Influence of Microbial Transglutaminase Modified Gelatin-sodium Caseinate, as a Filler, on the Subjective Mechanical and Structural Properties of Leather

by

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Abstract

This work investigated the influence of filling process, using gelatin-sodium caseinate modified by microbial transglutaminase (MTG) on the subjective, mechanical, and structural properties of leather. The results indicate that in comparison with the control (without filling process), the MTG modified gelatin-sodium caseinate as the fillers improved the subjective properties such as grain tightness, grain smoothness, and fullness, but did not significantly affected the mechanical properties such as tensile strength, elongation at break, and tongue tear. The scanning electron microscopic analysis indicates that the microstructure of the leather treated with the fillers became more regular and tighter compared with the control. The results obtained here may be useful for the application of MTG in the leather processing in improving certain subjective and structural properties of leather.

Resumen

Este trabajo investigó la influencia del proceso de "relleno", utilizando gelatina-caseinato de sodio modificado por transglutaminasa microbiana (MTG) en las propiedades subjetivas, mecánicas y estructurales del cuero. Los resultados indican que en comparación con la muestra de control (sin proceso de relleno), la gelatina-caseinato de sodio modificada por MTG como rellenante mejoró las propiedades subjetivas tales la firmeza de flor, la lisura de la flor, y la plenitud, pero no afectó significativamente las propiedades mecánicas, tales como la resistencia a la tracción, la elongación a la rotura, y al desgarro. El análisis microscópico de barrido electrónico indica que la micro-estructura de la piel tratada con el relleno se hizo más regular y más compacta con respecto al testigo. Los resultados obtenidos aquí pueden ser útiles para la aplicación de MTG en el tratamiento del cuero en la mejora de ciertas propiedades subjetivas y estructurales del cuero.

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Introduction

After tanning, the leather is retanned, dyed, fat-liquored and may be further treated extended in special operations like filling to make a tough, old hide into a soft, full and supple material. In the filling process, fillers are introduced into the voids that exist between the fibers of the leather to smooth the veins or other irregularities on the leather surface.[1] Traditionally, various natural products like tannin, glucose, flour, and gum are used as fillers. Recently, synthetic polymers like acrylic resin, polyurethane, and amino resin are widely used in the tanneries. Enzymatically or chemically modified protein filler products have been developed recently.[2] With structure similar to that of leather fiber, the gelatin, casein, and whey proteins are highly compatible with the collagen fibres. Gelatines modified with glutaraldehyde were able to fill the leather and remained bound to the leather during washing steps.[3] However, glutaraldehyde is potentially toxic and its application has been limited. With this background, the enzymatically modified protein fillers have been receiving increasing interests.[4] Transglutaminase (Tgase, E.C.2.3.2.13) can catalyze the formation of intra- or inter-molecular ε-(γ-glutamyl)-lysine cross-linking via an acyl-transfer reaction and finds wide applications in food and wool industries.[5-13] There are many reports regarding the influence of microbial transglutaminase (MTG) modification on the physico-chemical properties of proteinaceous industrial byproducts such as gelatins, casein, and whey proteins.[14-22] Taylor et al. reported that proteinaceous byproducts like gelatin, casein and whey protein isolate (WPI) modified by MTG can be used as fillers in leather processing, and the use of theses fillers can significantly improve several subjective properties of the leather and reduce environmental pollutions.[23-26] However, the above filling process was conducted after a neutralization step, and there were no consideration of the influence on leather properties resulting from the filling operation, which was conducted after the other post-tanning processes such as retanning, dying, and fat-liquoring. Finally, to investigate whether the chromium used in tanning of hides may interfere with the activity of the MTG, Taylor et al. prepared gelatin solution containing chromium and treated the solution, as well as a control with no chromium, with MTG, and it was found that the physical properties of gelatin were similar. They reported that the chromium used to tan the hides does not affect the activity of MTG.[27]

