Chiang Mai J. Sci. 2017; 44(3) : 881-890
http://epg.science.cmu.ac.th/ejournal/
Contributed Paper

Thermal Inactivation Kinetics of Peroxidase and Polyphenol Oxidase in Long Bean (*Vigna sesquipedalis*) and the Changes in their Activities during Storage

Lee Ting Hun [a], Shalom Wangrangsimakul [a], Waseem A. Wani [a], Ee Ling Yong [b], Ardawati Adnan [a], Eddie Ti Tjih Tan [c], Lim Chew Ching [a], Ooi Siew Yin [a], Azila Abdul-Aziz [a], Hesham A. El-Enshasy [a], Ramlan Aziz [a]

[a] Institute of Bioproduct Development, Universiti Teknologi Malaysia, 81310 Skudai, Johor, Malaysia.

[b] Faculty of Civil Engineering, Universiti Teknologi Malaysia, 81310 Skudai, Johor, Malaysia.

[c] Food Technology Department, Faculty of Applied Sciences, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia.

*Author for correspondence; e-mail: lee@ibd.utm.my

Received: 21 May 2015
Accepted: 21 September 2015

ABSTRACT

The thermal inactivation kinetics by hot water blanching of peroxidase and polyphenol oxidase in long beans (*Vigna sesquipedalis*) was investigated. Kinetic parameters such as the inactivation rate constant, activation energy and decimal reduction time were also determined. Peroxidase exhibited a biphasic inactivation behavior at 45-85°C with activation energies of 35.07 and 89.86 kJ/mol, respectively. However, polyphenol oxidase was monophasic at all temperatures with an activation energy of 98.25 kJ/mol. After 6 months of storage at -18, 0 and 5°C, the peroxidase activities of unblanched samples with respect to the level before storage were 42.20, 34.96 and 29.13 %, respectively, whereas the polyphenol activities were 68.44, 65.38 and 60.36 %, respectively. For blanched samples, the changes in peroxidase and polyphenol oxidase activities were approximately zero for storage at -18°C, whereas at the other temperatures, it experienced an increase of less than 4 %. Overall, this research gives insights into the long time storage of long beans. The blanching of long beans at 95°C for 12 min inactivates both these enzymes, and thus may increase the storage life of long beans.

Keywords: blanching, enzymatic browning, peroxidase, polyphenol oxidase, post-harvesting, enzyme activity

1. INTRODUCTION

In tropical countries such as Malaysia, long bean (*Vigna sesquipedalis*) is one of the most harvested vegetables due to its high export values. Handling and transportation can deplete the freshness of long beans before reaching the consumer. Therefore, post-harvest preservation techniques are critical to ensure the freshness and attractiveness of the

products before reaching the local and international markets. For this purpose, freezing techniques are commonly applied. However, the freezing techniques do not stop enzyme activities which aggravate the deterioration of the vegetable that results in significant reduction of quality through changes in texture, color, flavor and nutritional value [1]. In order to inactivate the enzymes, blanching of the long beans prior to freezing is generally employed [2].

Among the different enzymes responsible for the deterioration of the quality of vegetables, peroxidase (POD), polyphenol oxidase (PPO), catalase and protease are the most common ones. POD (E.C. 1.11.1.7) and PPO (E.C. 1.10.3.1) are the two enzymes found in long beans and their presence cause the formation of undesirable flavors and colors, as well as the reduction of nutritional values [3]. POD is an iron-porphyrin organic catalyst which involves the reduction of hydrogen peroxide and is regarded as one of the most heat resistant enzymes. Therefore, the inactivation of this enzyme might indicate that other enzymes are likely to be inactivated as well. PPO is a copper containing enzyme which has the ability to convert *o*-dihydroxyphenols to *o*-benzoquinones using oxygen as the secondary substrate that polymerizes non-enzymatically to brown pigments [4]. To date, the thermal kinetics and inactivation profiles of POD and PPO in long bean and the effects of cold storage on their activity are lacking. Therefore, the thermal kinetics and inactivation profiles of POD and PPO in long bean, and the effects of cold storage on their activity profiles were studied. This work might help to extend the shelf life of long beans and consequently increase their economic value.

