



Sustainable Enzymatic Dehairing of Cowhide Using Pineapple-Derived Bromelain

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Abstract: The leather industry traditionally employs lime and sodium sulfide for unhairing, generating highly alkaline wastewater and significant environmental pollution. This study evaluates the potential of bromelain extracted from different parts of pineapple (*Ananas comosus*) crown, fruit, peel, and leaves as an eco-friendly alternative for enzymatic unhairing of cowhide, and compares its performance with the conventional chemical method. Cowhide samples were treated with crude bromelain extracts under controlled conditions, and their performance was assessed based on weight loss, hair removal efficiency, chemical oxygen demand (COD), total dissolved solids (TDS), and pH changes in the treatment liquor. The conventional process showed a weight loss of 10.83% and COD of 7285 mg/L, with complete and easy hair removal. Among enzymatic treatments, leaf extract without purification demonstrated the highest weight loss (22.98%) and effective hair removal; however, excessive weight loss may indicate partial collagen degradation. Purified extracts generally exhibited lower enzymatic activity and reduced unhairing efficiency. Enzymatic treatments operated under mildly acidic conditions (pH 4.08–5.60) compared to the highly alkaline conventional process (pH 12.58), indicating improved environmental compatibility. The results suggest that crude bromelain, particularly from pineapple leaves; can serve as an effective and sustainable alternative to sulfide-based unhairing in leather processing.

Keywords: Bromelain, cleaner production, enzymatic dehairing, pineapple waste valorization, proteolytic enzymes, sulfide-free dehairing, sustainable leather processing

1 INTRODUCTION

The pineapple plant is rich with a mixture of proteolytic enzymes that have the ability to break down the proteins into amino acids by hydrolyzing their peptide bonds. Protease distribution varies among pineapple tissues depending on plant maturity, extraction conditions, and cultivar. The major proteolytic enzymes present in pineapple are cysteine proteases. Bromelain is a mixture of cysteine proteases present in pineapple tissues [1]. The leather industry is one of the most significant sectors in many countries due to its economic and cultural significance. The global market for leather products, according to a report by Grand View Research was estimated to be valued USD 242.85 billion in 2022 and is expected to grow at a CAGR of 6.6% from 2023 to 2030. There are several steps involved in the production of leather, including curing, soaking, dehairing, fleshing, deliming, pickling, and tanning. Among these steps, dehairing is an important step that removes hair and other unwanted substances from the hide [2].

The traditional unhairing process includes lime ($\text{Ca}(\text{OH})_2$) and sodium sulfide (Na_2S) that ultimately produces hazardous effluent containing sulfide ions that present serious environmental and health risks.

Disposal of lime-sulfide effluent waste is difficult and generates environmental issues of soil alkalization, water contamination, and release of hydrogen sulfide gas. Because of the environmental risks, the traditional unhairing process is not sustainable. Protease enzymes extracted from pineapple can be applied as an alternative to conventional chemical unhairing.

Eco-friendly enzymatic unhairing has become a viable alternative to conventional chemical unhairing to solve these issues. It is an effective technique that utilizes enzymes to break down and dissolve the proteins in the hair, leaving the hides clean and ready for tanning. Enzymes are biodegradable, and their use in the leather industry can reduce pollution caused using harsh chemicals. The most used unhairing enzymes are proteases such as bromelain because they hydrolyze peptide bonds in non-keratinous proteins associated with hair follicles and epidermal tissues, in animal hide [2].

2 METHODOLOGY

This study was conducted to extract bromelain enzyme from different parts of pineapple and evaluate its potential application in the unhairing process of the leather industry. The extracted enzyme was tested for its effectiveness in hair removal from cowhide, and several physicochemical parameters were analyzed to assess the treatment process. The methodology adopted for the study is described below.

a) Extraction of bromelain enzyme from pineapple

The fruit, crown, peel and leaves of pineapple were separated, cleaned and dried properly. The samples were then cut into small pieces separately for the extraction of enzyme. Then each part was weighed and 200 g of each pineapple part was blended with 250mL of phosphate buffer (0.05M, pH 7). After blending, the mixture was filtered using cheese cloth. The filtrate was then centrifuged at 4000 rpm for 20 min at 4°C. supernatant containing crude bromelain extract was collected and stored at -20°C for further use [4].

