PURIFICATION AND CHARACTERIZATION OF AMYLASE EXTRACTED FROM LOCAL WHEAT

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ABSTRACT

This study was aimed to test four types of different plants for the purpose of selecting the optimal plant as a source of the amylase enzyme, including wheat, barley, rice, and potatoes. Among these plants, wheat was selected as the best plant source for enzyme extraction because it possessed the highest effectiveness of the enzyme specific activity (3.0 U/mg protein). Furthermore, sodium acetate buffer (0.2 M, pH 6.0) was determined to be the optimal extraction buffer with a 1:5 (w:v) ratio after 75 min, and it provided 68 U/mg protein. The enzyme was concentrated with sucrose before being purified by gel filtration chromatography using Sephadex G-150. The results demonstrated a 1.8-fold increases in final purification folds with a 332% yield of enzymes. At pH 6.0, the purified enzyme showed its highest activity and stability. The activity of the purified enzyme was most effective at 45 °C and remained stable up to 37 °C. At concentrations of 5 and 10 M, various ionic and chemical substances had an impact on amylase.

Keywords: Extraction, amylase, optimum conditions, Sephadex G-150, stability

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تنقية وتوصيف الاميليز المستخلص من نبات الحنطه المحلية

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المستخلص

هدفت الدراسه الحاليه الى اختبار اربعه انواع من نباتات مختلفه لغرض اختيار النبات الامثل كمصدر لانزيم الاميليز شملت الحنطه والشعير والأرز والبطاطا، ومن بين هذه النباتات، تم اختيار الحنطه كأفضل مصدر لاستخلاص الانزيم ، حيث امتلك اعلى فعاليه نوعيه لإنزيم الأميليز (3.0 وحدة / ملغم بروتين). كما تم اختيار محلول خلات الصوديوم (0.2 M، 6.0 pH) كأفضل محلول استخلاص بنسبه استخلاص1: 5 (و: ح) بعد 75 دقيقة، حيث بلغت الفعاليه النوعيه 68 وحدة / ملغم بروتين. تم تنقية الإنزيم بواسطة كروماتوجرافيا الترشيح الهلامي باستخدام هلام سيفادكس ج–150 بعد التركيز بالسكروز. أظهرت النتائج زيادة في عدد مرات التنقية النهائية بمقدار 1.8 مرة بحصيله انزيميه مقدارها 332٪ . وأظهر الإنزيم المنقى أقصى نشاط وثبات عند درجة الحموضة 6.0. وكانت درجة الحرارة المثلى لنشاط الإنزيم المنقى 45 درجة مئوية ومستقر عند 37 درجة مئوية. تأثر الأميليز ببعض المركبات الأيونية والكيميائية بتركيز 5 و 10 ملم.

كلمات مفتاحيه: استخلاص، اميليز، ظروف مثلى، سيفادكس ج–150، ثباتيه

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INTRODUCRION

Amylase enzyme (EC 3.2.1.1) is an endo-hydrolyase that operates on (1,4)-glucosidic linkages in starch and other similar oligo- and polysaccharides, releasing malto-oligosaccharides such as dextrin and smaller polymers made of glucose units and glucose on the anomeric form. It may come from a variety of sources, including plants, animals, and microbes, amylase is essential for starch breakdown during seed germination in plants (15,24). According to Muralikrishna and Nirmala (25), cereal amylases are recognized as enzymes derived from cereal grains including wheat, barley, maize, rye, etc. It is crucial to the metabolism of starch during the growth and germination of grains. It comes in a variety of forms and is synthesized in response to plant growth hormones (8). Additionally, it is effectively employed in the processing industry to hydrolyze starch into low weight products (17). Due to its cumulative effect on the quality of final products such as bread (11), beer (14), supplemental foods (16), and glucose syrup (25), in the technical processing of grains, it is seen as a crucial requirement (10). Because of their technological role in the final products in a variety of industries, including food, fermentation, and the starch processing industry, researchers have been interested in the characterization of cereal amylases from an enzymological point of view for many years (1). Such studies primarily aim to determine the ideal conditions for processing starch hydrolysis utilizing the amylases from grains. Knowledge of the features of amylases from various cereal grains allows for more efficient selection of cereal amylases that suit the requirements of a certain application (13). Because of importation, microbial amylases from fungal or bacterial sources are costly in many impoverished nations. As a result, if grain amylases are sufficiently stable and abundant, they might be an acceptable alternative for microbial amylases (1). Furthermore, the low cost of cereal amylases generated from local cultivars in developing countries may stimulate their usage as an appropriate alternative source for exporting microbial amylases (1, 12). Such study requires a thorough knowledge of the properties of amylase derived from such a source, as well as the industrial contexts in which it would be employed (13). Different amylases from various plants have been purified and characterized using both conventional and classical approaches. The fundamental issue in the purification of enzymes from grains is their presence in numerous forms as isoenzymes. However, this difficulty does not exist in dicotyledonous plants, where the number of isoforms is usually less than two (22). The goal of this work was to purify and characterize amylase enzyme extracted from diverse local cereal plants under ideal circumstances in order to investigate its properties in the future.

