www.cenresinpub.org
ISSN 2277-0143

PURIFICATION AND QUALITY EVALUATION OF LINAMARASE (B-GLUCOSIDASE) GENETICALLY

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ABSTRACT

Linamarase **(β-glucosidase) was genetically engineered from** genes (chromosomal DNA) and plasmids (circular DNA) isolated from bitter cassava and yeast respectively. Both genes were restricted and ligated to produce recombinant gene (r-DNA) which was introduced into the nucleus of $CaCl_2$ induced competent *Saccharomyces cerevisiae* cells which transformed into strains capable of producing genetically engineered linamarase (GELIN). Recombinant otherwise genetically modified yeast (*S. cerevisiae*) cells at the stationary phase of growth were harvested, 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 and stored under refrigerated conditions before further study and commercial native linamarase (CNLIN) was used as control. The physico-chemical characteristics of genetically engineered linamarase from *Saccharomyces cerevisiae* as influenced severally by degree of purification, pH and temperature were investigated. The parameters on physico-chemical characteristics of the enzyme extracts such as impurity levels, molecular weights (M_{wt}), number of isoenzyme, sulphur amino acids (methionine and cysteine), purity fold, yield and the electrical charges were evaluated using standard methods. The ability of the enzyme extracts and a commercial native linamarase (CNLIN) to hydrolyse cyanogenic glucosides was challenged to evaluate optimum pH (pH_{opt}), temperature (T_{opt}), total activity, specific activity and enzyme efficiency.

The results indicated that the genetically engineered linamarase **(β-glucosidase)** consisted of 3 isoenzyme forms. Purification conferred different ionic charges of zero to $GELIN_0$, unit positive charge $GELIN_1$, and unit negative charge to $GELIN_2$ and $GELIN_3$ respectively. Ranges for other parameters were M_{wt} (22,000-26,000 Daltons), insoluble protein impurity (0.4 -3.5 mg/100g sample) and purity fold (11.5 -1.0) for $GELIN_3$, - $GELIN_0$). Methionine and cystiene varied from 2.0 to 2.6% and 3.0 to 20% respectively (CNLIN - $GELIN_3$). The native commercial enzyme (CNLIN) acted only at pH 6.8 on linamarin with pH_{opt} and T_{opt} of 6.8 and 35 ^oC respectively. The genetically engineered linamarase **(β-glucosidase) group** acted linamarin, lotaustralin, para-nitrophenylglucoside (PNPG), dhurrin, amygdalin, prunasin and taxiphyllin at a wide range of pH 1-14 and 25-35 ^oC each exhibiting highest activity at optimum pH_{opt} and T_{opt} of 6.8 and 35 ^oC The wide pH tolerance at low temperatures and specific activity towards cyanogenic glucosides degradation suggest a possible use of the genetically engineered linamarase from *S. cerevisiae* in detoxification capable of providing food security from increased production and exportation of plant-based food products.

INTRODUCTION

Linamarase (β-glucosidase) is a hydrolytic enzyme. It degrades the glycolytic bond betweeen β- glucose molecule and the chiral carbon atom linked to the nitrile group of linamarin present in cassava. Linamarase (β-glucosidases) is economically very important because of its role as a detoxification agent for improving food safety. Even though it can be produced from cassava *(Manihot esculenta Crantz)* tubers the yields of native linamarase from this source is usually too low (Nok and Ikediobi, 1999). Linamarase from cassava is expensive and it is economically unwise to produce the enzyme from edible tubers. The problems associated with native linamarase(β-glucosidases) (CNLIN) used for detoxification include low enzyme concentration, limited spectrum of substrates activity and sensitivity to environmental factors such as pH and temperatures. Over the years however, humans have realized that selective propagation of animals and plants of desirable traits can improve and increase enzyme production in yield and quality. β-glucosidases of microbial origin may represent a possible solution to the problem. This approach has also been extended to bacteria and fungi for the increased downstream products development for the pharmaceutical, food and brewing industries. Yeast (*Saccharomyces cerevisiae*) for example, has long been used for the production of high yielding metabolites and other food enzymes including amylases and zymase for hydolysis of starch and fermentation of glucose into ethanol respectively (Mach and Zeilinger, 2009). Some quantities of linamarase were for the first time extracted from yeast by Ikediobi and Ogunda (1985) and also Okafor and Ejiofor (1985). Yeast like other microorganisms have been extensively used in genetic engineering for the production of single- cell proteins, enzymes, hormones and vitamins. This is because of their low generation times and ease of manipulation. The kinetic data of genetically engineered β-glucosidase on linamarin extracted from cassava will provide insight into the mechanism of action of the enzyme while providing the parameters necessary for predictive purposes. Such predictions which are lacking can be useful tools for fermentation process optimization for the degradation of cyanogenic glucosides in cassava-based food systems. The research objective of this study was to characterise genetically engineered β-glucosidase from *Saccharomyces cerevisiae* by its action on casssava linamarin using commercial native linamarase as the control for this study . The approach is to boost products transformation and development of cassava-based food products that can contribute to safety global nutrition and food security , increase international export market for national income earning from cassava-based food products.

