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Extraction conditions of polyphenol oxidase from banana peel

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Abstract:

Polyphenol oxidase (PPO) is an enzyme containing copper, presents in various fruits and vegetables. It is responsible for the browning reactions when the cells are damaged during handling. The best conditions for extraction of polyphenol oxidase from banana peel was by using an extraction buffer containing phosphate buffer (0.05 M, pH 7), 0.01 M ascorbic acid and 0.5% polyethylene glycol, with extraction ratio 1:4 (w:v) for one minute by using blender. The enzyme activity was measured spectrophotometrically at 425 nm. PPO was studied to prevent the browning of banana peel which results in the loss of their marketability. The aim of this study was to determine the optimum conditions for polyphenol oxidase extraction from banana peel.

Key words: Polyphenol oxidase; Banana peel; Extraction.

Introduction:

Polyphenol oxidase (PPO) (EC 1.14.18.1) is a nuclear encoded enzyme that catalyzes the phenolic compounds oxidation forming corresponding quinone intermediates that polymerize to form undesirable pigment [1]. Two types of the oxidative reaction are catalyzed involving molecular oxygen: the hydroxylation of monophenols to o-diphenols and the oxidation of O-diphenols to O-quinones, leading to black or brown pigments formation. Polyphenol oxidase is a copper-containing enzyme which is probably present in all plants [2]. PPO is a widely distributed enzyme which is involved in biosynthesis of melanins in animals and in browning of plants. PPO plays a great role in plant tissues browning have been purified from a number of fruit tissues and their properties studied. In order to determine how to prevent the browning which results in the loss of banana peel marketability, PPO has been widely studied in many fruits and vegetables. PPO levels in a plant increase when a plant is wounded or infected [3]. Multiple forms of enzyme are shown to exist from many sources. Banana is an important food sources in the developing world and one of the most important worldwide crop. It is good nutritional and health value. This enzyme is found almost in all living

organisms including plants, animals and microorganisms. PPO is involved in plant defense mechanism. When a plant bruises or cut, phenolic compounds are oxidized in the presence of oxygen forming polymeric structure preventing microbial contamination [4]. PPO has been also shown to have important applications such as using in valuable added products synthesis like the substituted catechol, L-DOPA for the Parkinson's disease treatment. Other catechols have found applications as starting materials or as fine chemicals for pharmaceutical drug synthesis. PPO plays an important role as an efficient reagent for cleaning polyphenols-containing wastewater. Considering 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 [5]. The aim of this study was to characterize PPO from banana peel in terms of best source, extraction ratio, extraction time, pH and type of extraction buffer.

Materials and Methods:

Chemicals:

Catechol, polyethylene glycol (PEG), Coomassie brilliant blue, bovine serum albumin were obtained from Sigma Co. other chemicals were supplied by BDH Chemicals.

Enzyme Extraction:

Banana fruits were purchased in the yellow-green stage of ripening a local market. All experimental procedures were carried out at 4 °C. preparation of crude extract was as follows, 25 grams of banana peel were homogenized in 100 mL of phosphate buffer (0.05M, pH 7) containing ascorbic acid (0.01 M) and (0.5%) polyethylene glycol, and extraction by using blender for 1 min. Crude extract was filtered, and then the filtrated was centrifuged at 10000 rpm for 15 min at 4 °C. and suspension was used as crude enzyme [6].

Estimation of PPO Activity:

Catechol was used as substrate to determine enzyme activity. The cuvette containing the sample contained 2.9 mL of (0.01 M) substrate in phosphate buffer (0.05 M, pH 7.0) and 0.1 ml of the enzyme. 3 mL of substrate solution was used as blank sample. One unit of PPO activity was defined as the amount of enzyme that caused an increase in absorbance of 0.001/min. Calculation of polyphenol oxidase activity was used by the following equation:

Activity of PPO ($U.ml^{-1}$) = [(A2 sample – A1 sample) – (A2 blank – A1 blank)] / (0.001× t)

Where, A2 sample is the final absorbance of the sample, A1 sample is the initial absorbance of the sample; A2 blank is the final absorbance of the control, A1 blank is the initial absorbance of the control, and t is the reaction time in minutes (3 minutes) [1].

