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Effects of certain factors on radish amylase activity

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Abstract
Radish root is a rich source of amylase. In the present investigation detection of amylase activity, progression of degradation of starch by amylase, and certain factors which affect radish amylase activity have been studied. The factors that have been studied are the effects of enzyme concentration, pH and temperature. The iodine reagent test and the dinitrosalicylic acid (DNS) reagent method have been used. Using the iodine reagent test the progression of degradation of starch was studied colorimetrically. The increase in the amount of sugars as a result of amylase action on starch has been shown colorimetrically using the DNS reagent method. The results show that young radish roots are richer in amylase than full-grown roots. As the enzyme concentration was increased the time taken to reach the end point decreased, and the amount of sugars formed increased up to a certain concentration. The optimal pH for radish amylase was 6.2. Interestingly, the amylase was stable up to 90ºC. In fact, both the iodine reagent test and the DNS reagent method showed that the enzyme activity was far more at 50º to 90ºC as compared to 31ºC. When the effect of temperature on amylase activity was studied by the DNS reagent method it was observed that the enzyme activity increased as the temperature was increased from 33ºC (room temperature) to 50ºC; surprisingly the amylase activity increased slightly at 60ºC when compared to the activity at 50ºC, and at 90ºC when compared to the activity at 60ºC. The results show that radish amylase is thermostable.

Keywords: radish root, amylase, iodine reagent, dinitrosalicylic acid (DNS) reagent

Introduction
Amylases are hydrolytic enzymes found in bacteria, fungi, plants and animals. The fungal and bacterial amylases are very valuable commercially and find several industrial applications (John 2017, Ahmad *et al* 2019) [9, 1]. Certain species of *Bacillus* are known to produce thermostable α-amylase (John 2017) [9]. However, there has been a search for acid-stable and thermostable α-amylases from extremophilic microbes (Satyanarayana 2016) [14]. In higher plants three families of α-amylase genes have been recognized, and each family of α-amylase is predicted to be directed to a cellular compartment, namely the extracellular compartment, the cytosol and the plastids (Stanley *et al* 2005) [16]. Screening of agro-industrial wastes for amylase-producing bacteria and fungi has been fruitful (Okunwaye *et al* 2021, Pandeeswari & Kiruthiga 2022) [11, 12]. Radish root has been shown to have amylolytic activity (Hara *et al* 2009, Wang *et al* 2000) [5, 20], and specifically shown to contain α-amylase (Cho *et al* 2009) [4] and β-amylase **(**Jahan *et al* 2009, 2011, 2014; Takahashi *et al* 2012) [7, 6, 8, 18].
Alpha-amylase is an endoamylase acting on starch at random on α(1→4) glycosidic bonds to release limit dextrins (intermediate oligosaccharides), and some maltose and glucose. Beta-amylase is an exoamylase acting on α(1→4) glycosidic bonds from the non-reducing end of starch removing one maltose at a time and yielding maltose and dextrins (Buchanan *et al* 2000) [3]. The alpha-amylase molecule is a calcium metalloenzyme requiring at least one calcium ion for stability; salivary and pancreatic amylases are alpha-amylases (Tiwari *et al* 2015) [19]. Salivary amylase initiates the digestion of starch in the mouth (Sella *et al* 2018) [15]. Radish roots, ripe banana fruits, and sprouted mungbeans and wheat grains are rich in amylase and aid in the digestion of starch in the food consumed (Subramanyan & Chaudhary 2017) [17].
Amylase activity is detected by the iodine reagent test (Bajracharya 1999) [2] and the dinitrosalicylic acid (DNS) reagent method (Sadasivam & Manickam 1992) [13]. The reaction between starch and iodine reagent gives the characteristic blue-black colour. The intensity of the blue-black colour determines the quantity of starch. As the starch degradation by amylase progresses, the blue-black colour fades and finally disappears which is the end point. Dinitrosalicylic acid (DNS) is an aromatic compound that reacts with reducing sugars and other reducing molecules to form 3-amino-5-nitrosalicyclic acid which absorbs light strongly at 540 nm. The amount of 3-amino-5-nitrosalicylic acid formed and, therefore, the intensity of colour developed would depend on the amount of available reducing sugars. Using a colorimeter the starch-iodine complex formed by the iodine reagent test, and the reducing sugars formed as a result of amylase activity can be quantified by the DNS reagent method.

In the present study radish roots have been used to detect amylase activity, to study the progression of degradation of starch because of amylase activity, and to study the effects of certain factors, namely enzyme concentration, pH, and temperature, on amylase activity. In all experiments after transferring the radish cylinders to calcium chloride solution, the test tubes were shaken and incubated for 15 minutes. All experiments were replicated at least three times and for each replicate one radish was used to obtain all the cylinders.

Materials and Methods

Detection of Amylase Activity by Iodine Reagent Test

Fresh roots of radish (*Raphanus sativus* Linn.) were bored with the help of a cork-borer and 1-cm-long cylinders were prepared and placed in a Petri dish lined with moist filter paper. Five mL of calcium chloride (10 mM) solution was pipetted into test tubes (Table 1). Four radish cylinders were added to the solution with the help of a pair of forceps and the test tubes were shaken and maintained for 15 minutes. Calcium chloride was added to activate amylase. Then 5 mL of starch (0.1%) was added into the test tubes followed by 4 drops of iodine reagent. The test tubes were shaken. Control tubes were maintained. The time taken to reach the end point, i.e., when the blue-black colour disappeared completely, was recorded (Table 1). The experiment was repeated four times and the average time to reach the end point was calculated for all the tubes.