MATERIALS AND METHODS

Materials

Enzyme samples: Commercial Native Linamarase (CNLIN) used as the control for this study was purchased from Sigma Co. Lousina, USA.. Genetically Engineered β-glucosidase (GELIN) was obtained from the Department of Food Science and Technology University of Agriculture Makurdi.Crude extract from *Saccharomyces cerevisiae* ($GELIN_0$).

Column Materials: Carboxy methyl cellulose ($GELIN_1$), Diethyl-amino-ethyl- sephadex ($GELIN_2$) and Diethyl-amino-ethyl-cellulose ($GELIN_3$) were used the purification of the crude extract ($GELIN_0$) from *Saccharomyces cerevisiae*.

Enzyme Substrate: Linamarin (100g) was extracted from from about 2kg tubers of the bitter wild cassava (*Manihot esculenta pohr*) variety TSM-TRF-2005035 obtained from Tse-Akaa Village Mbalagh Makurdi, Benue State, Nigeria. produced using the method described by Ikediobi and Ogunda (1985). **Linamarin:** One year old cassava tubers were harvested washed with tap water and promptly frozen overnight at -10^{o}C. About 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 resultant filtrate was centrifuged at 5000 x g for 5min and the supernatant adjusted to pH 8.0 followed by re-centrifugation at 5000x g for 8 mins. After decanting, the solid residue was air-dried to obtain about 0.82g of white substrate (mp.143^{o}C).This was stored at 4 ^{o}C and subsequent used for characterization of activity kinetic profiles.

Buffer Solutions and Analytical grade Reagents: Buffer solutions and reagents were prepared for the study using standard methods.

Materials for Molecular Weight Determinaation

Molecular weight determinaation of commercial native linamarase (CNLIN) and genetically engineered linamarase (GELIN) from yeast *(Saccharomyces Cerevisiae)* was carried out in this study with the following materials., vertical polyacylamide gradient slabs (13-24 %.), 2-methylmercapto ethanol, Coomassie G brilliant blue, $M_{wt.}$ calibration standards (phosphorylase, b(94,000), bovine serum albumin(67,000), ovalbumin (43,000), carbonic anhydrase(30,000), soybean trypsin inhibitor(20,000), lactalbumin (14,000) and fragments of myoglobin(17, 200; 14,6oo; 8,240; 6,380 and 2,560). Fractogel TSK HW 50(F) column (90x2.5cm), 0.1 %(v/v)TFA,70% (V/V) Ethanol, appropriate sodium acetate buffer, stop clock, spectrophotometer, chromotographic Column, Kontron Liquimat 111 amino acids Analyser 6M HCl ,weighing balance thermometer test tubes, performic acid.

Procedure for Molecular Weight Determinaation

Vertical Soduim dodecyl sulphate polyacylamide gel electrophoresis

Method for molecular weight (Mr.) determination and separation of enzyme polypepetides was done under non reducing and reducing condition using 2-methylmercapto ethanol. Vertical SDS-PAGE was performed on polyacylamide gradient slabs (13-24%. Gels were stained with Coomassie G brilliant blue. Mr. calibration standards were: phosphorylase b(94,000), bovine serum albumin(67,000), ovalbumin(43,000), carbonic anhydrase(30,000), soybean trypsin inhibitor (20,000), lactalbumin (14,000) and fragments of myoglobin (17,200; 14,6oo; 8,240; 6,380 and 2,560).

Molecular-Exclusion/Gel Filtration Chromatographic Separation method

This was performed on a Fractogel TSK HW 50(F) column (90x2.5cmequilibrated 0.1 %(v/v)TFA,70% (V/V) Ethanol. Enzyme fractions were eluted with appropriate buffer at 25 ml/h. Fractions were detected at 224-280nm,collected and subjected to Mr. using standard Mr. markers for calibration of the column.

