Characterization of Gelatin and Casein Films Modified by Microbial Transglutaminase and the Application as Coating Agents in Leather Finishing

by

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Abstract

This work aims to characterize the mechanical and water-resistant properties of gelatin-casein (GC) films modified by microbial transglutaminase (MTG), and to further evaluate the potential application of the mixture of the modified GC films and polyurethane (PU) as coating agents in leather finishing. MTG can improve the mechanical properties (tensile strength and elongation at break) of GC films at the ratios of 2:2, 1:3 and 0:4, and reduce the water absorption. With the increase of MTG concentrations, the elongation at break also increased, but the tensile strength did not change much. The largest elongation at break of the films treated with MTG was obtained at a concentration of 10 U/g-protein. With the increase of PU composition in the GC system, the mechanical properties and water resistance of the films were improved significantly. The PU-GC system was applied as coating agents in leather finishing. The results indicated that the PU-GC11 system (the ratio of gelatin to casein is 1:1) at a ratio of 2:2 (the ratio of PU to GC) was the best coating agent for leather finishing.

Resumen

Este trabajo tiene como objetivo caracterizar las propiedades mecánicas y de resistencia al agua de la película de caseina-gelatina (GC) modificada por la transglutaminasa microbiana (MTG), y para continuar evaluando la aplicación potencial de la mezcla de películas GC modificadas y de poliuretano (PU) como agentes de cobertura en el acabado del cuero. MTG puede mejorar las propiedades mecánicas (resistencia a la tracción y alargamiento a la rotura) de las películas de GC en las proporciones de 2:2, 1:3 y 0:4, y reducir la absorción de agua. Con el aumento de las concentraciones de MTG, el alargamiento a la rotura también aumenta, pero la resistencia a la tracción no cambia mucho. El mayor alargamiento a la rotura de las películas tratadas con MTG se obtuvo a una concentración de 10 U/g de proteína. Con el aumento de la composición de PU en el sistema GC, las propiedades mecánicas y de resistencia al agua de las películas se han mejorado de manera significativa. El sistema PU-GC se aplicó como agentes de cubrimiento en el acabado del cuero. Los resultados indicaron que el sistema PU-GC11 (la relación entre la gelatina y la caseína es 1:1) en una proporción de 2:2 (la relación entre PU y GC) fue el mejor agente de recubrimiento para el acabado del cuero.

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INTRODUCTION

Finishing is an important step in leather processing. The natural grain side of the leather usually appears irregular and thus the applications are restricted. With finishing, the leather could be more regular, beautiful, and durable.[1] The leather finishing agents consist of film-forming agents, colorants, auxiliaries and solvents. The film-forming agents like acrylic resin, polyurethane, nitrocellulose, and proteins are the main components of finishing agents. Casein, gelatin and plant proteins, particularly casein, are popularly used for their thermostability, anti-organic solvent and pressurization. The films of these proteins also can be polished to keep the natural grain, natural leather feeling, good air permeability and high water vapor permeability. However, usually the films have poor water resistance and wet rubbing fastness, low elongation and poor resist-twist.[1] At present, several methods that can modify the proteins to improve their properties and overcome the disadvantages such as poor film-forming and strong hydrophilic properties have been developed in processing.[2] For example, the water resistance, hardness, elongation and resist-twist of the protein film can be improved with the modification of acrylic resin.[3,4] With the utilization of polyurethane, the water resistance, resist-twist, plasticity, low temperature resistance and glossiness can be improved.[5] Other agents such as organic silica and melamine resin also have been used for the protein modification.[6] But the use of these chemical agents can cause heavy environmental pollution, and in the finishing processing, formaldehyde that is used to crosslink the proteins for the increase of wet and dry fastness is toxic.[7]

