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Effect of heat and thermosonication on kinetics of peroxidase inactivation and vitamin C degradation in seedless guava (*Psidium guajava* L.)

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Abstract: This study aims to evaluate the effect of heat and the simultaneous application of heat (80-95°C) and ultrasonic waves (thermosonication) on the inactivation kinetic of peroxidase and vitamin C degradation in seedless guava. Ultrasonic wave's amplitudes except 25 and 100% had significant ($P<0.05$) effect on peroxidase inactivation rate. The thermal and thermosonication inactivation of peroxidase was described well by first-order kinetics ($R^2> 0.98$). In the heat blanching process, the peroxidase inactivation rate constant increased from 1.1×10^{-2} to 4.6×10^{-2} s^{-1}. However, the inactivation rate of peroxidase was increased by 1.5–3 times in the temperature range 80–95ºC, with the 50 and 75% ultrasonic wave amplitudes, respectively. Decreases in vitamin C contents due to blanching treatments were found. Blanching processes at high temperature and short time resulted in higher vitamin C retention. It was found that thermosonication treatment inactivates seedless guava peroxidase at less severe blanching conditions and consequently retains vitamin C content at higher levels. 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 thermally treated product.

Keywords: Blanching, seedless guava (*Psidium guajava* L.), thermosonication, peroxidase inactivation, vitamin C

Introduction

Guava, *Psidium guajava* L., belongs to the family of Myrtaceae, believed to originate from the Caribbean. Guavas contain micronutrient such as vitamins C, A, B and also a rich source of soluble, fiber, phosphorous and nicotinic acid. Most of the guava produced around the world is consumed fresh. However, processed guava such as juice, puree, nectar, jams and jellies exists in the market (Morton, 1978; Kaur *et al*., 2009). Guavas like other tropical fruits are highly perishable, and often processed by heat treatment prior to further processing such as freezing, drying and canning to extend the shelf life and make the fruit available throughout the year.

Blanching in hot water or steam commonly carried out to a wide range of fruits and vegetables allowing stabilization and commercialization of product. The benefits of heat treatment in enzymes inactivation and vegetative microbial cells destruction have been addressed in literature (Canet, 1989). In some cases, the presence of some high heat resistance enzymes such as peroxidase makes heat treatments itself a problem not a solution (Gonçalves *et al*., 2007). The effectiveness of blanching process has been assessed by using peroxidase due to the highest thermo stability of this enzyme in plant-based foods. The other advantages such as simple and inexpensive activity measurement were also reported (Yemenicioğlu *et al*., 1998; Forsyth *et al*., 1998; Icier *et al*., 2006). Thermal process can negatively modify some food properties like sensorial (undesired texture, color and flavor changes) and nutritional (namely vitamin C) attributes (Murcia *et al*., 1999; Oboh, 2005). It has been reported that presence of oxygen, degree of thermal treatment, exposure to light, action of metals and enzymatic oxidation are all adversely affected content of vitamin C (Deman, 1990). Several studies were carried out on effect of thermal treatment on vitamin C content of amla, orange juice, cupuaçu nectar, tomatoes, strawberry, drumstick, swiss chard and broccoli which concluded that thermal process decrease the vitamin C content dramatically (Johnson *et al*., 1995; Vieira *et al*., 2000; Dewanto *et al*., 2002; Nisha *et al*., 2004; Castro *et al*., 2004; Bineesh *et al*., 2005; Aguero *et al*., 2005; Wambui Munyaka *et al*., 2010).

The growing interest in searching for alternative methods of enzyme inactivation was motivated by heat elimination or the heat input reduction of

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traditional technologies (Mertens and Knorr, 1992; Vercet *et al*., 2002). Among different proposed potential alternative methods to thermal processing, application of ultrasound creates novel and interesting methodologies. It was found that ultrasound combined with classical techniques (heat) reduced processing time and increased efficiency of enzymes inactivation process (Lopez *et al*., 1994; Lopez and Burgos, 1995; De Gennaro *et al*., 1999; Cruz *et al*., 2009). The majority of ultrasonic wave effects are caused by cavitation phenomena (physical effect) and/or generation of free radicals (chemical effect) (Floros and Liang, 1994; Vercet *et al*., 2001).

