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Abstract

The use of conventional lime/alkali system in beamhouse involves a series of complex operation units, which bring a lot of sulfide, suspended solids and ammonia nitrogen pollution. In this work, dispase was used for unhairing/opening collagen fiber-bundles/bating after alkali pre-swelling to replace lime/alkali process to simplify the unit operations in beamhouse. As a result, dispase one-bath beamhouse using alkali pre-swelling process has good effects of unhairing and fiber opening. Especially, NaOH pre-swelling dispase process, rapidly hydrolyzes the proteoglycans in bovine hides, while the collagen hydrolysis is very little. Besides, collagen fibers dispersion, grain smoothness and the fluffy effect of collagen fibers of tanned leather from dispase one beamhouse using NaOH pre-swelling process are the best. This work might provide a reference for simplifying the unit operations in beamhouse, reducing the environmental pollution and the development of high-quality leather.

Keywords: Alkali swelling; Dispase; Leather making; Unhairing; Opening collagen fiber-bundles

1. **Introduction**

Leather with excellent properties were made using few materials and simplified way by our ancestor, which was often used to keep warm, make armor and ornaments, et al [1-3]. However, the development of leather technology so for, hundreds of materials have been used during leather processing, especially, beamhouse such as the auxiliaries of unhairing, liming and bating, etc [4, 5]. They not only increase the cost of leather making but also bring environmental pollution, which attribute to the complexity of the beamhouse operations and various chemical materials used[6, 7]. Hence, simplified the beamhouse unit operations and reduced the use of chemical materials to realize emission reduction and lightweight of leather making is very urgent for the sustainable development of leather.

In this work, dispase was chose for unhairing/opening fibers/bating in one-bath. To provide a natural environment for dispase, the bovine hides must be adjusted natural after alkali pre-swelling by ammonium salts such as ammonium sulfate ($(NH_4)_2SO_4$). After one-bath operation, the hydrolyzed results of elastin, proteoglycans and mucopolysaccharides in bovine hide were investigated by Verhoeff's Van Gieson (EVG) staining. Morphological structures of grain surface and cross section of crust leather were observed by scanning electron microscope (SEM). This technological design of one-both beamhouse using dispase provides a guidance for simplifying conventional beamhouse operations and reducing cost and pollution.

2. **Material and Methods**

2.1 Materials

Wet salted bovine hides were provided from Henan Prosper Skins & Leather Enterprise Co. Ltd, China. Sodium hydroxide (NaOH, analytical reagent (AR)), calcium hydroxide ($Ca(OH)_2$, AR), ammonium chloride (NH_4Cl, AR), phenol (AR), dispase (biochemical reagent, 50 u/mg), casein (biochemical reagent (BR)), tannic acid (98%) and sodium chloride (NaCl, AR) were purchase from Macklin Inc., Shanghai, China.

2.2 De-salting of bovine hide

Salted wet bovine hides were added into a drum of tanning machine (DJDØ350, Xishan, Beitang Leather Machinery Factory, Jiangsu, China) with 500 wt% water (based on salted wet bovine hide in weight) by at room temperature for desalting. The water was changed every hour to completely remove the NaCl in bovine hides, which was verified by dropping in a little silver nitrate solution (0.02 mol/L) with no white precipitation appeared. After desalting, the bovine hides were washed with distilled water and stored at 4 ℃ for subsequent use.

2.3 Conventional unhairing (liming), reliming, deliming and bating

A piece of de-salted bovine hides (15 g) was carried out using $Na_2S/Ca(OH)_2$, $Ca(OH)_2$, NH_4Cl and dispase for unhairing(liming), reliming, deliming and bating respectively, and their detailed experimental procedure was referred to the previous work (Liu et al., 2021).

