J Food Sci Technol (January–February 2010) 47(1):67–72

ORIGINAL ARTICLE

Kinetic study of thermal inactivation of potato peroxidase during high-temperature short-time processing

Bo Yu · Zhengyu Jin · Li Deng · Xueming Xu · Lifeng He · Jinpeng Wang · Yaoqi Tian · Hanqing Chen

Revised: 13 October 2008 / Accepted: 9 June 2009

Abstract Thermal inactivation curves for peroxidase in potato extracts were determined in the range of 100 to 140°C for 10 to 100 sec. The capillary tube method was used to obtain isothermal conditions. The come-up time for the capillary tubes was accurately calculated by analysis method by which thermal inactivation kinetics of enzymes in relation to high temperature processing would be more easily detected. Heat inactivation of potato peroxidase followed first-order reaction kinetics and yielded a curved Arrhenius plot for the temperature dependence at high temperatures. Kinetics parameters, k and E_a, were calculated for potato peroxidase. At temperature range of 100–140°C, the activation energy of peroxidase was lower than that in the range of 78–84°C. It could be elucidated by the scheme of thermal inactivation pathway.

Keywords Thermal inactivation · Peroxidase · High-temperature short-time · Potato

Introduction

Deterioration of raw or processed fruit and vegetable products is mainly attributed to residual enzymatic activity. These changes can affect the colour, flavour, texture, and nutrient content (Adams 1991, Ramaswamy and Fakhouri 1998, Kaur and Kapoor 2001, Premakumar and Khurdiya 2002, Jayakumar et al. 2007). However, the temperature range of traditional heat treatment is between 60 and 100°C, and this process always takes long time to inactivate these enzymes. A number of nutrients are lost during the processing. Haase and Weber (2003) estimated ascorbic acid losses during various steps in the processing of French fries and observed that loss of ascorbic acid was highest during blanching. Therefore, it is desirable to keep the heat treatment to a minimum but sufficient to completely inactivate these enzymes.

A new heat treatment – high-temperature short-time (HTST) has been introduced into the food industry. Drake and Carmichael (1986) demonstrated that HTST stream blanching produced high quality vegetables and imparted distinct quality parameters to the frozen vegetables as compared with water-blanched vegetables. To monitor the heat treatment, peroxidase (POD, EC 1.11.1.7) is often used as an indicator enzyme to assess the degree of inactivation. Its residual activity has adverse effect on the quality of processed plant products, resulting in changes such as browning, off-flavour and loss of vitamins. Moreover, it is one of the most heat-stable enzymes and its inactivity can indicate that other enzymes are unlikely to be active. Peroxidases catalyze the oxidation of a large variety of substrates through the reaction with hydrogen peroxide. It typically catalyzes a reaction of the form:

$$H_2O_2 + AH_2 \text{ (electron donor)} \longrightarrow A + 2\,H_2O \quad (1)$$

Based on this feature, peroxidase is easily assayed to evaluate the degree of inactivation.

Studies in thermal inactivation kinetics of peroxidase in the range of 60 to 100°C have clearly shown biphasic curves which are thought to depend on the presence of isoenzymes with different thermal stabilities (Wang and Luh 1983, Powers et al. 1984, Ganthavorn et al. 1991, Forsyth et al. 1999). An inactivation biphasic model was proposed by Ling and Lund (1978) to describe the inactivation thermal

Yu B.[1,2] · Jin Z.[1,2] · Deng L.[1] · Xu X.[1,2] · He L.[1,2] · Wang J.[1,2] · Tian Y.[1,2] · Chen H.[2]
[1]The State Key Lab of Food Science and Technology, Jiangnan University, Wuxi - 214 122, China
[2]School of Food Science and Technology, Jiangnan University, Wuxi - 214 122, China

Zhengyu Jin (✉)
E-mail: jinlab2008@yahoo.com

kinetics of an enzyme system formed by a heat-labile fraction and a heat-resistant fraction, both with first-order inactivation kinetics. The difference between kinetic parameters for heat-labile and heat-resistant isoenzyme fractions from several sources (Ling and Lund 1978, Günes and Bayindirh 1993) indicated the need and importance of determining the kinetics of POD in different vegetable extracts.

