Preparation of Protein Retanning Agent by Grafting Modification of Collagen Hydrolysate Extracted from Chrome Shavings

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Abstract: The leather industry represents a significant environment encumbrance and it generates a great quantity of chrome shavings, which often determine the economic efficiency of the tannery. Therefore the implementation of clean technologies and waste processing is of vital importance. It's cleaner and more economical to isolate collagen hydrolysate (CH) from chrome shavings which can be chemically modified to reuse in the leather processing. A grafted protein retanning agent (PRA) was prepared by modifying CH with acrylic monomers such as acrylic acid， acrylamide， n-butyl acrylate and acrylonitrile. Results of ninhydrin reaction, FTIR and DSC analysis showed that acrylic monomers were grafted onto the polypeptide chains of collagen hydrolysate with covalent bond, and graft copolymerization can improve thermal stability of collagen hydrolysate. Application experiments showed that the protein retanning agent can improve the absorption of chrome agent and provide some good organoleptic properties such as grain tightness, fullness and good selective filling performance for wet-blue leather.

Key words: chrome shavings; collagen hydrolysate; protein retanning agent; acrylic monomers; characterization; application

1 Introduction

The production of chromium-containing solid waste including chrome shavings and tanned splits in tannery has been recognized as a problem for many years, but recent years, pressure from environmental authorities has given the problem increasing urgency. In past decades, significant efforts have been made to decrease the amount of chrome shavings, but with more than 90% of tanneries adopting chrome tanning [1], chrome shavings from leather industry are unavoidable [2].

Chrome shavings primarily consist of chromium and protein, which could be treated to give the potential resources of collagen protein and chromium [3]. These wastes can be utilized with or without the presence of chromium. Attempts have also been made to reduce potassium dichromate using chrome shavings directly to give a chrome tanning agent product, usable in the tanning or retanning processing of leather industry [4-6]. Prior research has demonstrated that it's an effective way to acid hydrolyze chrome shavings into a chromium-containing protein hydrolysate which can also be reused in retanning processing [7]. It's found that it's cleaner and more economical to separate the protein-bound chromium by the treatment of alkali or enzyme and use the protein and chrome cake or chrome sludge for several applications [8]. A way was made to use protein products isolated from chrome shavings for food or biomaterial, but it was banned by authorities for its high chrome content, so utilization of protein products in food chain is not the best chance until the problem of superscale of chromium content is solved. If protein products can be chemically modified to reuse in the leather processing such as retanning, fatliquoring and finishing, superscale of chrome content will not be confronted, and there will bring considerable economic efficiency and environmental profits because of chrome shavings

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circulation in leather industry.

Research over several years in our laboratory has been designed to fully utilize chrome shavings, and a complete set of technological alternative based on separating the protein-bound chromium by the treatment of sodium hydroxide for reutilization of these waste has developed, including modifying collagen hydrolysate to give a retanning agent and using chrome cake or chrome sludge as a reductant for potassium dichromate to give a chrome tanning agent. The main objectivities of this work are to prepare a retanning agent by grafted modifying CH with acrylic monomers, to characterize the structure and properties of grafted products, and to evaluate the behavior of protein retanning agent grafted by acrylic monomers.

2 Experimental

2.1 Materials

Fresh chrome shavings were obtained from a commercial tannery and were kept at room temperature. Acrylic acid, acrylamide，n-butyl acrylate, acrylonitrile, sodium hydroxide and ammonium persulfate were purchased from Chengdu Kelong Chemical Plant.

2.2 Preparation of PRA

A mixture of chrome shavings with sodium hydroxide (2%) and non-ionic surfactant (0.05%) in water (500% float) was tumbled for 24 hours at room temperature. Then sodium hydroxide (4%) was added and the mixture was heated at 100℃ for 2 hours. After filtering, collagen hydrolysate solution was concentrated to a higher concentration.

The modification of collagen hydrolysate by acrylic monomers were carried out in a four-neck glass reactor equipped with a mechanical stirrer, a thermometer, and a nitrogen inlet. 125 grams 0f 25% collagen hydrolysate solution was immersed with 0.18g sodium dodecyl sulfate and 0.16g OP-10, and tumbled at 40℃ for half an hour. Required amount of initiator $(NH_4)_2SO_4$(99.8%) was dissolved in water, and distilled acrylic monomers was mixed for 30 minutes with 0.09g sodium dodecyl sulfate and 10mL water. Then the monomers and initiator were added and the reaction was carried out in N_2 for 2h. The reaction was stopped by hydroquinone at 40℃ and pH of the latex was adjusted to about 6 by sodium hydroxide(0.1mol/L), and the crude grafted protein retanning agent (PRA) was collected and kept at room temperature.

2.3 Purification

In order to prove whether the reaction between collagen hydrolysate and acrylic monomers is graft copolymerization, and to investigate the structure and properties of grafted product, the retanning agent must be purified and some substances such as collagen hydrolysate, acrylic polymer and other impurities should be removed.

