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Polyphenol Oxidase Activities and Properties in Some Potato (*Solanum tuberosum*) Varieties Produced in Saudi Arabia

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ABSTRACT. Since polyphenol oxidase (PPO) plays the key role in enzymatic browning of potatoes, five potato varieties produced in Al-Kharj region, Kingdom of Saudi Arabia, were evaluated for PPO activity. Wide variations in PPO activities among the different potato varieties were found. Ajax potato variety was very low in PPO. Extensive cultivation of low PPO potato varieties should be encouraged. Characteristics of potato PPO extract were also investigated. The temperature and pH optima for the enzyme were 30-40°C and pH 7, respectively. Heat stability studies indicated that a complete inactivation of PPO occurred at 80°C for 30 min. Regarding the effect of inhibitors on the enzyme, sodium metabisulfite and ascorbic acid were the most effective, whereas sodium chloride and EDTA were the least.

Enzymatic browning is a serious problem in potato processing, caused by the catalytic action of polyphenol oxidase (EC 1.14.14.1, monophenol, dihydroxyphenylalanine: oxygen oxidoreductase; EC 1.10.3.1, 1,2-benzenediol: oxygen oxidoreductase). Browning results not only in undesirable color formation, but also in loss of nutrient quality and undesirable taste (Matheis 1987a).

Browning in potato has been correlated with PPO activity and the concentrations of PPO substrates (Matheis and Belitz 1978, Brudzynki and Zawidzka-Okoniewska 1979, Stark *et al.* 1985, Sapers and Douglas 1987). The correlation between the rate of browning and the concentration of potato PPO was studied in different potato varieties at the stage of maturity (Sweeney and Simadle 1968). the concentration of potato constituents and , consequently, the rate of browning affected by variery of entrinsic factors such as fertilizers, phytohormones, fungicides, insecticides, nematodicide, rainfall (or irrigation) and storage time and temperature (Matheis 1987b).

No previous studies have been made on the polyphenol oxidase and enzymatic browning of potato varieties grown in Saudi Arabia conditions. Accordingly, this work was directed towards comparative study on the PPO activities of local varieties of potatoes grown in Saudi Arabia conditions. Isolation of PPO from such varieties and investigation of some characterization mainly with respect to pH, heat and chemical inactivation of the enzymes was the second purpose of the present study.

Materials and Methods

A. Material:

Five potato cultivars (*Solanum tuberosum*): Korrigan, mirka, diamont, spunta, and ajax of 1992 crop were obtained from Ministry of Agriculture Research Station in Al-Kharj city.

B. Methods:

Extraction and preparation of potato PPO: The enzyme extraction and preparation procedure was a modification of a method described by Park *et al.* (1980) as follows: About 100 - 125 g potato samples were diced to small pieces and thoroughly homogenized with 250 ml of ice-cold 0.02 M phosphate buffer of pH 6.0 containing 1 M KCl. After agitation for 1 h, the mixture was centrifuged at 12,000 xg for 15 min. The supernatent was then frozen for 12 h. After thawing (30 min), the clear supernatent was precipitated by cold acetone fractionation (1.2 V/V). The precipitate obtained by centrifugation was dissolved in 100 ml phosphate buffer and used as a source of enzyme.

Polyphenol Oxidase Assay:

The enzyme activity was determined by measuring initial rates of the increase in absorbance at 410 nm with a Bursh and Lamb Spectronic 20. The sample cuvette contained 3 ml catechol in a 0.1 M phosphate buffer, pH 6.0, 0.2 ml of 0.2 M sodium acetate buffer pH 5.6, 3.0 ml distilled water. 0.5 ml enzyme extract, and total value was completed to 5.0 ml with the phosphate buffer. Under these conditions. The initial increase in absorbance was a linear function of time for at least 2 min. One unit of PPO activity was defined as the quantity of enzyme that increased absorbance by 0.001 /min (Park *et al.* 1980).

Effect of enzyme concentration:

To determine the effect of enzyme concentration, different volumes of enzyme extract preparation ranging from 0.4 to 2.0 ml were used in the reaction mixture.