Peroxidase activity was measured in potatoes after blanching treatment at 30, 50, 70 and 85°C (Wang and Luh 1983, Powers et al. 1984, Ganthavorn et al. 1991, Tijskens et al. 1997, Forsyth et al. 1999, Anthon and Barrett 2002). Heat denaturation of enzymes could be described using an exponential decay (first order reaction). The temperature dependence of reaction rates could be described by Arrhenius'law (Tijskens et al. 1997). Anthon and Barrett (2002) reported that peroxidase in potatoes was the most resistant and its activity gave simple first-order inactivation kinetics but yielded a curved Arrhenius plot for the temperature dependence. However, the early researches in the thermal inactivation kinetics of peroxidase from potatoes focus on the rules or mathematical modeling between low temperature and enzyme inactivation. We report here more detailed inactivation kinetic data for peroxidase of potatoes during HTST processing.

Materials and methods

Potatoes (*Solanum tuberosum* variety 'Favorita') were purchased from local market. Hydrogen peroxide (30%), o-phenylenediamine, potassium dihydrogen phosphate and dipotassium hydrogen phosphate were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). All chemicals were of analytical grade. The phosphate buffer was prepared with monopotassium phosphate and dipotassium phosphate in distilled water obtaining a molar concentration of 0.1M and pH 6.0. The buffer solution was cooled to 4°C prior to use.

Preparation of crude extract: Potatoes were rinsed thrice with distilled water, peeled with a surgical blade to prevent damage of outer pericarp tissue and cut into 0.4±0.1 cm thick disks. The sample (100 g) was homogenized in 100 ml of 0.1 M phosphate buffer (pH 6.0) in a Waring commercial blender (Philips, Holland) and blended for 3 min at high speed. The homogenate was filtered through 2 layers of cheesecloth and then centrifuged for 20 min (4°C, 16000xg) in a Sigma 3K30 laboratory centrifuge (Steinheim, Germany). The supernatant was desalted by passage through Sephadex G-25 equilibrated with 0.1 M phosphate buffer (pH 6) using the centrifugation method of Helmerhorst and Stokes (1980). This desalting step was included to remove phenolic compounds that caused potato homogenates to brown rapidly. The resulting crude POD extract was kept in ice-water bath (0°C) prior to heat treatment.

Thermal inactivation experiments: Early studies on thermal inactivation kinetics of peroxidase in plants adopted the method of capillary tubes (Resende et al. 1969, Wang and Dimarco 1972, Adams 1978, Bhirud and Sosulski 1993, Rodrigo et al. 1997). In this study heat inactivation experiments of POD extracts were conducted following the capillary tube method. In order to achieve a quasi-isothermal condition, the heating-up time must be as short as possible. Enzyme samples (30 µl) were transferred to capillary tubes (1 mm i.d., 50 µl total volume) through siphonage. Capillary tubes were immediately sealed on the flame of alcohol blowtorch, quickly cooled in ice-water bath and stored in refrigerator until heat treatment.

Capillary tubes with 30 µl of enzyme extracts were immersed in a thermostatic oil bath (Model HY020, Shanghai Laboratory Instrument Works Co., Ltd., China) with controlled temperature. Heat inaction was studied for holding temperatures range from 100 to 140°C at exposure time between 10 and 100 sec. In order to cool the samples as soon as possible, capillary tubes were quickly transferred to ice-water bath (0°C). Then enzyme extracts were collected in 1.5 ml centrifuge tubes and centrifuged for 5 min (4°C, 3800xg) in a Sigma 3K30 laboratory centrifuge.

Heat conduction calculation: Capillary tubes are regarded as infinite cylinder. The come-up time for the capillary tubes is accurately calculated based on the theory of heat transfer.

$$\Theta = 2\sum_{n=1}^{\infty} \frac{BiJ_0(\lambda_n x)}{(\lambda_n^2 L^2 + Bi^2)J_0(\lambda_n L)} \exp(-\lambda_n^2 L^2 F_0) \quad (2)$$

where, Θ is excess temperature, L is computing sizes, Bi is biot value, $Bi = \frac{h_{fp}L}{k}$, h_{fp} is convective heat transfer coefficient, k is heat conductivity, J_0 is the zeroth-order primal Bessel function, λ_n is instability analytical calculation eigenvalue and it is in accord with the following equation.

$$\frac{J_0(\lambda_n L)}{J_1(\lambda_n L)} = \frac{\lambda_n L}{Bi} \quad (3)$$

where, J_1 is the first-order primal Bessel function.

