BEYOND LEATHER: FUNCTIONAL PROTEINS FOR AGRICULTURE AND LEATHER PROCESSING

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ABSTRACT

In the context of climate imperatives and the transition from fossil-based inputs, the Trumpler Group has developed an advanced biotechnological platform for valorizing chrome-tanned leather waste. Through controlled enzymatic hydrolysis followed by nanofiltration, hydrolyzed collagen peptides of high purity and functional integrity are recovered, achieving up to 90% protein yield from chrome shavings.

This biopolymer serves dual applications: as a bio-based retanning agent replacing petrochemical derivatives, and as a bioestimulant in agriculture. In the latter, collagen-derived peptides function as signaling molecules, modulating plant metabolic pathways and enhancing tolerance to abiotic stressors such as drought and salinity. Additionally, they contribute to soil health by promoting beneficial microbial activity and improving soil structure.

Compared to conventional acid or alkaline hydrolysis, the enzymatic process operates under milder conditions, minimizes secondary pollutants, and offers substrate specificity, yielding peptides with preserved bioactivity. Nanofiltration further concentrates low-molecular-weight peptides, enhancing their efficacy and broadening application potential.

The process is integrated within a low-carbon infrastructure utilizing photovoltaic solar arrays and biomass-based thermal energy, significantly reducing Scope 1 and 2 emissions. A closed-loop water management system enables internal recycling of process water, eliminating effluent discharge and aligning with zero-liquid-discharge (ZLD) principles.

This model exemplifies circular economy and industrial symbiosis, converting hazardous solid waste into high value biochemicals. Life Cycle Assessment (LCA) confirms the environmental and economic viability of the process, demonstrating how precision biotechnology and green engineering can drive sustainable transformation in traditionally resource-intensive industries.

Keywords: collagen hydrolysate, nanofiltration, leather waste, protein recovery, circular economy, biostimulant

1. Introduction

The Trumpler Group has developed a biotechnological process to recover hydrolyzed collagen from chrome shavings, addressing the dual challenge of waste valorization and fossil resource dependency. This process, based on enzymatic hydrolysis and nanofiltration, yields a high-purity hydrolysate with applications in leather processing and agriculture. Compared to chemical hydrolysis (Holloway1978), the

enzymatic route is more selective, environmentally friendly, and energy efficient. The integration of renewable energy and water reuse further enhances the sustainability of the process.

Protein-based chemical derivatives, particularly those derived from the controlled hydrolysis of collagen (Taylor1992), have been extensively utilized in leather processing, primarily as functional retanning agents (Escabrós2014). Advances in hydrolysis technology now enable precise control over the molecular weight distribution of collagen hydrolysates, facilitating the development of high-performance polymeric formulations. These amphoteric macromolecules, characterized by defined isoelectric points, exhibit strong affinity and reactivity with both chrome- and vegetable-tanned leathers, enhancing fiber interaction and fixation. Furthermore, these biopolymers present significant environmental and toxicological advantages: they are inherently formaldehyde-free, demonstrate excellent biodegradability, and exhibit high biocompatibility with human skin and mucosal tissues. Their structural flexibility enables chemical modifications, allowing them to be used across a broad spectrum of retanning applications. This, in turn, enhances leather quality, improves process efficiency, and supports overall sustainability.

This paper defines the technical feasibility at a small scale for concentrating the target product through nanofiltration was confirmed, achieving up to 20-30% dry matter. This process included a preliminary ultrafiltration step to remove large molecules and a final reverse osmosis step for polishing the effluent (Pezeshk2019; Pouliot2000). The main objective of the current project is to study the nanofiltration stage at a pre-pilot scale, focusing on both the concentration evolution of the sample and the membrane fouling behavior, as well as the required cleaning protocol (Namla2025).

The second part of this study investigates the agronomic potential of collagen hydrolysates derived through a specific enzymatic process, with a focus on their application as biostimulants in agricultural systems. Emphasis was placed on evaluating the effects of amino acid-based and foliar biostimulant formulations on selected horticultural and fruit crops (Shafeek2020; Wang2019). Specifically, the study encompassed tomato (*Solanum lycopersicum*), potato (*Solanum tuberosum*), and plum trees (*Prunus domestica*), representing a diverse range of crop types and physiological responses.

Field trials were conducted under practical cultivation conditions to assess key agronomic parameters, including vegetative development, plant health, fruit yield, and quality. The findings revealed significant improvements in crop performance and economic viability, suggesting that collagen-derived biostimulants may serve as effective supplements to conventional fertilization strategies. This integrated approach contributes to the growing body of knowledge on sustainable agricultural inputs and supports the development of more resilient and productive cropping systems.

