APPLICATION OF DHURRIN FOR KINETICS AND THERMODYNAMIC CHARACTERIZATION OF LINAMARASE (Β-GLUCOSIDASE) GENETICALLY ENGINEERED FROM *SACCHAROMYCES CEREVISIAE*.

Ikya, J.K[1]., Ariahu, C. C.[1] and Ayatse, J.O.I. [2]

[1]Department of Food Science and Technology, University of Agriculture, P. M. B. 2373, Makurdi.

[2] Department of Biochemistry, University of Agriculture, P. M. B. 2373, Makurdi.

*[1]Corresponding Author: E-mail: aveyina2012@gmail.com

ABSTRACT:

***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 of $GELIN_0$ resulting in $GELIN_1$, $GELIN_2$ and $GELIN_3$, respectively. The ability of the enzyme extracts and a commercial native linamarase (CNLIN) to hydrolyse cyanogenic glucosides was challenged using dhurrin from sorghum as substrate. Precisely, the actions of commercial native linamarase (CNLIN) and the genetically engineered linamarase (β-glucosidase) from* Saccharomyces cerevisiae *on dhurrin as influenced by degree of its purification, pH (6.8) and temperature(30-45^oC) were investigated and the data derived were applied for kinetics and thermodynamic characterization of the enzymes. Enzymic degradation kinetics of the dhurrin were evaluated using a 4 x 6 x 8 B/W design comprising of 4 enzyme types (GELINo, $GELIN_1$ $GELIN_2$ and $GELIN_3$,, 6 temperatures (30, 32, 35, 37, 40, 45 °C) and 8 time intervals(0-70 min.). Data obtained from the residual HCN with time were fitted into zero, first and second order kinetics models to derive reaction rate constant ($Kmin^{-1}$) values which were analyzed using the Arrhenius and absolute reaction rate models Thermodynamic parameters were obtained including; activation energies (E_a), frequency factor(K_o), enthalpy (ΔH) and entropy ($\Delta S^{\#}$) that characterized the reactions on dhurrin catalyzed by commercial native linamarase (CNLIN) and the genetically engineered linamarase (β-glucosidase) from* Saccharomyces cerevisiae*. The results showed that the best fitted order based on higher coefficient of linear regression (r^2) values > 0.998 and linearity of curves was the first order kinetics model and not either zero or second order models. $Kmin^{-1}$ values ranged from $GELIN_O$- $GELIN_3$ 0.03-0.07 µmol/min while the derived D-values from K-values were in the range of 24-65 min. The frequency factors (Ko) increased with enzyme purity from $GELIN_0$ to $GELIN_3$ corresponding to K_o (min^{-1}) of 22.585 to 56.462. The energy of activation Ea (KJ/mol) generated 60.0995 to150.6900 corresponding to enzymes $GELIN_0$ to $GELIN_3$ followed the same pattern with frequency factor for breaking of bonds in dhurrin molecules. At pH 6.8 CNLIN showed no action on dhurrin. The high correlation coefficient values of (r^2= 0.97 to 0.99) indicated the best fit of the Arrhenius and the absolute reaction rate models. The entropy change (ΔS) increased with enzyme purity from 0.588 J/mol.deg. to 1.4625Jmol degree. The enthalpy change KJ/mol followed the same pattern whereby increases influenced by enzyme purity ranged from 1892 KJ/mol to 13104KJ/mol.**

Contact us for full article. Email: info@agrosciencejournal.com