Effect of Sodium Chloride on Structure of Collagen Fiber Network in Pickling and Tanning

by

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Abstract

Tannery wastewater usually contains a high salinity due to the use of sodium chloride (NaCl) in curing and pickling. Although some no-pickle tanning and salt-free pickling technologies were developed, few of them have been widely used due to relatively poor mechanical and bulk properties of the resultant leathers. Therefore, the role of NaCl in pickling and tanning should be investigated in the first place. In this study, bated pelts were pickled by salt-free pickling and conventional salt-assisted pickling processes, respectively, and then tanned by chrome tanning agent. The hierarchical structures of collagen fiber network of the pickled pelts and leathers were observed by optical microscope and SEM, while the porosity of leathers was measured. The results showed that the fiber bundles of the pelt pickled in the presence of NaCl were more sufficiently dispersed compared with those of salt-free pickled pelt. Both of the chrome tanned leathers had a regular arrangement of collagen fibers, but the leather with salt-assisted pickling presented remarkably higher degree of fiber dispersion, as well as larger porosity. Moreover, the role of NaCl in organic tanning using an amphoteric organic tanning agent was investigated. The results also showed that the presence of NaCl in tanning could improve the opening up of collagen fiber network and the porosity of the leather. In general, NaCl used in leather processing presented a positive effect in consideration of leather quality.

Introduction

Sodium chloride (NaCl) is one of the commonest chemicals used in leather industry. It is mainly used in curing, soaking and pickling processes. However, with the increase of people's environmental awareness, chloride is regarded as one of the most concerned pollutants in tannery wastewater. It is reported that the content of chloride in effluent could be reduced by 30%-40% when salt-free pickling technology was used.[1] Therefore, some salt-free or salt-reduced pickling processes and no-pickle tanning techniques have been developed to minimize chloride impact from the origin of leather making.[2-5] However, few of them have been widely used due to the fact that these technologies produce leathers with relatively poor mechanical and bulk properties in comparison with the conventional process. NaCl is regarded to dehydrate and prevent swelling of pelt during pickling in classical theories of leather manufacture,[6-7] but none have mentioned its effect on leather properties. Therefore, to understand the reason of the phenomenon above, the role of NaCl in pickling and tanning processes should be investigated in the first place. We know that the hierarchical structure of leather is described as a network in three dimensions that is woven by collagen fiber bundles (20-200 μm). The bundles are assembled by elemental fibers (10 μm), and the elemental fibers are regularly arrayed by fibrils (0.01-0.5 μm), which are self-assembled by collagen molecules with a parallel staggering.[8-9] Some research indicated that a good quality of leather will be achieved when the collagen fiber bundles were dispersed adequately in beamhouse processes.[10-12] This means that fibrous network structure has an important influence on the mechanical and morphological properties of leather. In our preceding work,[13] the role of neutral salt in the assembly behaviors of collagen molecules was investigated, and it was found that NaCl dehydrated collagen molecules and induced collagen molecules to assemble into collagen fibers. On the basis of these observations, we speculated that NaCl would lead to formation of wider spaces between collagen fibers during pickling and tanning, and therefore affect fibrous network structure of resultant leather. However, this assumption was based the research on collagen molecules rather than leather. In this work, the effect of NaCl on fibrous network of pelt/leather in pickling and tanning (chrome tanning and amphoteric organic tanning) was investigated. The hierarchical structures of fibrous network were observed by optical microscope and scanning electron microscope, and the porosity of leather was measured to explore the role of NaCl in pickling and tanning processes.

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Experimental

Materials

Raw materials used in experiments were cowhides from Queensland, Australia. They were soaked, limed, split (thickness of upper layer 2 mm), weighed, delimed and bated conventionally. The chemicals used for leather processing were of commercial grade, and the chemicals used for analysis were of analytical grade. Salt-free pickling auxiliary mainly composed of sulfone sulfonic acid was from Dowell Science & Technology Inc. (Sichuan, China). Chrome tanning agent (Cr_2O_3 content 24%, basicity 33%) was from Minfeng Chemical Co., Ltd. (Chongqing, China). Amphoteric organic tanning agent (TWT) was supplied by Tingjiang New Material Co., Ltd. (Sichuan, China).

