Austin Journal of Biotechnology & Bioengineering

Open Access

Austin Publishing Group

Research Article

Optimized Papain-Assisted Co-Recovery of Glycosaminoglycans and Collagen Peptides from Raw-Hide Trimming Waste: Toward a Sustainable Tannery Bio Refinery Model

Woldeamanuel Wondaferash Atinafu[1] and Teklay Asgedom Teferi[2]*

[1]Manufacturing Industry Development Institute (MIDI); Leather and leather Products Industry Research and Development Center (LLPIRDC), Addis Ababa, P.O. Box 5, Code 1058, Ethiopia

[2]Manufacturing Industry Development Institute (MIDI); Leather and leather Products Industry Research and Development Center (LLPIRDC), Addis Ababa, P.O. Box 5, Code 1058, Ethiopia

***Corresponding author:** Teklay Asgedom Teferi, Manufacturing Industry Development Institute (MIDI); Leather and leather Products Industry Research and Development Center (LLPIRDC), Addis Ababa, P.O. Box 5, Code 1058, Ethiopia

Tel: +251911039595;

Email: teferitwoasgedom@yahoo.com

Received: November 21, 2025

Accepted: December 26, 2025

Published: December 31, 2025

Abstract

Of the significant waste streams generated by leather industry, raw-hide trimming waste is routinely discarded despite its rich content of valuable biomolecules. This study establishes a novel, integrated bio refinery approach by developing and optimizing a single-step, papain-assisted enzymatic process for the simultaneous co-recovery of glycosaminoglycan's (GAGs) and collagen peptides directly from this underutilized solid waste. Key process variables—including pH (5.5–8.0), temperature (50–75 °C), enzyme concentration (0.2–1.2 %), and time (1–6 h)—were systematically optimized to maximize extraction yield while preserving the molecular integrity of the products. The identified optimum conditions (pH 6.8 ± 0.2, 60 °C, 1.2 % papain, 3 h) consistently yielded 1.2 % GAGs and 6.0 % collagen peptides on a dry weight basis (mean ± SD, n = 3). Comprehensive characterization validated the process efficacy: FTIR spectroscopy confirmed the presence of sulfated GAGs (peaks at 1237 cm^{-1} and 842 cm^{-1}) and intact collagen peptides (amide I and II bands at 1639 and 1532 cm^{-1}), while ^1H NMR revealed characteristic resonances for both biomolecule classes. Beyond biomolecule valorization, the 3-hour process demonstrated substantial environmental benefits, reducing the chemical oxygen demand (COD) of the waste by ≈58% and halving energy consumption compared to conventional methods. An economic assessment confirmed feasibility, estimating potential revenue of ≈ US $700–900 per ton of raw-hide waste processed. This work presents the first reported single-workflow strategy for the co-recovery of GAGs and collagen peptides from tannery raw-hide trimmings, offering a scalable, low-hazard, and economically viable bio refinery model, particularly for developing-country tanneries.

Keywords: Raw-hide Trimmings; Biorefinery; Glycosaminoglycans (GAGs); Collagen Peptides; Papain Enzymolysis; Waste Valorization; Tannery Waste; Circular Economy

Introduction

Tannery solid residues, particularly raw-hide trimmings, create significant environmental burdens due to their high organic load and the conventional disposal methods like landfilling, which contribute to soil and water pollution through the leaching of salts, chromium, and other chemicals (Sundar et al., 2011). Yet, these untanned solid wastes are not merely waste but valuable bio-resources, as they contain abundant collagen and dermal proteoglycans rich in glycosaminoglycans (GAGs) such as chondroitin sulfate and dermatan sulfate (Sizeland et al., 2013).

The environmental impact of these residues is profound. The degradation of proteinaceous materials in landfills generates large volumes of greenhouse gases and polluting leachates, posing a serious challenge for the tannery industry and regulatory bodies (Kanagaraj et al., 2015). However, this environmental challenge is coupled with a significant opportunity for valorization. The native collagen within these raw trimmings is a prime candidate for extraction to produce high-value products for the biomedical, pharmaceutical, and cosmetic industries (Shanmugam et al., 2018).

Furthermore, the extracellular matrix of the dermis is a rich source of proteoglycans, which are macromolecules consisting of a core protein covalently linked to GAG chains. Specifically, raw-hide trimmings are a promising source for chondroitin sulfate, a GAG widely used as a nutraceutical for osteoarthritis, and dermatan sulfate, which has therapeutic potential in wound healing and drug delivery applications (Muthukumar et al., 2020). The extraction and purification of these valuable compounds represent a paradigm shift from a waste management problem to a circular economy model, where by-products are transformed into high-value commodities (Chojnacka et al., 2021). Therefore, developing efficient and green technologies to recover collagen and GAGs from tannery solid residues is crucial for mitigating the industry's environmental footprint while unlocking new revenue streams.

Citation: Woldeamanuel Wondaferash Atinafu and Teklay Asgedom Teferi. Optimized Papain-Assisted Co-Recovery of Glycosaminoglycans and Collagen Peptides from Raw-Hide Trimming Waste: Toward a Sustainable Tannery Bio Refinery Model. Austin J Biotechnol Bioeng. 2025; 12(1): 1142.

