

THE ROLE OF NEUTRAL SALT FOR THE HYDROLYSIS AND HIERARCHICAL STRUCTURE OF HIDE FIBER IN PICKLING

by

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ABSTRACT

Pickling process carried out for adjusting the skin to the desired pH for tanning, in which neutral salts were added to avoid skin fiber from the acid swelling. This research study aimed at investigating the contribution of neutral salt to the hydrolysis and the fibrous structure of collagen fiber in acid solutions. Collagen fiber and bovine hide in sulfuric acid solutions with sodium chloride or sodium sulfate were investigated. The total protein concentration in the solutions was determined. The hierarchical structures of bovine hide in different acid and neutral salt systems were investigated by optical microscope, TEM and SEM. This study suggested the importance of neutral salt for the hydrolysis and the dispersion of collagen fiber in pickling. It implied that the osmotic swelling by acid could not reach the inner scale of fibril. The decrease in mechanical properties for the leather tanned with salt free pickling may have been caused by osmotic swelling destroying the interactions among the fibril and fiber bundles

INTRODUCTION

The most abundant protein of hide and skin is type I collagen, a fibrous protein, which is featured by its triple-helical conformation, formed by three left-handed polyproline II like helices twisted in a right handed triple helix with a length of about 300nm.¹⁻³ The hierarchical structures of hide and skin from molecular level to visible scale are consisting of collagen molecules, fibril, fiber and fiber bundle networks.⁴⁻⁵ The collagen molecules, having lengths of 300nm with approximately 1.5nm in diameter, are self-assembled by a parallel staggering to form fibrils. The fibrils then regularly arrayed to form fiber bundles by a matrix rich in proteoglycans into fiber and fiber bundles, and the fiber bundles then weaved into network in three dimensions to form the corium layer of hide and skin. To obtain a good quality of leather, the fiber bundles of hide and skin should be dispersed or opened up adequately in beamhouse.⁶⁻⁹ For example, to open up the fiber bundles, lime was used in the liming process, and enzymes were used in the bating process.

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Pickling, a process immediately before chromium tanning in leather making, is to treat the hide and skin with acid and neutral salt solutions.⁶ The main purpose of pickling is to adjust the collagen fiber of hide and skin to the desired pH for tanning. Furthermore, acid has the purpose to disperse the collagen fiber bundles and to impart the finished leather with good quality as well. The hide and skin will be swollen in acidic solutions due to the osmotic pressure. Adequate amount of neutral salt such as sodium chloride was added in the acid solution to avoid the swelling. The concentration usually is 5% of sodium chloride based on the water in the solution. It was reported that pickling process contributes to about 35% total dissolved solids (TDS) in the effluents.¹⁰ To overcome the environmental problems of TDS related to neutral salts in the effluent, several of the novel processes have been developed.¹¹⁻¹³ Salt free pickling using naphthalene sulfonic acid is well known and extensively studied.¹¹ Oxazolidine based materials have been developed to displace neutral salt for salt-free pickling process.¹² Recycle/reuse of pickle liquor for the subsequent batches as well as resorting to pickle free alum-chrome combination tanning systems have been studied.¹³ However, the effect of neutral salts to the skin collagen fiber structure and the hydrolysis of collagen in pickling were seldom reported. In this paper, bovine hide was treated with various concentrations of acid and neutral salt solution, in which the hydrolysis of collagen fiber was analyzed and the hierarchical structures of collagen fiber were investigated by transmission electron microscopy (TEM), scanning electron microscopy (SEM) and optical microscopy to illustrate the role of neutral salt for collagen fiber in acid solutions.

EXPERIMENTAL PROCEDURES

Materials

The bovine hide used for this experiment was from Sichuan, China. The preparation of acid relaxed collagen fiber was described elsewhere.¹⁴ Sulfuric acid, sodium sulfate and sodium chloride used in this experiment were chemical pure reagents. Chrome tanning agent is KMC-2 (Tingjiang Co. China), a self-basified chrome powder with 23% chrome oxide (Cr_2O_3) content and 33% basicity.