The microbial transglutaminase (MTGase, 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.[8] Many proteins such as gelatin, casein, soybean, whey protein isolate, gluten and egg white protein can be the substrates for MTGase. The work of Taylor et al. indicated that the mechanical properties and water resistance of gelatin-cast film modified by MTG can be improved.[9] The mechanical and water vapor permeability (WVP) of gelatin and casein films at different ratios treated with MTG were examined by Chambi et al.[10] Compared with the film formed by only gelatin or casein, the gelatin-casein film had greater elongation values. The gelatin: casein (25:75) formulation with MTG showed the lowest WVP value (5.06 ± 0.31 g mm/m^2 d kPa). When treated with MTG, the high molecular weight protein components in the film forming solutions were increased.[10] It was also indicated that the finishing properties of casein with MTG treatment were improved.[11]

In this work, the influence of MTG treatment on the mechanical properties (tensile strength and elongation at break) and water resistance (solubility and water absorption) of gelatin-casein (GC) and polyurethane-gelatin-casein (PU-GC) films was investigated. The influence of GC mixed with PU as coatings on the properties of leather, applied at different ratios in leather finishing, was also examined.

MATERIALS AND METHODS

Material

Leather material and enzyme

The dyed bovine leather was full grain shoe upper crust supplied by Wuxi Lanxess Energizing Chemistry (China). Activa MTG (100 units/g) was obtained from our lab via batch fermentation by *Streptomyces hygroscopicus* at 30ºC and pH 7.0. The agitation speed and aeration rate were controlled at 450 rpm and 1.0 vvm, respectively. The MTG product was obtained by treating the fermentation broth by ethanol precipitation, cation exchange resin (Fractogel EMD SO_3^-), and spray drying. Gelatin (G) (Type B from bovine skin, Bloom = 75±10, ash ≤ 2%, moisture content ≤ 14% on a dry weight basis, pH 5.0-7.0), casein (C) (ash ≤ 1.5%, moisture content ≤ 10%, total nitrogen = 14.5-15.5% on a dry weight basis, pH 3.0-5.0), polyurethane (PU) (water soluble polyurethane resin as the top coating agent) and other chemicals were all supplied by Lanxess Energizing Chemistry.

Methods

Preparation of MTG modified gelatin-casein (GC) and polyurethane-gelatin-casein (PU-GC) coatings

The solution of gelatin and casein were prepared at a concentration of 10% (w/v). The gelatin was suspended in deionized water and swollen for about 2 h at room temperature (RT), and then was stored overnight at 4ºC, and subsequently was placed in a bath at 65ºC until dissolved. The casein was dispersed in deionized water at room temperature and aqueous ammonia was added under constant magnetic stirring until the pH was at 7.5-8.0. Then the mixed solution was stirred and heated to 85ºC in a bath until dissolved. The glycerol (3%, w/v) was added to both gelatin and casein solutions. Different volumes of the two solutions were mixed to get the desired ratios (V (gelatin): V (casein) = 4:0, 3:1, 2:2, 1:3, 0:4) of each component, and the total volume of mixture was 20 ml. The pH of the mixture was adjusted to 7.5, and 5 U/g-protein MTG was added to the GC solutions. Different concentrations of MTG (5, 10, 15, 20, 25 U/g-protein) were added to the mixture at the ratios of 2:2 and 1:3. The controls without MTG addition were also prepared. The reaction was carried out at 50ºC in a shaker bath for 2 h and then heated at 90ºC for 10 min to inactivate the MTG. The GC solutions at the ratios of 2:2 and 1:3 modified by 10 U/g-protein MTG were then mixed with PU to get the desired ratios (V (PU): V (GC) = 4:0, 3:1, 2:2, 1:3, 0:4).

Preparation of GC films and PU-GC films modified by MTG

All the GC and PU-GC solutions were poured immediately into the PTFE plates (120×120 mm) and the films were cast. The films were dried at room temperature for 48 h, and then were placed in a constant temperature (23 ± 2ºC) and relative humidity (50 ± 3%, RH) room for at least 48 h prior to testing. The mechanical properties (tensile strength and elongation at break) and water resistance (solubility and water absorption) of the films were then determined.

Application of MTG modified PU-GC as coatings in leather finishing

The formula for finished leather is shown in Table 1. Leather samples were sprayed twice using a spray gun with the base coat materials to make a film thickness of about 100 μm and hung up at 56ºC for 10 min, and then sprayed twice with the prepared PU-GC mixtures at different ratios in the same manner.

