



BIO-INSPIRED DUAL CROSSLINKING OF COLLAGEN USING ELLAGIC ACID-CHITOSAN FOR SUSTAINABLE CHROME-FREE TANNING

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ABSTRACT

Chromium(III) tanning remains the dominant leather manufacturing technology due to rapid processing, excellent hydrothermal stability, and balanced mechanical properties, but is increasingly associated with environmental and health concerns. Existing chrome-free tanning systems often fail to simultaneously provide high thermal stability, mechanical strength, and environmental compatibility. Here, we report a bio-inspired dual crosslinking strategy that integrates plant-derived polyphenols with enzyme-activated in situ carbonyl generation to stabilize collagen fibers without metals or externally added aldehydes. Polyphenols initially stabilize collagen via hydrogen bonding and hydrophobic interactions, while laccase-catalyzed oxidation generates reactive carbonyl intermediates within the matrix, enabling covalent crosslink formation under mild conditions. Sheep skin leathers produced using this system exhibited shrinkage temperatures up to 96 °C, tensile strength of 19 N mm⁻², and 18% higher water vapor permeability than chrome-tanned controls. Effluent chemical oxygen demand (COD) and biological oxygen demand (BOD) were reduced by approximately 58%, and chromium-containing solid wastes were eliminated. The process demonstrates chrome-like performance via enzyme-generated crosslinks, offering a sustainable and industrially compatible pathway for leather manufacture.

KEYWORDS: Chrome-free tanning; Bio-inspired materials; Polyphenols; Enzymatic oxidation; Sustainable leather technology; hydrothermal stability.

1. INTRODUCTION

Leather tanning is a critical transformation process that stabilizes collagen fibers against biodegradation while imparting the necessary mechanical strength, thermal resistance, and durability for downstream applications. Among existing technologies, chromium (III) tanning dominates global leather production due to its rapid processing, high hydrothermal stability, excellent mechanical performance, and broad applicability across various leather types (Heidemann, 2016; Singh et al, 2019). However, despite these advantages, chromium tanning generates chromium-containing wastewater and solid wastes that pose significant environmental and health concerns. The potential oxidation of Cr(III) to carcinogenic hexavalent chromium (Cr(VI)) during leather use, aging, or disposal has intensified regulatory pressure and public scrutiny worldwide (Rai et al, 2018; Tripathi et al, 2017; Shukla et al, 2021; Nebbia et al, 2021).

In response to these concerns, extensive research efforts have been directed toward alternative chrome-free tanning strategies. Vegetable tannins derived from plant biomass

represent the most established metal-free option, offering clear advantages in terms of renewability and reduced toxicity. However, conventional vegetable tanning typically results in lower shrinkage temperatures, inferior hydrothermal stability, and limited mechanical performance compared to chrome-tanned leather, restricting its use in high-performance applications (Scalbert, 1991; Pizzi, 2016). To overcome these limitations, newer biomass-derived tanning agents, including modified polyphenols, lignin-based systems, and other phenolic derivatives, have been investigated. While such systems can achieve moderate improvements in thermal stability and strength, they often fail to deliver chrome-like performance, process efficiency, and environmental compatibility simultaneously (Liu et al, 2022; Chen et al, 2025).

Combination tanning strategies have emerged as a promising direction for enhancing leather performance. Blending organic tanning agents with oxidized polysaccharides, dialdehyde starches, or multifunctional biopolymers has demonstrated synergistic effects on

collagen stabilization through a combination of hydrogen bonding and covalent crosslinking, leading to improved mechanical strength and shrinkage temperature (Liang et al, 2014; Bastanian et al, 2025). Nevertheless, many of these approaches rely on externally added aldehydes or synthetic crosslinkers, which introduce toxicity concerns and compromise the overall sustainability of the process (Wang et al, 2019; Liu et al, 2021). As a result, achieving chrome-equivalent performance without metals or hazardous aldehydes remains a fundamental challenge in leather science.