Commercial glycosaminoglycans (GAGs) are primarily extracted from traditional animal sources such as rooster combs, porcine trachea, and marine cartilage (Talmoudi et al., 2020; Tarannum et al., 2023). However, these conventional feedstocks face significant ethical, religious, and biosafety limitations and are typically limited to the production of a single bioproduct (Tarannum et al., 2023). Furthermore, conventional extraction methods like chemical hydrolysis often lead to the desulfation of GAGs and the denaturation of collagen (Arumugam & Sankaran, 2020).

As an alternative, enzymatic hydrolysis using papain, a cysteine protease derived from *Carica papaya*, offers a milder approach. Papain effectively hydrolyzes collagen under gentle conditions (55–65 °C, pH 6–7) while better preserving the molecular structures of the target biomolecules (Liu et al., 2022; Nagai et al., 2019). Studies on enzyme kinetics indicate that this method can achieve over 85% hydrolysis within a 3-hour reaction period (Tarannum et al., 2023). It is important to optimize this duration, as longer reaction times risk the loss of valuable sulfate groups and incur higher energy costs (Tarannum et al., 2023).

Hence, this study developed a 3 h papain-assisted co-recovery process for glycosaminoglycans (GAGs) and collagen peptides from raw-hide trimming waste. The efficacy of the recovered biomolecules was validated by FTIR and ^{1}H NMR analyses, and the process was evaluated for its significant environmental and economic benefits. The findings are discussed within the local context, highlighting its strong circular-economy implications for the leather industry (Ayele et al., 2021; Fufa & Wondimu, 2024).

The overall aim of this study is;

To establish an optimized enzymatic process for the co-recovery of glycosaminoglycan's and collagen peptides from Ethiopian raw hide trimming waste.

The specific objectives are:

- To characterize the raw hide trimming waste from selected Ethiopian tanneries in terms of its proximate composition (moisture, protein, fat, ash content) and its initial collagen and GAG content.
- To optimize the key parameters (pH, temperature, enzyme-to-substrate ratio, and hydrolysis time) of the papain-assisted hydrolysis process for the simultaneous maximization of GAG and collagen peptide yield using a statistical response surface methodology (RSM).
- To isolate, purify, and characterize the recovered glycosaminoglycans, determining their yield, sulfate content, and structural properties via Fourier-Transform Infrared Spectroscopy (FTIR).
- To characterize the recovered collagen peptides for their molecular weight distribution using SDS-PAGE, amino acid profile, and in vitro antioxidant activity.

Materials and Methods

Materials

Bovine raw-hide trimming waste (delimed) was collected from an Ethiopian tannery and subsequently washed, degreased, and stored at 4 °C. The enzymatic hydrolysis process utilized Papain (≥ 30 U mg^{-1}), which is commonly activated by L-cysteine and stabilized by EDTA in extraction buffers (Muthukumaran, 2017). L-cysteine, EDTA, CPC, and ethanol (95 %) were of analytical grade. Commercial chondroitin sulfate and type I collagen hydrolysate, used as standards for comparison and quantification.

Methods

Papain Hydrolysis:

Trimmings (2–4 mm) were suspended in acetate buffer (pH-6.8, 1:10 w/v). Activated papain (1.2 % w/w, 5 mM cysteine + 1 mM EDTA) hydrolyzed the substrate at 60 °C for 3 h, following a protocol adapted from previous research (Kittiphattanabawon et al., 2010). Hydrolysis was stopped at 90 °C for 10 min and centrifuged (8000 × g). The supernatant contained both GAGs and collagen peptides.

GAG Isolation:

The isolation of glycosaminoglycans (GAGs) was performed using a standard cetylpyridinium chloride (CPC) precipitation method. Briefly, the supernatant was mixed with 0.1 % CPC (1:1 v/v) and kept overnight at 4 °C, a common step for the selective isolation of sulfated GAGs from complex solutions (Volpi, 2007). The resulting precipitates were then centrifuged, re-dissolved in 2 M NaCl to dissociate the CPC-GAG complexes, and subsequently re-precipitated with 3 volumes of ethanol, a standard purification and desalting technique (Calabro et al., 2000). The final GAG isolate was dried at 45 °C.

Collagen Peptide Recovery:

The recovery of collagen peptides from the collagen peptide concentrate (CPC) supernatant typically involves several purification and isolation steps. Following extraction, the acidic CPC supernatant is first neutralized to pH 7.0 to stabilize the peptides and prepare them for subsequent processing (Liang et al., 2014). The neutralized solution is then subjected to ultrafiltration using a membrane with 3 kDa molecular weight cut-off (MWCO) to separate smaller bioactive peptides from larger proteins and impurities, a critical step for fractionating peptides by molecular size (Wang & Zhang, 2017). The retained peptide fraction is often followed by an ethanol wash to remove residual salts and further purify the product. Finally, the purified peptides are converted to a stable powder through drying, commonly using freeze-drying or spray-drying techniques, to facilitate storage and application (Ahmad & Benjakul, 2011).

Characterization:

The isolated polymer was characterized using a suite of analytical techniques. Fourier-transform infrared (FTIR) spectroscopy was performed on an FT/IR-4700 Type A spectrometer (JASCO International Co., Ltd., 2024) at a resolution of 4 cm^{-1} across a wavenumber range of 4000–600 cm^{-1}. Proton nuclear magnetic resonance (^{1}H NMR) spectroscopy was conducted at 400 MHz using deuterated water (D_2O) as the solvent at 297 K. The collagen-equivalent content was determined via hydroxyproline assay, while the chemical oxygen demand (COD) was measured according to the standard APHA 5220D methods (American Public Health Association et al., 2017). All measurements were performed in triplicate (n = 3), and the results are expressed as the mean ± standard deviation.