Effect of pH:

The enzyme activity was determined in 0.1 M citrate buffer and 0.2 M phosphate buffer at different pH values ranging from 2.0 to 8.0.

Effect of Reaction Temperature:

The PPO activity as a function of reaction temperature was determined at various temperatures from 10 to 90°C.

Heat Stability:

To study the heat stability of PPO, a 10 ml of average extract was kept for 30 min at constant temperature ranging from 0-90°C in 10°C intervals.

Enzyme Inhibitors:

The effect of inhibitors on the enzyme was demonstrated by measuring enzyme activity in a reaction mixture containing 1 ml of various concentrations of different chemical inhibitors.

Results and Discussion

PPO activities in the different varieties grown in Saudi Arabia:

Different potato varieties at the stage of maturity are known to differ in their ratios of browning (Matheis 1987b). The correlation between the rate of browning and the concentration of PPO was also evidenced by Sweeney *et al.* (1968). Data in Table 1 illustrate the potency of PPO activities of different potato varieties grown in the same conditions in Al-Kharj region, Saudi Arabia. As can be seen in Table 1 and Fig. 1, there are wide variations in PPO activities among the different potato varieties grown under the same conditions. The enzyme activity of korrigan variety was the highest followed by mirka, spunta, ajax, and diamont in decreasing order. Ajax and diamont potato varieties have only about 30 and 40% of PPO activity of korrigan cultivar, respectively. Therefore, ajax and diamont can be expected to show the lower susceptibility for browning. It would be desirable to breed potato varieties with low concentration of PPO. However, it is well known that there are other entrinsic factors contributing to the rate of browning in addition to the variety. These factors including fertilizers, fungicides etc., have been reviwed by Matheis (1987b).

Table 1. Composition of PPO activity for different potato cultivars

Potato cultivar	PPO activity unit/g
Korrigan	242
Mirka	206
Diamont	72
Spunta	180
Ajax	98

Properties of potato polyphenol oxidase:

The data compiled here fefer to crude enzyme extract composite samples obtained from the five potato varieties. The crude enzyme extracts were only partially purified by acetone precipitation.

Effect of enzyme concentration:

The relationship between PPO activity and concentration of partially purified potato PPO extract are shown in Fig. 2. A linear relationship between the enzyme activity and its concentration can be seen. This linearity illustrates: (1) that the method employed to follow the reaction rate truly reflected the velocity of substrate-to-product conversion at all enzyme concentrations used; (2) that there was no inhibitory or toxic impurity present in the reaction mixture that would partially inactivate the enzyme present; and (3) that the concentration of substrate and any required factor were greatly in excess of enzyme concentration. Actually this experiment was necessary to demonstrate that the present method is appliable with partially purified potato PPO extract, where the presence of interfering substances coul distort results. Therefore, the present method was judged to be satisfactory for determination of PPO activity of the potato extract since it gave a limear relationship between the concentration of PPO extract and the absorbance.

Effect of hydrogen-ion concentration:

Variations in activity of PPO from potato extract with pH are shown in Fig. 3. Maximum potato PPO activity occurred at pH 7.0. The enzyme activity appeared to be more sensitive to higher pH values than to acidic conditions, since the curve in the alkaline side of the optimum pH is very steep. At pH 6, the enzyme had about 76% of the maximum activity. At pH 4, the enzyme had only about 13% of its maximum activity. Therefore, it is clear that potato PPO activity can be minimized by decreasing the pH down to 4 or below.