In this work, the influence of filling operation on the subjective (grain, softness, fullness, and color), mechanical (tensile strength, elongation, tongue tear, and thickness), and structural properties of the leather was investigated. The gelatin and casein mixtures were used as protein fillers and MTG was used as the cross-linker. Scanning electron microscopic (SEM) analysis was conducted to examine the influence of filling process on the micro-structure of the leather. To the best of our knowledge, this is the first report regarding the influence of MTG treatment in post-tanning processes on the subjective, mechanical, and structural properties of the leather, and the results obtained here may be useful for the application of MTG in leather processing to improve some subjective and structural properties of leather.

Experimental

Leather material and enzyme

The wet blue stock supplied by Wuxi Lanxess Energizing Chemistry (China) was tanned by 7% Chromosal B (chrome sulfate with 33% alkaline and approximately 26% chrome oxide). Activa MTG (100 units/g) was obtained from our laboratory via microbial fermentation. Gelatin, sodium caseinate and other chemicals were all supplied by Lanxess Energizing Chemistry.

MTG modified gelatin-sodium caseinate preparation and characterization

Gelatin-sodium caseinate solutions (5% gelatin mixed with 0-5% sodium caseinate) were prepared by suspending the protein powders in deionized water and swollen for about 2 h at room temperature (RT), then stored overnight at 4ºC, and subsequently placed in a bath at 65ºC until dissolved. The pH was adjusted to 7.0-7.5 with 1 mol/l $NaHCO_3$. 5 U/g protein MTG was added to the gelatin-sodium caseinate solutions. The controls without MTG were also prepared. The reaction was carried out at 40ºC in a shaker bath for 4 h. The enzyme was inactivated at 90ºC for 10 min. Then all the samples were cooled to room temperature and chilled for 17h at 10ºC in a constant temperature bath. Gel strength of the samples was determined at last.

Application of MTG, gelatin-sodium caseinate, and MTG modified gelatin-sodium caseinate as the fillers in filling process

The pH of leather samples were adjusted to 6.5-7.0 during neutralization, and then the filling treatment A (treated with MTG), B (treated with MTG and gelatin-sodium caseinate), and C (treated with gelatin-sodium caseinate) were started. The leather processing without filling operation is referred as the control. The filling operation of neutralized blue stock was as follows: 5% MTG (based on the weight of wet blue stock, calculated to be 5 units/g wet blue stocks) and/ or 5% gelatin-sodium caseinate with 100% water was added to the test samples. 100% water alone was added to the control. All the samples were drummed for 1h at ambient temperature and then for 4 h at 40ºC. The pH was then adjusted to 3.0-3.5 with 3% formic acid. The floats were drained and the samples were washed twice for 10 min at 50ºC (200% float). As shown in Figure 1, Treatment A, B, and C were used to compare the effects of MTG, gelatin-sodium caseinate, and MTG modified gelatin-sodium caseinate on the subjective, mechanical, and structural properties of the leather. Eight pieces of blue stock

(2 pieces/drum, ~80g each) were placed in four Dose drums (Model 30036, Dose Maschinenbau GmbH, Germany) and were further washed twice with water (300%) by drumming each time for 30 min at 40ºC. The filled and control samples were placed in two Dose drums, and were retanned, colored and fat-liquored as shown in Figure 2.

Application of MTG modified gelatin-sodium caseinate as the fillers in the post-tanning processes

Figure 1 also shows treatment D, E, and F with MTG modified gelatin-sodium caseinate as the fillers in the post-tanning processes. Eight pieces of blue stock (2 pieces/drum, ~80g each) were placed in four Dose drums (Model 30036, Dose Maschinenbau GmbH, Germany) and were further washed twice with water (300%) by drumming each time for 30 min at 40ºC. In treatment D, the filling operation with MTG modified gelatin-sodium caseinate as the fillers was conducted after neutralization, and in Treatments E and F, the filling operation with the same fillers as in Treatment D was conducted after coloring and after fat-liquoring, respectively. The filling operation in treatment D, E, and F was the similar as treatment A, B, and C.