Preparation of Pickled Pelts

A piece of bated pelt (approx. 2 kg, on the back of the pelt) was cut along the backbone in halves and pickled using salt-free process and conventional process, respectively (TABLE I). Next day ran for 30 min and collected the samples from matched areas near the backbone for further analyses.

Preparation of Tanned Leathers

Chrome Tanned Leathers

The pickled pelts obtained above were tanned using 6% chrome tanning agent in 50% pickle liquor (based on weight of limed pelts, the same below) for 2 h, respectively. Then they were basified to pH 3.8-4.0 using sodium bicarbonate. 200% water was added and ran for another 2 h at 40°C, then left overnight. Next day ran for 30 min and collected the samples from matched areas near the backbone for further analyses.

TWT Tanned Leathers

The pH of a piece of bated pelt (approx. 1 kg, on the back of the pelt) was adjusted to 6.0 using 0.1% formic acid in 50% water. Then the pelt was cut along the backbone and divided into two groups. Group 1 was directly tanned by 5% TWT for 4 h using no-pickle tanning and then basified to pH 8.0 using sodium carbonate. 200% water was added and ran for another 2 h at 40°C, then left overnight[14]. Next day ran for 30 min and collected the samples from matched areas near the backbone for further analyses. Group 2 was treated with 6% NaCl in 50% water for 3 h and left overnight (12 h). Next day it was tanned with TWT in the same conditions as group 1.

In addition, a piece of conventional pickled pelt (approx. 1 kg, on the back of the pelt) was depickled to pH 6.0 with sodium carbonate in 50% pickle liquor. Then it was also cut along the backbone and divided into group 3 and 4. Group 3 was immediately tanned with TWT in 50% depickling liquor in the same conditions as group 1. Group 4 was washed 6 times with 200% water for 10 min to remove neutral salts, and then tanned with TWT in the same conditions as group 1.

Histological Staining

Samples (1 cm × 1 cm) from pickled pelts, chrome tanned leathers and TWT tanned leathers were cut into 20μm thickness cross sections by freeze microtome (CM1950, Leica, Germany). It should be noted that the pickled pelts were fixed in 10% neutral buffered formalin for 24 h before cutting to prevent from acid swelling. The sections were stained with hematoxylin and then counterstained with eosin. The stained sections were observed by biological microscope (CX41, Olympus, Japan).

Scanning Electron Microscopy

Samples from pickled pelts, chrome tanned leathers and TWT tanned leathers were lyophilized by vacuum freeze dryer (LGJ-30F, XinYi, China). The cross sections of the samples were observed by Scanning Electron Microscope (SEM, Phenom Pro, Phenom-world, Netherlands) at low vacuum with an accelerating voltage of 5/10KV.

Table I
Pickling processes.

Salt-free pickling process			Conventional pickling process		
Water	**50%**		**Water**	**50%**	
Salt-free pickling auxiliary	6%	Run for 60 min, pH=2.6-2.8. Then run for 120 min and still overnight.	NaCl	6%	Run for 10 min.
			Formic acid	0.6%	Run for 30 min.
			Sulfuric acid	0.3%	Run for 30 min.
			Sulfuric acid	0.4%	Run for 30 min, pH=2.6-2.8. Then run for 120 min and still overnight.

The offers of materials were based on weight of limed pelts.

BET Surface Area Analysis

Samples from pickled pelts, chrome tanned leathers and TWT tanned leathers were lyophilized by vacuum freeze dryer and cut into 3 mm × 3 mm pieces. Then they were vacuum dried for 10 h at different temperatures (40°C for pickled pelts, 90°C for chrome tanned leathers and 60°C for TWT tanned leathers). The specific surface areas of the samples were determined using surface area and porosity analyzer (TriStar 3000, Micromeritics, USA).