Analyses

Measurement of polymerization

Polymerization was evaluated by SDS–polyacrylamide gel electrophoresis (SDS-PAGE). The concentration of acrylamide stacking gel was 5% (w/v), and the acrylamide running gel was 12% (w/v). Protein molecular weight marker (Beyotime, China) contains a mixture of seven proteins ranging in size from 14.4 to 116 kDa.

Eight microliter of samples (1mg/ml) was dissolved in 2 μl of sample loading buffer (5X) (Beyotime, China). The volume of 10 μl was loaded into each lane of gel. Electrophoresis was conducted at 70 V until the tracker dye reached the stacking gel, and then at 110 V until it reached the bottom of each gel. After electrophoresis, the gels were stained with commassie blue staining solution and destained with commassie blue staining destaining solution (Beyotime, China).

Mechanical properties of films

The thickness of each specimen was determined using a digital micrometer; five measurements were taken. The mechanical properties (tensile strength and elongation at break) of the gelatin-casein films were determined using a universal texture testing machine (LPX Plus) as previously described.[12] The samples were cut into specimens of 10×100 mm. The grip separation was set to 50 mm and the crosshead speed was 100 mm/min. Mechanical measurements were done in triplicate, and the average values and standard deviations were calculated.

Water resistance (solubility and water absorption) of films

According to method described by Pereda et al.,[13] six randomly selected pieces about 2×2 cm from each film sample were dried at 105ºC until constant weight and the dry mass was weighed as initial dry mass (W_0). The dried pieces were immersed into 30ml of distilled water for 24 h at 25ºC. The residual matter (W_1) was weighed and dried at 105ºC until constant weight, and then the dry mass was weighed as final dry mass (W_2). The percentage of water solubility (S) and the absorbed water (A) were calculated as follows:

$$S = (W_0 - W_2) \times 100\% / W_0 \quad (1)$$

$$A = (W_1 - W_2) \times 100\% / W_2 \quad (2)$$

Measurement of leather properties

The subjective properties (softness, transparency, tackiness and genuine leather feeling) of light leathers were measured. Measurement of water resistance of light leathers was conducted as previously described. [14, 15] Fastness to rubbing (wet and dry) of light leathers was determined on a Veslic Tester (Italy) according to the method DIN EN ISO11640, DIN 53339*, IUF450. Flexing endurance (wet and dry) of light leathers was measured on a Clamp Flexometer (Italy) in

TABLE 1
Formula of leather finishes

Base coat		Top coat					
		PU:GC	4:0	3:1	2:2	1:3	0:4
Euderm Black BN(g)	100	Bayderm Finish BDP3016 (PU)[a](g)	400	300	200	100	0
Euderm Compact NPDP 2002[b](g)	600	GC11[c]/GC13[d](g)	0	100	200	300	400
Tap water(ml)	300	Tap water(ml)	300	300	300	300	300
		XL 70[e](g)	10	10	10	10	10

[a] water soluble polyurethane resin
[b] acrylic resin with assistant
[c] V (gelatin): V (casein) = 2: 2
[d] V (gelatin): V (casein) = 1:3
[e] polyisocyanate cross linker.

accordance with the method IUP20. Glossiness of light leathers was measured using a Glossiness Tester (Italy) at an angle of 60° according to method, previously described[16] which were performed over a black matte standard plate and run in quintuplicate. The value from 0 to 3 (0 representing the worst and 3 representing the best) was assigned for each parameter.

RESULTS AND DISCUSSION

Influence of MTG on the molecular weight distribution of GC mixtures

Fig. 1 shows the molecular weight distribution of GC mixtures treated with MTG (lane 2-6), and the control (untreated sample) is also shown for comparison (lane 1). The band at about 30, 000 Da is for casein that is more reactive with MTG than gelatin, and disappears quicker with increasing MTG concentration. The band at 116,000 Da is only one of the bands representing gelatin. In most gel electrophoresis analysis it is very difficult to distinguish these bands. After modification by MTG, casein cross-linked with gelatin resulted in the formation of new polymer with a higher molecular weight, and the new polymer could not get into the gel. With the increase of enzyme concentrations, the cross-linking in the proteins increased especially at the concentrations above 10 U/g, and the molecular weight of GC was much higher than the control.

