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Original Article

Isolation, Purification and Partial Characterization of Intracellular Invertase from Palm Wine Yeast (*Saccharomyces* sp.)

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Abstract

Introduction: Invertase belongs to the class of enzymes called glycosidase. The enzyme is responsible for the catalytic hydrolysis of sucrose to release monosaccharides known as invert sugars. The aim of this study was the isolation, purification, and physicochemical properties of intracellular invertase from palm wine yeast (*Saccharomyces* sp.) as an alternative enzyme in several industrial applications.

Materials and Methods: The yeast was harvested from the palm wine through flocculation and the intracellular invertase was isolated from the yeast cell by mechanical grinding using acid washed sand. The intracellular invertase was purified using combination of ion-exchange chromatography on DEAE-trisacryl and gel filtration on Sephacryl S-300. The kinetics and other physicochemical properties of the purified enzyme were determined.

Results: The two-step purification scheme employed gave a final yield of 168% and a purification fold of 3.0. Denaturing polyacrylamide gel electrophoresis (SDS-PAGE) of the intracellular invertase gave six subunits with the molecular mass of 73.5 ± 2.1 kDa, 52.3 ± 8.1 kDa, 48.6 ± 3.0 kDa, 37.4 ± 4.8 kDa, 26.5 ± 3.6, and 24 ± 4.3 kDa, respectively, while non-denaturing PAGE revealed the presence of single entity invertase with the molecular mass of 267 kDa. Also, native molecular mass estimated on calibrated Sephacryl S-300 was 266 ± 28 kDa, revealing that the purified palm wine *Saccharomyces* invertase (PWSInv) is heterohexameric in nature. The K_m and V_{max} of the purified invertase were 30.8 ± 3.2 mM and 9672 ± 169.0 units/mg protein, respectively, leading to catalytic efficiency, k_{cat}/K_m of 1.43 × 10^6 M^{-1} s^{-1}. The optimum temperature and pH were 60 °C and 3.0, respectively. The activation energy (E_a) of the intracellular invertase for the hydrolysis of sucrose was estimated to be 280.42 kJ/mol.

Conclusions: The study established the presence of intracellular invertase from palm wine yeast and investigated some properties and characteristics of the purified invertase, which could be exploited for several biotechnological and industrial processes.

Keywords: Microbial Enzyme, Intracellular Invertase, High Fructose Syrup, *Saccharomyces* sp, Biotechnological Application

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Introduction

Invertase (EC.3.2.1.26) also known as beta-fructofuranosidase belongs to the class of enzymes called glycosidase. The enzyme is responsible for the catalytic hydrolysis of sucrose to release monosaccharides known as invert sugars.[1] It is widely distributed in nature, its presence has been reported in animal tissues, microorganisms, and even plants.[2] Invertase has been well characterized in microorganisms and plants.[3] In addition to microorganisms, enzymes from plant origins have been reported to have several industrial and biotechnological applications.[4] Invertase has found applications in food industries where fructose is required especially in the preparation of candies and jams.[5] The intracellular invertase of *Saccharomyces cerevisiae* is mainly found in a soluble form, while only minor amounts are found bound to the plasma membranes.[6] Intracellularly, the main protein form of invertase is largely localized in vacuoles whereas the small isoenzyme is largely present in the soluble cell fraction.[7] Generally, invertase exists as either intracellular or extracellular.[8] The former one has a molecular weight of 270 kDa whereas the latter variety has a molecular weight of 135 kDa.[9] Isoforms of invertase exist with various properties, subcellular locations, and diverse roles in plants.[10] Invertase is used for the manufacture of plasticizing agents in cosmetics, artificial honey, paper industries, and pharmaceuticals.[11] New potential application of the enzyme is the synthesis of fructooligosaccharides as prebiotics in functional foods and pharmaceutical formulations.[12] Invertase has been reported to be immobilized on solid supports to allow reusability and operational stability.[13] An increasing trend has been observed in the use of immobilized enzymes as catalysts in several industrial chemical processes.[14]

Microbial invertase has gained more patronage because of

its mass production capacity and the fact that microbes are easily manipulated to obtain enzymes of desired attributes.[15] Large amounts of money are spent on this commercially available invertase.[16] Microorganisms such as the *Saccharomyces* sp., and bacteria have been reported to be abundant in palm wine.[17] Palm wine is consumed for its nutritional effect because of its probiotic content as well as a good source of natural fermenting microorganisms.[18] Due to the inversion process of sucrose/sugar, invertase enzymes are vital to the confectionery industry, which uses sugar as one of the essential ingredients. Considering the important applications of invertase in several industrial processes, the discovery of novel invertase with high catalytic activity is highly desirable. Invertase is widely distributed among the biosphere. It is mainly characterized in plants and micro-organisms. *Saccharomyces cerevisiae* is the chief strain of microorganism used for the production of invertase commercially.[19] However, till now, there is the scarcity of information on the possibility of using palm wine yeast (*Saccharomyces* sp.) for invertase production. The usability of invertase is limited due to high cost of commercially available one. There is considerable interest in cheap and readily available sources with suitable catalytic properties to match the high demand of the enzyme in several industrial processes. We, therefore, focused on the isolation of intracellular invertase from palm wine yeast with high catalytic activity, in addition to providing information on its physicochemical properties which could be exploited for several industrial and biotechnological applications.