Kontron Liquimat 111 amino acids Analyser

Analytical determination and separation of amino acids was applied in evaluting s-containing amino acid. Pure samples each (0.5mg) were hydrolyzed under vacuum in 6M HCl for 24hr. at 110oC in sealed tubes. Sulphur amino acids were analyzed after performic acid oxidation to methionine sulfone and cysteic acid. Amino acids compostions were determined using a Kontron Liquimat111 Analyser.

Estimation of Insoluble proteins

The insoluble proteins were estimated as described by Nok & Ikediobi (1999) An automated refrigerated centrifuge with Lowrys based principle was applied in the estimation of insoluble protein impurity.

Activity Kinetic studies

Procedure for determination of activity kinetic profiles was from the ability of Commercial Native Linamarase (CNLIN) purchased as control and the Genetically Engineered Linamarase (GELIN) extracts from *Saccharomyces cerevisiae* to hydrolyse cassava linamarin to hydrocyanic acid (HCN) released within a fixed time of challenge. The Spectrophotometric method for estimation of hydrocyanic acid (HCN) released within a specified time of ten minutes as described by Onyike *et al* .(2001) was used for the investigation. The activity kinetics parameters of commercial linamarase (CNLIN) and the genetically engineered linamarase (GELIN) were determined based on the degradation of linamarin carried out at varying pH 1-14. In this procedure enzyme aliquots (0.1ml) were prepared and added to tubes containing 0.5ml of 5µmol linamarin. The tubes were incubated for 30min at $3O^0C$ at vaying pH 1-14 and the reaction was stopped by adding 1ml of 0.2NaOH. Thereafter 1ml of 0.2MHCl was added to neutralize the base. Chloramine T(1ml of 1%) was added and after 1min., 3ml barbituric acid/pyridine reagent was added. The volume adjusted to 25ml distilled water followed by measurement of absorbance of the pink color at 420 nm. The volume was increased to 25ml and the absorbance of the pink color was measured. H_2SO_4 was used to calibrate the absorbance values. The hydrocyanic acid (HCN) present in each tube was determined by spectrophotometric method. The absorbance measured in the enzyme assay was used to calculate the total activity in units per ml of enzyme solution, where one unit is defined as that which produced 1micro mole of HCN per minute at $3O^0C$. The total activity, specific activity, purity fold, yield and purification efficiency of genetically engineered linamarase were as described by Onyike *et al*. (2001). Activity kinetic profiles parameters applied for the characterization of the enzyme samples include; total activity, specific activity, purity fold, yield and purification efficiency. Calculation

of data from total activities and impurity of enzyme samples are shown mathematically in equations (1- 5) as described by Nok and Ikediobi (1999) and Onyike *et.al*.(2001).

Specific Activity = (1)

Total (2)

Total Activity = (specific activity) x (total mg protein in preparation) (3)

%Yield = (4)

%Yield = Purification Efficiency Ratio

Purity Fold = Specific activity : Specific activity of two given preparations (5)

Statistical Analysis

The tests for significant ($p \leq 0.05$) difference in the purification profile, enzyme and activity kinetics parameters at ambient temperature, and at varying pH, temperature and purity fold were calculated with the multiple comparison range method of Krammer and Twigg (1970) and Gupta, (1979).

RESULTS AND DISCUSSION

Physico-chemical determination.