Progression of Degradation of Starch

Ten test tubes were taken and set up for the experiment as given in Table 2. The absorbance of the reaction mixture was recorded at 620 nm keeping a time difference of 2 min in the incubation time between the tubes; the absorbance was recorded immediately after adding iodine reagent and shaking (0 min) in the first tube, 2 min from adding iodine reagent in the second tube, 4 min from adding iodine reagent in the third tube, and so on. The incubation time was continued till the blue-black colour faded completely and the absorbance of the reaction mixture became zero. A blank containing 5 mL calcium chloride, 3 radish cylinders, and 5 mL starch was used to set for zero absorbance at 620 nm (Table 2). The experiment was repeated three times and the average absorbance was calculated for each time interval.

Effect of Enzyme Concentration

The effect of enzyme concentration was studied using the iodine reagent as well as the DNS reagent. For each five test tubes were set up as given in Tables 3 and 4. The enzyme concentration was varied by varying the number of radish cylinders in the test tubes from zero to four. The first tube did not have any cylinder. The time taken to reach the end point, i.e., when the blue-black colour of starch-iodine complex disappeared completely, was recorded when the iodine reagent test was conducted. The experiment was repeated three times and the average time to reach the end point was calculated for all the tubes (Table 3).

When the DNS reagent was used after adding 5 mL of the substrate starch (0.1%) to all the tubes, the test tubes were shaken and incubated for 15 min. The contents of each test tube was decanted into a clean and dry labelled test tube. One mL of NaOH (2 M) was added into all the tubes and shaken to terminate the reaction between starch and amylase. Then 1 mL of DNS reagent was added to all the tubes, and the tubes were kept in a boiling water bath for heating for 5 min. The test tubes were shaken and the absorbance of the reaction mixtures were recorded at 540 nm. The blank was 5 mL calcium chloride, 5 mL starch, 1 mL NaOH and 1 mL DNS reagent, and the tube was heated in a boiling water bath for 5 min, cooled and used. The experiment was repeated thrice. The average absorbance was calculated for all the tubes (Table 4).

Effect of pH

The effect of pH was studied using the DNS reagent. The experiment was set as given in Table 5. Starch (0.1%) prepared in buffers of the desired pH was added into the labelled test tubes. The pH of the mixture in each test tube was recorded using broad and narrow range pH papers. The tubes were incubated for 15 min. The mixture in each test tube was transferred to a clean and dry tube, 1 mL of NaOH (2 M) was added into all the tubes and shaken to terminate the reaction between starch and amylase. Then 1 mL of DNS reagent was added to all tubes, and the tubes were kept in a boiling water bath for heating for 5 min. The test tubes were cooled, shaken and the absorbance of the reaction mixtures were recorded at 540 nm. The blank solution was 5 mL calcium chloride, three radish cylinders, 5 mL distilled water, 1 mL NaOH and 1 mL DNS reagent which had been heated for 5 min in a boiling water bath for 5 min and cooled. The experiment was repeated thrice and the average absorbance was calculated for all the pH treatments (Table 5).

Effect of Temperature

The effect of temperature was studied on the stability of amylase and on the activity of amylase. For studying the effect of temperature on the stability of amylase the iodine reagent test as well as the DNS reagent method were used in separate experiments. The test tubes were set up as given in Tables 6 and 7.

For the iodine reagent test three radish cylinders were transferred to each test tube containing calcium chloride (5 mL) and the test tubes were brought to the desired temperature and maintained at that temperature for 15 min. Then the test tubes were allowed to come to room temperature. Starch solution (5 mL) was added into each tube followed by 4 drops of iodine. The time taken to reach the end point was recorded. The experiment was repeated thrice and the average time taken to reach the end point was calculated for all temperature treatments (Table 6).

When the DNS reagent method was used, after incubating the test tubes at the desired temperature for 15 min and allowing the tubes to come to room temperature starch solution (5 mL) was added into each tube. All the tubes were maintained at room temperature for 15 min. Then the contents in each test tube without the radish cylinders were decanted into separate clean and dry labelled test tubes. The reaction between amylase and starch was terminated by adding 1 mL NaOH. Then 1 mL of DNS reagent was added to all the tubes, and the tubes were kept in a boiling water bath for heating for 5 min. The test tubes were shaken and the absorbance of the reaction mixtures were recorded at 540 nm. The blank was 5 mL calcium chloride, 5 mL starch, 1 mL NaOH and 1 mL DNS reagent, and the tube was heated in a boiling water bath for 5 min, cooled and used. The experiment was repeated thrice and the average absorbance was calculated for all the tubes (Table 7).

The effect of temperature on amylase activity was studied using the DNS reagent. Two sets of test tubes each with six tubes labelled as 5º, 20º, room temperature, 50º, 60º and 90ºC were readied. In the test tube labelled 5ºC in the first set calcium chloride (5 mL) was pipetted, and in the test tube labelled 5ºC in the second set starch solution (5 mL) was pipetted. Both the test tubes were brought to 5ºC and maintained at 5ºC. Three radish cylinders were transferred into the tube containing calcium chloride and the starch solution at 5ºC was decanted completely into the tube containing calcium chloride. The test tube was maintained at 5ºC for 15 min. After the 15 min reaction time between amylase in the radish cylinders and starch at the desired temperature, the reaction mixture was transferred to a clean and dry test tube. The reaction was terminated by adding NaOH (1 mL) followed by DNS reagent (1 mL). The remaining steps are identical to the previous experiment. Similar procedure was followed for the remaining five temperatures. The experiment was repeated thrice and the average absorbance at 540 nm was calculated for all the temperature treatments (Table 8).

