ARRHENIUS AND ABSOLUTE REACTION RATE MODELS FOR THERMODYNAMIC CHARACTERIZATION OF LINAMARASE (B-GLUCOSIDASE) USING LINAMARIN SUBSTRATE

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ABSTRACT

Thermodynamic characterization of linamarase (β-glucosidase)influenced by linamarin substrate purification, pH and temperature were investigated. In the study, recombinant Saccharomyces cerevisiae cells at the stationary phase of growth were recovered, homogenized and centrifuged to obtain crude extracts designated as $GELIN_0$. Carboxy methyl cellulose, diethyl amino-ethyl-sephadex and diethyl amino-ethyl-cellulose were used to purify the crude extracts resulting in $GELIN_1$, $GELIN_2$ and $GELIN_3$, respectively. Commercial native linamarase (CNLIN) was purchased and used as control. The ability of the GELIN extracts(β-glucosidase) and the commercial native linamarase (CNLIN) to hydrolyse cyanogenic glucosides was challenged using linamarin extracted from cassava as substrates. Degradation of linamarin was evaluated at optimum pH 6.8 using a 4 × 6 × 8 between and within factorial design comprising of 4 enzyme types ($GELIN_0$, $GELIN_1$, $GELIN_2$ and $GELIN_3$), and 6 temperatures (25, 27, 29, 31, 33, 35 ^oC, respectively) and 8 time intervals (0, 10, 20, 30, 40, 50, 60 and 70 min.). Data obtained from residual hydrocyanic acid with time were fitted with zero, first and second order kinetics, respectively, to determine the best fit order (based on r^2 and linearity). Arrhenius and absolute reaction rate models were applied to obtain activation energies (E_a), frequency factor (K_0) and enthalpy ($\Delta H^{\#}$), entropy ($\Delta S^{\#}$), respectively, that characterized the reactions. The results indicated that the degradation of linamarin by GELIN at the optimum pH 6.8 was best described by first order kinetics, Arrhenius and absolute reaction rate models showing high coefficient of linear regression (r^2>0.996) with reaction rate constant increasing from 0.0252 -0.0923 min^{-1} with enzyme purification ranging for $GELIN_0$ – $GELIN_3$. Frequency factor (K_o), E_a, $\Delta H^{\#}$ and $\Delta S^{\#}$ values decreased with enzyme purification. Activation energy (E_a) values for the degradation of linamarin ($GELIN_0$ – $GELIN_3$) ranged from 60.9 to 91.7 kJ/mol. Enthalpy values varied from 58 to 89 kJ/mol while $\Delta S^{\#}$ values varied from -92.8 to 4.1 J/mol. deg.) indicating spontaneous and irreversible degradation reactions which suggest a possible use of the purified linamarase (β-glucosidase) in detoxification process for foods containing linamarin.