EXTRACTION AND CHARACTERIZATION OF POLYPHENOL OXIDASE FROM APRICOT, APPLE, EGGPLANT AND POTATO

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ABSTRACT

Polyphenol oxidases (PPOs) from the fruits of apricot, apple, eggplant and potato tubers were extracted by homogenization with potassium phosphate buffer followed by precipitation with 1.5 volumes of cold acetone. Some properties of the isolated PPOs were studied. The optimum pH for potato PPO activity was found to be 6.4 while it was 7 for the other fruits PPOs. Optimum temperature of activity was 20^{o}C for apricot and apple PPOs, while it was 22^{o}C for eggplant and potato PPOs. Potato PPO was the most thermostable followed by eggplant, apricot and apple PPOs. The enzymes from the four sources were stable at neutral pH values at room temperature. Their stabilities decreased sharply at pH below 5. Storage of the enzymes solutions at 4 and -18^{o}C at pH 7 for 3 months indicated that potato PPO possessed the highest stability followed by apricot, eggplant and apple PPOs. K_m values toward the substrate catechol were 4, 4.16, 1.25 and 2.4 mM for PPOs of apricot, apple, eggplant and potato, respectively.

INTRODUCTION

Polyphenol oxidase (PPO) (monophenol, dihydroxyphenylalanine, oxygen oxidoreductase, E.C. 1.14.18.1) is a copper enzyme that catalyzes two distinct reactions involving molecular oxygen as a co-substrate, namely 1: the o-hydroxylation of monophenols to o-diphenols, (cresolase activity); and 2: the subsequent oxidation of o-diphenols to o-quinones, (catecholase activity) which are subsequently polymerized into red, brown or black pigments (Ziyan and Pekyardimci, 2003). This enzyme is almost found in all living organisms including plants, animals and microorganisms. In plant, it is involved in defense mechanism. When a plant gets a bruise or cut, certain phenolic compounds are oxidized in the presence of oxygen to form a polymeric structure which prevents microbial contamination (Whitaker, 1994). The catalytic action of polyphenol oxidase is connected to undesirable browning and off-flavor generation in stored and processed foods. On the other hand, PPO has been also shown to have important applications such as the use in the synthesis of valuable added products like the substituted catechol, L-DOPA for the treatment of Parkinson's disease (Pialis and Saville, 1998). A number of other catechols have found applications as fine chemicals or as starting materials for pharmaceutical drug synthesis (Halder *et al.*, 1998). PPOs also play an important role as efficient reagents for cleaning polyphenols-containing wastewater (Freire *et al*., 2002).

In the view of the increasing commercial applications of PPO in various fields and the development of more effective preservation conditions and methods in order to prevent the enzymatic browning, the properties of PPO from its various sources need to be studied. The objective of our study was to extract and characterize polyphenol oxidase from apricot, apple, eggplant and potato.

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MATERIALS AND METHODS

Fresh and mature fruits of apricot (*Prunus armeniaca* L.), apple (*Malus pumila*), eggplant (*Solanum melongena* L.) and potato tubers (*Solanum tuberosum* L.) were purchased from the local market. Catechol and sodium metabisulphite were purchased from Fluka Co. Potassium dihydrogen phosphate, dipotassium hydrogen phosphate and acetone were obtained from Riedel-DeHaen Co. All other chemicals were analytical grade.

Preparation of crude PPO extracts: The enzyme was extracted by homogenizing 20 gm of sliced fruits with 250 ml cold potassium phosphate buffer (0.2 M, pH 7). The homogenate was filtered through cheese cloth and centrifuged at 5000 g for 10 minutes. The enzyme was precipitated from the supernatant by adding 1.5 volumes of cold acetone (-5^{o}C) with gentle stirring for 60 minutes. The mixture was centrifuged at 10000g for 15 minutes and the precipitate was dissolved in 50 ml of potassium phosphate buffer. This crude enzyme extract was used for the enzyme characterization.

Enzyme activity assay: PPO activity was assayed by measuring the increase in absorbance at 420 nm using a spectrophotometer (Cecil 1021 UV/Vis). Catechol was used as the substrate following the method described by Shi *et al*. (2002). One unit of PPO activity is defined as the amount of enzyme that causes an increase in absorbance of 0.001/minute.

Determination of optimum temperature: The activity of PPO was measured at different temperatures ranged from 10 to 30^{o}C.

