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Thermodynamics and Thermal Inactivation of Endo-*β*-1,4-Glucanase Produced from *Aspergillus niger*

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ABSTRACT

The limited understanding of the thermal properties of endo-*β*-1,4-glucanase poses a significant obstacle to its widespread industrial use. This research was designed to explore the thermodynamic and thermal inactivation of endo-*β*-1,4-glucanase extracted from *Aspergillus niger* for industrial applications. In this study, grape bagasse was utilized as the carbon source. Endo-*β*-1,4-glucanase was derived from *Aspergillus niger* through a submerged fermentation process. *Aspergillus niger* endo-*β*-1,4-glucanase was partially purified to the extent of gel filtration chromatography (Sephadex G-100) with a 2.68% yield and a specific activity of 9.21 U/mg after ammonium sulphate precipitation (50% saturation) and dialysis. Endo-*β*-1,4-glucanase activity reached its optimal at a pH of 5.0. The enzyme exhibited an optimum activity at temperature of 55°C and a maximum thermostability with a half-life of 364.79 min. Thermodynamic parameters showed that the enthalpy of activation of denaturation (ΔH) was 26.12, 26.04, 25.99, 25.96, 25.92, 25.87, and 25.79 KJ/mol at 40ºC, 50ºC, 55ºC, 60ºC, 65ºC, 70ºC, and 80ºC, respectively. A z-value of 0.014ºC was obtained, with an activation energy (Ea) of 28.73 KJ/mol for the denaturation of the enzyme indicating the enzyme's responsiveness to a rise in temperature. The D-value of the enzyme ranged from 291.5 to 1212.1 min. The Gibbs free energy was negative ($\Delta G > 0$) at the temperatures studied, while the entropy change was positive ($\Delta S < 0$). The thermodynamic studies showed the thermal deactivation of the enzyme. These characteristics at the optimum conditions and stability of the enzyme make it a promising enzyme for industrial applications, particularly for bioethanol fermentation.

Keywords: Grape bagasse, Endo-*β*-1,4-glucanase, Characterization, Thermostability, Thermal inactivation

Introduction

Agricultural lignocellulose wastes are one of the large wastes created due to farming practices and by the wood product industry.[1] Grapefruit (*Citrus paradisi*) is a subtropical citrus tree known for its sour to semi-sweet fruit. The frequent use of grapefruits to produce juices, nectars, concentrates, jams, jelly powders, and flakes generates wastes in the form of grape peel and bagasse which may result in environmental pollution if not properly handled.[1] These wastes are composed of cellulosic and hemicellulose materials; if not managed properly, they constitute an environmental menace. The use of lignocellulosic wastes as a sustainable carbon source relies on advancing practical technologies for endo-*β*-1,4-glucanase production.[1] Endo-*β*-1,4-glucanases (EC 3.2.1.4) are a type of enzyme that breaks down *β*-1,4 linkage in cellulose to produce smaller *β*-D-glucose.[2] Cellulose degradation by cellulase involves the synergistic activities of endo-*β*-glucanase, exo-*β*-glucanase (cellobiohydrolase), and *β*-glucosidase.[1]

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Endo-*β*-1,4-glucanase is believed to begin a random assault on various internal sites within the cellulose compound, causing the polymer to become more susceptible to hydrolysis by other cellohydrolases.[3] The synergy involves an attack on amorphous parts of cellulose by endo-*β*-1,4-glucanase, creating areas for exoglucanase which then splits off cellobiose units from the cellulose chain. Lastly, *β*-glucosidases hydrolyse cellobiose to glucose. The hydrolysis of cellobiose prevents the accumulation of cellobiose because it can act as an inhibitor of exoglucanase activity.[4,5]

