Natural fiber biopolymer foam as potential carrier for enzyme immobilization

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Introduction: Biocatalytic processes, known for their operational simplicity and environmental and economic competitiveness, face challenges in catalyst recycling, posing limitations on process development. To address this, enzyme immobilization has been explored, necessitating carriers with specific physicochemical attributes that confer stability to enzymes while accommodating a broad operational range (Rodríguez-Restrepo and Orrego, 2020). In pursuit of eco-friendly strategies and resolution of agricultural waste issues, an underexplored approach involves the utilization of biobased epoxy composite foams (BPXF) filled with lignocellulosic residues as enzyme carriers. This innovative strategy, rooted in the chemically resistant and sustainably sourced nature of BPXF, aims to contribute to both environmental sustainability and agricultural waste management.

Methodology: The biopolymer foam was made from epoxidized pine oil, with a curing agent of polyamine-modified polyacrylates (1:0.5 ratio), using polymethylhydrosiloxane (PMHS) as foaming agent. The enzyme supports were made with the foams filled with dry ground fiber straw of ají rocoto cultivation (hot pepper) (*Capsicum pubescens*) (5% w/w). Rohalase®GMP and Lipase from *Candida Rugose* were immobilized on the carriers. Both biocatalytic systems were characterized by thermo-mechanical properties (TGA/DTG, FTIR and crushing strength), enzymatic activity, activity retention, and immobilized protein measurements.

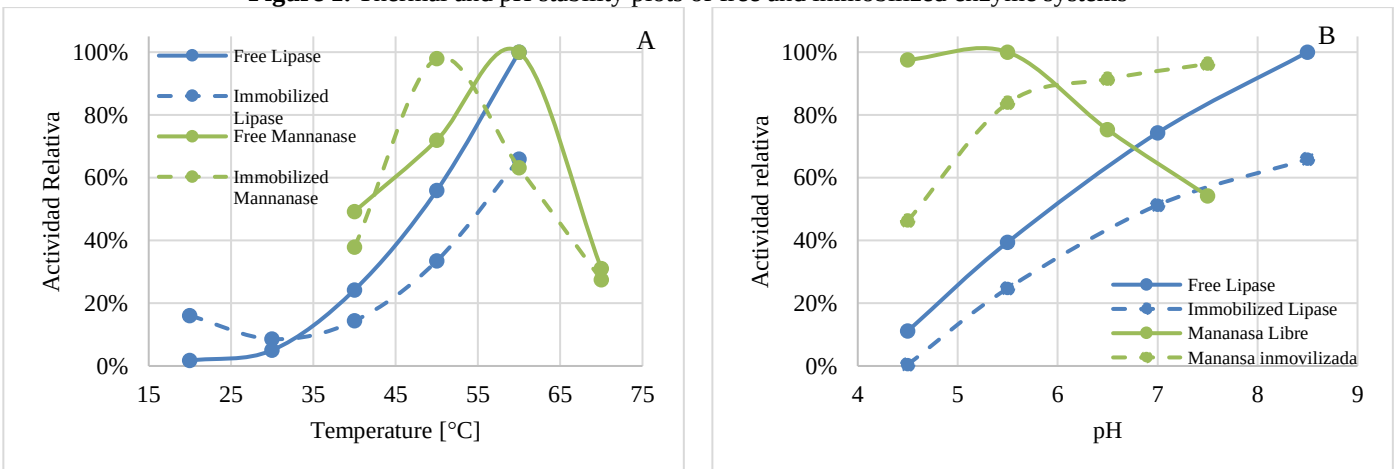
Results: Table 1 shows the results of protein load, catalytic activity and activity retention of the immobilized enzymes with respect to the free enzymes. Activity retention was good, with trypsin presenting the highest value (>95%). The Figure 1a demonstrates that immobilization slightly enhances the lipase performance at low temperatures. Meanwhile, in Figure 1b, there is no apparent effect on the lipase behavior with the pH change; however, mannanase shows improved stability at more basic pH levels. The catalyst exhibits an average breaking strength of 178.39 ± 31.77 N. Crushing strength of six commercial catalysts evaluated by (Wu, Zhou and Li, 2007) shown values ranging from 167.58 ± 42.92 to 335.16 ± 109.76 N.