Determination of optimum pH: The rate of catechol oxidation by PPO was estimated in the pH range of 5.8-7.9 using potassium phosphate buffer (0.2 M).

Thermal stability: The enzyme solution was held in a water bath at 40, 50, 60 and 70^{o}C for 60 minutes. The residual activity was measured at the optimum temperature.

pH stability: The enzyme solution was held at pH values ranged from 4 to 9. Citrate- phosphate buffer was used in the pH range of 4- 5.5, potassium phosphate buffer in the range of 6-7.5 and Tris-HCl buffer in the range of 8-9. At the end of the incubation period, the pH of all enzyme solutions was brought to 7 and their residual activities were estimated.

Michaelis constant (K_m): The enzymatic activity was measured using substrate (catechol) concentration range of 1 to 20 mM. K_m values were estimated from Lineweaver- Burk plot.

Storage stability: The enzyme solutions were stored at 4 and -18^{o}C for 3 months. PPO activity was measured every 4 days for refrigeration storage and 8 days for freezing storage.

RESULTS AND DISCUSSION

Optimum pH of polyphenol oxidase activity: PPO activity was measured in the pH range 5.8-7.9 using potassium phosphate buffer (0.2 M) with 20 mM catechol as the substrate. Optimum pH was found to be 7.0 for apricot, apple and eggplant PPO and 6.4 for potato PPO (Fig. 1). As shown in the Figure, apricot and apple PPOs showed good activity in the neutral and alkaline pH while the activity sharply

declined in the acidic pH. The pH-activity profile of eggplant PPO was a typical bell-shaped curve. Potato PPO showed a good activity in the acidic pH values while lower activity was observed in the alkaline conditions. In general, most plant polyphenol oxidases show a maximum activity at neutral pH (Benjamin and Montomery, 1973; Siddiq *et al*., 1992; Unal, 2007; Yue-Ming, 1999). The optimum pH of PPO activity may vary depending on some factors such as the enzyme source, maturity of the fruit, extraction method, temperature, substrate and type and concentration of the buffer (Whitaker, 1994; Ziyan and Pekyardimic, 2004).

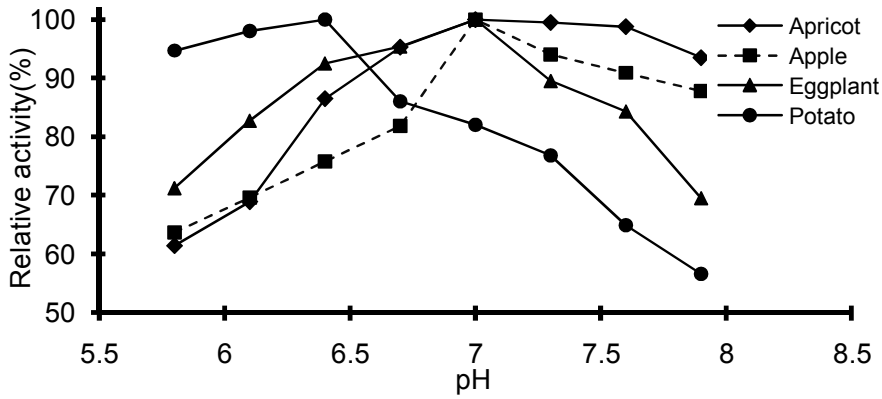

Fig. (1): Optimum pH of polyphenol oxidase activity.

Optimum temperature of polyphenol oxidase activity: PPO activity was assayed at temperature range of 10-30^{o}C. The activity was found to be remarkably affected by temperature (Fig. 2).

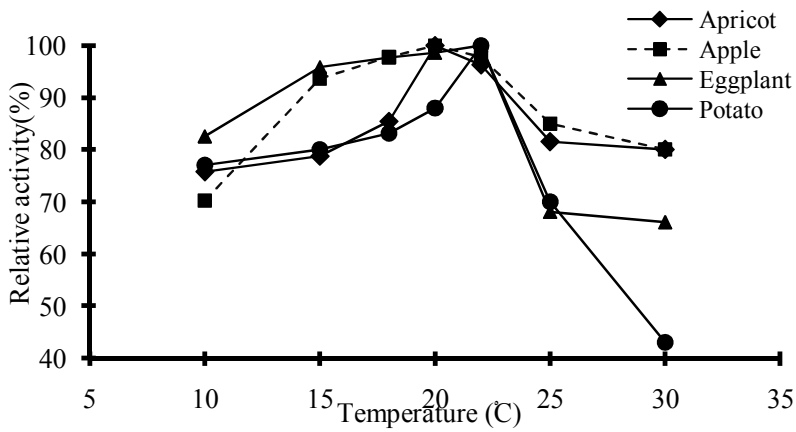

Fig. (2): Optimum temperature of polyphenol oxidase.

