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Thermostability at different pH levels of peroxidase extracted from four vegetables

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Abstract

The stability of crude peroxidase incubated for different period of time and at different pH and temperatures of four vegetables (potato, carrot, eggplant and tomato) was investigated. Peroxidases of high activity were extracted at pH 5.0 from potato and tomato while those of carrot and eggplant were extracted at pH 6.0. Potato tuber contained higher level of peroxidase whereas carrot had lower levels at all pH values. The results showed that the rate of loss of peroxidase activity from the vegetables increased with both increase in temperature and heating time. Biphasic inactivation curves were observed for the enzymes extracted from all samples, where the initial heat inactivation is rapid followed by much slower inactivation periods. The rate of loss of peroxidase activity was shown to be pH dependent. Potato peroxidase was observed to be more stable to heat. A less severe heat treatment is required to inactivate carrot, eggplant and tomato peroxidases. Complete inactivation of carrot peroxidase was accomplished within 4–10 min at 80ºC and within 2–10 min at 90ºC at pH 8.0, while peroxidase inactivation in eggplant required 8–10 min at 90ºC at pH 8.0. Complete inactivation of tomato peroxidase required 6–10 min at 90º C at pH 6.0.

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Introduction

Peroxidase (EC 1.11.1.7; donor:hydrogen-peroxide oxidoreductase, POD) is one of the key enzymes controlling plant growth, differentiation and development. The enzyme participates in construction, rigidification and eventual lignification of cell walls, biosynthesis of ethylene from 1-aminocyclopropane-1-carboxylic acid and H_2O_2, regulation of auxin level through auxin catabolism, protection of tissue from damage and infection by pathogenic microorganisms, the oxidation of indoleacetic acid (Dunford 1991; Wakamatsu and Takahama, 1993). *In vitro*, this enzyme is widely employed in microanalysis (Krell, 1991) and for the construction of enzyme electrodes (Gorton, 1995). More than 80% of immunoenzymatic kits contain peroxidase as labeling enzyme. Recently, peroxidases have been used for biotransformation of organic molecules (Adam *et al*., 1999). It has been well established that peroxidase as one of the most stable enzymes can contribute to deteriorative changes in quality of the processed products (Stanley *et al*., 1995). Enzymatic browning is one of the most important colour reactions that affect fruits and vegetables. It is catalyzed by polyphenoloxidases and peroxidases (Marshall *et al*., 2000). It was estimated that over 50 percent losses in fruits and vegetables occur as result of enzymatic browning (Whitaker and Lee, 1995). Marshall *et al*. (2000) reported that the rate of enzymatic browning in fruits and vegetables is governed by the active polyphenoloxidase and peroxidase content of the tissues, the phenolic content of the tissues, pH, temperature and oxygen availability with the tissue. Polyphenoloxidase had been reported to be heat sensitive enzyme (Ghafoor and Choi, 2012). Lee *et al*. (1990) reported that the relationship of the rate of browning to phenolic content and polyphenoloxidase activity could be positively related to discolouration of peaches. According to Khan and Robinson (1993) the peroxidase is directly responsible for enzymatic browning in mangoes. In developing countries fruit and vegetable processing

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is among the most important agricultural activities, without questions, this activity plays an important role in the world of food economy by supplying wholesome, safe, good quality and acceptable food to consumers throughout the year. Dauthy (1995) claimed that the deterioration reaction in fruits and vegetables could be due to enzymatic, chemical and biological changes. It is well-known that the presence of residual endogenous enzyme in both raw or processed fruit and vegetable product may cause a loss of quality during storage. The high thermal stability of peroxidase can be seen as either an advantage or a problem in food industry. On one hand, it provides natural margin of safety in that if peroxidase is inactivated, it is a reasonable assumption that other quality–related enzymes have also been inactivated and on the other hand, the reliance on peroxidase as an indicator may lead to an excessive heat treatment of the product and cause other quality problems (Anthon and Barrett, 2002). There is an increasing interest in the study of peroxidases, not only in order to establish their physiological, but also for their possible industrial and analytical application. The objective of this work was to study the effect of pH and heat on peroxidase activity and to extend the knowledge of vegetables peroxidase in particular.

Materials and Methods

Sample collection and preparation

Fruits of potato, carrot, eggplant and tomato were collected from Department of Horticulture, Faculty of Agriculture, University of Khartoum, Sudan. The collected fruits were analyzed on the same day. All chemicals used for the experiments were of reagent grade.