2. Materials and Methods

Filtration

The process consists of three main stages: 1. Ultrafiltration (30 kDa): Removes large molecules. 2. Nanofiltration (500 Da): Concentrates the hydrolysate, targeting peptides and proteins.3. Reverse Osmosis: Polishes the permeate for water reuse (Picot2010).

The filtration system was adapted to accommodate larger membrane modules 2540 (2.5 inches in diameter and 40 inches in length, equivalent to 6.35 cm in diameter and 1 meter in length), and various membranes

were tested. Analytical parameters included pH, conductivity (CE), dry matter (MS), total nitrogen (NT), and chemical oxygen demand (COD). Cleaning protocols involved NaOH and acid washes to mitigate membrane fouling.

Membrane	**Ultra filtration**	**Nanofiltration**	**Reverse Osmosis**
Molecular Cut-off	(30 kDa)	(300 - 500 Da)	Reverse Osmosis
Material	Polyacrylonitrile (PAN)	Poly Piperazine	Polyamide
Area	2.7 m²	2.3 m²	2.4 m²
Maximum Inlet Pressure	8.3 bar	41 bar	41 bar
Maximum Temperature	55ºC	45ºC	45ºC
pH Range	3 – 10	1 – 12	1 – 12
Recommended Recirculation Flow	1.4 m³/h	1.4 m³/h	1.4 m³/h

Table 1 shows the characteristics of the membranes.

Plant Bioassays

Tomato Plant Bioassay

Locations and Soil Types: The tomato trials were conducted in two locations within Zhejiang Province, China. Jiashan County (Jiaxing): Characterized by rice soil and Wenling City (Taizhou): Characterized by slightly coated neutral soil. Crop Varieties: Jiashan: Dongsheng 1329 and Wenling: Hainer 178. Experimental Design: A randomized block design was employed with three replicates per treatment. Treatments: Three treatment groups were established: T1: Conventional fertilization, a widely used agronomic practice to ensure baseline nutrient availability. T2: Conventional fertilization + water spray (control for foliar application). T3: Conventional fertilization + amino acid fertilizer (0.3% dilution) applied three times during the growing season.

Potato Plant Bioassay

Crop and Planting Details: The potato variety used was Fontane (28–35 mm). Planting was carried out on July 16, 2024, with a spacing of 30 cm between plants. Harvesting occurred on October 4, 2024. Fertilization Regime: All treatments received a base fertilization consisting of: 170 kg N from animal manure (equivalent to 102 kg active N), 400 kg of 13-0-17 fertilizer, 200 kg of Greenhouse 27, 17 applications of 2.3N from spray urea.

Treatment Groups Control (Untreated): Standard fertilization only. Collagen Hydrolysate Treatment: Standard fertilization + six applications of 3 L/ha Collagen Hydrolysate, a biostimulant known to enhance crop vigor and stress tolerance.

Plum Tree Bioassay

Location:The trial was conducted in Albatàrrec, Lleida, Spain (UTM: 31N ETRS89; 302416.0 E, 4604790.0 N; 167.2 m a.s.l.). Experimental Design: A total of six trees were monitored, with three trees receiving treatment and three serving as controls. The treatment was applied using an air-assisted sprayer at a 0.3% concentration. Measurements and Data Collection: Shoot Foliage Expansion (SFE, m²/tree): Assessed using UAV-based RGB image analysis on days 0, 10, and 25 post-treatment. Greenness Index (IV): Calculated from RGB analysis of 30 leaves per tree. Average Leaf Size (TPH, cm²/leaf): Measured on the same leaf samples used for greenness index analysis.

3. Results and Discussion

Nanofiltration

This study presents a three-stage membrane filtration process designed to purify and concentrate collagen from a complex aqueous matrix. The process begins with ultrafiltration using a 30 kDa membrane, aimed at removing high-molecular-weight impurities. Approximately 300 liters of sample were processed, targeting a 95% permeate recovery rate. Samples from the feed, permeate, and concentrate were collected for comprehensive physicochemical characterization.