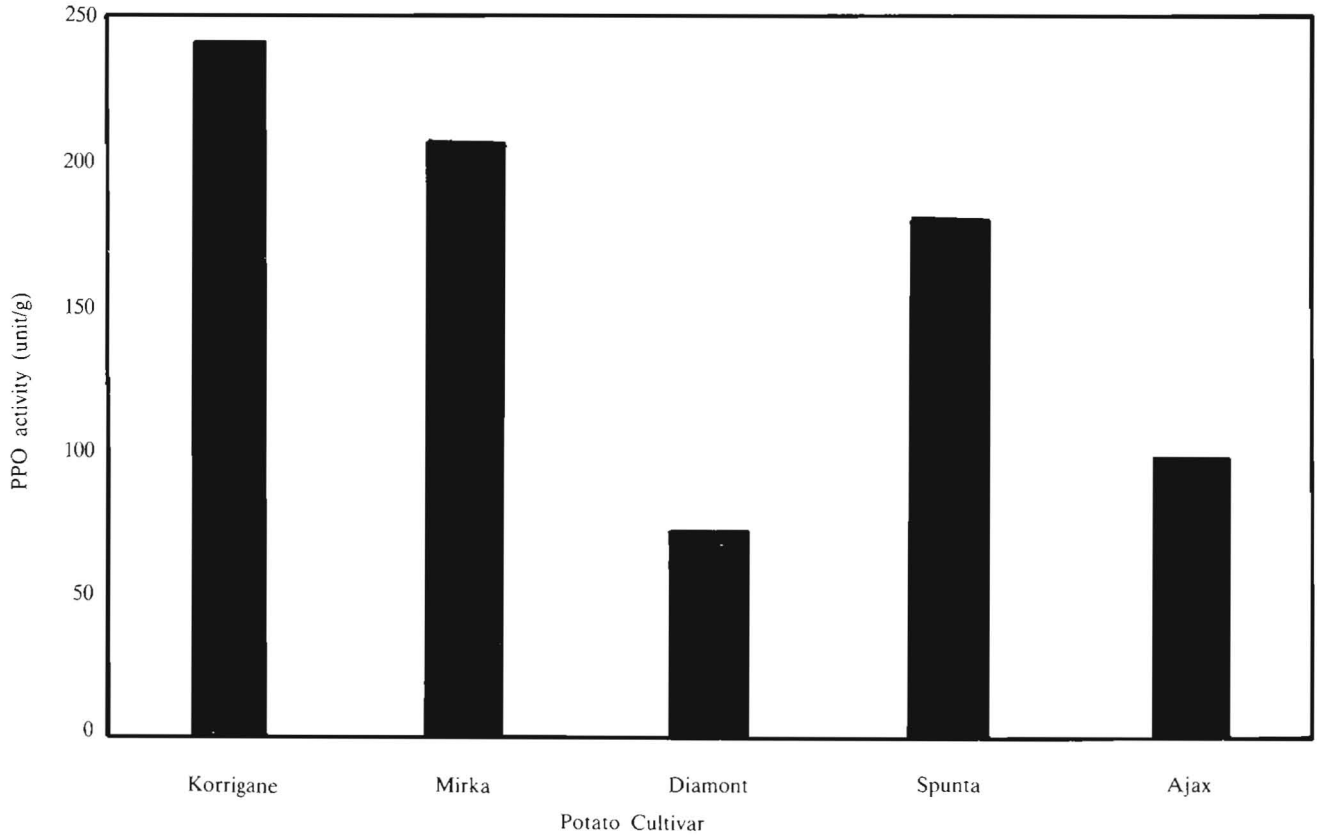

Fig. 1. Comparison of PPO activity for different potato cultivars.

