Inhibitory Effect of Protein Filling Agent from Bovine Hair on the Oxidation of Cr(III)

by

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Abstract

In consideration of the fact that the hydrolysate of hair contains antioxidant and free radical scavenging groups, the inhibitory effect of protein filling agent prepared from bovine hair (HPFA) on the oxidation of Cr(III) was investigated. The results demonstrated that HPFA possessed remarkable reducing ability to transfer Cr(VI) to Cr(III) in solution when pH was lower than 6.0. The reduction reaction was enhanced with rise of temperature, extension of reaction time and increase of HPFA dosage. Meanwhile, the HPFA also presented a high activity to inhibit the oxidation of Cr(III) in chrome liquor in the presence of unsaturated fish oil fatliquor. More interestingly, the HPFA achieved satisfactory inhibitory effect on Cr(III) oxidation in leather in the temperature range from 20°C to 100°C. Its inhibitory activity was much higher than that of commercial protein filling agent made from collagen hydrolysate. All the results suggested that bovine hair could be used as a potential resource for preparing protein filling agent with both filling and Cr(III) oxidation inhibiting properties.

Introduction

Chrome tanning is widely used in leather industry due to the excellent and comprehensive performances of chrome tanned leather.[1] However, the Cr(III) in leather may be oxidized into Cr(VI) under adverse conditions, such as high temperature, pH and humidity, and light. In addition, some leather chemicals such as fatliquoring agents with unsaturated bonds and processing steps such as neutralization may cause the formation of Cr(VI) in leather.[2-6] Medical research has shown that Cr(VI) is harmful to human health and is associated with many clinical diseases, such as nausea, diarrhea, dermatitis, internal hemorrhage, kidney and liver damage.[7-9] Thus, many efforts have been made to find agents which are capable of inhibiting the oxidation of Cr(III) in leather.[10-12]

In traditional leather processing, hide and skin hairs were dissolved in water by sulfide, which led to high content of chemical oxygen demand (COD) in wastewater. With increasing concerns of environmental protection, hair-saving unhairing technique, to a greater extent, is used in tanneries, which can eliminate dissolution of hair and remarkably alleviate impact of wastewater pollution.[13-16] However, a large amount of hair, as solid waste, is generated.[17] Therefore, more research has been focused on the recycling of tannery hair. Previous studies indicated that the hydrolysate of waste bovine hair could be used as protein filling agent in leather industry.[17] Meanwhile, it was found that this kind of protein filling agent can enhance the uptake of chromium salt in chrome tanning and retanning processes.[18] On the other hand, as we know, hair is mainly composed of abundant keratin-containing functional groups, such as sulfhydryl groups and amide linkages, which implies that it has the potential to be used as a natural resource for preparing biological agents with physiological functions. Our recent study demonstrated that the hydrolysate of bovine hair possessed remarkable biological activity in scavenging free radicals and inhibiting lipid-peroxidation.[19] These results strongly inspired us to explore the method of preparing protein filling agent with good inhibitory activity on Cr(III) oxidation from tannery waste hair.

To the best of our knowledge, there is no information about the inhibitory activity of protein filling agent from waste hair on the oxidation of Cr(III). Therefore, in the present work, effort was made to prepare a satisfactory protein filling agent using tannery waste bovine hair, and then its inhibitory activity on Cr(III) oxidation was investigated.

Experimental

Materials and Chemicals

The bovine hair generated from hair-saving unhairing process was provided by a local tannery. It was washed with distilled water and air-dried before use. The specimen (voucher number: 2014-0126) was preserved at the Key Laboratory of

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Glycerol triglycidyl ether used as modification reagent in preparation of protein filling agent was of commercial grade. Chrome tanning agent (basicity 33%, Cr_2O_3 24%), mimosa extract, commercial protein filling agent (CPFA, made from collagen hydrolysates), sulfited fish oil fatliquor (iodine value 167.5±3.8 g I_2/100g fatliquor) and other leather chemicals were all industrial products. Wet blue tanned with 5% chrome powder was provided by Haining Leather Star Co., Ltd., Zhejiang, China. The reagents used for analysis were of analytical grade.