$h_{fp} \to \infty$, $Bi \to 0$. The Eq. 2 could be expressed by the following equation:

$$\Theta = 2\sum_{n=0}^{\infty} \frac{J_0(\lambda_n r / D)}{\lambda_n J_1(\lambda_n)} \exp(-\lambda_n^2 L^2 F_0) \quad (4)$$

Based on Eq. 4, the come-up time for the capillary tubes is accurately calculated and analyzed by means of computer programming.

Determination of peroxidase activity: POD activity was assayed using a modification of the spectrophotometric method of Rastogi et al. (1999) and Préstamo (1989). The sample cuvette contained 80 µl enzyme extract and a mixture composed of 2.6 ml potassium phosphate buffer (10 mM, pH 6.0) with 0.1 ml (1%, w/v) p-phenylenediamine as H-donor and 0.2 ml (0.3%, v/v) hydrogen peroxidase as

oxidant. An increase in absorbance at 430 nm was recorded automatically for 3 min at 30°C (Ultrospec 2100, Unico Co., USA). The enzyme extract was replaced by 0.1 ml potassium phosphate buffer (10 mM, pH 6.5) in the blank sample. Enzyme activity was calculated from the linear part of curve obtained by plotting absorbance against incubation time. One POD unit was defined as an increase of 0.1 in absorbance per min (Flurkey and Jen 1978). Thermal treatments of enzymatic extracts were done in triplicate.

Kinetic parameters calculation: For investigations above 100°C, reported data of thermal inactivation kinetics have showed linearity (log percent of remaining activity *versus* heating time) or simple first order kinetics. The rate constant k for first-order inactivation was determined from the slope of inactivation time curve according to Eq. 5:

$$\mathrm{Log}(A/A_0) = -(k/2.303)t \quad (5)$$

where, A_0 is the initial enzyme activity and A is the activity after heating for time t.

Slopes of these lines are determined by linear regression and calculated rate constants replot in Arrhenius plots. The temperature and denaturation constant are related according to the Arrhenius equation:

$$K = Ae^{-Ea/RT} \quad (6)$$

where, k represents the rate constant for the denaturation process, A is the Arrhenius constant, E_a is the apparent energy of activation, R is the gas constant (8.314 J/mol·K) and T is the temperature in K. Taking natural logarithms:

$$1\mathrm{n}(k) = -E_a / RT + C \quad (7)$$

Slopes and their standard errors are calculated by linear regression. Activation energies (E_a) are calculated from slopes of these Arrhenius plots according to Eq. 7.

In some cases inactivation is given as a D value, the time required to reduce the enzyme activity to 10% of its original value. The D values are calculated by regression analysis of the lines obtained by plotting the logarithm of residual peroxidase activity expressed as the percentage of initial peroxidase concentration against time. They correspond to the reciprocal of slope of those lines. According to the Bigelow model (Ball and Olson 1957), the D value is directly related to the inactivation rate constant k by Eq. 8:

$$D = 2.303 / k \quad (8)$$

Results and discussion

Heat penetration: It was critical to accurately control heating time in this experiment. The come-up time for the capillary tubes was determined by placing a thermocouple in the solution at the center of the tube and recording the time necessary for the solution in the tube to reach the set temperature of water bath or oil bath (Anthon and Barrett 2002, Cruz et al. 2006, Morales-Blancas et al. 2002). However, it was difficult to operate and easy to be error prone, especially in terms of high-temperature. The come-up time could be calculated by analysis method. The results are presented in the following figures obtained by computer programming.

