Partial Purification and Thermal Characterization of Peroxidase from Okra (*Hibiscus esculentum*)

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Thermal characteristics and substrate specificity of peroxidase (POX) from okra were determined. Crude POX was separated into eight fractions (P1−P8) by DEAE−cellulose chromatography. Heat inactivation of POX was biphasic and fitted to a first-order kinetic model. Inactivation rates of crude POX and eight POX fractions at 70 °C varied between (63.499 and 123.809) $\times$ 10^{-2} min^{-1} for the heat-labile fractions and (11.255 and 31.441) $\times$ 10^{-2} min^{-1} for the heat-stable fractions. Activation energies for the inactivation of crude extract were 37.0 kcal mol^{-1} with a *z*-value of 13.8 °C for the heat-labile fraction and 37.8 kcal mol^{-1} with a *z*-value of 13.5 °C for the heat-stable fraction. Optimum temperatures were between 30 and 35 °C for the crude extract, 60 °C for P1−P5 fractions, and more than 65 °C for P6−P8 fractions. Among substrates, guaiacol was oxidized primarily by P1−P6 fractions; whereas, pyrogallol and catechin were preferred by P7 and P8, respectively.

Keywords: *Peroxidase; okra; partial purification; thermal characterization; substrate specificity*

INTRODUCTION

Peroxidase (POX, donor:hydrogen peroxide oxidoreductase, EC 1.11.1.7) catalyzes the oxidation of phenols (guaiacol, *p*-cresol), aromatic amines (aniline, *o*-dianisidine), and some other organic compounds in the presence of hydrogen peroxide (Vámos-Vigyázó, 1981). Inactivation of POX has conventionally been used as an indicator of blanching adequacy in the processing of vegetables. This is because POX is one of the most heat-stable and widely distributed enzymes in the plant kingdom. Moreover, the detection of POX and measurement of its activity can easily be done in almost every fruit and vegetable. There is also an empirical relationship between the prevention of off-flavor development in frozen vegetables and inactivation of POX (Guyer and Holmquist, 1954; Burnette, 1977; Williams et al., 1986; Lopez and Burgos, 1995).

The heat inactivation of POX from many plant sources has been shown to be biphasic because POX has multiple isoenzymes with different heat stabilities (Yamamoto et al., 1962; Ling and Lund, 1978; Naveh et al., 1982; Miller et al., 1990). Since some of the POX isoenzymes are very heat stable, complete inactivation of POX may impair the quality (e.g., color, flavor, texture, and nutrients) of processed vegetables (Müftügil, 1985; Ganthavorn and Powers, 1988; Hemeda and Klein, 1991; Romero and Barrett, 1997). However, it may not be appropriate to assume that POX from all vegetables exhibits extreme heat stability. In fact, POX from asparagus and soybeans (Ganthavorn et al., 1991) and pinto beans (Yemenicioğlu et al., 1998) were shown to be less heat-stable than lipoxygenase (LOX) from the same sources. Therefore, blanching to inactivate POX in some vegetables may not be sufficient to prevent off-flavor formation during frozen storage.

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In place of POX, LOX has been suggested as a blanch indicator for some vegetables, including English green peas and green beans (Williams et al., 1986), sweet corn (Theerakulkait and McDaniel, 1995), asparagus (Ganthavorn et al., 1991), and soy beans (Sheu and Chen, 1991). However, there are some problems associated with using LOX as a blanch indicator. First of all, LOX activity cannot be easily measured in plant tissues. In fact, there is no rapid method currently used by the frozen food industry to measure LOX activity (Romero and Barrett, 1997). A rapid semiquantitative potassium iodide−starch (KI−S) method has been developed by Williams et al. (1986) for measuring LOX activity. However, with this method, LOX activity cannot be accurately measured in carotene-containing fruits and vegetables. Romero and Barrett (1997) evaluated three potentially rapid methods for sweet corn and green beans: KI−S, carotene bleaching (CB), and methylene blue bleaching (MBB). Among these methods, only MBB method was effective for both vegetables, but it is a quite laborious procedure. Therefore, POX will continue to serve as a blanch indicator in the processing of vegetables (Halpin et al., 1989; Lopez and Burgos, 1995; Rodrigo et al., 1997).

No published data has been found in the literature, which discusses the thermal characteristics of POX from okra. Therefore, this study was undertaken to determine the thermal characteristics of crude and partially purified POX enzymes from okra.

