Optimization of thermostable inulinase production from *Aspergillus niger* NRRL 3122, purification, and characterization

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ABSTRACT

Inulinase is a versatile glycoside hydrolase enzyme that targets the -2, 1 linkage of fructopolymers. The objective of the study was to produce inulinase using a low-cost carbon source from *Aspergillus niger* NRRL 3122, which was successful in producing a high titer of inulinase on agave. The Plackett-Burman design and Box-Behnken design were used to optimize the production of inulinase, which resulted in a high inulinase titer of 2170.22 U/ml, which is 2.83 times higher than the screening. The optimal levels of agave, NaNO3, and KCl were found to be 10 g/L, 4 g/L, and 0.3 g/L, respectively. The molecular weight of the enzyme was around 50 KDa. The enzyme showed maximum performance at 50°C and pH 6.0, and temperature stability up to 70°C, while pH stability was observed between 4-6. The pure inulinase could only hydrolyze inulin and sucrose, and not cellobiose and soluble starches. The Km and Vmax values for inulin were found to be 0.76 mg/mL and 100,000 U/mg, respectively.

Keywords: *Aspergillus niger* NRRL 3122; inulinase; agave; Plackett-Burman, Box-Behnken purification, characterization

1. INTRODUCTION

Inulin is a type of dietary fiber that is found in many fruits, vegetables, and herbs. It is a polysaccharide that consists of repeating fructose units and belongs to a class of carbohydrates known as fructans. Inulin is not digested in the small intestine, but rather, it passes to the large intestine where it is fermented by gut bacteria. In this way, inulin can provide various health benefits, such as promoting the growth of beneficial gut bacteria, improving digestion, and supporting immune function. In addition to its health benefits, inulin is also used as a food ingredient and can be used as a sugar substitute due to its sweetness and low calorie content (Popoola-Akinola et al.,2022). Inulin is reported to be used for the production of bioethanol, single cell protein, single cell oil, citric acid and other chemical productions (Chi 2011)

According to Singh and Chauhan (2017), inulin can be hydrolyzed by two types of enzymes: exoinulinase and endoinulinase. Exoinulinases break down inulin into fructose, while endoinulinases break down inulin into fructooligosaccharides, as reported by Das *et al*. (2019).

Inulinases are applied in high-fructose syrups manfacture, as sweetner for diabetic patients (Yang *et al*., 2011; Saber and El-Naggar (2009). Additionally, inulinases was used for synthesis of inulo-oligosaccharides, which are beneficial for microbiota (Das *et al*., 2019). Bioethanol production is another application of inulinases (Gencheva *et al.*, 2012). Additionally, organic acids were produced using inulinases (Yazici *et al*., 2021).

As reported by Nath *et al*. (2022), fungal strains are known to be effective producers of inulinase and are favored for their ability to grow on low-cost media. Similarly, Mohamed *et al*. (2015) noted the potent inulinase production capability of certain fungal strains, likely due to their ability to thrive on inexpensive substrates. Trivedi *et al*. (2012) reported that due to the high cost of inulin, researchers are exploring the use of more affordable raw substrates as alternative sources of inulin.

In this study, we screened different fungi producing inulinase enzyme, we optimized inulinase enzyme production on an inexpensive substrate like agave using response surface methodology (RSM), we also purified and characterized the produced inulinase enzyme.

2. MATERIALS AND METHODS

2.1. Cultures and media

Two strains of fungi, namely *Aspergillus niger* NRRL 3122 and *Rhizopus oryzae* NRRL 3563 were obtained from the ARS (Agricultural Research Service), Illinois, USA. *Agave tequilana* leaves (blue agave) were obtained from research garden of the El-Sadat City University, washed, air dried, and ground into fine powder.

To maintain these cultures, they were subcultured every four weeks on potato dextrose agar (PDA) slants and stored at a temperature of 4°C. For the preparation of a spore suspension, spores were harvested from 7-day-old PDA slants that had been incubated at 30°C. The spores were then mixed with 10 ml of distilled water to achieve a final concentration of approximately 2×10^6 spores/ml spores/ml.