Preparation of Protein Filling Agent from Bovine Hair

Bovine hair (100 g) was suspended in NaOH solution (2000 mL, 2.5%, *w/v*) and hydrolyzed at 100°C for 2 h, and then it was centrifuged at 10,000 rpm for 15 min. The pH of supernatant was adjusted to 5.5 using HCl solution (0.5 mol/L). The supernatant was further reacted with glycerol triglycidyl ether (1.6%, *w/v*) at 55°C for 5 h. Then, the mixtures were dried using a spray dryer (Shanghai Guigo Industrial Co., Ltd., China) to obtain the hair protein filling agent, named HPFA. HPFA was a yellowish brown powder with the M_w of 14439 determined by Gel Permeation Chromatography (Malvern 270 max GPC, Malvern Instruments, UK).

Cr(VI) Reduction Experiment

Effect of Reaction Time on Cr(VI) Reduction by HPFA

2 g of HPFA was dissolved in 500 mL of Cr(VI) solution (0.6 mg/L), and the pH of the solution was adjusted to 2.0 by phosphoric acid aqueous solution (1:1, *v/v*). Then the solution was stirred in 50°C water bath for 2, 4, 8, 10 and 14 h, respectively. The reaction system without HPFA was used as blank control. After reaction, the Cr(VI) content in the solutions was determined.

Effect of Temperature on Cr(VI) Reduction by HPFA

2 g of HPFA was dissolved in 500 mL of Cr(VI) solution (0.6 mg/L), and the pH of the solution was adjusted to 2.0 by phosphoric acid aqueous solution (1:1, *v/v*). Then the solution was stirred for 4 h at 20, 30, 50, 70 and 90°C, respectively. The reaction system without HPFA was used as blank control. After reaction, the Cr(VI) content in the solutions was determined.

Effect of pH on Cr(VI) Reduction by HPFA

2 g of HPFA was dissolved in 500 mL of Cr(VI) solution (0.6 mg/L), and the pH of the solution was adjusted to 1.0, 2.0, 4.0, 6.0 and 8.0, respectively, by phosphoric acid aqueous solution (1:1, *v/v*). Then the solutions were stirred in 50°C water bath for 4 h. The reaction system without HPFA was used as blank control. After reaction, the Cr(VI) content in the solutions was determined.

Effect of Dosage of HPFA on Cr(VI) Reduction

0.5, 1.0, 2.0, 3.0 and 4.0 g HPFA were dissolved in 500 mL of Cr(VI) solution (0.6 mg/L) respectively, and the pH of the solutions was adjusted to 2.0 by phosphoric acid aqueous solution (1:1, *v/v*). Then the solutions were stirred for 4 h at 50°C. The reaction system without HPFA was used as blank control. After reaction, the Cr(VI) content in the solutions was determined.

Inhibitory Effect of HPFA on Cr(III) Oxidation in Chrome Liquor

Effect of Fatliquor on Cr(III) Oxidation

Sulfited fish oil fatliquor is rich in unsaturated bonds (iodine value 167.5±3.8 g I_2/100g fatliquor), implying that it is prone to produce free radicals under certain conditions. Therefore, it was used for determining the effect of fatliquor on Cr(III) oxidation. 200 mL chrome tanning agent solution (100 g/L) was mixed with 16 g sulfited fish oil fatliquor. The pH of the solution was adjusted to 5.4 with 1 mol/L H_2SO_4 solution. Then, the mixture was stirred at 80°C for 5 h under air. The sample without sulfited fish oil fatliquor was used as control. The effect of fatliquor on Cr(III) oxidation was evaluated by measuring the content of Cr(VI) in the solution after reaction.

Effect of pH on Cr(III) Oxidation in the Presence of Fatliquor

200 mL chrome tanning agent solution (100 g/L) was mixed with sulfited fish oil fatliquor (16 g) and the pH of the solution was adjusted to 5.4 and 6.4 with 1 mol/L H_2SO_4, respectively. The concentration of Cr(VI) in the solutions was detected after stirring for 5 h at 80°C under air.