Treatments of Cattle Hide in Acid and Salt Solutions

The fleshed and unhaird bovine hide was cut into 20×20 cm size. The cut was soaked in 0.1mol/L H_2SO_4 solution with various concentration of neutral salt with continuous shaking at 25°C. The 0.1% bromocresol green solution was used to determine the penetration of the acid, after the acid was penetrated thoroughly into the hide, stop shaking and over night. The swelling rate of the samples was determined according to the accumulation of its thickness as well as its weight. After 24h, a small piece was taken out and fixed with 4% formaldehyde (pH 7.4) for TEM and histological section investigations. The remaining part of the sample was tanned

by 6% KMC-2 chromium tanning agent. The mechanical properties of tanned sample were determined by GT-AI-7000S Universal tensile testing machine (Gotech, China). All the experiments were done at least twice.

Treatments of Acid Relaxed Collagen Fiber in Acid and Salt Solutions

50mg freeze-dried collagen fiber was soaked in 100mL acid and salt solution of set concentration with continuous shaking for the set length of time. The concentration of sulfuric acid is from 0.1 to 0.5 Mol/L, in which the concentration of neutral salt is 0, 0.05, 0.1, 0.5 and 1.0 Mol/L respectively. The concentration of the hydrolyzed protein in the solution during the process was determined by Lowry method¹⁵. After treatment, the unsolved samples were fixed with 4% formaldehyde (pH 7.4) for SEM and TEM investigations.

Histological Investigation

The samples fixed in 4% formaldehyde (pH 7.4) over 24h were cut into 20um thickness sections by a freeze microtome (Leica, Germany). The cut section slides were stained with picric acid and fuchsin acid, with which the collagen fiber was stained into red. The stained sections were observed by optical microscope and recorded by a digital camera (Olympus, Japan).

Scanning Electron Microscopy (SEM) of Un-hydrolyzed Collagen Fiber

Samples of collagen fiber were attached to alum SEM stubs using carbon tabs. Specimens were sputter coated with gold prior to examination using a JSM-5900LV SEM (Japan Electron Optical Laboratory Co., LTD, Japan).

TEM Investigation

The unsolved collagen fiber was fixed in 4% formaldehyde solution and post-fixed in 1% Osmium tetroxide solution. After that the samples were gradient dehydration with acetone and embedded in epoxy resin. The Ultra-thin sections stained with uranyl acetate and lead citrate were observed with Hitachi H600-IV electron microscope at 75 kV (Hitachi, Japan).

RESULTS AND DISCUSSION

The Effect of Neutral Salts to Collagen Hydrolysis in Acid-neutral Salt Systems

The commonly using neutral salt in leather making, sodium chloride and sodium sulfate were applied for this experiment. The sulfuric acid solutions were adjusted by various concentration of salt to create acid and neutral salt solutions, in which the collagen fiber was treated to a set length of time. The concentration of protein hydrolyzed into the solution was determined by Lowry method. The OD value is positive related to the concentration of the protein in solution.

The Concentration and the Type of Neutral Salt

The concentration of the neutral salt in 0.1 Mol/L H_2SO_4 solutions was set at 0, 0.05, 0.1, 0.5 and 1.0 Mol/L respectively. The hydrolysis profiles of collagen fiber in these solutions were shown in Figure 1. Sodium chloride and sodium sulfate showed different features to the hydrolysis of collagen in acid and neutral salt solutions. For sodium sulfate, 0.1 Mol/L of which in H_2SO_4 solution would reduce the hydrolysis rate of collagen fiber, while the hydrolysis rate would be depressed much more when the concentration of sodium sulfate in the solution increased to 1.0 Mol/L. For sodium chloride, when the concentration was 0.1 Mol/L in the solution, it would improve the hydrolysis rate of collagen fiber. However, when its concentration was 1.0 Mol/L in the solution, the hydrolysis rate would be depressed. These results could be interpreted by the salting in and salting out principles¹⁶⁻¹⁸. Sodium chloride showed salting in effect at low concentration while would show salting out effect at high concentration. Sodium sulfate showed better salting out effect than sodium chloride. The excellent dehydration effect of sodium sulfate plays a critical role in reducing the hydrolysis rate of collagen fiber in acid solution.

