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Utilization of vegetable tannins for stabilization of collagen fibers by using supported process enzyme

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Abstract

Tanning is the process of treating animal skins or hides to produce leather, which is more durable and less susceptible to decomposition. The leather industry has been pushed to develop tanning systems that utilize natural products due to environmental concerns. Vegetable tanning is one of the oldest methods, but its usage is limited due to the high organic load in the effluent generated. This load is difficult to degrade and results in high biological and chemical oxygen demand. This study aimed to develop an eco-friendly tanning process for protein fibers using proteolytic enzymes. The goal was to improve the exhaustion of vegetable tannins by increasing their uptake during the tanning process. The optimized experimental process, which involved enzymatic treatment of protein fibers, resulted in an exhaustion of 97% compared to 83% in conventional vegetable tanning processes. Enzyme treatment before tanning also led to a slight improvement in hydrothermal stability, as well as slightly better physical properties of the resulting leathers compared to conventionally tanned leathers. The enzymatic process has the added benefit of reducing total solids (TS) and chemical oxygen demand (COD) loads in the identified streams by 80% and 33%, respectively.

Keyword: Leather Industry; Vegetable Tanning; Acid Protease; Total Solids; Chemical Oxygen Demand

1. Introduction

Skin collagen is used in leather making, clothing, footwear, industrial, and biomedical applications. To prepare skin for leather processing, unwanted material is removed and the skin is stabilized through pretanning operations. Post tanning stages are used to add aesthetic properties. To stabilize leather, traditional methods involve several stages including curing, soaking, liming, deliming, pickling, depickling, and tanning. This process can be accomplished with vegetable tannins or oligomers of various metals and aldehydes [1]. Tanning or stabilization strengthens collagen fibers, protecting them against biodegradation.

For millennia, vegetable tannins have been utilized to transform animal hides into leather. However, the advent of chrome tanning in 1858 began to displace vegetable tanning, due in large part to the versatility of basic chromium sulfate. Today, the majority of tanning processes utilize chromium salts. Basic chromium sulfates (BCS) are used to tan over 90% of global leather production, but the current commercial chrome tanning process only achieves approximately 50% to 70% chromium uptake [2]. The low chromium uptake leads to both material waste and ecological imbalances. Discharging wastewater with a chromium concentration exceeding 0.1-2 ppm violates international regulations [3], and even high-exhaust chrome tanning systems cannot achieve such low concentrations.

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There are concerns about the toxicity of chromium (III) [4], as well as the potential for its conversion to chromium (VI) in certain soil conditions [5]. Additionally, disposing of solid wastes and sludge containing chromium is proving to be a significant challenge [6].

In recent years, the widespread use of chrome tanning has come under inspection by environmental authorities in industrialized nations due to growing concerns about its impact on health and the environment. This has prompted a search for more ecologically friendly tanning materials and methods. As a result, tanning processes that utilize vegetable tannins are once again gaining attention and importance.

Vegetable tanning agents used for processing various types of leather are extracted from water-soluble, non-crystalline plant materials such as wood, bark, leaves, and roots [7]. These agents, known as vegetable tannins, have a distinctive astringent quality. Chemically, tannins consist of a mixture of several molecular species [8], and tannin extract is a complex blend of polyphenols. Tannins typically have a molecular weight range of 500 to 3,000 Daltons [9] and contain a sufficient number of hydroxyl and other functional groups, such as carboxyl, to effectively form strong complexes with proteins and other macromolecules [10-11]. Despite being natural materials, vegetable tanning agents have been reported to be slow to biodegrade. New processing technologies, such as rapid drum tannages, have been developed to accelerate the vegetable tanning process for the production of medium and light vegetable-tanned leathers. While drum processes use less water than pit processes, they generate effluent that contributes to high levels of biological oxygen demand (BOD), chemical oxygen demand (COD), and suspended solids due to the high concentration of tanning liquor used. Due to the limited biodegradability of tannins, treated wastewater can retain residual color, leading to concerns from the public. Additionally, the effluent can contain phenolic compounds, which present further environmental challenges. Due to increasingly strict regulations on effluent discharge both in Sudan and globally [3], it has become necessary to find ways to fully utilize vegetable tannins and reduce the environmental impact of the tanning process. Several methods have been proposed to achieve this goal, such as pickle less vegetable tanning [12], oxidative degradation of tannin liquors using hydrogen peroxide [13-14], precipitation using zinc sulfate [15], and oxidative detoxification of bark extracts [16]. These methods aim to reduce the pollution load in effluents and promote a more sustainable tanning industry. The methods reported so far have not achieved complete exhaustion of vegetable tannins or near-zero emissions. Therefore, there is a need to develop effective strategies for improving the exhaustion of vegetable tannins in the stabilization tanning process and addressing the associated environmental issues.