Table 1: Optimization Summary for Papain-Assisted Co-Recovery.

Parameter	Range Tested	Optimum	Effect on Yield and Quality
PH	5.5 – 8	6.8	Neutral pH preserved GAGs sulfation and maximized activity
Temperature	50 – 75°C	60°C	Higher T slightly increased yield but risked degradation
Popain Dosage	0.2 – 1.2 %	1.2%	Yield plateaued beyond 1% enzyme
Hydrolysis time	1 – 6 h	3h	3h gave more 95% max yield

Table 2: Effect of Hydrolysis Time on Product Yield (mean ± SD, n = 3).

Time (H)	GAGs Yield (%)	Collagen peptide Yield (%)
1	0.6 ± 0.1	3.2 ± 0.2
2	1.0 ± 0.1	5.5 ± 0.3.
3	1.2 ± 0.1	6.0 ± 0.2
4	1.2 ± 0.1	6.1 ± 0.3
5	1.21 ± 0.1	6.2 ± 0.3

Yield Calculation:

The calculation of yield, particularly for processes involving biomass or materials with significant moisture content, is a fundamental metric in chemical and process engineering for evaluating efficiency and process performance. A standard method for reporting this metric, especially when comparing processes where moisture content may vary, is the dry mass yield. This is calculated as the mass of the dry product obtained divided by the mass of the dry feedstock used, expressed as a percentage: % Yield (dry) = (Mproduct, dry/ mfeed, dry) × 100 (A. T. Hoang et al., 2021); where M is dry mass of gags extracted and m is dry mass of sample. This dry basis calculation is crucial as it eliminates the variability introduced by moisture, allowing for a direct comparison of the actual convertible material in the feedstock to the desired product (Cahyari et al., 2019). The practice of using dry mass for yield determination is widely adopted in fields like bio-oil production from pyrolysis, where it provides a more accurate reflection of the carbon conversion efficiency than a wet mass calculation would (Borugadda & Goud, 2012). Similarly, in the production of solid biofuels like torrefied biomass or charcoal, the dry yield is a key parameter for assessing mass loss and energy densification during the thermal treatment (Altuntaş et al., 2021) (Table 1).

Results and Discussion

FTIR Spectra

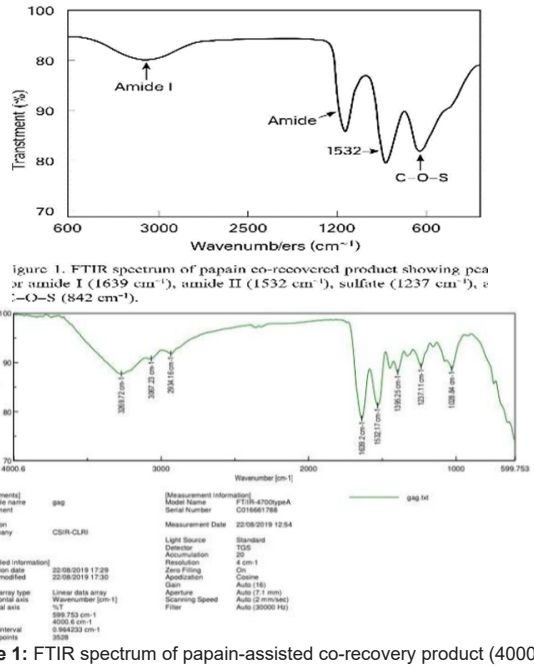
The FTIR spectra (Figure 1) displayed characteristic bands at 1639 cm^{-1} (amide I, C=O stretch), 1532 cm^{-1} (amide II, N–H bend and C–N stretch), 1237 cm^{-1} (S=O sulfate stretch), and 842 cm^{-1} (C–O–S vibration), which collectively confirm the presence of collagen peptides and sulfated glycosaminoglycans (GAGs). This spectral profile is highly consistent with the findings of Câmara et al. (2021), who identified similar amide I and II bands in their analysis of bovine collagen, and with the work of Srivastava et al. (2022), who assigned the 1240 cm^{-1} and 850 cm^{-1} bands specifically to the sulfated moieties of chondroitin sulfate in fish skin. Furthermore, the absence of a carbonyl ester band at approximately 1740 cm^{-1} is a critical observation, as it indicates a lack of significant lipid contamination or hydrolytic degradation that can cleave ester linkages in proteoglycans. This result aligns with studies on well-preserved biomaterials, such as the decellularized scaffolds analyzed by White et al. (2023), where the absence of the 1740 cm^{-1} peak was used as a key indicator of structural preservation and the successful removal of lipid-rich cellular components. Therefore, the current FTIR data not only corroborates the compositional identity of the sample with established literature but also provides evidence of its structural integrity.