MATERIALS AND METHODS

Amylase enzyme extraction Plants

In this investigation, plants were employed as the source of material, and they were easily available in Iraqi market locally. Include wheat (*Triticum aestivum*), barley (*Hordeum vulgare* L.), rice (*Oryza sativa*), and potato (*Solanum tuberosum*).

Extraction and recovery of plant-based amylase enzyme: At pH 7.0, amylase was extracted from wheat, barley, rice, and potatoes using phosphate buffer (0.02M). A gram of each ground material was mixed with 1:10 (w:v) phosphate buffer in a mortar for 15 minutes under ambient conditions. The resultant paste was subjected to filtration using gauze in order to eliminate cellular components, followed by centrifugation at a speed of 10,000 revolutions per minute for a duration of 15 minutes. This process yielded a transparent supernatant, which was used to measure amylase activity. This supernatant is commonly known as the crude amylase extract (18).

Determination amylase assay

The hydrolytic activity of amylase was routinely assayed using starch as substrate as described by Bernfeld (6) which depend on the standard curve of maltose decomposition throughout the release of reducing sugars by the enzyme. The maltose percentage (w:v) in the reaction wells was estimated using the corrected absorbance of each test and the calibration curve equation with maltose dilutions ranging from 360 to 1800 µg/ml. By combining 0.8 ml of starch solution with 0.2

ml of enzyme solution (plant extract) for 20 min in a water bath at 37 °C, the enzyme reaction was started. One ml of 3,5-Dinitrosalicylic acid (DNSA) was then added to stop the reaction. which was then cooled and added along with 10 ml of D.W with vortex. The increases in absorbance at 540 nm was utilized to detect amylase activity. Instead of enzyme, distilled water is added to the previous reaction mixture to create the blank. Enzymatic activity unit defined as the amount of enzyme that under optimal conditions releases 1 µmole of reducing carbohydrates (glucose and maltose) per minute. The concentration of proteins was determined using the Bradford method (7).

Amylase extraction optimization

The amount of amylase obtained from certain plants is subject to various processes, and these mechanisms were optimized individually to maximize the yield of amylase. The extraction of amylase has four essential variables: buffer type, buffer concentration, extraction duration, and extraction ratio.

Extraction buffer type

The most active plant from the preliminary screening was chosen to test the buffer's influence on extraction. The chosen plant samples were homogenized for 15 minutes at 30 degrees Celsius in 0.02 M sodium acetate (99% $C_2H_3NaO_2$, BDH, England) (pH 4, 5, and 6), 0.02 M phosphate-buffered saline ($C_{l2}H_3K_2Na_3O_8P_2$, BDH, England) (pH 7), and 0.02 M Tris-base ($C_4H_{11}NO_3$, Hi-Media, India) (pH 8, and 9) at 1:10 (w:v) (3). Protein content, activity of enzyme, and specific activity were all evaluated in each experiment. The experiments involving the extraction buffer were conducted in duplicate (6,7).

Extraction buffer concentration

Extraction concentration courses of acetate buffer (0.01, 0.02, 0.03, 0.05, 0.1, and 0.2) M were performed using a mortar to find the optimal extraction buffer concentration (19). Treatment included 15 minutes of centrifugation at 10,000 rpm and filtering through paper. The supernatant was evaluated for activity of enzyme, protein content, and specific activity (6,7).

