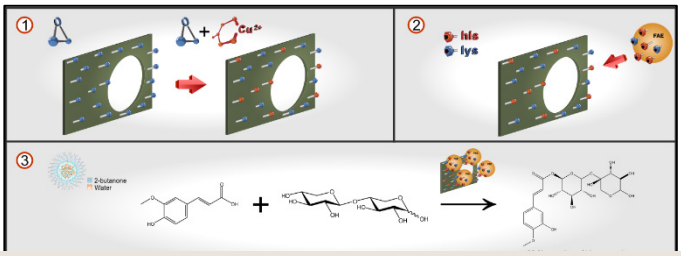

BIOCATALYTIC ACYLATION OF CARBOHYDRATES TO PRODUCE FERULOYLATED OLIGOSACCHARIDES AND CARBOHYDRATE FATTY ACID ESTERS

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Abstract

Enzymatic acylation of saccharides with bioactive moieties has attracted attention as a possible route to produce value-added products from low cost renewable sources. This research work investigated the potential of immobilized carboxylic ester hydrolases to catalyze the synthesis of compounds with potential bioactive properties in non-conventional media. Namely, the feruloyl esterase (FAE)-catalyzed ferulic acid acylation of oligosaccharides in surfactantless microemulsions and the C_{12}-C_{14} fatty acid acylation of fructose and fructooligosaccharides (FOS) in low-solvent reaction media. FAE from *Humicola insolens* showed broader acyl acceptor specificity in the media composed by *n*-Hexane/2-Butanone/Water. Modification of epoxy immobilization supports to include charged reactive groups or metal-chelate reactive groups led to higher hydrolytic FAE activity/gram of support. The feruloylation capacity of immobilized FAE was found to be dependent on the immobilization support. Optimization by response surface methodology (RSM) of the FAE immobilization onto metal-chelate-epoxy mesoporous supports resulted in immobilization conditions that caused an increase of the enzymatic activity yield and retention of specific activity yield, when compared to unoptimized immobilization conditions. An increment of support pore size improved hydrolytic activity and esterifying efficiency of the immobilized FAE. It was determined that the optimally immobilized and stabilized macroporous FAE retained up to 92.9 % of the feruloylation activity of the free enzyme. Enzymatic fatty acid acylation of fructose was performed in a mixture of C_{12} or C_{14} fatty acid/*tert*-butanol (90/10; v/v). Novozym® 435 reactions attained a bioconversion yield and productivity of 19.7 % and 9.45 μmol/L min, respectively, were obtained with a ball-milled fructose concentration of 0.2 mol/L. An increase in reaction media fatty acid content adversely affected esterification. The enhancement of polar substrate solubility and an increase in acylation reaction productivity were significant benefits of the combined low-solvent and mechanical milling of reaction substrates. Acyl acceptor chain length was determined to be inversely proportional to the reaction yield while inclusion of an unsaturated fatty acid in the reaction media decreased the viscosity and the melting point of the media but also decreased the esterification yield of the reaction. Maximum fructose myristate yield (22 %) was obtained using 0.2 mol/L of fructose and 5 % (w/v) of Lipozyme® RM IM in a reaction media composed of 10 % tert-butanol, 10 % oleic acid, and 80 % myristic acid, using RSM based on a 5-level and 3-factor central composite design. Mass spectrometry confirmed the production of mono- and diesters, and the selectivity of this lipase for saturated

About the Candidate

Juan holds a B.Eng. degree in Food Engineering from ESPOL Polytechnic University (Ecuador). After 6 years of industry experience in the food additives sector he decided to pursue higher studies at McGill as a non-thesis M.Sc. student in the Food Science Department. Within one year he switched to the M.Sc. thesis program under the supervision of Dr. Salwa Karboune, fast-tracking to the Ph.D. program the following year.