2. MATERIALS AND METHODS

2.1 Vegetable Materials

The long beans (*Vigna sesquipedalis*) were obtained from a local market in Skudai, Johor, Malaysia. The beans were washed, the ends snipped off, and the strings removed. They were finally cut into small pieces and stored in a freezer at -20°C.

2.2 Chemicals

Phosphate and McIlvaine buffer solutions, polyvinylpolypyrrolidone (PVPP), and hydrogen peroxide were obtained from Merck Chemicals (Merck, Darmstadt, Germany). *p*-phenylenediamine (PPD) and catechol were supplied by Sigma-Aldrich (Sigma-Aldrich, USA). All chemicals were used without further purification.

2.3 Preparation of Homogenates

The refrigerated long beans were cut into small pieces, homogenized using a fruit juicer (M3-70M Juicer, National Co., Tokyo, Japan) and the extract was immediately transferred to an ice bath. The homogenates were prepared before each experiment.

2.4 Thermal Inactivation

1 ml homogenate was transferred to a test tube and was kept in ice bath before being heated in a circulated water bath (WNB 22, Memmert, Schwabach, Germany) at temperatures ranging from 45-95°C. The temperature of the water bath was verified using a thermometer (Hanna Instruments, HI 9043). To inactivate POD and PPO, the samples were blanched at 95°C for 6 min and 12 min, respectively. Following the heating process, samples were cooled in ice bath and immediately warmed to room temperature (25°C) in the water bath prior to analysis.

2.5 Enzyme Extraction and Assay

2.5.1 Peroxidase

The POD extraction was carried out by

mixing 1 ml of the homogenate with 5 ml of 0.05 M phosphate buffer (pH 7.0) containing 10 g/l of PPD. The mixture was centrifuged at 6000 rpm for 10 min at 4°C. 25 µl of the supernatant was mixed with 2.7 ml of 0.05 M phosphate buffer of pH 7.0, 200 µl of 10 g/l PPD and 100 µl of 1.5 % hydrogen peroxide (H_2O_2) solution. The activity was measured by using a UV-Vis spectrophotometer (Genesis 10 UV; Shimadzu Corp., Tokyo, Japan) at 485 nm (ΔAbs_{485nm}) at 25°C. A blank solution consisting of 2.7 ml of 0.05 M phosphate buffer at pH 7.0, 200 µl of 10 g/l PPD and 100 µl of 15 ml/l of H_2O_2 was prepared and the enzyme activity was deducted from this blank solution. One unit of POD activity was expressed as the amount of enzyme, which catalyzes the production of 1 mmol of 2,5-diamino-N, N'-di-(4-aminophenyl)-2, 5-cyclohexadiene-1, 4-diimine in 1 min at 25°C at 7.0 pH of [5].

2.5.2 Polyphenol oxidase

1 ml aliquot of the homogenate was mixed with 1 ml of McIlvaine buffer of pH 6.5 containing 5 % v/v PVPP. The mixture was centrifuged at 6000 rpm for 30 min at 4°C, and the supernatant was collected. 75 µl of supernatant was mixed with 3 ml of 0.05 M catechol and its activity was measured spectrophotometrically at 410 nm and 25°C. This measurement was done against blank which consisted of 3 ml of catechol without enzyme mixture. One unit of PPO activity was defined as the amount of enzyme which catalyzes the production of 1 mmol of benzoquinone in 1 min at 25°C at a pH 6.5 [4].

2.6 Determination of Heat Inactivation Kinetic Parameters

The enzyme activity was determined by taking the slope of the graph of Δ_{Abs} against time. Subsequently, the percentage of residual enzyme activity was calculated by taking the ratio of enzyme activities of blanched and unblanched samples (Equation 1).

$$Residual\ activity = E/E_0 \times 100 \quad (1)$$

Where E and E_0 are the activities of blanched and unblanched samples, respectively.

The deactivation rate constant, k, at different temperatures was determined from the slope of ln (E/E_0) *vs* blanching time (t) (Equation 2).

$$\ln E/E_0 = -kT \quad (2)$$

The activation energy (E_a), was obtained from the slope of the plot ln k *vs* $1/T$ (Equation 3).

$$\ln k = -/RT + \ln C \quad (3)$$

Where R is the gas constant (8.314 J/mol/K) and T is temperature in K.

