E 61 IMMOBILIZATION OF α-GALACTOSIDASE BY HYDROPHOBIC INTERACTION AND COVALENT ATTACHMENT TO SEPHAROSE-4B

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α-Galactosidase is used in the food industry to hydrolyse the raffinose family of sugars[1]. In this study purified α-galactosidase from coconut[2] was immobilized using a hydrophobic gel and by covalent attachment to Sepharose-4B.

α-Galactosidase was immobilized by binding to the hydrophobic gel Sepharose-4B-Capranilide[3] with a retained enzyme activity of 59%. The loss of activity after 30 days storage at 6°C was 34%. Immobilized α-galactosidase (60 units) on a Sepharose-4B-Capranilide column (2.5 x 20 cm) hydrolysed 43% of a 1% raffinose solution eluted at a flow rate of 75ml/h in 4h. However, 2.5 units of free enzyme hydrolysed 36% of a 1% raffinose solution in 6h indicating the low efficiency of α-galactosidase immobilized on a hydrophobic gel.

α-Galactosidase covalently bound to Sepharose-4B-caproic acid did not lose any activity when stored at 6°C for 30 days. Optimum conditions for covalent attachment was determined by varying the CNBr content used for activation of Sepharose-4B from 25mg to 200mg per 100mg of gel. Enzyme activity of the α-galactosidase immobilized gels varied from 5 to 48 milliunits/ml gel while the maximum retained activity of 32% was obtained with a CNBr content of 100mg per 100mg of gel.

References

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