Extraction, Purification and Characterization of Poly Phenol Oxidase from Potato and Apple and Inhibition of Enzymatic Browning

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KEYWORDS

Polyphenol oxidase, Browning, Apple, Potato, Characterization, Isolation.

ABSTRACT

The purpose of this study was to determine the optimum conditions for the extraction of polyphenol oxidase (PPO) from potato and apple. PPO is found in a variety of fruits and vegetables and is the enzyme that causes browning reactions when handling damaged cells. At 405 nm, the enzyme activity was determined using spectrophotometry. PPO was researched to stop potato and apple from browning, which reduces their marketability. The outcomes showed that using an extraction buffer containing phosphate buffer (0.05 M, pH 6.6) was the optimum condition for the extraction of polyphenol oxidase from potato and apple. In addition, the results showed that as the temperature increased, the (PPO) activity increased, and the activity reached its peak at a temperature of 35 °C. The optimum pH value of (PPO) activity was 6.5 and we observed during the research that (PPO) decreased in strong acid condition and alkaline environments. Moreover, the results showed that the highest activity of polyphenol oxidase enzyme extracted from apple and potato was by using (O-dihydroxybenzene) as a substrate. As the concentration of the substrate increased, the (PPO) activity increased, reaching the optimum substrate concentration of 0.8%, and then its activity decreased. Also, we found that when using ascorbic acid, citric acid, and Na2SO3 as inhibitors, the activity of the polyphenol oxidase enzyme decreased with an increase in the concentration of ascorbic acid, citric acid, and Na2SO3. Sodium thiosulfate, 0.01 M ascorbic acid and 0.5% polyethylene glycol were extracted with an extraction ratio of 1:4 (w/v) for three minutes by using a blender. The purpose of study was to determine the optimum conditions for polyphenol oxidase extraction from potato and apple.

1. Introduction

Polyphenol oxidases are copper-containing oxidoreductase enzymes that catalyze the presence of molecular oxygen during the oxidation and hydroxylation of phenolic groups. Enzymes known as polyphenol oxidases are present in many different types of animals, plants, bacteria, and fungi (28).

It functions as a defense mechanism in plants. Certain phenolic compounds are oxidized in the presence of oxygen when a plant is cut or bruised, creating a polymeric structure that shields the wound from microbial contamination (24). Unwanted browning and the development of off flavors in foods that have been processed and stored are linked to the catalytic activity of polyphenol oxidase.

Polyphenol oxidase, also referred to as tyrosinase, phenolase, phenol oxidase, and catechol oxidase, is a copper enzyme. that catalyzes two distinct reactions when oxygen is present. (7). In the first reaction, monophenols are converted to o-diphenols (monophenolase activity), and in the second reaction, o-diphenols are converted to o-quinones (diphenolase activity), which are then polymerized to create brown, red, or black pigments. (9).

Browning causes some fruits to lose up to half of their volume. In many fruits and vegetables, melanin formation caused rapid browning due to bruises, cuts, and other mechanical damage that allows O2 penetration. The consumer finds the appearance of unhealthy products unacceptable, as well as their off flavor and diminished nutritional value. Fruits and juices such as bananas, strawberries, peaches, and grapes, as well as potato, some lettuce, and other leafy vegetables, turn brown when exposed to air. (23).

Understanding the features of the enzyme is crucial for managing PPO's activity. The PPO enzyme from potato and apple was to be isolated, purified, and studied in this study. The PPO enzyme's characterization may contribute to the creation of improved techniques for preventing browning in

apple and potato.

2. Methodology

Fresh and mature apple (*Malus pumila*) and potato (*Solanum tuberosum L.*) were purchased from the local market, while chemicals were purchased from Aljumhuria Co. All chemical materials were analytical grade.

Preparation of crude PPO extracts: 20 gm of raw material were homogenized with 250 ml of potassium phosphate buffer (0.2 M, pH 7) to extract the enzyme. Homogenate the extract and filtered and also centrifuged at 5000 g for 10 minutes. By stirring for 60 minutes while adding 1.5 volumes of cold acetone (-5C^{o}), the enzyme was precipitated from the supernatant. The mixture was centrifuged at 10,000 g for 15 minutes, and the precipitate was then dissolved in 50 ml of potassium phosphate buffer. This unrefined enzyme extract was utilized for the enzyme characterization method described by (11).

Enzyme activity assay: PPO activity was evaluated by measuring the increase in absorbance at 420 nm using a spectrophotometer O-dihydroxybenzene was used as the substrate following the method described by (20).

Determination of optimum temperature: PPO activity was measuring at a variety of temperatures, as described (19).

Determination of optimum pH: The rate of O-dihydroxybenzene oxidation by PPO was calculated in the pH range of 5.8-7.9 using potassium phosphate buffer (0.2 M) the method described by (5).

Thermal stability: The enzyme solution was incubated for 60 minutes at 40, 50, 60, and 70 ^{o}C in a water bath. At the optimum temperature, the residual activity was measured the method described by (1).

pH stability: They preserved the pH of the enzyme solution between 4 and 9. pH ranges for citrate-phosphate buffer, potassium phosphate buffer, and Tris-HCl buffer were 4–5.5, 6-7.5, and 8–9, respectively. After all enzyme solutions were brought to a pH of 7, their residual activities were calculated of the incubation period the method described by (12).