The decimal reduction time (D) at different temperatures was calculated by using Equation 2.

$$D = 1/k \quad (4)$$

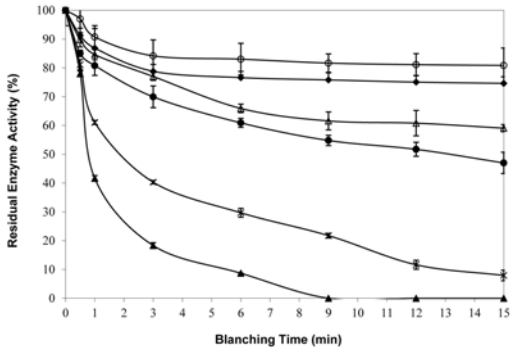
2.7 Storage of Samples

The samples were blanched at 95°C for 12 and 9 min to inactivate PPO and POD, respectively. The inactivated samples were subsequently stored in a biomedical freezer at -18, 0 and 5°C (MDF-136, Sanyo Electric Biomedical, Japan). The storage temperatures were chosen based on the industrial practice for fruits and vegetables where -18°C, 0°C, and 5°C is the long term, intermediate transport and retailing temperatures, respectively.

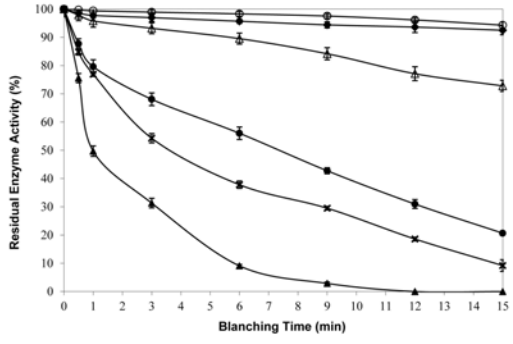
3. RESULTS AND DISCUSSION

3.1 Thermal Inactivation of POD and PPO

The POD and PPO residual activities of the samples during blanching at temperatures ranging from 45-95°C are shown in Figure 1 A and Figure 1 B, respectively. Both the enzymes showed signs of inactivation after blanching. The degree of inactivation was found to increase with rising temperature. After 15 min, POD activity reduced by a maximum of 50 % and 8 % at temperatures below 75°C and 85°C, respectively. The complete inactivation of POD was achieved at 95°C after 9 min.



(A)



(B)

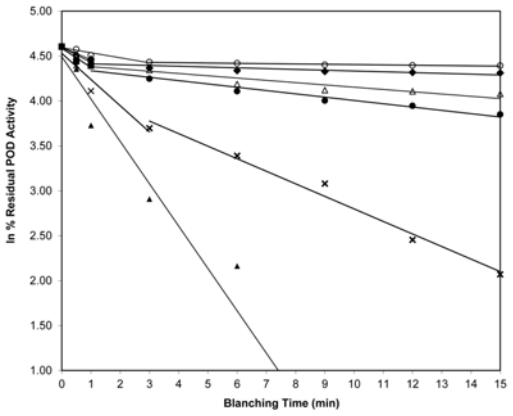
Figure 1. Enzyme residual activity (%) vs. blanching time (min) for (A) POD and (B) PPO at various blanching temperatures (○ 45°C, ♦ 55°C, Δ 65°C, • 75°C, X 85°C and ▲ 95°C).

Although PPO residual activities (Figure 1 B) followed a similar trend of inactivation as that by POD, the temperature at which more than half of the activity was reduced was 75°C and above. The complete inactivation of the enzyme was achieved at 95°C in 12 min. Thus, it can be concluded that PPO was more heat resistant compared to POD. The results of this work are in fair agreement with those for pineapple, apple and strawberry [6-8]. However, contrary results have also been reported in green coconut juice when microwave was used to inactivate POD and PPO [9]. Thus, it is safe to say that the thermal stability of POD and PPO is influenced both by plant types and the inactivation method applied.