MATERIALS AND METHODS

Materials. Fresh okra (*Hibiscus esculentum* cv. Sultani), purchased from a local market, was washed, drained, packed in polyethylene bags, and stored at −35 °C until the enzyme extraction. The selected Sultani cultivar is a local cultivar and is very suitable for freezing and canning. The average weight and measurements of okra used in this study are as follows: weight, 3.5 g; length, 4.4 cm; and diameter, 1.2 cm.

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Table 1. Summary of Partial Purification of Peroxidase from Okra

purification step	vol (mL)	total activity (units 10^{-3})	total protein (mg 10^2)	specific activity (units mg^{-1} 10^{-3})	purity (fold)	recovery (%)
crude extract	232	501	10 700	4.7	1	100
0−90% $(NH_4)_2SO_4$ precipitation and dialysis	55	289	2300	12.6	2.7	58
DEAE-cellulose chromatography						
P1	5	6.5	0.01			2.2
P2	5	8.4	0.43	1953	416	1.7
P3	5	7.2	17.6	40.9	8.7	1.4
P4	5	6.8	5.1	133.3	28	1.4
P5	5	5.9	14.1	41.8	8.9	1.2
P6	5	3.3	21.6	15.3	3.3	0.7
P7	5	2.3	0.44	523	111	0.5
P8	10	4.4	0.3	1467	312	0.9

DEAE-cellulose (fast flow), catechin, 4-methyl catechol, and bovine serum albumin were purchased from Sigma Chemical Co. (St. Louis, MO). Pyrogallol, catechol, guaiacol, and tris [tris(hydroxymethyl)aminomethane] were obtained from Merck (Darmstadt, Germany). All other chemicals used in this study were of reagent grade.

Preparation of Acetone Powder. Acetone powder was prepared according to the method of Coseteng and Lee (1987) and used as the enzyme source for this study. Unless otherwise indicated, all extraction, fractionation, and purification procedures, as well as heat inactivation experiments, were carried out in 0.01 M tris-HCl buffer, pH 7.0.

Enzyme Extraction. The acetone powder (3−4 g) was suspended in 250 mL of tris-HCl buffer at +4 °C containing 1 M NaCl and 1 g of polyamide as phenolic scavenger. The suspension was continuously stirred with a magnetic stirrer for 24 h at +4 °C and filtered through eight layers of muslin cloth to remove the solid particles. The filtrate was centrifuged immediately at 7000*g* for 1 h to obtain the clear crude enzyme extract which was directly used for partial purification. For the thermal characterization study, the crude extract was dialyzed against three 2 L portions of tris-HCl buffer at +4 °C before use.

Partial Purification. *Ammonium Sulfate Precipitation.* The crude enzyme extract was brought to a 95% saturation by adding solid $(NH_4)_2SO_4$ slowly with continuous mixing on a magnetic stirrer for 24 h at +1 °C. The resulting precipitate was separated by centrifugation at 7000*g* for 1 h. The precipitate obtained on centrifugation was dissolved in a minimum volume of tris-HCl buffer and dialyzed against three changes (2, 4, and 4 L) of the same buffer for 72 h at +4 °C.

Ion-Exchange Chromatography. For further purification, the $(NH_4)_2SO_4$ precipitated and dialyzed enzyme extract was loaded onto a DEAE−cellulose column (2.5 × 15 cm) preequilibriated with tris-HCl buffer at +4 °C. Elution was performed with a linear discontinuous gradient of 0 to 1 M NaCl in tris-HCl buffer at +4 °C. Fractions of 5 mL were collected during elution, analyzed immediately for POX activity and measured for their absorbances at 280 nm.

POX Activity. Enzyme activity was determined spectrophotometrically by the method of Heil et al. (1988) with minor modifications. First, 0.2 mL of POX extract was mixed with 2 mL of tris-HCl buffer in a test tube. The content of tube was then incubated for 5 min at 30 °C and transferred to a 3 mL quartz cuvette. The reaction was initiated by adding 0.2 mL of 1% H_2O_2 and 1% guaiacol (v/v, prepared in 50% ethanol) into the quartz cuvette. The increase in absorbance at 410 nm was recorded manually at 15 s intervals using a PYE UNICAM SPG-550 spectrophotometer equipped with a constant temperature cell holder at 30 °C. Enzyme activity was calculated from the slope of the initial linear portion of an absorbance versus time curve and was expressed as the change in absorbance per minute milliliter or unit. One unit of POX activity was defined as 0.001 unit change in absorbance for 1 mL of enzyme solution in 1 min.