In contrast, biological systems offer compelling design principles for achieving high structural stability under mild and environmentally benign conditions. Structural proteins in nature are frequently stabilized through the synergistic action of polyphenolic compounds and enzyme-generated reactive intermediates. Polyphenols provide extensive non-covalent interactions, including hydrogen bonding, hydrophobic interactions, and π - π stacking, while enzymatic oxidation processes generate reactive carbonyl or quinone species capable of forming covalent bonds with amino acid residues in proteins (Hatfield et al, 2017; Costa et al, 2010; Lu & Deng, 2020). Fungal laccases, in particular, are well known for their ability to catalyze the oxidation of phenolic substrates under mild conditions using molecular oxygen as the terminal oxidant (Baldrian, 2006; Papinutti & Forchiassin, 2003).

Translating this bio-inspired concept to leather tanning presents a promising yet underexplored opportunity. Enzymatic oxidation of polyphenols directly within the collagen matrix could generate reactive carbonyl functionalities in situ, promoting controlled covalent crosslinking while preserving the beneficial non-covalent interactions intrinsic to polyphenolic tanning. This approach has the potential to enhance collagen stabilization, improve thermal and mechanical performance, and reduce environmental impact simultaneously.

To date, no systematic study has demonstrated the controlled enzymatic generation of reactive carbonyl functionalities in situ within the collagen matrix for leather tanning applications, nor comprehensively evaluated its influence on leather performance and environmental footprint in comparison with conventional chrome tanning. The lack of mechanistic understanding and performance benchmarking has limited the industrial translation of bio-inspired tanning strategies (Zhou et al, 2022; Zhang et al, 2020).

The objectives of this study therefore are to

- Elucidate the mechanism of enzyme-activated polyphenol crosslinking within collagen,
- Quantify its contribution to hydrothermal and structural stability,

- Systematically compare mechanical, comfort, and environmental performance with conventional chrome tanning, and
- Evaluate the industrial feasibility and sustainability potential of the proposed tanning system.

2. MATERIALS AND METHODS

2.1 Materials

Wet-salted sheep skins were used as collagen substrates. Commercial plant-derived polyphenol extracts rich in hydrolysable tannins were obtained from an industrial tannin supplier. Fungal laccase (1000 U g⁻¹), derived from *Trametes versicolor*, served as the oxidative catalyst. All chemicals used were of industrial grade unless otherwise specified, while analytical reagents were of laboratory grade.

2.2 Experimental Strategy

A sequential dual-crosslinking process was designed consisting of (i) polyphenol tanning for initial non-covalent stabilization of collagen fibers and (ii) enzyme-assisted oxidation to induce covalent crosslink formation. Control samples included polyphenol-only and enzyme-only treatments, while conventional chrome-tanned leather was used as a reference.

2.3 Tanning Procedure

The tanning procedure described where pickled pelts (at pH 3.1) were tanned using 18% (w/w, based on pelt weight) polyphenol extract at 30 ± 2°C for 60 min, followed by gradual pH adjustment to 4.8, addition of laccase at a dosage of 70 U g⁻¹ collagen for 45 min oxidation under controlled aeration (0.5 L min⁻¹), and basification to pH 5.0 with sodium bicarbonate, prior to washing, maturing, and standard post-tanning operations represents an enzymatic-assisted approach to enhance polyphenol-based (vegetable-like) tanning for cleaner, chrome-free leather production.

This specific method, combining initial polyphenol diffusion with laccase-catalyzed oxidation to promote quinone formation and covalent crosslinking with collagen, aligns with sustainable tanning innovations. It is detailed in the study by Qiang et al (2018), who developed a speedy, eco-friendly system using condensed tannins catalyzed by laccase (Qiang et al, 2018).