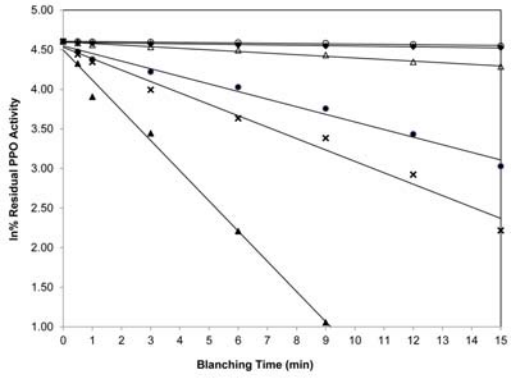
The logarithmic graphs of POD and PPO residual activities have been displayed in Figure 2A and 2B, respectively. Two linear stages were observed in Figure 2 A whereas Figure 2 B has only one. The two linear stages demonstrated that POD followed a biphasic inactivation behavior, which has also been observed in other fruits and vegetables such as strawberry, watercress and coconut water [8, 10, 11].

The first stage represents rapid inactivation of POD followed by a slower inactivation indicating the presence of iso-enzymes of different thermal stabilities [12]. This coincides with the physical and catalytic properties of POD which can be classified into heat labile and heat resistant fractions. The heat labile POD has lower thermal stability than the heat resistant fraction. However, at 95°C, POD inactivation followed a monophasic first-order kinetics and this may be due to the inactivation of the heat labile fraction of POD that was not able to be measured using the current experimental setup. Enzymes are basically proteins, and it is a well-known fact that the enzyme structures unfold or denature on

thermal treatment or on exposure to certain chemicals. This denaturation often leads to the loss of activity (inactivation).



(A)



(B)

Figure 2. ln % residual activity vs. blanching time (min) for (A) POD and (B) PPO at various blanching temperatures (○ 45°C, ♦ 55°C, Δ 65°C, • 75°C, **X** 85°C and ▲ 95°C).

From Figure 2 B, the thermal inactivation curves of PPO showed simple first-order inactivation at all temperatures. The monophasic curve demonstrated the presence of a dominant iso-enzyme or isozymes with similar inactivation characteristics at the studied temperature range. The findings were in agreement with previous studies on the inactivation of PPO in apple, watercress and horseradish [10, 13, 14]. However, in the work of Yemenicioglu and Cemeroglu [15], a biphasic curve for PPO inactivation in apricots was observed. Their findings might be associated with the environmental factors and the presence of specific proteases, which determined the conformation changes responsible for the biphasic curve.

3.2 Kinetic Parameters of POD and PPO Inactivation

3.2.1 Deactivation rate constant and decimal reduction time

The k value indicates the thermal stability of enzymes while the *D-value* represents the time required to reduce 90 % of enzyme activity, and both are used to measure the effectiveness of the blanching process. The k and *D-values* for POD and PPO are presented in Tables 1 and 2.

Tables 1 and 2 show the k and *D-values* for the thermal inactivation of POD and PPO, respectively. For both enzymes, the k values increased with temperature while the converse was true for *D-values*. In Table 1, the k values of heat labile fraction were higher than that of the heat resistant fraction, whereas the *D-values* displayed the opposite trend. PPO was more stable than POD at 55, 65 and 95°C while at other temperatures, it was less stable. Fortea *et al.* [16] demonstrated that the thermal inactivation of enzymes occurs through various mechanisms each with its own k and *D-values* and associates high E_a with protein unfolding.

Table 1. *k* and *D-values* of the thermal inactivation of POD.

k (min^{-1})		D-value (min)	
Heat Labile	Heat Resistant	Heat Labile	Heat Resistant
0.0563 ± 0.0105	0.0035 ± 0.0005	17.77 ± 3.33	289.04 ± 44.26
0.1409 ± 0.0239	0.0088 ± 0.0030	7.09 ± 1.20	113.32 ± 38.17
0.1671 ± 0.0281	0.0254 ± 0.0049	5.98 ± 1.09	39.43 ± 7.66
0.2134 ± 0.0640	0.0368 ± 0.0037	4.69 ± 1.40	27.16 ± 2.69
0.2868 ± 0.0487	0.1396 ± 0.0110	3.49 ± 1.40	7.16 ± 0.56
N/A	0.4004 ± 0.0589	N/A	2.50 ± 0.37

Table 2. *k* and *D-values* of the thermal inactivation of PPO.