The fermentation medium contained various components including inulin, Na2HPO4,

2.2. Culture screening for enzyme production

CaSO4.7H2O, KNO3, NaCI, MnSO4.7H2O, and (NH4) H2PO4, and had a pH of 6.5. A spore suspension of *Aspergillus niger* NRRL 3122 (2×106 spores/ml) and/or *Rhizopus oryzae* NRRL 3563 was inoculated into the medium, which was then incubated at 30°C with 250 rpm of agitation for 8 days. Inulinase analysis was conducted on the 2nd, 4th, 6th, and 8th days.,

2.3 Carbon sources

Different sugars and agricultural residues were used as carbon source such as sucrose, aewwgave, peel, wheat bran, glucose, inulin, orange peel, fructose, oat bran. They were used at concentration of 1%.

2.4. Response surface methodology

2.4.1. Plackett-Burman design

A Plackett-Burman design to test the effects of nine different factors (agave, K_2HPO_4, $MgSO_4.7H_2O$, $NaNO_3$, KCI, FeSO4.7H2O, $NH_4H_2PO_4$, temperature, and pH) on inulinase activity in 12 different experiments (Plackett *et al*., 1946**).** The levels of each factor are shown in Table 1**.** Inulinase activity was used as the dependent variable, and a first-order equation was used to model the relationship between the independent variables (the levels of each factor) and inulinase activity. The equation was

$$\mathbf{Y=\beta o + \Sigma\, \beta i Xi,}$$

where Y is inulinase activity (measured in U/mL), βo is the intercept of the model, βi is

the linear coefficient for each factor, and Xi is the coded level of each independent variable.

2.4.2. Box-Behnken design

The Box-Behnken design (Box *et al*., 1960) is a statistical approach that involves three factors and three levels. In this study, the data obtained from the experiment were fitted into an equation with multiple terms. The data were fitted into the following equation:

$$Y=\beta o + \Sigma\, \beta iXi + \Sigma\, \beta ijXi\, Xj + \Sigma\, \beta iiXi$$

Y (the dependent variable), βo (the model intercept), βi (the linear coefficient for each factor), and Xi (the coded independent variable representing the level of each factor). Additionally, the equation also included terms such as βij (the interaction coefficient between two factors) and βii (the quadratic coefficient of a factor).

2.5. Analytical methods

A 1% inulin suspension was used to test the inulinase activity. The reducing sugar was measured using DNS according to (Miller *et al*., 1959). The amount of total protein was calculated using (Bradford *et al*., 1976).

2.6. Purification of inulinase.

For enzyme purification, ammonium sulphate is used to concentrate the enzyme to 80% saturation. Then, ultrafiltration is performed using Amicon Ultra centrifugal filters with a molecular weight cutoff (MWCO) of 50 kDa to separate the inulinase from other molecules that are smaller than 50 kDa.

2.7. Characterization of inulinase.

The molecular weight of inulinase was estimated by 12% sodium dodecyl sulphate-polyacrylamide gel (SDS-PAGE) using standard protein markers (IRIS11Prestained Protein Ladder (IRIS11#PMI11-0500), (3–260) KDa, Bio-Helix, USA. The gel was stained with Coomassie Brilliant Blue R-250 (Laemmli,1970).

To determine optimum temperature, inulinase activity was measured at temperatures ranging from 30 °C to 90 °C in 0.2 M sodium acetate buffer at pH 6.5. To determine the optimum pH, inulinase activity was measured at 50 °C in sodium acetate buffer at pH 3 to 6 and phosphate buffer at pH 7.0 and 9.0. Temperature stability was determined by measuring the residual activity after incubating the enzyme in 0.2 M sodium acetate buffer pH 6.5 at various temperatures (30–90 °C) for 120 min. The pH stability was determined by measuring the remaining activity after incubating the enzyme in series of buffer at pH range of 3 to 9 at 4 °C for 24h.

Substrate specificity for purified inulinase was investigated by incubation of 0.5 mL of appropriately diluted enzyme solution with 0.5 mL of each substrate in 0.2 M sodium acetate buffer pH 6.5 at 50^{O}C for 30 minutes. Then measurement of total amount of reducing sugars released from 1% polysaccharides (inulin, and soluble starch) and glucose released from 10 mM (sucrose, and cellobiose) were done by DNS. The initial hydrolysis rate of inulin at different substrate concentrations (0.5, 1, 2, 5 and 10 mg/mL) prepared in 0.2 M sodium acetate buffer pH 6.5 at 50 °C was used to study the enzyme kinetics. The Michaelis constant (Km) and maxi-mum velocity (Vmax) values were calculated according to Lineweaver and Burk by linear regression from double-reciprocal plots (Lineweaver and Burk,1934).