Endo-*β*-1,4-glucanases are synthesized by a wide range of microorganisms during their growth in cellulolytic substrates.[3,6-8] Fungal endo-*β*-1,4-glucanase is an inducible enzyme used in the textile, food, pulp and paper, laundry, and biofuel industries.[9-11] *Aspergillus sp* has been reported to secrete large amounts of extracellular endo-*β*-1,4-glucanases. The structure of fungal endo-*β*-1,4-glucanase comprises a prominent catalytic region (CR), and a smaller cellulose-binding region (CBR), interconnected by glycosylated peptides.[3] The catalytic region (CD) houses the active site responsible for cellulose degradation, while the cellulose-binding region (CBR) plays a crucial role in facilitating the breakdown of cellulose.[3]

The selection of fungal endo-*β*-1,4-glucanase is becoming increasingly intriguing due to its diverse range of species, the convenient retrieval of the enzyme product, and its capacity to produce enzymes that can withstand extreme environmental conditions. Extensive evidence has shown that conducting thermal stability and heat inactivation studies is a crucial aspect in characterizing commercial enzymes, serving both scientific and industrial purposes.[14]

In the quest to obtain green and sustainable development,[15] this research was designed to search for eco-friendly and cheaper carbon sources for endo-*β*-1,4-glucanase production. A good heat stability of enzymes is crucial in enhancing the efficiency of their products.[16] The limitation in the industrial use of endo-*β*-1,4-glucanase is the limited understanding of the thermal properties of endo-*β*-1,4-glucanase, which may hinder the eco-friendly production of endo-*β*-1,4-glucanase. To explore locally sourced microbial heat-stable endo-*β*-1,4-glucanase, and to explore the structure–stability relationship of this enzyme, this study focused on endo-*β*-1,4-glucanase production, thermodynamic and thermal inactivation of endo-*β*-1,4-glucanase from *Aspergillus niger* strain grown on grape bagasse. This study also contributed to the global effort to convert agricultural waste to useful products by using grape bagasse as an economical source for endo-*β*-1,4-glucanase production.

Materials and Methods

Chemicals

Chemicals used in this study were of analytical grade, and were purchased from British Drug House Chemical Limited (England), Merck (Germany), May and Baker Limited (England), and Sigma Chemical Company (USA) unless otherwise mentioned.

Sample collection

Grapefruits were obtained from the University of Nigeria, Nsukka Staff Quarters in June 2021. A pure culture of *Aspergillus niger* MCCAN24 was obtained from the Microbial Culture Collection Unit, Department of Microbiology, University of Nigeria, Nsukka.

Grape bagasse preparation

Grape bagasse was obtained following the peeling and removal of the interiors of the grapefruits. The baggase was washed with distilled water, and then treated with 70% ethanol. The treated bagasse was rinsed with distilled water, and dried in the sun. The sun-dried bagasse was pulverized using a milling machine (Universal milling machine, Ashu Enterprises, Batala, Punjab, India), and sieved with a 3 mm mesh.

Organism and inoculum preparation

Pure culture of *Aspergillus niger* was grown on potato dextrose agar (PDA) at a temperature of 25°C. The inoculum was cultivated on $FeSO_4.7H_2O$ (0.1%), NH_4NO_3 (0.1%), $NH_4(H_2PO)_4$ (0.1%), $MgSO_4.7H_2O$ (0.1%), and agar-containing medium in a 1 L solution of sodium acetate buffer (pH 7.0). The broth was sterilized at 121ºC for 15 min, after which the broth was poured into Petri dishes and left to solidify. From the peripheral zone of the pure culture, a disc (2 mm) of fungal culture was taken and transferred into Petri plates containing the above medium.