Storage stability: The three months that the enzyme solutions were kept at 4 and -18^{o}C had been indicated. Every eight days for freezing storage and every four days for refrigeration storage, PPO activity was measured. Followed by the method described by (17).

Substrate Specificity

Making use of (O-dihydroxybenzene) substrate solutions at five different concentrations (0.2, 0.4, 0.6, 0.8, and 1) %, the substrate specificity was measured in a pH 6.5 0.1 M citric acid–0.2 M disodium phosphate buffer. At 420 nm, PPO activity was measured as previously mentioned (4).

Effect of Inhibitors

PPO activity was measured in the presence of 3 different inhibitors

(Ascorbic acid, citric acid, sodium thiosulphite)

Ascorbic acid and sodium thiosulphite concentrations (0.5, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.45) % and citric acid

concentrations of (0.3, 0.6, 0.9 ,1.2, 1.5, 1.8, 2.1) %.

PPO activity was assayed as described earlier and the results were described as percent O-dihydroxybenzene inhibition at 420 nm the method described by (26).

3. Result and Discussion

Determining the optimal pH for the activity and stability of the enzyme:

We use (O-dihydroxybenzene) at a concentration of 0.8% to study the effect of the pH value on the activity of the polyphenol oxidase enzyme at a temperature of 25 °C. The optimum pH is related to the source of the enzyme from apple and potato, and a sharp decrease in the activity of the enzyme in the acidic medium is observed at pH = 2 and at a rate of 95, 96 % of the original activity of each of the apple and potato, respectively, as the copper has been separated and the enzyme has been inactivated These results were similar to those obtained by (2).

The purified enzyme was stable in a pH range between (5-7) and maintained 80% of its original effectiveness, but it was unstable at pH below 4 – 9.

In the alkaline medium, it was found that the enzyme lost 90% of its activity at pH = 9 for both apple and potato, while at pH = 10, a total inhibition of the enzyme extracted from apple and potato was observed, as the copper was separated into copper hydroxide and a decrease in the enzyme's activity. Polyphenol oxidase is more active around pH equal 7, where the activity of the enzyme extracted from apple was 90% and that extracted from potato was 85% the pH values for effectiveness and stability vary according to the source of the enzyme and the base material used to estimate the enzyme activity.

The reason for the decrease in the enzymatic activity at the extreme values of pH is due to the occurrence of obvious changes in the enzyme structure or the active site, which leads to a loss of enzymatic activity as a result of losing double and triple form, where the change in the pH values affects the effective ionizable groups present in the enzyme molecule or on the ionic state of the base substance as described (8). It is also noted from the figure (1) the optimum pH for the activity of the polyphenol oxidase enzyme equal to 6.5 and this result agreed with what was reached

(22).

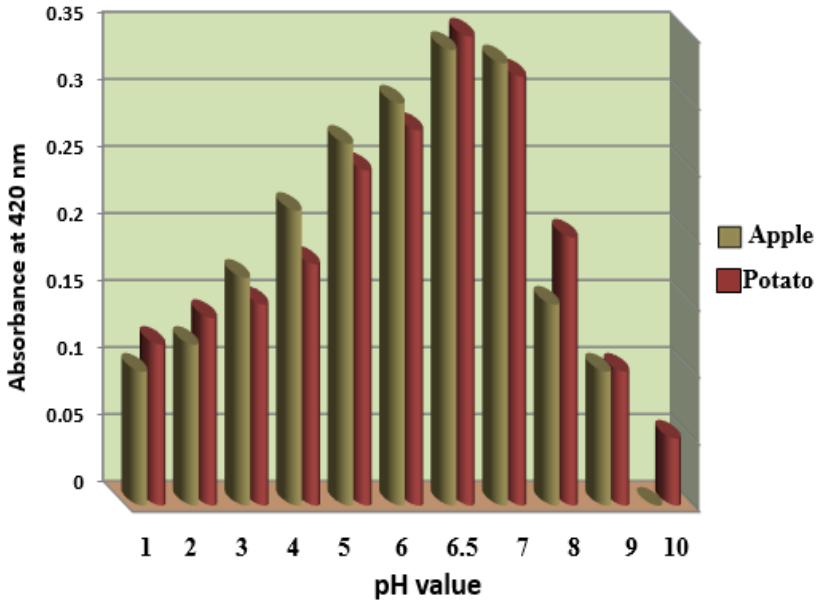

Figure 1. effect of the pH value on the activity of the polyphenol oxidase enzyme for both apple and potato.

Temperature's effects on an enzyme's stability and activity

Using a phosphate regulator solution pH 6.6 and (O-dihydroxybenzene) at a concentration of 0.8% to study of effect of temperature on the activity of the PPO enzyme for both potato and apple. It reaches its peak when the temperature at 35 °C, and when the temperature increases, the activity of the enzyme polyphenol oxidase becomes less.

Increasing temperature leads to obvious changes in the reaction components that include each of the enzymes and the base material, which leads to a continuous increase in the effectiveness of the enzyme due to the increased chance of collisions as a result of the increase in the kinetic energy of the reactant molecules due to the influence of temperature, the increase in temperature. Because of its negative impact on the reaction's components, the optimum temperature for the reaction causes an enzymatic activity decrease. It is also noted from the figure (2) that the rate of loss in the activity of the enzyme (PPO) increases with the increase in the temperature of the heat treatment. The polyphenol oxidase enzyme extracted from potato and apple loses 50% of its activity at a temperature of 80 °C. By increasing the temperature, instability occurs in the enzyme's activity. It is noted that the heat treatment at 90 °C for 5 minutes leads to an inhibition of 94, 93% of the original activity of the enzyme extracted from both apple and potato, respectively. It completely deactivated (8).

