

Sequential Low-Enzyme Hydrolysis of Untanned Leather Trimming Waste for the Production of Functional Peptide Fractions

Untanned leather trimming waste is a collagen-rich by-product generated during manufacturing that represents an underutilised renewable protein resource. Hydrolysis offers a sustainable route for producing bioactive peptide fractions for high-value applications, supporting a circular bioeconomy residue whose valorisation could contribute to more circular and resource-efficient material production. However, conventional enzymatic hydrolysis often requires high enzyme dosages and prolonged processing times, limiting its economic and industrial feasibility. This study investigated a low-enzyme sequential hydrolysis strategy for recovering functional peptide fractions from untanned leather trimming waste for potential use in sustainable material formulations.

Following alkaline pretreatment at 90 °C for 2 h, leather residues were hydrolysed with 0.2 or 0.3% Alcalase at 60 °C for 6 h. Degree of hydrolysis, soluble protein, antioxidant activity, molecular-weight distribution and mass conversion were evaluated. Both low-enzyme treatments achieved hydrolysis degrees of approximately 86% ($86.42 \pm 2.32\%$ and $86.08 \pm 3.52\%$ for 0.2 and 0.3% Alcalase, respectively), compared with $75.17 \pm 1.14\%$ for a reference hydrolysate produced using alkaline pretreatment at 90 °C 2 h, 10 % Alcalase for 6 h. Soluble protein concentrations reached 82.10 ± 1.86 and 84.26 ± 2.59 mg/mL, while ORAC activities were 89.87 ± 5.24 and 89.09 ± 5.24 $\mu\text{mol Trolox equivalents/mL}$, for 0.2 and 0.3% Alcalase, respectively. Molecular-weight profiling showed that the final hydrolysates were predominantly composed of peptide fractions within the 3-5 kDa range.

The remaining solid fraction after the 0.2% Alcalase treatment was subsequently re-hydrolysed under the same conditions, increasing the overall cumulative yield across both hydrolysis stages to $86.11 \pm 0.68\%$

These results demonstrate that sequential low-enzyme hydrolysis can maximise protein recovery while reducing enzyme consumption and processing time. The recovered antioxidant peptide fractions are promising renewable ingredients for sustainable coatings and textile materials, supporting leather by-product valorisation within a circular bioeconomy.