Capillary Flow Porometry[15-16]

Samples from pickled pelts, chrome tanned leathers and TWT tanned leathers were lyophilized by vacuum freeze dryer and then cut into Φ25 mm circular pieces. The thicknesses of the samples were determined using a thickness gauge with 100 g pressure. Porometry was performed using a capillary flow porometer (POROLUX™ 1000, POROMETER, Germany). A sample was first soaked in Porofil wetting liquid of low vapor pressure (0.4 kPa), low reactivity and low interfacial tension (0.016 N/m) that can be assumed to fully wet the samples[16-17]. Then it was placed in the sample chamber and sealed. Pressure was increased from 0 to 2.5 bar to obtain two gas flow curves (wet curve and dry curve) as a function of pressure. The mean flow pore size of the sample could be calculated according to the curves. In addition, the linear dry curve can be expressed as:

$$F = \frac{AK}{u\eta}\Delta P \qquad (1)$$

where F is the gas flow rate (m^3/s), A is the area of the sample (m^2), u is the thickness of the sample (m), K is the air permeability coefficient of the sample (m^2), η is the dynamic viscosity of gas flow (Pa·s), and Δp is the pressure differential between the two sides of the sample.

Darcy air permeability ($\frac{K}{\eta}$, m^2/(Pa·s)), which is related to the porosity and pore size distribution of the sample, could be obtained according to the dry curve. The bigger value of Darcy air permeability indicates better air permeability of the sample.

Measurement of Porosity of Pelt/leather[18-19]

Samples from pickled pelts, chrome tanned leathers and TWT tanned leathers were lyophilized by vacuum freeze dryer and then cut into 20 mm × 3 mm pieces. The porosities of the samples were measured as follows. Firstly, over 100 mL of benzyl alcohol was added to a special volumetric flask (the neck of the flask has 0-10 mL tick marks), and the volume (mL) was recorded as V_1. Secondly, pelt/leather pieces were added into the flask and soaked for 48 h, and the volume (mL) was recorded as V_2. Then the pelt/leather pieces and benzyl alcohol were all poured out of the flask, and the surfaces of the pelt/leather pieces were slightly dried by filter paper. Over 100 mL of fresh benzyl alcohol was once again added into the flask. The volume (mL) was recorded as V_3. Finally, the pelt/leather pieces were added into the flask, and the volume (mL) was recorded as V_4. The porosity of pelt/leather was calculated as:

$$porosity = \frac{(V_4 - V_3) - (V_2 - V_1)}{V_4 - V_3} \times 100\% \qquad (2)$$

Results and Discussion

Effect of NaCl on the Fibrous Network in Pickling

Bated pelts were pickled using salt-free and conventional salt-assisted processes, respectively. Figure 1 shows the histological stained cross sections of the pickled pelts. It can be seen from Figure 1a that collagen fiber bundles of salt-free pickled pelt, particularly those in reticular layer, were mainly in the form of thick bundles due to the lack of opening up. However, the thick collagen fiber bundles were separated into thin bundles in the reticular layer of conventional pickled pelt (Figure 1b), which suggested that the fibrous network was more sufficiently dispersed in the presence of NaCl. In order to observe finer fibrous structure, SEM analysis of the cross sections of pickled pelts was performed, as shown in Figure 2. It is known that in the hierarchical structures of hide/leather, fiber bundles (20-200 μm) are composed of elemental fibers (10 μm), which can be further divided into even finer fibrils (0.01-0.5 μm). Figure 2a shows that the fiber bundles of salt-free pickled pelt were cemented together. It was even difficult to observe them clearly as the weave of fibers was relatively disordered. Furthermore, the collagen fibers in the magnified reticular layer (Figure 2a) were stuck together in clumps. On the contrary, Figure 2b displays that fiber bundles were well distinguished and arranged in order when pickled with NaCl. What's more, they were divided into individual elemental fibers that were separated from each other and quite distinct in the reticular layer. This phenomenon implies the increase of gaps and pores in fibrous network. The surface area measurement will give the concrete proof for fiber dispersion. Unsurprisingly, the BET surface area of the conventional pickled pelt (TABLE II) was much larger, indicating better fiber dispersion performance than that of salt-free pickled pelt. Moreover, lower mean flow pore size, larger value of Darcy air permeability and higher porosity was found for pelt pickled using conventional salt-assisted pickling process (TABLE II). These results suggested that the presence of NaCl benefits the opening up of fibrous network, which is in accordance with the microscopic observation. To confirm this hypothesis, more investigations were carried out in the following sections.