It is noted from figure (2) that a total inhibition of the enzyme activity (PPO) occurred at a temperature of 100 °C for 5 minutes. Of enzyme as well as on the occurrence of partial or total denaturation of the protein. As heating of 30 °C for 30 minutes did not affect the enzyme's effectiveness, while raising the temperature caused a clear decrease in the enzyme

activity, as temperatures (60-40) °C for 30 minutes affected the enzyme activity negatively, causing a decrease of (17,52) % Of the original effectiveness, while the enzyme lost 50% of its original effectiveness when exposed to a temperature of (80, 70) C for (1.5, 6.5) minutes respectively. And this result agreed with (15).

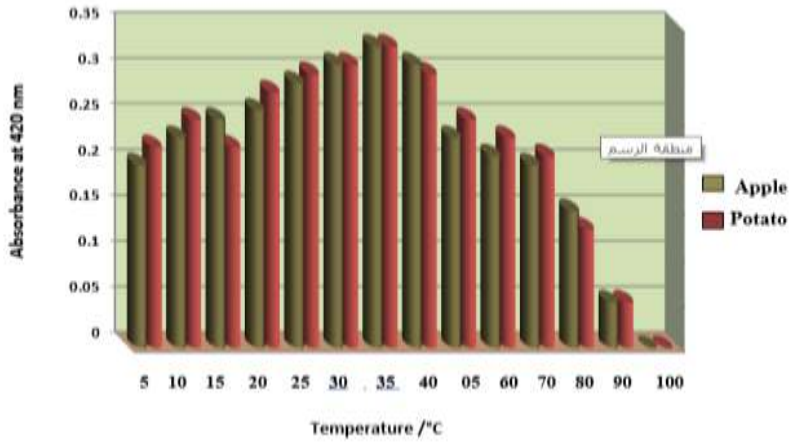

Figure 2. effect of temperature on the activity of polyphenol oxidase enzyme for both apple and potato.

Thermal stability: Figure (3) displays the results of the PPO's thermal inactivation from the two sources (A, B). The thermal stabilities of the enzyme different depending on where it came from. It was discovered that potato PPOs were more stable than apple PPOs. After 60 minutes at 60 oC, they retained 40 % and 50 % of their binitial activities, respectively. While potato and apple PPOs had completely inactivated under the same conditions, at 65oC, they retained 40 and 25% of their initial activities over the course of 30 minutes of incubation, respectively.

PPOs potato were generally found to have better thermal stability than fruit PPOs apple. It appears

that heat treatment for 15 minutes at 65 °C for apples and 75 °C for potatoes is adequate to deactivate their PPOs and stop enzymatic browning, which could occur during production and storage. It has been discovered that polyphenol oxidases have multiple thermal stabilities. The enzyme potato PPO is thermolabile. Above 40 °C, it completely deactivated (14). Apple PPO's thermal stability is mediocre. At 50 degrees Celsius, its half-life was 20 minutes (3). PPO from potatoes is more stable. After 30 minutes of incubation at 55°C, it maintained all of its activity.

The enzyme's structure underwent significant changes that impeded the availability of the active sites, potentially leading to denaturation of the enzyme itself, which could account for the decrease in activity at higher temperatures and this result agreed with (27).