The mathematical modeling of enzyme inactivation and quality changes gives invaluable information about the order and constant of reaction, and the activation energy which is necessary to evaluate the different thermal processes effects on enzyme activity and predict quality losses without performing numerous trial runs (Gonçalves *et al.*, 2007). Despite of continuous worldwide research in this area, so far, no previous study has been found on the feasibility of thermosonication treatment to increase the rate of peroxidase inactivation and its effect on nutritional changes of seedless guava which constitute high proportion of people diet in tropical and subtropical regions. This study therefore seeks to assess the peroxidase inactivation kinetics and vitamin C degradation during traditional blanching conditions and with combination method of heat and ultrasonic waves (thermosonication) of seedless guava.

Materials and Methods

Raw materials and chemicals

Fresh seedless guavas (*Psidium guajava* L.) were obtained from a local market at Serdang, Malaysia on daily basis prior to each set of experiments. Fruits were chosen at commercial maturity according to their similarity of color, size, absence of surface defects and ripening grade (around 8ºBrix). Before each experiment, fruits were washed, peeled and then cut into cubes (2 cm^3). Monopotassium dihydrogen phosphate (Merck) and dipotassium hydrogen phosphate (Merck) in distilled water were mixed to obtain potassium phosphate buffer (0.1 mol/L and pH 6.5).

Heat and thermosonication treatments

Fresh fruits were blanched in hot water at different temperatures (80, 85, 90 and 95˚C) using agitated water bath (Memmert, WNE14. Memmert GmbH Co. KG, Germany). The temperature-time profile was obtained in previous study (Ganjloo *et al*., 2009). Cooled water (2-5˚C) was used to stop blanching treatments after the blanched samples were removed from the medium. The digital thermometer (Ellab CTD-85, Ellab, Denmark) and a thermocouple (1.2 mm needle diameter constantan-type T) were used to verify the temperature of the water bath and cooled water. Non-blanched samples were taken as a control.

In second set of experiment, the combination of heat/ultrasound (thermosonication) was applied to the fruit cubes for the same range of temperatures. The samples were processed with an ultrasonic processor (Sonics and Materials Inc., Model VC505, Danbury, CT, USA), set at 500 W, 20 kHz and fitted with a 13 mm diameter titanium probe. Thermosonication was carried out at 25, 50, 75 and 100% (31 μm: 1.56 W/cm^2; 62 μm: 3.1 W/cm^2; 93 μm: 7.37 W/cm^2; and 124 μm: 8.35 W/cm^2, respectively) amplitude of ultrasonic wave. The temperature was maintained by reducing the water bath temperature to avoid overheating during thermosonication. Temperatures of water bath at 79, 83, 88 and 93°C were used for 80, 85, 90 and 95°C treatments, respectively. Sonotrode was immersed in water near sample (1-2 cm distance) in the experiment. Each experiment was run in triplicate.

Extraction of crude peroxidase

Peroxidase was extracted by mixing cold phosphate buffer in the proportion of 3:25 w/v with the blanched samples and then homogenized for 1 min at 13,500 rpm under chilled condition. The filtrate which was obtained using filter paper (Whatman No.1) centrifuged at 6,000×g and 4°C for 20 min. The supernatants were kept on ice until the analysis (<5 min).

Determination of peroxidase activity

Peroxidase activity was determined spectrophotometrically at 470 nm using an UV/vis spectrophotometer (Shimadzu Corporation, UV-mini 1240, Japan) according to Morales-Blancas *et al*. (2002). A 0.12 mL of enzyme extract was mixed with 3.48 mL of substrate solution (0.1 mL guaiacol, 0.1 mL hydrogen peroxide (30%) and 99.8 mL phosphate buffer (0.1 mol/L, pH6.5)). The absorbance was automatically monitored for 20 min at 5-s intervals at 25°C. Initial linear portion of the absorbance vs. time curve's slope is equal to enzyme activity. All the experiments were done in triplicate. Residual enzyme activity is expressed as a fraction of initial activity (C_0):

$$\text{Residual enzyme activity} = C/C_0 \times 100 \quad (1)$$

Vitamin C determination

About 10 g of seedless guava cubes were homogenized with 100 mL of 3% metaphosphoric acid and then filtered through Whatman (no.4) filter paper. About 5 mL of filtrate was titrated with 2, 6-dichlorophenol iodophenol (DCPIP) indicator according to AOAC 967.21 (AOAC, 1995). Vitamin C content was expressed as mg vitamin C per 100 g of sample. Vitamin C content measurement was done in triplicate.