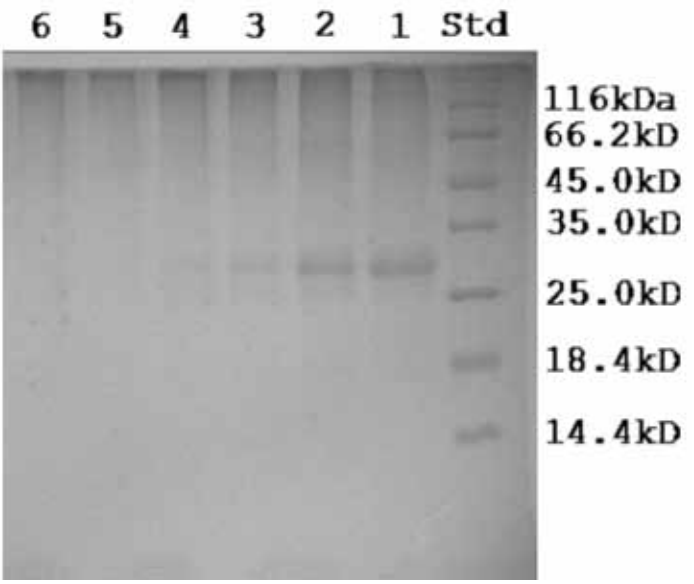

Fig. 1: SDS-PAGE of gelatin-casein mixture with varying enzyme concentrations. V (gelatin): V (casein) = 1:3, MTG concentration (lane 1-6): 0, 5, 10, 15, 20, 25 U/g-protein.

Influence of MTG on the mechanical properties of GC and PU-GC films

Fig. 2(a, b) and Fig. 3(a, b) show the influence of MTG on the mechanical properties (tensile strength and elongation at break) of GC films. As shown in Fig. 2(a, b), the tensile strength and elongation at break of GC films unmodified by