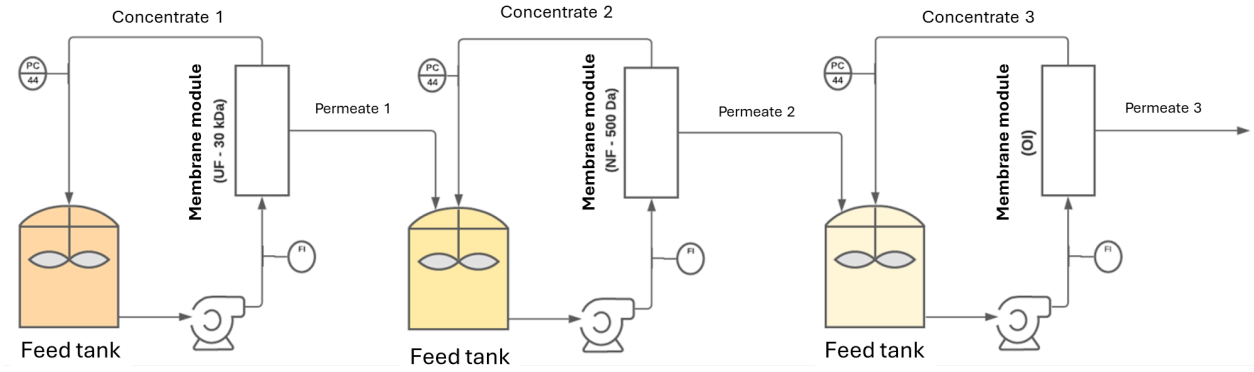


Figure 1 shows the flow chart for the filtration process.

The second stage employs nanofiltration with a membrane cut-off between 300 and 500 Da. This step is critical for concentrating collagen to a target of approximately 20-30% dry matter. Throughout the nanofiltration process, concentrate samples were periodically analyzed to monitor nitrogen content and dry matter evolution, providing insight into filtration efficiency and collagen retention. Again, samples from all streams were collected for detailed analysis.

In the final stage, the nanofiltration permeate undergoes high-rejection reverse osmosis. This step ensures the removal of remaining low-molecular-weight solutes, producing a purified permeate suitable for either valorization or environmentally safe discharge. As with previous stages, all streams were sampled for physicochemical evaluation.

This integrated membrane approach enables selective separation and concentration of collagen while minimizing waste and preserving product quality. The systematic sampling and analysis at each stage provide robust data for process optimization and scalability.

	Units	Initial Sample	UF Per.	UF Conc.	NF per.	NF Conc.	OI Per.	OI Conc.
Weight	kg	300,7	291	9,8	247,5	42,4	213,2	32,8
pH	-	8,27	8,22	8,05	8,15	8,42	8,95	7,92
CE	µS/cm	7,56	7,31	7,98	5,13	6,81	0,24	26,7
MS	%	4,76	4,66	5,33	0,54	30,96	0,02	3,66
DQO	g/L	46,8	40	51,78	3,18	211,39	0,05	15,86
COT	g/L	17,52	15,73	21,2	1,31	87,27	0,02	8,33
NT	g/L	6,964	7,045	8,832	0,523	41,629	0,023	4,082
N-NH_4	g/L	0,621	0,606	0,825	0,166	2,902	0,016	1,023
Cl^-	mg/L	992	967	896	887	862	24,2	6841
$SO_4^=$	mg/L	1940	1812	1922	363	6749	<40	2560
Ca	mg/L	1479	1520	1524	334	5867	<5	1900
Mg	mg/L	333	372	408	165	2151	<10	1377
P	mg/L	0,442	0,483	0,899	0,792	1,18	<0,05	4,2

Table 2 detailed analysis of the flows obtained in the different filtrations

Nanofiltration Permeate: Exhibits a significant reduction in organic load (COD, TOC), nitrogen species, and mineral content, indicating effective purification. This stream may be suitable for reuse or further treatment. Regarding nanofiltration concentrate: Shows a substantial increase in solids, organic matter, and nutrients, confirming the membrane's high retention capacity. This fraction is enriched in collagen peptides and other valuable compounds.