3. RESULTS AND DISCUSSION

3.1. Screening for inulinase producing strains.

In the screening process for inulinase producing strains, Table 2 shows that on the 6^{th} day of incubation, *A. niger* NRRL 3122 exhibited the highest inulinase activity with a measurement of 760 U/ml, while *R. oryzae* NRRL 3563 showed the lowest activity at 250 U/ml. These findings support previous research indicating that Aspergillus species

tend to produce more inulinase than other species. Aspergillus is known to be one of the most effective fungi in producing inulinase according to previous reports (Rawat et al., 2021; Ilgın *et al*., 2020). Lopes (2011) also stated that *Aspergillus niger* has the potential to produce various enzymes including glucose oxidase, pectinase, α-amylase, and inulinase.

3.2. Effect of different carbon sources

In the current study, the effect of different carbon sources on the production of inulinase was investigated. Figure (1) illustrates the comparison of inulinase production using various carbon sources at a concentration of 10 g/L. The results showed that the highest production of inulinase was observed in agave, which yielded 990 U/mL, followed by inulin, which yielded 760 U/mL. Agave is known to contain fructan polymers, which are composed of 3 to 29 fructose units, and previous research has shown that it can enhance the production of inulinase (Huitron *et al*., 2013). In addition, inulin was found to be a potent and selective substrate for inulinase, which may explain the high level of inulinase synthesis observed in this study (Singh *et al*., 2010).

3.3. Plackett-Burman design

The wide variation of inulinase activity measured in the Plackett-Burman experiment, ranging from (260.66 to 1330.33 U/mL) is shown in Table 3, which highlights the significance of modifying medium composition to achieve higher inulinase activity. The linear correlation model that describes the correlation between the nine factors (agave, K_2HPO_4, $NaNO_3$, KCL, $MgSO_4$, $FeSO_4$, $NH_4H_2PO_4$, Temp, and pH) and the inulinase activity as follows

Inulinase activity (U/ml) =950 + -41. 083*Agave + 50. 555*K2HPO4+ 132.33*NaNO3+-148.809*KCL+-12.962*FeSO4 +-8.24*NH4H2PO4+6.32*Temp+-27.2*pH

Variables having a confidence level above 95% ($P< 0.05$) were regarded as significant factors in the Plackett-Burman statistical analysis. Table 4 shows that the most impacting factors were agave, KCl, and NaNO3, with P values of 0.0027, 0.0398, and 0.0352, respectively. The concentrations of agave, KCl, and NaNO3 were determined to be the major significant factors studied on the synthesis of fungal inulinase based on the Plackett-Burman design results, and they were chosen for further optimization using the Box-Behnken design.

3.4. Box-Behnken design

Box-Behnken design matrix is shown in Table 5. The experimental inulinase activity and the predicted inulinase activity were compared after each factor was evaluated at three different levels (-1, 0, and 1).

The wide variation of inulinase activity measured in Box-Behnken experiment, ranging from (1140-2170 U/mL) highlights the significance of different levels of agave, and NaNO3 to achieve higher inulinase activity.

The following equation displays the Box-Behnken design's second-order polynomial model

Inulinase activity (U/ml) =1582.6667+ 390. 375*Agave + 15. 125*NaNO3+-49.25*KCL+71.25(Agave*NaNO3) +9 (Agave*KCL)-29.5 ($NaNO_3$*KCL) +-94.45833(Agave*Agave)

The optimal conditions producing 2170.22 U/ml were Agave, 10 g/L; NaNO3, 4 g/L; KCL, 0.3 g/L; K2HPO4, 1 g/L, temp, 30°C, and pH,6.5

Table 6 contains the calculated coefficients for the linear, quadratic, and interaction factors. Model and coefficients were deemed significant if $P <0.05$. agave and NaNO3 both have significant P values of. <.0001 and 0.0082, respectively. The sole significant

interaction term was agave *NaNO3. A significant quadratic term was agave * agave. Additionally, the model's P value was less than 0.0001, demonstrating the model's the model's accuracy in predicting inulinase activity.