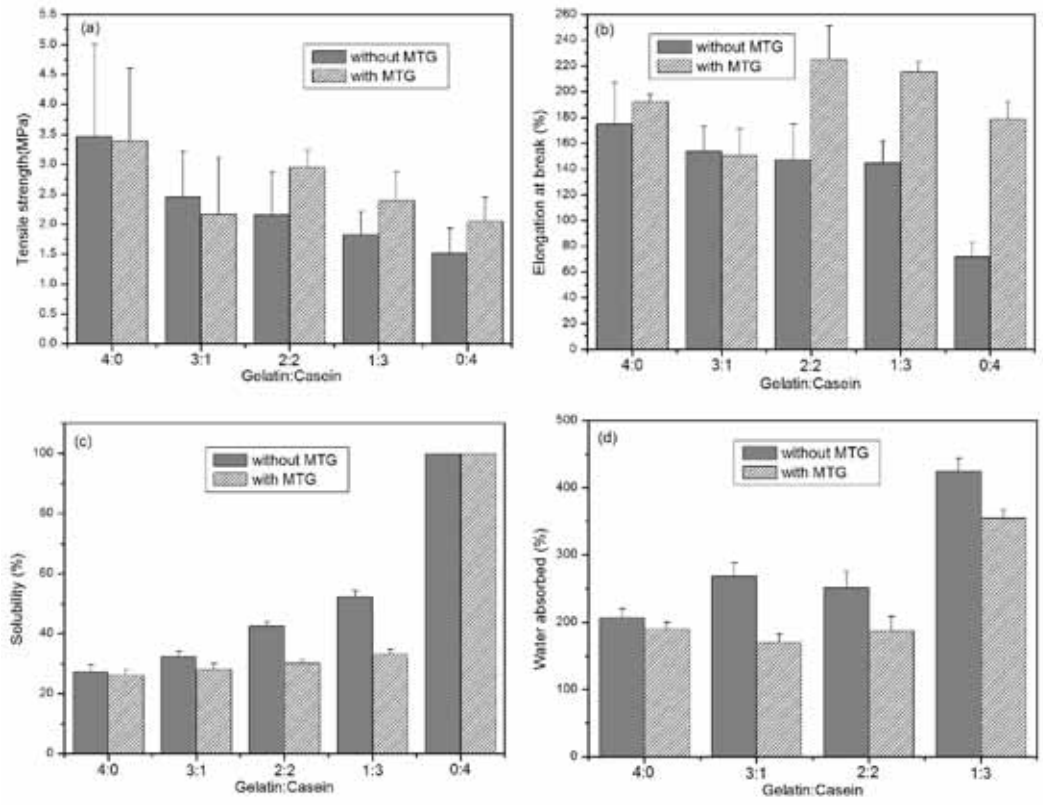

Fig. 2: Mechanical properties and water resistance of GC films at different ratios with MTG (5 U/g-protein).

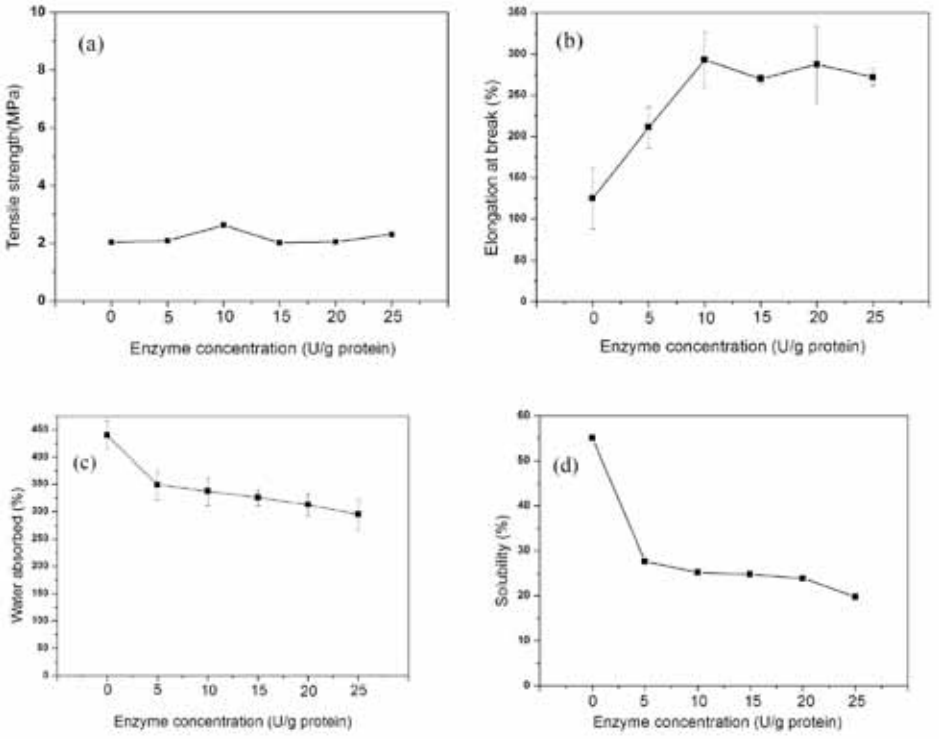

Fig. 3: Mechanical properties and water resistance of GC films (w (gelatin): w (casein) = 1:3) with varying enzyme concentrations.