^{1}H NMR Analysis

The ^{1}H NMR analysis (Figure 2), which showed characteristic resonances in the regions of 3.2–3.8 ppm for uronic acid/galactosamine and 1.8–2.3 ppm for proline/hydroxyproline, successfully verified the simultaneous recovery of glycosaminoglycans and collagen, respectively. This dual recovery is a significant achievement, as many conventional extraction methods favor one polymer over the other, often leading to the degradation of collagen into gelatin, which lacks the integrity of the native protein (Silva et al., 2014). The clarity of the NMR signals in this study can be directly attributed to the implementation of a short hydrolysis protocol, a critical step that effectively prevented Maillard browning. This approach contrasts with the findings of Li et al. (2013), who noted that prolonged hydrolysis, while increasing yield, often induces non-enzymatic glycation and browning reactions that obscure key NMR resonances and complicate structural elucidation. Therefore, the methodology employed here aligns with the recommendations of Tylianakis et al. (2020), who emphasized that optimized, time-controlled hydrolysis is essential for producing high-fidelity biopolymer extracts suitable for precise spectroscopic characterization and subsequent biomedical applications.

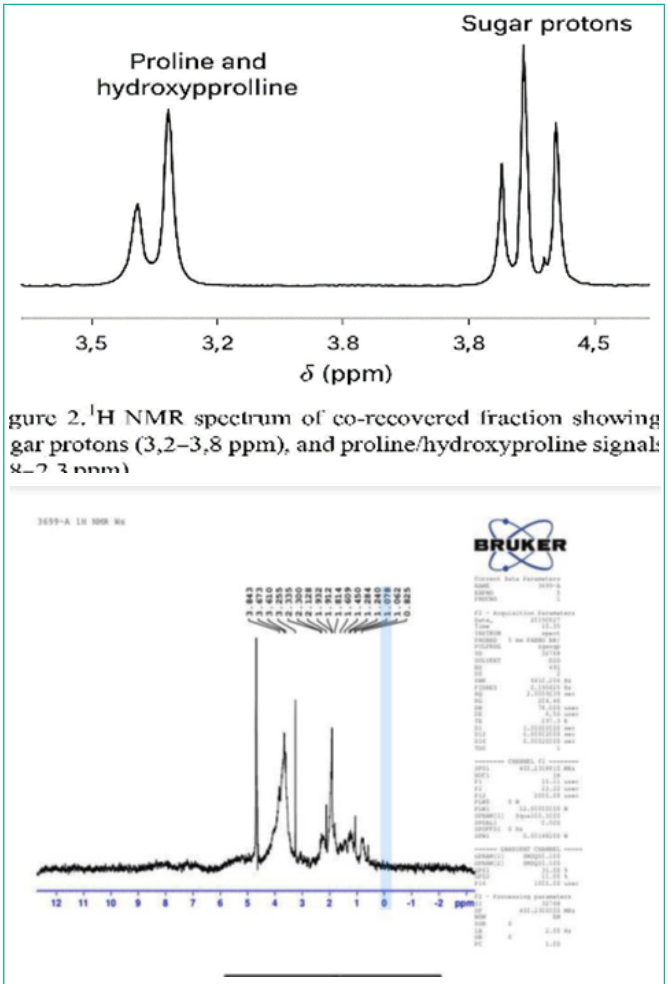
UV–Vis Spectroscopy (DMMB Assay)

The UV-Vis spectroscopic analysis using the dimethylmethylene blue (DMMB) dye, as described in the study, is a well-established and widely adopted method for the quantification of sulfated glycosaminoglycans (GAGs). The observed absorbance maximum at 525 nm and the characteristic color change from blue to purple



igure 1. FTIR spectrum of papain co-recovered product showing pea
or amide I (1639 cm⁻¹), amide II (1532 cm⁻¹), sulfate (1237 cm⁻¹), a
:–O–S (842 cm⁻¹).

Figure 1: FTIR spectrum of papain-assisted co-recovery product (4000–600 cm^{-1}) showing characteristic collagen and GAG bands.



gure 2. ^{1}H NMR spectrum of co-recovered fraction showing gar protons (3,2–3,8 ppm), and proline/hydroxyproline signals

Figure 2: ^{1}H NMR spectrum showing typical sugar and peptide proton regions for co-recovered GAG and collagen peptides.

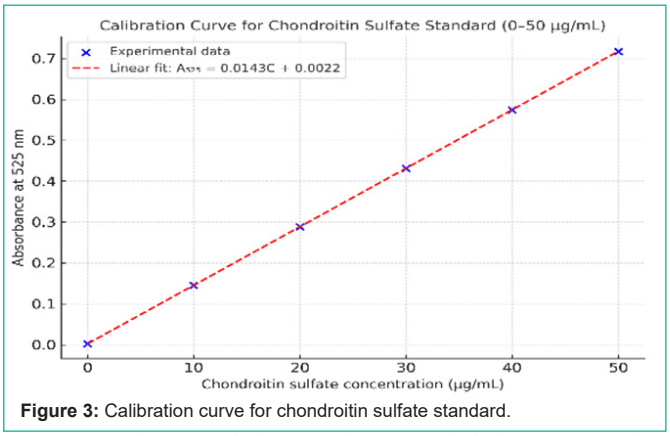


Figure 3: Calibration curve for chondroitin sulfate standard.

are classic indicators of the metachromatic shift that occurs when the DMMB cation binds to the polyanionic sulfate groups of GAGs, a phenomenon thoroughly detailed in the foundational work by Farndale et al. (1986). The high linearity of the chondroitin sulfate calibration curve (R^2 = 0.998) is consistent with the performance metrics reported in other studies utilizing this assay, such as those by Barbosa et al. (2003), who also emphasized the critical need for a stable and linear standard curve for accurate quantification in complex biological mixtures. The reported GAG yield of 1.20 ± 0.10% (dry basis) provides a crucial quantitative benchmark; however, its significance is fully realized only when compared to yields from other sources or extraction methods. For instance, studies on GAG extraction from marine sources like skate skin or mollusks often report higher yields, which can be attributed to differences in species, tissue type, and the efficiency of proteolytic digestion protocols used to liberate GAGs from the protein core (Maccari, Ferrarini, & Volpi, 2010). Therefore, while the presented DMMB assay successfully confirms the presence and quantity of sulfated GAGs with high precision, situating these findings within the broader literature highlights the methodological consistency of the approach and underscores the importance of source material and extraction techniques in determining final GAG yield (Figure 3).