The crude product was extracted in alcohol at 75℃ for 24h, then extracted by acetone at 80℃ for 12h, and dipped in dimethylformamide (99% ,Kelong Chemical) for 12h, and finally extracted by boiling water and acetone for 12h again, then dried in vacuum at 60℃, and purified grafted polymer (PGP) was collected.

2.4 Characterization

2.4.1 Ninhydrin reaction of PGP

Since collagen hydrolysate can be hydrolyzed completely in 6N HCl in 24 h and acrylic polymer hardly lost weight under the same condition, PGP was hydrolyzed in 6N HCl for 24 h to release the acrylic polymer side chains, and wasn't dialyzed in distilled water until ninhydrin didn't change color when it reacted to the extravasate out of semipermeable membrane. Then ninhydrin solution was added into

reserved liquid in the semipermeable membrane, and observed the color change which can determine whether Acrylic monomers were grafted onto the polypeptide chains of collagen hydrolysate(CH) with covalent bond.

2.4.2 FT-IR Analysis

In order to investigate presence of graft copolymerization between collagen hydrolysate and acrylic monomers, FT-IR spectra of collagen hydrolysate(CH) , acrylic polymer(AP), CH/AP mixture and purified grafted polymer(PGP) was taken in KBr pellets, using a Perkin-Elymer Spectrum-one spectrometer.

2.4.3 DSC Analysis

Differential scanning calorimetry (DSC) was performed on PGP and CH, using a NETZSCH 200PC differential scanning calorimeter. The 3-mg samples were heated at a rate of 10℃/min with N_2 flow.

2.4.4 Analysis of grafted protein retanning agent (PRA)

The grafted protein retanning agent was analyzed for pH, moisture, protein, kinetic viscosity, placed stability and stability against acid and alkali. The pH of the 10% solution of the sample was measured using a digital pH meter. Moisture was determined by weight loss resulting from heating the sample at 105℃ for 6 hours. Protein content was calculated through Kjeldahl nitrogen estimation.

2.5 Retanning application

The prepared products (PRA), as well as a commercial congeneric protein retanning agent (PRF), were used as retanning agents in leather processing, and crust leather samples were assessed for color, softness, grain tightness and general appearance by hand and visual examination by the experienced tanners. The crust leathers were rated on scale 0-10 points for each functional property by three experienced tanners, and the average values were given. Higher points indicate better property.

The experiments were performed using 6 sides of shaved wet-blue goat leather (3 left sides and 3 right sides), and one left side and one right one were taken at random for PRA, another two sides were taken for PRF, and the last two sides were taken for control sample (without retanning agent). All datas were calculated at average, and PRA and PRF were added after neutralization, then dyeing and fatliquoring processing were performed.

In order to investigate whether PRA can increase the absorption of chromium, an experiment was designed, adding PRA after chrome retanning. Chromium content of spent liquor (w_s) and chromium content of initial added chromium (w_i) were examined by an ICP instrument (OPTIMA 2100DV, Perkin Elymer), so absorption ratio of chromium(ARC) can be calculated as following formula:

$$ARC\% = \frac{w_i - w_s}{w_i} \times 100$$

3 Results and Discussion

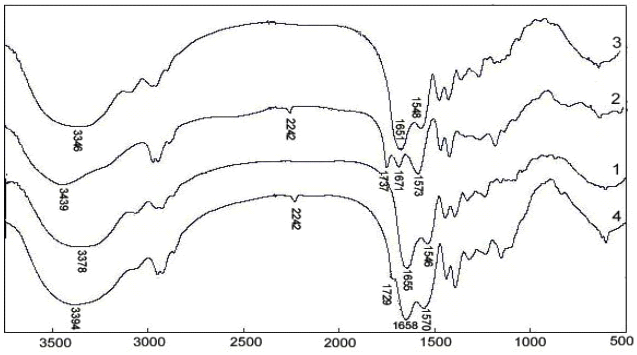
3.1 Results of ninhydrin reaction

Ninhydrin clearly showed a blue-violet color change when ninhydrin was added into boiling reserved liquid in the semipermeable membrane. Collagen polypeptide bone of grafted polymer was hydrolyzed into dissoluble small molecular amino acids at high temperature, which can penetrate the semipermeable membrane, and grafted side chains were dissolved in the reserved liquid which can't penetrate the semipermeable membrane because of theirs big molecules. Ninhydrin reaction showed a blue-violet color change, indicating the existence of dissociative amidocyanogen in the end of acrylic

polymer, which can prove acrylic monomers were grafted on the collagen polypeptide.