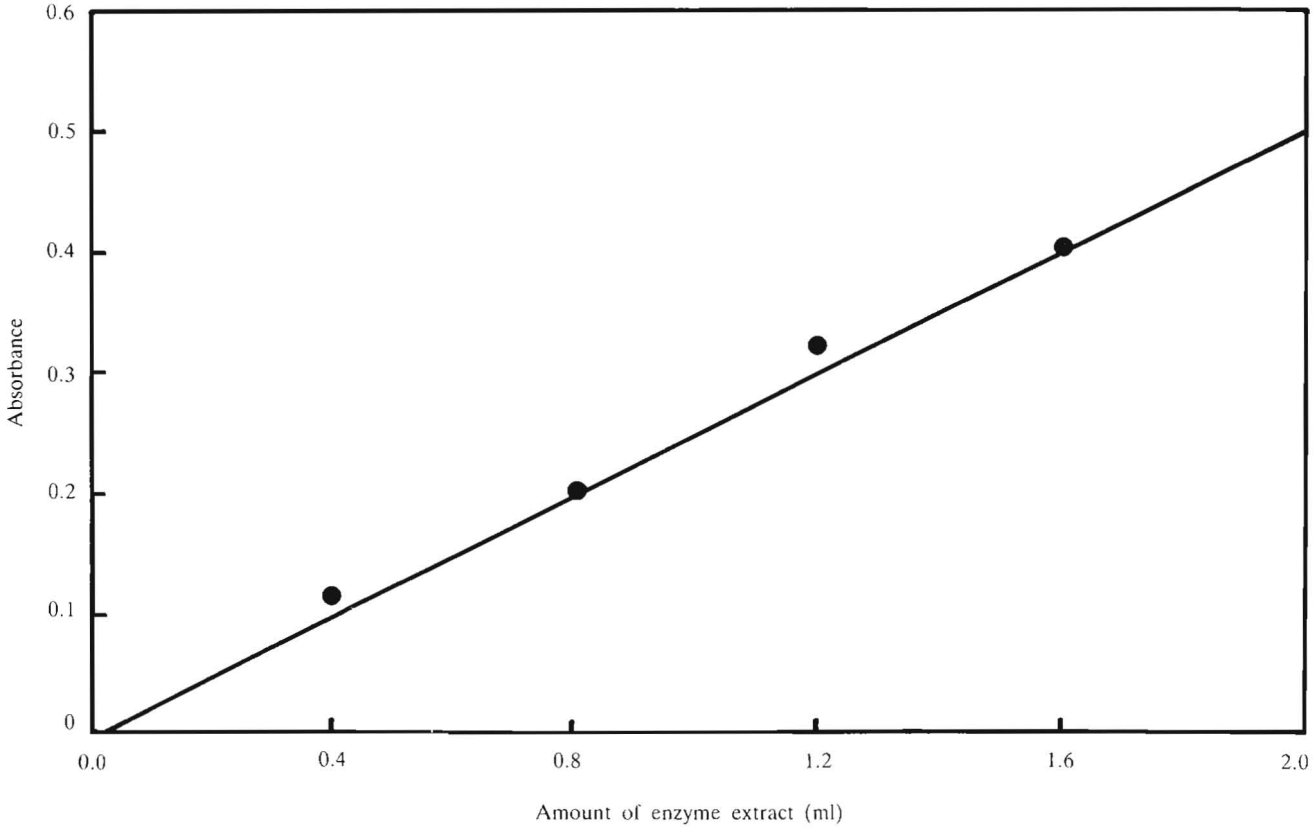

Fig. 2. Relationship between PPO activity and concentration of potato PPO extract. Reaction time = 2 min