the enzyme isolated from apricot and apple showed maximum activity at 20^{o}C and for both eggplant and potato at 22^{o}C. Above these temperature values, the activity was rapidly decreased. Oktay *et al*. (1995) and Ziyan and Pekyardimic (2004) had reported optimum temperatures of 18 and 20^{o}C for apple and pear PPOs, respectively. Optimum temperature of polyphenol oxidase may change depending on the type of the used substrate. Ziyan and Pekyrdimic (2004) studied the effect of seven different substrates on the optimum temperature of Ankara pear PPO. They found that these temperatures ranged from 20^{o}C (for catechol as substrate) to

55°C (for D-tyrosine). The relatively high activity of PPO at low temperature and the availability of other conditions such as the presence of oxygen and mechanical damages in the crops may explain the occurrence of enzymatic browning in fruits and crops which are stored at these low temperatures.

Thermal stability: The results of the thermal inactivation of the PPO from the four sources are presented in Figure (3: A, B, C and D). The enzyme showed different thermal stabilities according to its source. Potato PPO was found to be the most stable followed by eggplant, apricot and apple PPOs. They retained 60, 36, 24 and 12% of their original activities after 60 minutes of incubation at 60°C, respectively. At 65°C, potato and eggplant PPOs retained 40 and 25% of their original activities