Materials and Methods

Materials

Fresh palm wine was collected directly into a sterile container from palm wine tappers in farms around Owo, Ondo State, South western Nigeria.

Chemicals

Sodium acetate, sodium hydroxide pellet, hydrochloric acid, acetic acid, sodium-potassium tartrate, sodium chloride, sucrose, glucose, copper sulphate, anhydrous sodium hydrogen carbonate, concentrated sulphuric acid, ammonium molybdate, dinitrosalicylic acid, glycine, methanol, blue dextran, trizma base, trizma HCl, citric acid, lysozyme, α-chymotrypsinogen A, albumin (BSA), ovalbumin, sodium phosphate monobasic ($NaHPO_4$), sodium phosphate dibasic (Na_2HPO_4), N,N,N^1,N^1-tetramethylethylenediamine (TEMED), ammonium persulphate, sodium dodecyl sulphate (SDS), phosphoric acid, Coomassie brilliant blue R-250, and Coomassie brilliant blue G-250 was obtained from Sigma, St Louis, USA. Standard markers for SDS-PAGE from Carl Roth, Germany. Sephacryl S-300, SP-Sephadex C-50, DEAE tris acryl were also from Pharmacia Fine Chemicals, Sweden. All reagents were of analytical grades.

Isolation of Yeast from Palm Wine

The freshly collected palm wine sample was left for 24 h at ambient temperature to allow optimum yeast growth and flocculation. The flocculated yeast was collected by centrifugation at 10,000 x *g* at 4 ℃ for 30 min. The supernatant was discarded and the yeast pellet was weighed and stored at 4 ℃ for further use.

Assay for Invertase

Invertase activity was determined by measuring the reducing sugar end released upon incubation of invertase with sucrose using dinitrosalicylic acid (DNSA), according to Almeida et al.[20] with modification. The assay mixture contained 0.5 ml of 1% (w/v) sucrose in 0.05 M acetate buffer, pH 4.5, 0.1 ml of the enzyme, and 0.9 ml of distilled water. The mixture was incubated for 10 min at 30 ℃. One millilitre (10 ml) of DNSA was added and the tube was placed in boiling water for 10 min. After cooling to 30 ℃, the volume was made up to 5 ml. Absorbance was measured at 540 nm in a spectrophotometer. Boiled enzyme blanks were included as a control.

One unit of invertase activity is the amount of enzyme that catalyses the hydrolysis of sucrose to produce 1 µmol of glucose per min at 30 ℃. The amount of glucose produced was estimated from a glucose standard curve prepared alongside the experiment.

Extraction and Activity of Intracellular Invertase from Palm Wine Yeast Cell

Due to the low level of extracellular invertase in the palm wine yeast, extraction of intracellular invertase from the yeast cells was conducted according to the method of Zhang et al.[21] Briefly, two grams (2 g) of acid washed sand was added to 2 g of the palm wine yeast pellet. The mixture was ground at 4 ℃ for 30 min using mortar and pestle. Thereafter, 2 ml of 0.02 M Tris-HCl buffer, pH 7.0 was added, and the suspension was further homogenized at 4 ℃ for 10 min. The resulting homogenate was subjected to centrifugation at 12,000 rpm for 30 min at 4 ℃ to obtain clear supernatant taken to be crude intracellular invertase. The activity of the crude enzyme was carried out as earlier described.

Determination of Protein Concentration

Protein concentrations were determined using the method of Bradford,[22] with BSA as standard. Briefly, 10 µg/ml BSA was prepared from a stock solution of 100 µg/ml BSA, which was varied to obtain a standard curve with a blank containing 1.6 ml distilled water and 0.4 ml of Bradford reagent. The mixture was measured at 595 nm on a spectrophotometer. The protein concentration in the samples was obtained by extrapolation from the standard curve.

Enzyme Purification

The enzyme was purified using ion exchange chromatography and gel filtration chromatography.

Purification by Anion Exchanger on DEAE-Tris-Acryl

The crude enzyme was layered on a DEAE-Tris-Acryl ion exchange column (2.5 × 10 cm) previously equilibrated using 20 mM Tris buffer, pH 7. The elution was done with the equilibration buffer, and fractions of 1 ml each were collected at a flow rate of 12 ml/h. Bound proteins were eluted gradient using 0-1 M NaCl in the elution buffer. The protein profile of the fractions was determined at 280 nm and each fraction was assayed for invertase activity. Active fractions were added together and lyophilized.