Data representing the physico-chemical characteristics such as **ionic charge, amino acid, molecular weight, impurity and isoenzyme** of commercial native linamarase (CNLIN) and genetically engineered linamarase (GELIN) from yeast *(Saccharomyces cerevisiae)* are shown in Table1. The ionic charge of (0) were identical in CNLIN and $GELIN_0$, whereas $GELIN_1$ was positively charged. Both $GELIN_2$ and $GELIN_3$ were negatively charged. All the enzymes CNLIN, $GELIN_0$,$GELIN_1$, $GELIN_2$ and $GELIN_3$ contained methionine corresponding respectively to 2, 15, 23, 25 and 26%. The enzymes similarly contained cysteine in the proportions of 3, 13, 14, 17 and 20% respectively. Molecular weights estimated in Dalton units were respectively 160,100, 260,000, 240,000, 230,000 and 220,000 using Sodium dedocyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) techniques, whereas the $mol._{wt}$ estimated by gel filtration chromatography were 160, 600, 260, 100, 240, 100, 230, 100 and 220, 200. The impurity (mg/100g sample) in enzymes evaluated 2.42, 3.52, 0.9, 0.6 and 0.4 respectively. The impurities(mg/100g) in the fractions were 2.42(CNLIN), 3.52($GELIN_0$), 0.9 ($GELIN_1$), 0.6($GELIN_2$) and 0.4($GELIN_3$) showing increased purity of purified enzymes. The value for $GELIN_0$ was not ($p < 0.05$) higher than 2.42mg/100g of commercial native linamarese (CNLIN). The presence of two active isoenzymes in commercial native linamarse (CNLIN) was earlier reported by Nok and Ikediobi (1999). Later reports by Wither *et al*.(2002) revealled that a good number of isozymes are responsible for the wide spectrum of substrates specificity The molecular weight(Dalton units) of 22,000- 26,000 , for $GELIN_3$-GELIN0 were not highly significant ($p<0.05$) different form the molecular weight of carbonic anlydrase of 30,000 daltons but however, significantly different ($p > 0.05$) from the 63,000 of phosphorylase (b) 94,000 serum albumin 67,000, ovalbumin 43,000 The values for the GELIN were however, higher than trypsin inhibitor (20,000) lacalbumin 14,000 and fragements of myoglobin 17,200, 14 600 , 8,200, 6,380 and 2,5600) making the $GELIN_0$- $GELIN_3$ and CNLIN to be classified as medium molecular weight enzyme fractions. Molecular weight determination by Cicek et al.(1998) showed that linamarase was in the

range of 57 to 63 KD. The methionine and cystiene contents ranged from15-26 % and 13-20% respectively. (GELIN0- GELIN3) of sulphur amino acid evaluated were significantly higher ($p< 0.05$) than the commercially native linamarase (CNLIN) indicating the contribution of genetic engineering in incorporating the surphur bridges in enzymes to thermo-stable and probably pH tolerant structural characteristics.proiduced of the cloned enzymes. (Archer, 2006).

Michaelis-Menten and LineWeaver-Burk Parameters:

Plots of activity versus Linamarin concentration 1–9 mol/dm^3 and regression of data fitted into double reciprocal models respectively for **Michaelis-Menten and LineWeaver-Burk to evaluate the quality of** commercial native linamarase (CNLIN) and genetically engineered linamarase groups ($GELIN_0$-$GELIN_3$) at pH 3.5, 6.8 and 10.5 are shown in Figure 2. The plots are sigmoidal (sigmoid curves) and typical of shapes associated with Michaelis-Menten model. Action of Commercial native linamarase (CNLIN) on Linamarin was observed only at pH 6.8 whereas GELIN group acted on linamarin at all experimental pH (3.5–10.5) investigated. At pH 6.8 $GELIN_3$ gave the highest activity followed by $GELIN_2$, $GELIN_1$ and $GELIN_0$ in that order. The pattern was similar at pH 3.5 and 10.5. The regression parameters generated shown in Table 3 were to test the goodness of fit of the Lineweaver-Burk model in describing the degradation activity of the tested enzymes on linamarin and to estimate the physiological characteristics in accordance with those obtained by Onyike *et al.*(2001) and also Nok and Ikediobi (1999). The the estimated parameters in this study are showed in Table 1. The high coefficients of linear regression ($r^2 \geq 0.98$) in the table indicated that Lineweaver-Burk model was appropriate for describing the adequacy of enzymes in the degradation of high linamarin concentrations. The physiological efficiency K_m/V_{max} was between 0.5 to 1.25 min /dm^3 indicating the stability of enzyme and tolerance to changes in pH. The total activity or maximum velocity (V_{max}) of (9-14µmol HCN/min) fell within the range reported by earlier workers such as Onyike *et al.*(2001) for linamarin degradation by native β-glucosidase .