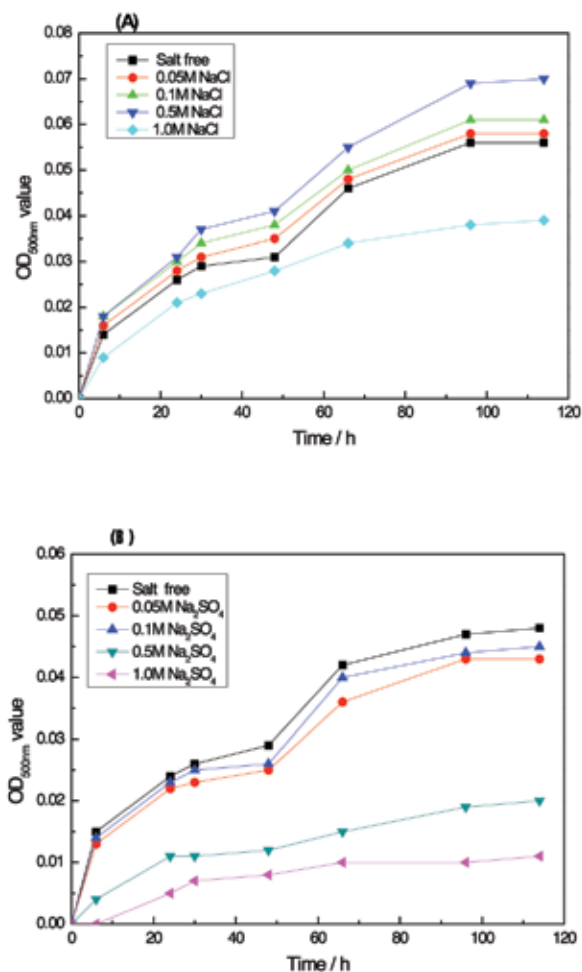


Figure 1. The hydrolysis profiles of collagen fiber in 0.1M H_2SO_4 & neutral salt systems at 25°C(A) 0.1Mol/L H_2SO_4 + NaCl; (B) 0.1Mol/L H_2SO_4 + Na_2SO_4 .

The Concentration of Sulfuric Acid

The influence of neutral salt to the hydrolysis of collagen fiber in different concentration of H_2SO_4 from 0.1 Mol/L to 0.5 Mol/L was investigated. The hydrolysis profiles of collagen fiber in these solutions at 30°C were shown in Figure 2. The results showed that the hydrolysis rate of the collagen fiber would be increased according to the concentration of acid increasing. For different concentration of H_2SO_4 , the low concentration of sodium chloride still showed salting in effect and the higher concentration of sodium chloride showed salting out effect. 0.5 Mol/L of sodium chloride showed salting in effect for 0.1 Mol/L of H_2SO_4 , while would show salting out effect for 0.5 Mol/L of H_2SO_4 . Sodium sulfate showed salting out effect for all the concentration acid as well (data were not shown).

Temperature

The influence of neutral salt to hydrolysis rate of collagen fiber in acid solutions at different temperatures was shown in Figure 3. It was shown that the higher the temperature was, the quicker the hydrolysis rate would be. When the treatment was set at 25°C, the hydrolysis of the collagen was much less than at 30°C or 35°C for the same treating period. More collagen would be hydrolyzed into the acid solution at higher temperature even the concentrated neutral salt was added. When the treatment of collagen fiber was above 30°C, the collagen will be dramatically hydrolyzed into the solution, suggesting a role of temperature for the hydrolysis of collagen in pickling and for the quality of the finished leather.