2.4 Tanning

The bovine hides samples from dispase one-beamhouse and conventional processes were pickled in 300 wt% sulfuric acid solution (pH = 4) at 27 ℃ for 30 min with vibrations (100 r/min). After being tanned in 10 wt%, 15 wt% and 20 wt% tannic acid solution with the ratio of solid-to-liquid (w/w) of 1:3 at 27 ℃ for 24 h with vibrations (50 r/min) respectively, the samples were soaked in the same liquor at 27 ℃ for another 24 h. After being washed with 200 wt% water at 27 ℃ for 12 h (the water was changed every 3 h), the samples were air-dried at 30 ℃ for two days, and then stored in a desiccator for subsequent use.

2.5 Verhoeff's Van Gieson (EVG) staining

The samples of bovine hide from one-bath beamhouse and conventional processes after formalin fixation were respectively dehydrated using 15% (v/v) and 30% (v/v) sucrose solution at 4 ℃. The samples were embedded with opti-mum cutting temperature compound (OCT), and then cut into slices with a thickness of 8 ~ 10 μm by a freezing microtome (CRYOSTAR NX50, Thermo Fisher Scientific, USA). Then the slices were stained with EVG as described in the reported work[8]. The stained slices were observed by digital slice scanning system (DSSS) (Pannoramic 250/MIDI, Hungary).

2.6 Morphological analysis

The samples of tanned leather were cut into pieces sized 5×4×1 mm, which were fixed on the sample platform by conductive tapes and gold-sprayed under vacuum conditions for 30 seconds. The opening effect of collagen fiber-bundles and grain morphology of tanned leather were observed by scanning electron microscopy (SEM) (FEI inspect F50, USA) at 10 kV.

3. **Results and Discussion**

3.1 Dispersion of collagen fibers and dissolution of polysaccharide in bovine hides

As shown in **Fig. 1**, the dispersion of collagen fibers and dissolution of polysaccharide (including proteoglycans and mucopolysaccharides) in bovine hides during one-bath beamhouses were characterized by EVG staining, with the pink and brownish gray respectively representing collagen fibers and

polysaccharide. During one-bath beamhouse, the collagen fibers in NaOH pre-swelling bovine hides is more fluffy than that of $Ca(OH)_2$ pre-swelling bovine hides (**Fig.1** (a), (b) and (c)), while the polysaccharide in NaOH pre-swelling bovine hides is hydrolyzed more thoroughly than that of $Ca(OH)_2$ pre-swelling bovine hides(**Fig.1** (A), (B) and (C)). The results might be because the swelling ratio of NaOH pre-swelling bovine hides was bigger than that of $Ca(OH)_2$ pre-swelling bovine hides for improving the transmission of inside and outside substance in bovine hides. The dispersion of collagen fibers in bovine hides can affect the mass transfer of feed liquid system during leather making, while the NaOH pre-swelling bovine hide provides a good condition for the mass transfer.