Substrate Specificity. The substrate specificity of the POX fractions was determined by measuring the activity with various substrates. Guaiacol, pyrogallol, catechol, and 4-methyl catechol were all 2.5 mM and the catechin was 0.5 mM. The reaction mixture was prepared as described in the activity determination section. Two different blanks were prepared for the activity measurement. One blank was used to test the spontaneous absorbance increase without adding enzyme to the reaction mixture. In the other blank, H_2O_2 was excluded to test whether polyphenol oxidase (PPO) activity was present in the purified fraction.

Thermal Characterization. Heat inactivation studies were carried out according to the method of Yemenicioğlu et al. (1997) over the temperature range of 55−70 °C by using thermal inactivation time (TIT) tubes (i.d., 9 mm; wall thickness, 1 mm). To minimize the lag phase, TIT tubes containing 4 mL of tris-HCl buffer were preheated to a given temperature in a constant temperature (±0.2 °C) water bath. Once the temperature of buffer solution stabilized, 0.5 mL of enzyme extract was pipetted into each TIT tube, mixed with a vortex and immersed again into the water bath. After heating for a given period, the tubes were rapidly cooled by plunging into an ice−water bath. The contents of TIT tubes were assayed immediately for POX activities. The percent POX activity remaining after each treatment was calculated from initial activity.

Temperature profiles were determined by holding the enzyme solutions for 5 min at various temperatures. The inactivation experiment was carried out as described above.

For the determination of the optimum temperatures, activity measurements were carried out over the temperature range 20−65 °C.

Protein Content. Protein was determined by the method of Lowry using bovine serum albumin as a standard (Harris, 1987).

RESULTS AND DISCUSSION

Partial Purification. The summary of partial purification of POX from okra is given in Table 1. $(NH_4)_2SO_4$ precipitation and dialysis, with a recovery of 58%, resulted in a 2.6-fold higher specific activity compared to that of the crude extract.

Two main fractions were obtained from the DEAE-cellulose chromatography (Figure 1). The first fraction contained two peaks (P1 and P2), and the second three peaks (P3, P4, and P5). Three small peaks (P6, P7, and P8) came after the second fraction. Only the most active fractions of each peak were used in the study. As seen in Table 1, the specific activities of P1, P2, P7, and P8 were much higher than those of the others. This is due to the release of proteins between fractions 30 and 50. The most retained fraction, P8, was released from the DEAE cellulose column by changing the gradient after fraction 65.

Substrate Specificity. The results from the activity measurement with different substrates (Table 2) revealed that pyrogallol was oxidized without the addition

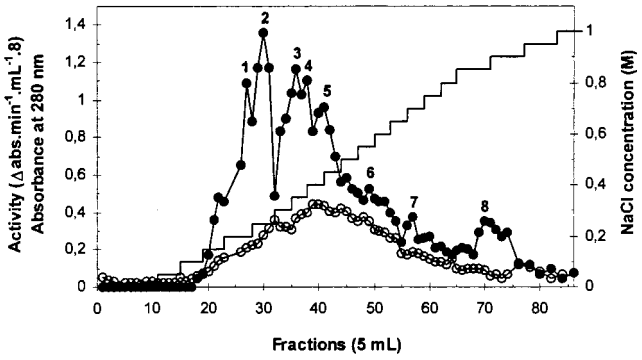

Figure 1. Elution profile of DEAE-cellulose chromatography of POX from okra: (○) Absorbance at 280 nm; (●) activity; and (—) NaCl concentration.