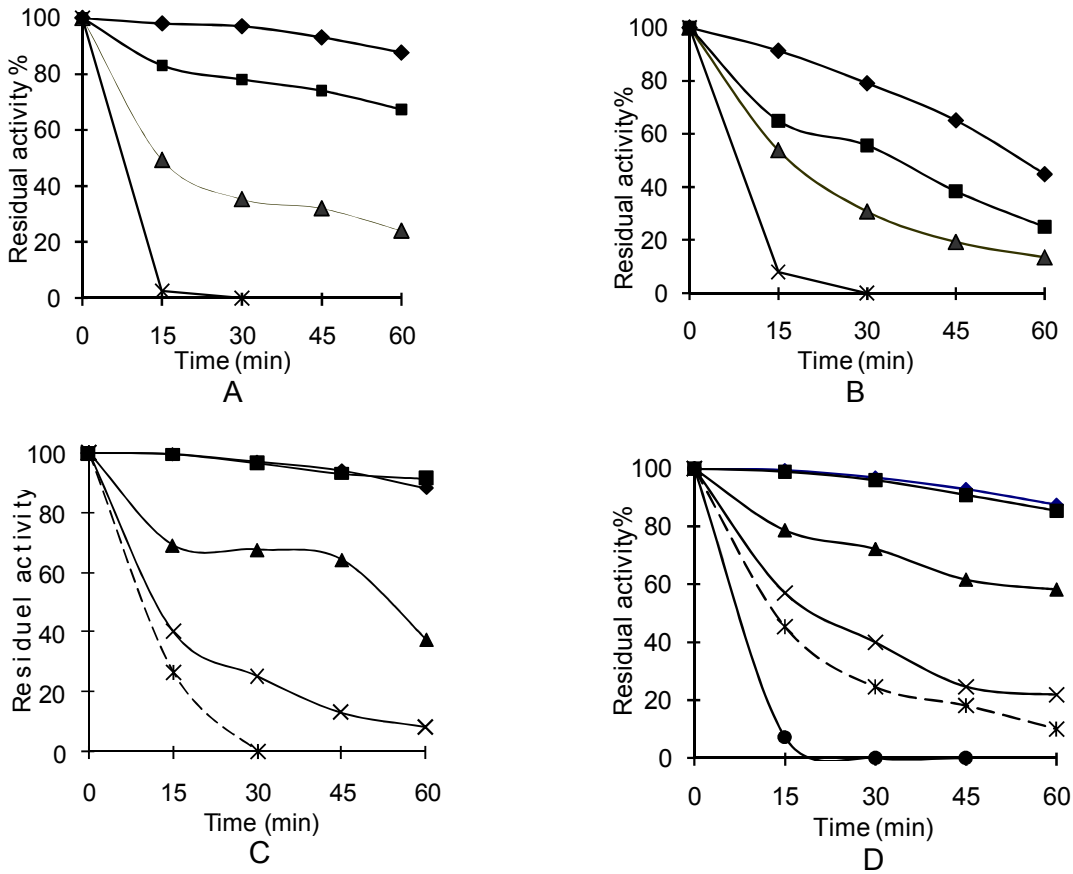

Fig. (3): Thermal stability of PPO (A: Apricot, B: Apple, C: Eggplant, D: Potato).
(–◆–40 °C, –■– 50 °C, –▲– 60 °C, –×–65 °C, –✳-70 °C, ● 75 °C)

over 30 minutes incubation, respectively, while apricot and apple PPOs had completely inactivated under the same conditions. Generally, it was noticed that vegetable PPOs (eggplant and potato) had better thermal stability than fruit PPOs (apricot and apple). It seems to be that the subjection of crops to heat treatment at 65°C for the fruits and 75°C for the vegetables for 15 minutes is sufficient to inactivate their PPOs and prevent enzymatic browning which may takes place through the manufacturing and storage operations of the crops.

Polyphenol oxidases from different sources were found to have different thermal stabilities. Allium PPO is a thermolabile enzyme. It was entirely inactivated above 40°C (Arslan *et al*., 1997). Longan fruit PPO has a moderate thermal

stability. Its half-life at 50°C was 20 minutes (Yue-Ming, 1999). Banana PPO is more stable. It retained its entire activity over 30 minutes of incubation at 55°C (Galeazzi *et al.*, 1981). The decrease in activity at elevated temperature degrees may be attributed to a dramatic change in the structure of the enzyme that hindered the availability of the active sites, with a possible denaturation of the enzyme itself (Kouassi *et al*., 2005).

pH stability: The crude PPOs of the studied fruits were kept at pH range 4- 9 for 60 minutes using suitable buffer solutions. Their residual activities were assayed at their optimum pH (Fig. 4).

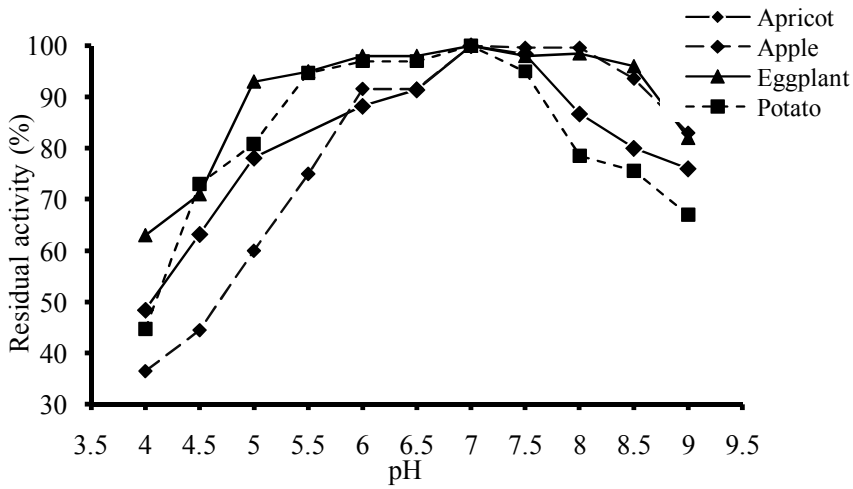

Fig. (4): pH stability of polyphenol oxidase.

The effect of pH on the stability of PPO was varied according to the source of the enzyme. Apricot PPO reached maximum stability at pH 7-7.5 and it showed a remarkable stability within the pH range of 5- 9 where it retained more than 75% of its activity. Apple PPO was stable in the pH range of 6-9 (80% activity retention). Eggplant PPO was characterized by a high stability in a wide pH range (5- 8.5). It retained about 90% of its original activity after 60 minutes of incubation. The optimum pH of stability for potato PPO was 7 with a good stability at pH values ranged between 5.5-7.5. However, its stability was sharply retarded in alkaline pH values. The effect of pH on the enzymatic activity and stability could be explained by the fact that the protein structure of an enzyme molecule is influenced by the alkalinity or acidity of the solution because its various amino acid residues are in different states of ionization (Kouassi *et al*., 2005). Enzyme from various sources may have different structures which cause different pH stabilities.