Calculation of kinetic parameters

Inactivation of peroxidase in seedless guava was descried using a first order equation (Eq. (2)):

$$\ln\frac{C}{C_0} = -k\times t \quad (2)$$

A straight line was obtained by plotting of the logarithm of the ratio C/C_0 against time which the negative of the slope is equal to the rate constant.

The D-value which is the time required for a decimal change in the property value at constant temperature is inversely related to the first order reaction constant (k):

$$D = \frac{2.303}{k} \quad (3)$$

An Arrhenius law was used to explain dependency of the rate constant to temperature:

$$\ln(k) = \frac{-E_a}{RT} \quad (4)$$

Statistical analysis

Statistical analysis was done using analysis of variance (ANOVA) to determine the effect of treatments on residual peroxidase activity and vitamin C degradation. Tukey test at 95% confidence level was used to compare means. The simple regression was used to fit the experimental data to the first order kinetics reaction equation. All statistical analysis was carried out using Minitab V. 14 statistical package (Minitab Inc., PA, USA)

Results and Discussion

Heat inactivation of peroxidase

Figure 1A-D presents the curves of thermal POD inactivation at the studied range of temperatures for heat and thermosonication treatments. The more intensive inactivation of POD is apparent with increasing temperature and treatment time. Linear

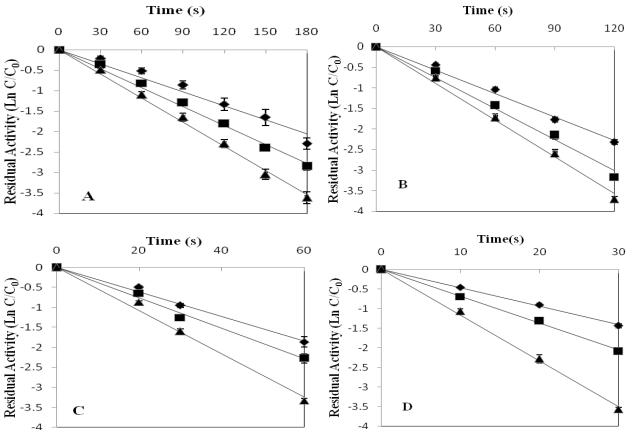

Figure 1. Thermal and thermosonication inactivation of peroxidase in seedless guava. Remaining peroxidase activity versus heating time.(A at 80°C; B at 85°C; C at 90°C; D at 95°C), Thermal treatment(♦); thermosonication at 50% (■) and 75% ultrasonic wave amplitude (▲).

curves at studied range of temperature were found by plotting residual activity versus treatment time (R^2>0.99) indicating the rapid enzyme's heat labile fraction inactivation during the first seconds of treatment (Figure 1A-D). Thus, observed kinetic represents the inactivation of heat resistant fraction. This result can be described well with monophasic first order kinetic reaction (Aguero *et al*., 2008; Ganjloo *et al.*, 2009) and it corroborates the findings of the previous works in this field (Serrano-Martínez *et al*., 2008; Anthon and Barrett 2002; Anthon *et al*., 2002; Soysal and Soylemez 2005; Gonçalves *et al*., 2007). The inactivation rate constants (k) varied from $1.1\times10^{-2}\pm0.0001$ s^{-1} at 80 ºC to $4.6\times10^{-2}\pm0.0004$ s^{-1} at 95ºC. The rate constants and activation energies are presented in Table 1.

Table 1. Reaction rate constants and activation energies of POD inactivation in seedless guava by heat and thermosonication treatments

Radiation (%)	Temperature (°C)	k (s^{-1})[a]	R^2	E_a (kJmol^{-1})[b]
Temperature (ºC)		Vitamin C retention (%)		
0	80	$1.1\times10^{-2a}\pm0.0001$	0.9819	
	85	$1.9\times10^{-2b}\pm0.0003$	0.9943	
	90	$3.0\times10^{-2c}\pm0.0007$	0.9938	
	95	$4.6\times10^{-2d}\pm0.0004$	0.9972	
				101.46±3
50	80	$1.6\times10^{-2a}\pm0.0001$	0.9968	
	85	$2.5\times10^{-2b}\pm0.0005$	0.9943	
	90	$3.8\times10^{-2c}\pm0.0008$	0.9948	
	95	$6.9\times10^{-2d}\pm0.0008$	0.9984	
				105.77±5
75	80	$2.0\times10^{-2a}\pm0.0002$	0.9971	
	85	$3.0\times10^{-2b}\pm0.0004$	0.9971	
	90	$5.5\times10^{-2c}\pm0.0010$	0.9958	
	95	$1.2\times10^{-1d}\pm0.0015$	0.9980	
				121.214±2

All measurements were replicated at least three times.
[a] k : rate constants for inactivation of seedless guava peroxidase.
[b] E_a: activation energy of seedless guava for peroxidase inactivation.
*Values of the reaction rate constants at each % of amplitude followed by different letters are significantly different ($p<0.05$).