Table 2. Substrate Specificity of the Various POX Fractions Obtained from DEAE-Cellulose Chromatography

	relative activity (%)							
substrate	P1	P2	P3	P4	P5	P6	P7	P8
guaiacol	100	100	100	100	100	100	63	46
catechin	48	41	44	46	45	41	60	100
catechol	9	10	15	12	17	13	13	12
pyrogallol	50	68	76	63	73	62	100	69
4-methylcatechol	27	34	37	42	47	21	37	35

of H_2O_2 into the reaction mixture. Although the level of oxidation is very little, this value was subtracted from the activity measurements with H_2O_2. The reason for the oxidation of pyrogallol without the presence of H_2O_2 in reaction mixture may be the presence of PPO activity in the purified fractions or the oxidative activity of POX which can occur in a limited manner in the absence of H_2O_2 and certain cofactors (Mn^{2+} and phenol—mostly 2,4-dichlorophenol) (Vamos-Vigyazo; 1981). The first seems more unlikely. This is because 4-methyl catechol, catechol, and catechin, considered to be the best substrates for PPO, were not oxidized in the absence of H_2O_2 in the reaction mixture. The data in Table 2 show that the POX fractions acted primarily on guaiacol, pyrogallol, and catechin. Among the substrates studied, catechol is the least preferred substrate. The P7 and P8 fractions acted mainly on catechin and pyrogallol, respectively, whereas the P1—P6 fractions acted primarily on guaiacol. Since most of the fractions showed high substrate specificity toward guaiacol, thermal characterization of crude extract and purified fractions were carried out by using guaiacol as substrate.

Thermal Characterization. The temperature profiles of eight POX fractions and the crude extract are shown in Figure 2. As seen in Figure 2a, while the temperature profiles of P1—P4 fractions were quite similar above 60 °C, they were different below 60 °C. Moreover, P1—P4 fractions lost almost 50% of their activity for 5 min heating at 60 °C. In contrast, P5—P8 fractions have different temperature profiles except between 65 and 70 °C. The inactivation of all fractions slowed above 70 °C. This indicates that only the heat-stable portions remain active at high temperatures.

The heat inactivation of crude POX between 50 and 70 °C (Figure 3) and eight POX fractions at 70 °C (Figure 4) were fitted to a first-order reaction model. The inactivation curves were biphasic, which indicated the presence of heat-labile and heat-stable portions (Yamamoto et al., 1962; Ling and Lund, 1978; Naveh et al., 1982; Miller et al., 1990).

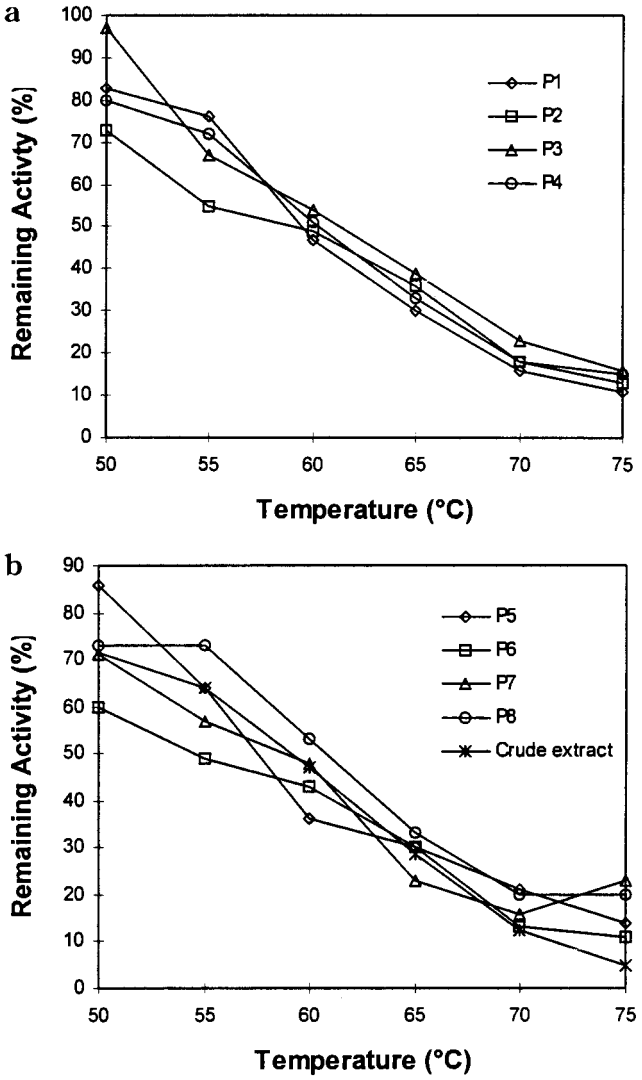

Figure 2. (a) Temperature profiles of partially purified peroxidase fractions. (b) Temperature profiles of partially purified peroxidase fractions and crude extract.