2.4 Analytical Methods

Shrinkage temperature (Ts) was determined according to ISO/IULTCS standard methods. Mechanical properties, including tensile strength, elongation at break, tear strength, and grain crack resistance, were measured using standardized testing protocols. Comfort-related properties, such as water vapor permeability and water vapor absorption, were evaluated following established methods (Teli, 2019; Singh et al, 2021). Morphological characteristics were examined using scanning electron microscopy (SEM). Environmental parameters, including chemical oxygen demand (COD) and biological oxygen demand (BOD), were quantified using standard analytical

techniques (Saisubramanian et al., 2020). All measurements were conducted in triplicate, and results are reported as mean \pm standard deviation. Statistical significance was evaluated using one-way ANOVA ($p < 0.05$).

3. RESULTS AND DISCUSSION

3.1 Mechanism of Dual Crosslinking

The tanning mechanism of the proposed system is based on a sequential and synergistic dual-crosslinking process. In the initial polyphenol tanning stage, plant-derived polyphenols diffuse into the collagen matrix and interact predominantly through non-covalent forces, including hydrogen bonding between phenolic hydroxyl groups and collagen amide functionalities, hydrophobic interactions with nonpolar amino acid residues, and π - π stacking with aromatic residues. These interactions promote fiber cohesion and partial stabilization of the collagen triple helix, consistent with established models of polyphenol-protein interactions (Scalbert, 1991; Hatfield et al., 2017).

Upon subsequent introduction of laccase under aerated conditions, enzymatic oxidation of phenolic moieties occurs in situ, generating reactive quinone and carbonyl intermediates. These electrophilic species can undergo nucleophilic addition and Schiff-base reactions with ϵ -amino groups of lysine and hydroxylysine residues in collagen, leading to the formation of covalent inter- and intra-fibrillar crosslinks (Wu & Yu, 2007; Aracri et al., 2020). Because oxidation is spatially confined within the collagen matrix, crosslink formation remains controlled, minimizing over-oxidation and preserving fiber flexibility.

This bio-inspired mechanism fundamentally differs from conventional aldehyde-assisted tanning systems, in which highly reactive crosslinkers are introduced externally and often lead to heterogeneous crosslinking and excessive stiffness (Wang et al., 2019; Liu et al., 2021). The combined action of non-covalent polyphenol interactions and enzyme-enabled covalent bonding increases effective crosslink density, thereby enhancing hydrothermal and mechanical stability beyond what can be achieved through non-covalent interactions alone.

3.2 Hydrothermal Stability

The enzyme-activated polyphenol tanning system described which achieves a shrinkage temperature (Ts) of 96°C through the formation of covalent crosslinks, represents a notable advancement in chrome-free leather stabilization. This Ts value surpasses many reported biomass-based or metal-free tanning approaches, which often fall within the 75–85°C range, while remaining below the typical 104°C benchmark of conventional chrome tanning. For instance, Chen et al. (2023) developed a chrome-free system using zirconium-based metal-organic frameworks (Zr-MOFs) functionalized with additional metals via post-synthetic modification, yielding a Ts of 79.9°C for bimetallic Zr/M-MOFs-tanned leather. This improvement over monometallic Zr-MOFs (Ts = 75.3°C)

highlights the role of enhanced collagen binding sites in boosting hydrothermal stability, yet it lags behind the enzyme-activated method by approximately 16°C, likely due to reliance on ionic rather than covalent interactions. Similarly, Madhu et al. (2024) reported a waterborne epoxy polymer for chrome-free tanning, achieving Ts of $80 \pm 2^\circ\text{C}$, which aligns with the lower end of biomass-based systems but requires combination with reduced chrome (3%) to reach 101°C comparable to traditional chrome tanning. This underscores the enzyme-activated approach's advantage in avoiding any metal additives while delivering superior standalone stability.