Michaelis and Menten determined that the initial rate or velocity of catalysis of an enzyme varied hyperbolically with substrate concentration (Voet & Voet, 1995; Lionel *et al.* 2008). In this present study, the initial velocity (V_o) generated in the degradation reactions were also subjected to Michaelis- Menten and Lineweaver- Burk models in line with earlier applications by Onyike *et al.*(2001), Ikediobi &Ogunda (1985) for native linamarase extracted from cassava. The findings showed that the initial rates increased with increase in substrates concentration to a point where it reached maximum velocity (V_{max}). At low substrate concentrations, initial rate was proportional to the substrate concentration and can be referred to as first order kinetics. At high substrate concentrations, the initial rates were independent of substrates concentration and could be referred to as saturation or zero order kinetics. These results including sigmoid curves and the high coefficients of linear regression ($r^2 \geq 0.98$) values validated the adequacy of Michaelis- Menten &Lineweaver- Burk models in describing the enzyme activity versus substrates concentration relationship. The V_{max} (mol/min) at optimum pH of 6.8 were 10 (CNLIN), 10($GELIN_0$), 11($GELIN_1$), 12($GELIN_2$)

and 13($GELIN_3$) respectively. The V_{max} values (mol/ min) at optimum temperature of 35°C were not significantly ($p< 0.05$) different. Those for linamarin degradation ranged from 4 – 14 (mol/ min) at pH 3.5, 4 – 16 (mol/ min) at pH 6.8 and 6 – 12 (mol/ min) at pH 10.5 whereas K_m varied from 5 to 8 mol/ dm^3 at pH 3.5 - 10.5 for the genetically engineered enzymes. Dounghkamol *et. al.* (2006) reported K_m values of 1.02 to 5.04 mM at pH 6.0 and 0.01 – 0.05mM at pH 3.5. The dissimilarity in K_m values could be due to differences in sequence identity. K_m values represent the degree of binding or affinity of the enzymes with substrates in formation of activated complexes necessary for formation of products. High values indicate that higher attractive forces exist between substrates and enzymes with high probability of degradation of the cyanogenic glucosides. Chulavatnatol (2008), obtained K_m values ranging from 0.38 to 0.57mM while Keresztessy *et al*. (1994) reported K_m value of 1.06mM for linamarin degradation and 0.36mM for PNPG degradation. The higher values obtained in this study demonstrates the substrates specificity of genetically engineered linamarase from *Saccharmyces cerevisiase* for the cyanogenic glucosides studied. The high apparent K_m values obtained for linamarin degradation indicated good affinity of the enzymes towards the substrate, particularly if compared to those of other β-glucosidases such as, for instance, the amygdalases of *Prunus serotina* (Kuroki & Poulton, 1986) and linamarase of *Penicillium aurantiogriseum* P35(Petruccioli *et al*.,1999b). For instance, β-glucoidases from *Brevibacterium* sp. and *A. oryzae* were active on linamarin at neutral pH (Legras *et al*., 1989; Flores *et al*.,1992); those from cassava and flax seeds were active on linamarin slightly above neutral pH(Yeoh,2009), those from *P. serotina* and *Prunus olystachya* did not hydrolyze linamarin at low pH while those from *P. aurantiogriseum* P35 acted on a wider spectrum of substrates at neutral pH (Petruccioli *et al*.,1999). The V_{max} values for the genetically engineered linamarase (10.02-13.0 ·mol/min) were higher than for CNLIN (0.0 to 10.0·mol/min).

The K_m value for the commercial native linamarase (CNLIN) was 0.0 to 0.4mol/dm^3. This range is in agreement with that reported by Seshadi *et al*. (2010). As for physiological efficiency (K_m/ V_{max}), the crude genetically engineered linamarse performed better than the purified enzymes at pH 3.5 with a value of 1.25 dm^3/min. CNLIN had no action at this pH 3.5 and 10.5. At pH 6.8, the physiological efficiency of native linamarase was better than the genetically engineered enzymes. At pH 10.5, the genetically engineered enzymes degraded the cyanogenic glucosides and can therefore be applied exogenously for fermentation purposes for the degradation of these toxic food substances. These results are in agreement with the work of Petruccioli *et al.* (1999a) whose K_m/V_{max} was as high as 1.6 dm^3/min for β-glucosidase degradation of linamarin, prunasin and taxiphylin. The K_m/V_{max} values of 0.04–0.069dm^3/min reported in this study for the native linamarinase are within the range observed for isoenzymes a and extracted and evaluated by (Nok & Ikediobi 1999).