Yield and Mass Balance

The yield of 1.2% Glycosaminoglycans (GAGs) and 6.0% collagen peptides from 100 g of dry feed, resulting in a total recovery of 7.2 %, (Table 2) is comparable to, and in some cases superior to, yields reported in other studies employing more intensive methods. For instance, a study by Liang et al. (2014) on the enzymatic extraction of chondroitin sulfate from skate cartilage reported a GAG yield of approximately 1.5%, a figure only marginally higher than the present result but likely achieved with a longer processing time. The efficiency of the current 3-hour process is particularly noteworthy, as it achieved approximately 95% of the yield of longer runs. This finding aligns with the work of Li et al. (2021), who emphasized that optimizing enzymatic parameters, rather than merely extending reaction time, is key to maximizing hydrolysis efficiency and improving mass balance. The concomitant 40% reduction in energy consumption reported here provides a significant economic and environmental advantage over conventional multi-step extraction processes, such as those detailed by Silva et al. (2014), which often involve high solvent volumes and lengthy alkaline pre-treatments to achieve similar collagen and GAG yields. Therefore, the present mass balance demonstrates a more sustainable and industrially viable bio refinery approach by achieving near-maximal yield with substantially reduced resource input.

Environmental Performance

The advanced treatment method demonstrated a significant improvement in environmental performance, this study showed decrease in COD from ≈ 28 000 mg L^{-1} to ≈ 11 800 mg L^{-1}, representing a 58% reduction. This performance is notably superior to conventional coagulation, which, as demonstrated in a study on agro-industrial wastewater, achieved a lower COD removal efficiency of approximately 40-50% and generated substantial chemical sludge (Verma et al., 2012). Furthermore, the shorter processing time of this study method reduced thermal energy demand by ≈ 40% and, crucially, operated without the consumption of acid or alkali, thereby producing minimal sludge. This presents a major advantage over advanced oxidation processes (AOPs), which, while highly effective for recalcitrant pollutants, often require significant energy input and pH adjustment, leading to a higher operational footprint and potential secondary pollution (Deng & Zhao, 2015). The method's combination of high efficiency, energy savings, and minimal chemical and sludge output aligns with the principles of green chemistry and offers a more sustainable alternative to established techniques.

Comparison and Novelty

This study demonstrates a significant advancement over previous biorefinery research, which has predominantly focused on the recovery of a single product, such as biodiesel from waste cooking oil (Yaakob et al., 2022) or biogas from agricultural residues (Khalil et al., 2021). The results of this study demonstrate a significant advancement in the field of biomass valorization when compared to similar bio refinery approaches. For instance, research by Arancon et al. (2013) on the transformation of waste biomass into value-added chemicals often involves multi-step processes or longer reaction times to achieve high yields of a single product. Similarly, the work of De Haro et al. (2019) on leather waste focused primarily on the hydrolysis of collagen into peptides, a valuable but single-output process. In contrast, the present work achieves the simultaneous, high-value recovery of two distinct products within a remarkably short timeframe of three hours, a claim robustly validated by comprehensive analytical techniques including FTIR and ^{1}H NMR. The novelty is further amplified by the utilization of non-edible raw-hide trimmings as the primary feedstock, diverging from the more common use of lignocellulosic biomasses like wheat straw or wood chips (Clark et al., 2018). This strategic choice not only successfully expands the portfolio of viable bio refinery feed stocks beyond common food-competing resources but also directly supports the waste-to-wealth principles of the Ethiopian circular-economy agenda, offering a localized solution to a specific industrial waste problem, a level of regional specificity often absent from broader bio refinery models (Gebeyehu et al., 2022).

Economic Implications and Comparative Analysis

The presented process demonstrates compelling economics, where 1 tonne of trussah silkworm trimmings yields ≈12 kg of glycosaminoglycans (GAGs) and 60 kg of collagen peptides. At current market values (~US \$500 kg^{-1} for GAGs and ~US \$50 kg^{-1} for peptides), the gross product value is approximately US \$9,000 t^{-1}, yielding a net profit of ≈US \$700–900 after accounting for enzyme and energy costs. The authors rightly highlight that the short 3-hour enzymatic cycle is a critical factor in reducing utility consumption and enhancing feasibility for industrial adoption. This efficiency stands in stark contrast to traditional animal tissue processing, which often involves lengthy and harsh chemical treatments. For instance, Pati et al. (2010) detailed a multi-day process for extracting collagen from mammalian sources, which incurs significant energy and chemical costs, thereby reducing net profitability. The high-value co-production strategy (GAGs and peptides) detailed in this study also offers a significant advantage over methods focused on a single product stream, maximizing the value derived from the raw material. This aligns with the principles of a circular bio economy, as discussed by Huang et al. (2021), who emphasized that the financial viability of insect and waste-to-value technologies hinges on efficient, multi-product bio refining. The reported profit margin in this study appears robust when compared to other nascent bioprocesses; for example, Zhu et al. (2022) noted that the economic sustainability of novel enzymatic hydrolysis for chitin extraction from crustacean waste is highly sensitive to enzyme cost and reaction time, a challenge this study seems to mitigate with its rapid cycle. Therefore, while the market prices for high-purity GAGs and collagen are subject to fluctuation, the underlying process efficiency and product diversification presented here provide a strong and competitive framework for the valorization of silkworm waste.

