Extraction and purification peroxidase enzyme from Zahdi date seeds

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ABSTRACT

Peroxidase enzyme was prepared from germinated Zahdi date seeds (nuclei) which passed to young radicals as a source for the production of peroxidase converted to acetone powder instead of extraction from palm leaves or other plant sources. Method of extraction characterized simple and low-cost.

The results obtained showed that roots of seedlings contain a twice enzyme activity effectiveness than peroxidase of palm leaves. Soluble peroxidase activity was 296 400 units / g in acetone powder of roots, while it was 156 750 units / g in acetone powder of leaves. As well as access of enzyme peroxidase in any season in the year.

Soluble peroxide purified by several steps, the number of purification steps was 7.52 time and enzymatic recovery 27.6 at the end of gel filtration stage, results of purification indicated that there is a high affinity of purified enzyme towards substrate consisting of orthodainzdin and hydrogen peroxide.

INTRODUCTION

Characterized enzyme peroxidase it spread wide in nature, the diversity of forms in the types of the plant kingdom and within the same species and characterized his specialty wide toward the substrates and thus multiple types of metabolic reactions and processes physiological involving the growth and development of various plants (Kelly & Latzko 1979), also became the peroxidase in many plants from reliable indicator of genetic discrimination in the detection of stress in plants as well as follow-up and study the mechanism of the effect of pathogens on plant families (Lojkowska & Holuhowska 1992; Hassni, et. al, 2004). Enzymes peroxidases became important industrially and medically, peroxidase were gained (peroxidase hours radish wild HRP) great economic importance through its uses in the number of diagnostic and analytical varied because of his qualities kinetic and physical and chemical suitable for such applications and perhaps the most important number is the number of ELISA (Enzyme -Linked Immuno Sorbent Assay) in labeling antibodies or antigens in the immune reactions by attaching these enzymes on solid surfaces as it is the specifications that qualify for that (Avrameas & Guibert, 1972) one of these properties the qualities high affinity toward the material basis, ease of detection effectiveness configures outputs of color do not need a process to measure the steps separating from substrates, high persistence during storage, low costs prepared and purified (Tijseen, 1985) as well as its importance in the analysis of the stigma blot assays and in pigmentation tissue (Belonogova & Mekler, 1996), it is also used in biochemical analysis to estimate the hydrogen peroxide generated by some systems, such as the oxidation of glucose, amino acids and cholesterol .... etc. through the electrode specialist for enzyme or with other enzymes (Lin, et. Al, 1990), also reflected the importance of these enzymes in the processing of fruits and vegetables by evaluating the content extracts of food stuff from antioxidants such as ascorbic acid, phenols, flavonoids and tannins, which working on multiple modifications during the manufacturing process and storage (Cano, et.al, 1996).

As well as the economic importance of the palm and its products (dates), the Palm side-off products have many uses, moreover the issue of the exploitation of the nuclei of the surplus dates for the need to produce materials with economic value be of great benefit and add value to palm and their products.

So it is appropriate to identify the nature of the peroxidase enzyme in roots and palm seeds and determine the appropriate conditions for the extraction and identification of suitability for analytical uses, where it is known that peroxidase of the palm roots was used in assessing activity of the glucose oxidase enzyme GOD, also used to measure the concentration of blood glucose and some other sugars in food samples with the participation glucose oxidase enzyme, also used to investigate the efficiency of the processing of canned fruits and vegetables, juices, by assessing the antioxidants content (Dalali,1983).

Hydrogen peroxide works as a recipient while working AII2 as donor and oxygen was not the result of the reaction, and that enzyme works includes four main types of activities: Peroxidatic, Oxidatic, Catalaitic and addition a hydroxyl group (Hydroxylation), peroxidase works on the oxidation of many phenolic compounds by its Peroxidatic activity like Guaicol, Res orcinol and Aniline ... etc. (Whitaker, 1972) when these compounds act as substrate. The Oxidatic activity were founded when the substrate occurred, such as acid dihydroxy fumaric or ascorbic acid or indole acetic acid (1AA) with oxygen (Helser, 2008), can also peroxidase added a hydroxyl group to the number of aromatic molecules such as tyrosine and phenylalanine and benzoic acid and salicylic acid (Buhler & Mason, 1961).

Peroxidase and its purified isomers from plant sources consist colorless glycoprotein which is associated with Ferriporphyrin each one molecule per in peroxidase owns one group of Ferriporphyrin 111 which distinguishes this group (prosthetic group) being strongly linked with protein part in the enzyme (Gaspar, et.al, 1982). Focused the vast majority of studies on the production of peroxidase from horseradish roots and wild rape and fig juicer fig (Paul, 1986), while not available for detailed studies of peroxidase palm.