Effect of NaCl on the Fibrous Network in Chrome Tanning

Chrome tanning was conducted in pickling bath, where the presence of NaCl may still influence the fibrous structure of leather during chrome tanning process. Figure 3 shows the histologically stained fibrous structure of chrome tanned leathers. With salt-free pickling, most of the collagen fibers in leather were adhered to each other and presented as thick bundles (Figure 3a). However, as for pickling with salt, the fibrous network of leather were dispersed better and displayed as thinner bundles (Figure 3b). Moreover, the fiber bundles in Figure 3 were denser and more robust compared with those in Figure 1, which should be due to the crosslinking/tanning effect of chrome tanning agent among the collagen fibers.

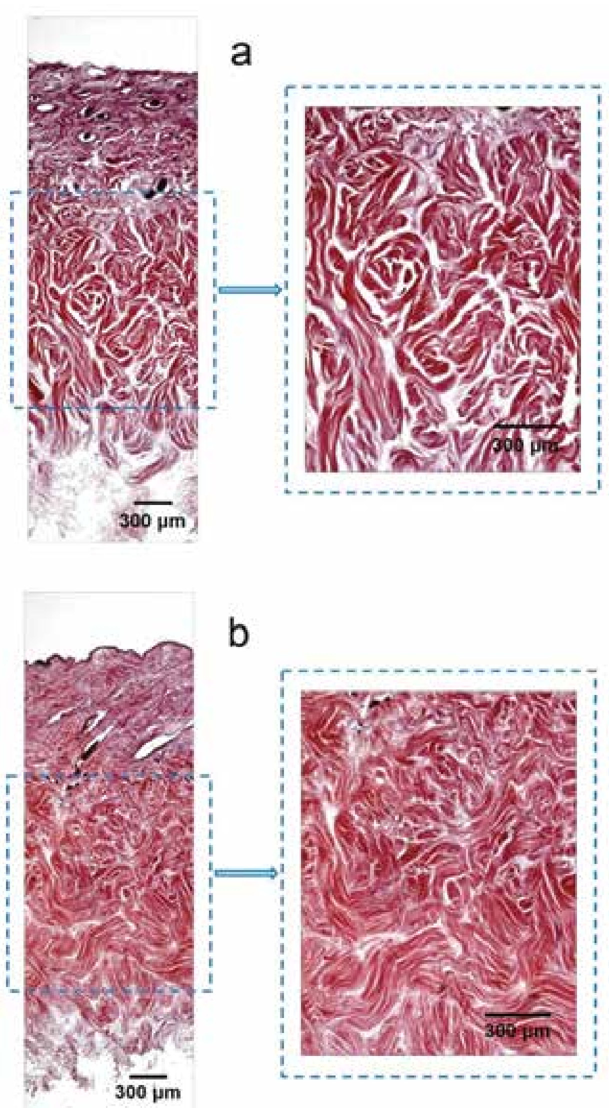

Figure 1. Histologically stained cross sections of (a) salt-free pickled pelt and (b) conventional pickled pelt.

On a more microscopic scale, the cross sections of chrome tanned leathers with different pickling processes were observed by SEM. Figure 4a shows the chrome tanned leather using salt-free pickling process, where most of the elemental fibers were adhered and assembled into fiber bundles. However, Figure 4b shows that the elemental fibers in the leather using conventional salt-assisted pickling process were not only assembled into fiber bundles, but also well separated from each other. This fact was also demonstrated by the porosity properties of chrome tanned leather (Table III). The BET surface area of chrome tanned leather using conventional pickling process was larger than that of leather using salt-free pickling process. The air permeability of conventional chrome tanned leather was better. The porosity of the chrome tanned leather using conventional pickling process was approx. 3% higher than that of using salt-free pickling process. In combination of the results in Section 3.1, we can infer that NaCl plays an important role in the dispersion of elemental fibers in fiber bundles during pickling and tanning processes.