When capillary tubes were placed in the oil bath (140°C), the temperature of inner wall of the capillary tubes reached 139°C in 0.4 sec, and they could be maintained 139.99°C in 0.7 sec (Fig. 1). When capillary tubes were placed in the oil bath (100°C), the temperature reached 99°C in 0.4 sec and was kept 99.99°C in 0.8 sec (Fig. 1). Thermal inactivation kinetics of peroxidase in relation to HTST processing of vegetables and fruits could be more easily detected by this method.

Thermal inactivation of POD: Conventional blanching processes involved temperatures ranging from 60 to 100°C and thermal inactivation experiments on potatoes were also carried out in the same temperature range (Anthon and Barrett 2002, Tijskens et al. 1997, Mukherjee and Chattopadhyay 2007). Conventional blanching can leach larger amounts of nutrients and solids and generate a higher waste load than HTST blanching. Sevilla and Luh (1974) observed that steam blanching leached fewer solids from beans than water blanching. The HTST blanching could produce high quality vegetables and impart distinct quality parameters to the frozen vegetables as compared to conventional blanched vegetables (Drake and Carmichael 1986). Generally, enzyme activities are dependent on temperature and heating time. However, this dependency would show differences among peroxidases from different sources. It was obvious that the residual potato peroxidase activity decreased sharply with increasing heating temperature in Fig. 2. The inactivation rate constant (k) was mainly determined by the temperature especially at higher temperatures.

The semilog plots of the residual activity *versus* heating time were linear at all high temperatures studied (Fig. 3). The inactivation velocity of peroxidase in potato crude extracts was enhanced with increase in heat treatment temperature. For some investigations above 100°C, the reported data have showed linearity or simple first order kinetics. Inactivation of heat-resistant peroxidase in sweet corn at 98–143°C followed a first-order reaction (Yamamoto et al. 1961). Asparagus peroxidase heated from 110 to 120°C reacted

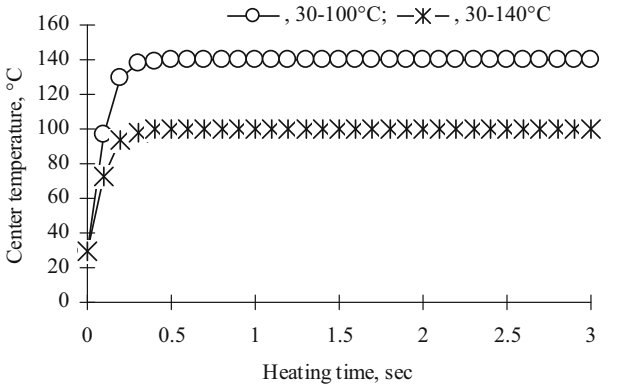

Fig. 1 The heating-up time curve for the capillary tubes

with first-order kinetics (Rodrigo et al. 1996, 1997). The inactivation rate constant (k) could be calculated at different temperatures according to Eq. 5 and the corresponding D values were obtained according to Eq. 7. These results are given in Table 1. There was significant correlation between the inactivation rate constants and temperatures. With the temperature increasing from 100 to 140°C, the inactivation rate constants increased by more than 2-fold. Meanwhile, time required for 90% inactivation at corresponding temperatures decreased by more than 2-fold.

The kinetic parameter of Arrhenius model, activation energies (E_a) for the potato peroxidase was determined by taking ln expression of Arrhenius equation. From the slopes of these lines (Fig. 3) inactivation rate constants were calculated and the plot of lnk *versus* 1/T is shown in Fig. 4. In the temperature range of 100 to 140°C, the plots could be approximated by straight lines. Activation energy of potato peroxidase was found from the slope of the curve as 27.114 kJ/mol (R^2=0.9543). There was apparent temperature-effect and time-effect in the thermal inactivation of peroxidase. In initial stage of HTST processing, inactivation rate constant was more affected by temperature-effect than time-effect. This result is also shown in Fig. 2. Most of peroxidase was inactivated in a short time. The growth rate of inactivation for potato peroxidase became smaller with prolongation of treatment time. Residual activation of potato peroxidase after treatment at 100 and 140°C for 10 sec was 49.0% and 16.3%, respectively. Activation energy of potato peroxidase in the range of 78–84°C was 478 kJ/mol (Anthon and Barrett 2002). In our experiments, activation energy potato peroxidase heated from 100 to 140°C was 27.114 kJ/mol. There was an explanation for this finding. Enzyme thermal inactivation was a complicated process that apparently may be described by simple kinetic models, but it consisted of many serial steps (Henley and Sadana 1986). The amount of potato peroxidase decreased obviously with treatment time at high temperature. The molecular structure and the secondary or tertiary stuctures of zymoprotein were destroyed. The thermal inactivation involved at least 2 steps, in the first step the zymoprotein unfolds reversibly from native structure (N) to yield an unfolded state (U) followed by an irreversible step that might be comformational scrambling, aggregation or a chemical process that resulted in an inactivated state (I) (Ahren and Klibanow 1985, Fágáin 1995). Therefore, the thermal inactivation pathway could be described by the following scheme:

$$N \Leftrightarrow U \rightarrow I \tag{9}$$

which is usually referred to as the Lumry and Eyring model (Lumry and Eyring 1954). In the first step there was a partial

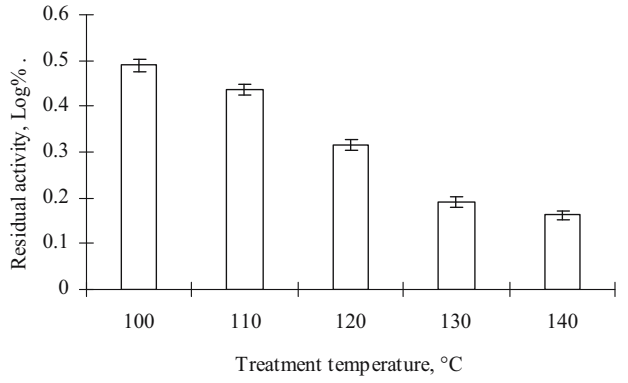

Fig. 2 Residual POD activity *versus* different heating temperature in 10 sec

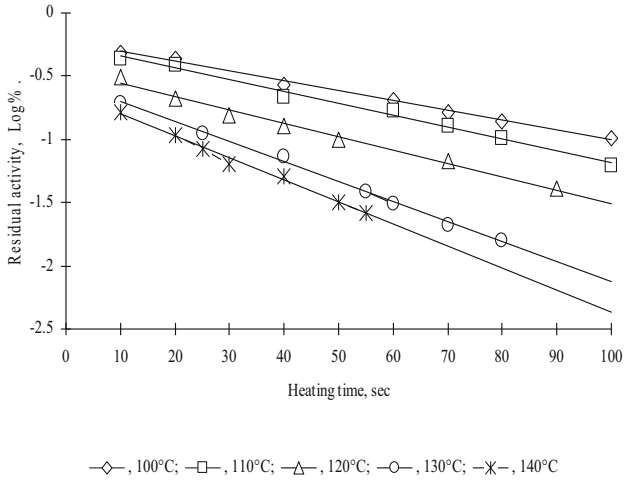

Fig. 3 Heat inactivation of peroxidase in potato crude extracts at different temperatures (100–140ºC)

Table 1 Inactivation parameters for potato peroxidase

Temp°C	K, s^{-1}	D values, s
100	0.017963	128.2051
110	0.021648	106.3830
120	0.024412	94.3396
130	0.036618	62.8931
140	0.039842	57.8035

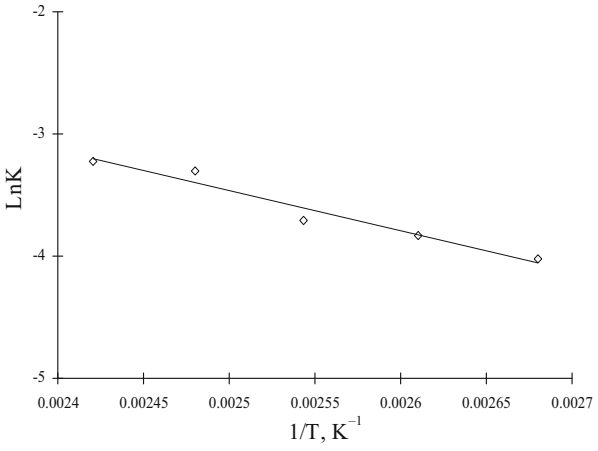

Fig. 4 Ahrrenius plot of the inactivation rates of potato peroxidase for thermal inactivation

