Supplementary Material

Optimization of a Single-step Enzymatic Beamhouse: Toward Eco-Friendly Leather Manufacturing

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Materials and methods

TG-DSC

The TG experiments were carried out using a TGA/DSC1 thermogravimetric analyzer (Mettler Toledo, Zurich, Switzerland) from room temperature to 600°C at the heating rate of 5 °C/min. The amount of sample used was around 6 mg. All the experiment were performed in a dynamic N_2 atmosphere. The flow rate of N_2 was 40 mL/min for the sample, and 20 mL/min for protection during the study.

Results and discussion

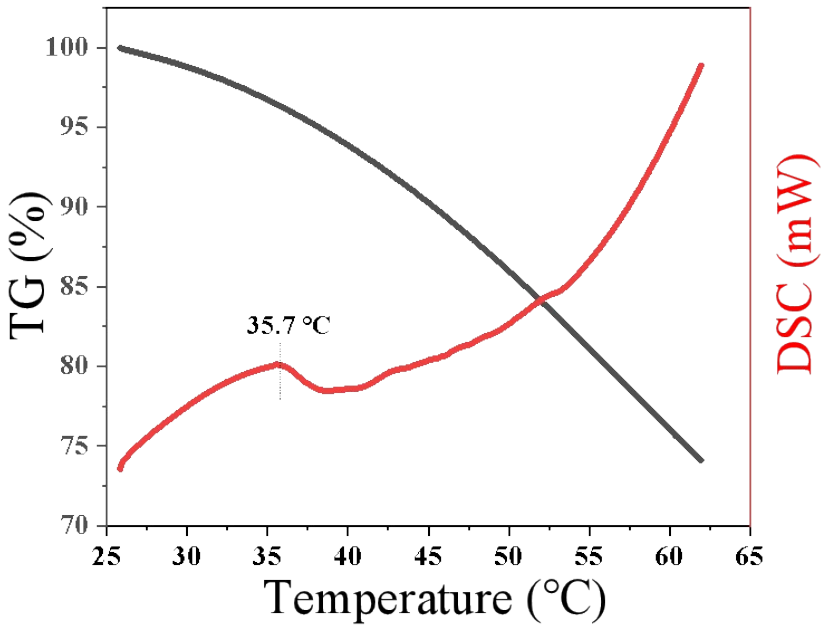

Fig. S1. The TG-DSC results of soaked rawhide.

The observed increase in reaction efficiency with temperature could be explained by two complementary mechanisms. Firstly, the rising temperature enhances the thermal motion of molecules, improving the distribution and flexibility of the enzyme molecular chain. This enhancement likely increases the possibility of enzyme's active ssite binding with the substrate, thereby accelerating the hydrolysis of proteins and polysaccharides. A further potential explanation is the thermal degradation or softening of the substrates at elevated temperatures, which makes them more susceptible to enzymatic attack. Supporting this theory, we conducted a TG-DSC test. The results, illustrated in **Fig. S1**, reveal an endothermic peak around 35°C. The peak is indicative of a glass transition, suggesting that the substrates become more amenable to reaction as their structural rigidity decreases. Beyond Tg, the increased mobility of chain segments likely facilitates greater exposure of active sites, contributing to the enhanced reaction efficiency observed with rising temperature.