Immobilized zinc affinity chromatography of pectin hydroxamic acids for purification of trypsin inhibitors from soybean and sweet potato

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Abstract

Commercial pectin (with a 94% degree of esterification, DE94) suspended in methanol was reacted with methanolic alkaline hydroxylamine at room temperature for 20 h to prepare pectin hydroxamic acids (PHAs). The prepared PHA was coupled to the epoxy-activated Sepharose 6B gel to get immobilized PHA resins. The immobilized PHA resin was then balanced in column with 2 mM ZnCl2 in 50 mM Tris-HCl buffer (pH 7.9) to test the immobilized Zn-PHA gel as solid phase for immobilized metal affinity chromatography for the purification of trypsin inhibitors (TIs) from soybean and sweet potato. Using TI activity staining, it was found that purified TIs from the commercial soybean and sweet potato after trypsin affinity column purification could be adsorbed onto an immobilized Zn-PHA affinity column and eluted by 100 mM EDTA in 10 mM Tris-HCl buffer (pH 7.9). The immobilized Zn-PHA affinity column was used for TI purifications from crude extracts of sweet potato. The recovery of TI activity for one step was 90%, with 19.74-fold purification increase.