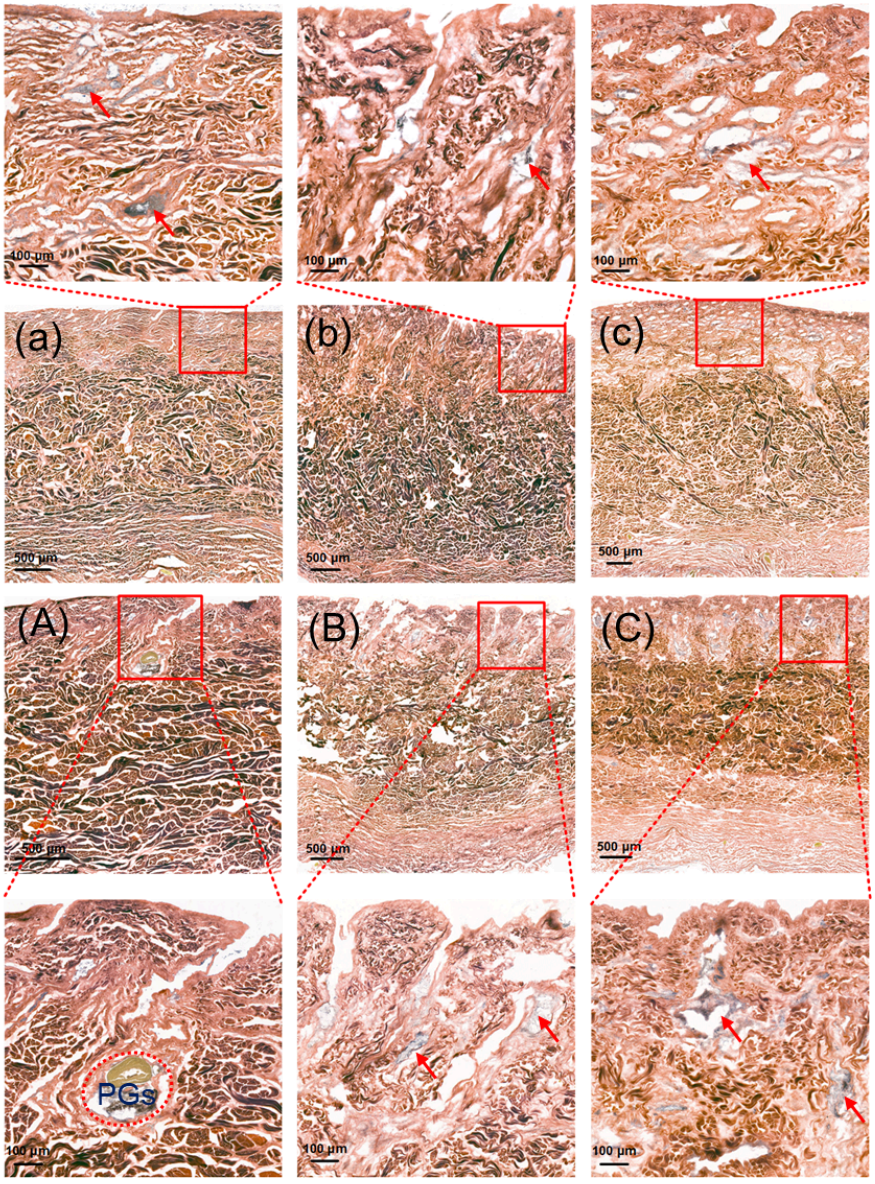


Fig.1 The cross-section digital scanning images of bovine hides by the EVG staining during one-bath beamhouse (using NaOH pre-swelling bovine hides for (a) 6 h, (b) 18 h and (c) 30 h; using $Ca(OH)_2$ pre-swelling bovine hides for (A) 6 h, (B) 18 h and (C) 30 h).

3.2 Cross-section and grain surface microstructure of tanned leather

The collagen fibers microstructure and grain surface morphology of tanned leather from conventional and one-bath beamhouse processes were observed by SEM, with their morphology shown in **Fig. 2**. The collagen fiber-bundles of tanned leather from conventional and one-bath beamhouse using $Ca(OH)_2$ pre-swelling bovine hides processes are thicker than that of one-bath beanmhouse using NaOH pre-swelling

bovine hides, which might be because the proteoglycans and mucopolysaccharides of collagen fibers gap did not be completely hydrolyzed owing to the low expansibility of $Ca(OH)_2$. The hydrolysis degree of grain surface of tanned leather from one-bath beamhouse using $Ca(OH)_2$ pre-swelling bovine hides is bigger than that from one-bath beamhouse using NaOH pre-swelling bovine hides, which might be because grain surface collagen was hydrolyzed by a lot of dispase attaching on the grain surface attribute to low swelling ratio of $Ca(OH)_2$ pre-swelling bovine hides. Thus, the fluffiness of tanned leather collagen fibrils relates to the dissolution of the proteoglycans and mucopolysaccharides of collagen fibers gap, while the high swelling ratio is beneficial for the penetration of dispase, further weakening the hydrolysis of dispase on the grain surface. So, using NaOH for pre-swelling bovine hides has more advantage than $Ca(OH)_2$.