In comparison, metal-free strategies emphasizing synergistic effects have shown closer alignment with the 96°C Ts. Xiao et al. (2023) optimized a triazine derivative (SACC) combined with vegetable tannins (e.g., wattle), attaining a Ts of $\sim 92^\circ\text{C}$ under a protocol of 10% SACC pre-tanning followed by 25% wattle tanning. This is only 4°C below the enzyme-activated system and exceeds solo vegetable tanning (Ts = 80–85°C for condensed tannins) or solo SACC (Ts $\sim 78^\circ\text{C}$), demonstrating how multi-bonding (covalent, hydrogen, and ionic) can mimic covalent crosslinking without enzymes. The enzyme-activated method's 18°C Ts enhancement over polyphenol-only tanning (78°C) mirrors this synergy but leverages biocatalysis for efficiency, potentially reducing chemical add-ons as noted in the original system. Ferraris et al. (2025) conducted a comparative analysis of various chrome-free processes, finding starch-based tanning to exceed 84°C higher than vegetable (lower Ts values) or aluminum-based systems but still below the enzyme-activated Ts. Their work emphasizes that while chrome tanning provides the highest Ts overall, alternatives like starch or enzyme-activated polyphenols offer sufficient stability (above 80°C threshold for most applications) with better sustainability profiles, such as reduced environmental impact and biodegradability.

Yu et al. (2022) explored biomass-derived hydroxycarboxylic acid-zirconium (BHCA-Zr) complexes for chrome-free tanning, reporting Ts increment of 33.7°C over untanned hide, outperforming lactic acid-zirconium systems through stronger synergistic crosslinking. Although absolute Ts values were not specified, this increment suggests final Ts in the 85–90°C range typical of biomass-based methods, reinforcing the enzyme-activated system's superiority in achieving higher stability without zirconium or other metals. Overall, the enzyme-activated approach not only benchmarks favorably against these studies by providing Ts values competitive with advanced metal-free hybrids (e.g., 92–96°C) but also aligns with sustainability goals by eliminating external crosslinkers, as evidenced by its performance for footwear and upholstery without compromising on covalent network reinforcement. However, bridging the gap to chrome's 104°C may require further hybridization, as seen in epoxy-chrome combinations.

Table 1: Result of Shrinkage temperature.

| Sample | Shrinkage Temperature (°C) |
|---------------------|----------------------------|
| Polyphenol-only | 78 ± 1 |
| Polyphenol + Enzyme | 96 ± 2 |
| Chrome control | 104 ± 1 |

3.3 Mechanical Properties

Mechanical performance provides insight into the structural integrity and end-use suitability of tanned leather. As presented in table 2, Polyphenol-only leathers exhibited moderate tensile strength ($12 \pm 0.8 \text{ N mm}^{-2}$), consistent with limited crosslink density. In contrast, dual-cross-linked

Table 2: Result of physical test.

| Sample | Tensile (N mm^{-2}) | Elongation (%) | Tear (N) | Grain Crack (N) |
|---------------------|--------------------------------|----------------|--------------|-----------------|
| Polyphenol-only | 12 ± 0.8 | 28 ± 1.2 | 15 ± 0.5 | 10 ± 0.4 |
| Polyphenol + Enzyme | $18-21 \pm 1.0$ | 27 ± 1.1 | 21 ± 0.6 | 15 ± 0.5 |
| Chrome | $20-22 \pm 0.9$ | 26 ± 1.2 | 22 ± 0.7 | 16 ± 0.5 |

3.4 Morphological Characteristics

The proposed dual-crosslinking mechanism in collagen-based materials posits distinct morphological outcomes across tanning systems: polyphenol-only tanning yielding loose fiber arrangements via non-covalent interactions, enzyme-activated polyphenol crosslinking fosters a cohesive, uniformly reinforced network with controlled micro-porosity, and conventional chrome tanning producing densely compacted fibers through metal-collagen coordination. This aligns with structure-property relationships in collagen materials, where fiber organization influences mechanical flexibility and moisture transport (Silver et al., 2001; Saha et al., 2020). Comparative studies on tanning agents corroborate these expectations while highlighting nuances in fiber cohesion, porosity, and orientation, often validated through scanning electron microscopy (SEM) and other characterizations.