Period of extraction

To find the most favorable duration for extracting amylase, a time course of (5, 15, 30, 45, 60, and 75) minutes was conducted using a mortar. Subsequently, the mixture was subjected to centrifugation at 10,000 revolutions per minute for 15 minutes (19). The filtrate was collected in order to assess activity of the enzymes, the amount of protein, and specific activity (6,7).

Ratio of extraction

Various buffer ratios were chosen to determine the optimal wheat ratio to extract the enzyme, including 1:5, 1:10, 1:15, 1:20, 1:25, and 1:30 (w: v), by mixing 1 gram of ground wheat plant with the optimal buffer for 15 minutes at each extraction ratio (19). Subsequently, the sample was subjected to centrifugation at a speed of 10,000 revolutions per minute (rpm) for a duration of 15 minutes, after filtration using a filter paper. The measurements activity of enzyme, quantity of protein, and specific activity were acquired (6,7).

Purification of amylase enzyme

After the ground wheat seeds was homogenized in a mortar with 0.2 M acetate buffer at pH 6, the amylase enzyme was extracted by centrifuging the plant at 10.000 rpm for 15 minutes. Two processes were required to complete the purification of the crude extract. First, the extract was concentrated with sucrose using a dialysis tube. Subsequently, the sample was subjected to Sephadex G-150 gel filtration in order to eliminate unwanted constituents. Gel filtration chromatography was conducted in accordance with the guidelines provided by the manufacturer (Pharmacia-Sweden). A suspension of approximately 20 grams of Sephadex G-150 was prepared in 0.5 liters of distilled water, which was mildly agitated, to facilitate the swelling of gel beads. This process was carried out at a temperature of 90 ^oC for a duration of 180 minutes. Following this step, the gel was stored overnight at 4 °C before being washed again with 0.1 M of Tris HCL buffer solution (pH 7.5). The concluding steps involved the suspension of the gel in the identical buffer, degassing it using a vacuum, and subsequently placing it into a glass column measuring 21 x 1.8 cm for the purpose of achieving equilibrium with the addition of the buffer solution. A 0.1 M Tris HCL buffer solution with a pH of 7.5 and a flow rate of 20 mL per hour were used to elute ten milliliters

of crude extract from the Sephadex G-150 column, yielding three milliliters each fraction. At 280 nm, each fraction's protein concentration was determined. These fractions' enzyme activities were determined, the effective fractions collected, the volume was measured, the activity and protein concentration were assessed, and finally the volume was concentrated, divided among tubes, frozen, and kept for use in future studies (3).

Properties of purified enzyme

Ideal pH for activity of enzyme: The activity of amylase was measured at different pH values using various buffers, sodium acetate (pH 4.0, 5.0, and 6.0), sodium phosphate buffer (pH7), and Tris-Base (8.0, 9.0) at 50 mM concentration for preparing the substrate. The activity was measured using the standard assay conditions and the relative activity plotted against different pH values.

Optimum pH for enzyme stability

The effect of pH on amylase stability was examined by pre-incubating the enzyme for 15 minutes at 37 °C with buffers ranging in pH from 4.0 to 9.0 at a ratio of 1:1. Immediately the samples were transported to an ice bath, then the residual activities were calculated at 37 °C and represented as a percentage of the starting activity, which was assumed to be 100%.

Ideal temperature

Amylase activity was measured at a range of temperatures: 25, 37, 45, and 50°C. The typical assay conditions were used to assess the activity, and the relative activity was displayed versus various temperatures (31).

Thermal Stability

Thermal stability of purified amylase extracted from wheat seeds accomplished by incubating the enzymes for 15 minutes at a variety of temperatures at ranges of (25- 50) °C. At the optimal temperature for enzyme activity, the activity was then measured. The remaining activity was subsequently calculated.

Effect of different metal ions and some chemicals on amylase activity: The effect of various metals and chemicals, such as Ca^{+}, Na^{+}, Zn^{+}, Hg^{+}, EDTA and Cysteine at concentrations of 5 and 10 mM on the activity of the purified amylase enzyme was investigated by pre-incubating the enzyme preparation with these chemicals for 15 minutes under optimal temperature and pH conditions. On the basis of the untreated enzyme, the remaining activity was measured with the tested metal (31).