Results and Discussion

Detection of Amylase Activity by Iodine Reagent Test

The blue-black colour of starch-iodine complex faded in test tube 1 which showed that amylase was present in the radish root cylinders (Table 1). Replicates 1 and 2 took very little time for reaching the end point compared to replicates 3 and 4. This shows that young radishes have more amylase than full-grown radishes. Test tube 2 is a control without amylase (radish cylinders) and, therefore, the starch was not degraded and the blue-black colour of starch- iodine complex persists. Similarly, test tube 3 is a control without starch and here the yellow colour of iodine reagent is observed. This yellow colour fades gradually. Test tube 5 has neither enzyme nor starch; hence the yellow colour of iodine reagent is observed. In test tube 4, the blue-black colour appeared immediately on adding starch and then faded instantaneously. This probably happens because boiling made the enzyme very active. The time taken to reach the end point is much more in tube 6 compared to tube 1. This clearly shows the important role of calcium chloride in the incubation medium. Absence of calcium ions in test tube 6 decreased the activity of amylase. It is known that calcium is an activator for alpha-amylase and chloride ions enhance the enzyme activity.

Table 1: Detection of amylase activity in radish

Test tube No.	$CaCl_2$ (mL)	Radish cylinders	0.1% starch (mL)	Distilled water (mL)	Iodine reagent (drops)	Time taken to reach end point Replicate No.*				
						1	2	3	4	Average
1	5	4	5	--	4	1 min 22 sec	40 sec	16 min	18 min	9.01 min
2	5	--	5	--	4	Blue-black colour persists				
3	5	4	--	5	4	Yellow colour appears and then fades after some time				
4	5	4 (boiled and cooled)	5	--	4	Blue-black colour appears and then fades instantaneously				
5	5	--	--	5	4	Yellow colour persists				
6	--	4	5	5	4	8 min	4 min	30 min	35 min	19.25 min

*For replicates 1 and 2 young (small) radishes were used whereas for replicates 3 and 4 full-grown (large) radishes were used.

Progression of Degradation of Starch

As the time period of incubation increased, the general trend was that the absorbance decreased (Table 2, Fig 1). This was because the blue-black color of the starch-iodine complex became less intense (lighter) due to the conversion of starch into dextrins and reducing sugar. And when all the starch was converted into dextrins and reducing sugar, the blue-black colour faded completely. As the period of incubation of the enzyme (radish cylinders) and the substrate starch increased, the breakdown of starch by amylase progressed and 18 min from incubation complete degradation of starch occurred. At this stage no more of starch was available for complexing with iodine, and the absorbance was zero.

Table 2: Progression of degradation of starch by amylase studied colorimetrically by the iodine reagent test

Test tube No.	$CaCl_2$ (mL)	Radish cylinders	0.1% starch (mL)	Iodine reagent (drops)	Time given (min)	Absorbance (620 nm) Replicate No.			
						1	2	3	Average
1	5	3	5	4	0	0.42	0.32	0.64	0.46
2	5	3	5	4	2	0.33	0.22	0.13	0.23
3	5	3	5	4	4	0.25	0.26	0.05	0.19
4	5	3	5	4	6	0.21	0.31	0.04	0.19
5	5	3	5	4	8	0.17	0.27	0.00	0.15
6	5	3	5	4	10	0.25	0.30	0.00	0.18
7	5	3	5	4	12	0.05	0.08	0.00	0.04
8	5	3	5	4	14	0.03	0.03	0.00	0.02
9	5	3	5	4	16	0.01	0.05	0.00	0.02
10	5	3	5	4	18	0.00	0.00	0.00	0.00