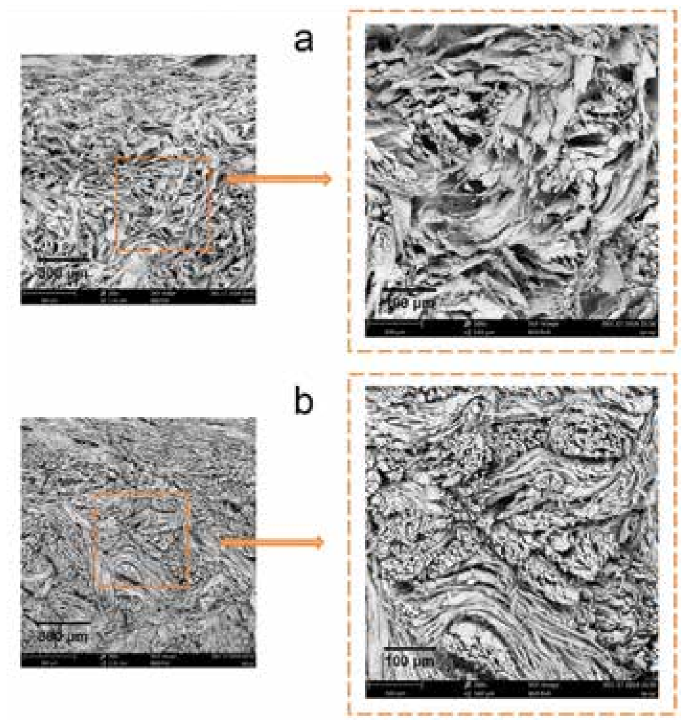

Figure 2. SEM images of cross sections of (a) salt-free pickled pelt and (b) conventional pickled pelt.

Table II
Porosity properties of pickled pelts.

Sample	BET surface area (m^2/g)	Mean flow pore size (μm)	Darcy air permeability ($m^2/(Pa{\cdot}s)$)	Porosity (%)
Salt-free pickled pelt	0.981 ± 0.053	5.752 ± 0.061	0.469×10^7	70.03 ± 0.95
Conventional pickled pelt	4.128 ± 0.039	1.599 ± 0.091	1.716×10^7	71.51 ± 0.67

Collagen has a large number of hydrophilic amino acid residues that combine with water through hydrogen bonds. NaCl can break the hydrogen bonds among collagen and water, leading to the damage to the hydrated layer and the dehydration of collagen. Therefore, in conventional pickling and tanning, NaCl is added to prevent pelt from absorbing an excessive amount of water so as to avoid the defect of acid plumping. The damage to the hydrated layer would improve the exposure and ionization of charged groups in collagen. The electrostatic interactions of these charged groups may result in better dispersion of collagen fibers. In addition, Hofmeister ion effects on protein stability arise repeatedly in protein research. Na^+ and Cl^- are believed as kosmotropes which lead to stabilization of protein by salting-out effect.[20-21] It has been found that NaCl can induce collagen molecules to assemble into collagen fibers and stabilize the fibrous network structure.[13] It is likely that this would be another reason for the orderly arrangement of fiber bundles in the presence of NaCl.