The Effect of Neutral Salts to the Hierarchy Structure of Hide Fiber in Acid and Neutral Salt Solutions

The bovine hide treated in 0.1 Mol/L H_2SO_4 solution with salt free was tanned by chrome tanning agent KMC-2. Its mechanical properties were compared with the chrome tanned leather by typical pickling. We noted that much longer time was needed for the chrome to penetrate thoroughly into the hide for salt free pickling. The content of Cr_2O_3 for leather treated by salt free pickling was 5.72%, which is higher than the typical pickling leather of 4.95% (Table I). It indicated that the swelling facilitated more of the chrome tanning agent to penetrate into the hide, although the swelling osmotic pressure hindered the penetration rate. The shrinkage temperature (T_s) could reach approximately 95°C for all the leather samples. It revealed that the leathers were tanned well enough by the two pickling methods. The tensile strength and tear resistance for salt free method's leather had the values of 8.38MPa and 11.73N/mm respectively which were much less than the values of the leather by typical pickling (15.64MPa and 24.12N/mm) (Table I). These results suggest that the collagen fiber networks should be partly destroyed when the hide was existed in the swelling state.

The bovine hide treated within 0.1Mol/L H_2SO_4 at 25°C without salt would be swelled to semi-translucent with absorbing water. When the salt concentration was gradually

increased to 1.0 Mol/L, the hide would be dehydration to non-swell state. The bovine hide treated in 0.1Mol/L H_2SO_4 solution with salt free for 48h (Fig. 4A), and its optical microscopy photos showed that collagen fiber in the papillary layer and the reticular layer become larger and bigger because