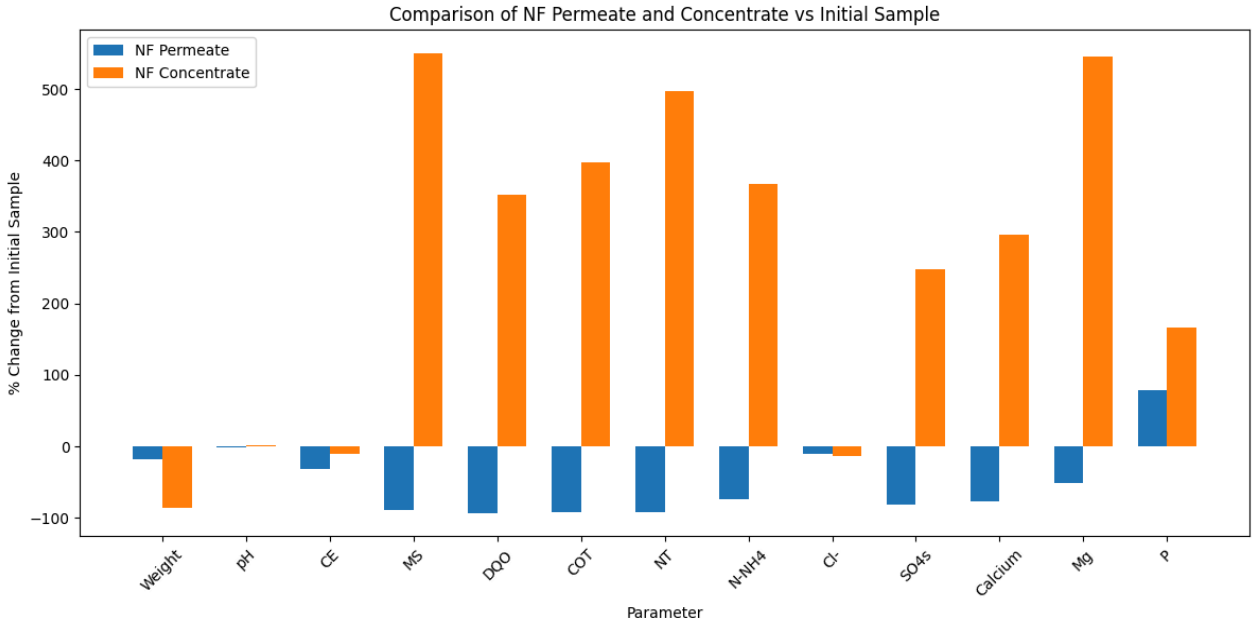


Figure 2 comparison of nanofiltration permeate and concentrate vs initial sample.

Nanofiltration effectively concentrates the collagen hydrolysate, enhancing the recovery of high-value components while producing a cleaner permeate. These results support the application of NF in the valorization of protein hydrolysates.

Plant Bioassays:

Tomato plant growth and yield**.** In the Jiashan region, treatment T3 demonstrated a notable improvement in tomato production metrics when compared to T1. Specifically, T3 increased the fruit count by 20.6% and the average single fruit weight by 8.4%, which collectively contributed to a 15.8% increase in overall yield. Similarly, in Wenling, T3 led to a 3.6% increase in plant height, a 7.2% rise in fruit count per plant, and a 2.5% enhancement in fruit weight relative to T1. Consequently, the total yield in Wenling increased by 10.3%. Furthermore, statistical analysis revealed that the differences observed between T3 and the other treatments were significant ($p < 0.05$) in both locations. In contrast, no significant differences were detected between T1 and T2, indicating that the improvements were specifically attributable to the T3 treatment.

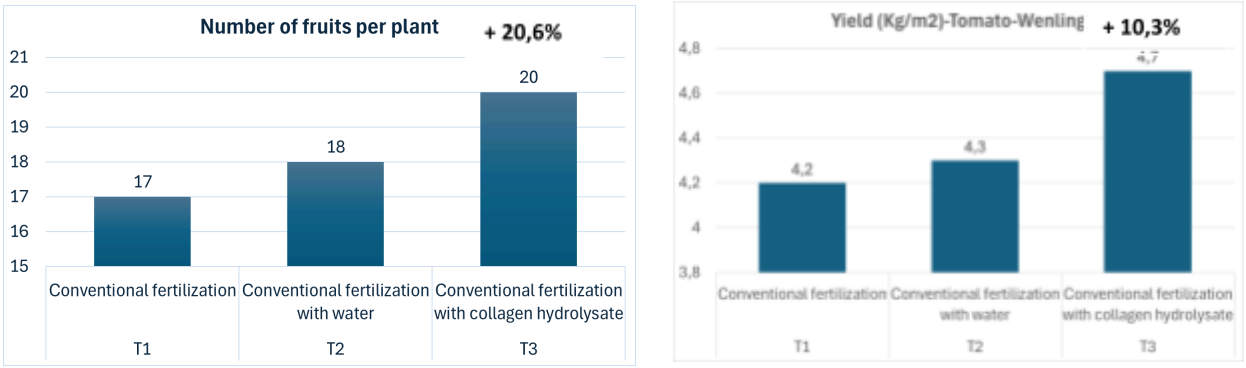
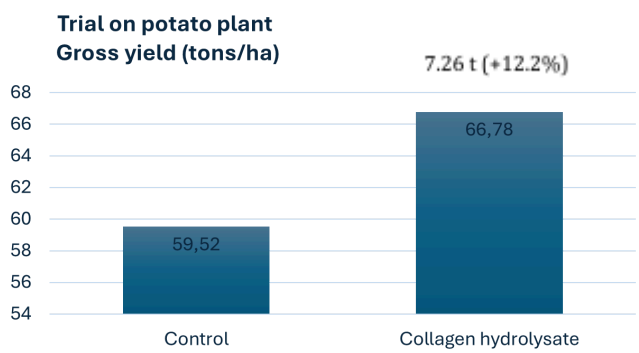