RESULTS AND DISCUSSION

Extraction of amylase from diverse plant materials: The plants chosen for experimentation were (wheat) *Triticum aestivum*, (barley) *Hordeum vulgare* L., (rice) *Oryza sativa*, and (potato) *Solanum tuberosum*. Wheat amylase enzyme exhibited the highest specific activity, 3.0 U/mg at pH 7 in 0.02 M phosphate buffer, according to the results. This was followed by barley, potato, and rice with specific activities of 2.4 U/mg, 2.0 U/mg, and 1.45 U/mg, respectively (Fig. 1). These results showed that wheat has a greater amylase content than the other plant materials investigated here, which might be useful in a variety of applications. Other research in this field reveals that the specific activity of amylase from germination of safflower seeds (*Carthamus tinctorius* L.) achieved a maximum after 5 days of development, reaching 1.8 U/mg protein (5).

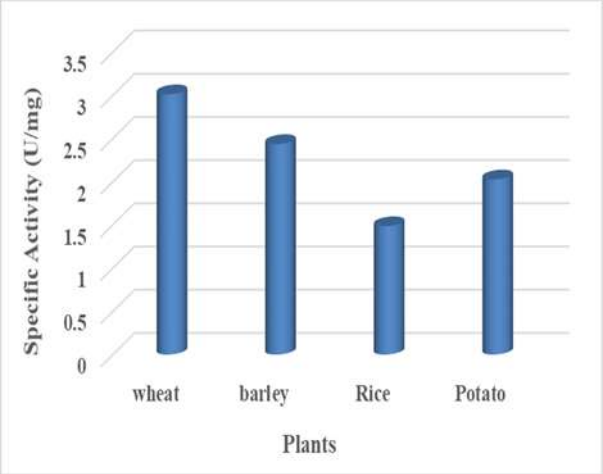

Figure 1. Influence of plant type on amylase extraction with 0.02 M phosphate buffer (1:10) (w:v) at pH 7.0 and 30°C for 15 minutes

Impact of the buffer type on the amylase extraction: Amylase was extracted from wheat using various chemical buffers, and the results were evaluated using enzyme specific activity, as shown in Fig. (2). Based on this, sodium acetate buffer (0.02 M, pH 6.0) was the best buffer, with specific activity of 16.6 U/mg whereas the other applied buffers all had lower specific activities. The pH of the

artificial system that mimic the natural environment plays a crucial role in determining the stability and activity of enzymes. Variations in the acidity or alkalinity of the sample solution lead to varying degrees of ionization for different amino acids, consequently impacting the efficiency of enzyme extraction (23). There were numerous interactions that altered the enzyme's operation. Enzymes exhibit an optimal pH at which they function most efficiently, while still retaining functionality within a pH range that encompasses this optimal pH. Furthermore, their stabilities vary when the pH rises towards the basic region, which may lead to denaturation. Other research have shown that amylase isolated from safflower seeds (*Carthamus tinctorius* L.) with an acetate buffer solution (0.1M, pH 6) had the highest specific activity (5).

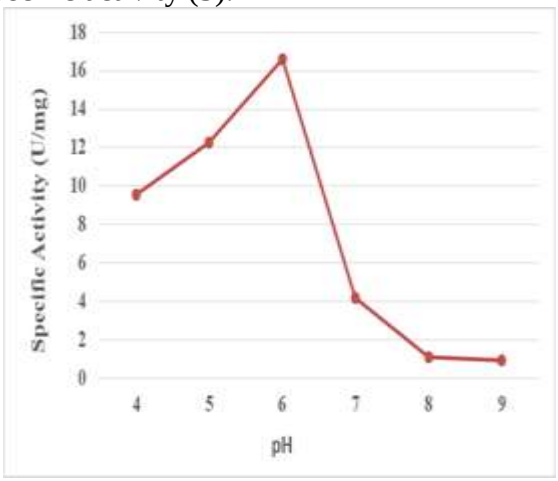

Figure 2. Influence of buffer types on wheat plant seeds amylase extraction at 30°C for 15 min at a ratio of (1:10) (w:v).