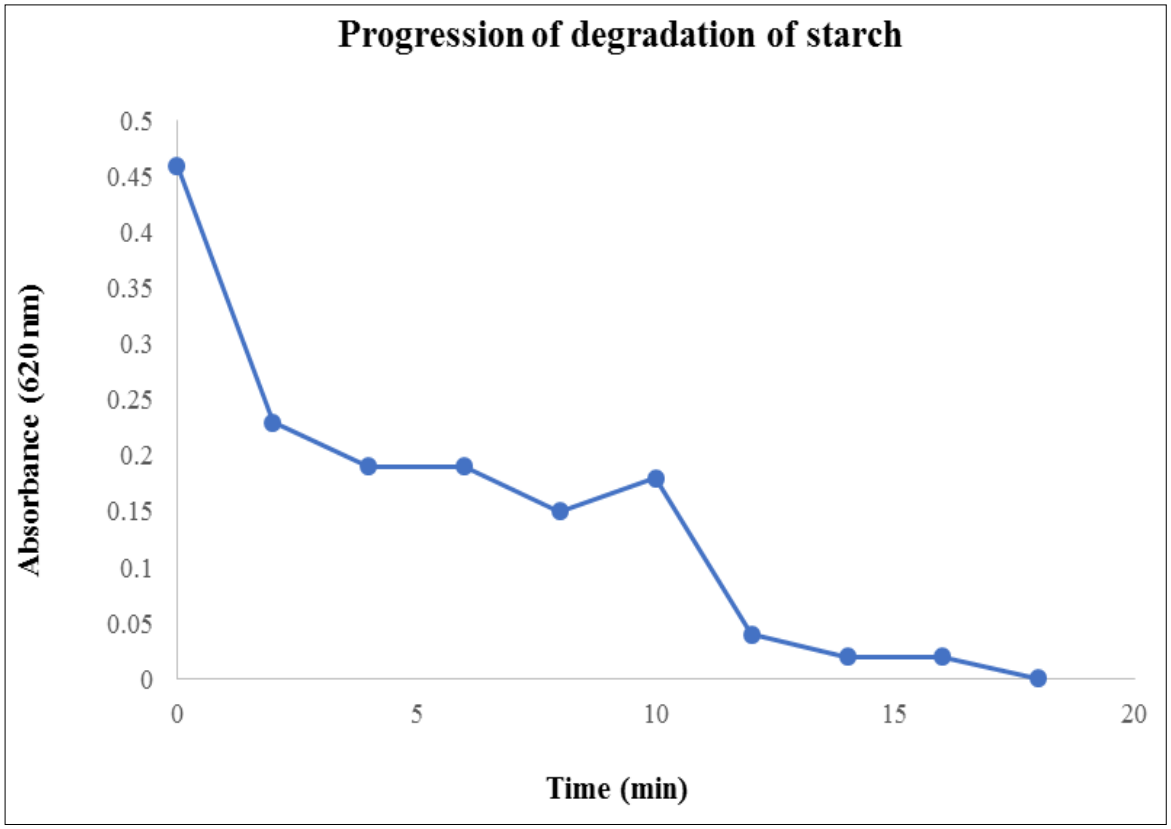

Fig 1: Progression of degradation of starch studied colorimetrically by the iodine reagent test

Effect of Enzyme Concentration

As the enzyme concentration increased from 1 to 4 cylinders, the time taken to reach the end point decreased when the iodine reagent test was performed (Table 3, Fig 2). The stage when the substrate become limiting was not observed. When the DNS reagent method was used, the amount of reducing sugar formed increased as the radish cylinders increased from 1 to 3, as reflected in the increase in the absorbance (Table 4, Fig 3). However, when 4 cylinders were used, the absorbance decreased, probably because the dextrins formed were not yet broken down to reducing sugars.

Table 3: Effect of enzyme concentration studied by the iodine reagent test

S. No.	$CaCl_2$ (mL)	Radish cylinders	0.1% starch (mL)	Iodine reagent (drops)	Time taken to reach end point Replicate No.			
					1	2	3	Average
1	5	0	5	4	Blue-black colour persists			
2	5	1	5	4	13 min	87 min	70 min	56.6 min
3	5	2	5	4	8 min	18 min	15 min	13.6 min
4	5	3	5	4	2 min	12 min	9 min	7.6 min
5	5	4	5	4	1 min 5 sec	5 min	5 min	3.6 min

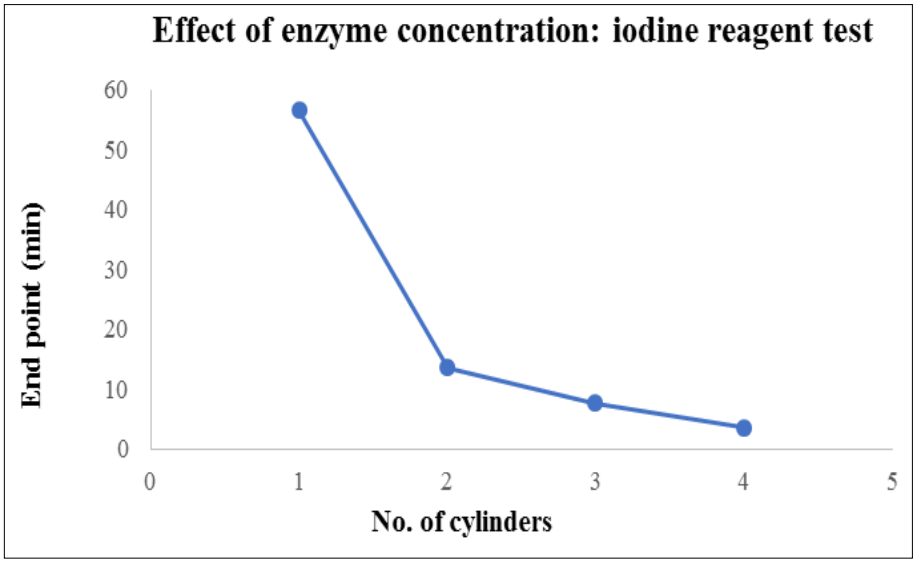

Fig 2: Effect of enzyme concentration studied by the iodine reagent test

Table 4: Effect of enzyme concentration studied by the DNS reagent method

S. No.	$CaCl_2$ (mL)	Radish cylinders	0.1% starch (mL)	NaOH (mL)	DNS (mL)	Absorbance (540 nm) Replicate No.			
						1	2	3	Average
1	5	0	5	1	1	0	0	0	0
2	5	1	5	1	1	0.12	0.02	0.08	0.07
3	5	2	5	1	1	0.26	0.08	0.16	0.17
4	5	3	5	1	1	0.57	0.72	0.62	0.64
5	5	4	5	1	1	0.39	0.48	0.40	0.42