When completed, all the samples were dehydrated by the Vacuum Drying Machine (Model SVIE, Officine Di Cartigliano S.P.A, Italy), and then left to dry at ambient temperature and humidity, staked twice by a Staking Machine (Model PAL BJ 1600, Officine Di Cartigliano S.P.A, Italy) and milled for approximately 24 h.

Gel strength determination

Gel strength was measured on a TA.XTplus Texture Analyzer (Stable Micro Systems, Surrey, UK) as described by Taylor.[19]

Increase in thickness (ΔT)

The thickness after the first neutralization step was firstly determined, and then the thickness after the filling process was determined. As described by Chen, the increase in thickness of leather samples was calculated using the following formula:

$$\Delta T(\%) = (T_{af} - T_{bf}) \times 100 / T_{bf} \qquad (1)$$

where T_{af} is the thickness after filling and T_{bf} is the thickness before filling.[3]

Subjective evaluation

The leather samples were treated as above, and then evaluated with respect to subjective properties including grain, softness, fullness and color. The value from 1 to 5 (1 representing the worst and 5 representing the best) was assigned for each parameter.

Mechanical properties measurement

According to ASTM D1610-01, the samples were stored in a conditioned room at 20°C and 65% RH. Thickness was measured according to GB 4689.4. Mechanical properties including tensile strength, elongation at 10N, elongation at break, and tongue tear were measured parallel to the backbone with a strain rate of 10 inches/min and a separation between the jaws of 4 inches (10 cm). The tensile strength is defined as the stress required to rupture the leather, elongation at 10N is defined as the strain with a load of 10N, and elongation at break is defined as the maximum strain at break. They were all determined according to ASTM D2209. The tongue tear, defined as the load at which the initial tear occurs in the sample, was determined according to ISO 3377-1. Four samples were run for each test of treated and untreated blue stock. The average was calculated, and from the mean and standard deviation (SD), error bars were determined.

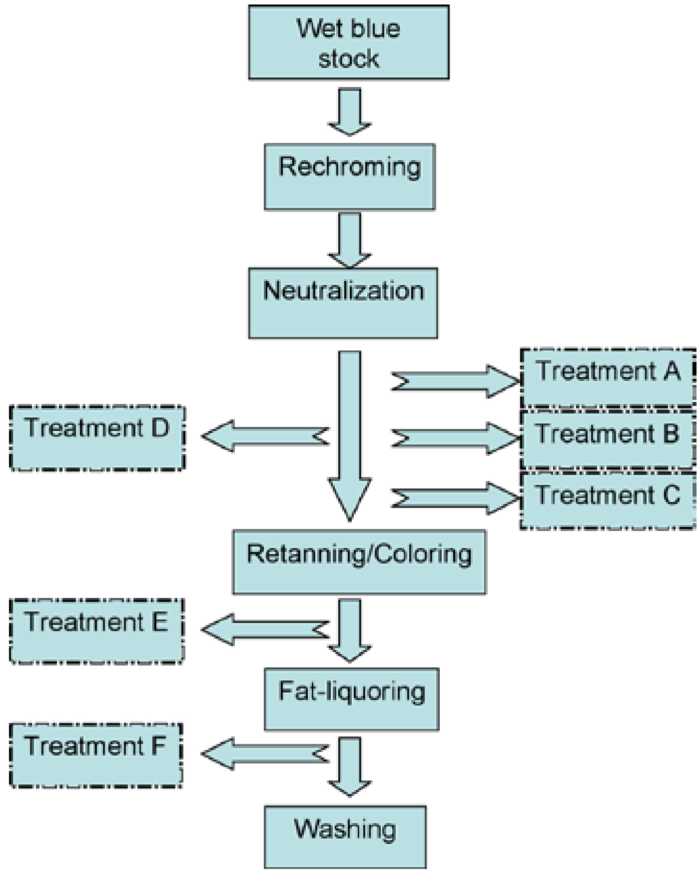

Figure 1: Schematic diagram for treatment, sampling, analysis of blue stock.