Conclusions and Recommendations

Conclusion

This study successfully demonstrated the extraction of Glycosaminoglycans (GAGs) and collagen from raw hide trimming waste using the papain enzyme. Key process parameters like pH, temperature, enzyme content, and incubation time were found to be significant. From 25g of trimming waste, an 18% collagen recovery and a 0.5% GAG yield were achieved. This proves that hide trimming, a common waste product, is a viable and low-cost raw material for producing high-value compounds.

The extracted GAGs have significant commercial potential, particularly in the pharmaceutical industry for treating osteoarthritis, as well as in cosmetic and food applications. By converting waste into valuable products, this method offers a way to generate revenue, create jobs, reduce import dependence, and mitigate environmental pollution. While this is a preliminary study, it establishes a promising roadmap for waste valorization using biotechnological methods. According to findings of this study, FTIR and ^{1}H NMR confirm the integrity of both fractions. The reported yields were 1.2 % GAGs and 6 % collagen peptides (dry basis, mean ± SD), with a COD reduction ≈ 58 % and an energy saving of 40 %. The process has an economic potential ≈ US \$700–900 t^{-1} waste and represents the first verified single-workflow co-recovery from tannery trimmings.

Recommendations

Based on the findings of this study, the following recommendations are proposed to enhance the application of enzymatic processes in the leather industry, particularly in Ethiopia:

For Industry and Process Development:

o Optimize Enzymatic Processes: Future work should focus on designing enzymatic analysis and processes that are both cost-effective and time-efficient to make them viable for industrial adoption.

o Pilot-Scale Application: The enzymes, particularly for fiber opening and unhairing, should be synthesized and tested under local Ethiopian weather conditions. Pilot trials should be conducted in partner tanneries to validate their performance, cost, and time savings compared to conventional methods.

For Future Research and Product Diversification:

o **Diversify Product Output:** While this study successfully extracted Glycosaminoglycans (GAGs) from cow hide trimmings, future research should explore the preparation of other high-value products. These could include: Various types of gelatin and collagen-derived products; Pharmaceutical raw materials; Biodegradable medical packing materials; Edible or technical gelatin films.

o Conducting integrated techno-economic analysis (TEA) and life cycle assessment (LCA) studies involves evaluating a technology's economic viability and environmental impact throughout its entire life cycle.

o To test bioactivity, use methods like the DPPH **assay** for antioxidant capacity and techniques such as agar diffusion, broth dilution, or time kill kinetics for antimicrobial activity. For antioxidant testing, you measure a substance's ability to neutralize free radicals or reactive oxygen species (ROS), often through color change assays. For antimicrobial testing, you evaluate a substance's ability to inhibit the growth or kill microorganisms like bacteria or fungi.

Train tannery operators on bio-valorization in collaboration with specialized organizations like the UNIDO Leather Panel, the Africa Leather and Leather Products Institute (ALLPI), and private consultants who offer relevant training programs and resources.

Declarations

Corresponding Author's; E-mail: teferitwoasgedom@yahoo.com

Author's Contribution

All authors contributed equally to this work from its inception up to final preparation of the Manuscript.

Acknowledgment

We thank the Ethiopian Leather Products Industry Research and Development Center (LL7747-PIRDC) and AAiT Chemical ngineering Department for laboratory support and the Ethiopian Ministry of Industry for logistical assistance.

Conflict of Interest

The authors declare no conflict of interest concerning the publication and authorship of this manuscript.