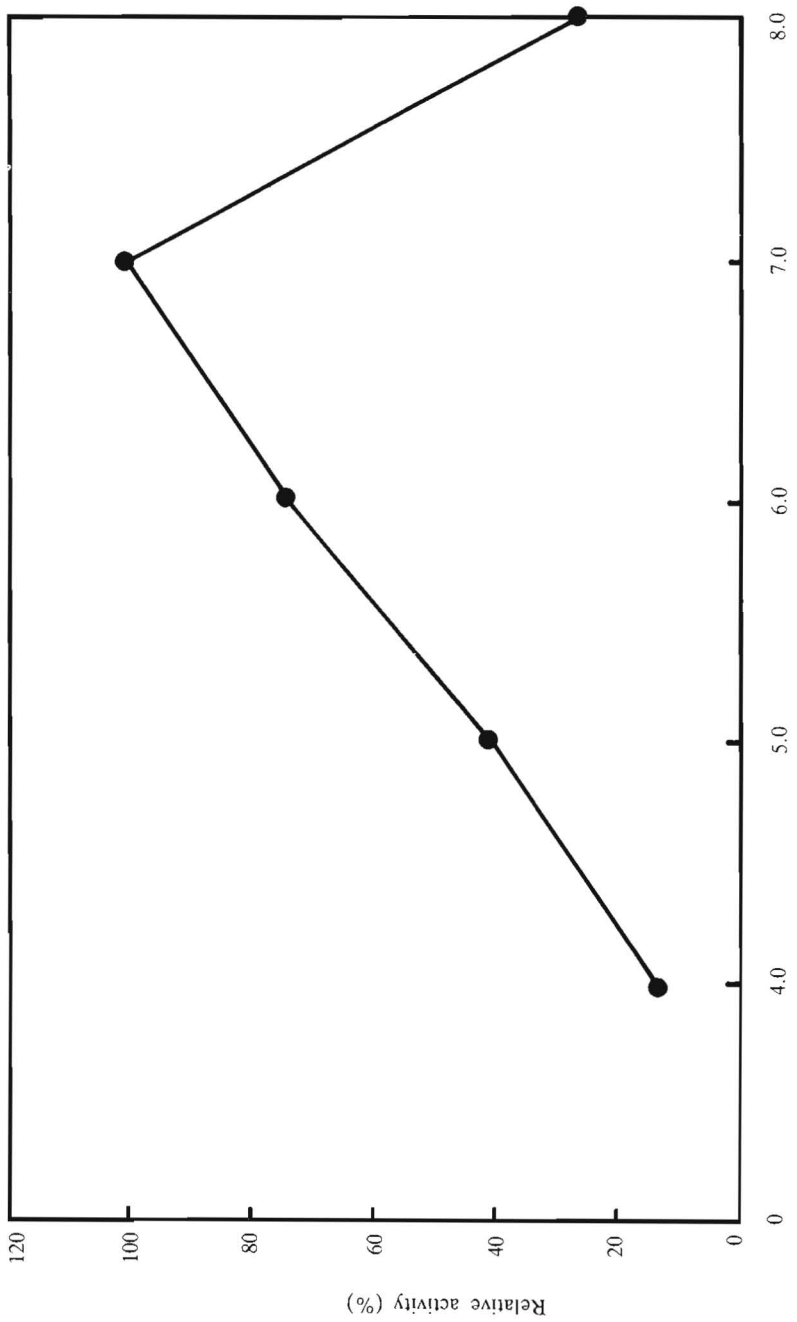

Fig. 3. Effect of pH on the activity of potato PPO extract.

For potato PPO enzyme preparations, the pH optimum depends on the phenolic substrate used. Therefore, our results showing an optimal pH at around 7 confirm the findings reported by Matheis, 1987 a who also used catechol as a substrate. The pH optimum is different when using other substrates as shown by studied reporting pH 5 as the optimum pH for potato PPO using chlorogenic acid as substrate, or pH 4.3 for cafferic acid as substrate (Matheis 1987 a).

The optimum pH 7.0 for potato PPO using catechol as a substrate (Fig. 2) was similar to that reported by Matheis (1987a) using tyrosine as substrate. Mahmoud and El-Shemy (1988) reported that the pH optimum can also be affected by the type of buffer and the purity of the enzyme.

Effect of reaction temperature:

To demonstrate the effect of temperature on potato PPO catalyzed catechol oxidation activities were measured at different reaction temperatures between 0°C and 90°C (Fig. 4). The enzyme activity increased with the raise of the temperature from 10 to 30°C. No change of the activity was noticed between 30 and 40°C. At 90°C the enzyme reached about 21% of its maiximum activity. On contrast, a sharp decrease in the enzyem activity was observed at temperature below its optimum temperature (30 -40°C), since only about 37% of its maximum activtiy was obtained at 10°C. It is clear that potato PPO is most active at a wide range of temperatures (between 20-60°C) with temperature optimum 30-40°C These reuslts are consistent with those reported by Matheis (1987a) in a review on properites of potato PPO.