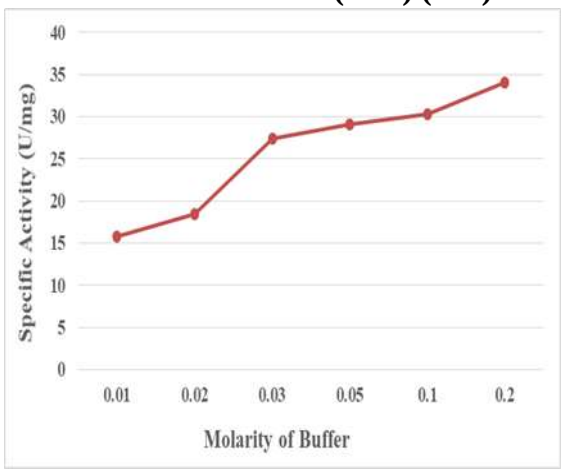

Figure 3. Effect of sodium acetate buffer concentration on the amylase extraction from wheat seeds at 30°C for 15 min with a ratio of (1:10) (w:v)

Concentration of extraction buffer

Six concentration of sodium acetate buffer were chosen to determine the best concentration of this buffer using to extract the amylase from wheat include (0.01, 0.02, 0.03, 0.05, 0.1 and 0.2) M in pH 6.0. From the result in Fig. (3), the maximum specific activity was observed for crude extract in 0.2 M, reaching 34.0 U/mg protein, whereas the lowest specific activity was found in 0.01 M, reaching 15.7 U/mg protein. Amid with his coworkers (2) found the best buffer for extract amylase from Dragon (*Hylocereus polyrhizus*) peel was 0.1 M acetate buffer pH=6.0.

Ratio of extraction

The optimal amylase extraction ratio was determined using sodium acetate buffer (0.2 M, pH 6.0) utilizing six extraction ratios: 1:5, 1:10, 1:15, 1:20, 1:25, and 1:30 (w: v). The crude extract with the 1:5 ratio had the greatest specific activity, which was recorded at 36.5 Unit/mg protein. Other ratios had specific activities of 33.8, 17, 12.5, 11.3, and 10.5 U/mg protein, respectively (Fig. 4). The quantity of herbal material employed in an extract, as measured by the native extract ratio, is variable. Because of this, the equivalent dry weight of an herb preparation may vary depending on how much of the active herbal component was actually utilized. In most cases, a significant yield of extractable material from the herbal source is indicated by a low native extract ratio (28).

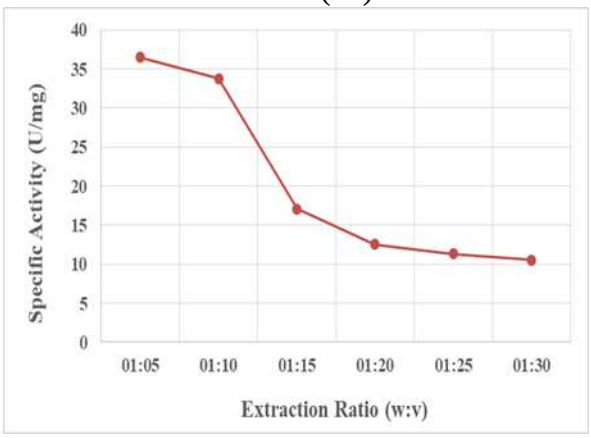

Figure 4. Influence of extraction ratio during 15 minutes at 30°C of amylase extraction from wheat seeds using 0.2M of sodium acetate buffer

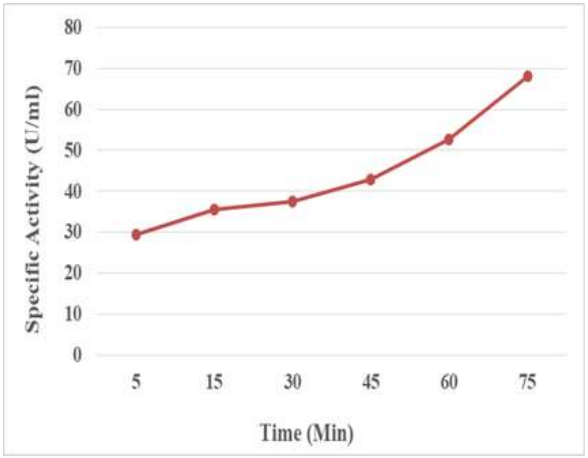

Figure 5. The impact of varying extraction durations on the extraction of amylase from wheat seeds at a temperature of 30°C using (1:5) (w:v) of 0.2 M of sodium acetate buffer