The common characteristic of the four studied PPOs was their low stability at pH values less than 5 where a fast denaturation of the enzyme molecules might take place. The same result was obtained by Lu *et al*. (2006) for Chinese water chestnut who suggested the immersion of the freshly cut fruits in acidic solution to inactivate PPO and prevent browning. The pH stability of an enzyme is considered as an important parameter for the determination of the conditions that should be available through isolation, purification, handling and storage of the enzyme. This stability is affected by some factors such as type, source and purity of the enzyme (Segel, 1976).

Storage stability: PPO solutions were stored at 4 and -18°C and the residual activity was measured to estimate the loss of activity during storage period (Fig. 5). Results indicated that potato PPO was the most stable followed by apricot, eggplant and apple PPOs. They retained 93, 93, 83 and 13.6% of their original activities over 32 days of storage at 4°C, respectively. At -18°C, the respective retained activities were 100, 99, 88 and 73% after the same storage period. Concerning the effect of

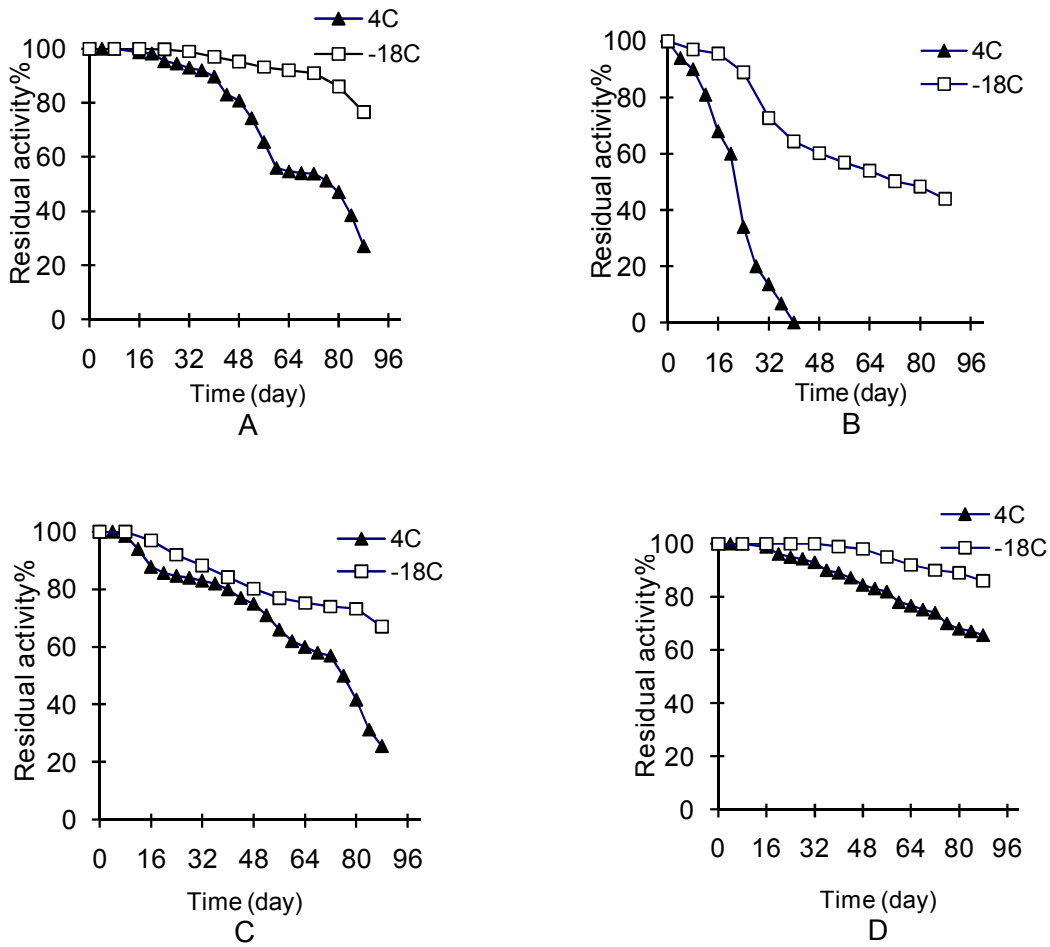

Fig. (5): Storage stability of PPO (A: Apricot, B: Apple, C: Eggplant, D: Potato).

storage temperature, freezing storage was found to maintain more activity in the storage period as compared with cooling storage.