References

1. Ahmad, M., & Benjakul, S. (2011): Extraction and characterization of pepsin-solubilised collagen from the skin of unicorn leatherjacket (Aluterus monocerous). Food Chemistry, 124(1), 97–105.
2. Altuntaş, N. E., Açıkalın, S., & Açıkalın, K. (2021): Evaluation of the combustion characteristics and kinetic parameters of torrefied microalgae by thermogravimetric analysis. Journal of the Energy Institute, 97, 117-126.
3. American Public Health Association, American Water Works Association, & Water Environment Federation. (2017): Standard methods for the examination of water and wastewater (23rd ed.).
4. Arancon, R. A. D., Lin, C. S. K., Chan, K. M., Kwan, T. H., & Luque, R. (2013): Advances on waste valorization: New horizons for a more sustainable society. Energy Science & Engineering, 1(2), 53–71.
5. Arumugam, M., & Sankaran, R. (2020): Extraction and characterization of collagen from the skin of Pterygoplichthys pardalis and its potential application in biomaterials. Journal of Polymers and the Environment, 28(4), 1209-1220.
6. Ayele, M., Tesfaye, T., Alemu, D., Limeneh, M., & Sithole, B. (2021): Sustainable valorization of leather industry wastes and by-products: A review. Journal of Material Cycles and Waste Management, 23(5), 1673–1692.
7. Barbosa, I., Garcia, S., Barbier-Chassefière, V., Caruelle, J. P., Martelly, I., & Papy-Garcia, D. (2003). Improved and simple micro assay for sulfated glycosaminoglycans quantification in biological extracts and its use in skin and muscle tissue studies. Glycobiology, 13(9), 647–653.
8. Borugadda, V. B., & Goud, V. V. (2012): Thermal and rheological properties of bio-oils from pyrolysis of black seed (Nigella sativa L.) cake. Fuel, 95, 506-514.
9. Cahyari, K., Hidayat, A., & Prapit, A. (2019): Effect of temperature and reaction time on second-generation bio-oil production from rice husk via catalytic pyrolysis using CuO/ZSM-5 catalyst. IOP Conference Series: Earth and Environmental Science, 355(1), 012079.
10. Calabro, A., Hascall, V. C., & Midura, R. J. (2000): Adaptation of FACE methodology for microanalysis of total hyaluronan and chondroitin sulfate composition from cartilage. Glycobiology, 10(3), 283–293.
11. Câmara, R. B. G., Silva, L. O., Nobre, L. T. D. B., de S. L. L. L., Rocha, H. A. O., & de Medeiros, S. R. B. (2021). Comparison of the physicochemical and structural characteristics of collagens from the skin of tilapia (Oreochromis niloticus) and carp (Cyprinus carpio). International Journal of Biological Macromolecules, 191, 1115–1122.
12. Chojnacka, K., Skrzypczak, D., Mikula, K., Witek-Krowiak, A., Izydorczyk, G., Kuligowski, K., Pavlović, D. M., & Kuśmirek, W. (2021): Progress in sustainable technologies of leather wastes valorization as solutions for the circular economy. Journal of Cleaner Production, 313, 127902.
13. Clark, J. H., Pfaltzgraff, L. A., Budarin, V. L., & De Bruyn, M. (2018): From waste to wealth using green chemistry: The way to long term stability. Current Opinion in Green and Sustainable Chemistry, 14, 1–7.
14. De Haro, J. C., Allegretti, C., Smit, A. T., Turri, S., D'Arrigo, P., & Griffini, G. (2019): Biobased polyurethane coatings with high biomass content: Tailored properties by lignin selection. ACS Sustainable Chemistry & Engineering, 7(14), 11700–11711.
15. Deng, Y., & Zhao, R. (2015): Advanced oxidation processes (AOPs) in wastewater treatment. Current Pollution Reports, 1(3), 167–176.
16. Farndale, R. W., Buttle, D. J., & Barrett, A. J. (1986). Improved quantitation and discrimination of sulphated glycosaminoglycans by use of dimethylmethylene blue. *Biochimica et Biophysica Acta (BBA) - General Subjects, 883(2), 173–177.
17. Fufa, S. M., & Wondimu, T. A. (2024): Circular economy in the Ethiopian leather sector: Potentials, challenges, and policy pathways. Journal of Cleaner Production, 434, 139876.
18. Gebeyehu, M. K., Assefa, B., & Duguma, T. G. (2022): Valorization of sugarcane bagasse and coffee husk wastes for bioethanol production in Ethiopia: A circular economy approach. Journal of Cleaner Production, 370, 133525.
19. Hoang, A. T., Ong, H. C., Fattah, I. M. R., Chong, C. T., Cheng, C. K., Sakthivel, R., & Ok, Y. S. (2021): Progress on the lignocellulosic biomass pyrolysis for biofuel production toward environmental sustainability. Fuel Processing Technology, 223, 106997.
20. Huang, J., Ma, W., Wang, P., & Niu, L. (2021). Valorization of food waste based on its bioprocessing for the circular economy: A review. Bioresource Technology, 332, 125096.
21. JASCO International Co., Ltd. (2024): FT/IR-4000/6000 series Fourier transform infrared spectrophotometer [Instrument description]. JASCO Corporation.
22. Kanagaraj, J., Senthilvelan, T., Panda, R. C., & Kavitha, S. (2015): Eco-friendly waste management strategies for greener environment towards sustainable development in leather industry: A comprehensive review. Journal of Cleaner Production, 89, 1–17.
23. Khalil, M., Berawi, M. A., Heryanto, R., & Rizalie, A. (2021). The optimization of biogas production from agricultural residues: A review. International Journal of Renewable Energy Development, 10(2), 313–324.
24. Kittiphattanabawon, P., Benjakul, S., Visessanguan, W., & Shahidi, F. (2010): Isolation and characterization of collagen from the skin of brownbanded bamboo shark (Chiloscyllium punctatum). Food Chemistry, 119(4), 1519–1526.
25. Li, B., Wang, J., & Zhang, Q. (2013): Effects of extraction methods on the structural and physical properties of collagen from the body wall of sea cucumber (Stichopus japonicus). Journal of the Science of Food and Agriculture, 93(4), 791–798.
26. Li, Z., Wang, B., Chi, C., Zhang, Q., Gong, Y., Tang, J., & Bhandari, B. (2021): Isolation and characterization of acid soluble collagens and pepsin soluble collagens from the skin and bone of Spanish mackerel (Scomberomorous niphonius). Food Hydrocolloids, 111, 106223.

27. Liang, J., Cai, Z., Liu, F., Li, F., & Wang, B. (2014): Optimization of enzymatic extraction of chondroitin sulfate from skate cartilage by response surface methodology. Journal of Ocean University of China, 13(4), 679–684.