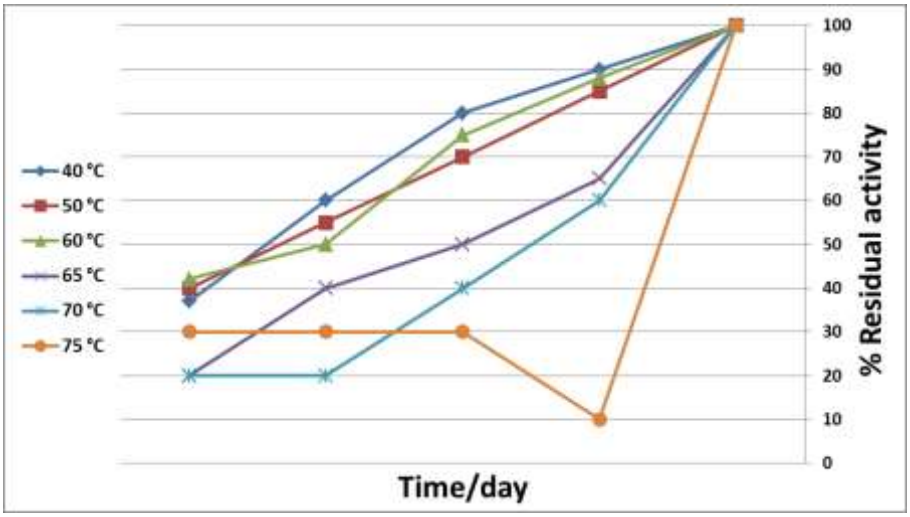

Figure 3A. Thermal stability of PPO of Apple

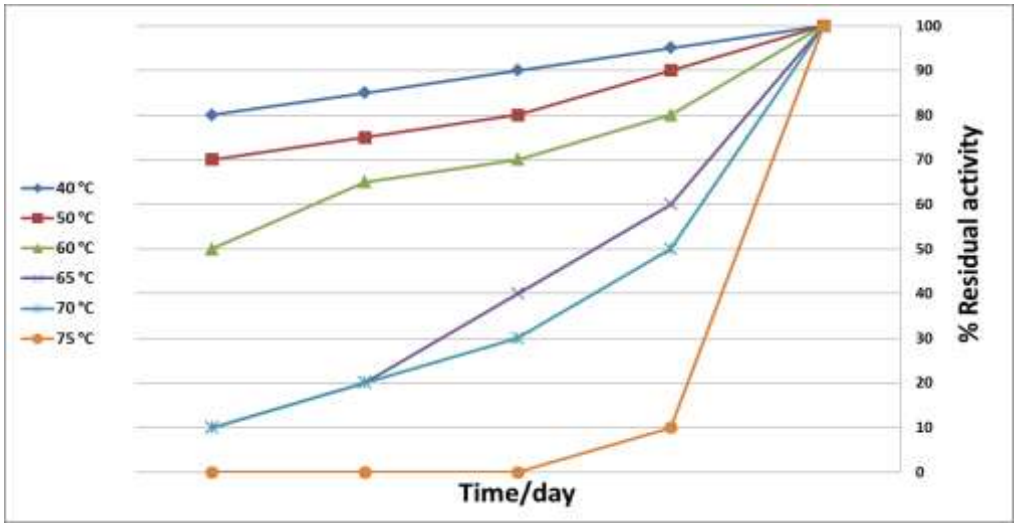

Figure 3B. Thermal stability of PPO of potato

pH stability: Using the proper buffer solutions, the crude PPOs of the studied were kept at a pH range of (4 – 9) for a duration of 60 minutes. At their ideal pH figure showed their residual activities were estimated. The stability of PPO was affected differently by pH depending on the enzyme's source. In the pH range of (6 – 9), apple PPO remained stable with 80% activity retention. Potato PPO showed good stability at pH values ranging from 5.5 to 7.5, with an ideal pH of 7. However, at alkaline pH values, its stability was markedly delayed as also noted from the figure (4).

Because an enzyme molecule's various amino acid residues are in varying states of ionization, the alkalinity or acidity of the solution can affect the molecule's protein structure, which in turn can affect

enzymatic activity and stability. This helps to explain why pH affects enzymatic activity and stability it completely deactivated (6). Different enzyme sources may contain different enzyme structures, resulting in varying pH stabilities.

The pH stability of an enzyme is regarded as a crucial factor in determining the ideal conditions for the enzyme's isolation, purification, handling, and storage. Various factors, including the type, source, and purity of the enzyme, influences on this stability and this result agreed with (21).

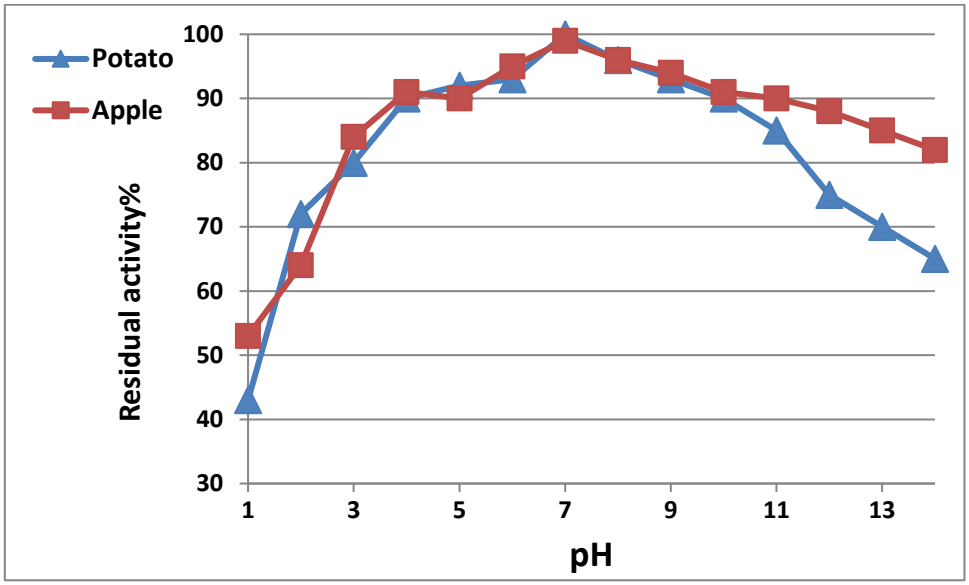

Figure 4. pH stability of PPO.

Storage stability:

It is noted from Figures (5, 6) that the purified enzyme completely lost its effectiveness after being stored for five days at a temperature of 18 °C, when it lost about 73% of its initial effectiveness after 21 days of storage at a temperature of 4°C, but it kept 84% of its initial effectiveness following a period of 21 days of freezing storage at -18°C.

Many overlapping factors determine the ability of the enzyme to be stable during the storage period, as the temperature and strength are the ionic buffer solutions used in enzyme storage have a significant role in the enzyme's stability under storage conditions due to the need for the active form of the enzyme molecule to have a specific ionic strength to ensure that it remains in an appropriate manner to complete the process of binding and analysis. Concerning the effect of storage temperature, freezing storage was found to maintain more activity in the storage period as compared with cooling storage.

It was shown in Fig (6) that storing the purified enzyme for 21 days at a temperature of 25 °C, a pH of 7, led to a loss of the enzyme was fully effective after 4 days, and at 4°C, the enzyme lost 20% of its activity during the first 5 days, while more than 80% of its effectiveness was lost after 12 days, while the stored enzyme was at a temperature of -15 C and this result agreed with (18).