Scanning electron microscopic (SEM) analysis

The samples were cut into small strips and freeze-dried. Five pieces were cut from each of the dry samples and mounted onto the surfaces of carbon adhesive tabs. The samples were pretreated with silver and gold paints with a Scancoat Six Sputter coater according to the method described by Taylor et al.[25] Samples were scanned with a scanning electron microscope (Quanta-200, FEI, Holland) at 5kV. Digital images were collected at 600 × magnification.

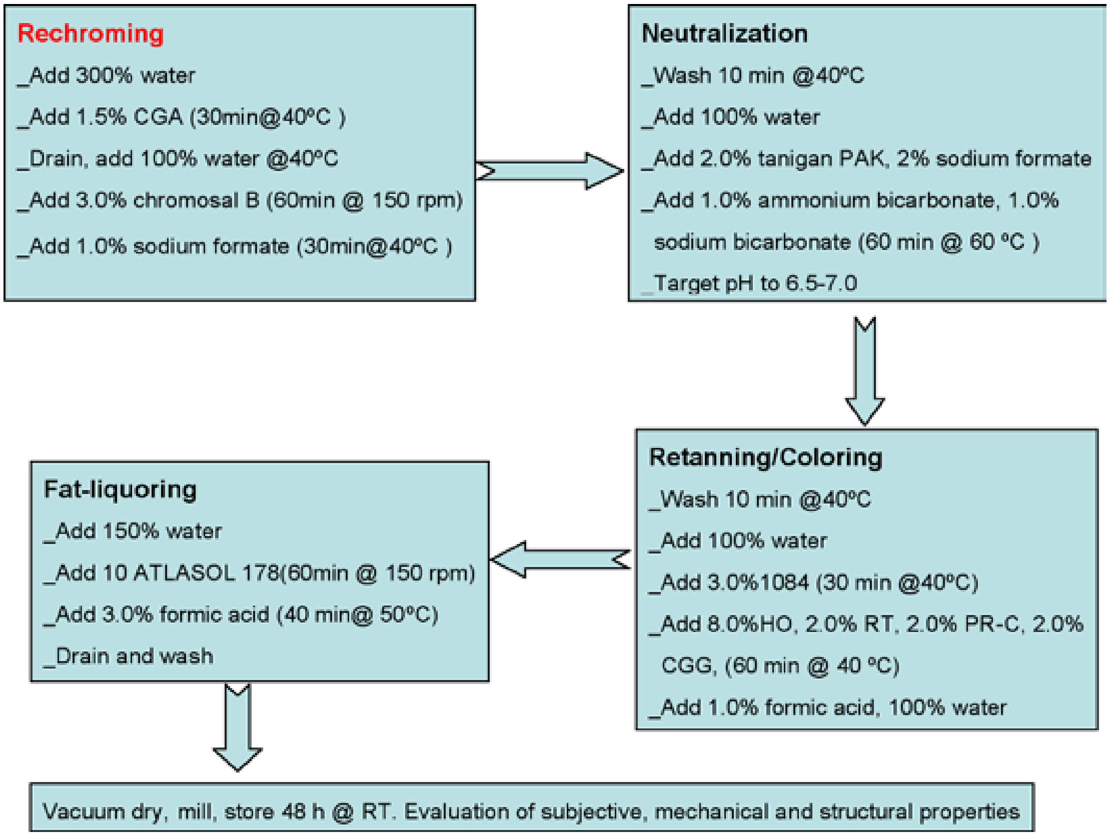

Figure 2: Flow diagram for retanning, coloring and fat-liquoring of control and treated samples of blue stock.

