An inhibitive determination method for heavy metals using bromelain, a Cysteine protease.

ABSTRACT

A heavy-metal assay has been developed using bromelain, a protease. The enzyme is assayed using casein as a substrate with Coomassie dye to track completion of hydrolysis of casein. In the absence of inhibitors, casein is hydrolysed to completion, and the solution is brown. In the presence of metal ions such as Hg2+ and Cu2+, the hydrolysis of casein is inhibited, and the solution remains blue. Exclusion of sulfhydryl protective agent and ethylenediaminetetraacetic in the original assay improved sensitivity to heavy metals several fold. The assay is sensitive to Hg2+ and Cu2+, exhibiting a dose-response curve with an IC50 of 0.15 mg 1(-1) for Hg2+ and a one-phase binding curve with an IC50 of 0.23 mg 1(-1) for Cu2+. The IC50 value for Hg2+ is found to be lower to several other assays such as immobilized urease and papain assay, whilst the IC50 value for Cu2+ is lower than immobilized urease, 15-min Microtox, and rainbow trout.

Keyword: Bromelain; Cysteine protease; Inhibitive determination method.