Heat Stability:

As evident form Fig. 5, the enzyme showed 17 and 31% loss of activity at 60°C and 70°C respectively, for 30 min. incubation time. A high rate of inactivation occured at higher temperature. Complete inactivation of PPO occurred at 80°C for 30 min. The enzyme showed similar stabilities at lower temperature. It retained about 53, 61, and 64% of its activty at 0-10, 30-40, and 40-50°C respectively.

Effect of inhibitors:

The effect of four inhibitors in different concentrations on potato polyphenol oxidase extract was studied and the results are shown in Table 2. Potato PPO underwent inhibition by ascorbic acid and sodium metabisulfite while on inhibitory effect was detected usign sodium chloride or EDTA, It is apparent that either sodium metabisulfite or ascorbic acid is the most effective inhibitor of potato PPO. while sodium chloride and EDTA were the least potent inhibitors. Both ascorbic acid and

sodium metabisulfite have been previously shown to inhibit the action of PPO (Wong *et al.* 1971, Wiseemann and Lee 1981, Park *et al.* 1980 and Mahmoud and El-Shemy 1988). EDTA has been found to be poor inhibitor for PPO while inhibition of PPO with sodium chloride has been found to be pH dependent (Luh and Phithakpol 1972, Mayer and Harel 1979).

Table 2. Effect of inhibitors on potato PPO extract

Inhibitor	Concentration	Inhibitor %
Ascorbic acid:		
	0.05 mM	48
	0.10 mM	57
	0.20 mM	65
	0.50 mM	100
Sodium metabisulfite:		
	0.03 mM	34
	0.05 mM	34
	0.07 mM	57
	0.10 mM	74
	0.50 mM	100
Sodium chlordie:		
	0.5 %	– a
	1.0 %	–
	2.0	–
	3.0	–
EDTA.		
	0.05 mM	–
	0.10 mN	–
	1.0 mM	–

* No inhibitor was detected.

