International Journal of Engineering and TechnologyVol. 1, No.4 , October, 2009
ISSN: 1793-8236

Modelling the kinetics of Seedless Guava (Psidium guajava L.) Peroxidase Inactivation due to Heat and Thermosonication Treatments

A. GANJLOO1*, R.A. RAHMAN1, 2, J. BAKAR1, A. OSMAN3 and M. BIMAKR2

Abstract—**Effect of heat and thermosonication on inactivation kinetics of peroxidase in seedless guava have been studied over a temperature range of 80-95°C. Application of thermosonication was studied at 25, 50 and 75% of radiation intensity at the same temperature range. Ultrasonic wave's intensity had significant (P<0.05) effect on peroxidase inactivation rate except the 25% intensity radiation. In both treatments, the enzyme kinetics showed a first-order kinetics model with monophasic behaviour. The activation energy, the rate constants were estimated which proving that the enzyme became more heat labile. Therefore, thermosonication can affect a process with reduced processing time and increased efficiency.**

Index Terms— **Peroxidase inactivation, Seedless guava, Thermal inactivation, Thermosonication.**

I. INTRODUCTION

Guava, Psidium guajava L., is native to the Caribbean and common throughout all warm areas of tropical America and in the West Indies. In Malaysia commercial guava production began in the mid 1980's and consumed as a fruit, has great amount of vitamin C (more than 3 times as much Vitamin C as an orange), vitamins A, B1 and B2. Guavas are useful sources of nicotinic acid, phosphorous, and soluble fiber.

Peroxidase (POD) is an enzyme commonly found in many plant-based foods and recognized as being one of the most heat-stable enzyme and its involvement in the oxidation of many organic compounds, leading to deterioration in flavor, color, and nutritional quality [1]. POD is also used as an index of the adequacy of fruit and vegetable blanching due to its presence in most plant tissues, its high thermal stability, and the simplicity of its measurement [2]. Heat treatment is commonly used to inactivate an active enzyme destroy vegetative microbial cells, allowing stabilization and product quality retention during storage [3].

The degree of heat treatment can have adverse effect on sensorial (excessive loss of texture and undesired colour changes) and nutritional quality attributes. Many researchers studied these alterations in different fruits. They have observed the dramatic blanching effect on the degradation of fruit and vegetables nutrient content (namely vitamin C and protein) and antioxidant properties [4]-[6]. For this reason many alternative methods have been developed, but the conventional hot water blanching is commonly used [7].

To minimize the adverse effects of heating on quality, alternative methods of blanching, or combinations of the conventional heat treatment with other physical factors, such as ohmic blanching [8] or microwave blanching [9] have been developed.

One of the alternative methods might be the thermosonication blanching, which is a combined treatment with heat and ultrasound. High energy ultrasound (18 kHz–100 kHz) has been applied for enzyme inactivation [10].

The majority of ultrasonic wave effects are caused by generation of microbubbles (cavities) that grow up to a critical size and then collapse (cavitational collapse) since ultrasonic waves propagate in a liquid medium [11],[12]. Therefore, ultrasound exerts its effects mainly through cavitation phenomena. The synergistic effect of the combined heat and ultrasound treatment allows inactivating several enzymes at lower temperatures and/or in shorter time [11]. However, research in this area is currently underway worldwide. Nevertheless, the economic viability of these emerging technologies still needs to be determined before they become common. The objective of this work was to study the kinetics of peroxidase inactivation of seedless guava during traditional blanching conditions and with combination method of heat and ultrasonic waves (thermosonication). The findings will help to evaluate the effectiveness of thermosonication blanching as a novel process to at least partly replace the classical heat treatment.

II. MATERIALS AND METHODS

A. Raw Materials

Seedless guava (Psidium guajava L.) fruits of commercial maturity used for the experiments were purchased from the local market in Serdang, Malaysia. The fresh samples were washed and peeled. Then, they were cut into cubes

A. GANJLOO is with the Department of Food Technology, Faculty of Food Science and Technology, Universiti Putra Malaysia 43400 Serdang, Selangor D.E., Malaysia

R.A. RAHMAN is with Department of Process and Food Engineering, Faculty of Engineering, Universiti Putra Malaysia 43400 Serdang, Selangor D.E., Malaysia

J. BAKAR is with the Department of Food Technology, Faculty of Food Science and Technology, Universiti Putra Malaysia 43400 Serdang, Selangor D.E., Malaysia

A. OSMAN is with the Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia 43400 Serdang, Selangor D.E., Malaysia

M. BIMAKR is with the Department of Process and Food Engineering, Faculty of Engineering, Universiti Putra Malaysia 43400 Serdang, Selangor D.E., Malaysia

approximately 20× 20× 20 mm sizes. All chemicals used in this study were of analytical grade.