Extraction period: To establish the optimal enzyme extraction time, six extraction periods (5, 15, 30, 45, 60, and 75) minutes were selected. The crude extract had the maximum specific activity after 75 minutes, reaching 68 U/mg protein, in comparison to lower specific activity after 5, 15, 30, 45, and 60 minutes, which were 29.4, 35.7, 37.4, 42.9, and 52.6 Unit/mg protein, respectively (Fig.5). Additionally, establishing the optimal time period is imperative due to the variability of the amylase enzyme extractor across different sources. This variability arises from variations in the materials present in each source, which can interfere with the enzyme's activity. Furthermore, the removal of impurities during the extraction process leads to the production of a protein extract that exhibits enhanced stability against decomposition (26).According to Amid *et al*. (2), the optimal extraction ratio for amylase from Dragon Peel (*Hylocereus polyrhizus*) was 1:4.

Enzyme purification: The methodology employed for the extraction and purification of the amylase enzyme from wheat seeds is illustrated in Figure 6. Initially, the enzyme extract was subjected to concentration using sucrose and subsequently passed through a Sephadex G150 gel column (21×1.8 cm) that had been pre-equilibrated with a Tris-HCL buffer of pH 7.5 and concentration of 0.1 M. The analysis demonstrates the presence of an individual protein peak in the eluted proportions obtained from the filtration process. Additionally, a second peak is observed, indicating the presence of amylase. This information is visually represented in Table 1, which aids in accurately collecting the appropriate portion of the filtrate. According to the data presented in Figure 6, fractions 10 to 22 exhibited the presence of active amylase. The observed activity of this enzyme was measured to be 123.6 U/mg protein. Consequently, the total yield obtained from these fractions was determined to be 328.44%, accompanied by a purification factor of 1.76. The amylase from germinative wheat plants has been purified using a two-step process using ammonium sulphate and affinity chromatography, with a purification yield of 81.5% and a purification fold of 131.52 (5). The apple (*Malus pumila*) amylase enzyme was successfully extracted and purified by Saini and colleagues (30), yielding 11% and 24 as a purification fold, respectively.

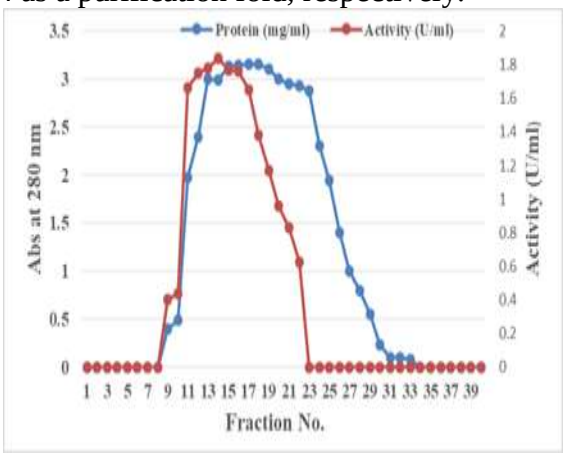

Figure 6. Amylase from the wheat plant was purified by gel filtration chromatography utilising a Sephadex G150 column (21 x 1.8 cm) and a 0.1 M, pH 7.5 Tris-HCL buffer at a flow rate of 20 ml/hr. 3 ml per fraction

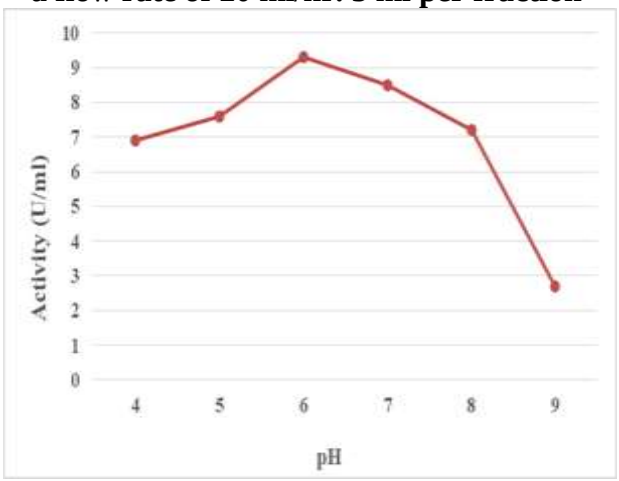

Figure 7. Effect of different pH values (4.0-9.0) on partial purified amylase activity from wheat plant