In this current study, an enzymatic pretreatment was employed before the vegetable tanning process using a commercial vegetable tanning agent (Garad) at an initial pH of 4.5. It has been reported that vegetable tannins have an optimal particle size for better penetration into the matrix at pH 4.5 to 5.0 [17-18].

The objective of this work is to enhance the exhaustion of vegetable tannins in the stabilization tanning process by treating the collagen fiber matrix with acid protease. This study compares the uptake of vegetable tannin, shrinkage temperature of the stabilized collagen, chemical oxygen demand (COD), Total solids (TS), and characteristics of stabilized collagen (leathers) with a control process.

2. Materials and methods

2.1. Materials

Cattle skins with more compact structure and of larger area were selected. Chemicals used for leather processing were of commercial grade, and the chemicals used for the analysis of spent tan liquors were of analytical grade. The acid protease enzyme used, which is of technical grade, was obtained from local chemical company.

2.2. Experimental methods

Table 1 display the experimental part carried out at different conditions of enzymatic treatment before tanning the collagen fibers, and subsequent to the enzyme treatment tanning process was carried out with 30% vegetable tannins of *Acacia nilotica* subspecies *tomentosa* pods (garad).

Six pieces of cattle skin were treated with acid protease enzyme at six different concentrations (0.10%, 0.20%, 0.30%, 0.40%, 0.50%, and 0.60% based on shaved weight of wet blue leather) at pH 4.5 for 40 minutes to evaluate the effect of enzyme concentration. Subsequently, all six pieces were tanned with 30% *Acacia nilotica* subspecies *tomentosa* pods (garad) for 4 hours. The pH of the pelts was adjusted to 3.7 using 0.4% formic acid, and the pelts were piled for 24 hours."

Six additional pieces of cattle skin were treated with 0.30% acid protease enzyme for 40 minutes, at different pH conditions (3, 3.5, 4, 4.5, 5, and 5.5%) to assess the influence of pH. Subsequently, these six pieces were tanned with 30% *Acacia nilotica* subspecies *tomentosa* pods (garad) for 4 hours, and the pH of the pelts was adjusted to 3.7 using 0.4% formic acid before being piled for 24 hours. To estimate the effect of time Six additional pieces of cattle skin were treated with 0.30% acid protease at different interval 20,30,40,50,60, &70 minutes. Subsequently, these six pieces were tanned with 30% *Acacia nilotica* subspecies *tomentosa* pods (garad) for 4 hours, and the pH of the pelts was adjusted to 3.7 using 0.4% formic acid before being piled for 24 hours. In the control process six pieces of cattle skins were subjected to conventional vegetable tanning process as mentioned in Table 1 without any enzymatic treatment.