b) Use of bromelain for unhairing in leather industry

The cowhide pieces were collected from the tannery. Then, the cowhide was cut into pieces measuring 3 cm × 3 cm and labeled as treatment I and treatment II using markings on the flesh side. The first treatment was a conventional method. In this process, a piece of hide was immersed in a solution containing distilled water, 5% calcium oxide (CaO) (w/w), and 2% sodium sulfide (Na₂S) (w/w). The second treatment was an enzyme treatment. In this treatment, Crude enzyme extract was added at a hide-to-liquor ratio of 1:10 (w/v). The ease of unhairing was checked after each hour of enzyme treatment and kept in the shaker for 6 h. For the control, a piece of hide was dipped in distilled water. After 6 h, all samples were removed and kept overnight. Then after 24 h, the hide pieces were taken out and checked the ease of hair loosening [5].

c) Determination of the weight loss

The percent weight loss of the silk fibers after degumming process was calculated using equation (1).

$$\% \text{ Weight loss} = (W_0 - W_1) / W_0 \times 100 \quad (1)$$

Where, W_0 and W_1 are the weight of the silk fiber samples before and after degumming.

d) Determination of pH value and TDS (Total Dissolved Solid) value

The pH meter was used to measure pH in prepared solution for unhairing treatment. The TDS meter was used to measure TDS (Total dissolved solid) value in solution used treatment. Their values were checked before and after unhairing treatment process.

e) Determination of COD (Chemical Oxygen Demand) value

The solution was prepared for fill the vial. For this, 12g of potassium dichromate was dissolved in 10ml of distilled water. 50ml of solution was taken and dissolved it with 150ml of concentrated sulfuric acid. Potassium dichromate was added to sulfuric acid. This mixture was kept at 1 hour in a container of cold

water. 5ml of this mixture was filled into well- cleaned vials. Then, 2ml from the sample was added to the vial. A vial was filled with distilled water to obtain a blank value. Then these vials were placed in the COD reactor at 150 °C for about 2 hours. The COD values were then measured using a colorimeter after cooling. COD analysis was performed according to APHA standard methods [5].

3 RESULTS AND DISCUSSION

The unhairing process is a crucial step in leather manufacture, involving the removal of hair and epidermal layers from cowhide. Conventionally, this is achieved using harsh chemicals such as sodium sulfide and lime, which are effective but environmentally harmful. The present study compares the conventional chemical unhairing method with enzymatic unhairing using crude and purified bromelain extracts obtained from different parts of the pineapple crown, fruit, peel, and leaves.

Table 1. Unhairing of cowhide with conventional and enzyme treatment

Sample	Before Weight (mg)	After Weight (mg)	Weight loss %	COD mg/L	Hair removing
Conventional	2.0509	1.8287	10.83	7285.0	Soft, hair can remove easy
Crown NP	1.5635	1.3151	15.89	3520.0	Hair can remove, but not easy as conventional sample
Crown P	1.206	1.1595	3.86	6545.0	Hair remove is difficult
Fruit NP	1.9075	1.6512	13.44	11100.0	Hair can remove easy
Fruit P	1.2476	1.2109	2.94	12355.0	Hair cannot remove
Peel NP	1.4045	1.3779	1.89	5215.0	Hair can remove, but not easy as conventional sample
Peel P	1.5466	1.5319	0.95	10410.0	Hair remove is difficult
Leaves NP	1.6583	1.2773	22.98	5150.0	Hair can remove easily than crown NP
Leaves P	1.472	1.4585	0.92	3185.0	Hair remove is difficult

a) Hair Removal Efficiency

The conventional sample showed complete and easy hair removal, producing a soft and clean hide surface. This effectiveness results from the strong chemical reaction of sulfide and lime that disrupts disulfide bonds in keratin and weakens hair follicle structures. The unhairing of cowhide using conventional and enzyme treatments was also analyzed and compared. Enzyme-treated samples showed more selective and milder action. Among the crude enzyme treatments, the fruit NP displayed the best unhairing ability, where the hair could be easily removed, closely resembling the conventional sample. The leaves NP extract also showed good hair removal, though slightly less efficient. The crown NP and peel NP extracts could remove hair but not as easily as the chemical treatment, indicating moderate enzyme activity. However, hair removal was generally more difficult in purified bromelain extracts. It is also possible that the purified extracts had lower activity under the specific pH or temperature conditions used [6, 7].