Extraction of peroxidase

Fresh fruits of potato, carrot, eggplant and tomato were washed thoroughly with distilled water and cut into pieces. The fruits were then homogenized with ice cold 10 mM sodium phosphate buffer of pH 5, 6, 7 or 8. The ratio of quantity of fruits taken to that of buffer was maintained constant at 1:1 (w/v). The crude extract was filtered through cheese cloth and centrifuged to remove traces of fibrous particles and cell debris. The supernatant was stored at 4°C and used as stock solution for further experiments.

Heat treatment

Heat inactivation of the enzyme extracted at different pH values was carried out at four temperatures (60, 70, 80 and 90°C). The crude extract of the enzyme was diluted (1/10, v/v) with 10 mM sodium phosphate buffer at each pH value in test tubes. The test tubes were then transferred to water bath set at the desired temperature for 10 minutes. At interval of 2 minutes the tubes were removed and rapidly cooled in ice water and held at -18°C. Then the enzyme activity was assayed at each interval, pH and temperature. Triplicate samples were used for each treatment.

Enzyme assays

Peroxidase activity was determined spectrophotometrically using Marangoni *et al*. (1995) method. About 10 µl of enzyme solution extracted at specific pH was added to 2 ml of 100 mM citrate-phosphate buffer, pH 5.5, containing 18.2 mM guaiacol and 4.4 mM H_2O_2 as substrates, and the absorbance change at 470 nm was monitored at 25°C. One unit of activity (U) was defined as the amount of enzyme that caused the oxidation of 1.0 µmole of substrate per min under standard conditions. One unit of peroxidase activity represents the amount of enzyme catalyzing the oxidation of 1 µ mol of guaiacol in 1 min. The activity at each time interval and temperature was expressed as percentage of total activity.

Statistical analysis

Each determination was carried out on three separate samples and analysed in triplicate on dry weight basis; the figures were then averaged. Data were assessed by the analysis of variance (Snedecor and Cochran, 1987). Comparisons of means for treatments were made using Duncan's multiple range tests. Significance was accepted at $P \geq 0.05$.

Results and Discussion

Peroxidase activity of vegetables

The activity of crude soluble peroxidase fraction from each vegetable, extracted at variable pH values, was determined and the results were summarized in Table 1. The results indicated clearly that peroxidase was found in all samples of vegetables investigated. The presence of peroxidase in various fruits and vegetables was observed by many workers (Miesle *et al*. 1991; Neves, 2002; Liano *et al*., 2003). A reasonable quantity of the enzyme was extracted and estimated at all pH values. Significant ($P \geq 0.05$) levels of peroxidase activities were extracted at pH 5.0 from potato and tomato while those of carrot and eggplant had significant ($P \geq 0.05$) levels at pH 6.0. Potato tuber was shown to contain the higher peroxidase levels at all pH values investigated whereas carrot had low peroxidase levels at all pH values. Carrot and egg-plant had significantly ($P \geq 0.05$) low peroxidases levels at pH 8.0. It has been

Table 1. Peroxidase activity at different pH vales of four vegetables

PH values	Peroxidase activity (U ml^{-1})			
	Potato	Carrot	Eggplant	Tomato
5.0	2.40^{a} (±0.04)	1.22^{b} (±0.15)	1.90^{a} (±0.21)	1.96^{a} (±0.09)
6.0	1.99^{b} (±0.11)	1.53^{a} (±0.07)	1.98^{a} (±0.13)	1.86^{b} (±0.07)
7.0	1.96^{b} (±0.06)	0.83^{c} (±0.08)	1.67^{b} (±0.08)	1.74^{b} (±0.04)
8.0	1.90^{b} (±0.07)	0.37^{d} (±0.02)	0.52^{c} (±0.03)	1.92^{a} (±0.13)

Values are means of three replicates (± SD). Means not sharing a common superscript(s) in a column are significantly different at $p \leq 0.05$ as assessed by Duncan's Multiple Range Test.

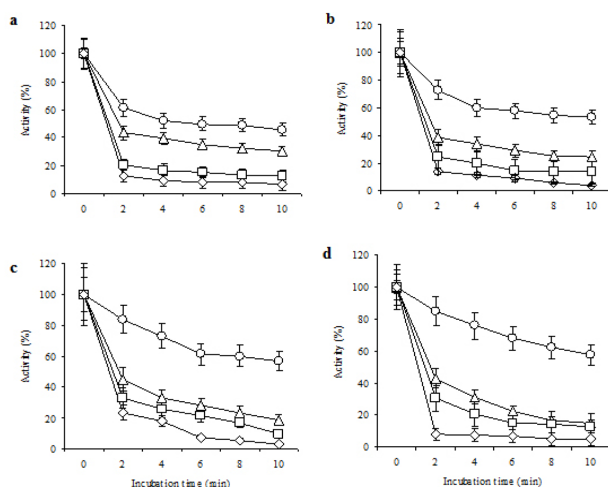

Figure 1. Activity of peroxidase of potato at (a) pH 5, (b) pH 6, (c) pH 7 and (d) pH 8 and incubated for different time at different temperatures. (o) 60°C, (∆) 70°C, (□) 80°C, (◊) 90°C.