3.5. Comparison of optimization of inulinase production by *Aspergillus niger* NRRL 3122 with other fungi

Aspergillus niger NRRL 3122 strain used in this study produced higher amounts of inulinase activity after medium optimization than most other organisms reported in literature (Table 7). *Aspergillus tamarii* AR-IN9 achieved 71.97 U/mL inulinase activity and with a productivity of 24 U/ml/day on 1% Dahlia tuber (Saber and El-Naggar 2009). Skowronek and Fiedurek 2004 optimized a medium and cultivation for *Aspergillus niger* 13/36, using simplex method, which re-sulted in a maximum activity of 80 U/ml and productivity of 20 U/ml/day. Abou-Taleb et al.,2019 used Taguchi orthogonal array (TOA) design to optimize production of inulinase from *Candida oleophila* cultivated on a medium with 1% chicory root and achieved an activity of 46.38 U/mL. More recently, Singh and Chauhan (2017) optimized production of inulinase from *Penicillium oxalicum* grown on 2% inulin and achieved an activity of 11.06 U/mL.

3.5. Purification of inulinase enzyme.

The inulinase enzyme was purified through precipitation in an 80% saturated NH4SO4 solution, followed by concentration via ultra-filtration. After purification, the enzyme activity reached 52130 U/ml, resulting in a 3.9-fold improvement in purity and a 60% inulinase yield (recovery) (Table 8). This purification method achieved a higher specific activity than most documented literature, measuring 78160 U/mg protein (Abu El-souod et al., 2014; Naidoo *et al*., 2015).

3.6. Characterization of partially purified inulinase enzyme

3.6.1. Molecular determination

The molecular weight of the inulinase enzyme was found to be approximately 50 kDa, as depicted in Figure 2. Previous studies have reported a wide range of molecular weights for inulinases, ranging from 30 to 175 kDa (Cho *et al*., 2002). In other studies, exoinulinase with a molecular weight of 66 kDa was obtained from *Aspergillus tamarii*-U4, while endo-inulinase with a molecular weight of 34 kDa was produced from *Aspergillus ficuum* JNSP5-06 using inulin as a carbon source. However, a study by Gill *et al*. (2004) reported a higher molecular weight of 176.5 kDa for inulinase.

3.6.2. Effect of temperature

The inulinase activity was found to be maximum at 50°C and decreased at higher temperatures (60, 70, 80, and 90°C), reaching 83%, 67%, 39%, and 21% of its peak activity, respectively (Figure 3). In comparison, exo-inulinase from *Aspergillus tamarii*-U4 had a maximum temperature of 60°C, and the exo-inulinase from Aspergillus niger CH-A-2010 showed the highest activity at 50°C (Huitron *et al*., 2013; Garuba et al., 2020). The inulinase enzyme remained stable at 40°C for 30 minutes, retaining 97% of its initial activity. However, at higher temperatures (50, 60, 70, 80, and 90°C), the enzyme activity decreased, retaining only 87%, 71%, 57%, 36%, and 17% of its initial activity, respectively (Figure 4). *Aspergillus tamarii* generated inulinase with a temperature stability of 75% at 50°C for 90 minutes, while the inulinase from *Aspergillus niger* retained only 21.8% of its activity at 60°C after 6 hours (Saber and El-Naggarm, 2009; Germec *et al*., 2022). Heat stability is associated with intermolecular linkages, and it is required in industrial applications, particularly for the commercial production of

fructooligosaccharides. Inulinases with an optimum temperature greater than 50°C are essential for industrial applications where high temperatures are required (Abdella *et al*., 2021).

3.6.3. Effect of pH

The optimal pH for the inulinase enzyme was found to be pH 6.0, with a decrease in activity observed at higher pH values. Specifically, the enzyme retained only 75%, 47%, and 18% of its maximum activity at pH 7.0, 8.0, and 9.0, respectively (Figure 5). This optimal pH range is consistent with previous studies (Torabizadeha *et al.*, 2018; Corrado *et al.*, 2021). Remarkably, the enzyme retained 100% activity after 24 hours of incubation at pH 4 (Figure 6), indicating that it is highly stable at acidic pH. In comparison to other inulinase-producing species, our strain showed greater pH stability in acidic conditions. For instance, the inulinase from *Aspergillus ficuum* JNSP5-06 was stable in the pH range of 5.0-8.0 (Chen *et al.*, 2009). The high stability of our inulinase enzyme at low pH values makes it an excellent candidate for industrial applications (Raveendran *et al.*, 2018).