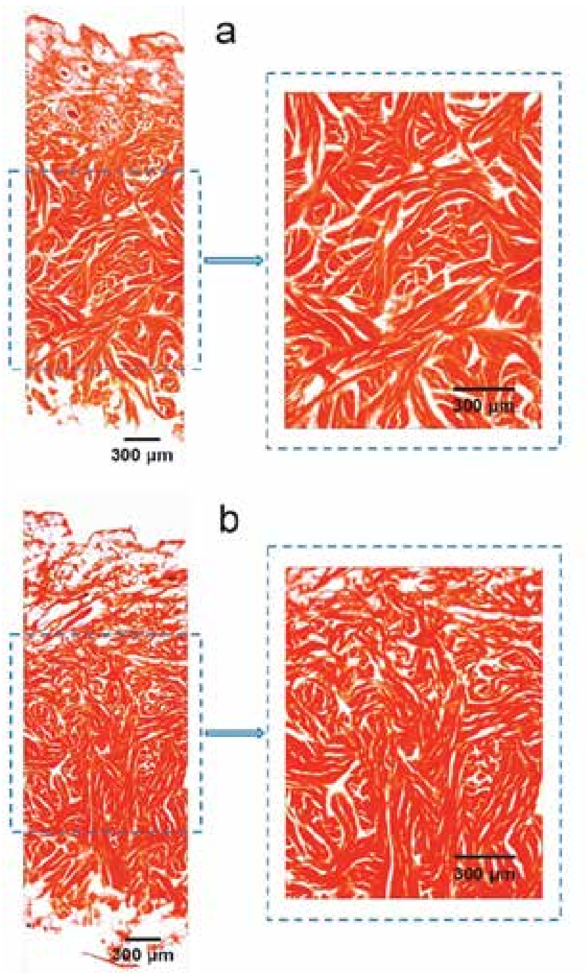

Figure 3. Histologically stained cross sections of (a) chrome tanned leather using salt-free pickling and (b) chrome tanned leather using conventional pickling.

Effect of NaCl on the Fibrous Network in Tanning with Amphoteric Organic Tanning Agent

Four groups of tanning trials using amphoteric organic agent (TWT) were performed according to Section 2.3.2. The histologically stained cross sections of all the TWT tanned

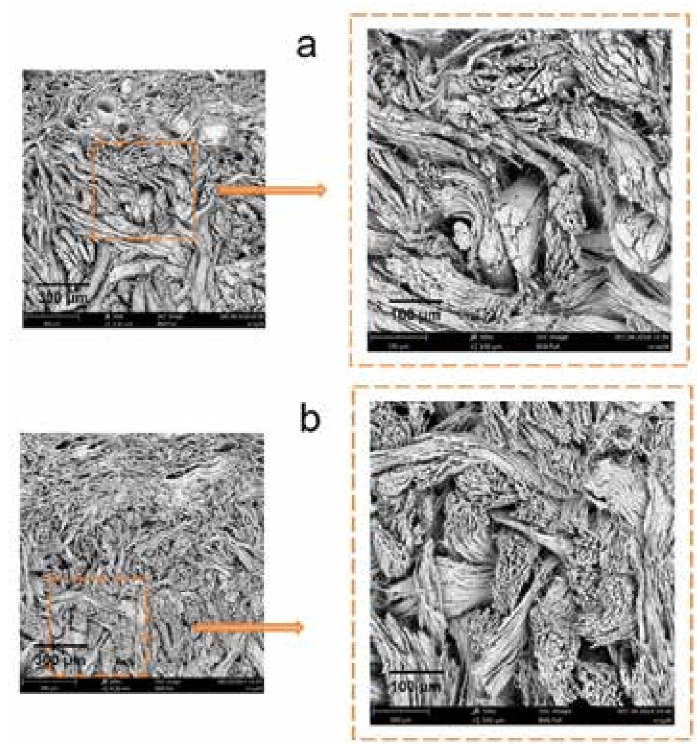

Figure 4. SEM images of cross sections of (a) chrome tanned leather using salt-free pickling and (b) chrome tanned leather using conventional pickling.

Table III
Porosity properties of chrome tanned leathers.

Sample	BET surface area (m^2/g)	Mean flow pore size (μm)	Darcy air permeability ($m^2/(Pa·s)$)	Porosity (%)
Chrome tanned leather using salt-free pickling	3.126 ± 0.068	1.502 ± 0.074	1.792×10^7	68.11 ± 0.31
Chrome tanned leather using conventional pickling	3.495 ± 0.023	1.563 ± 0.025	2.181×10^7	71.14 ± 0.21

leathers are shown in Figure 5. The fiber bundles of leathers using no-pickle tanning process (group 1 and 2) were thick and tightly woven together (Figure 5a and 5b). Even though group 2 was tanned in the presence of 6% NaCl, the dispersion extent of fiber bundles was not sufficient (Figure 5b). This fact was also confirmed by SEM images shown in Figure 6a and 6b. The elemental fibers were cemented together in grain layer and assembled into thick bundles with insufficient separation in reticular layer. Both groups did not exhibit any differences in the arrangement of fibrous network. It was found in histological staining and SEM images that the fiber bundles of leathers using pickling-depickling-tanning process (group 3 and 4) illustrated better dispersing performance (Figure 5c, 5d, 6c and 6d) than those of group 1 and 2 (Figure 5a, 5b, 6a and 6b). The fiber