The research aims to exploitation seeds Zahdi date remaining as by-products from factories of syrup and honey dates by germinated seeds then extract peroxidase of the seed roots and purified, due to the abundance and activity and possibility of getting it in any season of the year compared with peroxidase extracted from other plant sources.

MATERIALS AND METHODS

Materials

Seeds (nuclei's) of Zahdi date, sodium hydroxide solution (10 molar), large bowl covered with large size filter paper, then moisturizer with water, sought to spread 60-75 seed / 30 cm (diameter dish),sodium acetate buffer solution (1molar) with pH 6 .

Solution potassium phosphate buffer (0.005 molar), O - Dinazidine concentration solution (23 mM) and glucose oxidase enzyme solution.

Methods

- Create a seedling : Zahdi date seeds was germinated after being washed with distilled water and immersed in solution sodium hydroxide (10 molar) for two minutes and then washed with tap water running for the next day (Abdul - Wahab, et, al, 1976) and then put them in a large bowl (30-50 cm) covered with a large filter paper moisturized with distilled water and then covered with another layer of filter paper and incubated at 30-35 C° for a period of 4 weeks was then obtained during the seedling roots along at a rate of 10-15 cm approx.
- Preparation acetone powder: separate roots from seeds and then placed directly in acetone for crud extract, mixed roots with acetone by ratio 1:10 (w/v) then mixed for 5 minutes by blender, and repeated the process twice and equally acetone volume under cooling conditions then filtered mixture using a Buchner funnel and filter paper installed on the vacuum pump . Wash the precipitate by adding sufficient amount of cooled acetone. Then drying under vacuum for several hours and saved for the purpose of extraction peroxidase .
- Extraction peroxidase enzyme: peroxidase extracted from of acetone powder of the roots (crude extract) from the last step by mixing with sodium acetate buffer solution by ratio 1:20 (w / v) .
- Determination peroxidase activity: enzyme activity determined according to method (Whitaker & Bernhard, 1972) by reaction of substrate consisting of O-dinazidine and hydrogen peroxide with a tris solution, and then measure the beginning of the reaction by adding 0.1 ml of enzyme extract, followed the change in the absorption at a wavelength 436 nm every 30 seconds for a period of 3 minutes for 7 readings .
- Purification of peroxidase enzyme: crud extract of soluble peroxidase obtained from acetone powder was purified by several steps included precipitation by ethyl alcohol with ratio ranged between (40-75 %) and then done ion exchange chromatography for enzyme solution by using column of ion exchange (D- Ethyl Amino Ethyl – Cellulose) dimensions (1.6 × 20 cm), which preceded equilibrated with solution buffer of potassium phosphate (0.005 molar) pH 8, then recovered enzyme solution with balance solution for unlinked parts with exchanger and gradient saline solution of sodium chloride ranged from (0 – 1 molar).

- Using peroxidase enzyme in determination of glucose:

A . Prepared solutions of glucose solution ranged between (1 -2 mg / ml) of standard glucose.

B . Diluted solution of O-dinazidine by 100 times buffer solution (pH 6) and ionic forces (0.15 molar) according to the method used by (Eills & Morrison, 1982) and consisting of three types of buffer solutions: Sodium acetate, Sodium phosphate and Tris, with continuous stirring (Tzouwara-Karayanni &Crouch,1990).

C . Added 0.5 ml of glucose solution prepared previously, blood serum and grape juice after diluted to 2.4 ml with solution of O-dinazidine in cell reaction and then added 0.1 ml of purified peroxidase (enzymatic activity 6 units / ml) then added 0.2 ml of a solution of purified glucose oxidase enzyme (enzymatic activity 200 units / ml) and then read on the absorbance at wavelength 436 nm (Whitaker & Bernhard,1972).

RESULTS AND DISCUSSION

Followed different methods and means to extraction and purification peroxidase enzyme from its sources, results listed in Table 1 shown that extraction of soluble peroxidase by buffer sodium acetate solution (1 molar) at pH 6 from acetone powder of roots was the best method compared with others results of peroxidase activity in the crude extract was 296400 units / g of acetone powder of roots, while peroxidase activity was 156750 units / g of acetone powder of leaves. The superiority of this method used for extraction due to the high efficiency of the extraction due to the nature of the tissues of the palm and its fibrous composition which was characterized with high resistance to cracking and crushing (Al-Bakir and Whitaker, 1978). These results agreed with results obtained about low activity for peroxidase from date leaves and other plant tissues which extracted by buffer solutions with different ionic forces (Baaziz and Saaidi, 1988). Moreover, the high specific activity for enzymatic extracts of acetone powder of roots and leaves by increase the ionic strength of the solution extraction return to the nature of the links enzyme with cell components (Yoon, et.al, 1993), which requires increasing the ionic strength of the extraction solution to realized those linkages emerging between the enzyme and pictic substances or others cellular parts (Silva, et. al, 1990).

