# Study on the Microstructure of Crocodylus Niloticus Skins During Leather Making Process

by
Qiang Tao-Tao[1,2*] and Han Mi-Mi[1,2]
[1]*College of bioresources chemical and materials engineering, Shaanxi University of Science & Technology,*
Xi'an, Shaanxi, P. R. China 710021
[2]*Key laboratory of leather cleaner production, China national light industry, Ministry of Education, Shaanxi University of Science & Technology,*
Xi'an 710021, China

## Abstract

The variation on the microstructure of *Crocodylus niloticus* skins in the tanning process was observed and analyzed by using frozen section staining, which provide the reliable theoretical basis for *Crocodylus niloticus* leather production. The research on aldehyde fuchshin staining method showed that elastic fiber will receive a lot of damage in the bating and bleaching process. Through the observation of wet blue leather by scanning electron microscopy, the preparation work section and tanning process design are reasonable, also, inter-fibrillary substances had been well removed. The crosscut microstructure of *Crocodylus niloticus* hides before and after fatliquoring was observed by nile blue sulphate staining method, and the results showed that fatliquor could penetrate into the skin uniformly.

## Introduction

The analysis of the histology of leather mainly originated from biological tissue analysis of medicine and biology, usually by means of electron microscope and optical microscope.[1-8] Through sampling, fixing, sectioning, staining, light microscope and electron microscope, the histology of raw hides, semi-finished products and resultant leather was analyzed, which provided a reliable theoretical basis for the leather making.[9] With the improvement of scientific productivity and the lack of raw hides resources, using the optical microscopy technology to see through leather in depth, so the leather industry can be closer to leather making according to the properties of pelts, moreover, the energy-saving and emission-reduction and cleaner production are also achieved. Besides, optical microscopy technology is also a magic weapon to identify the quality of leather products.

Since 1970s, the histological and technical study on a variety of skins have been developed in succession. Liu Linling *et al.*[10] studied the histology of three kinds of mink skins by paraffin section staining. Xiao Shiwei *et al.*[11] analyzed the histology characteristics of Chengdu sheepskin by freezing section staining. Sun Danhong *et al.*[12] researched the histology of cautiflower snake skins by optical microscope. Internationally, crocodylus niloticus leather and other special leather have become an important part of the leather industry,[13] however, little research has been done on the microstructure of the tanning process, and it has no uniform or mainstream view. As a result, it brings a great inconvenience for studying crocodile leather. Therefore, in order to study the histology of crocodylus niloticus skins systematically, based on the acquaintance of histology of crocodylus niloticus skins, we can quantify appropriately processing materials and solve the thorny problems that may be encountered in the processing, which is conducive to develop high-grade crocodylus niloticus leather products more suited to market demand.

In this study, the trichrome-Sudan IV staining method, aldehyde-fuchsin staining method, nile blue sulphate staining methods and scanning electron microscope were used to study the variations of histology of *Crocodylus niloticus*, which provides a theoretical basis for the leather tanning process.

## Experimental

### Materials

*Crocodylus niloticus* wet salted skins, industrial products, were provided by Guangzhou Longze Leather Co., Ltd; acid fuchsin, AR, was supplied by Beijing procurement and supply station of China Pharmaceutical Company; Orange G, AR, was obtained

*Corresponding author e-mail: qiangtt515@163.com.
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by Sinopharm Chemical Reagent Co., Ltd; Molybdophosphoric Acid, AR, was supplied by Shanghai Chemical Reagent Company, Chinese Academy of Medical Sciences; Aniline blue, AR, was provided by Shanghai specimen model factory; Absolute alcohol, AR, was supplied by Tianjin Fu Yu Fine Chemical Co., Ltd; Sudan IV, AR, was obtained by Beijing 57601 organic chemical factory; Acetone, AR, was provided by Tianjin Fu Yu Fine Chemical Co., Ltd; Arabia gum, AR, was supplied by Tianjin Tianli Chemical Reagent Co., Ltd; Basic fuchsin, AR, was obtained by Shanghai Reagent No.3 factory; Concentrated hydrochloric acid, AR, was supplied by Kaifeng Dongda Chemical Co., Ltd; Potassium permanganate, AR, was provided by Wuqiao Xinghua Pharmaceutical Co., Ltd; Oxalic acid, AR, was supplied by Tianjin Hengxing Chemical Reagent Co. Ltd; Glacial acetic acid, AR, was obtained by Tianjin Fu Yu Fine Chemical Co., Ltd. Nile blue sulphate, AR, was supplied by Shanghai leap Biotechnology Co., Ltd.