Figure 3 trial on tomato plant.

Potato plant. The potato plant trial yielded compelling results. Collagen hydrolysates resulted in a significantly higher yield increase of 7.26 tons per hectare (a 12.2% increase). The superior performance of Collagen Hydrolysate is attributed to its high concentration of free amino acids and micro peptides. These components enhance plant metabolism by reducing the energy required for amino acid synthesis, thereby allowing the plant to allocate more energy toward growth and development. Additionally, it promotes root development, improving water and nutrient uptake



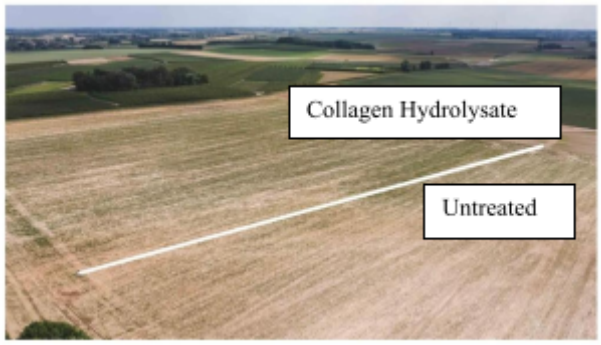


Figure 4 trial on potato plant.

Plum trees. Regarding specific leaf area, treated trees exhibited a greater increase in leaf area (an increase of 2.00 square meters) compared to control trees (an increase of 1.60 square meters). However, this difference was not statistically significant, as indicated by the multivariate analysis of variance (p-value greater than 0.05). Furthermore, no significant differences were observed in the greenness index or in the average leaf size across treatments or sampling dates. Lastly, the visual assessment revealed no signs of phytotoxicity, such as necrosis, chlorosis, or leaf abscission.

4. Conclusion

The pre-pilot study confirms the technical feasibility of recovering functional proteins from leather waste using membrane technology. Nanofiltration is the key step for protein concentration, though operational limits due to fouling and energy use must be managed. The recovered hydrolysate has potential applications in leather retanning and agriculture, supporting a circular and sustainable industry model.

This study demonstrates the effectiveness of a three-stage membrane filtration process, comprising ultrafiltration (UF), nanofiltration (NF), and reverse osmosis (RO). Among these, nanofiltration was identified as the most critical step, enabling the concentration of the hydrolysate with high quality and relatively low energy consumption for the purification and concentration of collagen from a complex aqueous matrix. The ultrafiltration stage successfully removed high-molecular-weight impurities, while nanofiltration significantly concentrated the collagen hydrolysate. The final reverse osmosis step effectively reduced residual low-molecular-weight solutes, yielding a purified permeate suitable for reuse or safe discharge.

In conclusion, nanofiltration is the pivotal stage in the membrane-based concentration process, offering the best balance between product quality, membrane longevity, and energy efficiency for industrial-scale collagen hydrolysate production.

Physicochemical analyses across all stages confirmed the system's high selectivity and retention capacity, particularly in the NF stage, where substantial enrichment of collagen peptides and organic matter was observed. The integrated membrane approach not only enhances product quality but also minimizes waste.

Furthermore, plant bioassays validated the agronomic potential of the collagen hydrolysate obtained with the enzymatic process. Tomato and potato trials showed statistically significant improvements in yield and plant development. Although plum trees exhibited an increased leaf area. Importantly, no phytotoxic effects were observed in any treatment, confirming the safety of the hydrolysate for agricultural use.

Overall, the combined membrane process and subsequent valorization through plant application present a sustainable and efficient strategy for collagen recovery and utilization.

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