3.6.4. Substrate specificity

The substrate specificity of the inulinase enzyme was studied, and the results showed that it was most effective against inulin, which contains -(21)-D-fructosyl-fructose linkages, with an activity of 51430 U/ml. The enzyme also showed high selectivity for sucrose, with an activity of 20570 U/ml. However, cellobiose was not hydrolyzed by the enzyme, and inulin was not found to undergo hydrolysis of the glycosidic bond. These findings are consistent with previous studies (Mensinka *et al*., 2015; Barclaya *et al*., 2012) (Table 9).

3.6.5. kinetic parameters of inulinase.

The Michaelis-Menten kinetic parameters of the inulinase enzyme were determined to be a Km value of 0.8 mg/mL and a Vmax value of 100,000 U/mg (Figure 7).. The Km value indicates the substrate concentration at which the enzyme reaches half of its maximum velocity, while the Vmax value represents the maximum velocity of the enzyme at saturating substrate concentration. The determination of these parameters is essential for understanding the enzyme's efficiency and for optimizing the reaction conditions in industrial applications. The results suggest that the inulinase enzyme has a high affinity for its substrate and a high catalytic activity.

4. Conclusion

In conclusion, while many microorganisms are capable of producing inulinase, there is still a need to develop an economical fermentation method that uses inexpensive feedstock for industrial use. Traditionally, the fermentation process is optimized by testing one variable at a time. However, by using Plackett-Burman experiments to identify key process factors and the Box-Behnken design to optimize their levels, *A. niger* has been able to produce more inulinase from agave in shake-flasks. This has resulted in an increase of 2.82-fold from 760 to 2170 U/ml. Moreover, this process can be further scaled up for industrial use by utilizing affordable agricultural waste such as agave as substrates.It is worth noting that the *A. niger* strain was able to produce a low molecular weight thermophilic inulinase enzyme that exhibited a high Vmax, significant affinity for inulin, and good stability at high temperatures and acidic pH. These characteristics make it a potentially valuable candidate for industrial applications, particularly in the production of fructooligosaccharides, where high temperatures are required. However, there is still a need for further research to optimize the

production process and improve the enzyme's stability and efficiency.

5. References

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Table 1. Variables and their levels employed in Plackett-Burman design for screening of Variables affecting inulinase production by *Aspergillus niger*.

Factor	Low level (-1)	High level (+1)
Agave	10 g/L	20 g/L
K_2HPO_4	0.5	2
$MgSO_4.7H_2O$	0.3	1
$NaNO_3$	0.5	3
KCI	0.3	1
$FeSO_4.7H_2O$	0.05	0.5
$(NH_4)\ H_2PO_4$	1	4
Temperature	25	35
pH	5.5	7.5

(−1) and (+1) are coded levels in Plackett-Burman design

Table 2. Screening of cultures for inulinase production.

Strain	Incubation Periods (day)	pH	Cell dry weight (g)	Protein (mg/ml)	Inulinase (IU/ml)
Aspergillus niger **NRRL 3122**	2	2.5	0.89	42	320
	4	3.41	1.11	63.1	500
	6	4.5	1.34	96.7	760
	8	5	1.32	95.1	740
Rhizopus oryzae **NRRL 3563**	2	5.5	0.93	8.7	180
	4	6.4	1.24	10.3	220
	6	7.5	1.31	10.5	250
	8	8.3	1.29	9.8	240