The impact of the ultrasonic wave's intensity on the residual activity was significant ($P<0.05$) for 50 and 75% amplitude of ultrasonic wave at the studied range of temperature. The peroxidase

inactivation in thermosonication treatments follows first order kinetics which supports the previous findings of Lopez and Burgos (1995) and Raviyan *et al.* (2005). It is apparent from Table 1 that thermosonication enhanced the inactivation kinetics of peroxidase in seedless guava compare with conventional thermal treatment. For example, in thermosonication treatment at 95ºC, inactivation rate increases two to three times as compared with the thermal inactivation rate at the same temperature ($p<0.05$). De Gennaro *et al*. (1999) demonstrated that inactivating capability of thermosonication depends on ultrasonic wave power and its frequency. Decimal reduction times for conventional thermal and thermosonication treatments are shown in Figure 2.

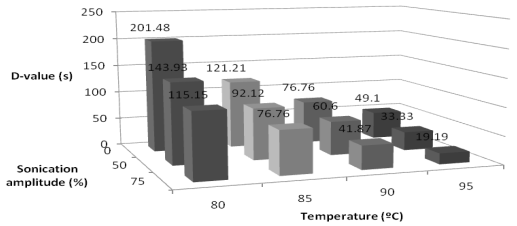

Figure 2. Effect of temperature and ultrasonic wave amplitudes on the D-value of seedless guava POD.

The ultrasound waves affect the enzyme inactivation through series of effects including thermal effect, generation of free radicals by water sonolysis, and the mechanical forces which caused by micro-streaming and shock waves (Price 1992; Vercet *et al*., 2002). These are, alone or in combination, can damage the integrity of the enzyme protein structure resulting in reduced enzyme activity (Suslick, 1988). Vercet *et al*. (1998) pointed out that at low temperatures the hydroxyl radicals production favored by ultrasound (Raso *et al*., 1999). According to Figure 1A, no faster peroxidase inactivation was found at lower temperature indicating that the enzyme inactivation is probably mediated by the mechanical effects not by free radicals.

Vitamin C retention

The content of vitamin C in fresh seedless guava was 122.31±3.60 mg/100 g of edible flesh. Although guava is a rich source of vitamin C, its level is subject to wide variations because of geographical location, horticultural practices, season and cultivar (Wilson 1980). Vitamin C retention in seedless guava cubes achieving 90% of POD inactivation is presented in Table 2. The ANOVA results showed significant differences ($P<0.05$) in vitamin C retention among all treated samples. It is apparent that longer processes at lower temperatures led to higher vitamin C losses (Aguero *et al.*, 2008) which is related to solubility of vitamin C in water and its instability at high temperature (Lee and Kader 2000; Liu *et al*., 2000; Oboh, 2005). Therefore, the vitamin C content would have destroyed and washed away during the course of blanching by temperature and water, respectively. Furthermore, the vitamin C reduction was probably related to the heat effect due to the short processing times (maximum 3 min). On the other hand, higher amount of vitamin C retained in thermosonically treated samples treated due to dissolved oxygen elimination which is essential for vitamin C degradation during cavitation (Mason 1991; Walkling-Ribeiro *et al*., 2007).

Table 2. Effect of heat and thermosonication treatments on retention of vitamin C in seedless guava

Temperature (ºC)	Vitamin C retention (%)		
	Heat	TS 50%	TS 75%
80	49.46^{a}±2.70	62.93^{a}±1.30	71.60^{a}±2.01
85	59.05^{b}±1.40	70.32^{b}±2.30	75.72^{b}±1.90
90	61.17^{c}±2.80	71.60^{b}±2.30	80.36^{c}±1.50
95	64.85^{d}±1.10	76.80^{c}±2.10	86.81^{d}±2.20

*Different letters at each column show significantly difference between treatments ($p<0.05$).