Protein concentration determination:

Protein concentration was determined according to the method of Bradford using the standard curve of bovine serum albumin [7].

PPO Source:

Different sources were used for extraction of PPO. These sources were banana pulp, banana peel, apple, Jerusalem artichoke, potato and kiwi. All sources were washed with tab water then extraction of enzyme. The enzyme activity and concentration of PPO were determination [8].

Extraction condition of enzyme:

Extraction ratio:

A 25 grams of banana peel were homogenized in different volumes of solution extract (0.05 M phosphate buffer pH 7, 0.01 M ascorbic acid and 0.5% polyethylene glycol) for extraction of PPO enzyme. The extraction ratios were 2:1, 4:1, 6:1, 8:1 and 10:1 (v:w).

The enzyme activity and protein concentration was determination.

Extraction time:

Used different time for extraction of PPO enzyme. Banana peel was homogenized in solution extract (0.05 M phosphate buffer pH 7, 0.01 M ascorbic acid and 0.5% polyethylene glycol) at proportion 4:1 (v:w) using blender for different periods, 0.5, 1, 3, 5 and 7 minutes. The enzyme activity and protein concentration were estimate in each period.

Type of extraction buffer:

Banana peel was homogenized with different types of buffers without ascorbic acid and polyethylene glycol for PPO extraction. These buffers are sodium acetate at concentration 0.1, 0.2 and 0.05 M at pH (4.5, 5 and 5.5 for each concentration), sodium phosphate 0.1, 0.2 and 0.05 M at pH (6, 6.5), potassium phosphate 0.1, 0.2 and 0.05 M at pH (7 and 7.5), and Tris-HCl 0.1, 0.2 and 0.05 M at pH (8, 8.5 and 9).Also used water tap for extraction of PPO enzyme from banana peel. The activity and concentration were estimate in each treatment [8].

The effect of poly ethylene glycol, ascorbic acid and polyvinylpyrrolidone on PPO enzyme extraction:

Banana peel was homogenized with potassium phosphate (0.05 M, pH 7), and one of each from the following solutions separately:

- Ascorbic acid 0.01 M
- Poly ethylene glycol 0.5 %
- Polyvinylpyrrolidone 0.5 %
- Poly ethylene glycol 0.5 % + Ascorbic acid 0.01 M
- Polyvinylpyrrolidone 0.5 % + Ascorbic acid 0.01 M

Then these solution were extracted at ratio 4:1 (v:w) using blender for 1 min. The enzyme activity and protein concentration were estimate in each solution.

Results and Discussions

PPO Source:

The sources were used in extraction of PPO showed different specific activities.as shown in Figure (1), the results of the effect of the plant source type on the specific activity of enzyme.

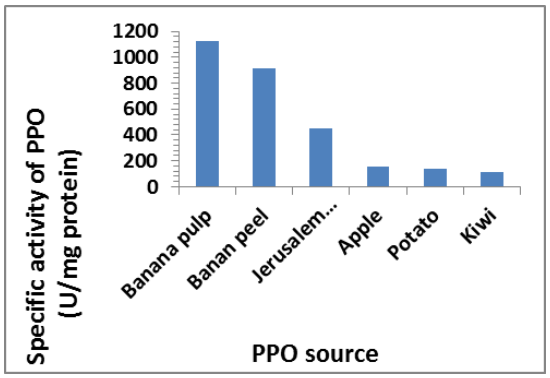


Fig. (1): Different courses for PPO extraction.

This figure shows that polyphenol oxidase from banana pulp gave highest specific activity 1127 U/mg, while banana peel, apple, jerusalem artichoke, potato and kiwi were 911.7, 157, 454, 137 and 112 U/mg, respectively. Banana peel was used as source of PPO because it's available, waste and inexpensive. Miyawaki, [9] found that the apple was best plant source of production PPO, while Kaviya, *et. al.*, [10] provide that banana peel was best source for PPO.