3.2 FTIR Analysis

Fig.1 shows the FTIR absorption spectra differences of collagen hydrolysate (CH), acrylic polymer (AP), purified grafted polymer(PGP) and mixture of CH and AP clearly. The FTIR spectrum of CH shows the characteristic absorption including the amide Ⅰ band(1652 cm^{-1}) and the amide Ⅱ band(1570 cm^{-1}), and the FTIR spectrum of AP shows absorption bands at 2242 cm^{-1}, 1737 cm^{-1}, 1671 cm^{-1} and 1573 cm^{-1}, corresponding to the functional groups of acrylic monomers such as acrylonitrile, n-butyl acrylate, acrylamide. The FTIR spectrum of PGP shows newer absorption of 2242 cm^{-1}, 1729 cm^{-1} than CH, characteristic of $-C\equiv N$ and ester linkage stretching and as well as those at 1658 cm^{-1}, 1570 cm^{-1} characteristic of amide bending vibrations. In addition, the FTIR spectrum of CH/AP mixture doesn't show the adsorption of functional groups of acrylic monomers. The presence of these intense absorption bands confirms the structure of PGP, and suggests that acrylic monomers were grafted onto collagen polypeptide of collagen hydrolysate.



1—CH 2—AP 3—CH/AP mixture 4—PGP

Fig. 1 FTIR Spectra of CH, AP, CH / AP mixture and PGP

3.3 DSC Analysis

The DSC curve of CH shows that an endotherm from evaporation of water in CH and a decomposition exotherm over a broad range of temperatures with the plateau centering 101.1°C. PGP, on the other hand, has a very sharp decomposition exotherm at a higher temperature of 122.1°C. It presents obviously that PGP exhibits much improved thermal stability than CH. It may indicate that the strong polar groups of acrylic monomers have improved interactional force of molecular chains, and graft of acrylic monomers and enlacement of molecular chains have increased thermal transition temperature of CH.

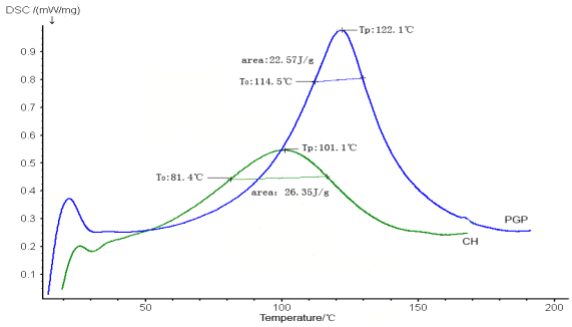


Fig. 2 DSC curve of CH and PGP

3.4 Characterization *of PRA*

Properties of PRA were investigated and results were showed in Tab.1. Grafted protein retanning agent is a kind of brown emulsion, containing 22-24% solid weight, and it has a high protein content ranged 48-60%. It was found that it can be placed at room temperature for 12 months. Demulsification and precipitation will be occurred when PRA is added into acidic solution whose pH value is less than 3.5, having a similar performance as acrylic retanning agent. On the other hand, PRA has a good performance of stability when pH value is more than 3.5, showing that it can be used in a wide range of pH.

Tab. 1 Characterization of grafted protein retanning agent

Parameter	**Measure**
Appearance	Brown emulsion
Solid weight	22-24%
Protein content	48-60%
pH	5.8-6.3
Kinetic viscosity	30 mPa·s
pH range of stability	pH≥3.5
Placed stability	A year

3.5 Results of retanning application

Retanning agent can improve the absorption of chrome agent and strengthen the combination of chromium and collagen fibre of crust leather, so calculating the absorption ratio of chromium is an effective way to evaluate the retanning capability of the retanning agent. Effect on the absorption of chromium of retanning agent was investigated and results were showed in Tab.2. It's clearly seen from Tab.2 that the use of PRA has an influence on the absorption of chromium. Comparing to the control procedure, absorption ratio of chromium increased from 65.09% to 90.54%. It is evident that PRA can form strong combination with chromium and collagen fibre, indicating excellent retanning properties.

Tab. 2 Absorption ratio of chromium

Samples	*Control*	*PRA*
ARC (%)	65.09	90.54

Tab. 3 Application Performances of PRA and PRF

Parameter	*Control*	*PRA*	*PRF*
Grain tightness	6	9.5	8
Fullness	5	9.5	8
Softness	8	8	9
Dyeing performance	10	9	9.5
Selective filling performance	—	9	8
Total scores	29	45	43

Application performances of PRA and PRF were studied and control experiment was designed. It's observed that adding of retanning agent can obviously improve subjective properties of crust leather, and PRA have a good retanning performance as PRF, a similar protein retanning agent, which has a good retanning performance bought from market. It's found that PRA has better capability especially on improvement of grain tightness, fullness and selective filling ability than PRF, when it is added between the processing of neutralization and fatliquoring.

4 Conclusions

In this study, we have demonstrated an efficient way of utilizing collagen hydrolysate extracted from chrome shavings. Graft copolymerization of collagen hydrolysate and acrylic monomers can produce valuable products used as protein retanning agent for leather making. Results of ninhydrin reaction, FTIR and DSC analysis showed that acrylic monomers were grafted onto the polypeptide chains of collagen hydrolysate with covalent bond, and graft copolymerization can improve thermal stability of collagen hydrolysate. Application experiments showed that the protein retanning agent can provide some good organoleptic properties such as grain tightness, fullness and good selective filling performance for wet-blue leather.

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