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KINETIC COMPENSATION EFFECT ON CASSAVA LINAMARIN DEGRADATION AT VARYING PH, OPTIMUM TEMPERATURE AND PURIFIED B-GLUCOSIDASE GENETICALLY ENGINEERED FROM SACCHAROMYCES CEREVISIAE.

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ABSTRACT

Heat degradation kinetics of linamarin were evaluated to obtain reaction rate constants $Kmin^{-1}$ at varying pH3.5, 6.8 and 10.5. Reaction rate Data obtained were analyzed using the Arrhenius and absolute reaction rate models to obtain activation energies (E_a), enthalpy (AH) and entropy ($AS^{\#}$).. The thermodynamic parameters were subjected to kinetic compensation analysis to obtain iso-kinetic temperatures (T_c), rate constants (k_c) and free energies (AG^W) related to the degradations of linamarin. at varying pH. The results showed that the Isokinetic Temperature (Tc) and Constant (Kc) for Degradation of at pH 3.5, Tc = 320k equivalent to 47^0C. The isokinetic constant was 1.25×10^{-3}/min. and the least square regression analysis which was applied produced a correlation coefficient r^2 value > 0.98. The same pattern was observed for degradation of Cassava linamarin at pH 6.8 and pH 10.5. Data obtained as isokinetic temperature (Tc) is very important and can be employed in control fermentation of foods containing Cassava linamarin. At this temperature, the fermentation processes are

independent of environmental conditions. These are derived from K_o vs E_a and ΔS vs ΔH relationships respectively and are independent of the environmental variables and presumably applied for predictive purposes. T_c range from 47.0-47.8 this value is usually compared with mean harmonic temperature which T_H which was computed in this study as 134^0C. From the results T_H was far greater than T_c. This observation validated true kinetic compensation effect where increase in K_o values Proportionally increased the E_a values and vice versa. Enthalpy of activation was plotted against the entropy of activation of heat degradation of Cassava linamarin produced 93.8KJ/mol of free energy of activation (ΔG). The enthalpy of activation varies from 274-302KJ/mol, the plot of this value against enthropy change produce the calculated ΔG value. Both entropy and enthalpy exhibited marked compensation effect in which increases in ΔH was accompanied by ΔG. The computed r^2 = 0.98 value calculated by least square regression analysis indicated the fitness of kinetic compensation models in the prediction of environmental conditions.. It was concluded in this study that true kinetic compensation occurred in the degradation of cassava linamarin at varying pH. This study on kinetic compensation can be used for predictive purposes in controlled fermentation of foods in our localities.

Keywords: *Kinetic compensation, mean harmonic temperature, Isokinetic Temperature (Tc) Controlled fermentation, pH*

INTRODUCTION

Natural fermentation involving native Linamarase from microorganisms applied for detoxification of Linamarin a

cyanogenic glucoside found in cassava is uncontrolled and lengthy.(Nok, . and Ikediobi, 1999., Onyike *et. al.*.2001)
This approach most often results in high amounts of residual cyanide in the finished products. Apart from the stated concern, other factors that prevent adequate degradation and detoxification of linamarin by native Linamarase include its' low concentration, limited spectrum of substrates activity and sensitivity to environmental inactivating factors especially changes in pH and temperature which occur in the fermenting containers. Controlled fermentation using extrogenous β-glucosidase can reduce fermentation periods while assuring efficient hydrolysis and detoxification of cyanogenic glucoside linamarin. The enzyme can be genetically engineered. Genetic engineering can be employed to produce robust and versatile enzymes for increased turnover and wider substrates specificity. This approach will improve on the efficiency and substrate spectrum of the native Linamarase to boost raw material transformation and products development from crops such as cassava, sorghum almond, stone fruits, lima beans and bamboo shoots.