Table 1. Characterization of the free and immobilized enzyme systems

Catalytic system	Protein load (mg/g carrier)	Activity × 10^3 (U/mg enzyme)	Activity retention (%)
Free Rohalase®GMP		650±90	
**Free Lipase*		2.23±0.29	
BPXF+Fiber+ Rohalase®GMP	30.46±2.18	480±40	77.62±3.40
**BPXF+Fiber+Lipase*	25.93±1.93	1.92±0.23	85.69±2.57

± standar desviation (n=3). U= amount of enzyme that can produce 1 µmol of reducing sugars (mannose base) per 1 min. U=amount of enzyme capable of hydrolysing 1 µmol of pNP per min.*

Figure 1. Thermal and pH stability plots of free and immobilized enzyme systems

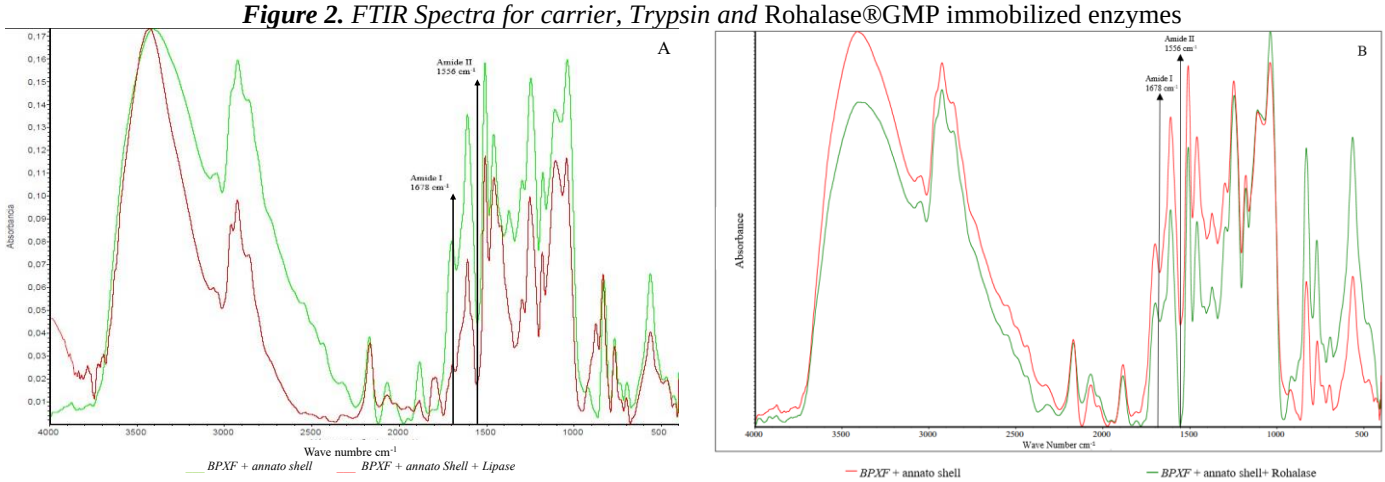


The thermogravimetric profiles of the carrier and immobilized enzymes were similar (Data not shown). A DTG maximum is at 233 °C for the carrier, and immobilized enzymes maximums were between 244 and 254 °C maybe due to the depolymerization of hemicellulose and the scission of weak bonds in lignin in the lignocellulosic residues used as fillers of carrier (Dorez *et al.*, 2013). Total weight loss for all systems were after 350°C, higher temperature than used in enzymatic process.

In Figures 2a and 2b, appear broad absorption bands at 3400 cm^{-1} and 2920 cm^{-1} that correspond to the -OH stretching vibration and the aliphatic -CH stretching, respectively. In the immobilized enzyme spectra these bands present lower intensity that can be related to- OH and -CH stretching from the support and immobilized enzyme. There are many characteristic absorption bands of in the FTIR spectra of proteins, among which the amide I band (1600 cm^{-1}- 1700 cm^{-1}) and the amide II band (1480 cm^{-1}–1600 cm^{-1}) are used to quantitatively analyze the effective immobilization and the alteration of secondary structure after the enzyme attachment to the support (Liu *et al.*, 2021)

Polyamides are used in the manufacture of the fiber-filled biopolymeric foam that was used as a support. So, the amide I (1678 cm^{-1}) and amide II (1556 cm^{-1}) peaks appear in support spectrum. However, when the enzyme is immobilized, this absorption peak is intensified, which is evidence of the presence and integrity of the enzymes bound to the support.

Figure 2. *FTIR Spectra for carrier, Trypsin and* Rohalase®GMP immobilized enzymes



Conclusions: Highly active biocatalysts with excellent activity retention (>70%) were produced. Thermogravimetric analyses indicate that these immobilized enzyme systems exhibit thermal stability within the typical temperature range for enzymatic reactions (< 100 °C). The similar biopolymer matrix in all analyzed samples suggests that shifts in DTG maxima may be linked to the strength of the enzyme-support bond. However, the technique used cannot precisely detect small shifts, limiting our ability to assess the binding strength. FTIR tests revealed the presence of amide I and amide II bands on both the support and the immobilized enzymes. Incorporation of the enzyme into the carrier led to noticeable increases in the infrared wavenumbers of amide I and amide II bands, as observed in the absorption spectra. The catalysts shown good mechanical properties measured trough breaking strength.

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