Production of endo-β-1,4-glucanase

Endo-*β*-1,4-glucanase production was carried out using the submerged fermentation (SmF) technique as described by Darabzadeh *et al.*(2019).[17] Grape peel bagasse (1%) in 100 mL of sterile cultivation medium was placed in a 250 mL Erlenmeyer flask containing 0.5% NH_4NO_3, 0.5% $NH_4(H_2PO)_4$, 0.5% $MgSO_4.7H_2O$, 0.1% $FeSO_4{\cdot}7H_2O$, 0.3% $CaCl_2.2H_2O$, and 0.75 g protease peptone. The medium was adjusted to pH 6.8 and autoclaved at 121ºC for 15 min. Using a cork borer with a diameter of 10 mm, four discs of the *Aspergillus niger* isolate were introduced into the medium for fermentation. The broth was incubated for five (5) days at room temperature on an Aluminium Alloy Rectangular Rotary Shaker (VTRS-1, India) to get homogenous growth. The microbial biomass was harvested, filtered, and centrifuged using BioSan Microspin 12 mini centrifuge (BIOSAN_22010, Berlin, Germany) at 10000 rpm for 15 min at 25°C. The supernatant was then used as the crude enzyme. The percentage yield was calculated using the method described by Ibrahim *et al.* (2021)[18] using the equation below

$$\frac{\textbf{\textit{Total enzyme activity}}}{\textbf{\textit{Starting Total activity}}} \times 100 \quad \text{..... (1)}$$

Endo-β-1, 4-glucanase activity assay

The endo-*β*-1,4-glucanase activity was determined using the DNS (3,5-dinitrosalicylic acid) method using Sodium Carboxymethyl Cellulose (Na-CMC) as substrate.[18] The reaction mixture consisted of 0.1 mL enzyme solution, 0.5 mL sodium acetate buffer (0.05 M, pH 5.5), and 0.5 mL carboxymethyl cellulose (2%). The reaction mixture was incubated at 50ºC for 30 min, the reaction was stopped by adding 1 mL of DNS reagent. The mixture was then boiled for 10 min followed by the addition of 1 mL of 25% Na-K Tartarate to maintain the colour stability. Finally, the reaction mixture was cooled on ice. The absorbance of the mixture was measured at 540 nm using Infitek double beam UV-visible spectrophotometer (SP-LUV-1920, Jinan, Shandong, China). One unit of Endo-*β*-1, 4-glucanase activity was quantified as the quantity of enzyme capable of hydrolyzing Na-CMC and liberating 1 µmol of glucose in a 1 min reaction.

Determination of protein

The protein content of the crude enzyme was assessed as described by Lowry *et al.* (1951)[19] using Bovine serum albumin (BSA) as a reference.

Purification of Aspergillus niger endo-β-1,4-glucanase

Endo-β-1,4-glucanase from *A. niger* was purified according to the method described by Ain *et al*. (2022).[20] Briefly, the crude enzyme extract (580 mL) was precipitated with 50% saturated solution of ammonium sulphate $(NH_4)_2SO_4$ at 0°C, and kept overnight at 4°C. The resulting precipitate was centrifuged at 10000 rpm for 15 min. The supernatant (20 mL) containing the enzyme was subsequently dialyzed using a dialysis bag for 12 h against a sodium acetate buffer (0.05 M, pH 5.5) to remove the salts. The dialysate was introduced into a Sephadex G-100 column (2.4 cm × 26.0 cm) pre-equilibrated with 0.05 M sodium acetate buffer (pH 5.5). Endo-*β*-1,4-glucanase activity was assessed by collecting enzyme fractions at a flow rate of 5 mL/15 min. The fractions with active enzyme activity were put together and stored at -10°C for further studies.

Characterization of Aspergillus niger endo-β-1,4-glucanase

Determination of optimal pH

The optimal pH for endo-*β*-1,4-glucanase activity was ascertained by adjusting the pH of 50 mM sodium acetate buffer (pH 3.5 – 5.5), 50 mM sodium phosphate (pH 6.0 – 7.5), and 50 mM Tris-HCl (pH 8.0 – 10.0). A portion (0.1 mL) of the enzyme solution was added to 0.5 mL each of the varied pH solution, and the pH was determined using a Benchtop pH meter (infitek PH-B300F, Jinan, Shandong, China) the mixture was incubated with 0.5 mL of 2% carboxymethyl cellulose for 30 min using a laboratory incubator (Zenithlab IB-9023A, Jintan, China). Endo-*β*-1,4-glucanase activity at these various pH intervals was assessed by employing the method outlined by Ibrahim *et al.* (2021).[18]