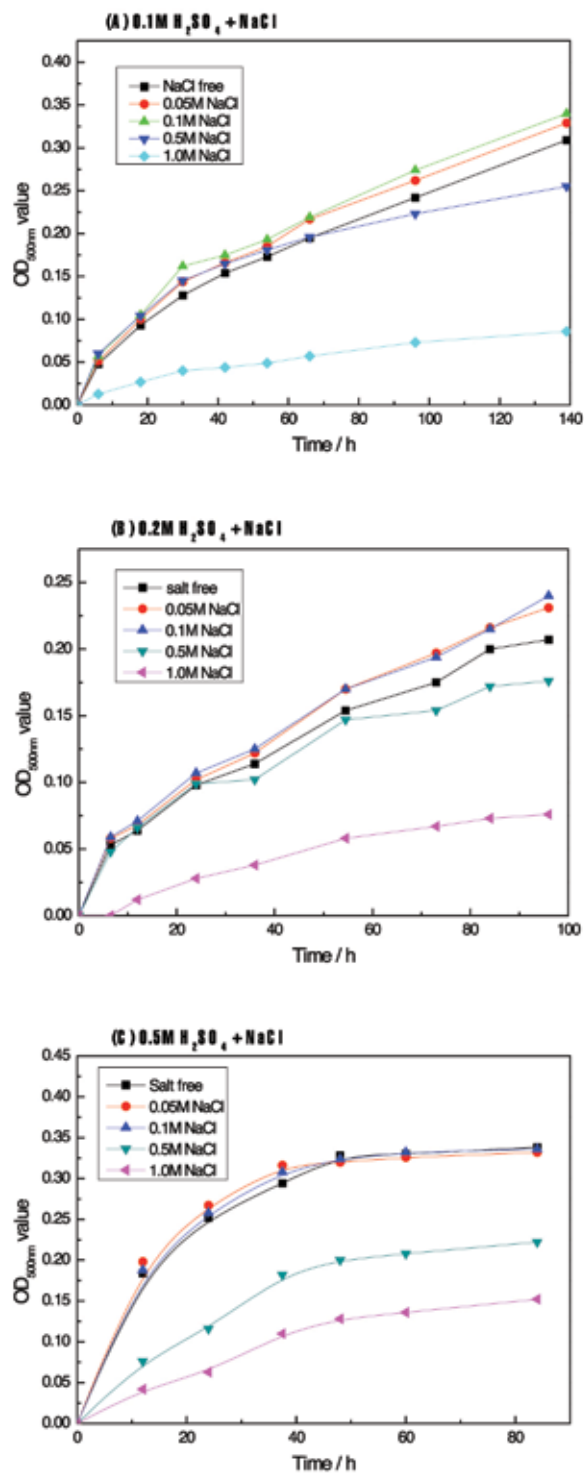


Figure 2. Hydrolysis profiles of collagen fiber in different concentration of H_2SO_4 and sodium chloride solutions at 30°C. (A): 0.1 Mol/L H_2SO_4 & NaCl; (B): 0.2 Mol/L H_2SO_4 & NaCl; (C): 0.5 Mol/L H_2SO_4 & NaCl.

of acid swelling. The fiber bundles were separated and accompanied with collagen hydrolysis. When the neutral salt was added into the acid solution, with increasing salt concentration, the diameter of the collagen fiber became smaller and the distance among the fiber bundles also became closer (Fig. 4B, 4C). Fibers in the papillary layer showed compactly appearance, while the concentration of the salt raise up to 1.0 Mol/L, the linkage region between the papillary and the reticular layer were compacted adjacently, which means that the swelling of the hide was reduced completely. With the same concentration of neutral salt, the higher concentration of the acid was, the better separation of collagen fiber would be.

The acid relaxed collagen fiber was treated with 0.1Mol/L H_2SO_4 solutions, the unsolved fibers were observed by SEM and TEM (Fig. 5). The SEM photos showed that the unsolved collagen fiber still maintained the fibrous structure perfectly, with evenly fiber diameter and smoothly surface, which was different to collagen fiber in alkali solutions¹⁹. The lower magnify pictures showed that the morphology of the fiber in acid solution with salt free has a large swelling tendency, and

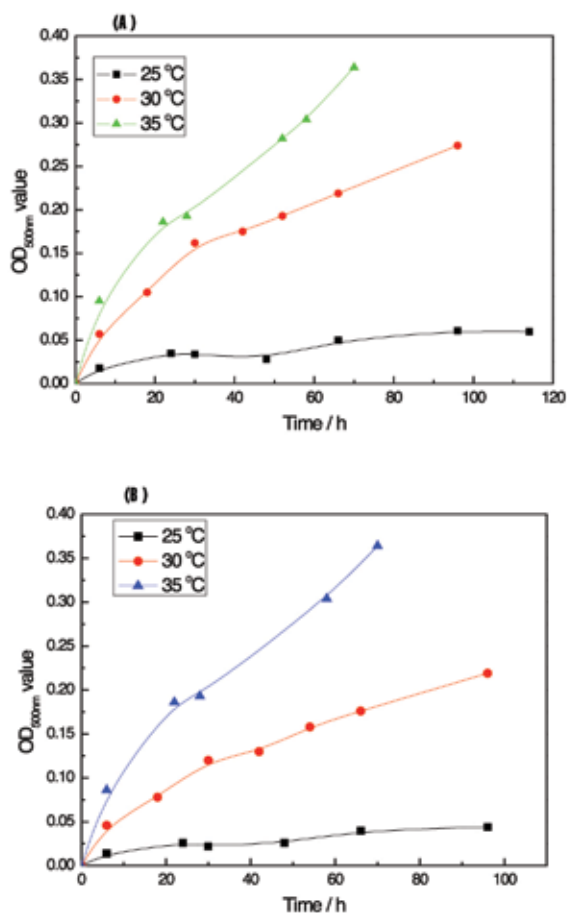


Figure 3. Hydrolysis profiles of collagen fiber in H_2SO_4 & neutral salt solutions at different temperatures. (A) 0.1Mol/L H_2SO_4 - 0.1Mol/L NaCl; (B) 0.1Mol/L H_2SO_4 - 0.1Mol/L Na_2SO_4 .