Inhibitory Effect of HPFA on Cr(III) Oxidation in the Presence of Fatliquor

200 mL chrome tanning agent solution (100 g/L) was mixed with 16 g sulfited fish oil fatliquor. Then, 20 g HPFA was added to the solution and the pH was adjusted to 6.4 with 1 mol/L H_2SO_4. The mixture was stirred at 80°C for 5 h under air. The sample without HPFA was used as control. The content of Cr(VI) in the solution was measured to reflect the inhibitory effect of HPFA on the oxidation of Cr(VI) in the presence of fatliquor.

Inhibitory Effect of HPFA on Cr(III) Oxidation in Leather

The crust leather used for determination was prepared as shown in Table 1. Three wet blue samples (cut from backbone area) were weighed, rewetted, rechromed and neutralized in drum. Then, they were filled using mimosa extract, hair protein filling agent (HPFA) and commercial protein filling agent (CPFA), respectively, followed by fatliquoring with 8% sulfited fish oil fatliquor. Finally, the crust leather samples were washed and hang dried for 24 h.

Previous studies have shown that the conversion of Cr(III) to Cr(VI) is fairly difficult in natural state.[5] So the crust leathers

filled by the three filling agents were induced to facilitate the formation of Cr(VI) in leather under the following conditions: i) exposed to 20, 40, 60, 80 and 100°C for 6 h, and ii) exposed to 80°C for 0, 3, 6, 9, 12, 15 and 18 h. Then the Cr(VI) contents in the crust leathers were determined.

Determination of Cr(VI) Content

10 mL of each reacted solution above was pipetted into a 100 mL beaker, and then a certain amount of zinc hydroxide coprecipitator (20 g/L NaOH solution + 80 g/L $ZnSO_4 \cdot 7H_2O$ solution, 1:1, *v/v*) was added to fully precipitate Cr(III). After being filtered using 30 μm quantitative analysis filter paper to remove Cr(III) [20], the Cr(VI) content in solution was detected using diphenylcarbazide spectrophotometry.[21] The absorbance at 540 nm was measured by UV-visible spectrophotometer (UV-1800PC, Shanghai Mapada Instruments Co., Ltd., China). In addition, Cr(VI) content in leather was determined according to the standard procedure of IULTCS.[22-23]

TABLE I
Post-tanning processes.

Operation	Material	Temperature (°C)	Dosage[a] (%)	Time (min)	pH
Rewetting	Water	40	200		
	Formic acid		0.3		
	Non-ionic degreasing agent		0.3	90	3.5
Rechroming	Water	40	150		
	Chrome tanning agent		4×2	60×2	
	Sodium bicarbonate		0.5×3	15×3	4.0
Washing	Water		200	5	
Neutralization	Water	35	150		
	Neutralizing retanning agent		2		
	Sodium formate		2	20	
+	Sodium bicarbonate		0.3×3	20×3+60	6.4
Washing	Water		200	5	
Filling	Water	30	100		
	Mimosa extract /HPFA/CPFA		10	20	
+	Mimosa extract /HPFA/CPFA		10	120	
+	Water	35	100	10	4.2
+	Formic acid		0.3	30	4.0
Fatliquoring	Water	50	150		
	Sulfited fish oil fatliquor		8	60	
+	Formic acid		0.5	30	3.7
Washing	Water		200	10	

a: based on wet blue weight (*w/w*)

Results and Discussion

Reduction of Cr(VI) by HPFA

The satisfactory filling property of HPFA for leather has been proven. (The thickness increase after filling was 32%, and the physical properties of the filled crust leather were comparable to that of conventional crust.) In fact, it has been practically used in tanneries. Herein, we concentrated on the investigation of antioxidant activity of HPFA.