Microorganisms have been extensively used in genetic engineering for production of single cell proteins, enzymes, hormones and vitamins. This is because of their low generation times and ease of manipulation. In enzymes production, efforts are on the use of bacteria cultures. In vitamins and protein production, yeast cells have been employed. Unfortunately, most of the bacteria such as Escherisia coli are potential pathogens and hence unsafe in food systems.
Saccharomyces cerevisiae is non-pathogenic yeast which is safe to use in food systems (Larone 2006). Saccharomyces cerevisiae is the conventional species used in baking, brewing

and wine production technology. The success of this study depends on obtaining appropriate Biotechnology and using non-pathogenic microorganisms such as *Saccharomyces cerevisiae* as vehicles. For the production of genetically engineered linamarase for accelerated and adequate degradation of Cassava linamarin contained in some food stuffs. However, Information on a specific Biotechnology for the production of genetically engineered linamarase from *Saccharomyces cerevisiae* for controlled fermentation action for accelerated and adequate degradation of Cassava linamarin is lacking. In this study genetic engineering was employed as a manipulation tool for production of β-glucosidase from the yeast *Saccharomyces cerevisiae* for food processing especially controlled fermentation action on Cassava linamarin. The kinetics and activities of the purified genetically engineered β-glucosidase from the yeast were evaluated to provide an insight into the mechanism of their actions which provided the parameters necessary for predictive purposes. Such predictions can be useful tools for fermentation process optimization for the degradation of Cassava linamarin in food systems. This study therefore, will highlighted the genetic techniques for industrial production of genetically engineered β-glucosidase (Linamarase) from yeast (*Saccharomyces cerevisiae*) for the degradation of Cassava linamarin present in certain food crops.

This study will provide the parameters which can be useful tools necessary for prediction of fermentation process related to the degradation of Cassava linamarin in food systems leading to optimization of raw material transformation and products development. The study will provide new technology for the production of residual cyanide

free food products for consumption that are safe from chronic cyanide toxicity and as a contribution for improving the nutritional and health status of consumers. The objective of this study was to Evaluate the effects varying pH on isokinetic temperature and kinetic compensation effect for heat degradation Linamarin.

MATERIALS AND METHODS

The Method of Itoh-Nashida *et. al.* (2007) Ikya et al. (2012a&b) were applied for the production of purified linamarase (ß-glucosidase). Linamarin was extracted from cassava tuber paranchyma tissues using the method of Ikediobi and Ogunda (1985) and Ikediob and Onyike, (2002). One year old fresh cassava tubers were harvested and immediately washed clean and frozen overnight at -10^{0}C. Essentially, 800g of frozen cassava parenchyma tissues were sliced with stainless steel knives and homogenized with 160ml of chilled 0.1M phosphoric acid solution. The resultant slurries were filtered rapidly using glass wool and the filtrate centrifuged (1000 rpm) for 5min. The resulting filtrate was centrifuged at 5000 xg for 5min. The resulting supernatant was adjusted to pH 8.0 and re-centrifuged at 500gxg for 8 mins. A total of 0.82g of white substrate mp.143^{0}C was obtained from a total of 800g of fresh cassava tissues. This was stored at 4 ^{0}C for subsequent use in the assays of Linamarase (ß-glucosidase) genetically engineered from Saccharomyces cerevisiae.

Heat degradation kinetics of linamarin were evaluated to obtain reaction rate constants Kmin^{-1} at varying pH3.5,6.8 and 10.5. Reaction rate Data previously obtained by Ikya et al 2012a&b were analyzed using the Arrhenius and

absolute reaction rate models in equations 1- 5 below to obtain activation energies (E_a), enthalpy (AH) and entropy ($AS^{\#}$).. The thermodynamic parameters were further subjected to kinetic compensation analysis by related plots and regression to obtain iso-kinetic temperatures (T_c), rate constants (k_c) and free energies (AG^W) related to the degradations of linamarin. at varying pH. Theoretical consideration: Kinetic compensation models applied were:

Arrhenius model, $\ln K = \ln K_o - Ea/R(1/T)$ ....Equation 1

$\ln K_o = \ln kc + 1/RT_C E_a$ ..... Equation 2

from which a plot of $\ln K_o$ VS E_a apllied and evaluated isokinetic temperature(T_C)

Absolute rate model:

$\ln K/T = \ln Ks/hc + \Delta S /R - \Delta H /R(1/T$.....Equation 3

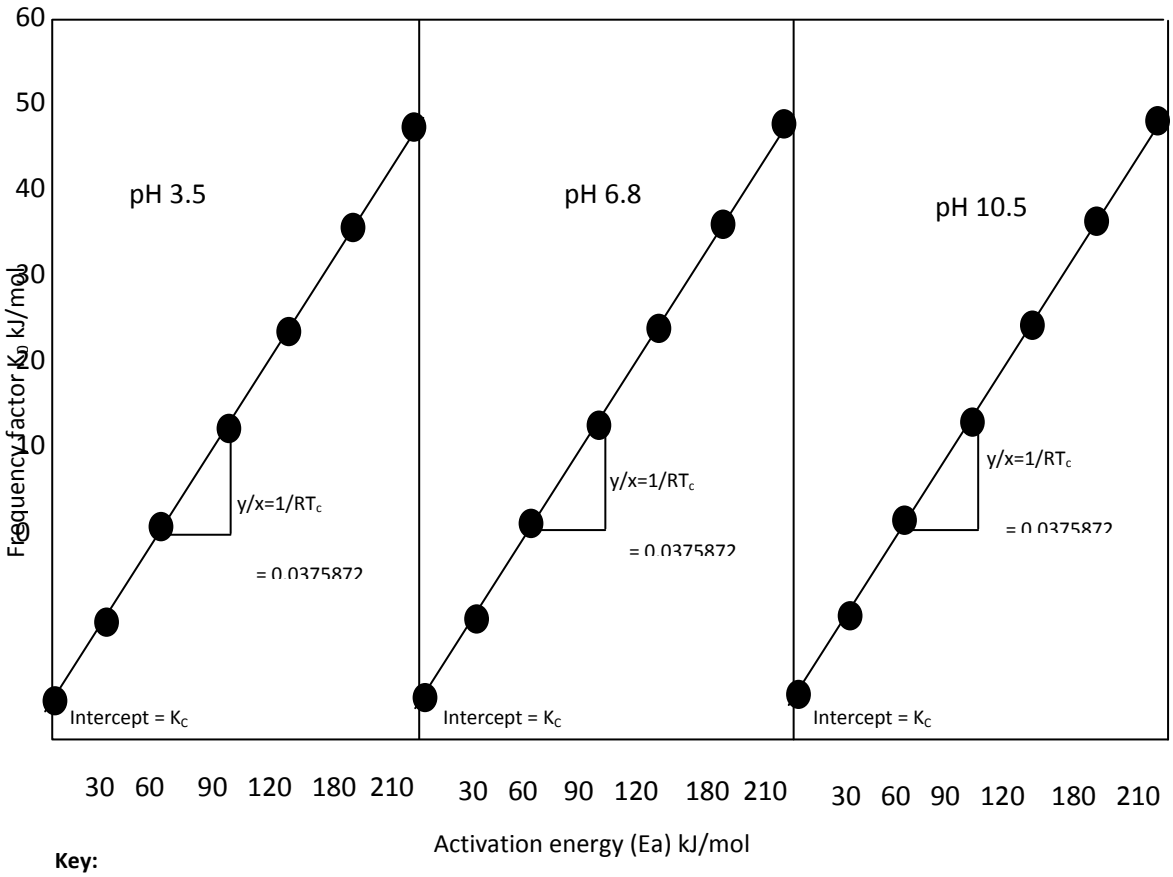
$\Delta G = \Delta H - T\Delta S$..... Equation4

$\Delta S = -\Delta G/T + 1/T_c (\Delta H)$. ..... Equation 5

A plot of ΔS VS ΔH in Equation 5 was applied in determining isokinetic temperature (T_c)