Michaelis constant (K_m): To determine the effect of substrate (catechol) concentration on the PPO activity, the enzyme activity was assayed with substrate concentrations ranged from 1-20 mM. K_m values were calculated from Lineweaver-Burk plot and they are shown in the Fig. (6). They were found to be 4, 4.16, 1.25 and 2.4 mM for PPOs of apricot, apple, eggplant and potato, respectively.

As shown in the Figure, K_m values of apricot and apple PPOs were higher than those of eggplant and potato PPOs. This indicates that apricot and apple PPOs possessed lower affinity for catechol than eggplant and potato PPOs. The result for apple PPO K_m resembled that for Amasya apple PPO (4.6 mM) (Oktay *et al*,. 1995)

However, PPO isolated from another apple variety (Red Delicious) had a K_m value of 2.2 mM (Satjawatcharaphong *et al*., 1983).

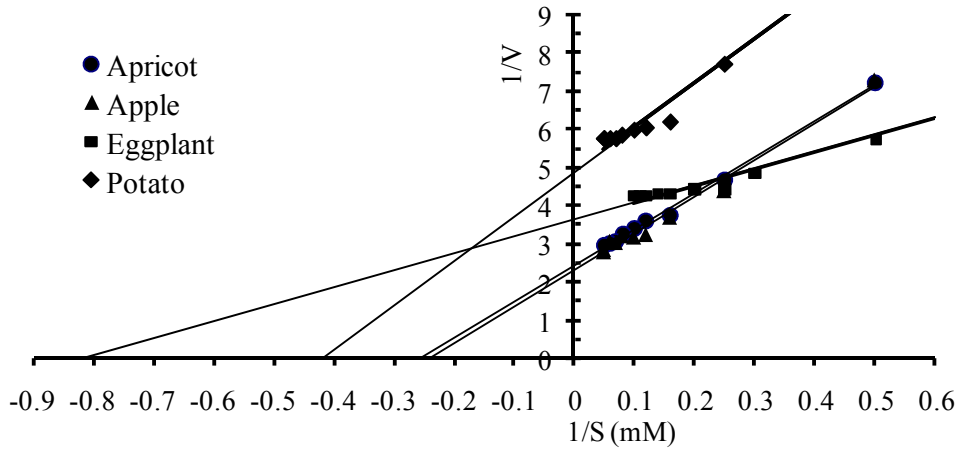

Fig. (6): Lineweaver-Burk plot of polyphenol oxidase.

إستخلاص وتوصيف إنزيم البولي فينول اوكسيديز من ثمار المشمش والتفاح والباذنجان والبطاطا

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الخلاصة

تم إستخلاص إنزيم البولي فينول أوكسيديز من ثمار كل من المشمش والتفاح والباذنجان والبطاطا بهرس الثمار مع محلول فوسفات البوتاسيوم الدارئ ثم رسب الإنزيم بإضافة حجم ونصف من الأسيتون البارد. تمت دراسة بعض صفات الإنزيم المعزول كان الاس الهيدروجيني الامثل لفعالية بولي فينول أوكسيديز البطاطا ٦.٤ ولبقية الثمار ٧.٠. بلغت درجة الحرارة المثلى لفعالية الإنزيم المعزول من كل من المشمش والتفاح ٢٠مْ ومن الباذنجان والبطاطا ٢٢مْ. لوحظ وجود ثبات حراري عالي للانزيم المستخلص من البطاطا تلاه الباذنجان فالمشمش واخيراً التفاح. أبدى الإنزيم المعزول من المصادر الاربعة ثباتاً جيداً عند الأس الهيدروجيني المتعادل بدرجة حرارة الغرفة وانخفض الثبات بصورة واضحة عند القيم التي تقل عن ٥. بينت نتائج خزن الانزيم بدرجتي حرارة ٤ و - ١٨مْ عند الأس الهيدروجيني المتعادل أن بولي فينول أوكسيديز البطاطا قد إمتلك أعلى ثباتية خلال الخزن تلاه المشمش فالباذنجان وأخيراً بولي فينول أوكسيديز التفاح. بلغت قيم ثابت ميكيلس (K_m) تجاه الكاتيكول (catechol) كركيزة للانزيم ٤ و ٤.١٦ و ١.٢٥ و ٢.٤ ملليمولر للإنزيمات المستخلصة من كل من المشمش والتفاح والباذنجان والبطاطا وعلى التوالي.

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