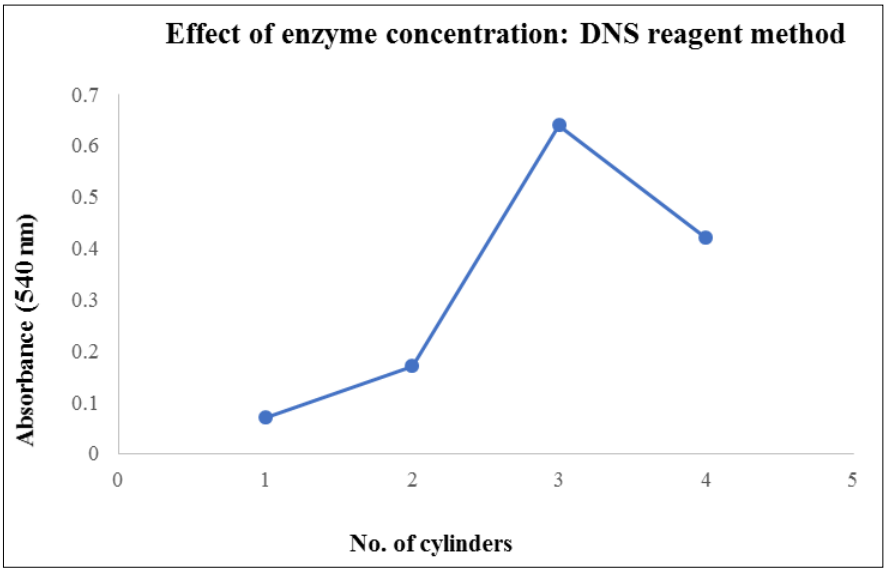

Fig 3: Effect of enzyme concentration studied by the DNS reagent method

Effect of pH

Amylase in radish shows activity in the wide range of pH tested (5.8 to 8.0). However, radish amylase is sensitive to pH (Table 5, Fig 4) and shows best activity at pH 6.2. Maximum absorbance was obtained in pH 6.2 which showed that the amount of reducing sugars formed in the reaction mixture was maximum when the pH was 6.2. The absorbance decreases as the pH is increased from 7.2 to 8.0. Therefore, radish amylase has an optimum pH of 6.2. The results are in agreement with the work of Jahan *et al* 2009 [7] who reported that purified β-amylase from radish root was stable in a pH range of 5.5 to 6.5 and the optimum pH for maximum starch hydrolysis was at pH 6.0, and of Cho *et al.*, 2009 [4] who reported that α-amylase activity in radish was stably maintained in a wide range of pH from 4.0 to 7.0. Radish seed α-amylase showed maximum activity in a pH range of 6 to 7, an optimum pH of 6.5, and total loss of activity at pH below 4.5 (Khare & Prakash 2019) [10].

Table 5: Effect of pH studied by the DNS reagent method

S. No.	$CaCl_2$ (mL)	Radish cylinders	pH of buffer used	0.1% starch in buffer (mL)	pH of mixture Broad range	pH of mixture Narrow range	NaOH (mL)	DNS (mL)	Absorbance (540 nm) Replicate No.			
									1	2	3	Average
1	5	3	5.8	5	6.0	5.5-6.0	1	1	0.17	0.20	0.19	0.19
2	5	3	6.2	5	6.0	6.0-6.5	1	1	0.22	0.28	0.22	0.24
3	5	3	7.2	5	7.0	7.0-7.5	1	1	0.15	0.19	0.18	0.17
4	5	3	7.8	5	7.8	7.5-8.0	1	1	0.10	0.09	0.16	0.12
5	5	3	8.0	5	8.0-9.0	8.0-8.5	1	1	0.09	0.08	0.11	0.09