Determination of optimal temperature

To ascertain the optimal temperature for endo-*β*-1,4-glucanase activity, the enzyme was incubated with 2% carboxymethyl cellulose at 30 - 90ºC, at intervals of 10ºC for 30 min at the optimum pH. The determination of endo-*β*-1,4-glucanase activity at these various temperature intervals was carried out following the method described by Ibrahim *et al.*(2021).[18]

Determination of thermodynamic stability

The thermal stability of the partially purified endo-*β*-1,4-glucanase was determined according to the method described by Salehi and Asoodeh (2022).[21] Purified endo-*β*-1,4-glucanase was incubated in a water bath pre-equilibrated at 40 – 80ºC (5ºC interval). Aliquots of the sample (0.1 mL) were collected periodically (0 – 120 min) and analyzed for endo-*β*-1,4-glucanase activity. The percentage residual activity for each incubation period was calculated, with the initial activity assumed to be 100%. The first-order inactivation constant (*k* value) was assessed by analyzing the slope of the first-order enzyme inactivation equation according to Eze *et al.* (2010)[22] as follows:

$$Log(\%\ residual\ activity = -\left(\frac{k}{2.303}\right)t \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

The half-life ($t_{1/2}$) of the enzyme inactivation was calculated using equation (3) below;

$$t_{1/2} = \frac{ln(2)}{k} \ldots\ldots\ldots\ldots\ldots\ldots (3)$$

$$D - value = \frac{ln10}{k} \ldots\ldots\ldots\ldots\ldots\ldots (4)$$

$$k = Ae^{-Ea/RT} \ldots\ldots\ldots\ldots\ldots\ldots (5)$$

The following expression was employed to ascertain the value of the activation energy (E_a) and Arrhenius rate constant (k)

$$\Delta G\ (Gibbs\ free\ energy\ change) = -RTln\left(\frac{kh}{K_bT}\right) \ldots\ldots\ldots\ldots\ldots\ldots (6)$$

$$\Delta S\ (entropy\ change) = \frac{\Delta H - \Delta G}{T} \ldots\ldots\ldots\ldots\ldots\ldots (7)$$

$$\Delta H(enthalpy\ change) = Ea - RT \ldots\ldots\ldots\ldots\ldots\ldots (8)$$

Where;
t = Time of enzyme inactivation
E_a = Activation energy
A = Arrhenius constant,
R = Universal gas constant (8.314 J/mol.K)
T = Absolute temperature
K_b = Boltzmann's constant (1.3806 x 10^{-23} J/K),
h = Plank's constant (6.6260 x 10^{-34} J.s)

Results and Discussion

Endo-*β*-1,4-glucanase was produced from *A. niger* MCCAN24 using a submerged fermentation system incorporated with grape bagasse after 5 days of incubation. The crude enzymes obtained gave a specific activity of 3.36 U/mg (Table 1). Bergquist *et al*. (2002)[11] maximized the fermentation media for the generation of cellulase by *Trichoderma reesei* and *Aspergillus awamori* fermented with rice straw and they produced a higher specific activity for *T. reesei* endo-*β*-1,4-glucanase at Day 5 (27.04 U/mg) when compared to *A. awamori* (15.19 U/mg). Our results indicated lower specific activity of 3.36 U/mg when compared to the findings of Bergquist *et al.*[11] The differences in the specific activity of the enzyme may be a result of the substrate (grape bagasse) used in this study which may have influenced the formation of biomass or production of the enzyme. The findings from the work of Kognou *et al*. (2022)[23] revealed that the highest level of cellulase production was attained after an incubation period of four (4) days.