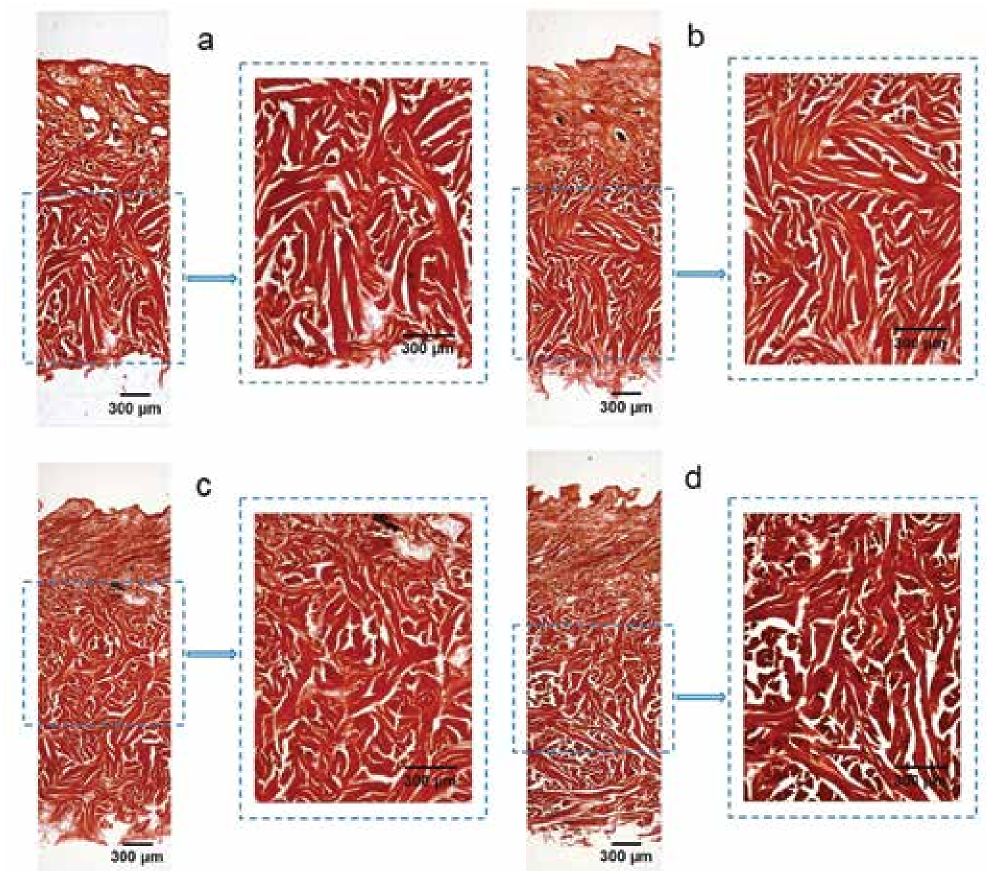

Figure 5. Histologically stained cross sections of (a) TWT tanned leather of group 1, (b) TWT tanned leather of group 2, (c) TWT tanned leather of group 3 and (d) TWT tanned leather of group 4.

Table IV
Porosity properties of TWT tanned leathers.

Sample	BET surface area (m^2/g)	Mean flow pore size (μm)	Darcy air permeability ($m^2/(Pa\cdot s)$)	Porosity (%)
TWT tanned leather of group 1	0.862 ± 0.006	2.176 ± 0.040	0.714×10^7	71.05 ± 0.59
TWT tanned leather of group 2	0.913 ± 0.037	2.288 ± 0.015	1.491×10^7	74.71 ± 0.44
TWT tanned leather of group 3	1.043 ± 0.008	1.928 ± 0.110	2.528×10^7	73.05 ± 0.28
TWT tanned leather of group 4	1.024 ± 0.017	3.442 ± 0.069	0.620×10^7	72.93 ± 0.55