b) Weight Loss Percentage

Weight loss during unhairing indicates how much of the hide material mainly proteins and fats were removed during the process. Excessive weight loss implies collagen degradation and lower leather strength,

while very low weight loss may indicate incomplete unhairing. The weight loss percentages for all enzyme treatments are shown in Table 1. The highest weight loss (22.98%) was observed in the leaves NP sample, suggesting that the crude enzyme from leaves acted strongly on both non-collagenous proteins and hair root structures. Although this enhanced hair removal efficiency, it may risk partial damage to the hide surface if not properly controlled. The crown NP (15.89%) and fruit NP (13.44%) samples also exhibited considerable weight loss, reflecting moderate enzymatic activity. These results suggest that crude extracts from different pineapple parts possess varying protease concentrations and substrate affinities. In contrast, the purified extracts produced very low weight losses, ranging from 0.92% (leaves P) to 3.86% (crown P). This indicates minimal degradation of the hide and high preservation of collagen structure. However, such low weight loss corresponds with poor hair removal efficiency, implying that the enzyme activity was insufficient to loosen the hair follicles effectively [7].

c) COD (Chemical oxygen demand) in Wastewater after treatment

COD (Chemical Oxygen Demand) measures the number of organic pollutants in wastewater, indicating its environmental load. The COD values for all samples obtained from conventional and enzyme-based unhairing of cowhide, using enzymes extracted from different parts of the pineapple, are presented in Table 1. The conventional process produced a COD of 7285 mg/L, reflecting the high organic and chemical contamination typically associated with sulfide-based unhairing. Such high COD values contribute to serious environmental pollution, requiring costly wastewater treatment. Enzyme-based unhairing showed significant differences in COD levels depending on the extract type. The lowest COD (3185 mg/L) was recorded for the leaves P (purified) extract, followed by crown NP (3520 mg/L). These values are far lower than those of the conventional process, demonstrating the potential environmental advantages under optimized conditions of enzymatic treatment. Since enzymatic unhairing releases intact hair instead of dissolving it, less organic matter enters the effluent. However, the fruit NP (11100 mg/L) and fruit P (12355 mg/L) samples recorded the highest COD values among all treatments. This unusually high COD might be due to the presence of residual proteins, peptide, and unreacted enzyme components released into the water [6,7].

d) pH change in unhairing of cowhide

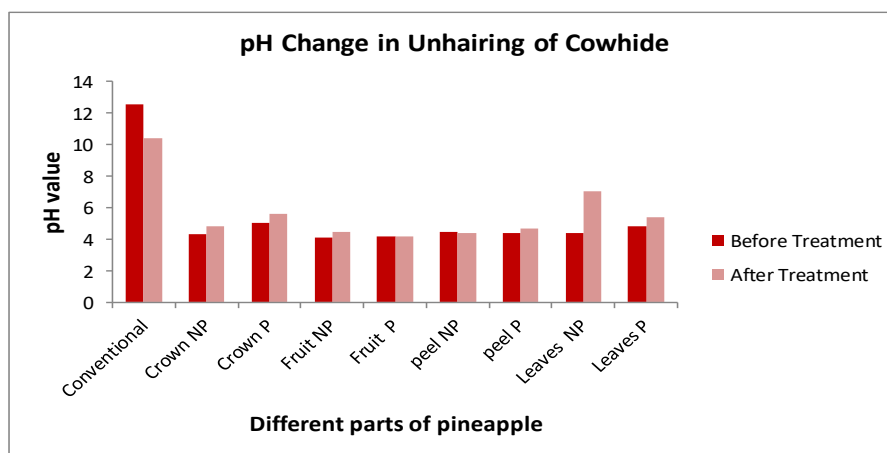


Fig. 1. pH change in Unhairing of cowhide with conventional and enzyme treatment
Table 2. pH change in Unhairing of cowhide with conventional and enzyme treatment

PH	Before Treatment	After Treatment
Conventional	12.58	10.44
Crown NP	4.34	4.83
Crown P	5.03	5.60
Fruit NP	4.08	4.45
Fruit P	4.16	4.18
peel NP	4.50	4.37
peel P	4.39	4.69
Leaves NP	4.39	7.04
Leaves P	4.86	5.43