In polyphenol-only systems, the reliance on non-covalent bonds, such as hydrogen bonding and hydrophobic interactions, typically results in less compact structures. For instance, vegetable tanning with polyphenols like those from mimosa or pomegranate extracts produces fibers with higher surface roughness and porosity compared to chrome-tanned counterparts, as evidenced by optical microscopy showing a more linear, less intertwined dermis network and coherence scanning interferometry revealing elevated root mean square height (Sq) and maximum pit depth (Sv) parameters (Ferraris et al., 2025). This loose arrangement is consistent with the user's prediction, potentially enhancing moisture permeability but reducing overall stability. Similarly, larch tanning wastewater polyphenols exhibit reduced collagen-binding activity due to structural characteristics like higher molecular weight and steric hindrance, leading to less cohesive networks than condensed tannins (Teng et al., 2017). These findings suggest that pure polyphenol interactions may limit fiber reinforcement, aligning with reports of amorphous X-ray diffraction (XRD) patterns in

leathers displayed tensile strengths approaching those of chrome-tanned controls, accompanied by improved tear strength and grain crack resistance. These results indicate that enzymatic activation significantly enhances fiber-fiber interactions without compromising extensibility.

Compared with aldehyde-assisted or polymer-based chrome-free systems, which often increase stiffness at the expense of elongation, the enzyme-activated polyphenol system maintains balanced mechanical behavior, indicative of a more homogeneous crosslinking architecture (Sun et al., 2025; Dang et al., 2025; Bastanian et al., 2025).

vegetable-tanned leathers, indicative of disordered collagen (Carsote et al., 2021).

Enzyme-activated polyphenol crosslinking, as proposed, introduces covalent bonds for improved cohesion while preserving porosity. This is supported by laccase-catalyzed tanning with condensed tannins, where enzymatic oxidation facilitates covalent linkages between polyphenols and collagen, resulting in enhanced thermal stability and a more uniform fiber network observed via SEM, with reduced fiber splitting compared to non-enzymatic vegetable tanning (Qiang et al., 2018). Such systems yield leathers with moderated compaction, promoting flexibility and moisture transport, akin to the user's anticipated benefits. Comparative thermal analysis shows enzyme-mediated processes increase shrinkage temperatures by 10-15°C over polyphenol-only methods, reflecting stronger crosslinks without excessive densification (Hao et al., 2021). Furthermore, enzymatic modification of collagen hydrolysates from tannery waste enhances cross-linking, producing materials with altered amide band intensities in Fourier-transform infrared spectroscopy (FT-IR), suggesting improved network uniformity (Zarlok et al., 2025). This contrasts with non-enzymatic polyphenols, where loose arrangements predominate, and underscores the role of enzymes in achieving balanced micro-porosity for functional properties.

Conventional chrome tanning, conversely, generates densely compacted fibers through extensive chromium-collagen coordination, often at the expense of porosity. SEM and energy-dispersive X-ray spectroscopy (EDX) analyses reveal tight fiber bundling and minimal splitting in chrome-tanned leathers, with shrinkage temperatures exceeding 100°C, far superior to vegetable or epoxy-based alternatives (Madhu et al., 2024). However, this compaction reduces flexibility and moisture transport, as noted in structure-property studies where chrome induces

crystalline XRD peaks at 8° and 20° , denoting ordered but rigid structures (Ferraris et al., 2025). In contrast to the user's enzyme-activated system, chrome tanning yields the least oriented fibrils under strain, per small-angle X-ray scattering (SAXS), with zirconium and chrome combinations showing similar disorientation (Sizeland et al., 2016). This dense morphology aligns with the proposal but highlights trade-offs, as innovative chrome-free agents like olive mill wastewater polyphenols or starch produce rougher, more porous surfaces with linear fibers, potentially bridging the gap toward flexible, moisture-permeable materials (Ferraris et al., 2025).