HPFA is made from the hydrolysate of bovine hair. It is known that the hair hydrolysate is rich in sulfhydryl groups derived from cysteine residue.[19] This fact suggests that Cr(VI) may be reduced by sulfhydryl groups in HPFA. The effects of several factors on Cr(VI) reduction by HPFA are shown in Figure 1. It was found that the concentration of Cr(VI) in control group kept constant (0.6 mg/L) through the whole experiments. HPFA exhibited a strong reducing power towards Cr(VI). The concentration of Cr(VI) in test group decreased to 0.1-0.2 mg/L with the increase of reaction time, temperature or dosage of HPFA (Figure 1A, 1B and 1D). The reducing activity of HPFA was significantly influenced by pH (Figure 1C). The amount of reduced Cr(VI) was remarkably decreased when pH was increased from 2 to 4, and only a small amount of Cr(VI) was reduced in the pH range of 4 to 6. This phenomenon could be due to the fact that the oxidation ability of Cr(VI) declines with the rise of pH.[4] As we know, the post-tanning processing of leather production is commonly

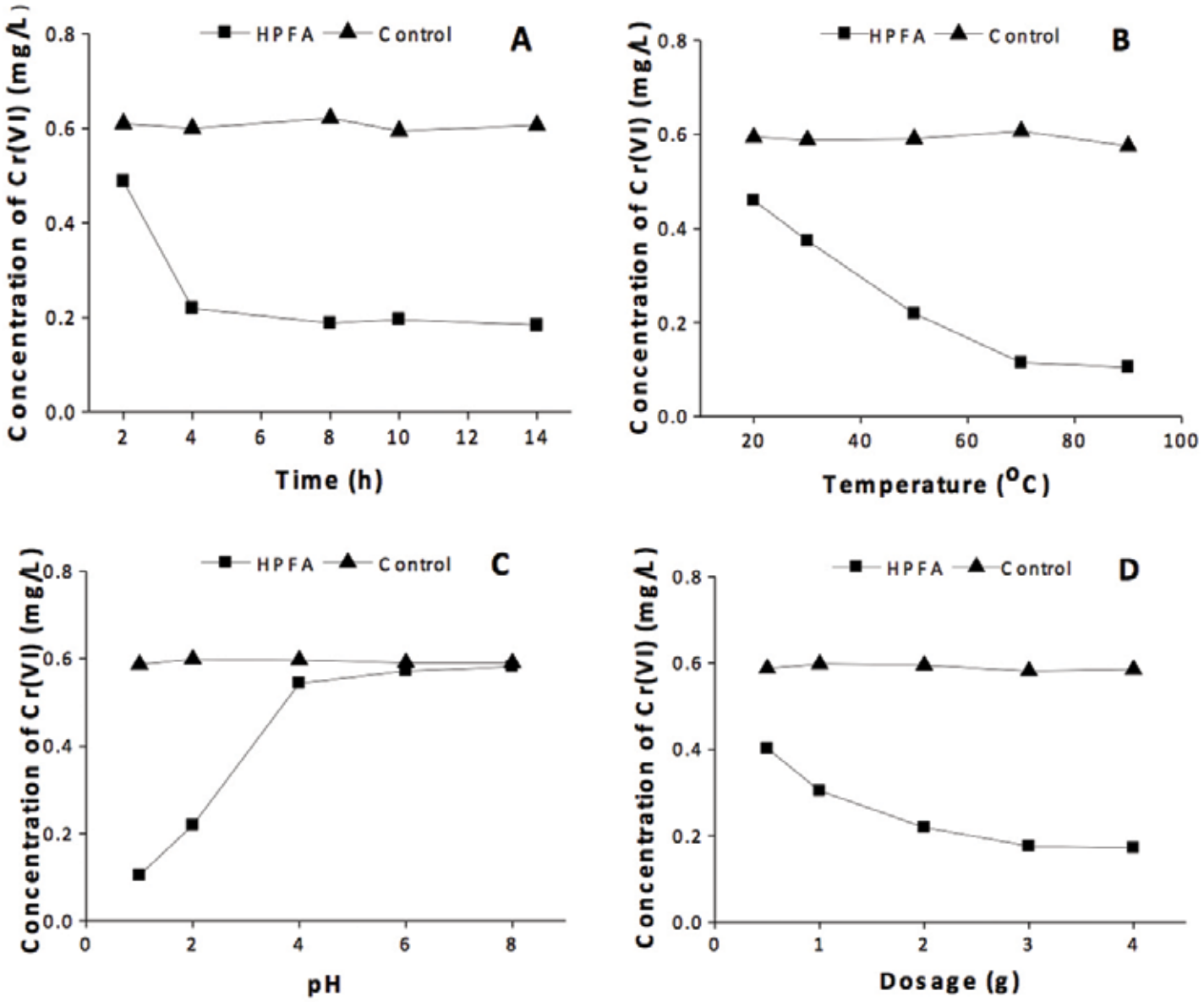

Figure 1. Effects of several factors on Cr(VI) reduction by HPFA: A. Reaction time (50°C, pH 2, HPFA 2 g); B. Reaction temperature (4 h, pH 2, HPFA 2 g); C. Reaction pH (50°C, 4 h, HPFA 2 g); D. Dosage of HPFA (50°C, 4 h, pH 2)

completed at pH 3.5-4.5. This implies that HPFA has reducing capacity towards the Cr(VI) adventitiously generated in leather.

Inhibitory Effect of HPFA on Cr(III) Oxidation in Chrome Liquor