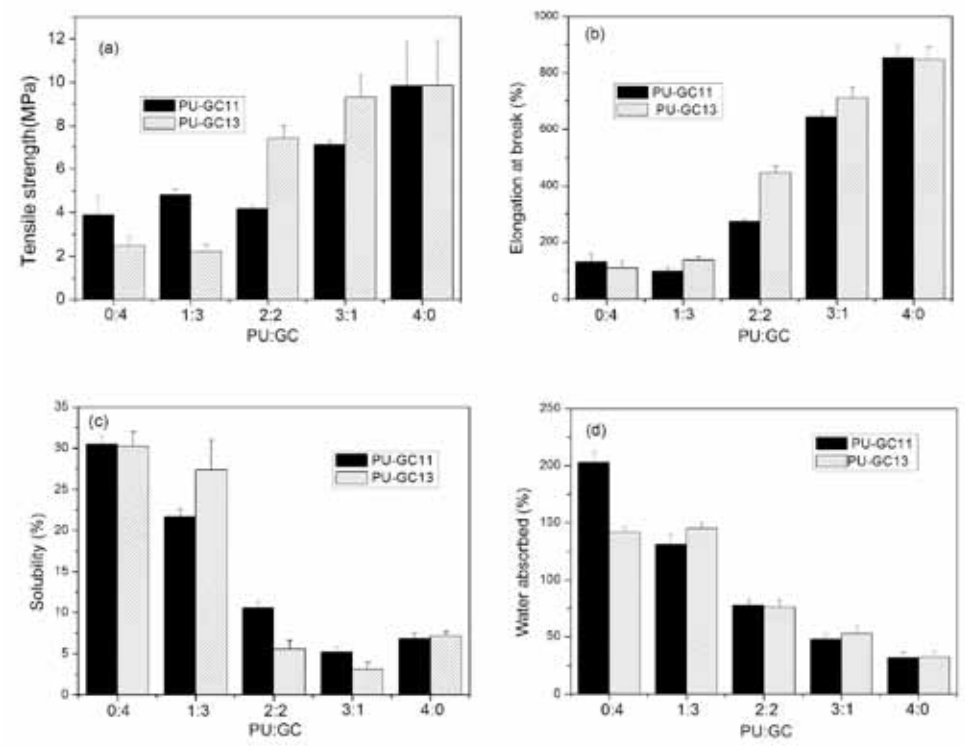

Fig. 4: Mechanical properties and water resistance of PU-GC11 and PU-GC13 films at different ratios with MTG (10 U/g-protein).

MTG was reduced with the decreasing ratio of gelatin. The tensile strength and elongation at break of GC films modified by MTG at the ratios of 2:2, 1:3 and 0:4 increased. As shown in Fig. 3(a, b), with the increase of enzyme concentrations, the elongation at break of GC films at the ratios of 2:2 and 1:3 (the data of 2:2 was not shown) was improved. And when the concentration was less than 10 U/g, the improvement was more obvious.

Fig. 4(a, b) show the mechanical properties of PU-GC films (PU-GC11 and PU-GC13). The mechanical properties of these films were improved significantly with the increase of PU composition.

Influence of MTG on the water resistance of GC and PU-GC films

Fig. 2 (c, d) and Fig. 3(c, d) show the influence of MTG on the water resistance (solubility and water absorption) of GC films. The water resistance of casein was worse than gelatin. Compared with the controls, the solubility of the treated samples did not decrease significantly except for the ratio of 1:3 (Fig. 2(c)). However, the water absorption of the treated samples except for casein film shown in Fig. 2(d) was reduced. As shown in Fig. 3(c, d), with the increase of enzyme concentrations, the solubility of the samples did not change much, but the water absorption was apparently reduced.

The water resistance of PU-GC11 and PU-GC13 films is shown in Fig. 4(c, d). The water resistance of polyurethane was much better than that of gelatin and casein even modified by MTG. The solubility and water absorption was gradually reduced with the increase of the polyurethane composition.