B. Heat and Thermosonication Treatments

Seedless guavas (Psidium guajava L.) were blanched in a circulating water bath (Memmert, WNE14. Memmert GmbH Co. KG, Germany) maintained at desired temperatures (±0.5°C). Heat inactivation was studied at temperatures ranging from 80 to 95˚C, with different times of exposure. After preset times, the samples were removed from the water bath and placed immediately in cooled water (2-5˚C) in order to stop thermal inactivation instantaneously. The temperature of the water bath and cooled water was monitored with a digital thermometer (Ellab CTD-85, Ellab, Denmark) and a thermocouple (1.2 mm needle diameter constantan-type T). Each experiment was replicated trice. An unblanched sample was taken as a control.

A second approach was the combination of heat/ultrasound applied to the seedless guava (Psidium guajava L.) cubes for the same range of temperatures. The samples were processed with an ultrasound horn (vibra-cell CV33; 13 mm dia) at 20 kHz and an ultrasound generator (vibra-cell, VC 505 Series, Sonics & materials, Inc, USA) radiating from 25-75% power.

C. Extraction of Peroxidase and its Assay

In order to determine the presence of peroxidase in seedless guava and ratio between sample weight (g) and the buffer solution volume (mL), preliminary experiments were carried out. Blanched samples were mixed with cold potassium phosphate buffer in the proportion of 3:25 w/v. Each sample was homogenized in an Ultra-Turrax T25 Janke & Kunkel for 1 min at 13,500 rpm under chilled condition. The homogenate was filtered using filter paper (Whatman No.1). The filtrate was centrifuged in a Beckman Coulter, Avanti J-25 centrifuge with a rotor no.JA14 at 6000 × g and 4˚C for 20 min with polypropylene tubes. The supernatants were kept on ice until the analysis.

Peroxidase activity was measured according to the method reported by (Morales-Blancas and others 2002). Peroxidase substrate solution was prepared daily by mixing 0.1 mL guaiacol, 0.1 mL hydrogen peroxide (30%), and 99.8 mL potassium phosphate buffer (0.1mol/L, pH 6.5). Peroxidase assays were conducted by pipetting 0.12 mL of enzyme extract and 3.48 mL of substrate solution in the 10 mm path-length quartz cuvette. Peroxidase activities were measured from the increase in absorbance at 470 nm using an UV/vis spectrophotometer (UV-mini 1240, Shimadzu, Japan). The reaction was monitored for 20 min at 5sec intervals at 25˚C. Enzyme activity was calculated from the slope of the initial linear portion of a plot of absorbance vs. time. All the experiments were replicated thrice. Residual enzyme activity (REA) in heat-treated samples is expressed as a fraction of initial activity (C0):

Residual enzyme activity (REA) = C/C0 ×100 (1)

Where C and C0 are ΔAbs./min after heat treatment for time t and native enzyme, respectively.

III. KINETIC MODELING

The first-order (Eq. (2)) equation was used to describe the enzyme inactivation in seedless guava:

$$C = C_0 \exp(-kt) \quad (2)$$

Where C is the measured value for residual peroxidase activity parameter, C0 the initial C, t the heating time and k is the reaction rate constant. The temperature dependence of the rate constant is normally described by an Arrhenius Law:

$$k = k_{ref} \cdot \exp\left[\frac{-E_a}{R}\left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right] \quad (3)$$

Where Ea is the activation energy and Kref is the rate constant at a reference temperature (Tref). Tref was taken as the medium temperature of the range in which this model was used to represent peroxidase inactivation (Tref = 87.5°C). The temperature effect can be directly included in quality factors prediction, by substitution of Eq. (3) into kinetic models.