Table 1. Purification steps of amylase from local wheat seeds

Sample	Volume (ml)	Activity (U/ml)	Protein (mg/ml)	Specific Activity (U/mg)	Total Activity (U/mg)	Purification Fold	Yield (%)
Crude	25	4.3	0.062	69.4	107.5	1	100
Sucrose	14	9.5	0.1	95	133	1.4	123.7
Sephadex G-150	42	8.5	0.069	123.2	357	1.8	332

Properties of partially purified enzyme

Optimum pH: The standard reaction mixture was used to measure the pH dependency of amylase activity throughout a pH range of 4-9. Enzyme activity was highest at pH 6.0 (Fig.7), with 9.3 U/mL when compared to other pH values. The decline in activity was also seen to be noticeably more pronounced in the alkaline medium as compared to the acidic medium. The pH value of a solution, specifically at a level of 9, has the potential to influence the ionization of functional groups within the active site of an enzyme, as well as the ionization of functional groups within the substrate (9). Additionally, it can impact the overall arrangement and organization of both the enzyme and substrate. Amylase isolated from *Malus pumila* was tested at several pH levels ranging from 4 to 11, and the enzyme had a pH optimal of 6.0 (30).

Optimum pH for enzyme stability

The stability of the enzyme was evaluated by subjecting it to pre-incubation in pH buffers ranging from 4 to 9. Analysis of the results presented in Figure 8 revealed that pH levels between 5 and 6 were optimal for maintaining the stability of the amylase enzyme. Specifically, the enzyme exhibited 100% activity retention at pH 6.0, 99% activity retention at pH 5.0, and approximately 97.7%, 95.3%, 94.1%, and 93.6% activity retention at pH 4.0, 7.0, 8.0, and 9.0, respectively. At both acidic and alkaline pHs, the enzyme's stability was limited. The findings could support the idea that an enzyme is more stable at a pH that is close to neutral. The impact of pH stability on enzyme structure is a significant factor that can lead to denaturation of the enzyme molecule or alterations in the ionic state of the enzyme's active site. Additionally, it can affect the secondary and tertiary structure of the enzyme. Consequently, this can result in a decrease in enzyme activity when pH values deviate from the optimal conditions (32).=

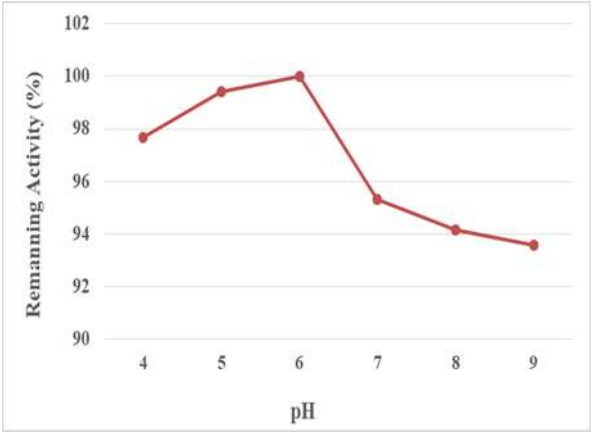

Figure 9. Influence of various temperature (25-50) ^{o}C on the partial purifying wheat amylase activity

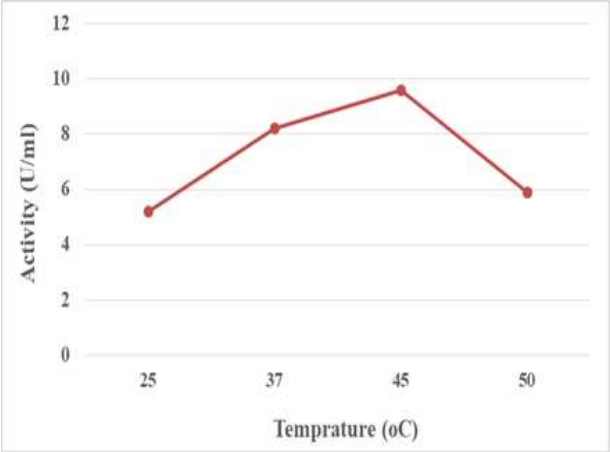

Figure 10. Impact of various temperature ranges on the stability of wheat partial-purified amylase