Table 3. Plackett-Burman experimental design

Pattern	Agave (g/L)	K_2HPO_4 (g/L)	$NaNO_3$ (g/L)	$MgSO_4$ (g/L)	KCL (g/L)	$FeSO_4$ (g/L)	$NH_4H_2PO_4$ (g/L)	Temp °C	pH	Inulinase (U/ml)
−+−−+−+++	20	2	0.5	0.3	1	0.05	1	25	7.5	280.33
+++++++++	10	2	0.5	1	0.3	0.5	1	35	7.5	660.66
−+++−−−+−	10	2	3	0.3	0.3	0.05	4	35	5.5	1330.33
−−−+−−+−+	20	0.5	3	1	0.3	0.05	4	25	7.5	400
+−+++−−−+	10	0.5	0.5	1	1	0.5	4	25	5.5	560.66
−+−+++−−−	20	2	0.5	0.3	0.3	0.5	4	25	5.5	260.66
−−+−+++−	10	0.5	3	0.3	0.3	0.5	1	25	7.5	1100
−−+−−+−++	20	0.5	3	0.3	1	0.5	1	35	5.5	560.66
+−−+−+++−	20	2	3	1	1	0.5	4	35	7.5	530.33
+−−−+−−+−	10	2	3	1	1	0.05	1	25	5.5	880.88
+++−−−+−−	20	0.5	0.5	1	0.3	0.05	1	35	5.5	300
++−−−+−−+	10	0.5	0.5	0.3	1	0.05	4	35	7.5	530.33

Table 4. Estimated effects, corresponding t ratios and p-values for the Plackett-Burman design experiment

Term	Estimate	Std Error	t Ratio	p-Value
Agave	-41.08	9.481	-4.33	0.0027*
$NaNO_3$	132.33	37.92	3.49	0.0398*
KCL	-148.8	135.44	-3.51	0.0352*
K2HPO4	50.55	63.20	0.80	0.4823
Temp	6.41	9.481	0.68	0.5471
pH	-30.41	47.406	-0.64	0.5668

Table 5. Box-Behnken factorial experimental design

Pattern	Agave (g/L)	NaNO3(g/L)	KCL	Experimental inulinase (U/ml)	Predicted inulinase (U/ml)
18440--	7.5	3	0.3	1720.32	1700.28
+0+	10	4	0.3	2040.05	2020
+0-	10	4	0.1	2140.6	2120.45
0	7.5	4	0.2	1800.26	1780.45
+-0	10	3	0.2	1980.75	1960.76
0+-	7.5	5	0.3	1690.72	1680.02
++0	10	5	0.2	**2170.22**	2150.04
-0+	5	4	0.1	1300.14	1280.83
0-+	7.5	3	0.1	1750.06	1730.3
0	7.5	4	0.2	1770.66	1750.88
0++	7.5	5	0.1	1850.61	1830.75
--0	5	3	0.2	1270.54	1260.29
0	7.5	4	0.2	1690.72	1690.72
-+0	5	5	0.2	1140.4	1120.86
-0-	5	4	0.3	1150.55	1530.99

Table 6 Parameter estimates and summary of fit for Box-Behnken design

Term	Estimate	Std Error	t Ratio	p-Value
Intercept	1582.6667	18.99898	83.30	<.0001*
Agave	390.375	11.63445	33.55	<.0001*
NaNO3	-49.25	11.63445	-4.23	0.0082*
KCL	15.125	11.63445	1.30	0.2503
Agave*NaNO3	71.25	16.4536	4.33	0.0075*
Agave*KCL	9	16.4536	0.55	0.6079
NaNO3*KCL	-29.5	16.4536	-1.79	0.1330
Agave*Agave	-94.45833	17.12545	-5.52	0.0027*

Table 7. Comparison of inulinase production from various substrates by *Aspergillus* and other fungi

Microorganism	Substrate	Inulinase (U/ml)	Productivity (U/ml/day)	References
Aspergillus niger NRRL 3112	1% agave	2170	361	**This study**
Aspergillus tamarii AR-IN9	1% Dahlia tuber	71.97	24	Saber and El-Naggar 2009
Aspergillus niger 13/36,	1% sucrose	80	20	Skowronek and Fiedurek 2004
Candida oleophila	1% chicory root	46.38	11.59	Abou-Taleb et al.,2019
Penicillium oxalicum	2 % inulin	11.06	2.5	Singh & Chauhan ,2017

Table 8. Purification profile of Inulinase enzyme through three different methods

	Volumetric activity U/ml	Total activity (U)	Protein conc (mg/ml)	Total protein (mg)	Specific activity (U/mg)	Purification folds	Recovery %
Crude enzyme	2170	433330	0.101	20.2	21480	1	100
80% Ammonium sulphate/dialysis	17370	347550	0.35	7	49640	2.3	80
Ultra-filtration	52130	260650	0.667	3.335	78160	3.63	60