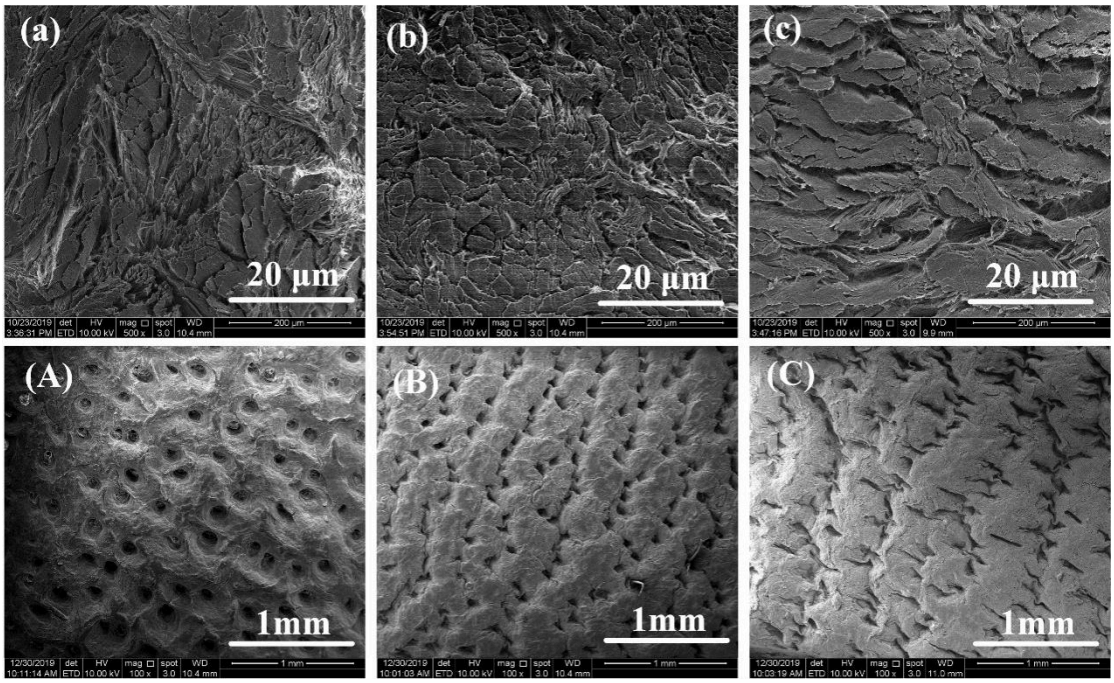


Fig. 2 SEM images of cross-section((a), (b) and (c)) and grain surface ((A), (B) and (C)) of tanned leather: (a) and (A) conventional process; (b) and (B) one-bath beamhouse using NaOH pre-swelling bovine hides; (c) and (C) one-bath beamhouse using $Ca(OH)_2$ pre-swelling bovine hides.

4. **Conclusion**

Simplified beamhouse with an enzyme of dispase one-bath unhairing/fiber opening/bating technology using NaOH and $Ca(OH)_2$ pre-swelling bovine hides was successfully developed and explored. The proteoglycans and mucopolysaccharides of NaOH pre-swelling bovine hides by the one-bath beamhouse process were dissolved more thoroughly owing to the excellent swelling of NaOH. For this reason, the dispersion and fluffiness of collagen fibrils from one-bath beamhouse using NaOH pre-swelling bovine hides were better than that of using $Ca(OH)_2$ pre-swelling bovine hides, which were comparable to that of the conventional process. This work provides theoretical and practical guidance for simplifying beamhouse, reducing the pollution of leather making, and renewing the technology of leather making.

5. **Acknowledgements**

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