IV. STATISTICAL ANALYSIS

Rate constants of seedless guava peroxidase inactivation were estimated by non-linear regression analysis, fitting the models of Eq. (2) to experimental data. The temperature effect on rate constants was described by the Arrhenius law (Eq. (3)). The pre-exponential factor and the activation energy were estimated directly from experimental data in one-step (quality factor versus time, at all temperatures), by performing a global non-linear regression analysis, merging the Arrhenius equation and the kinetic models considered [13]. The reference temperature used was the average value of the range considered (i.e. Tref = 87.5°C), aiming at improving parameter estimation. Parameters' precision was evaluated by confidence intervals at 95%, and the quality of the regression was assessed by the coefficient of determination (R2) [14], thus allowing best model selection. Statistica Version 6.0 Software was used for all regression analysis procedures (using least squares estimation and Levenverg–Marquart method, for minimising the sum of squares of the deviations between experimental values and the ones predicted by the mathematical model). An analysis of variance (one-way ANOVA with replication) was performed to determine significance of differences among treatments for peroxidase activity.

V. RESULTS AND DISCUSSION

A. Peroxidase Inactivation

In the hot water blanching study, it was observed that the time required for the inactivation of peroxidase changed with the temperature gradients applied (Fig. 1). The enzyme inactivation was significantly affected ($P < 0.05$) by the time and temperature of the blanching process. Inactivation kinetic models were tested for its applicability to the thermal inactivation data for seedless guava. Among them, the monophasic first-order kinetic model yield good R^2 values (above 0.97) in the range of temperatures tested.

Consequently, the monophasic first-order kinetic model was selected to model the kinetics of peroxidase inactivation in seedless guava. The model parameters (activation energy, the rate of the reaction at the reference temperature of 87.5°C) was estimated as, E_a = 101.46±3 kJmol^{-1} and $K_{87.5°C}$ = 0.023±7×10^{-3} s^{-1}, respectively (see Table 1).

Monophasic behavior of the enzyme inactivation at high temperatures could be due to the rapid inactivation of the heat-labile fraction of the enzyme during the first seconds of treatment, so the observed kinetics would correspond to the inactivation of the heat-resistant fraction of peroxidase. Similarly, the peroxidase inactivation in different vegetables, such as carrots, potatoes, tomato, green beans, green asparagus and pumpkin has been reported to follow a first-order model to describe the enzyme inactivation [15]-[20].

The application of thermosonication had a synergistic effect, since the enzyme activity decreased at a higher rate when compared to the traditional heat treatment (Fig. 1). The reduction of enzyme activity is related to the conformation changes in the tertiary structure, as in the active site three-dimensional structure affecting the enzyme–substrate interaction [21]. Thus, the modelling of the enzyme inactivation with thermosonication was important in this study, once it is in favour of less severe heat blanching conditions. Therefore, an inactivation monophasic first-order model was applied. The experimental data fitted well a first-order model (R^2 = 0.97) and the kinetic parameters estimated by the model were significant (a = 0.05). The activation energies, the rates of reaction at a reference temperature of 87.5 °C were, respectively, $E_{a50\%}$=105.77±5kJmol^1, $E_{a75\%}$=128.37±2, $k_{87.5°C(50\%)}$= 0.032±2×10^{-2}s^{-1} and $k_{87.5°C(75\%)}$=0.044±6×10^{-2} s^{-1} (Table 1).

Table 1 Kinetic parameters of thermosonication inactivation of peroxidase in seedless guava(*Pisidium gujava* L.)

Radiation (%)	Kinetic Parameters			
	C_0	$K_{87.5}$ (s^{-1})	E_a(kJmol^{-1})	R^2
0	1.012±0.018	2.3×10^{-2}±0.007	101.46±3	0.99
50	1.007±0.009	3.2×10^{-2}±0.029	105.77±5	0.99
75	1.004±0.004	4.4×10^{-2} ±0.061	128.37±2	0.97