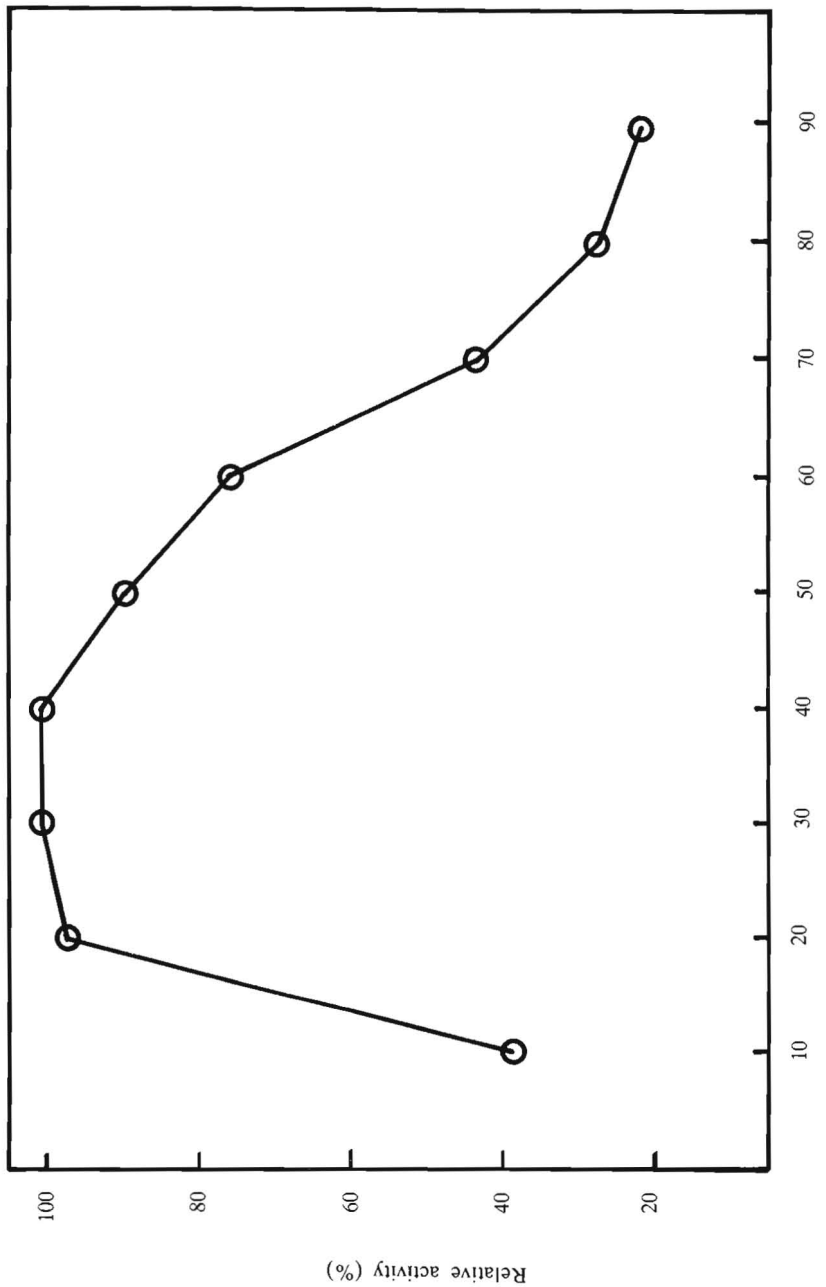

Fig. 4. Effect of reaction temperature on the activity of potato PPO extract.