Table 1 Experimental part of tanning and post-tanning methods

Process Parameter	**Quantity, %**	**Chemicals**	**Duration (min.)**	**Remark**
Pickling	100%	water		
	10%	Sodium Chloride	25	pH 4.5
	1%	Sulphuric acid	30	
Enzymes treatment				
Batch 1	Treatment at different concentrations 0.10%, 0.20%, 0.30%, 0.40%, 0.50%, and 0.60% at pH 4.5, 40 min			
Batch 2	treatment under six different pH conditions 3, 3.5, 4, 4.5, 5, and 5.5 at 0.30% acid protease enzyme for 40 minutes			
Batch 3	treatment under six different time 20,30,40,50,60, &70 minutes at 0.30% acid protease enzyme offer for 40 minutes			
Process Parameter	**Quantity, %**	**Chemicals**	**Duration (min.)**	**Remark**
Tanning	100%	Water	60	
	5%	Pretanning syntan	60	Check penetration/pH 3.7
	15%	Garad Tannin	180	
	15%	Garad Tannin	50	
	2%	Formic acid		
	20%	water		
The leathers were then a squeezed, split, and shaved to uniform thickness				
Process Parameter	**Quantity, %**	**Chemicals**	**Duration (min.)**	**Remark**
Stripping	100%	Water		
	1%	Sodium sulphate	40	Drain/wash/drain
	1%	Borax	40	
Bleaching	100%	Water	50	Drain/wash/drain
	1%	Oxalic acid		
Retanning/Dyeing/Fatliquring	100%	Water		Drain/wash/drain
	1%	Acrylic syntan	30	
	2%	Phenolic syntan	40	

	2%	melamine	40	
	3%	Formaldehyde syntan	50	
	2%	Synthetic Fatliquore	40	
	3%	Dye	40	
	3%	Semi Synthetic fatliquore	40	
	2%	Formic acid	20	
Leathers piled overnight; next day set, hooked to dry, staked, trimmed, buffed, and finished.				

2.3. Physical Analysis of collagen

2.3.1. Measurement of Hydrothermal stability

The thermal properties of tanned collagen fibers were investigated by using Theis's shrinkage meter. The temperature at which the collagenous fiber shrinks to one third of its original length is noted as the shrinkage temperature of the fiber. [19].

2.3.2. Measurement of Tensile strength and percent of elongation

Measurements of tensile strength, percent of elongation, tear strength, and grain crack strength were examined using an Instron testing machine with the standard procedures (IUP 6 2000; IUP 8 2000; IUP 9 2000). The specimen length was 1 cm and the elongation rate used was 0.5 cm min^{-1}. The tensile strength and percent elongation at room temperature were calculated.

Tensile strength = (Maximum breaking load)/ (Cross sectional area)

The Percent of Elongation at Break was measured according to the society of leather technologist and chemists [20].

Calculation

Elongation, %= (Final free length-Initial free length)/ (Initial free length)

2.4. Analysis of Spent Tan Liquor

The spent tanning liquors from both the experimental and control groups were collected and subjected to analysis for chemical oxygen demand (COD), chlorides, and total solids (TS). These analyses were carried out in accordance with the standard procedures outlined in [21], with the TS being dried at 103°C to 105°C for one hour.

2.5. Analysis of Exhaustion of Vegetable Tanning Spent Liquors

The percentage uptake of vegetable tannins in the spent tanning liquors was determined using the method outlined by [21]. After filtering the waste liquor, the amount of tannins present was analyzed using the same procedure mentioned above. The exhaustion was then calculated using the following formula:

$$\% \text{ exhaustion } = \frac{\text{amount of tannins offered } - \text{amount present in the effluent}}{\text{tannins offered}} \times 100$$

3. Results and Discussions

Garad pods (*Acacia nilotica* ssp *Tomentosa*) is mixed type of vegetable tannin (Condensed-Hydrolysable) widely used for the stabilization of collagen in leather making in Sudan. Mixed tannins of plants are phenolic polymers comprising catechin and gallic acid [22]. Garad pods polyphenols are shown to possess anticarcinogenic effects [23-24]. Mixed tannins comprise of a group of polyhydroxyflavan-3-ol oligomers and polymers linked by carbon-carbon bonds between

flavanol subunits may be different combinations of hydroxyl and Hydrogen groups that lead to different classes of flavanol polymers [9]. The polyphenolic compounds containing sufficient hydroxyls and other suitable groups (carboxyl) to form effectively strong complexes with protein and other macromolecules [10-11]. Tanning effect principally depends on the thermodynamic stability of the cross-linking bonds & degree of cross-linking between the collagen molecules [25]. Hides and skin of animal commonly have a considerable size dimension; hence, diffusion of tannins materials (penetration) is likewise vital for describing the course of interaction. Only complete penetration and uniform distribution of tanning materials along the hide's cross-section will lead to a satisfactory tanning effect.