Conclusion

In the current study, the effect of thermosonication treatment at different temperatures as an alternative to conventional heat treatment for peroxidase inactivation in seedless guava was evaluated. The ANOVA results revealed that the studied range of temperature affected the inactivation of peroxidase significantly ($P<0.05$). Moreover, thermosonication treatment were carried out at different levels of amplitude (25-100%) which no significant differences ($P<0.05$) were found for 25 and 100 % of ultrasonic waves amplitudes. The result of conventional heat and thermosonication treatments revealed the monophasic behavior of enzyme inactivation which followed the first order reaction kinetics model. Knowledge of kinetic parameter allows predicting the residual peroxidase activity. Further analysis showed that the application of thermosonication reduces the blanching times at the studied range of temperatures and retains more vitamin C (62-87%) compared to conventional heat treatment (49-64%). Thus, application of thermosonication is effective in peroxidase inactivation and the results allows designing the blanching conditions in order to reduce the severity of conventional thermal treatments and, therefore, improving the quality of the thermally treated product.

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References

AOAC. 1995. Official methods of analysis. Washington: Association of Official Analytical Chemists. Method 967.21.

Aguero, M.V., Ansorena, M.R., Roura, S.I. and del Valle, C.E. 2008. Thermal inactivation of peroxidase during blanching of butternut squash. LWT - Food Science and Technology 41: 401-407.

Anthon, G.E. and Barrett, D.M. 2002. Kinetic parameters for the thermal inactivation of quality-related enzymes in carrots and potatoes. Journal of Agriculture and Food Chemistry 50(14): 4119-4125.

Anthon, G.E., Sekine, Y., Watanabe, N. and Barrett, D.M. 2002. Thermal inactivation of pectin methylesterase, polygalacturonase, and peroxidase in tomato juice. Journal of Agriculture and Food Chemistry 50(21): 6153-6159.

Bineesh, N.P., Singhal, R.S. and Pandit, A.B. 2005. A study on degradation kinetics of vitamin C in drumstick (*Moringa olifera*) leaves during cooking. Journal of the Science of Food and Agriculture 85(11): 1953−1958.

Canet, W. 1989. Quality and stability of frozen vegetables. (Vol. 5). In S. Thorne (Eds). Developments in food preservation. New York: Elsevier Science Publishing Inc.

Castro, I., Teixeira, J.A., Salengke, S., Sastry, S.K. and Vicente, A.A. 2004. Ohmic heating of strawberry products: electrical conductivity measurements and vitamin C degradation kinetics. Innovative Food Science and Emerging Technologies 5: 27−36.

Cruz, R.M.S., Vieira, M.C., Fonseca, S.C. and Silva, C.L.M. 2009. Impact of thermal blanching and thermosonication treatments on watercress (*Nasturtium officinale*) quality: thermosonication process optimisation and microstructure evaluation. Food and Bioprocess Technology DOI: 10.1007/s11947-009-0220-0.

De Gennaro, L., Cavella, S., Romano, R. and Masi, P. 1999. The use of ultrasound in food technology I: inactivation of peroxidase by thermosonication. Journal of Food Engineering 39: 401-407.

Deman, J. 1990. Principles of food chemistry. New York: Van Nostrand Reinhold. pp. 350−351.

Dewanto, V., Wu, X., Adom, K.K. and Liu, R.H. 2002. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. Journal of Agricultural and Food Chemistry 50: 3010−3014.

Floros, J.D. and Liang, H. 1994. Acoustically assisted diffusion through membranes and biomaterials. Food Technology 79: 79-84.

Forsyth, J.L. and Robinson, D.S. 1998. Purification of Brussels sprout isoperoxidases. Food Chemistry 63(2): 227–234.

Ganjloo, A., Rahman, R.A., Osman, A., Bakar, J. and Bimakr, M. 2009. Kinetics of Crude Peroxidase Inactivation and Color Changes of Thermally Treated Seedless Guava (*Psidium guajava* L.). Food and Bioprocess Technology DOI: 10.1007/s11947-009-0245-4.

Gonçalves, E.M., Pinheiro, J., Abreu, M., Brandao, T.R.S. and Silva, C.L.M. 2007. Modelling the kinetics of peroxidase inactivation, colour and texture changes of pumpkin (*Cucurbita maxima* L.) during blanching. Journal of Food Engineering 81: 693-701.

