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Improving the stability and activity of horseradish peroxidase in betaine-based natural deep eutectic systems

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PURPOSE OF THE ABSTRACT

Applying enzymes as biocatalysts in industrial processes is of great interest due to their high selectivity. As profitable operations need to be ensured, several process parameters must be optimized, and the stability of the enzyme has to be assured [1]. The enzyme horseradish peroxidase (HRP) is widely used in chemical reactions, biosensors, and even wastewater treatment [2].

In this work, we propose the application of natural deep eutectic systems (NADES) as enhancers of the activity and stability of HRP. To that end, betaine and sugar-based systems were prepared in different combinations and molar ratios and used to incubate HRP at 37, 60, and 80 ºC for different periods. Densities, viscosities, and water activity of the prepared NADES were measured. The physical properties were successfully calculated using PC-SAFT [3], showing a qualitative agreement for water activity property that is known to be important in enzymatic activity [4]. After incubation, the impact on HRP's activity was assessed through enzymatic activity assays. Circular dichroism was used to evaluate the effect of the incubation conditions on the enzyme's secondary structure. Finally, the thermal stability of HRP in the presence of NADES was assessed with differential scanning calorimetry. Phosphate buffer saline (PBS, 100 mM, pH 7) was used as control throughout the work.

The results obtained so far show that the system composed of betaine, trehalose, glycerol, and water in a molar ratio of 2:1:3:5 is able to preserve HRP's activity, even upon 24h incubation at 60 ºC. Furthermore, this is the only system that maintains more than 50% activity upon 4h of incubation at 80 ºC. Thermal stability increase upon the addition of the NADES, is explained mainly by excluded volume effect in the heme pocket, while sugars such as trehalose, sucrose, and sorbitol use enthalpic interactions with amino acids from the protein backbone that can lead to a decrease in enzymatic activity for high sugar concentrations.

The possibility to extrapolate these findings to more relevant and sensitive molecules could open new opportunities for the pharmaceutical or cosmetic industries. Consequently, bioprocesses are significantly benefited by predictive methods that account for molecular interactions, reducing the experimental effort to find the appropriate components for each enzymatic reaction.

FIGURES

FIGURE 1

FIGURE 2

KEYWORDS

Natural deep eutectic systems | Horseradish Peroxidase | PC-SAFT | stabilization

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