Extraction ratio:

Five extraction ratios were examined to determine the best proportion for PPO extraction. Figure (2) shows that 4:1 (v:w) was the best ratio, with specific activity 988.5 U/mg, while 2:1 (v:w) ratio was 521 U/mg. Other ratios were 6:1, 8:1 and 10:1, (v:w) with specific activity 843.5, 333.3 and 164.7 U/mg respectively, because the enzyme was diluted. Rocha, *et. al.*,[11] found that 3:1(v:w) best extraction ratio for PPO extraction from apple, while Yagar, and Sagiroglu [12], found that best extraction solution ratio for PPO extraction from quince was 1:1 (v:w).

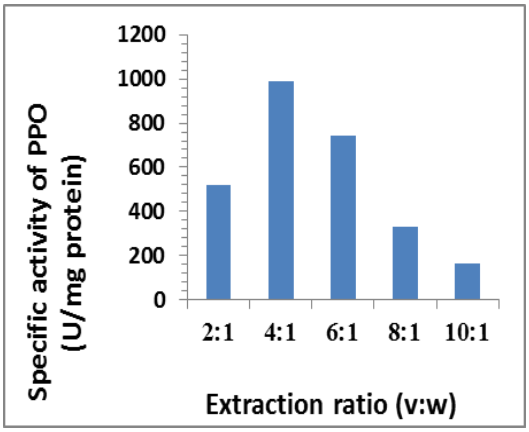


Fig. (2): The effect of extraction ratio for PPO extraction from banana peel.

Extraction time:

Different times for PPO extraction were determined (figure 3). The best time was after one minute with specific activity 981 U/mg, while in 0.5 min., the specific activity of enzyme was decreased and reduced to 688.4 U/mg, and the reason is the low release of enzyme from banana peel. While 3, 5 and 7 minutes showed low specific activity 503.9, 456.3 and 431.4 U/mg respectively, increasing temperature for blender, causes effect on total activity for PPO enzyme. The decrease in activity at elevated temperature degrees may change the structure of the enzyme that blocks the active sites, with denaturation of enzyme [13]. This result agrees with the results of Manohan and Wai [8].

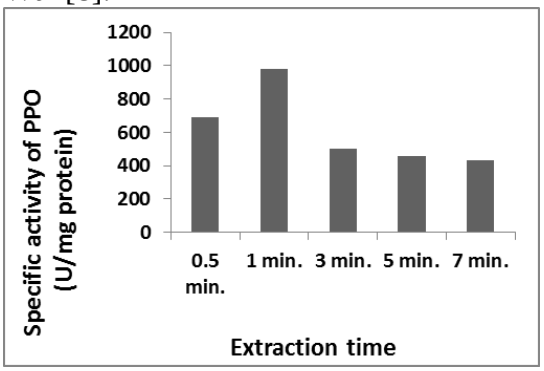
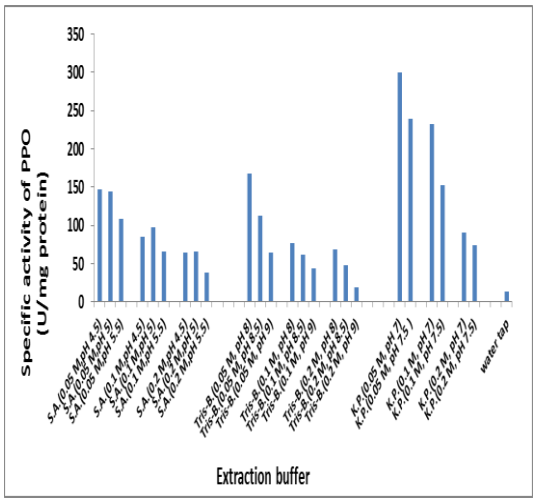


Fig. (3): The effect of extraction time for extraction of PPO from banana peel.