the fiber was deformed and bended, suggesting the inner stress of osmotic pressure. This kind of deformation may influence the mechanical properties of the finished leather. The fiber treated with typical pickling process showed that the morphology of the fibers was not deformed and thus no inner stress existed. In turn, the swelling reduce was in consistence to the mechanical properties of the leather. The TEM graph of the unsolved fiber in 0.1 Mol/L H₂SO₄ showed clearly typical collagen pattern D-periodic bands, and the diameter of the fibril was the same as the original collagen fibrils. These results indicated that the swelling of the hide in acidic solution would not affect the scales inside the fibril. The fibril structure of hide collagen was packed by paralleling 1/4 staggered to a dense crystal structure, water molecules may be difficult to penetrate inside it.

The hierarchical structures of the tanned samples were investigated by optical microscope and TEM (Fig. 6). The graphs showed that both kinds of pickling method have different morphology pattern. The fiber bundles of the sample for pickling with salt free were separated dramatically. The distance between the fibrils was dispersed widely, and the fibrils were distorted along the axial direction. Furthermore, it should be noted that the diameter of the fibril was unchanged, which implied that the swelling was only occurred at the fiber bundle and the fibril scales. For typical pickling process, the fiber bundles were arranged strictly, the distance of the fibrils showed no obvious change. These results also suggested the reason why the mechanical properties of the leather pickled with salt free were worse than the leather by typical pickling.

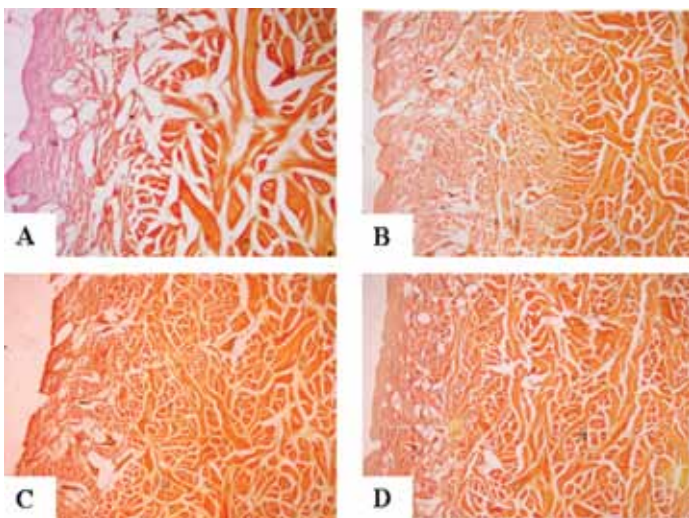


Figure 4. Histological graphs of cattle hide pickled in different salt concentration. The bovine hide was treated in 0.1mol/L H₂SO₄ with various concentration of NaCl at 25°C for 10h, after that, the samples were fixed with 4% formaldehyde for histological investigations. A: 0.0mol/L NaCl; B: 0.1mol/L NaCl; C: 0.5Mol/L; D: 1.0mol/L NaCl.