The Statistical aspect of kinetic modeling for Food Science problems of Van Boekel(1996) was applied to analyse data collected from the study

RESULTS AND DISCUSSION



Key:
R = Gas constant (0.08314)
T_c = Iso kinetic temperature (^{0}C)
K_c = Iso kinetic constant

Fig 1: Plots of Frequency factor K_o VS Activation energy E_a for evaluation of T_c

Fig 1: Shows Plots of Frequency factor K_o VS Activation energy E_a for evaluation of T_c . Effect of varying pH. on isokinetic temperature (Tc) and Isokinetic constant (Kc) for degradation of Cassava linamarin at varying pH 3.5 – pH 10.5 revealed that at pH 3.5, Tc = 320k equivalent to 47^0C. The Isokinetic constant (K_C) was 1.25×10^{-3}/min.

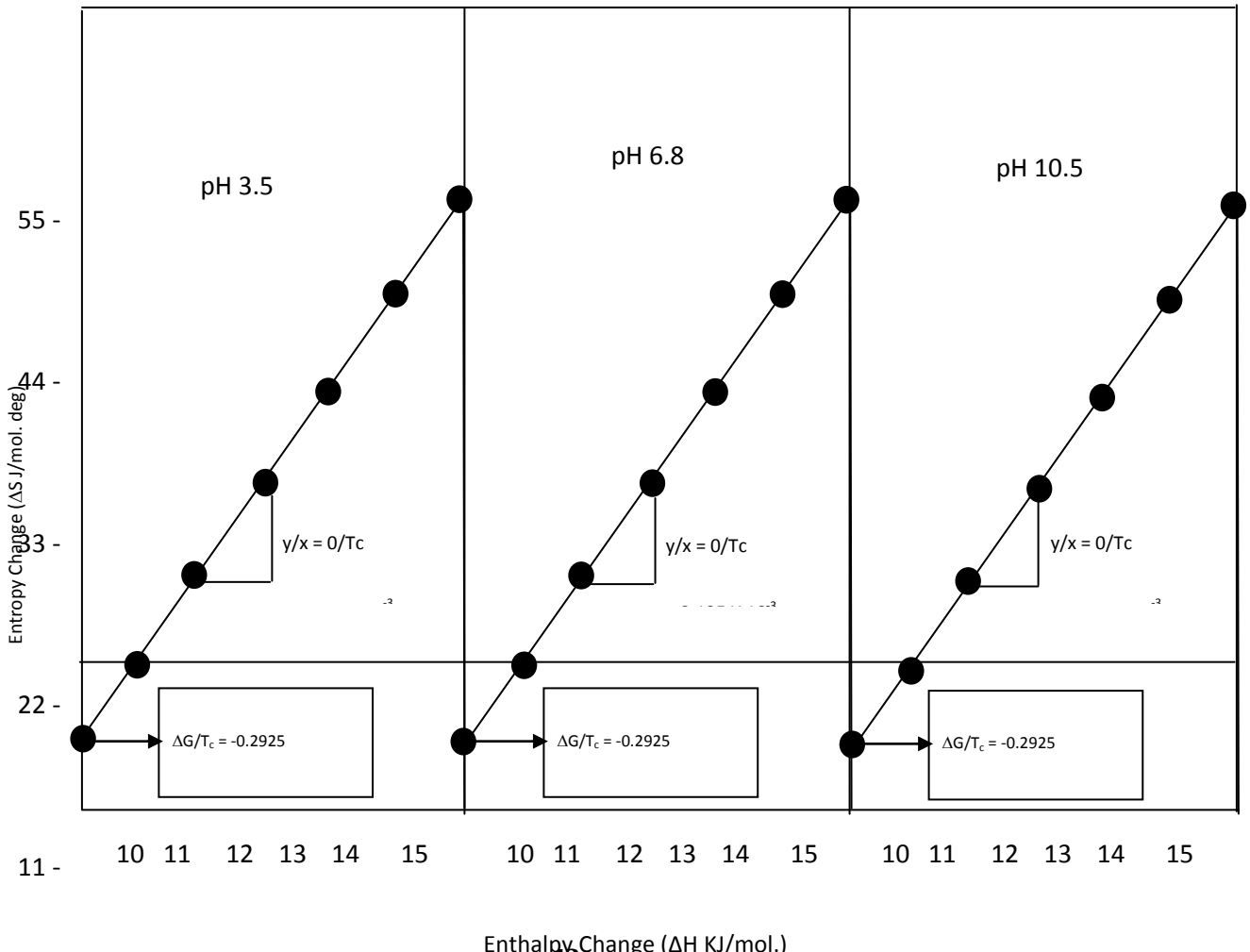
TABLE 1: ISO KINETIC TEMPERATURE (^0C) = T_C DERIVED FROM ARRHENIUS MODEL FOR EVALUATION OF KINETIC COMPENSATION EFFECT ON CASSAVA LINAMARIN DEGRADATION AT VARYING pH