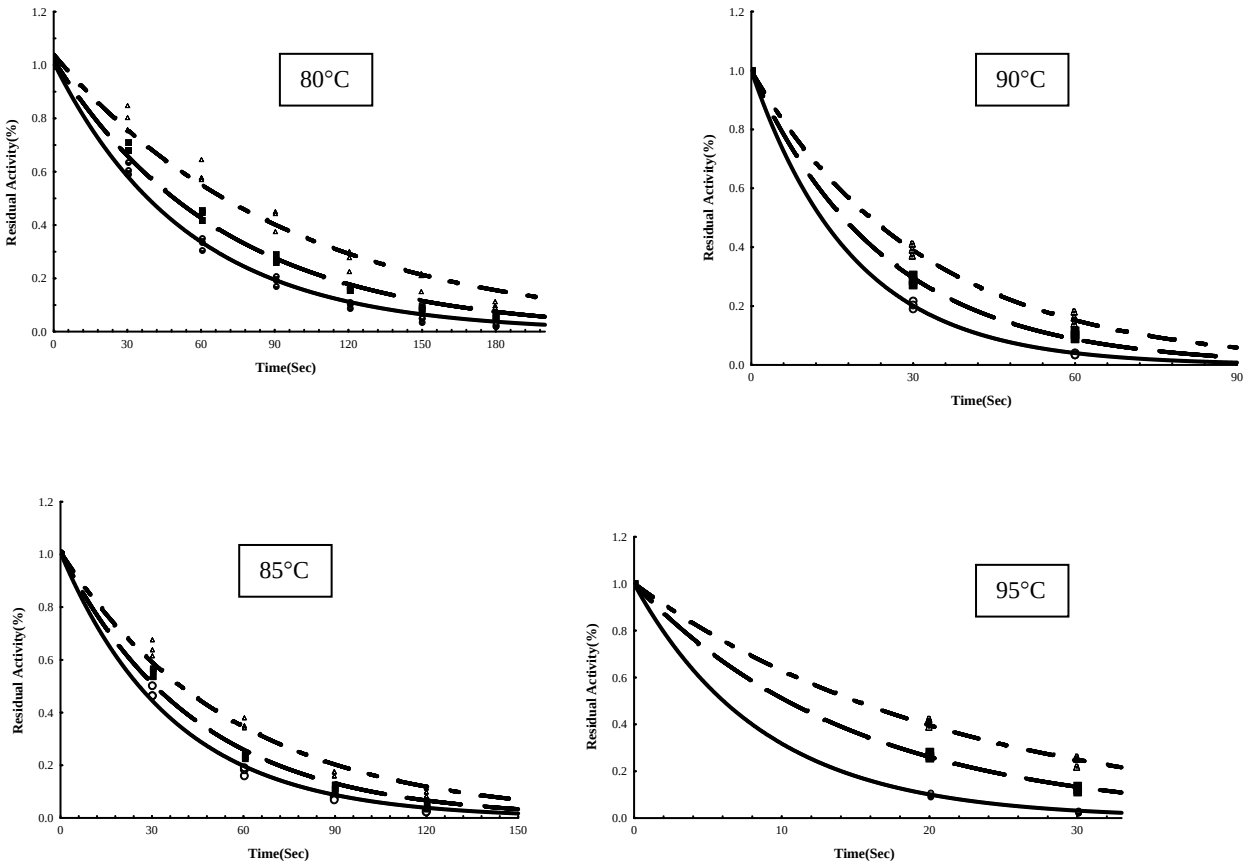

Fig. 1: Thermal and thermosonication inactivation of peroxidase in seedless guava. Thermal treatment (Δ); Thermosonication at 50% (■) and 75% intensity (○). The lines presented the monophasic first-order kinetic model.

VI. CONCLUSION

The monophasic first-order model fits well the

experimental data of the hot water and thermosonication blanching processes. With this model and the kinetic parameters determined, it is possible to predict the peroxidase activity as well as temperature and blanching process time. The application of thermosonication for the same blanching times, led to higher enzyme inactivation when compared with the hot blanching processes. These results allow the application of shorter blanching times at this range of temperatures, leading to a product with a higher quality, or minimized processing. Thus, the thermosonication treatments can be a good alternative to the traditional hot water blanching processes. The present findings will help to design the blanching conditions in order to reduce the severity of conventional thermal treatments and, therefore, improving the quality of the blanched product.