The earlier studies have shown that the formation of Cr(VI) in chrome tanned leathers is mainly due to reasons such as relatively high pH, photo-ageing or thermal ageing, and the use of fatliquors containing unsaturated fatty acids or natural fat.[2] As presented in Figure 2, the content of Cr(VI) in the chrome liquor without fatliquor (control) was 1.63 mg/kg after stirring at 80°C for 5 h, while the content of Cr(VI) in the chrome liquor with fatliquor was increased to 15.11 mg/kg. These results indicated that the addition of sulfited fish oil fatliquor significantly promoted the oxidation of Cr(III) in chrome liquor due to the oxidizing action of free radicals generated from oxidation of unsaturated fats, illustrated as follows: [2]

$$RH \xrightarrow{\text{UV or Heating}} R\cdot + H\cdot \quad (RH \text{ represents fatliquor})$$

$$R\cdot \xrightarrow{O_2} ROO\cdot$$

$$ROO\cdot + RH \rightarrow ROOH + R\cdot$$

$$2ROOH + R\cdot \rightarrow ROH + 2RO\cdot + \cdot OH$$

$$RO\cdot + 2\cdot OH + 3RH \rightarrow 3R\cdot + ROH + 2H_2O$$

In addition, the concentration of Cr(VI) in the chrome liquor containing fatliquor was increased from 15.11 mg/kg to 18.73 mg/kg when pH of the solution was adjusted from 5.4 to 6.4 (Figure 3). Obviously, raising the pH also promoted the oxidation of Cr(III) in solution, which was coincident with the observation of Cr(VI) formation in leather.[3, 24] As shown in Figure 4, the concentration of Cr(VI) in the chrome liquor containing both fatliquor and HPFA was clearly lower (9.72 mg/kg) than that without HPFA (18.73 mg/kg), which indicated that HPFA could considerably inhibit the oxidation of Cr(III) in chrome liquor. This could be due to the antioxidant and free radical scavenging activities of HPFA.