Kinetic model	Kinetic compensation parameter	pH		
		3.5	6.8	10.5
Arrhenius model	N	4	4	4
	r^2	0.99	0.98	0.98
	S.E	0.0032	0.0031	0.0032
	Intercept (K_c)	$1.25x10^{-3}$	$1.25x10^{-3}$	$1.25x10^{-3}$
	$K_C(Min^{-1})$	$1.25x10^{-3}$	$1.25x10^{-3}$	$1.25x10^{-3}$
	Gradient (1/RTc)	0.03758	0.03752	0.03750
$T_H(^0C)$	($n\Sigma 1/T$)	134	134	134
	$T_C{}^0C$	47.2	47.2	47.8

Key: n = Number of samples r^2 = Correlation coefficient T_c = Iso Kinetic temperature (^0C) S.E. = Standard error T_H = Harmonic mean temperature Kc = Iso Kinetic constant (per min) R = The gas constant (0.08314)

In Table1: the least square regression analysis was applied to produce correlation coefficient r^2 value > 0.98. The same pattern was observed for degradation of Cassava linamarin at pH 6.8 and pH 10.5.

Figure 2: shows the kinetic compensation parameters indicating effect of pH on isokinetic temperature (Tc) and free energy of activation (ΔG) ($KJmol^{-1}$) for Cassava linamarin degradation at varying pH 3.5 to pH 10.5. At pH 3.5, Tc=320°C equivalent to 47°C. The ΔG (KJ/mol) of 93.69 was calculated from the graph in Figure2 and by least square regression analysis as shown in Table2. The correlation coefficient (r^2) was > 0.98. The same pattern was observed for the degradation of Cassava linamarin at pH 6.8 and pH 10.5.



Key:
ΔG = Free energy of activation (kJ/mol)
R = Gas constant (0.08314)
T_c = Iso Kinetic temperature (°C)

TABLE 2: ISO KINETIC TEMPERATURE (^0C) = T_C DERIVED FROM ABSOLUTE RATE THEORY MODEL FOR EVALUATION OF KINETIC COMPENSATION EFFECT ON CASSAVA LINAMARIN DEGRADATION AT VARYING pH

Kinetic model	Kinetic compensation parameter	pH		
		3.5	6.8	10.5
	N	4	4	4
Absolute	r^2	0.99	0.98	0.99
Reaction	S.E	0.0025	0.0021	0.0020
Rate	Intercept ($\Delta G/T_C$)	-0.2925	-0.2923	-0.2925
Theory	ΔG(KJ/mol)	93.69	93.65	93.69
	Gradient (1/RTc)	$3.125x10^{-3}$	$3.125x10^{-3}$	$3.125x10^{-3}$
	$T_c(^0C)$	47	47	47
$T_H(^0C)$	$n\Sigma 1/T$	134	134	134