Gel-filtration (Size) Chromatography on Sephacryl S-300

The lyophilized enzyme from ion exchange was layered on a Sephacryl S-300 column (1.0 × 40 cm) equilibrated with 20 mM Tris buffer pH 7. Forty (40) fractions of 1 ml each were collected at a flow rate of 12 ml/h. Active fractions were pooled, lyophilized, and re-dissolved in a minimal volume of 20 mM Tris buffer pH 7.

Native Molecular Weight Determination by Gel-filtration Chromatography

The void volume (V_o) of a Sephacryl S-300 packed column (1.0 × 40 cm) was determined with blue dextran (2 mg/ml) from a plot of absorbance of fractions at 620 nm against fraction volume. The total volume (V_t) of the column was calculated from the dimension of the column. Standard protein solutions: Bovine serum albumin (BSA molecular weight 67 kDa; 7 mg/ml), Ovalbumin (molecular weight 45 kDa; 7 mg/ml), α-chymotrypsinogen A (molecular weight 25 kDa; 3 mg/ml), and lysozyme (molecular weight 14.4 kDa; 10 mg/ml) each was layered on the column and eluted in the same manner as with blue dextran at the rate 12 ml/h. The protein profile of the fractions in each case was monitored by measuring absorbance at 280 nm. A plot of absorbance at 280 nm against fraction number was used in determining the elution volume (V_e) of each of the standard proteins. The molecular weights of the standards were plotted versus their partition coefficient (K_{av}) from where the molecular weight of the invertase was calculated.

Homogeneity Test and Determination of Molecular Weight by Denaturing and Non-Denaturing Polyacrylamide Gel Electrophoresis

Both denaturing (SDS) and non-denaturing PAGE were carried out to ascertain the purity of the enzyme. The SDS-polyacrylamide gel electrophoresis was done on 10% (w/v) polyacrylamide (running gel) and 4% (w/v) stacking gel[23] while that of the non-denaturing PAGE was carried out with a system without SDS and mercaptoethanol. The bands were obtained following staining and destaining methods.

Kinetic Parameters of the Purified Invertase

Sucrose concentration (1-150 mM) was varied and the effect on the purified invertase was determined. The data were analysed with GraphPad Prism 5 and kinetic parameters were thereafter estimated.

Effect of Temperature on Invertase Activity

The temperature effect on purified invertase activity was carried out by incubating the enzyme at temperatures ranging from 10 to 70 °C. Aliquots were withdrawn at intervals and assayed for invertase activity.

Effect of Heat on the Stability of Invertase

The enzyme was incubated at varying temperatures. Aliquots were taken at 10 min intervals for 1 h. Invertase activity in each aliquot was determined under the standard assay conditions. The residual activities were plotted against the time of incubation.

Effect of pH on Invertase

The pH effect on the invertase activity was determined using pH range of 1-10 at 30 °C. The following buffer systems at the indicated pH ranges were used: 50 mM citrate buffer, pH 1-3; 50 mM acetate buffer, pH 4-5; 50 mM phosphate buffer, pH 6-8 and 50 mM borate buffer, pH 9-10. Relative activity was plotted against their respective pH values.

Effect of Metal Ions on Invertase

The effect of metal (monovalent, divalent and trivalent) ions such as Na^+, Mg^{2+}, Mn^{2+}, K^+, Ca^{2+}, and Al^{3+} on invertase activity was determined by the addition of the chloride salts of each metal ion in the assay mixture at concentrations of between 1-20 mM. Invertase activity at different salt concentrations was calculated and plotted against the concentrations of the respective metal ions.

Results

Extracellular and Intracellular Invertase Activity

The levels of extracellular and intracellular invertase are shown in Figure 1. The specific activity obtained for the extracellular invertase was 25 U/mg compared to intracellular invertase, which had a specific activity of 500 U/mg. The result formed the basis of our interest in the further investigation on the intracellular invertase from palm wine yeast.