Inhibitory Effect of HPFA on Cr(III) Oxidation in Leather

As HPFA presented significant reducing capacity on Cr(VI) and inhibitory effect on Cr(III) oxidation, the inhibitory effect of HPFA on Cr(III) oxidation in crust leather was further investigated. It should be noted that, in our experiment, the crust leather samples were treated with a large amount of sulfite fish oil fatliquor and exposed in extreme conditions so as to induce more considerable Cr(III) oxidation. So the results might be different from those in normal conditions, but would reflect the ability of HPFA in inhibiting Cr(III) oxidation in leather. Heat is one of the predominant factors that lead to oxidation of Cr(III).[4] The inhibitory effects of various filling

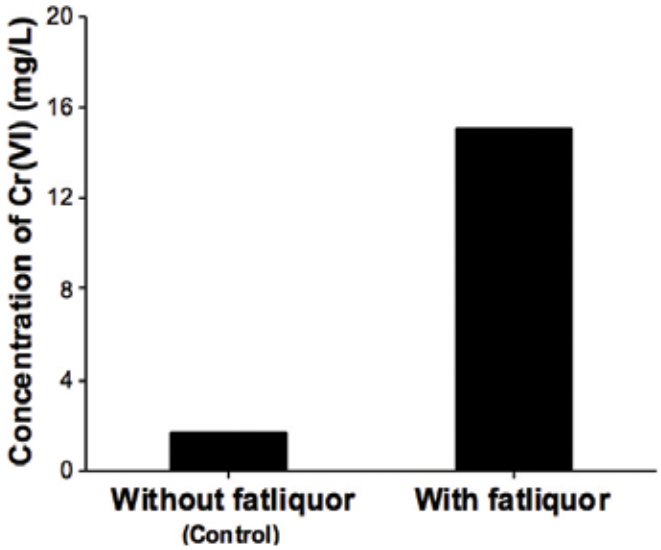

Figure 2. Effect of fatliquor on Cr(III) oxidation in chrome liquor (pH 5.4, 80°C, 5 h)

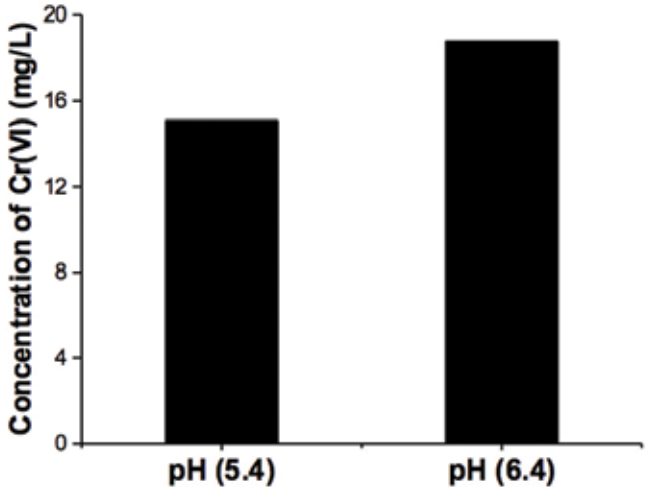

Figure 3. Effect of pH on Cr(III) oxidation in chrome liquor in the presence of fatliquor (80°C, 5 h)

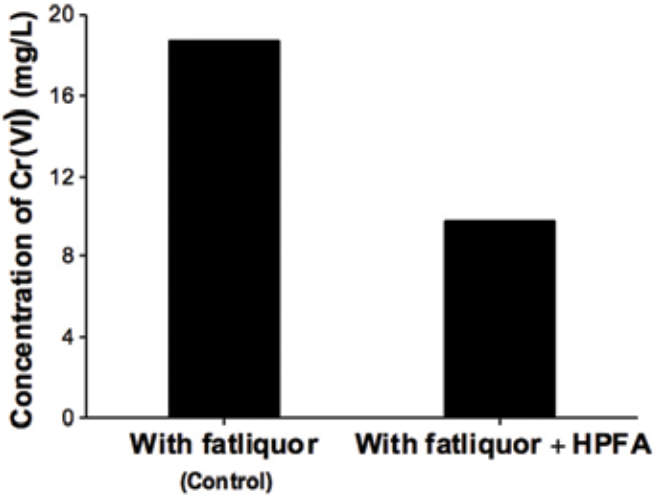

Figure 4. Inhibitory effect of HPFA on Cr(III) oxidation in chrome liquor in the presence of fatliquor (pH 6.4, 80°C, 5 h)

agents on Cr(III) oxidation after exposure to different temperatures were presented in Figure 5A. The content of Cr(VI) in leather filled with mimosa extract remained lower than 3 mg/kg (below the detection limits). The increase of temperature did not accelerate the formation of Cr(VI). This result demonstrates that mimosa extract has strong inhibitory effect on Cr(III) oxidation, which is consistent with the previous research.[4]