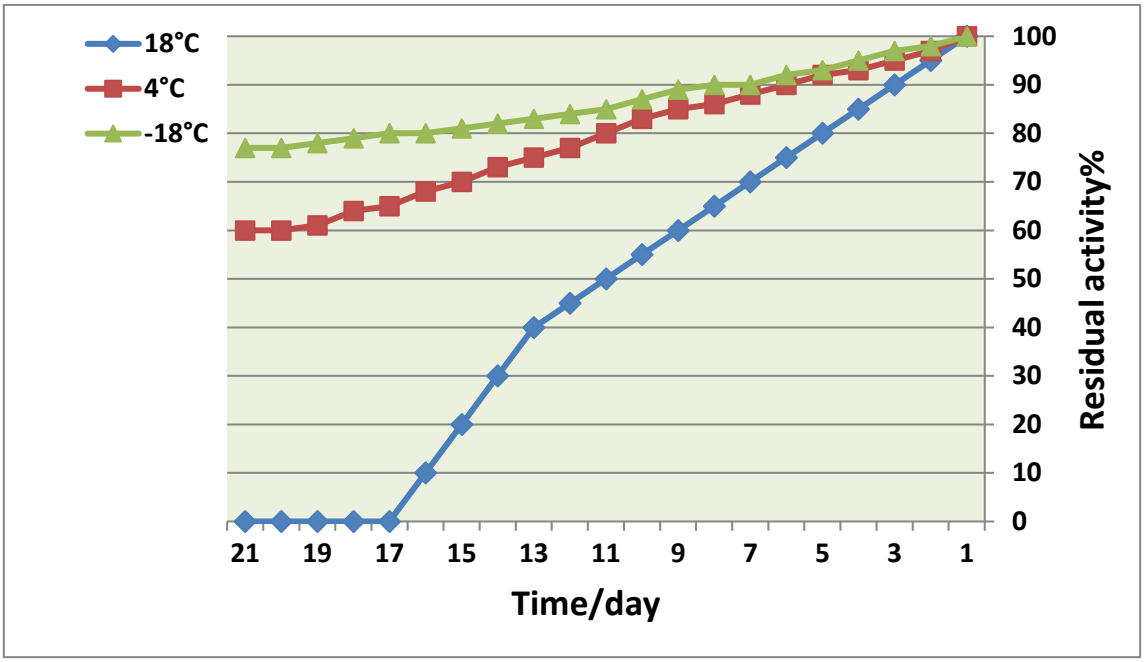

Figure 5. the effect of storage temperature on the activity of PPO enzyme purified from apple at temperatures 18°C, 4°C, -18°C.

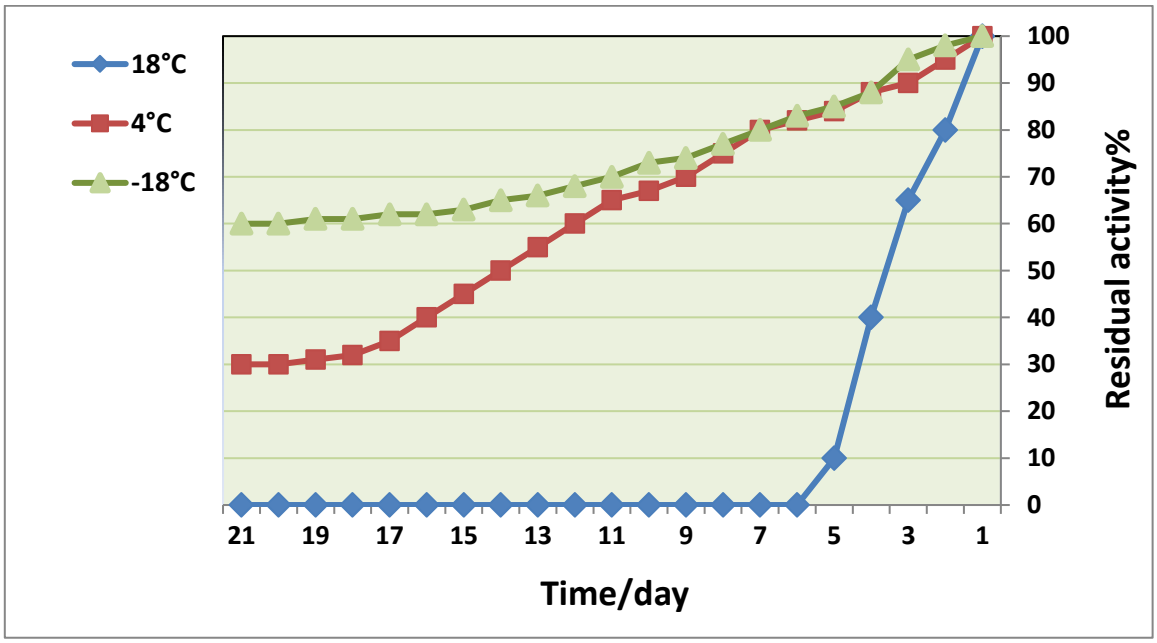

Figure 6. the effect of storage temperature on the activity of PPO enzyme purified from potato at temperatures 18°C, 4°C, -18°C.

