

Abstract

Glucosinolates are secondary products of plants, that are hydrolysed by the enzyme myrosinase. Hydrolysis of glucosinolates leads to a production of toxic compounds, such as isothiocyanates. These degradation products are involved in the defense system of the plant against external pathogens.

This bachelor thesis deals with monitoring of myrosinase enzymatic activity by capillary electrophoresis. As a substrate, 3-indolylmethylglucosinolate known by trivial name glucobrassicin, was selected. The enzymatic reaction was tested in both off-line and on-line setting by capillary electrophoresis. As the background electrolyte a solution containing 9 mM sodium tetraborate, 15 mM phosphoric acid and 10 mM hexadecyltrimethylammonium chloride (pH = 7.02) was used. The sample was injected hydrodynamically by pressure (5 kPa, 3 s). A voltage of -20 kV was applied to the capillary and solution in the capillary was simultaneously mobilized by a pressure 5 kPa. The optimized method was evaluated by measuring calibration curve, limit of detection, limit of quantification, repeatability of injection of glucobrassicin in off-line and on-line setting and also repeatability of on-line enzymatic reaction. The limit of detection was 0.011 mg ml⁻¹ and the limit of quantification was 0.035 mg ml⁻¹. In both off-line and on-line setting relative standard deviations were for peak areas less than 4 % and for migration time less than 1.5 %. The relative standard deviation for the enzyme reaction in the on-line setting was 0.76 % for the peak area and 0.54 % for the migration time.

Keywords

Myrosinase, glucobrassicin, capillary electrophoresis, enzyme activity