RESULTS AND DISCUSSION

Influence of MTG on the gel strength of gelatin-sodium caseinate mixtures

As shown in Figure 3, compared with the control, the gel strength of gelatin-sodium caseinate treated with MTG increased, especially when the concentration of sodium caseinate was higher than 2%.

Influence of MTG, gelatin/ sodium caseinate, and MTG modified gelatin-sodium caseinate as the fillers on the subjective properties of leather

The influence of filling process on the subjective properties of the leather was evaluated with respect to grain, softness, fullness, and color rated on a scale of 1 to 5, with 1 being the worst and 5 being the best. As shown in Table 1, the properties of the samples in Treatment A were similar to those of the control. The grain tightness and fullness of the samples in Treatment B and Treatment C were better than those of the control, and the fullness of the samples in treatment B were best. However, the softness of leather treated with filling operation was worse than that of the control. Table 2 shows the influence of filling operation with MTG modified gelatin-sodium caseinate as the filler in the post-tanning processes on the subjective properties of the leather. The results indicate that different treatments have different effects on the subjective properties of the leather. For Treatment D (the filling process conducted after neutralization), the grain tightness and fullness were improved in comparison with the control, and for Treatment E (the filling process conducted after coloring), the grain tightness and fullness were better than those of the control, and for Treatment F (the filling process conducted after fat-liquoring), the fullness, grain smoothness and evenness of dyeing were superior to those of the control. The color depth intensity of the samples by Treatments E and F was reduced compared with that of the control, possibly due to the protein fillers that combined with the collagen fibers and covered with the dyes.

Influence of MTG, gelatin/ sodium caseinate, and MTG modified gelatin-sodium caseinate as the fillers on the mechanical properties of leather

The increase in thickness (ΔT) of samples in treatment A, B, C and the control were separately 1.95±1.21%, 5.93±1.62%, 4.1±1.12%, and 2±1.57%. ΔT of the samples treated with MTG modified gelatin-sodium caseinate (treatment B) was the largest. Figure 4 and Figure 5 show the influence of different treatments on the mechanical properties (thickness, tensile strength, elongation at 10N, elongation at break, and tongue tear) of the leather. As shown in Figure 4, the elongation @

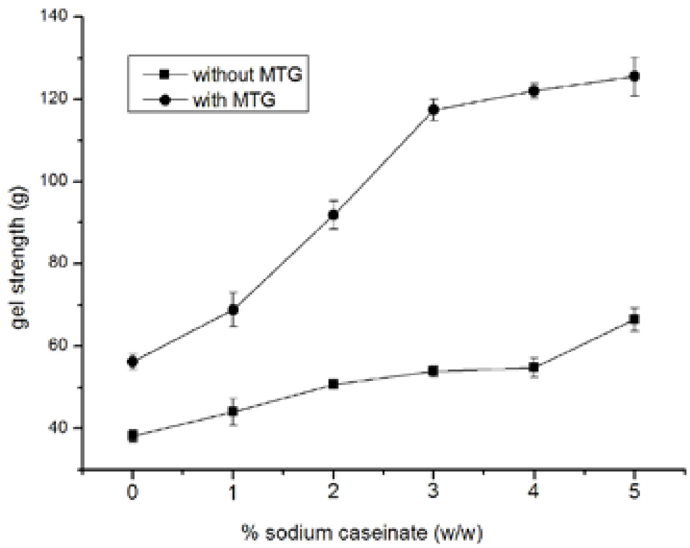

Figure 3: Gel strength of MTG modified protein matrices. Gelatin (5%), sodium caseinate (0, 1%, 2%, 3%, 4%, 5%), MTG (5U/g).

10N of the samples in treatment B and C was significantly decreased, while the other mechanical properties were almost the same as those of the control. Therefore, the addition of the protein fillers would not adversely affected the mechanical properties.