Enzyme Purification

On the anion exchanger, DEAE-Tris-acryl column, a single activity peak was recovered with a yield and purification fold of 338% and 2.1 respectively. In the final purification on Sephacryl S-300, a single peak of activity (Figure 2) was

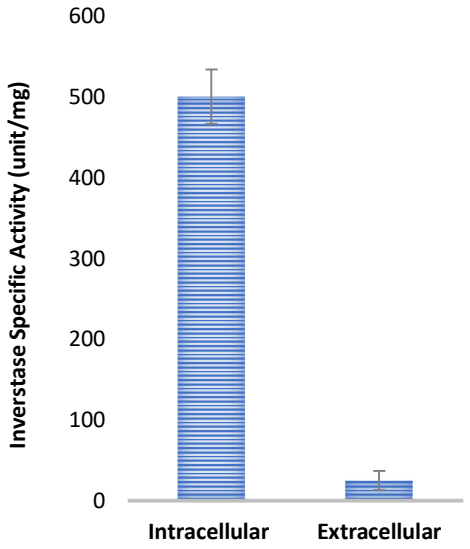

Figure 1. Specific Activities of Extracellular and Intracellular Invertase from Palm Wine Yeast.

achieved with a percentage yield of 168% and the purification fold of 3.0. Table 1 shows the purification summary. Activity in purified steps is more than the one in crude extract enzyme has almost double the enzyme units than the crude extract. This shows that there is a natural inhibitor present in this source, which has probably been removed during purification.

Homogeneity Test and Molecular Weight Estimation

SDS-PAGE of invertase from palm wine yeast (*Saccharomyces* sp) gave six bands with subunit molecular masses of 73.5 ± 2.12 kDa, 52.3 ± 8.06 kDa, 48.6 ± 3.04 kDa, 37.4 ± 4.81 kDa, 26.5 ± 3.61, and 24 ± 4.31 kDa, respectively (Figure 3a). The purified invertase is probably heterohexameric in nature. This was further investigated on non-denaturing PAGE. A single band with a high molecular mass of 267 kDa was obtained (Figure 3b). The bonds intercalating the subunits could not be cleaved due to the

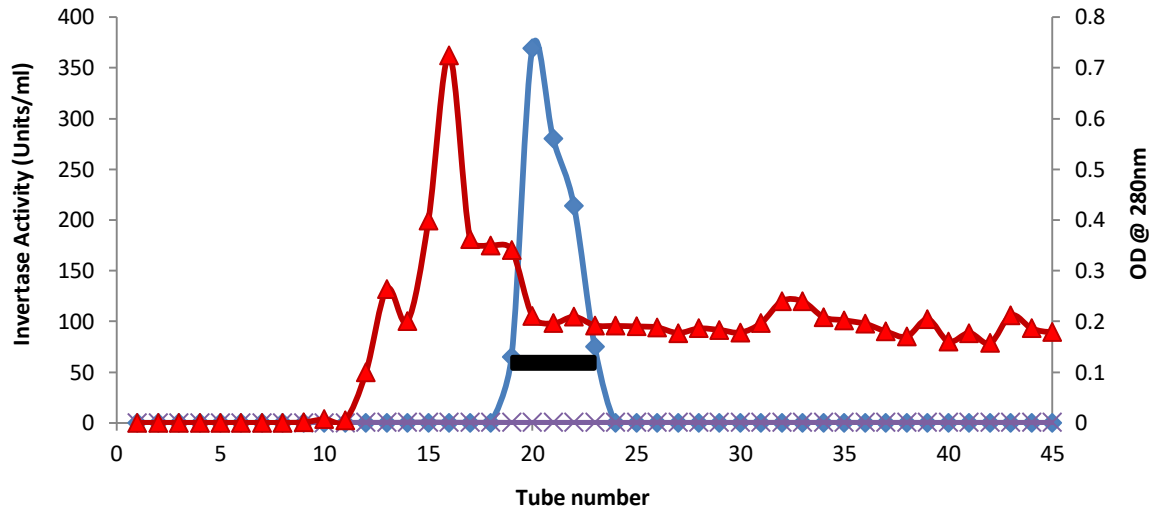

Figure 2. Elution Profile of Partially Purified Invertase from Palm Wine Yeast on Sephacryl S-300 Column. The DEAE-trisacryl pool obtained was free-dried and re-dissolved in minimal volume of 20 mM Tris buffer pH 7 and then layered on Sephacryl S-300 column (1.0 cm × 40.0 cm). The proteins were eluted with the equilibration buffer at a flow rate of 12 ml/h. Fractions of 1 ml each were collected and active fractions were pooled and concentrated. Invertase activity ( ), Absorbance @ 280nm ( ) and Fractions pooled: 19-23 ( ).

Table 1. Purification Summary of Intracellular Invertase from Palm Wine Yeast

Sample	Volume (ml)	Total Activity (units)	Total Protein (mg)	Specific Activity (units/mg protein)	% Yield	Purification Fold
Crude	30	23820	8.1	2941	100	1.0
DEAE-Tris-acryl	40	80480	13.4	6006	338	2.1
Sephacryl S-300	10	40130	4.5	8918	168	3.0

absence of SDS and mercaptoethanol. In addition, the estimation of the native molecular weight on calibrated Sephacryl S-300 column gave a single activity peak equivalent to an enzyme with a molecular weight of 266 ± 28 kDa. The data obtained on both non-denaturing PAGE and gel filtration validated that of the six bands on SDS-PAGE, revealing that the enzyme is a single entity but heterohexameric.