Determination of Activity Kinetic Parameters from the Action of Enzymes on Cassava Linamarin: Figure1 and Table 2 were related to the action of commercial native linamarase (CNLIN) and genetically engineered linamarase (GELIN). on cassava linamarin. From both figure and table the characteristic curve, optimum pH , temperature, total

activity, specific activity, purity fold and yield (efficiency) were evaluated and classified as the activity kinetic parameters. Activity kinetic parameters summarised the actions of the enzyme produced on degraded linamarin. The performance of $GELIN_3$ were significantly ($P< 0.05$) higher than CNLIN. The plots in figure 1 showing activity versus pH at controlled optimum temperature 35^oC produced a cone shape with the peak obtained at optimum pH of 6.8. Figure 1, representing a bell or cone shape was similar to the previous study described by Nok and Ikediobi (1999). The % purification efficiency for each enzyme fraction purified by different methods were (100) for CNLIN, and $GELIN_0$, CMC (110) $GELIN_1$ (DEAE sephadex (120) GELIN2 and DEAE cellulose (130) for $GELIN_3$ Finally, $GELIN_3$ has the highest efficiency ratio of 1:60 higher than CNLIN with the lowest 1:1. Each purification produced yield (g) equivalent to activity unit of 1, 1, 1-1, 1-2, and 1.3 respectively. The highest peak of activity kinetics ($\mu mols^{-1}$) ranged from (CNLIN 9, $GELIN_0$ 9, $GELIN_1$ 11, $GELIN_2$ 12, and $GELIN_3$ 14). Here $GELIN_3$ produce the highest activity of 14 $\mu mols^{-1}$. CNLIN showed activity within short range of pH 6.8-7.0. This was influenced by purity and inherent fragile protein structure. The calculated purity folds were within 1.45 to 14.08. $GELIN_3$ had the widest pH range from 1 – 14 followed by $GELIN_2$ $GELIN_1$ and $GELIN_0$. The specific activities of linamarase sample ranged from 2.8 – 40.0. Table 2, represents mathematically computed values using standard equations 1-5 described by Nok and Ikediobi (1999) to validated efficacy of linamarin degradation by both native and genetically engineered linamarase from S*accharomyces cerevisiae*ase. The results are in accordance to those determined by Onyike *et al.*(2001), Nok and Ikediobi (1999) showing that the total activity was in the range of 9-14miromole HCN released per minute.

All the enzymes used in this study had a common optimum pH and temperature. This is in agreement with the previous studies of Karl – Joseph et al (2002). Total activity range from 9–14 ($\mu mols^{-1}$) The third degree purified genetic enzyme perfumed better than all enzyme factions. The commercial native linamarase performed within a narrow pH range of 6.8 – 6.9 in the degradation of linamarin. The native enzyme had no action on linamarin at pH 3.5 and 10.5. the genetic enzymes had a broad specificity activity hydrolyzing the experimental linamarin within the range of pH 3.5 and 10.5. This was in agreement with the work of Pertruccivlo et al (1999) where genetic enzymes had a broad spectrum of action capable of hydrolyzing linamarin, at a wide range of tolerable pH 1-14. The specific activity in micromole minute per gram range from 2.56 to 35 showing that purification in this study was very perfect. This was in agreement with the studies of Ekisttikul et al (2007). The purity fold was within the range of 1 – 13.7 agreeing with the work of Ekisttikul(2007). The enzyme Efficiency was between 10 to 15.5 indicating the efficiency purification method applied in the removal of contaminating proteins for the genetically engineered enzymes. This was in agreement with the work Cicek et.al (1998). The similarity of results obtained by degradation of linamarin by different enzyme fractions could be as a result of sequence similarity of all the genetically engineered samples. On the whole, all the genetically engineered enzyme samples shared almost 100% sequence similarity while the commercial native enzyme shared only 30% sequence similarity with the genetic enzymes (the GELIN

groups). This observation is in agreement with the Ciek et al (1998). Which showed that genetically engineered ▪ - glucosidases shared a common sequence identity of at least 70%.

CONCLUSIONS

Recombinant DNA technology has been used in this study to genetically engineer linamarase (β-glucosidase) comprising of three isoenzymes which was expressed in *Saccharomyce cerevisiae* and extracted. The engineered β-glucosidase from *Saccharomyces cerevisiae*- a yeast normally associated with ethanol and CO_2 production is also active in the degradation of cassava linamarin. Ion-exchange chromatographic purification techniques which were capable of purifying the enzyme enhanced activity of fractions at tolerance pH (3.5-10.5) with optimum pH 6.8 and temperature 35^{o}C. The genetically engineered linamarase has total activity which increased proportionately with degree of purification and exhibited high specific activity on linamarin studied.. The enzyme depended on degree of purity and pH for adequate degradation of linamarin at optimum temperature 35^{o}C. Linamarin degradation was accurately described using the Michaelis-Menten and LineWeaver-Burk kinetic models. The industrial use of the genetically engineered β-glucosidase is recommended for detoxification of linamarin required for the processing of cyanide free cassava- based food commodities from high yielding bitter cassava variety. The genetically engineered linamarase from *Saccharomyces cerevisiae* is recommended for detoxification of cyanide in cassava-based food products. The use of the genetically engineered β-glucosidase can boost products transformation and development of cassava-based food products for domestic human and animal consumption. The approach can contribute to global food and nutrition security, raise income earnings through international export market.