REFERENCES

[1] P. Lopez, F.J. Sala, J.L. Fuente, S. Cardon, J. Raso and J. Burgos, 1994. Inactivation of peroxidase, lipoxigenase and phenol oxidase by manothermosonication. J. Agric. Food Chem. 42(2):253-256.
[2] G.E. Anthon, D.M. Barrett, 2002. Kinetic parameters for the thermal inactivation of quality-related enzymes in carrots and potatoes. J. Agric. Food Chem., 50(14):4119-4125.
[3] W. Canet, 1989. Quality and stability of frozen vegetables. Elsevier Science Publishing Inc.. In S. Thorne (Ed.). Developments in food preservation New York
[4] S.M. Castro, J.A. Saraiva, J.A. Lopes-da-Silva, I. Delgadillo, A.V. Loey, C. Smout and M. Hendrickx, 2007. Effect of thermal blanching and of high pressure treatments on sweet green and red bell pepper fruits (Capsicum annuum L.). Food Chem. 107:1436-1449.
[5] J.Y. Song, Gil-Hwan,C.-J. Kim, 2003. Color, texture, nutrient contents, and sensory values of vegetable soybeans [Glycine max (L.) Merrill] as affected by blanching. Food Chem. 83:69–74.
[6] J. Volden, G.I.A. Borge, G.B. Bengtsson, M. Hansen, I.E. Thygesen and T. Wicklund, 2008. Effect of thermal treatment on glucosinolates and antioxidant-related parameters in red cabbage (Brassica oleracea L. ssp. capitata f. rubra). Food Chem. 109:595-605.
[7] K.Z. Katsaboxakis, 1984. The influence of the degree of blanching on the quality of frozen vegetables. In P. Zeuthen, J. C. Cheftel, C. Eriksson, M. Jul,.
[8] F. Icier, H. Yildiz,T. Baysal, 2006. Peroxidase inactivation and colour changes during ohmic blanching of pea puree. J. Food Eng.. 74(3):424−429.
[9] M. Ihl, M. Monsalves,V. Bifani, 1998. Chlorophyllase inactivation as a measure of blanching efficacy and colour retention of Artichokes (Cynara scolymus L.). Lebensmittel-Wissenschaft und Technologie. 31:50−56.
[10] D. Knorr, M. Zenker, V. Heinz and D. Lee, 2005. Applications and potential of ultrasonics in food processing. Trends in Food Sci. Technol. 15(5):261−266.
[11] A. Vercet, J. Burgos, S. Crelier and P. Lopez-Buesa, 2001. Inactivation of proteases and lipases by ultrasound. Innovative Food Sci. Emerging Technol 2:139-50.
[12] K.S. Suslick, 1990. Sonochemistry. Journal of Food Science. 247:1439 -1445.
[13] [A. Arabshahi, D. Lund, 1985. Considerations in calculating kinetic parameters from experimental data. J. Food Proc. Eng. 7:239-251.
[14] C.G. Hill, R.A. Grieger-Block, 1980. Kinetic data: generation, interpretation and use. Food Technol. 34(2):56-66.
[15] C. Soysal, Z. Soylemez, 2005. Kinetics and inactivation of carrot peroxidase by heat treatment. J. Food Eng. 68:349-356.
[16] G.E. Anthon, D.M. Barrett, 2002. Kinetic parameters for the thermal inactivation of quality-related enzymes in carrots and potatoes. J. Agric. Food Chem. 50(14):4119-4125.
[17] G.E. Anthon, Y. Sekine, N. Watanabe and D.M. Barrett, 2002. Thermal inactivation of pectin methylesterase, polygalacturonase, and peroxidase in tomato juice. J. Agric. Food Chem. 50(21):6153-6159.
[18] V. Bifani, J. Inostroza, M.J. Cabezas and M. Ihl, 2002. Determinacion de parametros cineticos de peroxidasa y clorofila a en judias verdes (Phaseolus vulgaris cv. Win) y estabilidad del product congelado. Revista de Quimica Teorica y Aplicada, Febrero, LIX. 467:57-64.
[19] C. Ganthavorn, C.W. Nagel,J.R. Powers, 1991. Thermal inactivation of asparagus lipoxigenase and peroxidase. J. Food Sci. 56(1):47-49.
[20] E.M. Goncalves, J. Pinheiro, M. Abreu, T.R.S. Brandao and C.L.M. Silva, 2007. Modelling the kinetics of peroxidase inactivation, colour and texture changes of pumpkin (Cucurbita maxima L.) during blanching. J. Food Eng. 81:693-701.
[21] R.M.S. Cruz, M.C. Vieira, C.L.M. Silva, 2006. Effect of heat and thermosonication treatments on peroxidase inactivation kinetics in watercress (Nasturtium officinale). J. Food Eng. 72:8-15.