loss of activity due to the disruption of the non-covalent interactions maintaining the native conformation. There was a temperature point or an appropriate range of temperatures, which was assumed as T_{ref} in this paper. T_{ref} was a midpoint temperature, which was related to the mechanisms of peroxidase inactivation. At temperatures below the T_{ref} protein unfolding (N⇔U) was the rate-limiting step for the N-I transition. At high temperatures ($T > T_{ref}$) native protein molecules almost became unfolded. The rate of the reaction was determined by the nature of the U-I step, and such result led to the low activation energy. The regeneration of peroxidase activity after partial deactivation was observed (Lu and Whitaker 1974, Adams 1978). These mechanisms could also be elucidated by the scheme. After HTST, there was residual peroxidase (unfolded state) in samples. The residual peroxidase (unfolded state) would transform into the active peroxdase (native state) at low temperatures, which could affect the quality of products.

Conclusion

A new technology, high-temperature short-time (HTST), has been introduced into the food industry. High temperature is able to inactivate enzymes easily and preserve the precious natural food constituents like vitamins as much as possible. Peroxidase is one of the most heat-stable enzymes and often used as an indicator enzyme to assess the degree of inactivation of other enzymes. For successful predictions of residual potato actives at high temperatures, it is necessary to know the kinetic parameters (k and E_a). The findings of this study would be useful in determining the process parameters at high temperatures. Heat inactivation of potato peroxidase in the temperature range of 100–140°C was in accordance with the first-order model and the activation energy was 27.114 kJ/mol. The kinetics parameters of peroxidase inactivation at high temperatures were significantly different from that at low termperatures. The come-up time for the capillary tubes was accurately calculated by analysis method and thermal inactivation kinetics of enzymes in relation to the high temperature processing could be more easily detected by this novel method.

Acknowledgement We thank the National Natural Science Foundation of China (20436020**)**, Program of State Key Laboratory of Food Science and Technology, Jiangnan University (SKLF-MB-200804) and Nature Science Foundation of Jiangsu Province (BK2008003) for financial support.

References

Adams JB (1978) The inactivation and regeneration of peroxidase in relation to the high temperature-short time processing of vegetables. J Food Technol 13:281–297

Adams JB (1991) Enzyme inactivation during heat processing of food-stuffs. Int J Food Sci Technol 26:1–20

Ahren TJ, Klibanov AM (1985) The mechanism of irreversible enzyme inactivation at 100°C. Science 228:1280–1284

Anthon GE, Barrett DM (2002) Kinetic parameters for the thermal inactivation of quality-related enzymes in carrots and potatoes. J Agric Food Chem 50:4119–4125

Ball CO, Olson FCW (1957) Sterilization in food technology. McGraw-Hill, New York, p 1–150

Bhirud PR, Sosulski FW (1993) Thermal inactivation kinetics of wheat germ lipoxygenase. J Food Sci 58:1095–1098

Cruz RMS, Vieira MC, Silva CLM (2006) Effect of heat and thermosonication treatments on peroxidase inactivation kinetics in watercress (*Nasturtium officinale*). J Food Eng 72:8–15

Drake SR, Carmichael DM (1986) Frozen vegetable quality as influenced by high temperature short time (HTST) steam blanching. J Food Sci 51:1378–1379

Fágáin CO (1995) Understanding and increasing protein stability. Biochim Biophys Acta 1252:1–14

Flurkey WH, Jen JJ (1978) Peroxidase and polyphenol oxidase activities in developing peaches. J Food Sci 43:1826–1828

Forsyth JL, Apenten RKO, Robinson DS (1999) The thermostability of purified isoperoxides from *Brassica oleracea* var. *gemmifera*. Food Chem 65:99–109

Ganthavorn C, Nagel CW, Powers JR (1991) Thermal inactivation of asparagus lipoxygenase and peroxidase. J Food Sci 56: 47–49