Table 1 shows the tanned pickled skin and the results of optimization tests for parameters such as enzyme concentration, pH, and duration.

Table 2 shows the effects of acid protease treatment on the exhaustion of vegetable tannins and hydrothermal stability of tanned leather at various concentrations. It is observed that higher offers of acid protease result in greater uptake of vegetable tannins by the pelt. However, at 0.40% concentration, there is no significant improvement in the fixation of vegetable tannins or the hydrothermal stability.

Table 2 Exhaustion of vegetable tannins and Hydrothermal stability of the enzyme treated leathers

	Process Parameters						
	Control	**Concentration**					
		0.10%	0.20%	0.30%	0.40%	0.50%	0.60%
Vegetable tannins Exhaustion %	83±3.4	85±0.7	88±1.5	93±2.3	97±1.6	97±2.2	97±2.3
Hydrothermal stability, T_s (^{o} C)	81±2.1	81±0.9	83±1	85±2	87±2	87±2	87±2
		pH					
		3	3.5	4	4.5	5	5.5
Vegetable tannins Exhaustion %		81±0.8	85±1.6	90±0.7	93±0.9	90±1.3	89±1.4
Hydrothermal stability, T_s (^{o} C)		80±0.8	84±0.9	86±1.1	87±1.3	87±1.3	87±1.3
		Duration/minutes					
		20	30	40	50	60	70
Vegetable tannins Exhaustion %		91±0.5	93±0.9	95±1	97±1.1	97±1	97±0.9
Hydrothermal stability, T_s (^{o} C)		83±0.8	85±0.9	86±1	87±1	87±1	87±1

Higher concentrations of acid protease can be detrimental to the pelts and result in weaker leather. Therefore, a 0.40% offer is deemed sufficient for achieving maximum uptake of vegetable tannins and has been chosen as the optimized concentration for better exhaustion of vegetable tannins.

At this optimized concentration of 0.40%, the exhaustion of vegetable tannins was found to be 97% and the resulting shrinkage temperature of the leather was 87 °C.

Again Table 2 illustrates the exhaustion of vegetable tannins and shrinkage temperature, both increase gradually up to pH 4.5, but a further increase in pH causes a decrease in the exhaustion of vegetable tannins. The acid protease used in this study has a pH of 4.5, which is also the pH of its maximum activity. As a result, the highest uptake of vegetable tannins and shrinkage temperature are observed at pH 4.5. At the optimized pH of enzymatic treatment, acid proteases are able to open up the fiber structure and facilitate greater diffusion of vegetable tannins into the collagen matrix,

resulting in maximum uptake. Therefore, pH 4.5 at a concentration of 0.40% acid protease has been identified as the optimal combination for achieving better exhaustion of vegetable tannins.

Table 2 shows the percentage exhaustion of vegetable tannins at different time intervals and indicates that the uptake of vegetable tannins increases gradually with time. The table suggests that it takes a maximum of 50 minutes to achieve significant exhaustion in the tanning bath, while at higher time intervals, the fixation of vegetable tannins is more gradual.

To optimize the processing of acid protease, it is not ideal to increase the time intervals as it may lead to damage of the pelt. Therefore, it has been determined that a 50-minute enzymatic treatment is the best duration for processing. When acid protease was used for 50 minutes, it resulted in a vegetable tannins uptake of 97%. To achieve better exhaustion of vegetable tannins, the optimized pH, concentration, and time for acid protease treatment are pH 4.5 at 0.40% concentration for 50 minutes. Improved exhaustion of vegetable tannins will reduce pollution and result in high-quality leathers at a reduced cost of production. This study is based on the understanding that vegetable tannins are particulates with a higher molecular weight (ranging from 300 to 5,000 Daltons) and can penetrate rapidly after enzymatic treatment due to the opening up of the collagen fiber matrix in the pelt. The opened-up fiber matrix of the pelt, combined with the leather's pH, enables proper penetration of vegetable tannins while preventing surface fixation on the skins. To fix the well-penetrated vegetable tannins, a minimal amount of formic acid is used to adjust the pH to 3.5-3.7.