The pH changes before and after treatment indicates the chemical nature and effectiveness of the unhairing process. In the conventional method, the pH dropped from 12.58 to 10.44, showing a reduction in alkalinity due to reaction with skin proteins and chemicals used. In contrast, all enzyme-treated samples maintained near-neutral to slightly acidic pH values, ranging between 4.08 and 7.04, which are milder and environmentally safer. The slight pH increases after enzyme treatment (e.g., Crown P 5.03–5.60, Leaves NP 4.39–7.04) suggest enzymatic activity during unhairing without harsh chemical reactions [7,8]. The pH variation in conventional and enzyme treatments is illustrated in Fig. 1, while the corresponding values are presented in Table 2.

e) TDS (Total Dissolved Solid) change in unhairing of cowhide

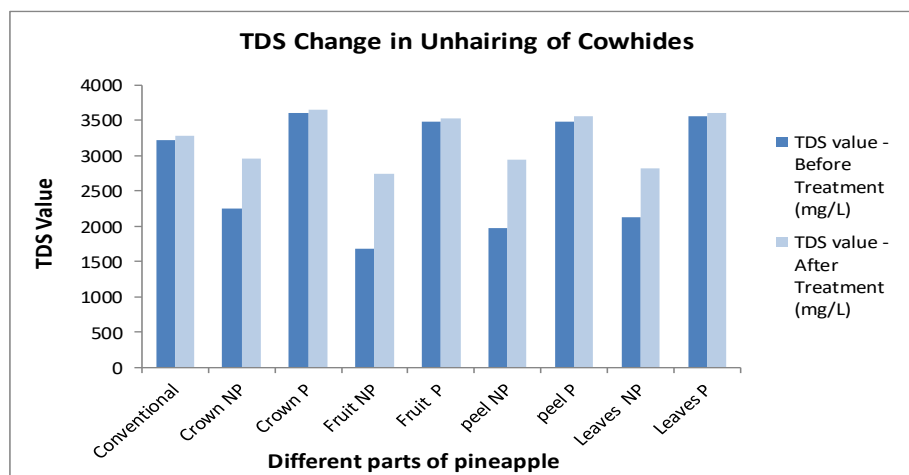


Fig. 2. TDS change in Unhairing of cowhide with conventional and enzyme treatment

The Total Dissolved Solids (TDS) values before and after unhairing show the level of dissolved substances in each treatment solution. In the conventional method, TDS increased slightly from 3212 to 3287 mg/L, indicating the release of hair residues, chemicals, and protein materials during treatment. In enzyme-based samples, TDS values also rose after treatment due to the enzymatic breakdown of skin proteins and organic matter. However, the increase was moderate compared to chemical methods, suggesting a cleaner process. Purified enzyme samples (Crown P 3597–3642.5 mg/L, Peel P 3487–3552 mg/L) showed controlled increases, confirming efficient unhairing with minimal waste load [6,7,8]. The variation in TDS values

during conventional and enzyme-based unhairing is illustrated in Fig. 2, while the corresponding data are presented in Table 3.

Table 3. TDS change during Unhairing of cowhide

Sample	TDS value -Before Treatment (mg/L)	TDS value -After Treatment (mg/L)
Conventional	3212.0	3287.0
Crown NP	2252.0	2960.0
Crown P	3597.0	3642.5
Fruit NP	1677.0	2741.0
Fruit P	3487.0	3530.0
peel NP	1968.0	2936.5
peel P	3487.0	3552.0
Leaves NP	2121.0	2819.0
Leaves P	3552.0	3597.0

4 CONCLUSION

This study demonstrated the potential effectiveness of bromelain extracted from different parts of pineapple (*Ananas comosus*) for enzymatic unhairing of cowhide. Among the tested samples, crude leaf extract demonstrated the highest weight loss and efficient hair removal, indicating strong proteolytic activity. Compared to the conventional lime–sulfide process, enzymatic treatments operated under milder pH conditions and showed potential for reducing environmental pollution. However, variations in COD and TDS values highlight the need for process optimization. Future research should focus on improving enzyme extraction efficiency, stabilizing enzyme activity, and optimizing treatment conditions such as concentration, temperature, and reaction time. Scaling up the process and evaluating its economic feasibility and long-term environmental benefits are also recommended to support industrial application of enzymatic unhairing.

5 REFERENCES

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