Temperature (°C)	*k* (min^{-1})	*D-value* (min)
45	0.0035 ± 0.0003	286.58 ± 23.61
55	0.0046 ± 0.0004	216.28 ± 20.12
65	0.0203 ± 0.0009	49.38 ± 2.16
75	0.0960 ± 0.0045	10.42 ± 0.48
85	0.1441 ± 0.0077	6.94 ± 0.37
95	0.3814 ± 0.0159	2.62 ± 0.11

3.2.2 Activation energy

The activation energy measures the thermal sensitivity of the enzyme and can be defined as the energy required for the enzyme to unfold (inactivate), where a larger E_a indicates that it is strongly influenced by any changes in temperature. POD showed E_a of 89.86±6.23 and 35.07±6.64 kJ/mol for the heat resistant and heat labile fractions, respectively. The E_a for the heat resistant fraction of POD was higher compared to the heat labile fraction and this was in agreement with the works of other researchers (Table 3).

Table 3. Activation energies for POD heat labile and heat resistant fractions in other fruits and vegetables.

Product	E_a (kJ/mol)		References
	Heat Labile	Heat Resistant	
Green Bean	57	77	[29]
Peas	41	75	
Carrot	89.6	148	[19]
Asparagus (stem)	61	53	[18]
Watercress	421	352	[10]

The E_a for the thermal inactivation of PPO was 98.24±9.00 kJ/mol. The enzyme inactivation of PPO represented a first-order reaction and PPO was more heat resistant than POD due to a higher E_a. Table 4 summarizes the E_a of PPO in other types of fruits and vegetables and these differences might be due to heat transfer related issues arising from differences in sample preparation procedures used for the thermal inactivation of enzymes causing different heat transfer rate [17].

Table 4. Activation energies for PPO heat resistant fractions in other fruits and vegetables.

Fruits/Vegetables	E_a (kJ/mol)	References
Pineapple (Puree)	23.7 ± 1.7 (40-70°C)	[8]
	82.8 ± 2.7 (70-90°C)	
Mushroom (*Agaricus bisporus*)	325.61 ± 9.40 (55°C)	[30]
Apple (Golden Delicious)	55.1 (68-78°C)	[14]
Apple (Granny Smith)	57. 4 (68-78°C)	

In the present study, the samples were prepared as homogenates to ensure that the heat transfer rate was uniform and not influenced by size or texture. In other studies, enzyme extracts from coconut water, broccoli, green asparagus and carrot were studied and different enzyme assay methods were used; which resulted in different heat transfers [9, 18, 19]. The stability of enzymes are dependent on the donor substrate, the method of enzyme extraction and preparation of the crude extract from fruits or vegetables [19]. The choice of donor is important for the inactivation of enzymes since the stabilities of the iso-enzyme vary with the species of the donor substrate.

3.2.3 Activities of POD and PPO at different storage temperatures

Figure 3 shows the changes in the residual percentage activity of POD in unblanched and blanched samples during storage under different temperatures. For unblanched samples, the samples stored at -18°C (18LDC), 0°C (0LDC) and 5°C (5LDC) experienced a decrease in POD activity by the end of the storage. Total losses of 42.20, 34.96 and 29.13 % were recorded, respectively. In blanched samples, the POD activity of the sample stored at -18°C (18X) showed no sign of increment, whereas the samples stored at 0°C (0X), and 5°C (5X), recorded an increase in POD activity by 2.52 and 3.62 % at the end of 6 months storage time, respectively. It can also be concluded that the POD activity in unblanched samples decreased by a smaller degree at higher storage temperatures, and blanching at 95°C for 12 min was sufficient to restrict POD activity to a level of less than 4 %.