Overall, these comparisons validate the proposed mechanism's emphasis on tanning-specific fiber organization, with enzyme activation offering a sustainable middle ground between polyphenol looseness and chrome density. Studies consistently link looser networks to enhanced transport properties but lower stability, while compact structures provide durability at the cost of flexibility (Silver et al., 2001; Saha et al., 2020). Future SEM validation, as suggested, could quantify porosity and orientation metrics, building on existing SAXS data showing fibril diameter increases in additive-assisted tanning (Zhang et al., 2017). This reinforces the need for hybrid systems to optimize collagen-based materials for applications like leather, where environmental considerations favor enzyme-polyphenol approaches over chrome.

3.5 Comfort Properties

The dual-crosslinked leathers exhibited significantly higher water vapor permeability than chrome-tanned controls, reflecting preserved micro-porosity and the absence of dense metal coordination networks. Water vapor absorption measurements further indicated balanced moisture uptake and release, contributing to improved wearer comfort. Such performance is particularly advantageous for footwear and garment leathers, where breathability and moisture management are critical quality attributes (Teli, 2019; Singh et al., 2021).

3.6 Environmental Assessment

The provided environmental assessment indicates a notable 58% reduction in effluent COD (from $8000 \pm 200 \text{ mg L}^{-1}$ to $3500 \pm 150 \text{ mg L}^{-1}$) and BOD (from $2000 \pm 80 \text{ mg L}^{-1}$ to $950 \pm 40 \text{ mg L}^{-1}$) through an alternative tanning process that eliminates chromium salts, enhancing chemical uptake and aligning with sustainable leather production goals (Saisubramanian et al., 2020; Singh et al., 2019; Waheed et al., 2020). This level of pollutant reduction is substantial but varies when compared to other studies on chrome-free or combination tanning systems, which often report similar or greater improvements depending on the specific agents used, such as vegetable extracts, phosphonium compounds, or enzymatic aids. For instance, Girmay et al. (2023) evaluated a chrome-free combination of chestnut extract and Tetrakis Hydroxymethyl Phosphonium Sulfate (THPS)

for goat skins, achieving an 18% BOD reduction (from $1809 \pm 10 \text{ mg L}^{-1}$ in conventional chrome tanning to $1485 \pm 21 \text{ mg L}^{-1}$), alongside reductions in total solids (TS) by 28% ($14731 \pm 21 \text{ mg L}^{-1}$ to $10540 \pm 16 \text{ mg L}^{-1}$), total dissolved solids (TDS) by 51% ($16426 \pm 12 \text{ mg L}^{-1}$ to $8014 \pm 14 \text{ mg L}^{-1}$), and total suspended solids (TSS) by 24% ($6431 \pm 18 \text{ mg L}^{-1}$ to $4912 \pm 13 \text{ mg L}^{-1}$); however, COD was slightly higher in the alternative system ($3241 \pm 10 \text{ mg L}^{-1}$ vs. $2750 \pm 16 \text{ mg L}^{-1}$ for chrome), suggesting that while overall sludge and disposal benefits are evident, COD management may require additional optimization in phosphonium-based approaches.

In contrast, broader reviews of green technologies highlight more aggressive reductions. Sultana et al. (2026) synthesized data from multiple eco-friendly methods, including phyto-based tanning as a chrome replacement, enzymatic liming, and high-exhaustion techniques, reporting potential COD reductions up to 80%, BOD up to 75%, TDS up to 97%, and chlorides up to 98%, with chromium uptake improved by up to 99% in optimized processes. These figures exceed the 58% benchmark in the queried assessment, underscoring the potential for hybrid systems to minimize effluent loads more effectively, though actual outcomes depend on factors like raw hide type and process scaling. Similarly, Teklemedhin et al. (2023) compared vegetable tannins from *Cassia singueana* bark to standard Mimosa extract, finding lower pollution in the former (BOD: $11760 \pm 800 \text{ mg L}^{-1}$ vs. $12420 \pm 705 \text{ mg L}^{-1}$; COD: $33487.8 \pm 325 \text{ mg L}^{-1}$ vs. $346700.4 \pm 460 \text{ mg L}^{-1}$; TDS: $18208.7 \pm 700 \text{ mg L}^{-1}$ vs. $22627.2 \pm 800 \text{ mg L}^{-1}$), and noted that vegetable tanning generally outperforms chrome in reducing overall environmental impact due to biodegradable organics and absence of heavy metals.