Analyzing the strength characteristics of leather after treatment with enzymes is vital because the opening up of the fiber structure may affect the leather's strength. To determine the effects of treatment on the leather's strength, matched pair control and optimized experimental crust leathers were subjected to tensile, tear strength, and grain crack tests both along and across the backbone line. Table 3 presents the average values of the various strength properties for both the optimized experimental leathers and the control leathers. While the optimized experimental leathers had slightly lower values, their strength properties were still comparable to those of the control leathers.

Table 3 Physical characteristics of the enzyme treated leathers and control

Samples	**Tensile strength, (Kg/cm^2)**	**Elongation at break, %**	**Tear strength, (Kg/cm)**
Control	210±5	60±4	50±6
Optimized Experiment	207±8	75±8	62 ±5

3.1. Role of Enzymes in Reducing Pollution Load

The spent vegetable tan liquor is highly polluting, containing significant amounts of COD, dissolved and suspended solids. Therefore, it is crucial to evaluate the environmental impact of both the conventional and enzymatic vegetable tanning processes. To evaluate the environmental impact of the tanning processes, a composite liquor was created by combining spent liquors from both the pickling and vegetable tanning processes. It is important to note that the optimized experimental vegetable tanning process included enzymes in the spent vegetable tan liquor. Parameters such as COD, TS, chlorides, and percentage uptake of vegetable tannin were chosen to assess the environmental impact.

Table 4 Environmental impact of the enzyme treated leathers and control

Parameters	**Control**	**Enzyme treated leathers**
Up take of tannins, %	83±3.4	97±2.2
Chemical oxygen Demand (COD)	24120±200	16100±300
Total Solids (TS)	94430±800	18600±900
Effluent Volume, liter	630±2	615±1
Emission Load (kg/metric ton of raw skin)		
Chemical oxygen Demand (COD)	15.5±0.8	11.6±0.3
Total Solids (TS)	52.4±2	9.7±1

liquor have significantly reduced when compared to the conventional tanning process. To better understand the consequences of Chemical oxygen Demand / Total Solids (COD/ TS) values, they have been converted into emission loads by multiplying them with the volume of effluent per metric ton of raw skins processed. The calculated emission loads and the Chemical oxygen Demand (COD) and Total Solids (TS) values are presented in Table 4. One of the main challenges faced by tanners is the large number of total solids emitted. The optimized experimental tanning process has resulted in a significant reduction in both Chemical oxygen Demand and Total Solids values of the spent tan liquor when compared to the conventional tanning process.

The conventional processing of 1 metric ton of raw skins produces about 50 kg of total solids (TS) through the partial pickling and vegetable tanning processes. However, the use of enzymatic vegetable tanning method can reduce the chemical oxygen demand (COD) and Total solids (TS) loads by 33% and 80% respectively. This reduction leads to a cleaner vegetable tanning process. Additionally, the optimized experimental process for vegetable tanning shows a higher uptake of vegetable tannin compared to the control process. Therefore, enzymatic vegetable tanning has the potential to revolutionize the tanning industry by promoting environmental sustainability through the use of natural materials for natural leathers.

4. Conclusions

Enzymes are used in tanning as an eco-friendly method to improve the exhaustion of vegetable tannins. This approach utilizes enzymes as biocatalysts to open up the fibrous collagen network, allowing for better diffusion of vegetable tannins into the collagen matrix. This also increases the contact surface area in the collagen matrix, promoting interaction with vegetable tannins. An optimal condition for the uptake of vegetable tannins and shrinkage measurements was achieved through the use of 0.40% enzyme at a pH of 4.5 for 50 minutes in the presence of 30% vegetable tannins. The resulting enzymatically processed stabilized collagen matrix demonstrated higher hydrothermal stability compared to conventionally processed materials. Compared to the control process, the enzymatic vegetable tanning method results in an 80% reduction in Total solids (TS) and a 33% reduction in chemical oxygen demand (COD) loads while achieving better uptake of vegetable tannins. This approach has great potential for decreasing pollution in the tanning industry.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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