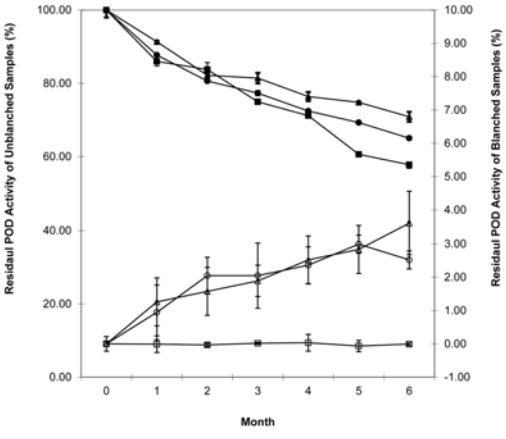


Figure 3. Changes of POD activity (%) during storage for unblanched samples under -18°C (■ 18LDC), 0°C (● 0LDC) and 5°C (▲ 5LDC) and for blanched samples (95°C for 9 min), targeted to inactivate POD at -18°C (□ 18X), 0°C (○ 0X) and 5°C (Δ 5X).

The changes in the residual percentage activity of PPO in unblanched and blanched samples are shown in Figure 4. The percentage of residual enzyme activity for PPO of unblanched samples stored at -18°C (18LOC) decreased gradually throughout the storage period and recorded a 68.44 % drop. For samples stored at 0°C (0LOC) and 5°C (5LOC), PPO activity decreased by 65.38 % and 60.36 % of their original activity, respectively. It could be concluded that the percentage residual enzyme activities of unblanched samples at the end of the storage time decreased more at lower storage temperatures. For blanched samples, PPO activity increased gradually with the sample stored at -18°C (18Y). Whereas, the PPO activity increased to 2.17 % and 3.99 % for samples stored at 0°C (0Y) and 5°C (5Y), respectively. The results indicated that the enzyme activity increased at higher storage temperatures after blanching and that the blanching condition of 95°C for 12 min was sufficient to restrict the increase in PPO activity to less than 5 % of residual enzyme activity.

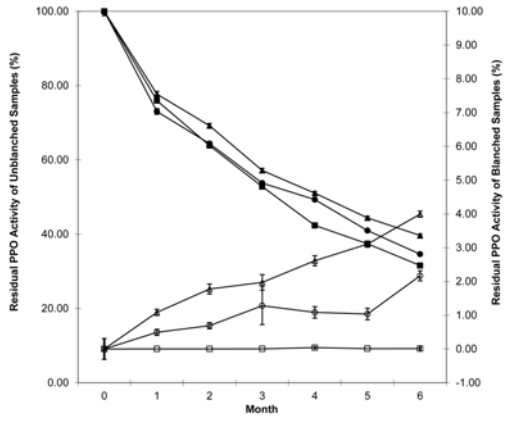


Figure 4. Changes of PPO activity (%) during storage for unblanched samples at -18°C (■ 18LOC), 0°C (● 0LOC), 5°C (▲ 5LOC) and for blanched (95°C for 12 min, targeted to inactivate PPO) samples at -18°C (□ 18Y), 0°C (○ 0Y) and 5°C (Δ 5Y).

Gokmen *et al.* [20] investigated the changes of POD activity in peas during frozen storage (-18°C) and found that the percentage of POD for unblanched samples were reduced by 40 %. In another study reported by Bahceci *et al.* [21], the percentage of POD activity for un-treated green bean samples decreased to about 35 % after 6 months of storage at -18°C. For PPO, Xu [22] studied the effect of low temperature storage (-20°C) on the activity of unblanched PPO in chestnuts and found that it decreased by 63 % after 6 months of storage. The greater reduction of PPO and POD activities of unblanched samples in lower temperatures may be due to greater long-term low temperature stress and instability, resulting in the rapid loss of activity during storage. The enzyme activities were strongly reduced by blanching but were not totally eliminated even at sub-zero temperature (-18°C).

