ABSTRACT

β-Galactosidase (EC 3.2.1.23) from ripe coffee beans was purified and characterized. Purification was carried out using $(NH_4)_2SO_4$ precipitation, ion-exchange chromatography on Cellex-CM and affinity chromatography on p-aminophenyl-β-D-thiogalactopyranoside-agarose. The enzyme displayed activity against PNPG (Km 0.33 mM), lactose (Km 40 mM), arabinogalactan and galactan. Activity was highest within a pH range of 2.5 - 6.0, with an optimum at pH 4.40. MW was estimated to be 2.9×10^4 by polyacrylamide gel electrophoresis in the presence of SDS. The enzyme was competitively inhibited by galactose (Ki, 0.26 mM), $CuSO_4$ and $HgCl_2$ inhibited the enzyme non-competitively (Ki, 1.17 and 0.33 mM respectively). p-chloromercuribenzoate at a concentration of 0.29 mM completely abolished activity.

The enzyme catalysed release of galactose from galactan and arabinogalactan; with pectin, the enzyme yielded free galactose only when polygalacturonase was

also present.

β-Galactosidase activity increased with the ripening of the coffee berry and the activity found in ripe berries was approximately 5.46-fold higher than in unripe fruit. It seems likely that this enzyme is involved in cell wall degradation such as occurs during ripening or seed germination.

Since ethylene is a major ripening hormone of most fruits, the possible inter-relationship between β-galactosidase activity and ethylene formation was examined. Galactose (a product of β – galactosidase activity) at concentrations varying from 10 - 25 mM, inhibited the production of ethylene. This was shown from studies in which coffee seeds were utilized in vitro to catalyse the conversion ACC to ethylene.