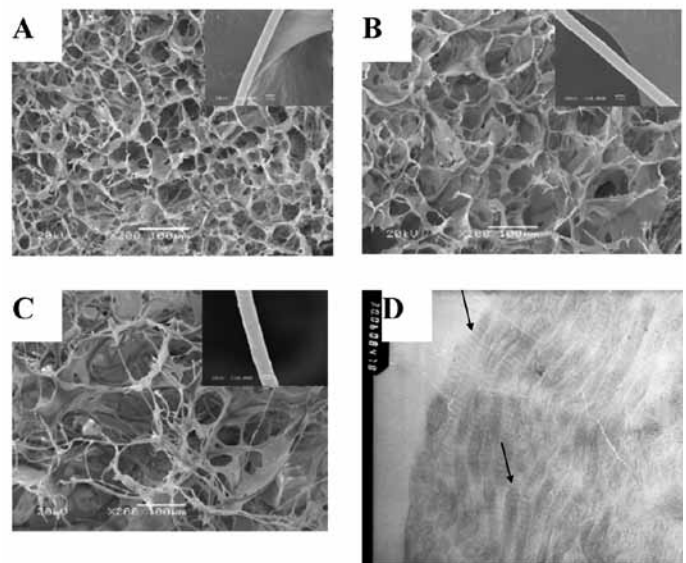


Figure 5. SEM & TEM pictures of collagen fiber treated in H₂SO₄ solutions with various Conc. of NaCl. The acid relaxed collagen fiber was treated in 0.1mol/L H₂SO₄ solutions with various concentration of NaCl at 25°C for 10h, the unsolved collagen fiber was freeze dried, then investigated by SEM(A, B, C) and TEM (D).,A: 0 Mol/L NaCl; B: 0.1 Mol/L NaCl; C: 1.0 Mol/L NaCl; D: 0Mol/L NaCl.

TABLE I
Properties of leather samples pickled in different salt concentrations.

Salt Conc. (mol/L)	Ts (°C)	Tensile strength (MPa)	Tear strength (N/mm)	Elongation at break (%)	Thickness swelling/%	Weight swelling /%	Cr ₂ O ₃ (%)
0	94.6	8.38	11.73	70.19	80.4	44.0	5.47
1.0	95.2	15.64	24.12	81.85	13.7	0.0	4.95

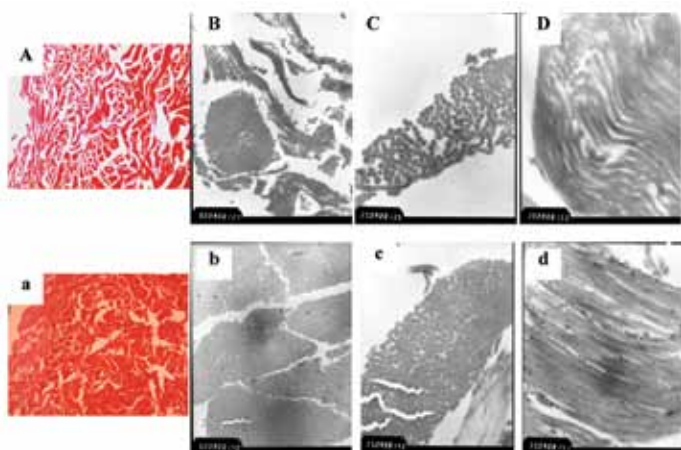


Figure 6. The optical microscope and TEM investigations of bovine hide pickled with typical pickling process (a, b, c, d) and without salt (A, B, C, D).

CONCLUSIONS

We examined the type and the concentration of neutral salt, the concentration of sulfuric acid and the temperature to the hydrolysis of collagen fiber in acid and neutral salt solutions. Sodium sulfate showed better inhibiting hydrolysis effect to collagen fiber than sodium chloride. More collagen would be hydrolyzed into the acid solution at the higher temperature even the concentrated neutral salt was added. The structures at the fiber scale were separated dramatically in acid solutions with salt free or with low concentration of salt since the osmotic stress among the collagen fiber was significant. While the concentration of sodium chloride was at 1.0 Mol/L, the fiber bundles would be packed closely and the swelling would be totally reduced. The results implied that the osmotic swelling by acid could not reach the inner scale of fibril. The mechanical properties were decreased for the tanned leather with salt free pickling, probably caused by osmotic swelling which destroyed the interactions among the fibril and fiber bundles.

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