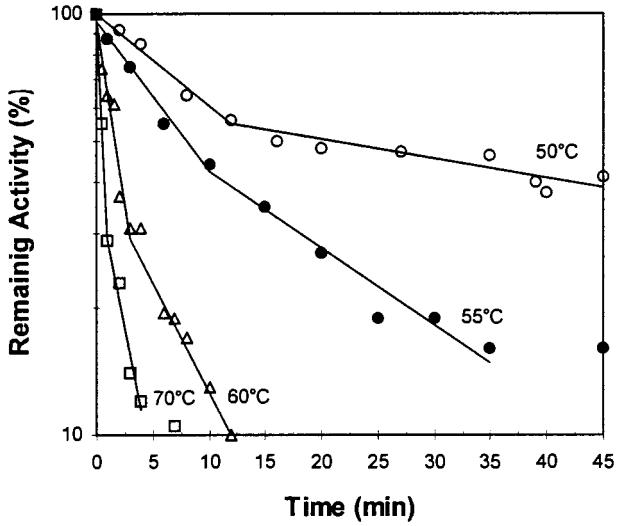

Figure 3. Heat inactivation of crude peroxidase from okra.

Table 3 shows the values of the heating times required to inactivate 90% of POX activity at a constant temperature (*D*-values) and the inactivation rate constants (*k*-values). It is obvious from these values that

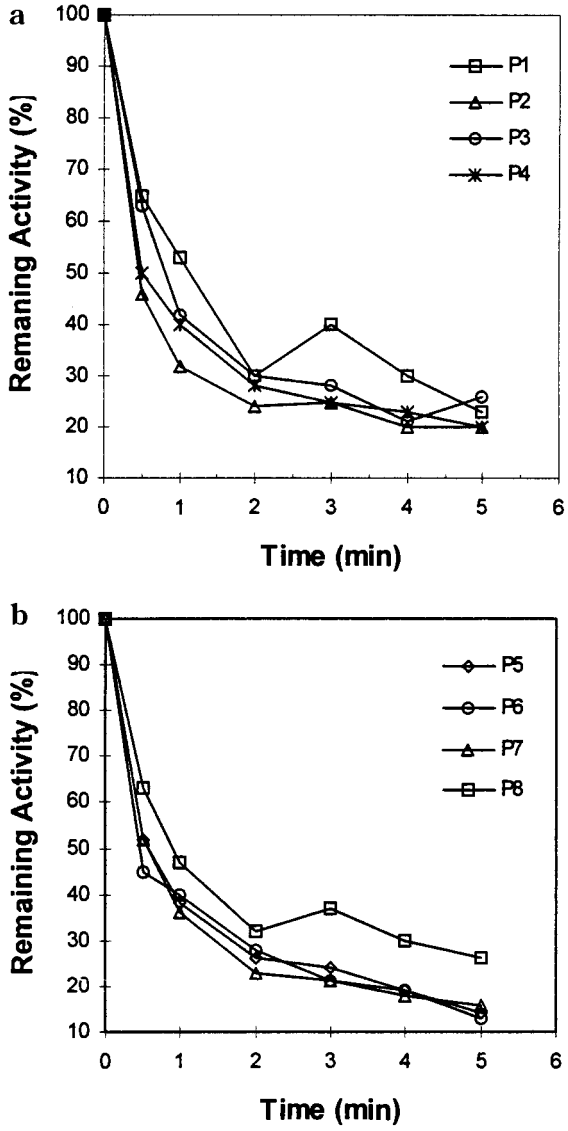

Figure 4. (a) Heat inactivation of partially purified peroxidase fractions at 70 °C. (b) Heat inactivation of partially purified peroxidase fractions at 70 °C.

Table 3. Heat Inactivation Parameters of the Crude POX from Okra[a]

temp (°C)	D (min)		k (min^{-1} 10^2)	
	HL	HS	HL	HS
50	45	247	5.098	0.932
55	28	56	8.207	4.139
60	5.8	17.8	39.392	12.972
70	1.9	7.3	123.809	31.441

[a] E_a(HL) = 37.0 kcal mol^{-1} (r = 0.978); z(HL) = 13.8 °C (r = 0.976); HL = heat labile. E_a(HS) = 37.8 kcal mol^{-1} (r = 0.962); z(HS) = 13.5 °C (r = 0.957); HS = heat stable.

the crude POX from okra is very heat labile. For this reason, the use of POX as an indicator of blanching adequacy of okra may not be safe. The heat-labile POX from various vegetables has been reported, including asparagus POX with D_{70} of 3 min (Ganthavorn et al., 1991), pinto bean POX with D_{70} of 8.2 min (Yemenicioğlu et al., 1998), and green pea POX with D_{70} of 0.5 min (Halpin et al., 1989).