A saturated solution of fifty percent (50%) ammonium sulphate was found to precipitate endo-*β*-1,4-glucanase with a specific activity of 4.0 U/mg, and a yield of 7.0% (Table 1). Mousa, *et al.*[24] reported that 60% ammonium sulphate was appropriate for inducing precipitation of endo-*β*-1,4-glucanase from irradiated *Arhromobacter spanius*. The use of a lower concentration of ammonium salt for the precipitation of endo-*β*-1,4-glucanase in the present study may be attributed to the presence of hydrophobic amino acid residues within the protein's active site. Ammonium sulphate precipitation serves as a way to concentrate proteins, offering a degree of purification by effectively separating proteins from non-proteins. In general, hydrophobic proteins tend to precipitate at lower salt concentrations compared to hydrophilic proteins.[25] This is because the solubility of proteins is influenced by the pH and the ionic strength of the solution.[25] The dialyzed enzyme was purified to 1.82 fold with a yield of 6.57% and a specific activity of 6.13 U/mg (Table 1). The increase in endo-*β*-1,4-glucanase-specific activity of the dialysate may be due to the removal of substances and ions with low molecular weight which might have interfered with the enzyme activity. Gel filtration chromatography purified the enzyme up to a yield of 2.68% with a specific activity of 9.21 U/mg resulting in a 2.59-fold increase in purification (Table 1). The elution profile derived from gel filtration of *A. niger* MCCAN24 endo-*β*-1,4-glucanase had a double peak of endo-*β*-1,4-glucanase activity (Figure 1). The double peak indicates that there are isoenzyme forms of the enzyme arising from anionic and physical heterogeneity. Also, double enzyme activity peaks are indicators of some levels of impurity of the proteins. The double peak exhibiting the highest endo-*β*-1,4-glucanase activity were merged for subsequent studies.

The relationship between pH, temperature, and the activity of purified endo-*β*-1,4-glucanase is shown in Figure 2. In this study, the specific of endo-*β*-1,4-glucanase increased as pH increased from 3.5 to 5.0, beyond which there was a decline in enzyme activity, making pH 5.0 the optimal pH for endo-*β*-1,4-glucanase activity (Figure 2). The loss of enzyme activity after the optimum pH is directly linked to the level of perturbations, which is influenced by the level of pH alteration. The presence of ionizable groups, such as carboxyl groups, in endo-*β*-1,4-glucanase, influences the enzyme's sensitivity to varying pH values. Consequently, this affects the protein's conformation, as well as the ionization state of both the enzyme and substrate.[15] Lee *et al.*[2] reported that *β*-glucosidase had optimum pH ranging from 4.0 to 5.0, Fagbohunka *et al.*[26] reported that *Amitermes eveuncifer* (Silvestri) exhibited its highest activity at pH 8.0, whereas Siddiqa *et al.*[27] found that *Bacillus licheniformis* S16 endo-*β*-1,4-glucanase showed optimal activity at alkaline pH levels. Additionally, Pham *et al.*[28] documented that endo-*β*-1,4-glucanase produced from coconut mesocarp has a wide pH in the range of 4.5 – 12. The varied pH optima reported for endo-*β*-1,4-glucanase activity may be attributed to the specific amino acid sequences of the protein, which undergoes protonation or deprotonation at varying pH values.

Table 1: Summary of the purification profile of endo-*β*-1,4-glucanase produced from *Aspergillus niger* MCCAN24

Purification Step	Total Volume (mL)	Total Protein (mg)	Total Activity (U)	Specific Activity (U/mg)	Purification Fold	Percentage Yield (%)
Crude Enzyme	580	3828	12876	3.36	1.00	100
50% $(NH_4)_2SO_4$ Saturation	41	225.5	902	4.0	1.19	7.06
Dialysis	30	138	846	6.13	1.82	5.17
Gel filtration chromatography	15	37.5	345.5	9.21	2.59`	2.59