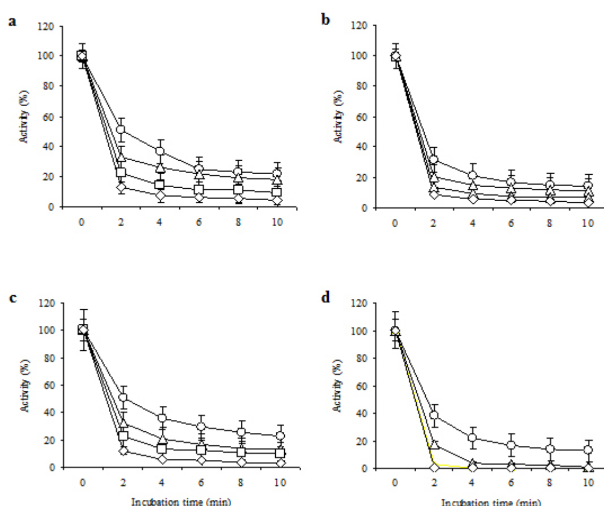

Figure 2. Activity of peroxidase of carrot at (a) pH 5, (b) pH 6, (c) pH 7 and (d) pH 8 and incubated for different time at different temperatures. (o) 60°C, (∆) 70°C, (□) 80°C, (◊) 90°C.

reported that the optimum pH values for detecting peroxidase activity was 5.0 for peach (Neves, 2002); 4.5–6.0 for lettuce (Bestwick *et al*., 1998) and 5.5–6.0 for papaya fruit (Silva *et al*. 1990).

Heat inactivation of peroxidase of vegetables

Potato peroxidase was subjected to thermal inactivation for varying heating temperatures and length of heating times under different conditions of pH and the results obtained are shown in Figure 1. The results obtained showed that the rate of loss of peroxidase activity increases with both increased temperatures and heating times. The initial heat inactivation of peroxidase enzyme is rapid followed by a much slower inactivation period. For instance, heating at 60°C for 10 min resulted in a loss of 54.5, 46.4, 43.1 and 42.5% of the enzyme activity at pH 5.0, 6.0, 7.0 and 8.0, respectively. This pattern was more or less true for other temperatures. It was observed that the stability of the enzyme to heat increased with increased pH values. Potato peroxidase was not completely inactivated when it was exposed to varying temperatures, length of heating times and at variable pH values. Figure 2 shows the effect of heating temperature and time on peroxidase activity of carrot at different pH levels. The results obtained showed that the rate of loss of peroxidase activity increases with both increased temperature and heating time. The initial heat inactivation of peroxidase enzyme was rapid followed by a much slower inactivation period. Heat inactivation of carrot peroxidase followed the usual pattern, which was also observed for potato peroxidase; however some variations were observed when the effects of the length of heating time were compared at the different pH values studied. Complete inactivation of carrot peroxidase was accomplished at 80°C for 4-10 min and at 90 º C within 2 to 10 min at pH 8.0. Eggplant peroxidase was subjected to thermal inactivation for varying heating temperatures and length of heating time under different conditions of pH. The results obtained are shown in Figure 3. The rate of loss of peroxidase activity increased with increase in temperature and heating time. The initial heat inactivation of peroxidase enzyme was rapid followed by much slower inactivation period, which is similar to the results obtained for potato and carrot. Complete inactivation of eggplant peroxidase required 8 to 10 min. at 90°C and pH 8.0, and therefore comes second to potato peroxidase in term of heat stability. Figure 4 shows the effect of heating temperature and time on peroxidase activity of tomato at different pH levels. The results obtained showed that the rate of loss of peroxidase activity increased with increase in temperature and heating time, a result which is similar to those of potato, carrot, and eggplant. The initial heat inactivation of peroxidase was rapid followed by a much slower inactivation period. Complete inactivation of tomato peroxidase requires 6 to 10 minutes at 90º C and pH 6.0. It was clearly observed that the patterns of heat inactivation of peroxidase of the vegetables investigated are similar. Potato peroxidase was observed to be more stable to heat