Table 4 shows the D- and k-values of the DEAE-cellulose-purified POX fractions at 70 °C. The comparison of the k-values for these fractions revealed that the P2 fraction contained the most heat-stable portion (D_{70} = 20.5 min). On the other hand, comparing the heat stabilities of heat-labile portions at 70 °C, P1 had the most heat-stable fraction. Moreover, P1 and P8 showed slight activation after heating for 3 min at 70 °C.

Table 4. D_{70} Values for the Various POX Fractions Obtained from DEAE-Cellulose Chromatography

fractions	D (min)		k (min^{-1} 10^2)	
	HL	HS	HL	HS
P1	3.6	*a*	63.499	*a*
P2	2.0	20.5	113.964	11.225
P3	2.7	17.5	86.766	13.161
P4	2.5	14.6	91.646	15.833
P5	2.4	10.0	96.776	23.111
P6	2.5	8.7	91.646	26.361
P7	2.3	12.3	102.184	18.673
P8	3.0	*a*	75.516	*a*

[a] Showed activation.

The temperature dependence of the heat inactivation of crude POX was determined by calculating the thermal inactivation time (TIT) and Arrhenius parameters for the heat-labile and -stable portions (Table 3). Our E_a-values compared well with the E_a of 37.6 kcal mol^{-1} for the heat-stable portion of pinto bean POX (Yemenicioğlu et al., 1998). However, our E_a values were much higher than the E_a of 21 kcal mol^{-1} for the heat-stable portion of corn POX (Naveh et al., 1982) and 22.1 kcal mol^{-1} for the heat-stable portion of horseradish POX (Ling and Lund, 1978). Our z-values were, on the other hand, lower than those reported by Williams et al. (1986) for pea POX (27 °C) and green bean POX (48.8 °C) and by Rodrigo et al. (1996) for asparagus POX (45.5 °C). Results of all these comparisons implied that the heat inactivation of POX from okra was relatively more dependent on temperature changes than the heat inactivation of POX from other vegetables.

The plots of activity versus temperature for eight POX fractions and the crude extract were given in Figure 5. The temperature dependence of the activities of purified POX fractions were quite different. The activity of P2 fraction which maintained more than 50% of its activity between 20 and 65 °C was not greatly affected from the temperature changes. In contrast, the activities of P7 and P8 fractions increased almost 10-fold as the temperature rose from 20 to 65 °C.

Comparing to the DEAE−cellulose-purified fractions, the crude POX had a completely different activity−temperature profile. The optimum temperature of crude POX was between 30 and 35 °C, and above 45 °C, the crude POX showed very little activity. In contrast, P1−P5 fractions exhibited maximum activity at 60 °C, and P6−P8 fractions at over 65 °C. The high-temperature optima obtained for the DEAE−cellulose purified fractions may be due to the activation which appears to begin between 50 and 60 °C (Figure 5). Therefore, the range between 60 and 65 °C may not be the true temperature optima for these fractions. This is because the fractions lost 50−60% and 60−75% of their activities within 5 min at 60 and 65 °C (Figure 2), respectively. The heat activation of various enzymes was reported by Yemenicioğlu et al. (1997) for apple PPO, by Lee et al. (1991) for cocoa PPO, by Vámos-Vigyázó (1981) for kohlrabi POX, and by Chan et al. (1996) for papaya ethylene-forming enzyme. The observed increase in activity by heat treatment is attributed to the release of latent enzymes (Yemenicioğlu et al., 1997) or the transformation of enzyme into a transient active form before inactivation (Vámos-Vigyázó, 1981).