In this study, temperature was observed to have a direct correlation with endo-β-1,4-glucanase activity, with an increase in activity up to the optimal temperature of 55ºC. However, beyond this point, the enzyme activity began to decline (Figure 2). The initial rise in temperature can be attributed to the increase in the frequency of contact between the enzyme molecule and the substrate. With the temperature increase occurring within the lower temperature range, the frequency of contact between enzyme molecules and substrate increases thus speeding up the enzymatic reaction and giving rise to the initial rise in activity. Lee *et al.*[2] reported that exoglucanase exhibited highest activity at an optimal temperature of 50ºC, while endo-*β*-1,4-glucanase and *β*-glucosidase showed optimal activity at temperature of 40ºC. Enzyme activity may decline after reaching the optimal temperature due to the denaturation of endo-*β*-1,4-glucanase primary and secondary structures. This denaturation could be caused by the dissociation of the enzyme's catalytic domain and cellulose-binding domain, leading to a loss of functionality. Both *β*-glucosidase and total endo-*β*-1,4-glucanase from different sources exhibited optimal activities at temperatures ranging from 45 - 60ºC.[26,27,29] The findings from the present study align with the results of previous studies as highlighted above. According to Sulyman *et al.*[1] report, purified endo-*β*-1,4-glucanase derived from *Aspergillus niger* cultured on *Arachis hypogaea* shells demonstrated optimal activity at pH and temperature values of 4.0 and 40ºC, respectively.

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Sulyman *et al.*[1] reported that a relatively high thermal stability is the desired characteristic of an enzyme for industrial application.

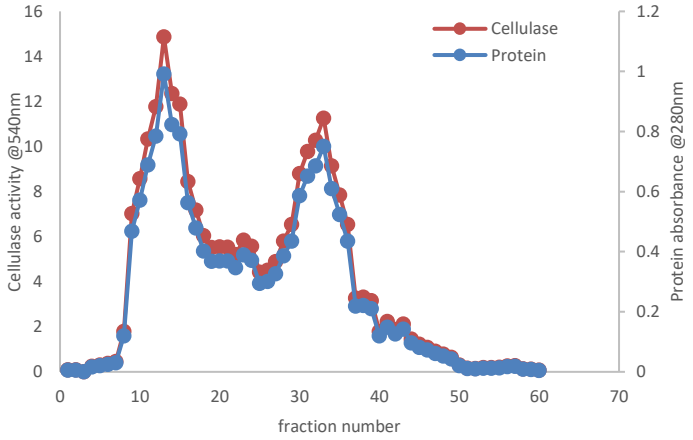

Figure 1: Gel filtration chromatogram for *Aspergillus niger* MCCAN24 endo-*β*-1,4-glucanase

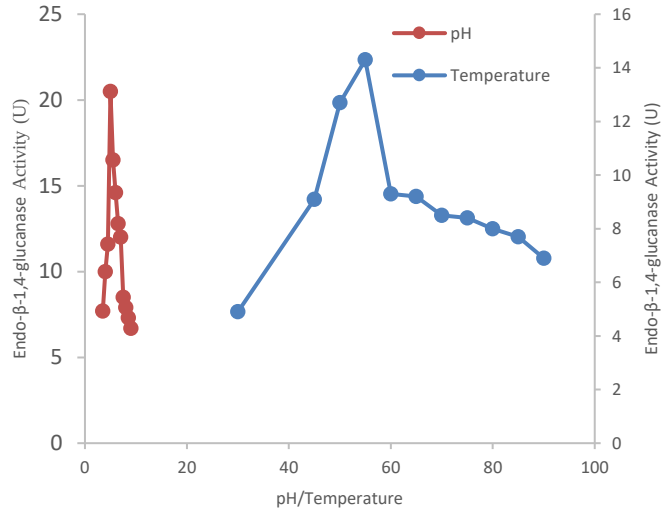

Figure 2: Effect of pH and temperature on *Aspergillus niger* MCCAN24 endo-*β*-1,4-glucanase activity