Type of extraction buffer:

The specific activity PPO was estimated after extraction using different buffer, and the results were illustrated in figure (4)**.** These results show that potassium phosphate buffer (0.05 M, pH 7) was best extraction buffer with specific activity 300 U/mg, while other buffer with different concentration and pH were given low specific activity. pH effect on enzymatic activity and stability can explained by the fact that protein structure of an enzyme molecule is influenced by the acidity or alkalinity of the solution because of the differences in ionization state of the various amino acid residues [14]. The pH of enzyme environment affects the activity of the enzyme in several ways. First each enzyme has its own optimum pH, at which the maximum enzyme activity, but the enzyme is stable within certain limits under and above the optimum. Secondly, enzyme stability is influenced by environmental pH, at extremes acidity or alkalinity the enzyme may be denatured. Thirdly, the reaction mixture pH may effects on association of the substrate with the enzyme [15].

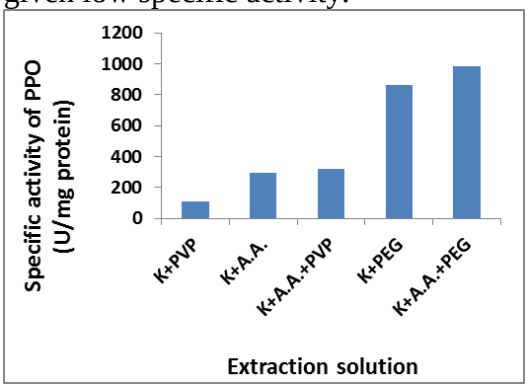


S.A.= Sodium acetate, Tris-B.= Tris-base, K.P.= Potassium phosphate.

Fig. (4): The effect the type of extraction buffer for PPO extraction from banana peel.

The effect of poly ethylene glycol, ascorbic acid and polyvinylpyrrolidone on PPO enzyme extraction:

Different types of extraction solutions were used for PPO extraction from banana peel. The results showed (figure 5) that potassium phosphate buffer contains ascorbic acid and polyethylene glycol was best extraction solution for PPO extraction from banana peel, with specific activity 985.7 U/mg, while other types of extraction solution were given low specific activity.



K= potassium phosphate. **A.A.**= ascorbic acid. **PVP**= Polyvinylpyrrolidone. **PEG**= polyethylene glycol.

Fig. (5): The effect extraction solution for extraction of PPO from banana peel.

Aydemir *et.al.* [**6**], provide that fresh sample of Jerusalem artichoke was homogenized in phosphate buffer containing polyethylene glycol and ascorbic acid, to give best crude enzyme. While Kaviya *et.al.* [**9**], found that best extraction solution for extraction PPO enzyme from banana peel was containing sodium phosphate buffer, Poly vinyl pyrolidone and Triton X-100.

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ظروف استخلاص انزيم بولي فينول اوكسيديز من قشور الموز

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الخلاصة:

بولي فينول اوكسيديز من الانزيمات المحتوية على النحاس والتي توجد في مختلف الفواكه والخضروات. هذه الانزيمات تكون مسؤولة عن ظهور اللون البني عندما تتعرض الخلايا الى الجروح خلال المحمل (النقل). تحديد الظروف المثلى لاستخلاص انزيم البولي فينول اوكسيديز من قشور الموز باستعمال دارئ الاستخلاص المتكون من دارئ فوسفات البوتاسيوم بتركيز 0,05 مولر ورقم هيدروجيني 7,0،وحامض الاسكوربيك اسد بتركيز 0,01 مولر و 0,5% بولي اثلين كلايكول وبنسبة استخلاص 1:4 (لحجم: وزن) لمدة دقيقة واحدة في الخلاط الكهربائي. تم قياس الفعالية الانزيمية باستعمال جهاز المطياف الضوئي وعلى الطول الموجي 425 نانوميتر. وقد دُرس انزيم البولي فينول اوكسيديز المستخلص من قشور الموز لتحديد كيفية منع ظهور اللون البني مما يؤدي إلى فقدان تسويقها. الهدف من هذه الدراسة هو تحديد الظروف المثلى لاستخلاص انزيم البولي فينول اوكسيديز من قشر الموز.

الكلمات المفتاحية: بولي فينول اوكسيديز، قشور الموز، الاستخلاص.