As shown in Figure 5, compared with the control, the thickness of the samples in treatments D and F was improved. The elongation @ 10N, and tongue tear were negatively affected, but the other mechanical properties of treatments D and F were similar with those of the control. There was no significantly difference between the treatment E and control in mechanical properties. Therefore, it seemed that the use of MTG modified gelatin-sodium caseinate as the fillers was not favorable for the improvement of mechanical properties in leather processing.

Influence of MTG, gelatin/ sodium caseinate, and MTG modified gelatin-sodium caseinate as the fillers on the structural properties of leather

The influence of filling process with MTG modified gelatin-sodium caseinate as the fillers on the structural properties of the leather was investigated by SEM analysis. Figure 6 (a-d) shows that the enzyme and protein have different actions on the collagen fibers. Compared with the control, the collagen fibres in samples cross-linked by MTG combined tightly and formed big bundles. Therefore, their structure was more open than that of the control. The diameter of fibres modified by gelatin-sodium caseinate mixtures appeared to be larger than that of the unmodified, maybe due to the proteins were cross-linked with the fibres. Figure 6 (e-h) shows the micrographs of the blue stock filled with MTG modified gelatin-sodium caseinate in the post-tanning processes, and the results

TABLE 1
Influence of different treatments on the subjective properties of leather[a]

Subjective Evaluation	Treatment A	Treatment B	Treatment C	Control
Grain tightness	2	4-5	4-5	2
Softness	3	1	2	4
Fullness	3	5	4	2

[a] Scale 1-5, 1=worst, 5=best.

TABLE 2
Influence of different treatments on the subjective properties of leather[a]

Subjective Evaluation	Treatment D	Treatment E	Treatment F	Control
Grain tightness	3-4	3	3	2
Grain smoothness	3	3	4-5	3-4
Softness	1	2-3	1-2	3-4
Fullness	4	3	4	2
Levelness of dyeing	2-3	3	3-4	3
Deepness of color	3-4	1-2	2	3

[a] Scale 1-5, 1=worst, 5=best.

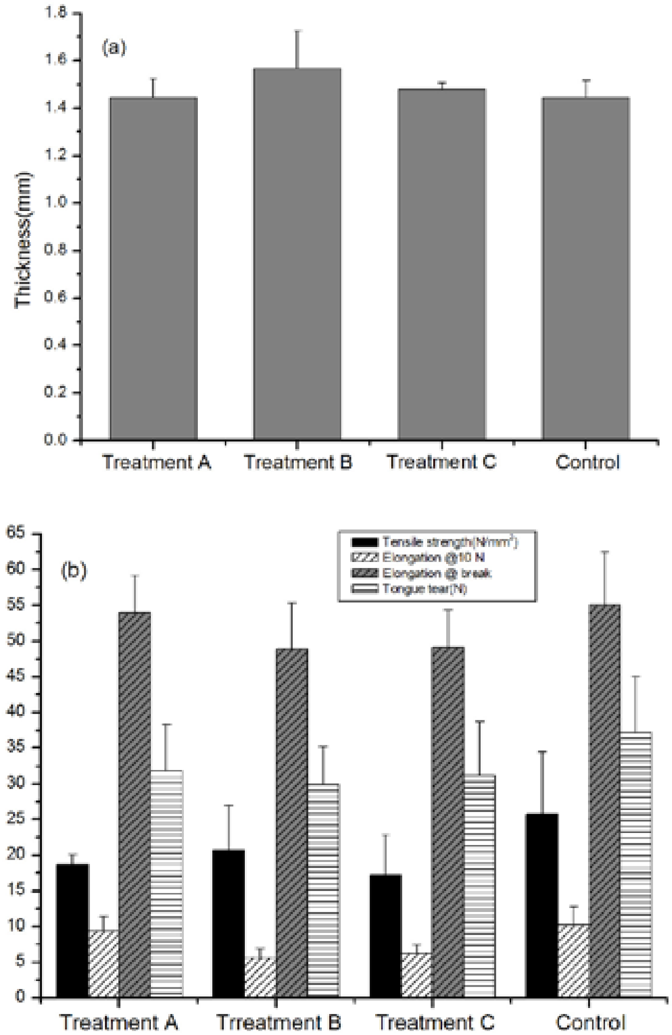

Figure 4: Influence of MTG, gelatin-sodium caseinate, and MTG modified gelatin-sodium caseinate as the fillers on the mechanical properties of leather