Substrate:

We use a phosphate buffer solution pH 6.6 to study the effect of the concentration of the substrate on the activity of the polyphenol oxidase enzyme at a temperature of 25 °C as shown in Figure (7). 0.8%, where the effectiveness reaches the peak, and we note that any increase in the concentration of the specialized reactant from the mentioned concentration leads to less effectiveness because all the enzyme molecules did not merge with the substrate.

Through the study, we observed that the highest activity of the polyphenol oxidase enzyme extracted from apple and potato was by using (O-dihydroxybenzene) as a substrate, due to its faster oxidation

in the presence of the enzyme (PPO) more than other phenolic substances.

Through the study, we note that the highest activity of the polyphenol oxidase enzyme extracted from apple and potato was by using (O-dihydroxybenzene) as a substrate, due to its rapid oxidation in the presence of the enzyme (PPO) more than other phenolic substances.

It was also noted that the activity of the enzyme extracted from apple and potato had a high activity by 98 and 97%, respectively, when using one of the catechol derivatives (4 methyl catechol), because the enzyme acts as a catalyst in the presence of phenols with a binary phenolic structure as a substrate and this result agreed with (25).

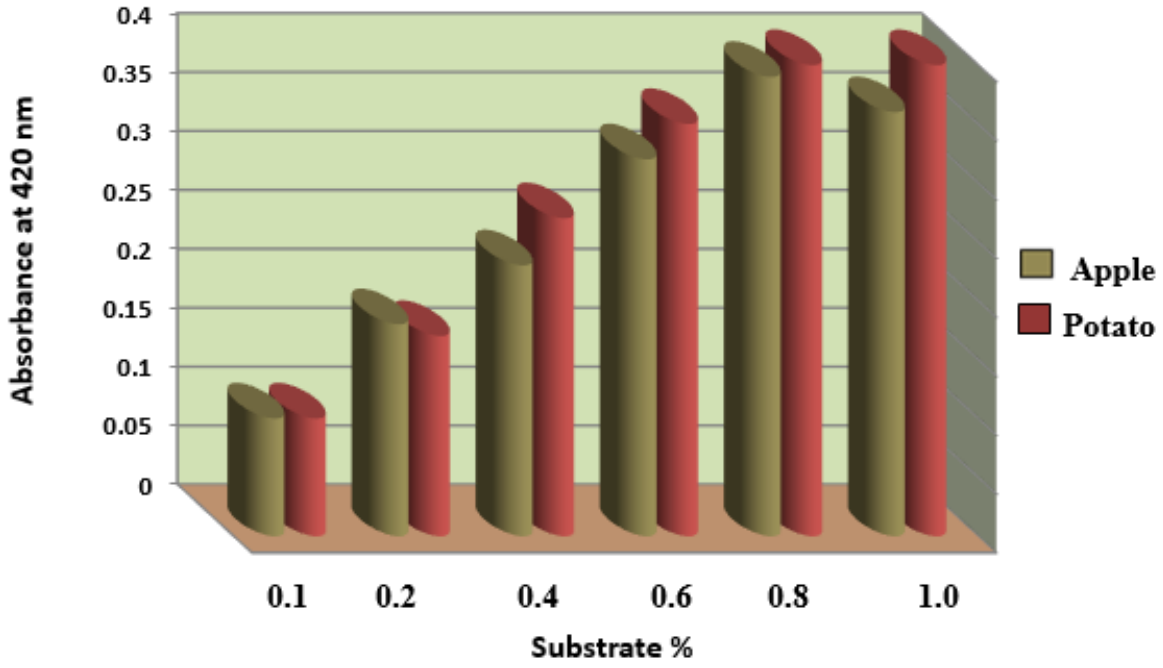

Figure 7. Effect of the concentration of the specialized reactant on the activity of the PPO enzyme for both apple and potato.

Inhibitors

Effect of ascorbic acid on the activity of the enzyme (PPO)

Results showed in Figure (8) that polyphenol oxidase enzyme's activity decreases as the ascorbic acid concentration increases until it reaches 0.3%. The activity of the enzyme reaches its peak and begins to decline as the concentration increases from the concentration above. When using ascorbic acid at a concentration of 0.1 leads to a inhibition of the activity of the PPO enzyme, the percentage of inhibition was 95, 94% for both apple and potato, respectively, and when using ascorbic acid at a concentration of 0.20, the percentage of inhibition was 70, 66% for each of apple and potato, respectively, and thus the optimal concentration of ascorbic acid to activate the activity of the enzyme polyphenol oxidase 0.3% and this result agreed with (16).