Kinetic Parameters of Purified Intracellular Invertase (PWSInv)

The K_m for sucrose hydrolysis of the purified invertase is 30.82 ± 3.2 mM, while that of V_{max} is 4672 ± 169.0 units/mg protein (Table 2) resulting in catalytic efficiency, k_{cat}/K_m value

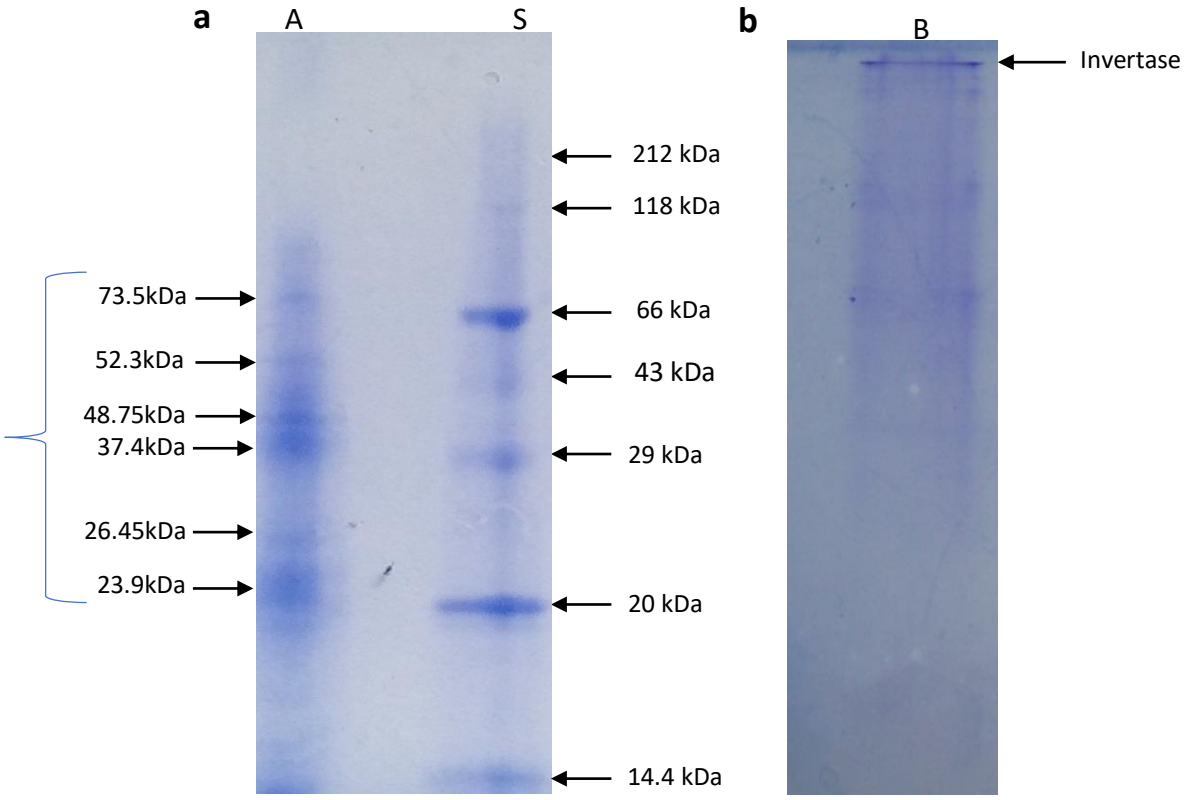

Figure 3. Electrophoretograms of the Purified Palm Wine *Saccharomyces* sp. Invertase. **(a)** SDS-PAGE of the invertase on 10% gel. Standard proteins: Myosin (212 kDa), β-galactosidase (118 kDa), BSA (66 kDa), Ovalbumin (43 kDa), Carbonic anhydrase (29 kDa), Trypsin inhibitor (20 kDa), and Lysozyme (14.4 kDa) are shown on lane S while the purified invertase is revealed to be heterohexameric (six subunits) in nature as shown on lane A. **(b)** The non-denaturing PAGE of the purified invertase. A high molecular weight single band equivalent to 267 kDa was obtained as shown on lane B. The bonds between the subunits could not be cleaved due to absence of SDS and mercaptoethanol.