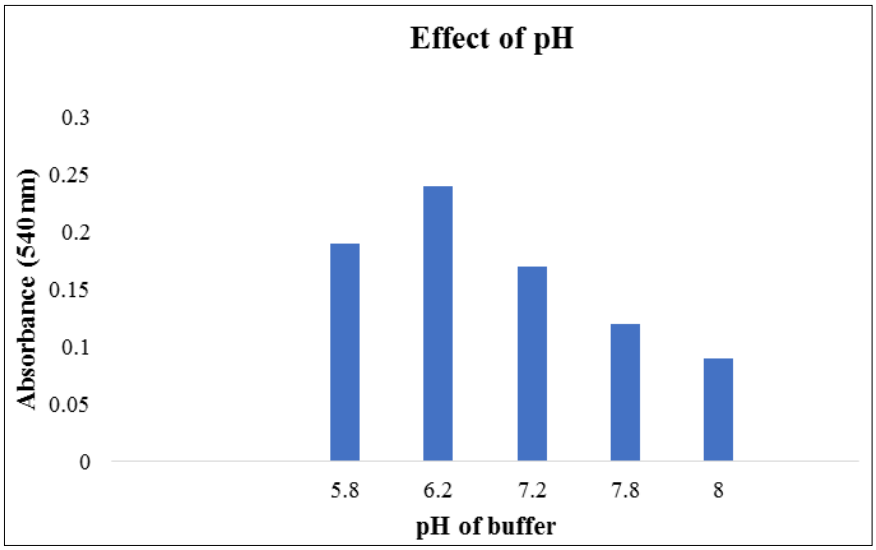

Fig 4: Effect of pH studied by the DNS reagent method

Effect of Temperature

The results of both the iodine reagent test and the DNS reagent method show that radish amylase shows activity in a wide range of temperature, i.e., 5º to 90º C, meaning thereby that the enzyme retains its stability in the temperature range studied. The enzyme is neither totally inactivated at 5ºC nor denatured at the high temperatures studied. In fact, when the radish cylinders had been given a treatment at 50ºC and then cooled to room temperature, the amylase activity was increased tremendously; it took only 25.3 sec to reach the end point when the iodine reagent test was conducted (Table 6, Fig 5). The DNS reagent method also shows a very high absorbance in the 50ºC treatment which means that a large amount of reducing sugar had been formed which had reacted with DNS reagent giving rise to the intense reddish brown complex (Table 7, Fig 6). These results clearly show that the 50ºC temperature treatment did not denature amylase. It is likely that the high temperature treatments probably changed the conformation of amylase making the enzyme more active.

Surprisingly, amylase activity increased further when the radish cylinders had been incubated at 60º and 90ºC for 15 min, cooled and then used to study amylase activity. The blue-black colour of starch-iodine complex was observed immediately on adding iodine. But the colour faded instantaneously (Table 6). The DNS reagent method results also showed an increase in the average absorbance in treatments at 60º and 90ºC compared to the absorbance at 50ºC (Table 7, Fig 6). The results clearly show that radish amylase is thermostable and its activity is not only retained but also increased when the radish cylinders are incubated at 50º, 60º, and 90ºC and cooled, and then used to study enzyme activity. Radish root α-amylase has been shown to be stably maintained at 25º-40ºC (Cho *et al* 2009) [4] and radish β-amylase has been reported to be stable in the temperature range 0º to 45ºC and showed maximum activity at 45º C (Jahan *et al* 2009) [7]. Purified α-amylase from radish seed showed high stability at a temperature of 60ºC, and even at 100ºC the enzyme retained 68% of its activity (Khare & Prakash 2019) [10].

Table 6: Effect of temperature on the stability of amylase studied by the iodine reagent test

S. No.	$CaCl_2$ (mL)	Radish cylinders	Temperature (ºC)	0.1% starch (mL)	Iodine reagent (drops)	Time taken to reach end point Replicate No.			
						1	2	3	Average
1	5	3	5	5	4	32 min	35 min	37 min	34.6 min
2	5	3	20	5	4	31 min	32 min	34 min	32.3 min
3	5	3	31 (room temperature)	5	4	30 min	29 min	26 min	28.3 min

4	5	3	50	5	4	30 sec	20 sec	26 sec	25.3 sec (0.42 min)
5	5	3	60	5	4	Blue-black colour appears, and then fades instantaneously.			
6	5	3	90	5	4	Blue-black colour appears, and then fades instantaneously.			

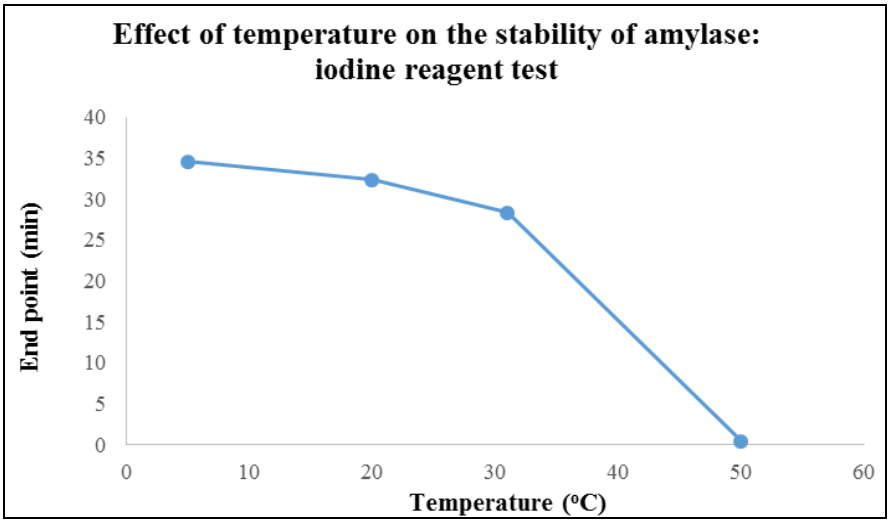

Fig 5: Effect of temperature on the stability of amylase studied by the iodine reagent test

Table 7: Effect of temperature on the stability of amylase studied by the DNS reagent method

S. No.	Temperature (°C)	$CaCl_2$ (mL)	Radish cylinders	0.1% starch (mL)	NaOH (mL)	DNS (mL)	Absorbance (540 nm) Replicate No.			
							1	2	3	Average
1	5	5	3	5	1	1	0.46	0.48	0.42	0.45
2	20	5	3	5	1	1	0.41	0.42	0.41	0.41
3	31 (room temperature)	5	3	5	1	1	0.42	0.41	0.51	0.45
4	50	5	3	5	1	1	0.84	1.03	1.20	1.02
5	60	5	3	5	1	1	1.10	1.07	1.16	1.11
6	90	5	3	5	1	1	0.99	1.00	1.24	1.08