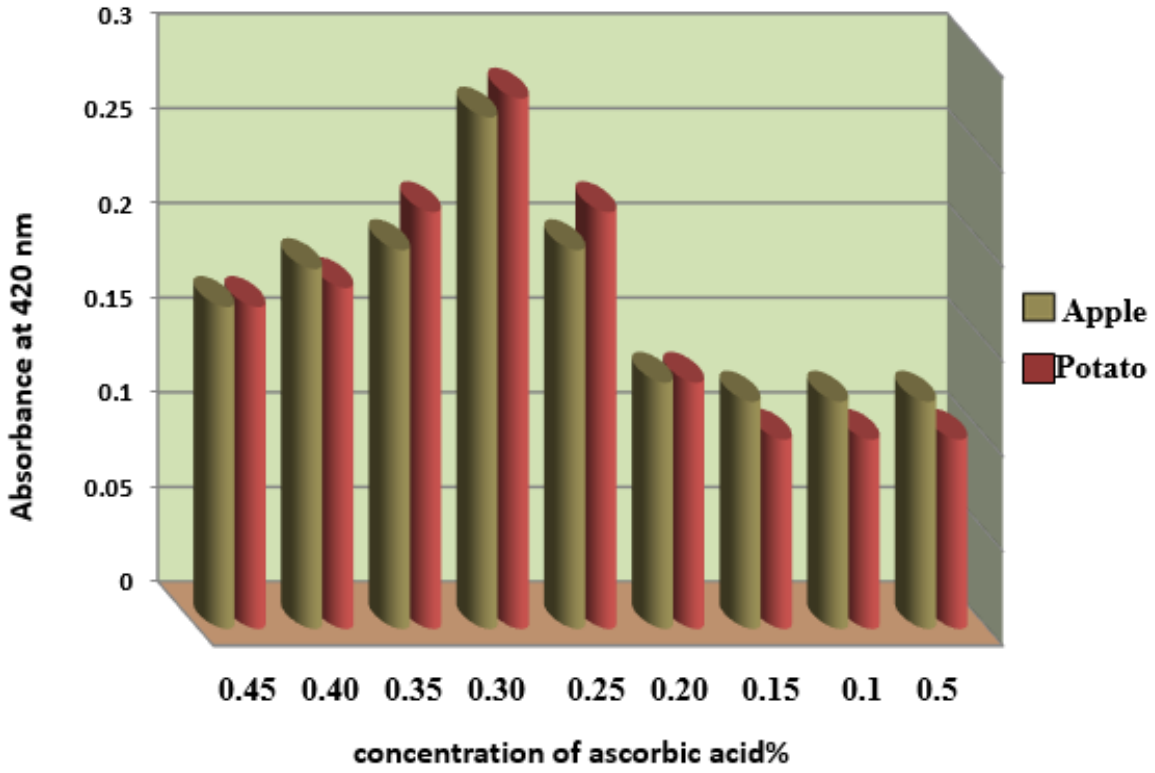

Figure 8. Effect of ascorbic acid concentration on the activity of PPO enzyme for apple and potato.

Effect of citric acid on the enzyme activity (PPO)

Figure (9) displays the extent to which Na_2SO_3 inhibits PPO activity. PPO activity falls off as concentration rises. At 0.25 Na_2SO_3 concentration, the inhibitory effect is at its strongest. The inhibitory effect holds steady as the concentration rises when the concretion exceeds 0.25%. Therefore, 0.25% is the ideal Na_2SO_3 concentration. Because of its potent inhibitory and color-protective properties, sulfur dioxide, also known as sulfite, is frequently used to stop apple and potato enzyme browning.

They have the ability to either act directly on the enzyme to decrease the catalytic reaction activity to monophenolase and diphenols, or they can integrate irreversibly with the quinines produced by the initial reaction to produce colorless matter. Meanwhile, the bleaching and microbial growth-inhibiting properties of Na_2SO_3 are present. Furthermore, and this result agreed with (13).

Submitted: 00-00-2024 Accepted: 00-00-2024 Posted: 00-00-2024, Vol. (XXIII)