Table 9. Substrate utilization

Substrate	Relative activity (%)
Inulin	**100**
Sucrose	**40**
Cellobiose	**0**
Soluble starch	**0**

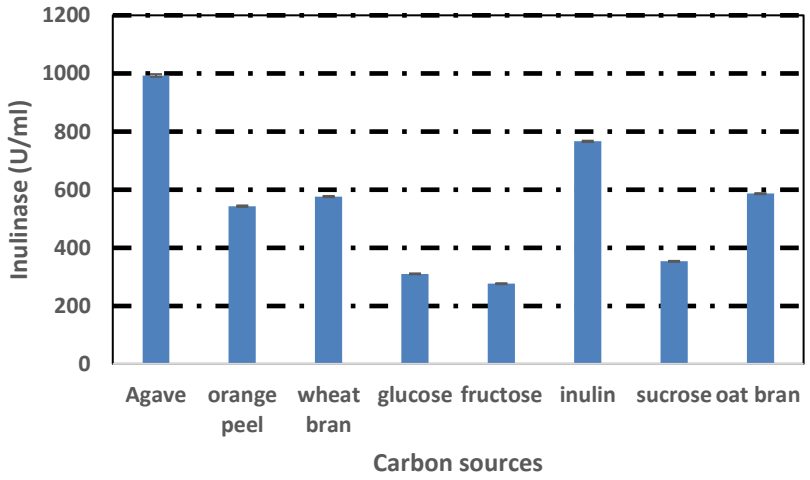

Figure 1. Production of inulinase enzyme on various carbon sources

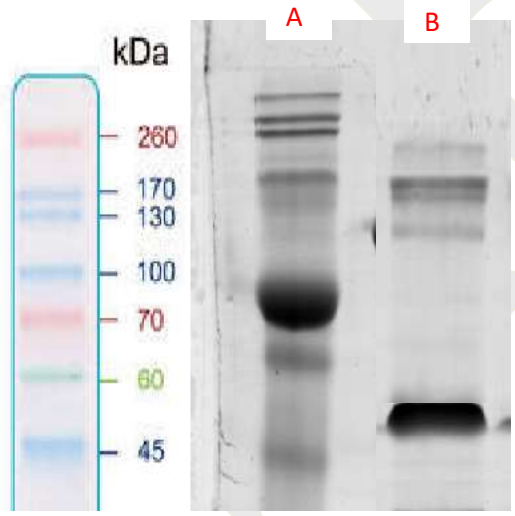

Figure 2. Cropped SDS-PAGE of inuliase enzyme
Lane A: Standard protein marker
Lane B: SDS-PAGE of the partially purified inulinase enzyme.