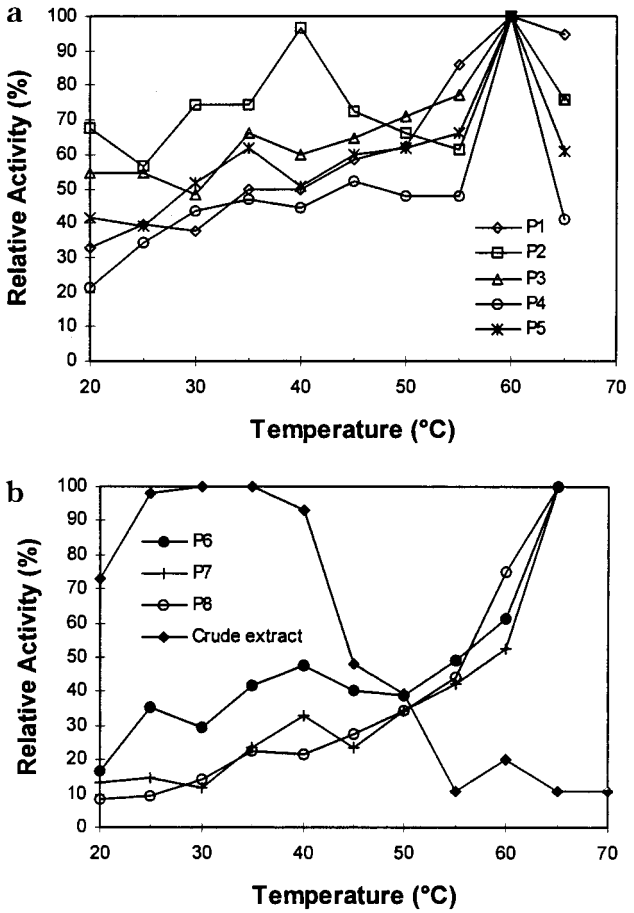

Figure 5. (a) Activity−temperature profiles for the partially purified peroxidase fractions. (b) Activity−temperature profiles for the partially purified peroxidase fractions and crude extract.

It is worth noting that the crude POX from okra did not show activation at high temperatures (Figure 5b). This might be due to the presence of some inhibitors which prevent activation or impurities, such as carbohydrates which are known to form a complex with POX (Lee and Kim, 1994). This complex may stabilize the enzyme (Adams, 1991) to prevent its activation, which appears to require a limited conformational change (Angleton and Flurkey, 1984; Moore and Flurkey, 1990). Most POX fractions showed a small rise in activity in the range between 35 and 40 °C before the obvious increase at higher temperatures defined as activation. These two temperatures are most likely the true temperature optima for the purified fractions.

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LITERATURE CITED

Adams, J. B. Enzyme inactivation during heat processing of food-stuffs. *Int. J. Food Sci. Technol.* **1991**, *26*, 1−20.

Angleton, E. L.; Flurkey, W. H. Activation and alteration of plant and fungal polyphenol oxidase isoenzymes in sodium dodecyl sulfate electrophoresis. *Pyhtochemistry* **1984**, *23*, 2723−2725.

Burnette, F. S. Peroxidase and its relationship to food flavor and quality: A Review. *J. Food Sci.* **1977**, *42*, 1−6.

Chan, H. T.; Maindonald, J. M.; Laidlaw, W. G.; Seltenrich. ACC oxidase in papaya sections after heat treatment. *J. Food Sci.* **1996**, *61*, 1182−1185, 1190.

Coseteng, M. Y.; Lee, C. Y. Changes in apple polyphenol oxidase and polyphenol concentrations in relation to degree of browning. *J. Food Sci.* **1987**, *52*, 985−989.

Ganthavorn, C.; Powers, J. R. Changes in peroxidase activity, hexanal, ascorbic acid and free sulfhydryl in blanched asparagus during frozen storage. *J. Food Sci.* **1988**, *53*, 1403−1405.

Ganthavorn, C.; Nagel, C. W.; Powers, J. R. Thermal inactivation of asparagus lipoxygenase and peroxidase. *J. Food Sci.* **1991**, *56*, 47−49, 79.

Guyer, R. B.; Holmquist, J. W. Enzyme regeneration in high temperature-short time sterilized canned foods. *Food Technol.* **1954**, *8* (12), 547−550.

Halpin, B.; Pressey, R.; Jen, J.; Mondy, N. Purification and characterization of peroxidase isoenzymes from green peas (*pisum sativum*). *J. Food Sci.* **1989**, *54*, 644−649.

Harris, D. A. Spectrophotometric assays. In *Spectrophotometry and Spectrofluorometry;* Harris, D. A., Bashford, C. L., Eds.; I. R. L. Press: Oxford, 1987.