The thermostability characteristics of endo-*β*-1,4-glucanase derived from *A. niger* MCCAN24 were investigated by subjecting the enzyme to incubation within a temperature range of 40ºC to 80ºC for 120 min. In this study, thermodynamic and thermal inactivation of *A. niger* endo-*β*-1,4-glucanase showed that there was a decrease in the rate of enzyme inactivation (K_d) until it reached the optimum temperature of 55ºC beyond which there was an increase in inactivation (Table 2). A decreased rate of enzyme inactivation (K_d) at temperatures below the optimum temperature was observed in this study. This indicates the inactivation of the enzyme; hence it would not react with the substrate at low temperatures. At elevated temperatures above the optimum temperature, there was a noticeable rise in the rate at which the inactivation of the enzyme occurred, thus indicating a decrease in thermal stability.[15] At the optimum temperature (55ºC), the half-life of the enzyme was 364.79 min (Figure 3). However, at 80ºC, the enzyme exhibited low stability and a half-life of 87.73 min under comparable circumstances (Table 2). This is suggestive of the fact that the enzyme is more thermostable at the optimum temperature.

In this study, the D-value for endo-*β*-1,4-glucanase produced from *A. niger* MCCAN24 decreased with an increase in temperature beyond the optimum temperature of 55ºC. At 80ºC, the D-value exhibited the lowest value (291.52 min) and the highest value (1212.11 min) at 55ºC. These results suggested that the enzyme exhibits thermal stability when maintained at its optimal temperature. The D-value (min) represents the duration of heat treatment at a specific temperature necessary to decrease enzyme activity by 10% of its initial value.[22] However, the thermal denaturation of enzymes can occur in two ways;[30]

$$Ne \leftrightarrow Ue \rightarrow De \ldots\ldots\ldots\ldots\ldots\ldots (9)$$

Where;
Ne = Native enzyme
Ue = Unfolded enzyme
De = Denatured enzyme

Thermodynamic constants such as Gibb's free energy, enthalpy, and entropy provide evidence of protein unfolding while undergoing heat inactivation.[15] In this study, endo-*β*-1,4-glucanase produced from *A. niger* strain was thermally stable, and the enthalpy of inactivation decreased from 26.12 to 25.79 KJ/mol as the temperature increased (Table 2). The change in enthalpy (ΔH) offers valuable information about the thermodynamic nature of the inactivation process, whether it is endothermic or exothermic. This determination is based on the extent of bond disruption that occurs during the inactivation process. The positive ΔH values in this study indicate an endothermic process, while the low enthalpy value reflects the enzymes' resistant nature.

Moreover, the observation of a high value of ΔG at 80°C (86.95) KJ/mol (Table 2) in this study suggests a strong resistance to thermal unfolding at elevated temperatures, indicating thermal stability as it can endure high energy levels.[15] The obtained positive Gibbs free energy of activation for denaturation (ΔG) in this study indicates that the enzyme is thermally stable at high temperatures, and the reaction it catalyzes is nonspontaneous.[25] Protein inactivation involves the distortions in the secondary, tertiary, or quaternary structures of proteins without covalent bond breakage.[31, 32] Entropy (ΔS) reflects the overall disorder of both the enzyme and solvent.[15] The thermal denaturation of the enzyme depends on the entropy (ΔS) of the reaction.[25] In this study, negative entropies were observed at all temperatures. At the highest temperature (80ºC), the change in entropy was -0.173 $J.mol^{-1}.K^{-1}$. The negative entropy (ΔS) of deactivation stated for endo-*β*-1,4-glucanase indicates that there was negligible disorderliness. The negative entropy values in this study indicate that as the temperature increases, the system experiences a rise in protein aggregation, which is accompanied by the formation of a limited number of intra-/inter molecular bonds. The entropy (ΔS) of a reaction depends on the thermal denaturation of the enzyme. On the contrary, a high or positive entropy typically suggests an irreversible reaction due to the substantial formation of intramolecular bonds.[22] The z-value of the study was found to be 0.014ºC (Figure 4). It has been observed that a low z-value indicates the protein or enzyme's sensitivity to temperature elevation.[25] Likewise, the extent of the thermal destruction time (z-value) signifies the protein or enzyme's greater responsiveness to the length of time the heat treatment is applied.[33]

Table 2: Kinetic and Thermodynamic properties of thermal denaturation of endo-*β*-1,4-glucanase from *Aspegillus niger* MCCAN24