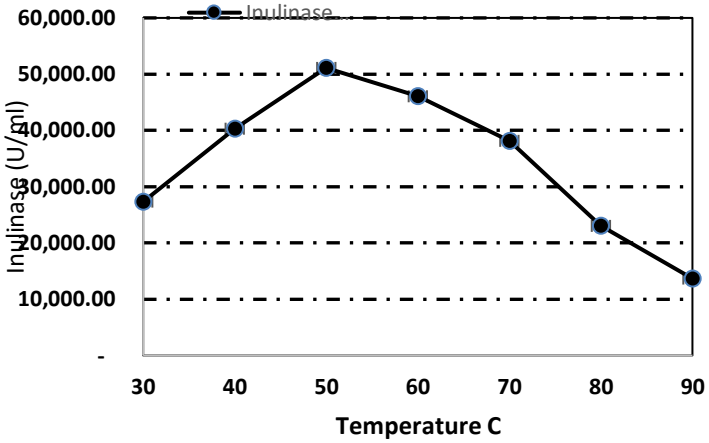

Figure 3. The optimal temperature range for the purified inulinase enzyme

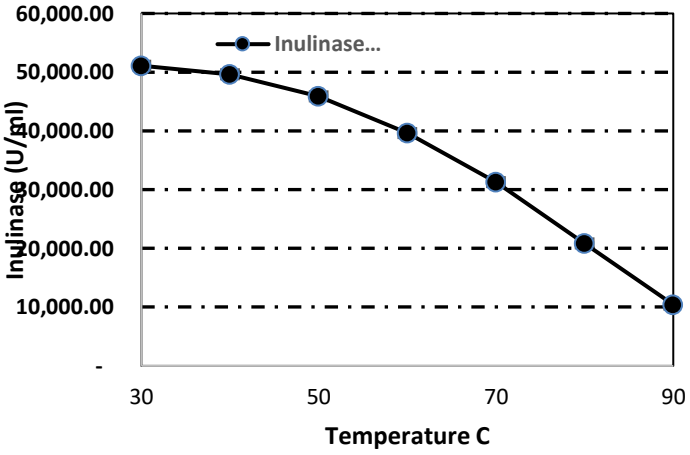

Figure 4. Thermostability of the purified inulinase

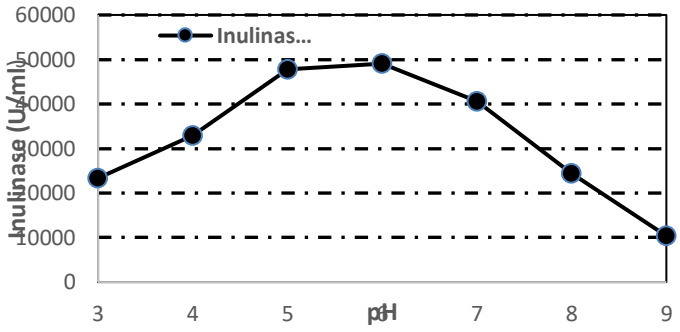

Figure 5. pH optima of the purified inulinase

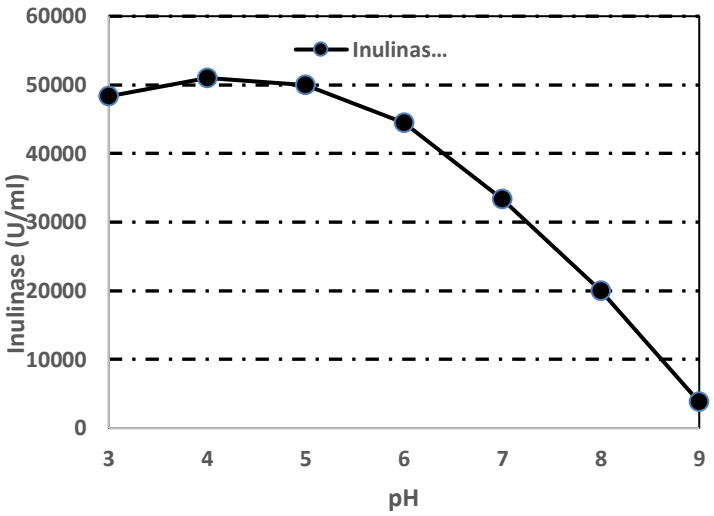

Figure 6. pH stability of the purified inulinase

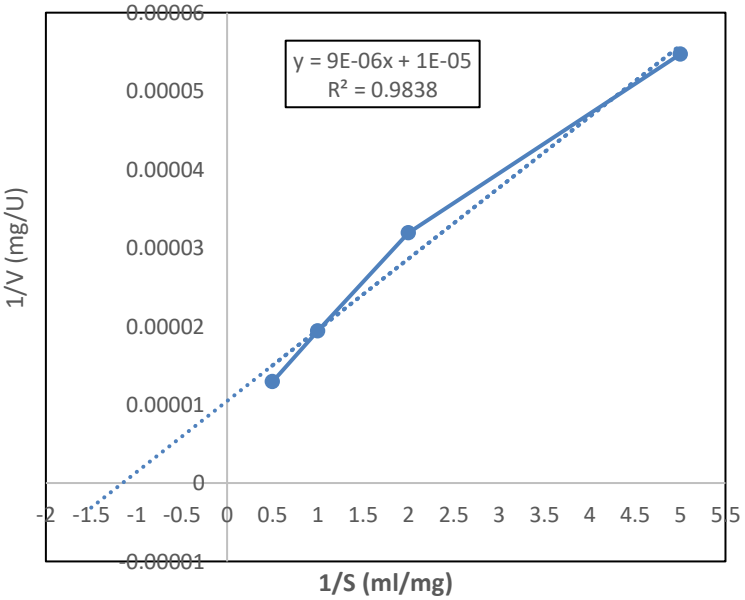

Figure 7. Lineweaver–Burk plot of inulinase