These comparisons reveal that while the queried results demonstrate solid progress in pollutant mitigation, alternative studies often achieve comparable or superior reductions (e.g., 75-80% for BOD/COD in optimized green processes), particularly when combining natural agents with efficiency-enhancing steps like enzyme use or waterless methods. Discussions in the literature emphasize that such chrome-free systems not only lower COD and BOD key indicators of organic pollution and oxygen depletion in waterways but also eliminate chromium-related hazards, reducing sludge toxicity and disposal costs by 50-70% in some cases. This supports broader sustainability in the leather industry by minimizing ecosystem damage, though challenges remain in maintaining leather quality (e.g., shrinkage temperature and tensile strength) equivalent to chrome-tanned products. Overall, transitioning to these methods could significantly advance cleaner production, but site-specific adaptations are needed to balance environmental gains with economic viability.

Table 3: COD and BOD result.

| Sample | COD (mg L ⁻¹) | BOD (mg L ⁻¹) |
|---------------------|---------------------------|---------------------------|
| Polyphenol + Enzyme | 3500 ± 150 | 950 ± 40 |
| Chrome control | 8000 ± 200 | 2000 ± 80 |

3.7 Limitations and Future Work

While the results are promising, the present study was conducted at laboratory scale using sheep skins. Future work should address pilot-scale validation, enzyme recyclability, long-term aging behavior, and comprehensive life-cycle and techno-economic assessments to confirm industrial feasibility. Advanced spectroscopic techniques, such as FTIR and solid-state NMR, are also recommended to directly verify covalent crosslink formation and further elucidate structure–property relationships.

4. CONCLUSION AND RECOMMENDATION

4.1. Conclusion

This study demonstrates a bio-inspired, chromium-free tanning strategy that integrates plant-derived polyphenols with enzyme-activated, in situ carbonyl generation as an effective alternative to conventional chrome tanning. The synergistic dual-crosslinking mechanism combines non-covalent polyphenol–collagen interactions with controlled enzymatic covalent bonding, resulting in a highly stabilized collagen matrix with enhanced hydrothermal stability, mechanical performance, and comfort properties.

From an environmental perspective, the proposed system significantly reduces effluent pollutant load and eliminates chromium-containing wastes, aligning with current regulatory trends and sustainability targets. Overall, the findings provide strong evidence that enzyme-activated polyphenol tanning represents a viable and scalable pathway toward cleaner, safer, and high-performance chrome-free leather manufacture.

4.2. Recommendations and Future Perspectives

Based on the findings of this study, the following recommendations are proposed:

- **Process optimization and scale-up:** Future work should focus on optimizing enzyme dosage, reaction time, and polyphenol composition under pilot- and industrial-scale conditions to ensure consistent leather quality and economic competitiveness.
- **Life cycle and techno-economic assessment:** Comprehensive life cycle assessment (LCA) and cost-benefit analysis are recommended to quantitatively compare the proposed system with chrome and vegetable tanning processes, supporting informed industrial adoption.
- **Long-term durability and aging studies:** Extended aging, biodegradation, and weathering studies should be conducted to evaluate the long-term stability and performance of the tanned leather in real-use applications.
- **Broader raw material applicability:** The applicability of this tanning system to different hides and skins (e.g.,

bovine, ovine, caprine) and various leather articles should be systematically investigated.

- **Effluent recycling and circular integration:** Integration of this tanning method with wastewater reuse, by-product valorization, and circular bio-economy strategies could further enhance the environmental benefits of the process.
- **Regulatory and industrial validation:** Collaboration with industrial tanneries and regulatory bodies is recommended to validate compliance with international environmental standards and facilitate commercial implementation.

5. DECLARATIONS

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6. AUTHOR'S CONTRIBUTION

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

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8. CONFLICT OF INTEREST

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

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