Table 2. Kinetic Parameters of Purified Intracellular Invertase from Palm Wine Yeast

Sample	K_m (mM)	V_{max} (units/mg protein)	k_{cat} (s^{-1})	k_{cat}/K_m (M^{-1} s^{-1})
Saccharomyces sp.	30.8 ± 3.2	9672.0 ± 169.0	4.30×10^4	1.43×10^6

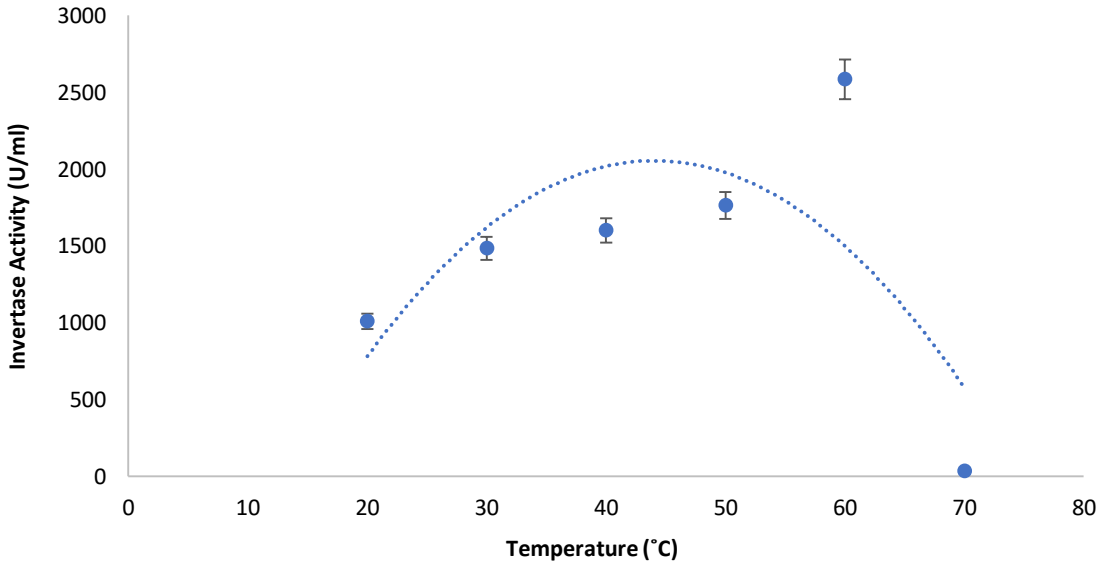

Figure 4. The Effect of Temperature on Purified Invertase from Palm Wine Yeast (*Saccharomyces* sp.). The optimum temperatue was 60 °C.

value of 6.72×10^5 M^{-1} s^{-1}.

Effect of Temperature on Purified Intracellular Invertase

The effect of temperature on purified intracellular invertase is as shown in Figure 4. The optimal temperature obtained was 60 °C. The purified enzyme was stable at 30-60 °C, but a sharp decline in activity at temperatures 70 °C and above. The activation energy required for the hydrolysis of sucrose by the invertase was estimated to be 2.8042 kJ/mol.

Effect of pH on the Activity of Purified Invertase from Palm Wine Yeast (Saccharomyces sp.)

When the activity of the purified invertase was determined in buffers with a pH range of 1-10, the optimum pH for catalysis by the enzyme was observed to be pH 3 (Figure 5). The enzyme was relatively stable at pH values between 2.5 to 5. A sharp decline was observed at pH 7 and extreme pH values of 1-2.5 and 8-11.

Effect of Metal Ions on Invertase Activity from Palm Wine Yeast (Saccharomyces sp.)

The effect of various metals on the activity of palm wine yeast intracellular invertase is as shown in Figure 6. In the presence of Na^+ and K^+, a drastic loss of enzyme activity was observed as the concentration of these ions increase. The activity of purified invertase increased gradually in the presence of aluminium chloride, up to 4 mM, there was sudden decline at 6 mM concentrations of aluminium chloride. There was a gradual increase in activity of invertase in the presence of magnesium chloride, even up to the highest concentrations used. The enzyme retained activity at 8 mM concentration of manganese but there was a complete loss of activity at 14 mM. In the presence of varying concentrations of calcium chloride, a steady loss of activity was observed above 5 mM $CaCl_2$.