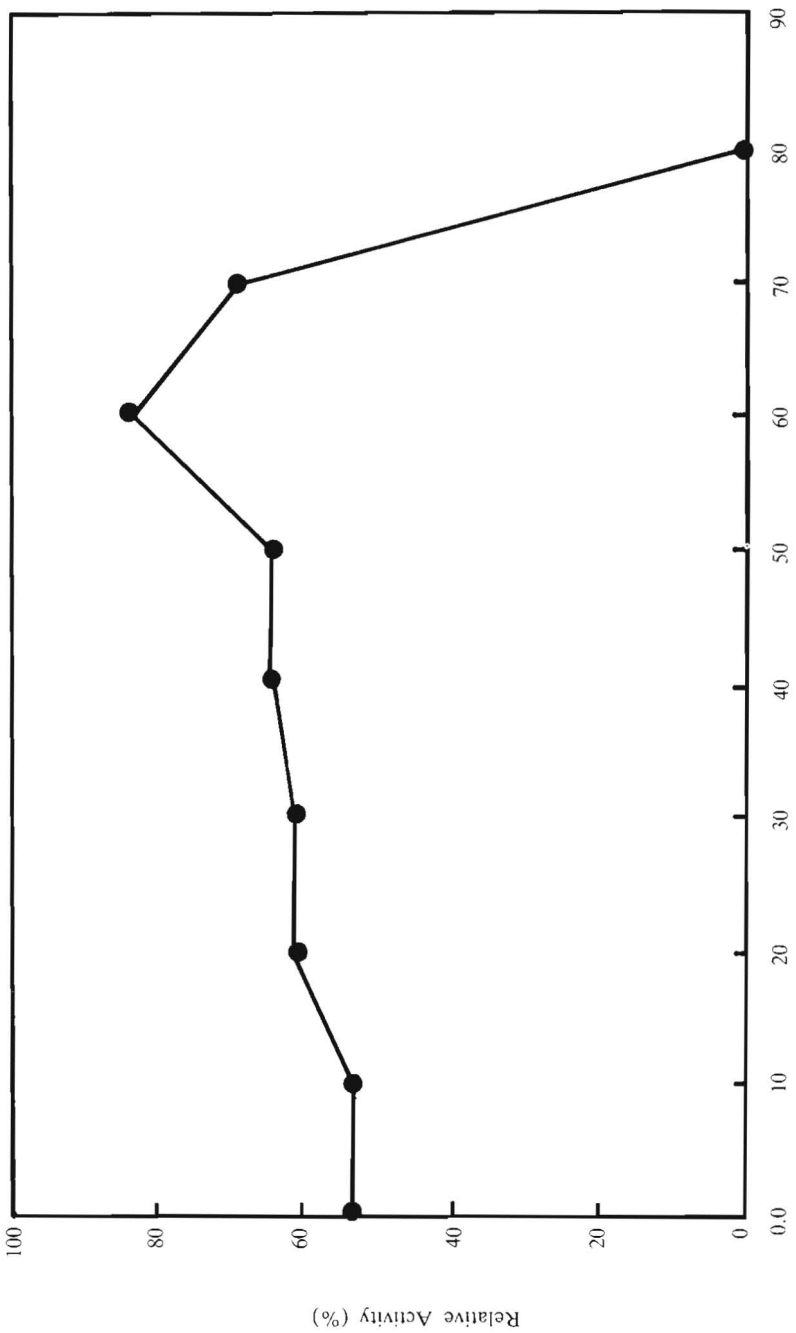

Fig. 5. Heat stability of potato for 30 min. incubation time.

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نشاط وخواص انـزيم البولي فينـول أكسديـز في بعض أصناف البطاطس المنتجة في المملكة العربية السعودية

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يعتـبر التلون البني الانزيمي النـاتج عن نشـاط انزيم البـولي فينـول أكسـديـز في البطاطس من المشاكل الرئيسية في تصنيع البطاطس لما ينتج عنه من تكـوين اللون غير المرغوب بالاضافة إلى ما يتسبب عنه من فقد في الجودة الغذائية والطعم غير المرغـوب ويختلف معدل التلون البني في البطاطس تبعاً للصنف وعدد من العوامل الأخـرى ولا توجد دراسات سابقة عن مدى نشـاط انزيمـات البولي فينـول أكسديـز ومعدل التلون البني في أصناف البطاطس المزروعة في المملكة العربيـة السعوديـة، ولذا فيتنـاول هذا البحث دراسـة مقارنـة لنشاط انـزيم البـولي فينـول أكسـديـز في أصنـاف البـطاطس المزروعة محلياً في المملكة العربية السعودية بالإضافـة إلى دراسة الخـواص المميزة لهـذا الانزيم وتركزت الدراسة على خمسة أصناف من البطاطس المزروعـة في منطقـة الخرج بالمملكة العربية السعودية حيث أسفرت النتائـج على أن هنـاك اختلافـات كبيرة بـين الأصناف في نشاط انزيم البولي فينول أكسديز وتميز الصنف أجاكس والدايامونـد بأقل درجة نشاط لهـذا الانزيم ممـا يشجع عـلى التركيـز على زراعـة هذين الصنفـين حيث بلغت درجة نشاط الانزيم فيهما ٤٠، ٣٠٪ فقط على التوالي من درجة نشاط الانـزيم لأصناف الكورسيجان والميركي اللذان تميزا بدرجة نشاط انزيمي عالي.

كما تم دراسة خواص مستخلص انزيم البولي فينول أكسديز لعينات البطاطس فكانت درجة الحرارة والرقم الايدروجيني المثلى ٣٠ - ٤٠ م° ورقم الحموضة ٧ على التوالي كما أسفرت دراسة مدى تحمل الانزيم للحرارة أن ٨٠ م° لمدة ٣٠ دقيقة كافية للتثبيط الكامل لهذا الانزيم، كما وجد أن بعض المثبطات الكيماوية مثل أملاح الصوديوم ميتاسلفيت وحمض الاسكوربيك ذات أثر فعال في تثبيط نشاط هذا الانزيم بينما كان التأثير المثبط لكلوريد الصوديوم وحمض الاثلين داي امين ترا أستيك ضئيل للغاية، ولذا فيوصى باستخدام أملاح الصوديوم ميتاسلفيت وحمض الاسكوربيك للحد من نشاط هذا الانزيم أثناء العمليات التصنيعية للبطاطس ويقلل من مدى أهمية استخدام كلوريد الصوديوم لهذا الغرض في كثير من العمليات التصنيعية المتبعة حالياً.