**Instruments**

Electronic balance, LT1001, Metter Toledo group; Fast freezing and paraffin section machine, HH-3658III, Jinhua City, Hua Hai teaching instruments factory; Microscope, UB100i, Chongqing Ao Pu Photoelectric Technology Co., Ltd; Optical Light microscope, DM2500M, Leica micro system (Shanghai) Trading Co., Ltd; Scanning electron microscope, S-4500, Japan Hitachi company.

**Leather Making Process**

Fleshing → group batching → soaking → degreasing, pre-liming → liming → brushing scales → deliming → combined bleaching → bating → shaving oil film → combined bleaching → degreasing → pickling → tanning → shaving → degreasing → neutralizing → retanning→ dyeing, fatliquoring → nailing → buffing → shaving → finishing.

**Sampling**

The both sides of the belly midline of belly tail, were chosen as the observation sampling site for histology. Sample site is shown in Figure 1; sample size is 5cm by 5cm. Samples were frozen for conservation after labeling and fixed separately after finishing all processes and sampling.

Figure 1. Sampling site for histology examination.

**Observation on Microstructure of Preparation and Tanning Section**

After soaking, degreasing, liming, deliming, bating, bleaching and tanning, the *Crocodylus niloticus* skin was sampled, sliced and dyed. The specific operation methods of Sectioning operation, Trichrome-Sudan IV staining method and Aldehyde-fuchsin staining method were referred to the study of Qiang.[14] The Nile blue sulphate staining method is as follows.

*Nile Blue Sulphate Staining Method:*

Preparation of 1% nile blue sulphate dye liquor: 1g nile blue sulphate wasdissolved in 100mL distilled water, then filtered before using.

Staining operation was similar to trichrome-sudan IV staining method, and the whole staining process was finished in the oven whose temperature was 62°C, specific staining steps were as follows:

Section washing→1% nile blue sulphate liquor for 10min, 60°C→quick washing with 60°C distilled water for 2 times→1% acetic acid aqueous solution for color separation 40s, 60°C→washing with distilled water for 3 times→Arabia gum sealing.

**Optical Light Microscope Observation**

After staining, sealing with one drop of Arabia gum, then covering with a cover glass, samples were placed on the microscope observation platform with objective multiples 4, 10, 20, 40, 100 times.

**Scanning Electron Microscope Observation**

The samples were dried at 50°C and spraying time of 30 s, then the *Crocodylus niloticus* raw hides and chrome tanning samples were analyzed by field emission scanning electron microscope.

## Results and Discussion

**The Variation of Histology at Preparation Stage**

Figure 2 is the histological structure of the *Crocodylus niloticus* skins after soaking and degreasing. As shown in Figure 2a, adipose layer and subcutaneous tissue layer have been removed, and compared with the raw materials under the same magnification, it was found that there are many gaps in the collagen fibers bundles of the reticular layer after soaking. It was proved that the raw material has been restored to the state of fresh skins and the part of inter-fibrillary substance has been removed after soaking.

It can be seen from Figure 2b and 2c, the epidermal layer is closely connected with the scale layer and is connected with the grain layer by unequal spacing fiber bundles. Owing to the difference between the dense scale layer and the grain layer, the

addition of 2% sodium sulfide in the degreasing process made the epidermal layer be affected by force and stretch the fiber bundles connecting the grain layer. Therefore, in the tanning process, reducing the mechanical strength should be paid more attention, especially for the tanning process which the collagen fibers were fixed, otherwise, the epidermal layer of resultant leather will produce a small piping or even bubble, grain crack.

Figure 3 is the histological structure of the *Crocodylus niloticus* skins after liming and bleaching. It can be seen from Figure 3a and 3b, the scale layer has been completely removed after liming, the grain layer and the epidermal layer still show a dense dark blue. There is a uniform gap in the collagen bundles of the reticular layer, and the lowest part of the reticular layer still has some red fats. The results showed that liming process can effectively remove the scale layer, and scales in the groove of the two adjacent scutellum will be completely removed. And then, the uniform expansion of collagen fiber bundles even proved that the process parameters of liming process are correct. After soaking, degreasing and liming, there is still a small amount of fat in the flesh side, and the degreasing process should also be strengthened in the subsequent processes.