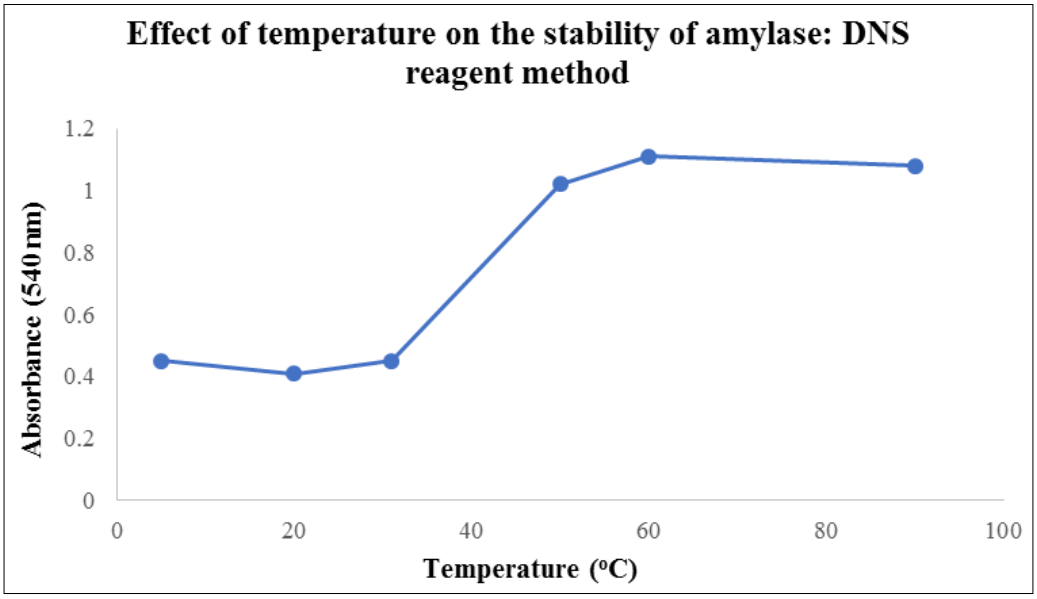

Fig 6: Effect of temperature on the stability of amylase studied by the DNS reagent method

Amylase activity was observed when the enzyme and substrate starch were allowed to react in a temperature ranging from 5° to 90°C (Table 8, Fig 7). The amount of reducing sugar formed increased as the temperature at which the enzyme + substrate reaction took place was increased from 5° to 90°C as is reflected in the absorbance values. The absorbance recorded when the reaction occurred at 20°C was almost double of that observed at 5°C.

A similar observation was recorded at 33ºC when compared to 20ºC. The absorbance increased considerably when the enzymatic reaction occurred at 50ºC, which increased slightly further at 60ºC. It is interesting that amylase activity increased even beyond 60ºC, and the activity increased further at 90ºC. The observations clearly show that radish amylase is thermostable.

Table 8: Effect of temperature on amylase activity studied by the DNS reagent method

S. No.	Temperature (ºC)	$CaCl_2$ (mL)	Radish cylinders	0.1% starch (mL)	NaOH (mL)	DNS (mL)	Absorbance (540 nm) Replicate No.			
							1	2	3	Average
1	5	5	3	5	1	1	0.10	0.12	0.12	0.11
2	20	5	3	5	1	1	0.21	0.21	0.21	0.21
3	33 (room temperature)	5	3	5	1	1	0.36	0.38	0.39	0.38
4	50	5	3	5	1	1	0.92	0.95	0.88	0.92
5	60	5	3	5	1	1	0.97	0.99	0.90	0.95
6	90	5	3	5	1	1	0.99	1.04	1.00	1.01

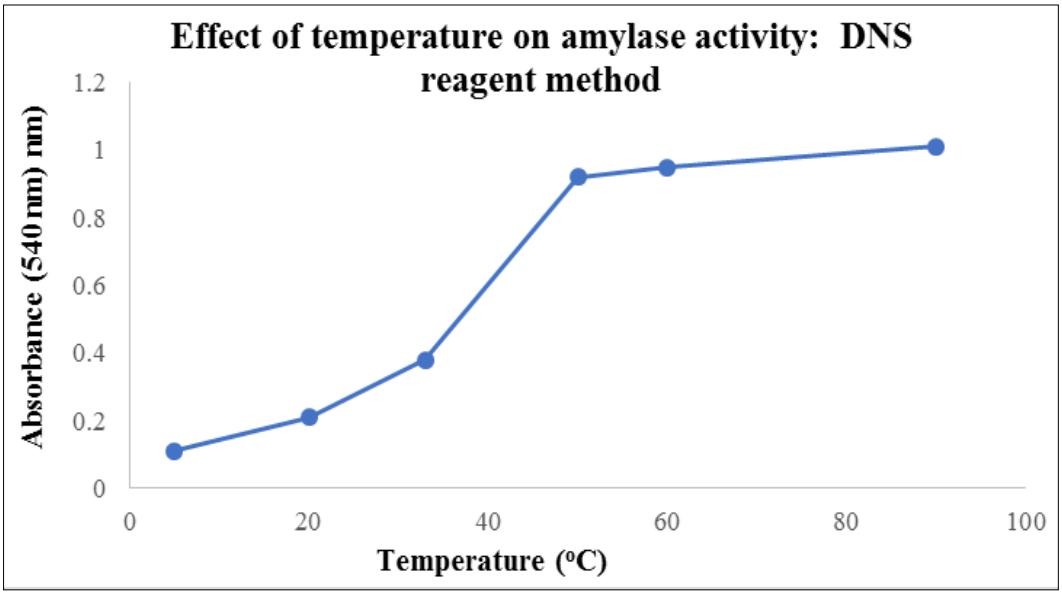

Fig 7: Effect of temperature on amylase activity studied by the DNS reagent method

