

In vitro α -amylase inhibitory assay

Maira Rubi, Maira Rubi Segura Campos

Abstract

The assay was carried out following the protocol reported by Dineshkumar et al. [8]. Starch (2 mg) was suspended in a tube containing 0.2 mL of 0.5 M Tris-HCl (Sigma-Aldrich, USA) buffer (pH 6.9) with 0.01 M calcium chloride as substrate. The tube was boiled for 5 min and then preincubated at 37 °C for 5 min. Plant aqueous extract (1 mg) was dissolved with 1 mL of 0.1% of dimethyl sulfoxide in order to obtain a concentration of 1,000 μ g/mL; then 0.2 mL of aqueous extract was added to the tube containing the substrate solution, 0.1 mL of porcine pancreatic amylase in Tris-HCl buffer (2 U/mL) was also added, and incubated for 10 min at 37 °C. Finally, the reaction was stopped with 0.5 mL of acetic acid (50% v/v) and centrifuged 5 min at 1,811 \times g and 4 °C. The assay was performed in triplicate. The α -amylase inhibitory activity was calculated using the formula $(Ac^+ - (Ac^- - (As - Ab)) / (Ac^+ - (Ac^-)) \times 100$, where Ac^+ , Ac^- , As , Ab are defined as the absorbance (595 nm) of 100% enzyme activity (only solvent with enzyme), 0% enzyme activity (only solvent without enzyme), test sample (with enzyme), and a blank (a test sample without enzyme), respectively.

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