As shown in Figure 3c and 3d, after bating and bleaching, the epidermal layer in the groove of the two adjacent scutellum became smoother under the action of enzyme, and no red fats existed in the flesh side. The results indicated that the impurities on the surface of the skin and the fats of the flesh side were further removed.

**The Variation of Elastic Fibers at Preparation Stage**

Figure 4 is the change in the elastic fibers of the *Crocodylus niloticus* skins during the preparation process, the background color is light purple, the elastic fiber is dark purple. It can be seen from Figure 4a, 4b and 4c, the elastic fibers were surrounded by the collagen fiber bundles, and the grain layer was deep purple and dense. In the reticular layer, the color of fiber bundles parallel to the cross-section was darker, and the dark purple elastic fibers were not reduced after soaking, degreasing and liming. As shown in Figure 4d, the dark purple elastic fibers in the grain surface significantly reduced, in the reticular layer, the elastic fibers around the collagen bundles were also significantly reduced. The results showed that the damaging effect of soaking, degreasing and liming on the elastic fibers was small, but the damaging effect of enzyme bating and bleaching on the elastic fibers was very obvious. Therefore, the damaging extent on elastic fibers can be adjusted according to the performance requirements of resultant leather, that is, adjusting the time of enzyme bating and bleaching.

**The Histology of Blue Wet Skins of *Crocodylus Niloticus***

There is more inter-fibrillary substance in the raw materials of *Crocodylus niloticus*, and the waving of collagen fibers is also

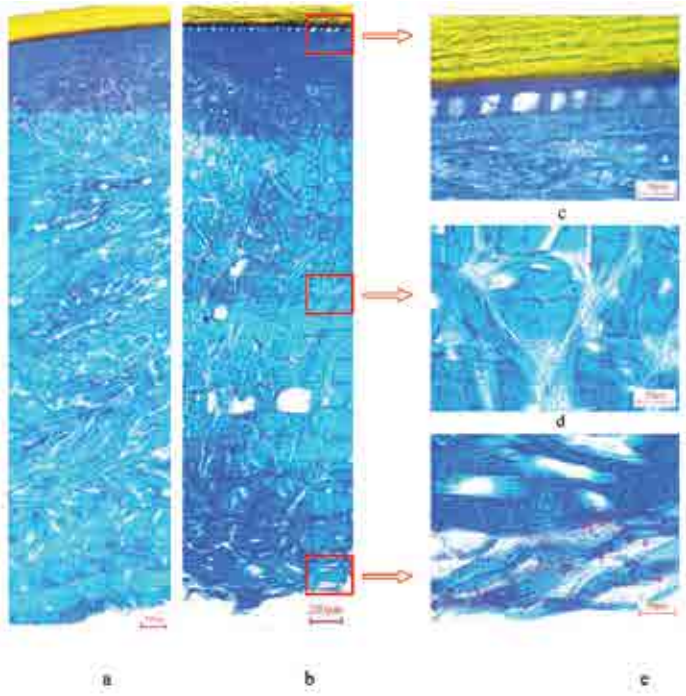


Figure 2. The crosscut microstructure of *Crocodylus niloticus* hides after soaking and degreasing (Trichrome-Sudan IV staining method.) a: after soaking, ×10 b: after degreasing,×10 cd and e:×40