Temperature (ºC)	K_d(min^{-1})	$t_{1/2}$ (min)	D-value (min)	$\Delta H^o_{(D)}$ KJ/mol	$\Delta G^o_{(D)}$ KJ/mol	$\Delta S^o_{(D)}$ $KJ.mol^{-1}.K^{-1}$
40	0.0026	266.58	885.77	26.12	76.79	-0.162
50	0.0025	277.24	921.20	26.04	79.31	-0.165
55	0.0019	364.79	1212.11	25.99	80.60	-0.166
60	0.0033	210.03	697.88	25.96	81.86	-0.168
65	0.0038	182.39	606.05	25.92	83.13	-0.169
70	0.0061	113.62	377.54	25.87	84.41	-0.171
80	0.0079	87.73	291.52	25.79	86.95	-0.173
Z-value (ºC)	0.014					

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Ea KJ/mol	28.73

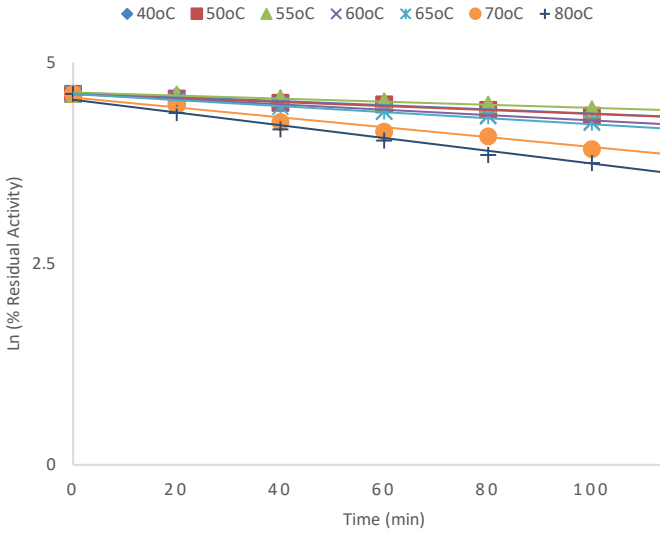

Figure 3: Plot of Ln (natural logarithm) % residual endo-*β*-1,4-glucanase activity against time

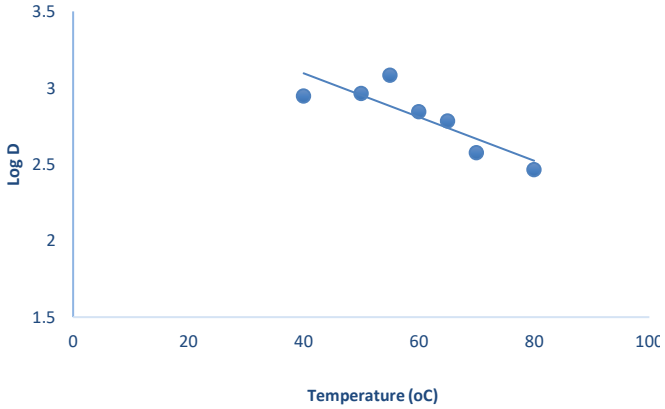

Figure 4: Temperature dependence of the decimal reduction time

Conclusion

Aspergillus niger MCCAN24 endo-*β*-1,4-glucanase exhibited optimum activity at pH and temperature of 5.5 and 55ºC, respectively. The thermal stability studies showed that *Aspergillus niger* endo-*β*-1,4-glucanase has a high thermostability with an ΔH of 25.99 KJ/mol, ΔG of 80.60 KJ/mol, and ΔS of -0.166 KJ.mol^{-1}.K^{-1}. The enzyme demonstrated a half-life of 364.79 min and a z-value of 0.014ºC, indicating its sensitivity to temperature changes. Additionally, the denaturation process of the enzyme was found to have an activation energy (Ea) of 28.73 KJ/mol. These findings showed that endo-*β*-1,4-glucanase from *Aspergillus niger* possesses excellent resistance to heat, thereby establishing its viability and economic sustainability as an enzyme of choice for industrial applications.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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