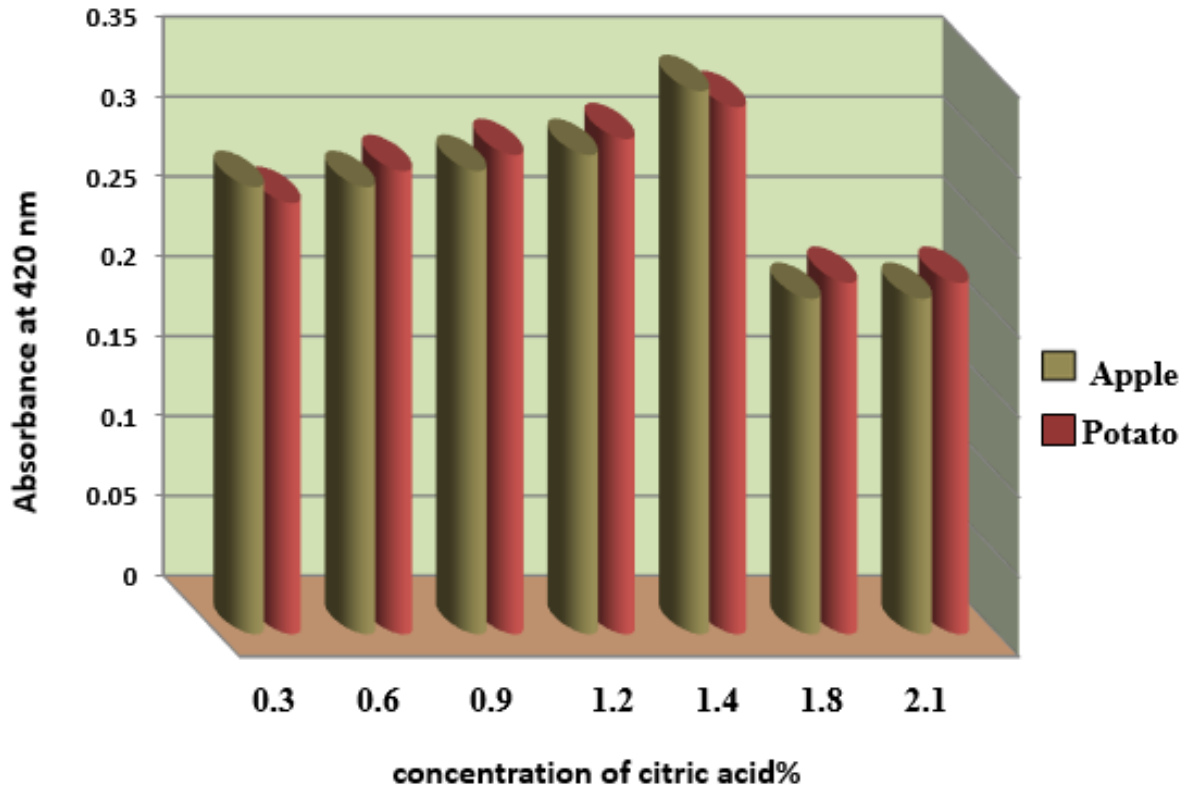

Figure 9. Effect of citric acid concentration on the activity of polyphenol oxidase enzyme for apple and potato

Effect of sodium thiosulfate (Na_2SO_3) on the enzyme activity (PPO)

As can be seen in Figure (10), Na_2SO_3 effectively inhibits PPO activity. With an increase in concentration, PPO activity falls. At 0.25 concentrations of Na_2SO_3, the inhibitory effect is at its strongest. The inhibitory effect does not change as the concentration rises when it is greater than 0.25%. Therefore, 0.25 percent is the ideal Na_2SO_3 concentration. Because of its potent

inhibitory and color-protective properties, sulfur dioxide, also known as sulfite, is frequently used to stop apple and potato enzyme browning.

They can either act directly on the enzyme to decrease the catalytic reaction activity of monophenolase and diphenols, or they can irreversibly integrate with quinines generated from the first reaction and produce colorless matter. Both bleaching and growth-inhibiting properties are exhibited by Na_2SO_3. Furthermore, and this result agreed with (10).

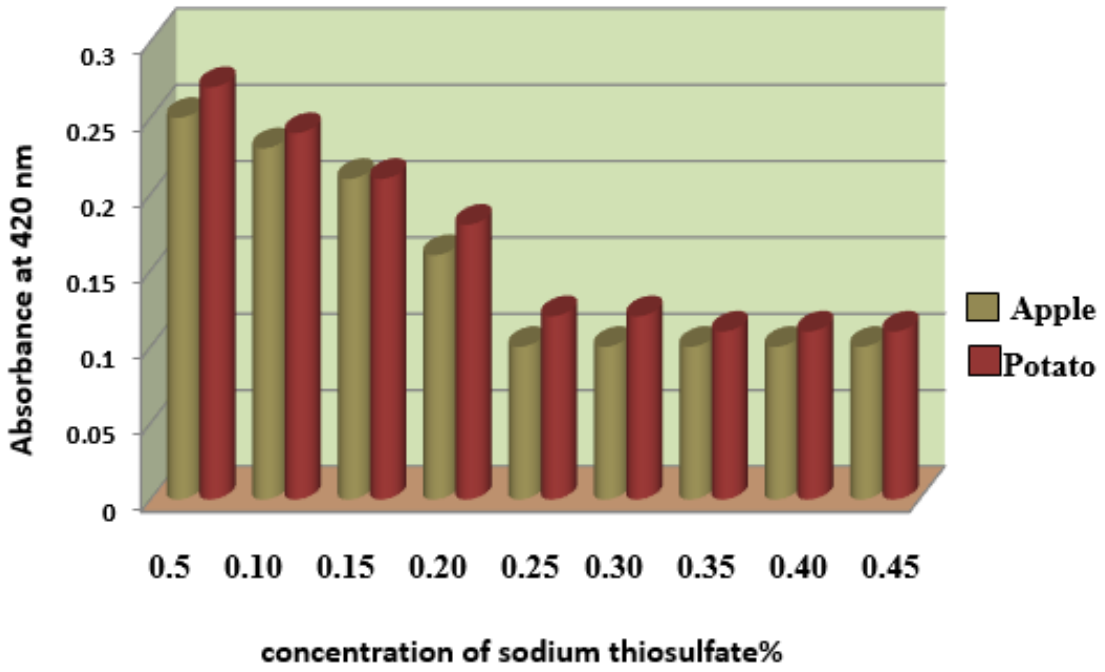

Figure 10. Effect of sodium thiosulfate concentration on the activity of polyphenol oxidase enzyme for apple and potato.

4. Conclusion and future scope

Through the experiment, the characteristics of the polyphenol oxidase enzyme were shown in both apple and potato, where the optimum temperature was 35 °C, the optimal pH value was 6.5, and the optimal concentration of the substance on which the enzyme worked was 0.8% (O-dihydroxybenzene), as the activity of the enzyme (PPO) was influenced by a Temperature and pH and the specialized reaction substance on which the enzyme works and some chemical compounds, and we take these factors into account to resist these enzymes that cause enzymatic browning and undesirable flavor in processed fruit products by adjusting the pH number from the optimum level for the enzyme to work by adding appropriate organic acids or using temperatures High to stop the activity of these enzymes when preparing fruits for manufacturing processes or by using some substances that inhibit the activity of these enzymes such as some sulfur compounds such as sulfur-bisulfite salts or meta-bisulfite for sodium salts, as well as using organic acids such as sodium thiosulfate, ascorbic acid NaCl and citric acid.

Conflict of Interest

There are no disclosed conflicts of interest for authors.

Acknowledgements

The author would like to thank the College of Agriculture, University of kufa for their facilities in using the food science laboratories.

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