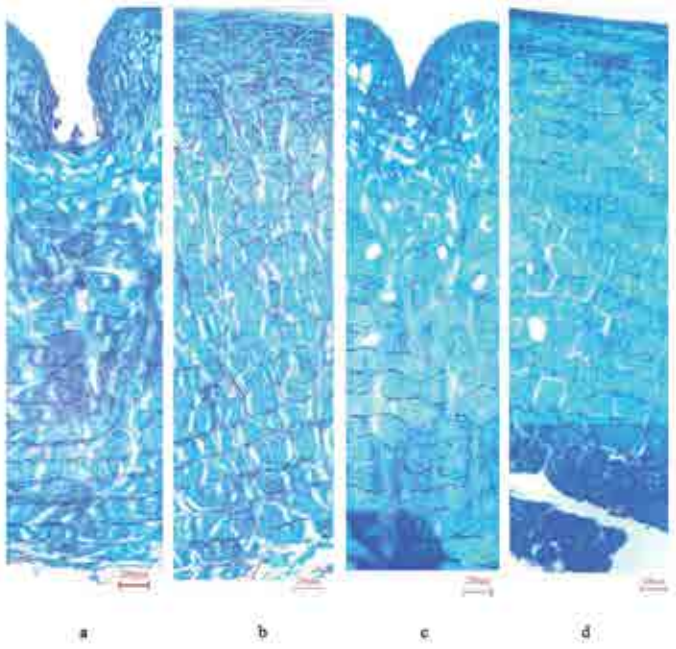


Figure 3. The microstructure of *Crocodylus niloticus* hides after liming and bleaching (Trichrome-Sudan IV staining method, ×10.) a and b: after liming c and d: after bleaching

dense, so whether the inter-fibrillary substance and fat can be removed totally or not is an important index of reasonable leather making technology. Therefore, the fibers of wet blue skins were observed by scanning electron microscope under higher magnification to determine whether the inter-fibrillary substance was removed, as shown in Figure 5.

Figure 5 is the scanning electron microscope diagram of blue wet skins of *Crocodylus niloticus*, it can be seen from Figure 5a, after the disposal of the preparation stage and tanning stage, it is clear to distinguish the crossing and waving collagen fiber bundles. As shown in Figure 5b, the inter-fibrillary substance around collagen fibers had been removed completely and the fiber bundles could be seen clearly. The results showed that the inter-fibrillary substance was removed well, and the design of tanning process was reasonable after the disposal of the preparation stage and tanning stage.

**The Variation of Histology Before and After Fatliquoring**

Nile Blue sulphate staining method is a common method for visualization of fatliquor in the resultant leather and fur. It can display fatty acids and neutral fats, after staining, the background is light blue, the fatty acids are dark blue and neutral fats are red. The *Crocodylus niloticus* skins are more compact, and whether the fatliquor enters smoothly and distributes evenly in the middle of hides or not is the necessary condition of successful fatliquoring and the key to soft extent of resultant leather. The crosscut microstructure of *Crocodylus niloticus* hides before and after fatliquoring is shown in Figure 6.

What can be seen from Figure 6a is that both the grain side and flesh side were dark blue, the middle part displayed light blue. As shown in Figure 6b, the dark blue color of the grain side and flesh side deepened, the middle part was blue, and the purple of uniform distribution was also displayed. A large number of carboxyl and phenolic hydroxyl groups of vegetable tannin

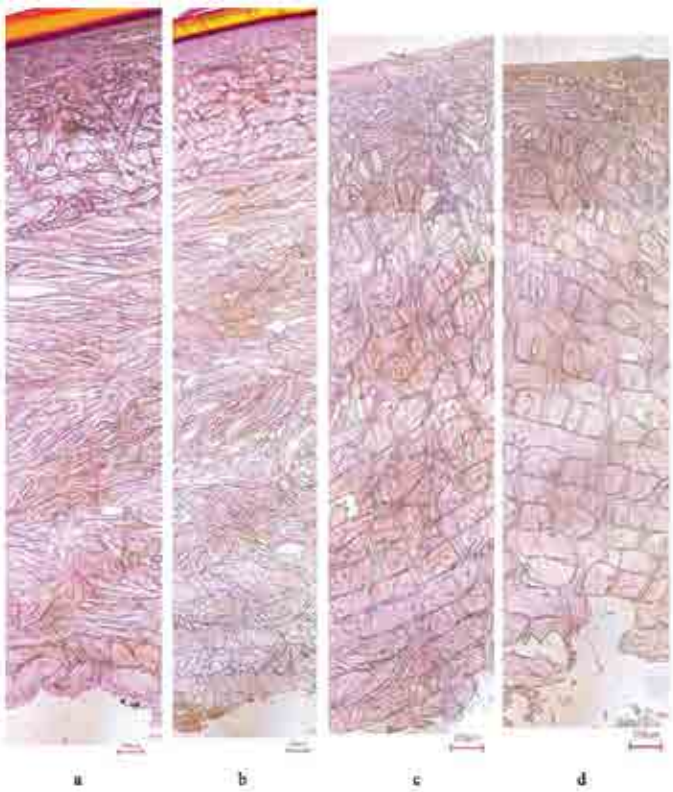


Figure 4. The crosscut microstructure of *Crocodylus niloticus* hides in beamhouse (aldehyde fuchshin staining method, ×10.)
a: after soaking b: after degreasing c: after liming d: after bleaching

