AN INNOVATIVE AND OPTIMIZED SOL GEL IMMOBILIZATION TECHNIQUE FOR GLYCOSIDES ENZYMATIC HYDROLYSIS

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In recent years there has been a growing interest for the health benefits of glycosides due to their important properties such as anti-oxidant, anti-inflammatory, anti-demential and anti-carcinogenic. The sugar residue is important for their activity, although in some cases deglycosylation improves the biological activity.

In this work a biocatalytic system was chosen to enable the deglycosylation of glycosides towards biomolecules, with improved biological activity. Naringinase (an enzyme complex consisting of *α*-L-rhamnosidase and *β*-D-glucosidase activity) was the enzyme used. Naringin, the substrate used in this bioconversion, and the product, its aglycone, naringenin, are healthy compounds with biological and pharmacological activities, such as anti-oxidant, anti-inflammatory and anti-cancer, showing a high potential in the pharmaceutical industry.

Studies regarding immobilization of naringinase on polymer matrices, k-carrageenan[1], calcium alginate[2], and the re-usability of the immobilized enzyme have been reported.

Sol-gel, an innovative and economical technique for naringinase immobilization in aqueous media was developed. Different sol-gel precursors (tetramethoxysilane, TMOS, methyltrimethoxysilane, MeTMOS, 3-Aminopropyl-trimethoxysilane, APTMOS, diglyceryl silane, DGS) at different aging time were tested in five consecutive re-utilizations. The best results were obtained with TMOS, 4 h aging time, TMOS and glycerol, DGS, 14 h aging time and TMOS/DGS, 4 h aging time. In order to optimize these four matrix, the effects of enzyme concentration, pH, reutilization and aging type system on immobilization efficiency were evaluated. These matrix were characterized regarding temperature, pH, naringin and naringenin partition coefficient and isotherms. The operational stability of bioencapsulated naringinase in the different sol-gel matrices was studied through fifty successive re-utilizations; 100% of residual activity remains constant for the best matrix obtained. Naringinase deactivation followed the Sadana model[3].

1. Ribeiro I.A., Ribeiro M.H.L. *J. Mol. Catalysis B: Enz.* 2008, 51, 10–18.
2. Pedro, H.A., Alfaia, A.J., Marques, J., Vila-Real, H.J., Calado, A.T., Ribeiro, M.H. L., *Enz. Microb. Technol*. 2007, 40, 442-446.
3. Sadana A, Henley JP. *Enzyme Microb Technol,* 1985, 7, 50-60.