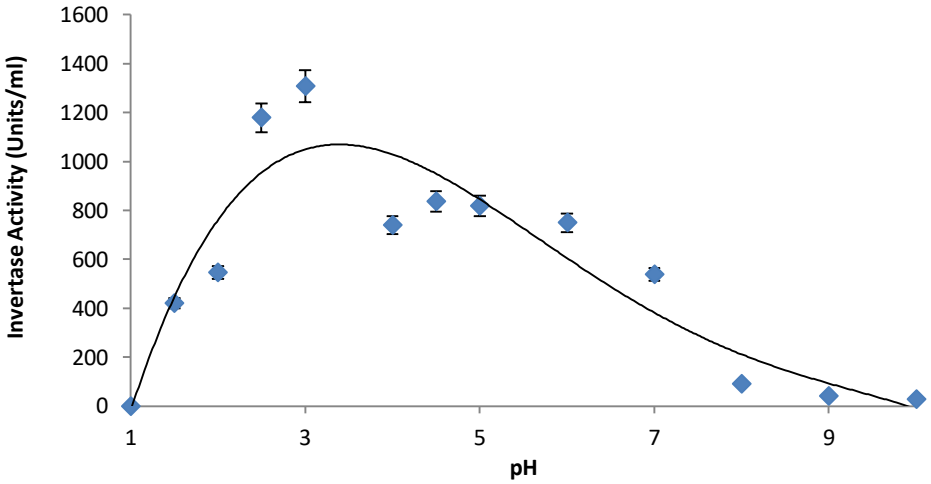

Figure 5. Effect of pH on Intracellular Invertase Activity from Palm Wine Yeast (*Saccharomyces* sp.). The optimum pH was 3.

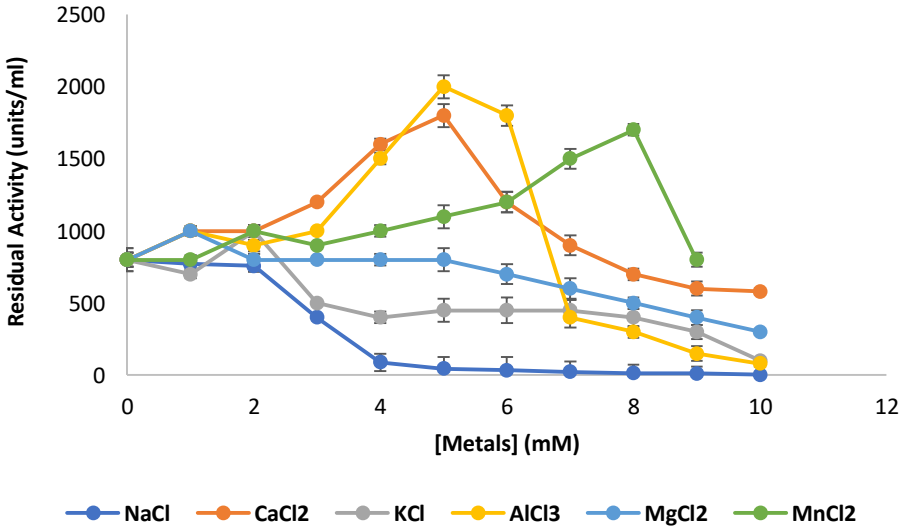

Figure 6. Effect of Various Metals on the Activity of Intracellular Invertase from Palm Wine Yeast.

Discussion

Isolation and development of suitable microbes for use in production processes of economic importance are crucial in biotechnology. Invertase is an industrial enzyme with several biotechnological applications. Commercially available invertase is produced chiefly by strains of yeast (*Saccharomyces cerevisiae*) and the predominant source of yeast (*Saccharomyces* sp.) is palm wine, accounting for 87% of the total population of yeast.[24] Palm wine is an alcoholic beverage that is very abundant in Nigeria. It is a traditional fermented drink that is appreciated throughout the world, especially in West Africa. In this work, the abundance of yeast production from palm wine was exploited for the isolation of invertase with the primary aim of finding a cost-effective source, with a high yield of invertase. The enzyme is usually present either as extracellular or intracellular in yeast.[25] The presence of high invertase in the intracellular milieu of the yeast cell in comparison with the extracellular level has been established. One possible reason for the different subcellular localization is that the yeast cells could be in a repressed state. There is a higher expression of intracellular invertase when cells of the organism source are in a repressed state. This result is in agreement with the report of Samarth et al.,[25] on the localization of invertase isoforms in cells. As enzymes have continued to attract the attention of industries and biotechnologists and new uses are developed, the development of a good purification method for enzymes is necessary.[26,27] After extraction and isolation, the intracellular invertase was purified using chromatography techniques. A single invertase activity peak was obtained on both cation and anion exchangers while final purification on Sephacryl S-300 also gave a single peak of activity leading to a percentage yield of 168% and purification fold of 3.0. The high yield obtained after purification was probably due to the removal of tightly bound proteinaceous or natural invertase inhibitors during the purification. The purification techniques were able to purify the intracellular invertase to a condition of near homogeneity as adjudged by polyacrylamide gel electrophoreses. Guimaraes et al.,[26] reported the purification of invertase from *Aspergillus ochraceus* on both DEAE cellulose and Sechacryl S-200 chromatography columns.