Heil, J. R.; McCarthy, M. J.; Merson, R. L. Influence of gluconic acid on enzyme inactivation and color retention in canned apricots and peaches. *J. Food Sci.* **1988**, *53*, 1717−1719.

Hemeda, H. M.; Klein, B. P. Inactivation and regeneration of peroxidase activity in vegetable extracts treated with antioxidants. *J. Food Sci.* **1991**, *56*, 68−71.

Lee, M. Y.; Kim, S. S. Characteristics of six isoperoxidases from Korean radish root. *Phytochemistry* **1994**, *35*, 287−290.

Lee, P. M.; Lee, K. H.; Karim, M. I. A. Biochemical studies of cocoa bean polyphenol oxidase. *J. Food Sci.* **1991**, *55*, 251−260.

Ling, A. C.; Lund, D. B. Determining kinetic parameters for thermal inactivation of heat-resistant and heat-labile isoenzymes from thermal destruction curves. *J. Food Sci.* **1978**, *43*, 1307−1310.

Lopez, P.; Burgos, J. Peroxidase stability and reactivation after heat treatment and manothermosonication. *J. Food Sci.* **1995**, *60*, 451−455, 482.

Miller, A. R.; Kelley, T. J.; Muyer, C. V. Anodic peroxidase isoenzymes and polyphenol oxidase activity from cucumber fruit: Tissue and substrate specificity. *Phytochemistry* **1990**, *29*, 705−709.

Moore, B. M.; Flurkey, W. H. Sodium dodecyl sulfate activation of a plant polyphenoloxidase. *J. Biol. Chem.* **1990**, *265*, 4982−4988.

Müftügil, N. The peroxidase enzyme activity of some vegetables and its resistance to heat. *J. Sci. Food Agric.* **1985**, *36*, 877−880.

Naveh, D.; Mizrahi, S.; Kopelman, I. J. Kinetics of peroxidase deactivation in blanching of corn on the cob. *J. Agric. Food Chem.* **1982**, *30*, 967−970.

Rodrigo, C.; Rodrigo, M.; Alvarruiz, A.; Frígola, A. Thermal inactivation at high temperatures and regeneration of green asparagus peroxidase. *J. Food Protect.* **1996**, *59*, 1065−1071.

Rodrigo, C.; Rodrigo, M.; Alvarruiz, A.; Frígola, A. Inactivation and regeneration kinetics ofhorseradish peroxidase heated at high temperatures. *J. Food Protect.* **1997**, *60*, 961−966.

Romero, M. V.; Barrett, D. M. Rapid methods for lipoxygenase assay in sweet corn. *J. Food Sci.* **1997**, *62*, 696−700.

Sheu, S. C.; Chen, A. O. Lipoxygenase as blanching index for frozen vegetable soybeans. *J. Food Sci.* **1991**, *56*, 448−451.

Theerakulkait, C.; Barrett, D. M.; McDaniel, M. R. Sweet corn germ enzymes affect odor formation. *J. Food Sci.* **1995**, *60*, 1034−1040.

Vámos-Vigyázó, L. Polyphenol oxidase and peroxidase in fruits and vegetables. *CRC Crit. Rev. Food Sci. Nutr.* **1981**, *15*, 49−127.

Williams, D. C.; Lim, M. H.; Chen, A. O.; Pangborn, R. M.; Whitaker, J. R. Blanching ofvegetables for freezing—which indicator enzyme to choose. *Food Technol.* **1986**, *40* (6), 130−140.

Yamamoto, H. Y.; Steinberg, M. P.; Nelson, A. I. Kinetic studies on the heat inactivation of peroxidase in sweet corn. *J. Food Sci.* **1962**, *27*, 113−119.

Yemenicioğlu, A.; Özkan, M.; Cemeroğlu, B. Heat inactivation kinetics of apple polyphenoloxidase and activation of its latent form. *J. Food Sci.* **1997**, *62*, 508−510.

Yemenicioğlu, A.; Özkan, M.; Velioğlu, S.; Cemeroğlu, B. Thermal inactivation kinetics of peroxidase and lipoxygenase from fresh pinto beans (*Phaseolus vulgaris*). *Z. Lebensm. Unters. Forsch.* **1998**, *206*, 294−296.

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