Furthermore, the activity of POD and PPO did not increase significantly at the storage temperature of -18°C compared to 0 and 5°C. This may be explained by enzyme aggregation and/or conformational changes. At storage temperatures of 0 and 5°C, there was a slight increase of POD activity of 2.52 % and 3.62 %, respectively. Schweiggert *et al.* [23] reported that there was a slight increase in POD activity stored at ambient temperature in spice powder (*Capsicum frutescens L.*) that was heat treated. Lopez and Burgos [24] investigated the stability and reactivation after heat treatment and manothermosonication of horseradish peroxidase. In their work, POD activity was observed after 6 months of storage after applying thermal treatment and they suggested that the activity can be restored to a certain extent. Feng *et al.* [25] indicated that PPO could be regenerated under certain conditions, and suggested that during PPO

denaturation, the changes in the protein tertiary structure and the reduction of internal disulfide bonds might result in the exposure of copper-binding regions on the surface of the protein. This conformational change could lead to the loss of some copper ions from the active sites and that the regeneration could occur at correct temperatures and in the presence of certain substrates. Overall, little research has been cited for the regeneration of enzymes during storage, and the increased inactivity after heat treatment is unusual for enzymes, although it is a well-recognized property of POD and PPO [21, 23]. Thongsook *et al.* [26] studied the reactivation of PPO in broccoli and other enzymes that can regenerate including pepsinogen [27] and ribonuclease [28].

4. CONCLUSIONS

The thermal inactivation kinetics of POD and PPO in long bean homogenates was investigated at blanching temperatures ranging from 45-95°C. The inactivation of POD showed a biphasic behavior, indicating the presence of heat resistant and labile fractions. The inactivation of the latter faction occurred at 95°C. On the other hand, PPO followed a single first-order inactivation kinetics. Besides, it was more heat resistant as compared to POD. Further, the enzyme activities were measured during storage at different temperatures over 6 months. Interestingly, the POD and PPO activities of blanched samples exhibited a slight increase at lower temperatures, whereas they experienced negligible change during storage at -18°C. Overall, the results obtained in this study give us a clue of the different conditions needed for the post-harvest storage of long beans. Long beans are very important legumes; and used as food in different parts of the world. These can be stored for long times by using the results obtained in this study. Our work recommends blanching of long beans at 95°C for 12 min to inactivate both enzymes, and consequently increase the storage life of long beans. The results in this study will be beneficent to both the sellers and consumers of long beans in Malaysia and other tropical countries.

CONFLICT OF INTEREST DISCLOSURE

All authors have no conflicts of interest to disclose in completing this manuscript.

ACKNOWLEDGEMENTS

This work was supported by the Ministry of Science and Technology Malaysia.

REFERENCES

[1] Weits J., van der Meer M.A., Lassche J.B., Meyer J.C., Steinbuch E. and Gersons L., *Int. J. Vitam. Nutr. Res.,* 1970; **40(5)**: 648-658

[2] Devece C., Rodriguez-Lopez J.N., Fenoll L.G., Tudela J., Catala J.M., de los Reyes E. and Garcia-Canovas F., *J. Agric. Food Chem.,* 1999; **47(11)**: 4506-4511. DOI 10.1021/jf981398+.

[3] Nicoli M.C., Elizalde B.E., Pitotti A. and Lerici C.R., *J. Food Biochem.,* 1991; **15(3)**: 169-184. DOI 10.1111/j.1745-4514.1991.tb00153.x.

[4] Soliva-Fortuny R.C., Elez-Martinez P., Sebastian-Caldero M. and Martin-Belloso O., *J. Food Eng.,* 2002; **55(2)**: 131-137. DOI 10.1016/s0260-8774(02)00027-4.

[5] Cano M.P., deAncos B., Lobo M.G. and Santos M., *Eur. Food Res. Technol.,* 1997; **204(1)**: 60-65. DOI 10.1007/s002170050038.

[6] Chutintrasri B. and Noomhorm A., *LWT-Food Sci. Technol.,* 2006; **39(5)**: 492-495. DOI 10.1016/j.lwt.2005.04.006.

[7] Manzocco L., Quarta B. and Dri A., *Innov. Food Sci. Emerg. Technol.,* 2009; **10(4)**: 506-511. DOI 10.1016/j.ifset.2009.02.004.

[8] Sulaiman A. and Silva F.V.M., *Food Control,* 2013; **33(2)**: 424-428. DOI 10.1016/j.foodcont.2013.03.008.

[9] Matsui K.N., Granado L.M., de Oliveira P.V. and Tadini C.C., *LWT-Food Sci. Technol.,* 2007; **40(5)**: 852-859. DOI 10.1016/j.lwt.2006.03.019.