Properties of finished leather

The properties of finished leather with PU-GC11 and PU-GC13 are shown in Table 2 and 3. Water resistance and wet rub fastness of the light leather finished by both PU-GC11 and PU-GC13 at the ratios of 4:0, 3:1 and 2:2 were best, and all the samples showed good dry rub fastness value. The samples coated with only GC, especially with GC13, showed low water resistance and wet rub fastness value, indicating the more hydrophilic nature of gelatin and casein. The samples coated with PU-GC11 showed better wet flexibility than those coated with only PU, and also had good dry flexibility. The wet flexibility of the samples coated with PU-GC13 was similar to those coated with only PU, however, the dry flexibility of which was very low when the composition of PU less than 1/2. It can be concluded that the bonding between PU-GC11 films and the leather is stronger than that between PU-GC13 films and the leather, which could not be easily weakened by flexing. The glossiness of the finished leather with PU-GC11 at the ratio of 3:1 and PU-GC13 at the ratio of 3:1 and 2:2 was very low. From the data in Table 2 and 3, it can be concluded that the samples coated with GC13 showed better glossiness than those with GC11. In our previous studies, we also found that the glossiness of the samples coated with casein was better than that with gelatin, but the water resistance of the samples coated with casein was worse than that with gelatin. That is why we choose the mixture system of gelatin and casein as the coating agents in this work. With the increase of the composition of GC in the PU-GC system, the softness, transparency and tackiness of the finished samples was gradually reduced, but the genuine leather feeling was improved apparently, indicating that the gelatin and casein can keep the finished leather natural grain and natural leather feeling.

TABLE 2
Properties of leather coated with PU-GC11[a]

PU:(GC11) / Evaluation	4:0	3:1	2:2	1:3	0:4
Water resistance	3	3	3	2	2
Wet rub fastness class (100 times)	3	3	3	2-3	2
Dry rub fastness class (1,000 times)	3	3	3	3	3
Wet flexibility	2,500-5,000	7,500	5,000-7,500	5,000-7,500	5,000-7,500
Dry flexibility	50,000	50,000	50,000	40,000-50,000	40,000-50,000
Glossiness	12.26	7.57	8.9	9.6	8.6
Softness	3	2-3	2	2	1-2
Transparency	3	3	2-3	2-3	2
Tackiness	3	2-3	2-3	2	1-2
Genuine leather feeling	1	1-2	2-3	2-3	3

[a] Scale 0-3, 0=worst, 3=best

TABLE 3
Properties of leather coated with PU-GC13[a]

PU:(GC13) / Evaluation	4:0	3:1	2:2	1:3	0:4
Water resistance	3	3	2-3	2	1-2
Wet rub fastness class (20 times)	3	3	2-3	1-2	1
Dry rub fastness class (1,000 times)	3	3	3	3	3
Wet flexibility	2,500-5,000	2,500-5,000	2,500	2,500	2,500-5,000
Dry flexibility	50,000	50,000	50,000	8,000	8,000
Glossiness	12.26	7.25	7.45	11.93	11.23
Softness	3	2-3	2-3	2	1-2
Transparency	3	3	3	2-3	2-3
Tackiness	3	2-3	2-3	2	1-2
Genuine leather feeling	1	1-2	2-3	2-3	3

[a] Scale 0-3, 0=worst, 3=best

CONCLUSIONS

The mechanical properties of GC films modified by MTG at the ratio of 2:2, 1:3, and 0:4 were improved due to the cross-linking between gelatin and casein catalyzed by MTG. Treated with MTG at a concentration of 10 U/g-protein, the elongation at break of the films turned to the largest, but tensile strength did not have much change. The water solubility of the films modified by MTG did not change much, but the water absorption was reduced especially when MTG concentrations increased. The water resistance of gelatin film was stronger than that of casein film. Mixed with PU, the mechanical properties and water resistance of the GC films modified by MTG were greatly improved. With the increase of PU content, the properties such as water resistance, wet rub fastness, dry flexibility, softness, transparency and tackiness were improved, but the genuine leather feeling significantly decreased. The glossiness of finished leather with PU-GC11 at the ratio of 3:1 and PU-GC13 at the ratio of 3:1 and 2:2 was very low. Therefore, it is better to apply the PU-GC system in leather finishing as coating agents than only PU or GC. Compared with the samples coated with PU-GC13, the water resistance, wet rub fastness and flexibility of the samples coated with PU-GC11 were better, indicating that PU-GC11 system is more suitable as coating agents in leather finishing. Comprehensively considering various factors, the PU-GC11 system at a ratio of 2:2 was the best coating agents for leather finishing.

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