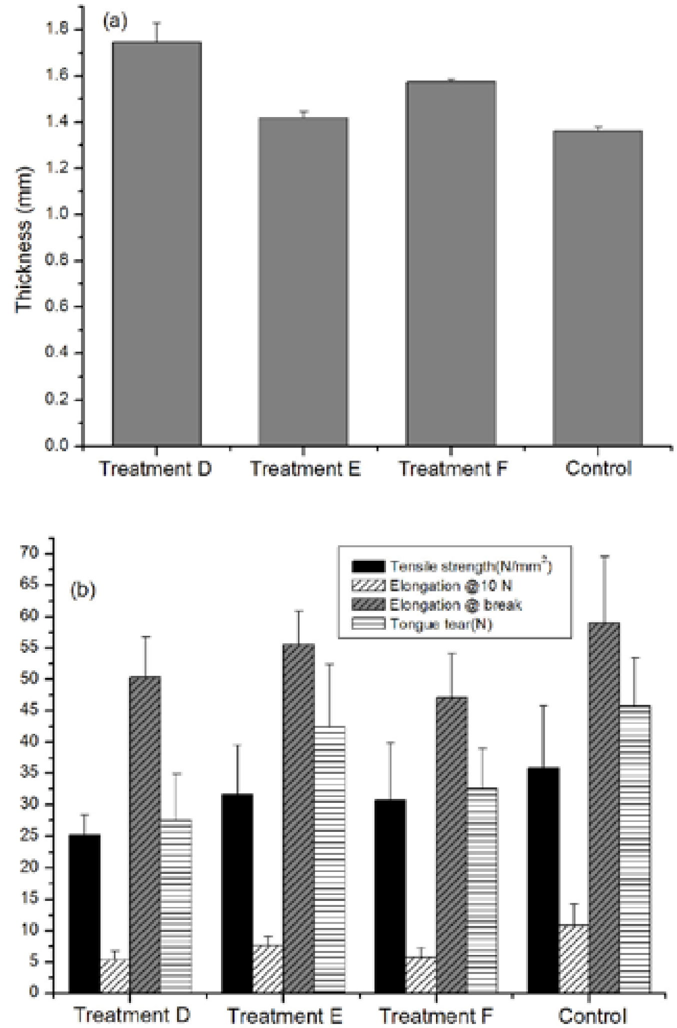

Figure 5: Influence of MTG modified gelatin-sodium caseinate as the fillers on the mechanical properties of leather

indicate that the addition of fillers had a significant influence on the micro-structure of the leather. The structure of the filled leather was more regular and tighter than that of the control. Particularly, the fibers of the samples filled after coloring (Figures 6(f)) and fat-liquoring (Figures 6(g)) were cross-linked most tightly and formed a blocky structure. Agents added in the retanning, coloring or fat-liquoring processes may play a function of a surfactant, which made the enzyme and proteins distribute into the inside of the stock more easily and enhanced the cross-linking degree between the proteins and fibers.

CONCLUSIONS

The influence of filling process with MTG modified gelatin-sodium caseinate as fillers on the subjective, mechanical, and structural properties of the leather was investigated. Generally speaking, the use of MTG modified gelatin-sodium caseinate as the fillers was favorable for the improvement of thickness and subjective properties like grain tightness, fullness, and grain smoothness. Most part of the mechanical properties was nearly not negatively affected by the addition of gelatin-sodium caseinate fillers modified by MTG, except for the elongation @ 10N of the samples in treatment B, C, D, and F. The SEM analysis indicated that the micro-structure of the filled sample was more regular and tighter than that of the control without filling treatment. The results obtained here may be useful for the application of MTG modified protein fillers in the post-tanning processes to produce the leather with superior subjective and structural properties.

