	442.05±0.01
Liquor 2	10.32±0.30	2.65±0.10	42.45±21.63	317.87±99.38	9.12±1.13	257.44±58.02
Liquor 3	9.85±0.03	1.18±0.05	53.14±18.72	27.20±16.00	6.32±0.56	498.46±58.02
Liquor 4	12.56±0.20	1.48±0.10	63.94±30.65	24.94±14.00	14.45±1.22	1226.67±43.51
Liquor 5	12.60±0.03	2.53±0.17	69.06±37.88	707.20±159.79	28.59±0.46	1047.18±65.27
Liquor 6	13.08±0.04	3.13±0.15	45.68±12.81	907.20±24.00	52.59±3.33	911.20±25.38
Liquor 7	13.03±0.02	1.95±0.10	56.31±22.16	325.87±16.65	16.72±1.70	621.50±159.55
Liquor 8	13.08±0.02	2.25±0.07	34.96±10.55	237.87±24.44	11.52±0.80	549.7±36.26
Liquor 9	12.25±0.11	2.45±0.07	78.85±9.79	544.53±16.65	106.72±2.26	1001.03±65.27

- No detection

Table 4. Changes of biochemical parameters during lime-free beamhouse processing

	pH	Be°	Protease activity (U)	Protein concentration (mg/mL)	4-Hyp concentration (μg/mL)	Glycosaminoglycan content (μg/mL)
Liquor 1	8.72	2.2	13.7±9.2	467.20±78.79	3.92±0.57	454.87±10.88
Liquor 2	9.75	3.0	37.4±6.0	323.20±39.46	9.92±0.80	1236.92±14.50
Liquor 3	9.54	1.6	47.5±4.5	101.87±39.46	5.52±0.57	475.38±10.88
Liquor 4	12.02	2.2	34.0±27.5	165.87±12.22	9.92±0.80	436.92±94.28
Liquor 5	11.49	1.8	169.4±1.8	195.20±21.17	38.32±1.70	826.67±108.79
Liquor 6	12.31	3.8	163.8±2.5	1355.20±125.73	86.72±1.13	760.00±7.28
Liquor 7	12.01	4.1	128.2±8.7	1336.53±96.77	123.92±1.70	1093.33±246.58
Liquor 8	11.82	4.0	140.7±21.5	1989.87±281.29	184.32±4.52	1195.90±500.41
Liquor 9	12.44	4.7	142.7±6.2	2235.20±531.44	337.92±1.13	888.21±36.26
Liquor 10	12.29	4.9	132.0±17.0	1920.53±36.95	377.52±2.42	1339.49±43.51
Liquor 11	12.62	4.7	145.0±6.0	2203.20±309.94	335.92±7.35	2136.92±511.29
Liquor 12	12.53	5.0	132±17.0	2075.20±319.60	353.12±5.66	1870.26±141.42
Liquor 13	12.45	5.0	124.0±11.6	2917.87±299.58	370.32±6.22	1903.59±420.64
Liquor 14	12.73	5.0	123.0±7.6	2376.53±331.43	355.52±6.79	1072.82±29.01
Liquor 15	12.73	4.9	93.3±15.9	2000.53±110.37	363.92±7.35	2131.79±54.39
Liquor 16	12.66	3.2	64.5±7.2	1149.86±28.10	184.32±7.92	1260.00±576.56

5. References

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