Conclusions

The radish root is a rich source of amylase; the young roots are richer in amylase than full-grown roots. The amylase activity is best at pH 6.2. The enzyme is stable and retains its activity when already treated at high temperatures before adding the substrate; the activity is more at 50º to 90ºC when compared to the room temperature 31ºC. Amylase activity increased as the temperature was increased from 33ºC (room temperature) to 50ºC; the activity increased slightly at 60ºC when compared to the activity at 50ºC, and at 90ºC when compared to the activity at 60ºC. The results show that radish amylase is thermostable and has potential for use in the food industry.

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References

1. Ahmad MA, Isah U, Raubilu IA, Muhammad SI, Ibrahim D. An overview of the enzyme: amylase and its industrial potentials. Bayero Journal of Pure and Applied Sciences,2019:12(1):352-358.
2. Bajracharya D. Experiments in Plant Physiology, New Delhi. Narosa Publishing House, 1999.
3. Buchanan BB, Gruissem W, Jones RL. Biochemistry & Molecular Biology of Plants, New Delhi. IK International Pvt. Ltd, 2000, 850-852.
4. Cho E, Choi A-R, Choi SJ, Kim S, Lee GS, Lee SS, *et al*. α-Amylase activity of radish and stability in processing. Journal of Korean Society of Food Science and Nutrition,2009:38(6):812-815.

5. Hara M, Ito F, Asai T, Kuboi T. Variation in amylase activities in radish (*Raphanus sativus*) cultivars. Plant Foods for Human Nutrition,2009:64:188-192.
6. Jahan MGS, Pervin MS, Belal Uddin M, Sana NK, Rahman MH. Screening of some enzymes and nutrients in radish (*Raphanus sativus* L.) root. Biochemistry: An Indian Journal,2011:5(5):296-300.
7. Jahan MGS, Pervin MS, Habib MR, Nikkon FF, Rahman MH. Purification and characterization of β-amylase from radish *(Raphanus sativus* L.) root. Journal of Applied Sciences Research,2009:5(12):2225-2233.
8. Jahan MGS, Pervin MS, Rana AM, Shovon MS, Sharma SD, Karim MR, *et al*. Correlation between β-amylase activity and starch content in different cultivars of radish *(Raphanus sativus* L.). BioTechnology: An Indian Journal,2014:9:298-302.
9. John J. Amylases--bioprocess and potential applications: a review. International Journal of Bioinformatics and Biological Science,2017:5(2):41-50.
10. Khare S, Prakash O. Purification and biochemical characterization of α-amylase from radish (*Raphanus sativus* L.) seeds using response surface methodology. Proceedings of the National Academy of Sciences, India, Section B, Biological Sciences,2019:89:79-88.
11. Okunwaye T, Uadia PO, Okogbenin BO, Okogbenin EA, Onyia DC, Obibuzor JU. Amylase-producing fungi and bacteria associated with some food processing wastes. Nigerian Journal of Biotechnology,2021:38(1):74-82.
12. Pandeeswari N, Kiruthiga N. Studies on the optimization of cultural conditions for the production of amylase by Bacillus sp. International Journal of Botany Studies,2022:7(2):185-189.
13. Sadasivam S, Manickam A. Biochemical Methods for Agricultural Sciences, Wiley Eastern Limited, New Delhi, & Tamil Nadu Agricultural University, Coimbatore, 1992.
14. Satyanarayana T. In search of acidstable and thermostable α-amylases. The Botanica,2016:66:1-4.
15. Sella N, Manchanda D, Gupta I, Bundela P, Bansal S, Aggarwal S, *et al*. Use of the smartphone as a handy colorimeter. DU Journal of Undergraduate Research and Innovation,2018:3(2):56-63.
16. Stanley D, Farnden KJF, MacRae EA. Plant α-amylases: functions and roles in carbohydrate metabolism. Biologia Bratislava,2005:60(16):65-71.
17. Subramanyan J, Chaudhary N. Food enzymes. In: Abstracts One-day Indo-Hungarian Symposium on Recent Advances in Chemistry and Biology (INHCAB-2017), Department of Chemistry, Miranda House, University of Delhi, in association with Department of Pharmaceutical Chemistry, University of Debrecen, Debrecen, Hungary, organized at Miranda House, 2017, 36.
18. Takahashi I, Kuboi T, Fujiwara T, Hara M. Overexpression of an extraplastidic β-amylase which accumulates in the radish taproot influences the starch content of *Arabidopsis thaliana.* Plant Biotechnology,2012:29(5):447-455.
19. Tiwari SP, Srivastava R, Singh CS, Shukla K, Singh RK, Singh P, *et al*. Amylases: an overview with special reference to alpha amylase. Journal of Global Biosciences,2015:4(1):1886-1901.
20. Wang L, Zhang W, Wei L, Xu C. A study of peroxidase and amylase isoenzymes in different radish cultivars during winter storage. Journal of the Science of Food and Agriculture,2000:80(12):1767-1771.