[10] Cruz R.M.S., Vieira M.C. and Silva C.L.M., *J. Food Eng.,* 2006; **72(1)**: 8-15. DOI 10.1016/j.jfoodeng.2004.11.007.

[11] Matsui K.N., Wilhelms Gut J.A., de Oliveira P.V. and Tadini C.C., *J. Food Eng.,* 2008; **88(2)**: 169-176. DOI 10.1016/j.jfoodeng.2008.02.003.

[12] McLellan K.M. and Robinson D.S., *Food Chem.,* 1981; **7(4)**: 257-266

[13] Lu A.T. and Whitaker J.R., *J. Food Sci.,* 1974; **39(6)**: 1173-1178. DOI 10.1111/j.1365-2621.1974.tb07347.x.

[14] Yemenicioglu A., Ozkan M. and Cemeroglu B., *J. Food Sci.,* 1997; **62(3)**: 508-510. DOI 10.1111/j.1365-2621.1997.tb04417.x.

[15] Yemenicioglu A. and Cemeroglu B., *J. Agric. Food Chem.,* 2003; **51(8)**: 2371-2379. DOI 10.1021/jf025988q.

[16] Fortea M.I., Lopez-Miranda S., Serrano-Martinez A., Carreno J. and Nunez-Delicado E., *Food Chem.,* 2009; **113(4)**: 1008-1014. DOI 10.1016/j.foodchem.2008.08.053.

[17] Lee T.H., Chua L.S., Tan E.T.T., Yeong C., Lim C.C., Ooi S.Y., Aziz R..A., Aziz A. and bin Sarmidi M.R., *Food Sci. Biotechnol.,* 2009; **18(3)**: 661-666.

[18] Morales-Blancas E.F., Chandia V.E. and Cisneros-Zevallos L., *J. Food Sci.,* 2002; **67(1)**: 146-154. DOI 10.1111/j.1365-2621.2002.tb11375.x.

[19] Soysal C. and Soylemez Z., *J. Food Eng.,* 2005; **68(3)**: 349-356. DOI 10.1016/j.jfoodeng.2004.06.009.

[20] Gokmen V., Bahceci K.S., Serpen A. and Acar J., *LWT-Food Sci. Technol.,* 2005; **38(8)**: 903-908. DOI 10.1016/j.lwt.2004.06. 018.

[21] Bahceci K.S., Serpen A., Gokmen V. and Acar J., *J. Food Eng.,* 2005; **66(2)**: 187-192. DOI 10.1016/j.jfoodeng. 2004.03.004.

[22] Xu J.S., *Postharvest Biol. Technol.,* 2005; **38(1)**: 91-98. DOI 10.1016/j.postharvbio.2005.05.011.

[23] Schweiggert U., Schieber A. and Carle R., *Innov. Food Sci. Emerg. Technol.,* 2006; **7(3)**: 217-224. DOI 10.1016/j.ifset.2006. 03.003.

[24] Lopez P. and Burgos J., *J. Food Sci.,* 1995; **60(3)**: 451-455. DOI 10.1111/j.1365-2621.1995.tb09801.x.

[25] Feng W., Wang M., Cao J., Sun J. and Jiang W., *Process Biochem.,* 2007; **42(7)**: 1155-1159. DOI 10.1016/j.procbio.2007.05.010.

[26] Thongsook T., Whitaker J.R., Smith G.M. and Barrett D.M., *J. Agric. Food Chem.,* 2007; **55(3)**: 1009-1018. DOI 10.1021/jf062242.

[27] Herriott R.M., *J. Gen. Physiol.,* 1938; **21(4)**: 501-40.

[28] Anfinsen C.B., Haber E., Sela M., White Jr., F.H., *Proc. Natl. Acad. Sci. U.S.A.,* 1961; **47(9)**: 1309-1314.

[29] Gunes B. and Bayindirli A., *LWT-Food Sci. Technol.,* 1993; **26(5)**: 406-410.

[30] Weemaes C., Rubens P., DeCordt S., Ludikhuyze L., VandenBroeck I., Hendrickx M., Heremans K. and Tobback P., *J. Food Sci.,* 1997; **62(2)**: 261-266. DOI 10.1111/j.1365-2621.1997.tb03981.x.