Soluble peroxidase enzyme was purified by steps included concentrated by precipitation with ethyl alcohol (40-75 %), results listed in table 2 showed that the number of purification times was 1.89 and enzymatic recovery increase to 44.4% . Next step was dialysis of enzymatic solution versus distilled water to discard of the remnants of alcohol and some insoluble compounds reaching the number of the purification times to 2.9 with enzymatic recovery 34.4 %, followed by the step of ion exchange chromatography by column of di- ethyl amino ethyl cellulose (20 × 1.6 cm) enzymatic solution was recovered by balance buffer solution of potassium phosphate (0.05 molar) at pH 7.8, where increased the number of purification times to 4.92, with enzymatic recovery 29.6%, then concentrated and filtered through column Sephadex - G25, reached to the number of purification times 7.5 and enzymatic recovery 27.6 % .Linked enzyme recovered by linear gradient of saline solution of sodium chloride concentrations ranged from (0 - 1 molar).

Figure 1 shown appearance one peak of the protein and enzymatic activity in parts unlinked with Di diethyl amino ethyl - cellulose (Wash) and this indicate exit enzymatic solution of soluble peroxidase and similar proteins to the charge of exchanger, and the appearance of several peaks of protein and one peak and some enzymatic isomers which oboists charged to exchanger, as the peak of the protein be identical with enzymatic activity peak of the parts unlinked with exchanger be considerable as indicator for enzyme purity (Whitaker, 1972).

Peroxidase was used in estimating glucose concentration as an easy, fast method sharing with the glucose oxidase enzyme (GOD), and due to the high affinity shown by purified soluble enzyme towards O- Dinazidine that encouraged their for using in the estimation sugar enzymatically in the blood and food (Dalali, 1983).

CONCLUSIONS

1. Possibility of benefiting of the seeds dates (by-products) after manufacturing dates syrup and honey dates, for preparation peroxidase enzyme in large quantities .
2. Adoption method for preparation acetone powder of palm seeds roots as optimal method to obtained for extracts with high content of enzymes .
3. Using enzymatic methods as one of the methods used in determination of sugars which found to be the best of coloring methods specialized with sugar, such as enzyme glucose oxidase and peroxidase.
4. Results showed that purified peroxidase from plant sources besides the extract from the roots of palm seeds have high affinity towards O-dnazidine (as a substrate) and hydrogen peroxide where Quicken enzymatic reaction .
5. The possibility of exploiting the seeds roots of date (Zahdi date) as a source for the production of peroxidase enzyme in large quantities for analytical purposes and diagnostic immunohistochemistry (ELISA)) under controlled laboratory conditions as an alternative for local horseradish peroxidase HRP is available from one of the byproducts of the palm .

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Tables

Table 1: Comparison between specific activity (unit/gm) for soluble peroxidase enzyme and for extracted of roots and leaves for Zahdi date palm.

Code Method	Specific activity for soluble peroxidase enzyme from roots (unit/gm)	Specific activity for soluble peroxidase enzyme from leaves (unit/gm)
A	42180	23810
B	88920	34723
C-1	67260	38691

Code Method	Specific activity for soluble peroxidase enzyme from roots (unit/gm)	Specific activity for soluble peroxidase enzyme from leaves (unit/gm)
C-2	132240	77382
C-3	164160	82343
C-4	245100	156759
C-5	296400	113693
	188100	65477

- A: Direct extraction by buffer acetate solution (0.5 M).
- B : Direct extraction by buffer acetate solution (1.0 M).
- C-1: Extraction by sodium acetate buffer solution (0.1 M) of acetone powder of roots.
- C-2: Extraction by sodium acetate buffer solution (0.1 M),sodium chloride solution (0.5M) .
- C-3: Extraction by sodium acetate buffer solution (0.6 M) of acetone powder of roots.
- C-4: Extraction by sodium acetate buffer solution (0.8 M) of acetone powder of roots.
- C-5: Extraction by sodium acetate buffer solution (1.0 M) of acetone powder of roots.
- C-6: Extraction by sodium chloride solution (1.0 M) of acetone powder of roots.