bundles were separated into elemental fibers both in grain and reticular layers, and there were more gaps and pores in the fibrous network, particularly for the leather tanned in the presence of NaCl (group 3). We know from the results above that the presence of NaCl benefits the separation of fiber bundles. Meanwhile, acid used in pickling process had broken some chemical bonds among collagen fibers, and this action contributed to the opening up of the collagen fibers as well.[7] Furthermore, the crosslink action of TWT tanning agent seemed to tighten the fiber bundles. Therefore, under the influence of these factors, the porosity properties of TWT tanned leather illustrated some interesting rules, as shown in TABLE IV. As expected, the BET surface area and porosity of leather tanned without pickling and in the absence of NaCl (group 1) was the lowest among all the groups. Leathers treated with NaCl (group 2 and 3) showed higher BET surface area and porosity compared to group 1. Meanwhile, it was found that leathers of these two groups had better air permeability. This fact once again demonstrates that NaCl plays an important role in the opening up of fibrous network of leather.

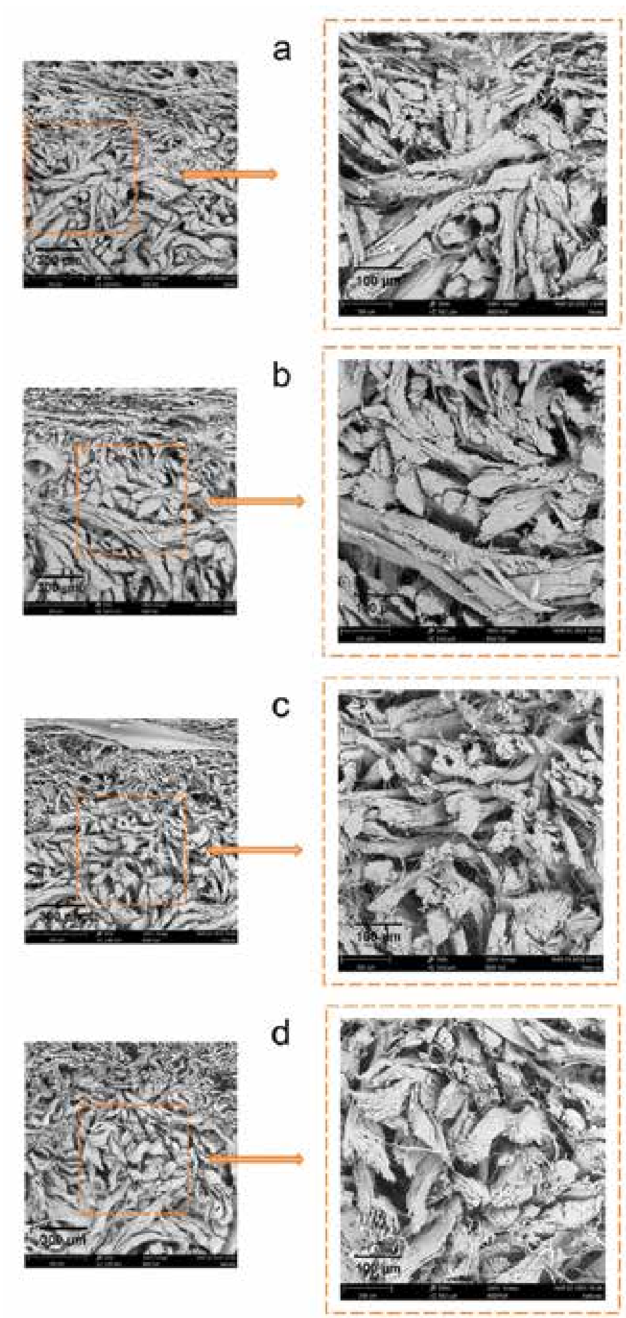

Figure 6. SEM images of cross sections of (a) TWT tanned leather of group 1, (b) TWT tanned leather of group 2, (c) TWT tanned leather of group 3 and (d) TWT tanned leather of group 4.

Conclusion

The presence of NaCl in pickling and tanning processes improves the dispersion of elemental fibers, orderly arrangement of fiber bundles and formation of a porous fiber network due to the dehydration and salting-out effects of NaCl. This should be an important factor for acquisition of excellent mechanical and organoleptic properties of leather made by using salt-assisted pickling process. Correct understanding of the role of NaCl in leather manufacture might be favorable for us to further develop novel chemicals and practical technologies aiming at reducing chloride pollution as well as guaranteeing leather quality.

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