The purified intracellular invertase from palm wine yeast is heterohexameric in nature. This was observed on SDS-PAGE, revealing six subunits for the purified enzyme. The result was validated with data obtained on non-SDS PAGE where a single band of high molecular weight (268 kDa) was obtained. Further experimental analysis on gel filtration chromatography where a native molecular weight of approximately 266 ± 28 kDa was also obtained finally established the. Many proteins and enzymes exist and function as multimeric complexes, in which subunits noncovalently interact with each other. Our result is similar to the work of Sainz-Polo et al.,[28] where octameric *Saccharomyces* invertase from *Escherichia coli* with eight subunits was reported. Oda and Tonomura[29] earlier reported tetrameric invertase from *Torulaspora pretoriensis* with native molecular weight of 530 kDa. Gel filtration and SDS-PAGE analyses revealed that *Aspergillus terreus* invertase has a dimeric structure composed of two monomers of 32 kDa.[20] The multi-subunit structural complexity of the enzyme would constitute a convenient toolbox for improved biocatalysis and versatility in several applications.

The kinetics of the intracellular invertase for sucrose utilization were investigated. The initial velocity of the purified invertase produced a hyperbolic curve with increasing substrate concentration. At low concentrations of the substrate, first-order kinetics was observed as the initial rate was directly proportional to substrate concentration. However, at very high substrate concentrations, saturation or zero-order kinetics was observed. The K_m of 30 .8 ± 3.2 mM and a V_{max} of 9672 ± 169.0 units/mg protein were obtained, which led to catalytic efficiency, k_{cat}/K_m of 1.43×10^6 $M^{-1}s^{-1}$. The high K_m value should be of industrial and biotechnological interest. For industrial enzymes, high K_m for substrates is highly beneficial, it showed that the active site can accommodate large sucrose concentration for conversion into fructose and glucose. In addition, the somewhat high catalytic efficiency could be exploited for sucrose conversion efficiency. These data compare well with the literatures on the kinetic properties of yeast invertase. The Michaelis-Menten (K_m) value reported by Vu and Le[30] for invertase is usually around 30 mM. The kinetic parameters obtained in this work is in agreement with the report of Sivakumar et al.,[31] on invertase from *Saccharomyces cerevisiae* strain isolated from grapes.

The effect of temperature on the enzymatic activity of purified invertase, that the optimum temperature is 60 °C. The activity of palm wine yeast intracellular invertase increased gradually up to 60 °C and declined at higher temperatures which may be due to denaturation. This is in good agreement with the work of Qureshi et al.,[32] who reported maximum activity for invertase from *Mucor geophillus* EFRL 03 at temperatures between 60-70 °C. Many researchers have also been able to affirm that invertase exhibits high catalytic activity in temperatures ranging from 35-75 °C on the basis of the source of enzyme[33].

The stability and activity of enzymes are highly dependent on the pH of the reaction medium and the environment. The effect of pH on the invertase activity for the conversion of sucrose was investigated. The highest activity of purified palm wine yeast invertase was found to be pH 3. A sharp decline was observed at pH 7 to alkaline pH, in addition to extreme acidic pH of 1 and 2. Mona and Mohammed[34] reported optimum pH ranges of 5 to 7 for invertase from *S. cerevisiae*. Similarly, maximum invertase activity from

Mucor geophillus was observed at pH 5.[32] Invertase from *Aspergillus terreus* had the highest activity at pH 2 and was stable over a range of pH 3-5.[35] It is evident that the palm wine intracellular invertase is an acidic enzyme and this low optimum pH would be beneficial for inversion of sucrose for fructose production as it will prevent the undesired colour formation and microbial contamination.

The effect of different electropositive metals on the activity of palm wine intracellular invertase was investigated. All the divalent metal ions (Ca^{2+}, Mg^{2+}, and Mn^{2+}) were tolerated by the enzyme as close to 100% activity was maintained even at concentrations up to 8 mM. In contrast, the monovalent metals, Na^{+} and K^{+} inactivated the enzyme. There was a total loss of activity in the presence of 4 mM Na^{+} metal. It was observed that the intracellular invertase activity was activated by chlorides of Al^{3+} at concentrations of 4-6 mM.

Conclusion

Both the extracellular and intracellular invertase activities were investigated for an in-depth understanding of invertase expression in palm wine yeast due to its application in several industrial processes. The specific activity of the intracellular invertase activity was twenty-fold higher than that of extracellular. The two-step purification scheme adopted resulted into good yield and purification fold. The purified intracellular invertase had a high catalytic efficiency suggesting its high affinity for sucrose conversion to invert sugars. The purified enzyme was active and stable at high temperature, acidic pH and in the presence of some metal ions. The combination of properties of the purified intracellular invertase from palm wine yeast could be of interest in industries for the production of invert sugars and high fructose syrup.

Authors' Contributions

OFA carried out the experiment, contributed chemicals and wrote the paper. OSI conceived the research, contributed chemicals and revised the paper.

Conflict of Interest Disclosures

The authors declare that they have no conflicts of interest.

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