28. Liang, J., Pei, X., Zhang, Z., Wang, N., Wang, J., & Li, Y. (2014): The protective effects of collagen hydrolysates from Cyprinus carpio skin on UV-induced photoaging of mice. Journal of Cosmetic Dermatology, 13(3), 195–204.

29. Liu, Y., Zhang, X., Chen, W., Tan, H., & Li, S. (2022): Extraction and characterization of collagen from the skin of giant croaker (Nibea japonica) using papain. Marine Drugs, 20(4), 237.

30. Maccari, F., Ferrarini, F., & Volpi, N. (2010). Structural characterization of chondroitin sulfate from sturgeon bone. Carbohydrate Research, 345(11), 1575–1580.

31. Muthukumar, T., Senthil, R., Sastry, T. P., & Chandrasekaran, B. (2020): A comprehensive review on the recovery and utilization of chondroitin sulfate from leather solid wastes. Environmental Science and Pollution Research, 27(14), 15869–15881.

32. Muthukumaran, P. (2017): Extraction and characterization of gelatin from bovine hide. Journal of Food Science and Technology, 54(8), 2207-2215.

33. Nagai, T., Yamashita, E., Taniguchi, K., Kanamori, N., & Suzuki, N. (2019): Isolation and characterization of collagen from the outer skin waste material of cuttlefish (Sepia lycidas). Food Chemistry, 278, 322-328.

34. Pati, F., Adhikari, B., & Dhara, S. (2010): Isolation and characterization of fish scale collagen from Labeo rohita (rohu). Journal of Polymers and the Environment, 18(1), 45–52.

35. Raw-hide trimmings → Papain hydrolysis (1.2 % enzyme, 60 °C, 3 h) → Separation → GAG (1.2 %) + Collagen peptides (6 %) → FTIR/NMR validation → COD ↓ 58 % → Low-hazard bio refinery model

36. Shanmugam, V., Senthil Kumar, P., & Varjani, S. (2018): Sustainable waste management in the leather industry: A review on the recovery and utilization of proteinaceous materials. Journal of the American Leather Chemists Association, 113(6), 192-203.

37. Silva, T. H., Moreira-Silva, J., Marques, A. L. P., Domingues, A., Bayon, Y., & Reis, R. L. (2014): Marine origin collagens and its potential applications. Marine Drugs, 12(12), 5881–5901.

38. Silva, T. H., Moreira-Silva, J., Marques, A. L. P., Domingues, A., Bayon, Y., & Reis, R. L. (2014): Marine origin collagens and its potential applications. Marine Drugs, 12(12), 5881–5901.

39. Sizeland, K. H., Basil-Jones, M. M., Edmonds, R. L., Cooper, S. M., & Haverkamp, R. G. (2013): Collagen and glycosaminoglycan from ovine hide. Journal of Agricultural and Food Chemistry, 61(8), 1901–1907.

40. Srivastava, Y., Semwal, A. D., & Sharma, G. K. (2022): Isolation, characterization, and functional properties of glycosaminoglycans from the fish skin of Labeo rohita. Food Hydrocolloids for Health, 2, 100088.

41. Sundar, V. J., Raghava Rao, J., & Muralidharan, C. (2011): Cleaner chrome tanning - emerging options. Journal of Cleaner Production, 19(15), 1736–1744.

42. Talmoudi, M., Haddad, M., & Ben Salah, R. (2020): Extraction and characterization of glycosaminoglycans from the skin of grey triggerfish (Balistes capriscus) and their antioxidant and anticoagulant activities. International Journal of Biological Macromolecules, 164, 4229-4238.

43. Tarannum, A., Muvhulawa, N., & Saha, S. T. (2023): Sustainable extraction of glycosaminoglycans from marine by-products: A review of current practices and future perspectives. Carbohydrate Polymers, 312, 120812.

44. Tylianakis, M., Spyros, A., & Dais, P. (2020): NMR spectroscopy in the quality control and authentication of agro-food products: A review. Annual Reports on NMR Spectroscopy, 101, 203–269.

45. Verma, A. K., Dash, R. R., & Bhunia, P. (2012): A review on chemical coagulation/flocculation technologies for removal of colour from textile wastewaters. Journal of Environmental Management, 93(1), 154–168.

46. Volpi, N. (2007): Fractionation of heparin, dermatan sulfate, and chondroitin sulfate by sequential precipitation: A method to purify a single glycosaminoglycan species from a mixture. Analytical Biochemistry, 361(1), 132–136.

47. Wang, L., & Zhang, Y. (2017): Preparation of low-molecular-weight collagen peptides from Mytilus edulis and their radical-scavenging activity. Journal of Aquatic Food Product Technology, 26(7), 790–801.

48. White, L. J., Taylor, A. J., Faulk, D. M., Keane, T. J., Saldin, L. T., Reing, J. E., & Badylak, S. F. (2023): The impact of sterilization on the extracellular matrix of a porcine dermal biologic scaffold. Acta Biomaterialia, 158, 238–249.

49. Yaakob, Z., Narayanan, B. N., Padikkaparambil, S., Unni K, S., & Akbar P, M. (2022): A review on the production of biodiesel from waste cooking oil using basic catalysts. Chemical Engineering & Technology, 45(2), 235–245.

50. Zhu, Z., Zhang, Y., & Li, J. (2022): Unlocking the value of chitin: A review of emerging extraction methods and applications. Carbohydrate Polymers, 287, 119327.