Key:
n = Number of samples r^2 = Correlation coefficient
ΔG = Free energy of activation (KJ/mol)
T_c = Iso Kinetic temperature (^0C) S.E. = Standard error
Mean harmonic temperature (T_H) = $n\Sigma 1/T$ for Degradation of substrates by enzymes

The kinetic compensation data derived from Mean harmonic temperature (T_H) = $n\Sigma 1/T$ and isokinetic are presented in Table2 where the least square regression analysis was applied to produce correlation coefficient r^2 value > 0.98. The same pattern was observed for degradation of Cassava linamarin at pH 6.8 and pH 10.5. In Figure 2 Enthalpy of activation plotted against the entropy of activation of heat Degradation of Cassava linamarin produced 93.8KJ/mol of free energy of

activation (ΔG). The enthalpy of activation varied from 274-302KJ/mol, and the plot of these values against entropy change produced ΔG value. Both entropy and enthalpy exhibited marked compensation effect in which increases in enthalpy (ΔH) and entropy(ΔS) was accompanied by increases in ΔG(KJ/mo). The computed r^2 = 0.98 value in Table 2 by least square regression analysis indicated the fitness of kinetic compensation models in the prediction of environmental condition especially Iso Kinetic temperature (^0C). They may however, not present the same results. The discrepany in the T_c determined by different methods was reported by Rhim *et al* (19900) in whey for protein denaturisation. According to the authors, the methods for calculating isokinetic temperatures are usually not exactly consistent because they are derived from different kinetic and thermodynamic models. Again computational errors may sometimes arise giving different isokinetic temperatures (Tc). Inequalities of T_c and T_H ($T_H \# T_c$) whereas T_H is far greater than T_c ($T_H >>> T_c$) was in agreement with the significant difference in the two values in the degradation of chlorophyll to chrorophylides described by Canjura *et al* (1999) and Krug *et al* (1976). The thermodynamic parameters (ΔH, ΔS, K_o, E_a,) characterized degradation of enzyme catalyse reactions. The data are influenced by environmental conditions especially isokinetic temperature of 47^0C that is optimum for fermentation in our environment. The isokinetic temperature of 47^0C was close to experimental temperatures of 30-450C but not the same, these observation was reported by Zsako (1978) that the isokinetic temperatures are not the same with the experimental temperatures Rhim *et al.* (1989) demonstrated that the isokinetic temperature for acid catalysed hydrolysis of dissacharides was not in the range of

selected experimental temperatures. Kinetic compensation effects Zsako (1978), demonstrated that the kinetic compensation effect can be validated in thermally treated systems. Rhim, *et.al.* (1990) described the existence of true kinetic compensation effect in the heat denaturation of whey protein. Rhim, et.al.(1989) showed that a kinetic compensation effect in the acid-catalysed hydrolysis of disaccharides can appeare when examined at different incubation temperatures. Aguerre,*et.al.*. (1986) demonstrated the existence of the enthalpy-entropy compensation in sorption phenomena which can be applied in the the prediction of the effect of temperature on food isotherms. These were derieved from K_o vs E_a and ΔS vs ΔH relationships respectively and are independent of the environmental variables and presumably applied for predictive purposes.

CONCLUSIONS AND RECOMMENDATIONS

Mean harmonic temperature (T_H) the arithmetric means of the reciprocal of the experimental temperatures when compared with isokinetic temperatures (T_C) the result showed that T_H is far more than T_C . This result suggests validation of true compensation effect on the degradation of linamarin at varying pH. Thermodynamics data can be applied to evaluate isokinetics parameters for describing catalytic degradation of Cassava linamarin. This study on kinetic compensation can be used for predictive purposes in controlled fermentation of foods in our localities and data obtained on isokinetic temperature (Tc) is very important and can be employed in control fermentation of foods containing Cassava linamarin. At this temperature, the fermentation processes are independent of environmental conditions

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