Table 2: Purification steps of soluble peroxidase enzyme extracted from seeds root of Zahdi date.

Enzymatic recovery	Purification times	Total activity (unit)	Specific activity (unit/ml)	Protein (mg/ml)	Enzymatic activity (unit/ml)	volume (ml)	Purification steps
100	1	561600	34361.4	0.454	15690	36	Crud extract
44.4	1.89	249600	65271.9	0.478	31200	8	Precipitation with alcohol (40-70%)
34.4	2.91	193200	100311.5	0.321	32200	6	Dialysis against dis.water
29.6	4.92	166500	168965	0.058	9800	17	Ion exchange
2.24	0.21	12600	7200	0.070	501	25	Recovery
27.6	7.52	156017.5	258375	0.08	352	15	Gel filtration Sephadex G25

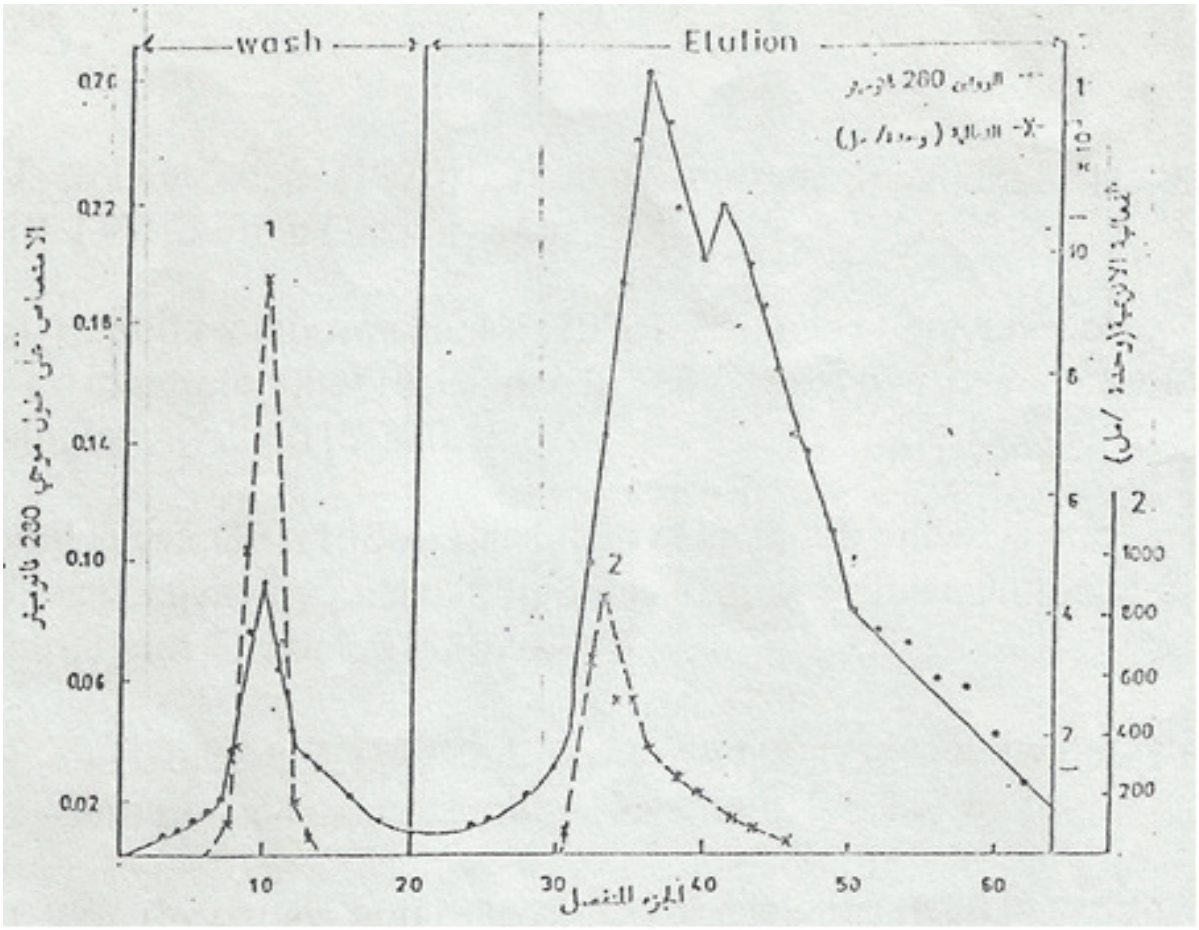

Figure 1: Ion exchange step