The content of Cr(VI) in the leathers filled with HPFA and commercial protein filling agent CPFA (made from collagen hydrolysate) were both increased with the rise of temperature. After exposure at 100°C for 6 h, the Cr(VI) content in HPFA filled leather and CPFA filled leather was increased to 30 mg/kg and 80 mg/kg, respectively. Generally, the Cr(VI) content in the HPFA filled leather was always lower than that in the CPFA filled leather after exposure at different temperatures, which indicated that the addition of HPFA could obviously inhibit the oxidation of Cr(III) in leather, although not as good as mimosa extract.

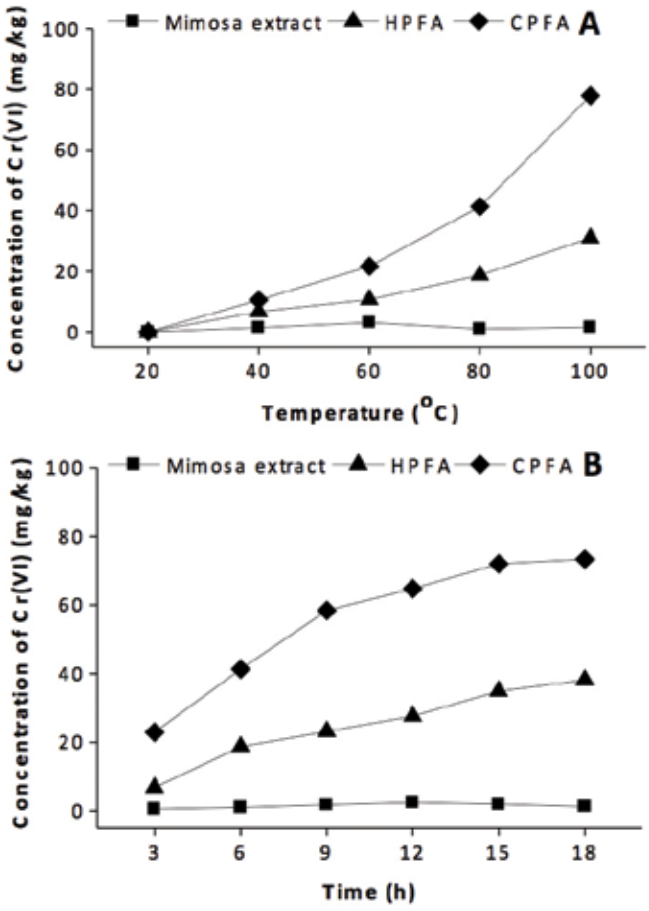

Figure 5. Inhibitory effects of various filling agents on Cr(III) oxidation in leather: A. Effect of temperature on Cr(III) oxidation (6 h); B. Effect of time on Cr(III) oxidation (80°C)

Figure 5B shows the inhibitory effects of various filling agents on Cr(III) oxidation in leather after exposure at 80°C for different times. It was also found that, in each period of exposure, the Cr(VI) content in the HPFA filled leather was higher than that in the mimosa filled leather, but remarkably lower than that in the CPFA filled leather. All the results above suggested that HPFA, the protein filling agent based on hair hydrolysate possesses considerable inhibitory effect on formation of Cr(VI) in leather, probably due to the fact that it contains rich antioxidative and free radical scavenging structures, such as sulfhydryl groups.

Conclusions

The protein filling agent made from tannery waste bovine hair is able to reduce Cr(VI) to Cr(III) in solution, and inhibits the oxidation of Cr(III) in chrome liquor and in leather, which suggests that the reuse of tannery waste hair as a raw material for producing protein filling agent would be a good option.

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