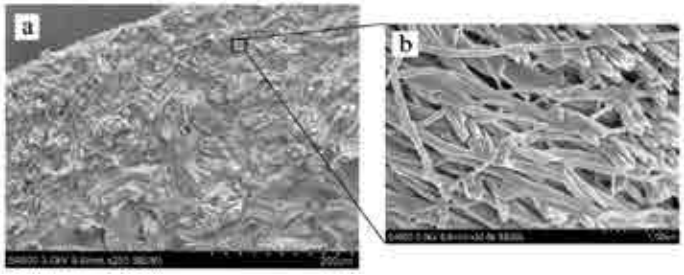


Figure 5. The structure of chrome tanned *Crocodylus niloticus* leather. (a:×250 b:×30000)

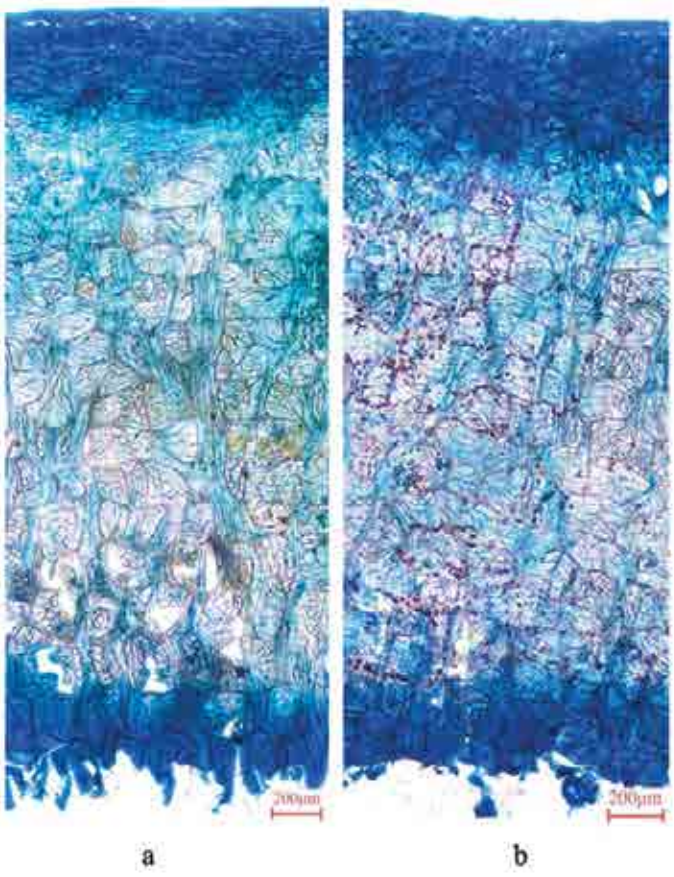


Figure 6. The crosscut microstructure of *Crocodylus niloticus* hides before and after fatliquoring.
(Nile Blue sulphate staining method, ×10)

extracts can combine with Nile blue sulphate, and fat revealed blue when combined with Nile blue sulphate, so the blue color of the grain side and flesh side deepened. In the pelt, the background was light blue, the fatty acid was blue and neutral fat was purple. Also, the distribution of the upper, middle and bottom parts of the pelt was more uniform, which showed that the greasing process and the mechanical action were reasonable, and the fatliquor could distribute uniformly. In addition, as shown in Figure 6a, vegetable tannin extracts couldn't penetrate into the pelt completely and the depth of penetrating into the grain side was deeper than that of flesh side, which indicated that the fiber waving of grain side was looser than that of the reticular layer.

## Conclusions

The microstructure of pelt in the leather making was observed and analyzed by trichrome-Sudan IV staining method, aldehyde-Fuchshin staining method, nile blue sulphate staining method and scanning electron microscope. The conclusions are as follows.

(1) The research on aldehyde-Fuchshin staining method showed that elastic fiber will be a lot of damage in the bating and bleaching process.

(2) Through the observation of wet blue leather by scanning electron microscopy, the preparation work section and tanning process design were reasonable, also, the inter-fibrillary substances had been removed well.

(3) The crosscut microstructure of *Crocodylus niloticus* hides before and after fatliquoring was observed by nile blue sulphate staining method, and the results showed that fatliquor could penetrate into the skin uniformly.

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