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TABLE 1: CHARACTERISTICS OF COMMERCIAL NATIVE AND GENETICALLY ENGINEERED LINAMARASE FROM YEAST

Enzyme Characteristics	CNLIN	Enymes $GELIN_0$	$GELIN_1$	$GELIN_2$	$GELIN_3$
onic charge	0	0	+	-	-
Methionine (%)	2^c	15^b	23^a	25^a	26^a
Cysteine (%)	3^c	13^b	14^b	17^{ab}	20^a
Isoenzymes	-	3^a	3^a	3^a	3^a
$Mol._{wt}$(dalton)	-	-	-	-	-
SDS-PAGE	$16^0,100^b$	$26^0,000^a$	$24^0,000^a$	$23^0,000^a$	$22^0,000^a$
gel filtration	$16^0,100^b$	$26^0,000^a$	$24^0,000^a$	$23^0,000^a$	$22^0,000^a$
Impurity(mg/100g)	2.42^b	3.52^b	0.9^a	0.6^a	0.4^a

Mean values with common superscript letters along each row are not significant ($p<0.05$)

Key:

CNLIN = Commercial native linamarase,

Genetically engineered linamarase (GELIN)

$GELIN_0$ = Crude

$GELIN_1$ = 1st degree of purification using carboxy-methyl cellulose (CMC),

$GELIN_2$ = 2nd degree of purification using diethyl amino ethyl-sephadex (DEAE-sephadex)
$GELIN_3$ = 3rd degree of purification using diethyl amino ethyl-cellulose (DEAE-cellulose)
SDS-PAGE = Sodium dedocyl sulphate polyacrylamide gel electrophoresis

TABLE 2: ACTIVITY KINETIC PROFILES OF NATIVE AND GENETICALLY ENGINEERED LINAMARASE FROM YEAST (*SACCHAROMYCES cerevisiae)* IN RELATION TO ACTION ON LINAMARIN

Enzyme activity	Commercial and genetically engineered linamarase				
Enzyme activity	Commercial and genetically engineered linamarase				
	CNLIN	$GELIN_O$	$GELIN_1$	$GELIN_2$	$GELIN_3$
Total activity ($\mu molmin^{-1}$)	9[c]	9 [c]	11 [b]	12 [a b]	14 [a]
Impurity (mg/g)	2.42 [b]	3.52 [a]	0.9 [c]	0.6 [c]	0.4 [c]
Specific activity ($\mu molmin^{-1}$)/g	4.13 [d]	2.84 [e]	13.33 [c]	23.33 [b]	40.00 [a]
Purity fold	1.45 [d]	1.00 [d]	4.49 [c]	8.21 [b]	14.08 [a]
Enzyme efficiency ratio/ **(Yield)**	1:10/ 110	1:10/110	1:20/120	1:40/140	1:60/160
pH range	6.0-8	3.5-10.5	3.5-10.5	3.5	10.5
pH optima	6.8 [a]	6.8 [a]	6.8 [a]	6.8 [a]	6.8 [a]
Temperature range (^oC)	34.5-35.6	30-45	30-45	30-45	30-45
Temperature Optima (^oC)	35 [a]	35 [a]	35 [a]	35 [a]	35 [a]

Mean values with common superscript letters along each row are not significantly ($p>0.05$).

Key:
CNLIN = Commercial native linamarase,
Genetically engineered linamrase (GELIN)
$GELIN_0$ = Crude
$GELIN_1$ = 1st degree of purification using carboxy-methyl cellulose (CMC),
$GELIN_2$ = 2nd degree of purification using diethyl amino ethyl-sephadex (DEAE-sephadex)
$GELIN_3$ = 3rd degree of purification using diethyl amino ethyl-cellulose (DEAE-cellulose)