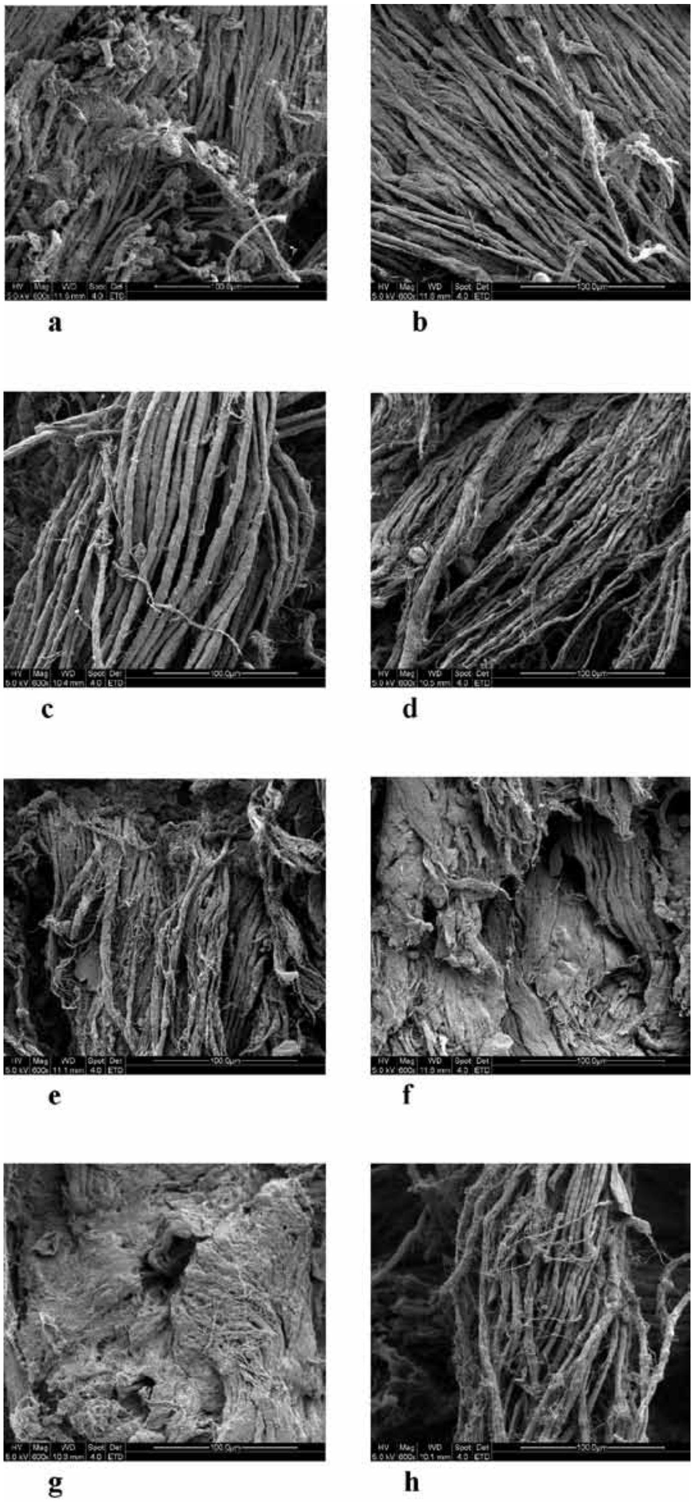

Figure 6: SEM of treated samples in 600 × magnification. a-d: treatment A, B,C, and control. e-h: treatment D, E, F, and control.

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