Günes B, Bayindirh A (1993) Peroxidase and lipoxygenase inactivation during blanching of green beans, green peas and carrots. Lebensm Wiss Technol 26:406–410

Haase NU, Weber L (2003) Ascorbic acid losses during processing of French fries and potato chips. J Food Eng 56:207–209

Helmerhorst E, Stokes GB (1980) Microcentrifuge desalting: a rapid, quantitative method for desalting small amounts of protein. Anal Biochem 104:130–135

Henley JP, Sadana A (1986) Deactivation theory. Biotechnol Bioeng 28:1277–1285

Jayakumar V, Pandey MC, Jayathilakan K, Manral M (2007) Development and evaluation of thermally processed pearlspot (*Etroplus suratensis*) fish curry. J Food Sci Technol 44: 350–352

Kaur C, Kapoor HC (2001) Effect of different blanching methods on the physico-chemical qualities of frozen french beans and carrots. J Food Sci Technol 38:65–67

Ling A, Lund D (1978) Determining kinetic parameter for thermal inactivation of heat-resistant and heat-labile isozymes from thermal destruction curves. J Food Sci 43:1307–1310

Lu AT, Whitaker JR (1974) Some factors affecting rates of heat inactivation and reactivation of horseradish peroxidase. J Food Sci 39:1173–1178

Lumry R, Eyring H (1954) Conformational changes of protein. J Phys Chem 58:110–120

Morales-Blancas EF, Chandia VE, Cisneros-Zevallos L (2002) Thermal inactivation kinetics of peroxidase and lipoxygenase from broccoli, green asparagus and carrots. J Food Sci 67: 146–154

Mukherjee S, Chattopadhyay PK (2007) Whirling bed blanching of potato cubes and its effects on product quality. J Food Eng 78:52–60

Powers JR, Costello MJ, Leung HK (1984) Peroxidase fractions from asparagus of varying heat stabilities. J Food Sci 49: 1618–1619

Premakumar K, Khurdiya DS (2002) Effect of microwave blanching on the nutritional qualities of banana puree. J Food Sci Technol 39:258–260

Préstamo G (1989) Peroxidase of Kiwifruit. J Food Sci 54: 760–762

Ramaswamy HS, Fakhouri MO (1998) Microwave blanching: Effect on peroxidase activity, texture and quality of frozen vegetables. J Food Sci Technol 35:216–222

Rastogi NK, Eshtiaghi MN, Knorr D (1999) Effect of combined high pressure and heat treatment on the reduction of peroxidase and polyphenoloxidase activity in red grapes. Food Biotechnol 13:195–208

Resende R, Francis FJ, Stumbo CR (1969) Thermal destruction and regeneration of enzymes in green bean and spinach puree. Food Technol 23(1):63–66

Rodrigo C, Rodrigo M, Alvarruiz A, Frígola A (1996) Thermal inactivation at high temperatures and regeneration of green asparagus peroxidase. J Food Prot 59:1065–1071

Rodrigo C, Alvarruiz A, Martinez A, Frígola A, Rodrigo M (1997) High-temperature short-time inactivation of peroxidase by direct heating with a five-channel computer-controlled thermo-resistometer. J Food Prot 60:967–972

Sevilla UL, Luh BS (1974) Several factors influencing color and texture of canned red kidney beans. Proc. IV Int. Congress Food Sci Technol 1:130

Tijskens LMM, Rodis PS, Hertog MLATM, Waldron KW, Ingham L, Proxenia N, Dijk C (1997) Activity of peroxidase during blanching of peaches, carrots and potatoes. J Food Eng 34: 355–370

Wang SS, Dimarco GR (1972) Isolation and characterization of the native thermally inactivated and regenerated horseradish peroxidase isozymes. J Food Sci 37:574–578

Wang Z, Luh BS (1983) Characterization of soluble and bound peroxidases in green asparagus. J Food Sci 48:1412–1417

Yamamoto HY, Steinberg MP, Nelson AI (1961) Kinetic studies on the heat inactivation of peroxidase in sweet corn. J Food Sci 27:113–119