Optimum temperature

Amylase activity was calculated for 15 minutes at several temperature ranges, including 25, 37, 45, and 50°C. The findings in Fig. (9) shows that the amylase activity increased at 45 °C, reaching a maximum of 9.6 U/ml, and subsequently decreased as the temperature increased up to 45 °C, reaching a low of 5.9 U/ml at 50 °C. However, below 37 °C, amylase activity also dropped. Different aspects of the enzymatic process are impacted by temperature, including pH, enzyme-substrate affinity, and the ionization of the system's prosthetic group (33). According to

Mahmood *et al.* (21), 40 °C was the ideal temperature for *Aspergillus niger*-produced amylase activity.

Thermal stability

The enzyme's thermostability was determined by incubating a purified enzyme solution at varying temperatures (20-50 °C) for 15 minutes. The results demonstrated that the enzyme was stable at 37 degrees Celsius and attained full activity. Enzyme activity decreased over time as the temperature progressively increased (Figure 10). In general, the rate of any enzymatic reaction will be drastically reduced if the temperature is below or above the optimal range. This could be due to the enzyme becoming denatured or losing its three-dimensional structure, when a protein is denaturized, its hydrogen bonds and other non-covalent bonds are destroyed. (34). Mahmood *et al*. (21) found the optimal temperature for amylase produced from *Aspergillus niger* was 40 °C.

Impact of metallic ions and some chemicals

Metallic ions, Ca^{2+}, Na^{+}, Zn^{2+}, and Hg^{2+}, as well as EDTA and Cysteine, were examined for their ability to activate or inhibit amylase at concentrations of 5 and 10 mM. The presence of Ca^{2+} was found to exhibit dual effects, activating and stabilizing, as evidenced by an observed increase in activity. Conversely, Na^{+} demonstrated no impact on amylase activity at a concentration of 5 mM, but exhibited a significant inhibitory effect of 76.4% at a concentration of 10 mM (Table 2). This may mean that when the concentration was raised, the enzyme activity altered. It is widely known that Ca^{2+} contributes to the stability and structural integrity of the amylase (27). Ca^{2+} ions increase amylase activity by interacting with negatively charged amino acid residues, such as aspartic and glutamic acids, resulting in enzyme conformation stabilization and maintenance (29). However, partial enzyme inactivation was observed in the presence of Zn^{2+} and Hg^{2}. Based on the results of a recent study, it has been observed that the impact of ions on specific type and concentration. Furthermore, it has been noted that the influence of these ions on enzyme activity tends to increase as their concentration increases. The formation of complexes with the enzyme is responsible for the decline in activity; these complexes hinder the enzyme from attaching itself to the substrate and converting it into product (31), which in turn leads to the decline. $HgCl_2$ was able to block the enzyme at concentrations of 5 and 10 mM, showing that there were SH groups in the enzyme active area that were being oxidized by the presence of $HgCl_2$. Furthermore, by forming a compound with the enzyme, $HgCl_2$ in the substrate processing solution may prevent substrate binding and creation of products (20). This metal may inhibit enzyme activity by binding to catalytic residues or by dislodging Ca^{2+} from the enzyme's substrate-binding site. EDTA was used to evaluate the inhibitor's influence on amylase activity. Based on the assessment of the residual enzyme activities, it was observed that the enzyme exhibited reduced enzymatic activity alongside elevated inhibitor concentration. Additionally, the experiment demonstrated that the enzyme under investigation is classified as a metallo-enzyme, with divalent ions being crucial for its catalytic activity. Consequently, EDTA was employed to explore the impact of inhibitors on the amylase activity. When an enzyme is treated with cysteine, disulfide bonds in its structure and conformation are weakened, resulting in fragmentation of the protein into its constituent units and a decrease in its activity. These results suggested that the investigated enzyme contained disulfide bonds (4).

Table .2 Effect of metal ions (5mM and 10 mM) on purified wheat α-amylase activity (represented as % activity).

Chemicals	5mM	10mM
NaCl	98.8	76.4
$CaCl_2$	110	100
$ZnSO_4$	89.9	12.7
$HgCl_2$	6.0	87.4
Cysteine	85.6	99.1
EDTA	85.6	14.4

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