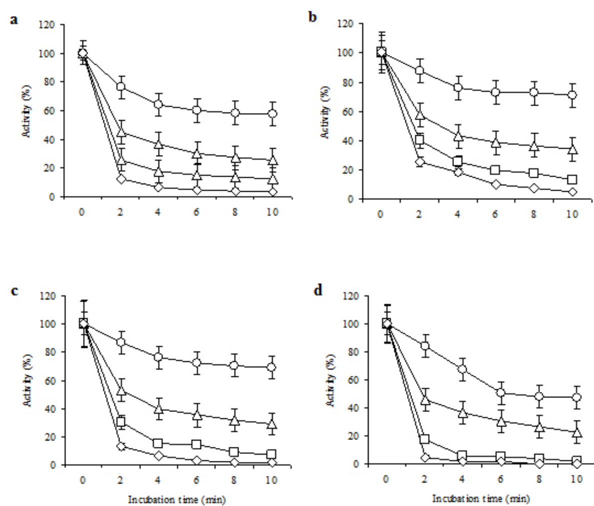

Figure 3. Activity of peroxidase of eggplant at (a) pH 5, (b) pH 6, (c) pH 7 and (d) pH 8 and incubated for different time at different temperatures. (o) 60ºC, (Δ) 70ºC, (□) 80ºC, (◊) 90ºC.

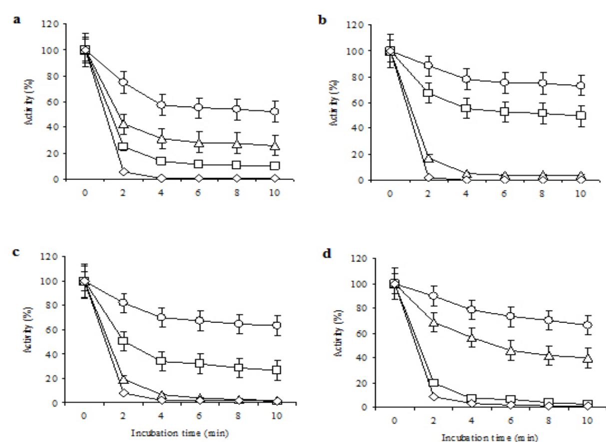

Figure 4. Activity of peroxidase of tomato at (a) pH 5, (b) pH 6, (c) pH 7 and (d) pH 8 and incubated for different time at different temperatures. (o) 60ºC, (Δ) 70ºC, (□) 80ºC, (◊) 90ºC.

and therefore a less severe heat treatment is required to inactivate carrot, eggplant and tomato. Peroxidase is reported to be one of the most heat stable enzyme in plant, hence can influence the flavour, texture and colour in row and processed fruit and vegetables (Clemente and Pastore, 1998). Anthon and Barrett (2002) stated that as peroxidase is very resistant to thermal inactivation; it is widely used as an index of blanching and other heat treatments. Yemenicloglu *et al*. (1999) and Neves (2002), have reported that the initial heat inactivation of peroxidase enzyme was rapid followed by a much slower inactivation period and have concluded that the heat inactivation process is biphasic. The results obtained in this study, showed that, potato peroxidase is thermally more stable than carrot, eggplant and tomato peroxidase. In addition, rates of heat inactivation were found to be pH dependent. Deepa and Arumughan (2002) reported that the resistance to heat treatment depends on the source of the enzyme as well as the assay condition, especially pH and nature of substrate employed. Neves and Lourenço (1998) reported that peach peroxidase, soluble and bound, showed distinct heat lability, that fact was also observed for isolated enzyme from papaya (Silva *et al*., 1990). Deepa and Arumughan (2002) observed that the thermal stability of oil palm fruit peroxidase was greater than that reported for cotton by Triplett and Mellon (1992) and strawberry by Civello *et al*., (1995). It has been shown that the thermal stability of peroxidase was due to the presence of a large number of cysteine residues in the polypeptide chain (Deepa and Arumughan, 2002).

Conclusions

All vegetables investigated contained peroxidase enzyme. The activity of the enzyme depends on the pH value of the medium. Potato tuber had higher peroxidase level at all pH values investigated, whereas carrot peroxidase had lower levels under the same conditions. The rate of loss of peroxidase activity increased with both increase in temperatures and heating time in all vegetables. Heat inactivation of peroxidase enzyme is biphasic, i.e. the initial heat inactivation of peroxidase enzyme is rapid followed by much slower inactivation period.

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