Icier, F., Yildiz, H. and Baysal, T. 2006. Peroxidase inactivation and colour changes during ohmic blanching of pea puree. Journal of Food Engineering 74(3): 424–429.

Johnson, J.R., Braddock, R.J. and Chen, C.S. 1995. Kinetics of vitamin C loss and nonenzymatic browning in orange juice serum: Experimental rate constants. Journal of Food Science 60(3): 502−505.

Lee, S.K. and Kader, A.A. 2000. Preharvest and postharvest factors influencing vitamin C content of horticulture crops. Postharvest Biology and Technology 20(3): 207−220.

Liu, R.H., Dewanto, V., Wu, X. and Adom, K. 2000. Effect of thermal processing on the nutritional values of tomatoes. *http://ift.confex.com/ift/2000/techprogram/paper-4046.htm.*

Lopez, P. and Burgos, J. 1995. Lipoxygenase inactivation by manothermosonication: Effects of sonication physical parameters, pH, KCl, sugars, glycerol, and enzyme concentration. Journal of Agricultural and Food Chemistry 43(3): 620-625.

Lopez, P. and Burgos, J. 1995. Peroxidase stability and reactivation after heat treatment and manothermosonication. Journal of Food Science 60(3): 551-553.

Lopez, P., Sala, F.J., Fuente, J.L., Cardon, S., Raso, J. and Burgos, J. 1994. Inactivation of peroxidase lipoxigenase and phenol oxidase by manothermosonication. Journal of Agricultural and Food Chemistry 42(2): 253-256.

Mason, T.J. 1991. Practical sonochemistry user's guide to application in chemistry and chemical engineering. New York: Ellis Horwood.

Mertens, B. and Knorr, D. 1992. Developments of nonthermal processes for food preservation. Food Technology 46: 124-133.

Morales-Blancas, E.F., Chandia, V.E. and Cisneros-Zevallos, L. 2002. Thermal Inactivation Kinetics of Peroxidase and Lipoxygenase from Broccoli, Green Asparagus and Carrots. Food Chemistry and Toxicology 67: 146-154.

Morton, J. 1978. In: Fruits of warm climates, Miami, FL.

Murcia, M.A., Lopez-Ayerra, B. and Garcia-Carmona, F. 1999. Effect of processing methods and different blanching times on broccoli: proximate composition and fatty acids. LWT - Food Science and Technology 32(4): 238–243.

Nisha P., Singhal, R.S. and Pandit, A.B. 2004. Study on degradation kinetics of vitamin C in amla (*Phyllanthus emblica* L.) during cooking. International Journal of Food Sciences and Nutrition 55: 415−422.

Oboh, G. 2005. Effect of blanching on the antioxidant properties of some tropical green leafy vegetables. LWT - Food Science and Technology 38(5): 513–517.

Price, G.J. 1992. Current trends in sonochemistry. Cambridge: Royal Society of Chemistry.

Raso, J., Manas, P., Pagan, R. and Sala, F.J. 1999. Influence of different factors on the output power transferred into medium by ultrasound. Ultrasonics Sonochemistry 5: 157-162.

Raviyan, P., Zhang, Z. and Feng, H. 2005. Ultrasonication for tomato pectinmethylesterase inactivation: Effect of cavitation intensity and temperature on inactivation. Journal of Food Engineering 70: 189−196.

Serrano-Martínez, A., Fortea, M.I., Del Amor, F.M. and Núñez-Delicado, E. 2008. Kinetic characterisation and thermal inactivation study of partially purified red pepper (*Capsicum annuum* L.) peroxidase. Food Chemistry 107: 193–199.

Soysal, C. and Soylemez, Z. 2005. Kinetics and inactivation of carrot peroxidase by heat treatment. Journal of Food Engineering 68: 349-356.

Suslick, K.S. 1988. Homogeneous sonochemistry. In: Ultrasound. Its chemical, physical and biological effects. New York: VCH Publishers Inc.

Vercet, A., Burgos, J., Crelier, S. and Lopez-Buesa, P. 2001. Inactivation of proteases and lipases by ultrasound. Innovative Food Science and Emerging Technologies 2: 139-50.

Vercet, A., Lopez, P. and Burgos, J. 1998. Free radical production by manothermosonication. Ultrasonics 36(6): 15-18.

Vercet, A., Sanchez, C., Burgos, J., Montanes, L. and Buesa, P.L. 2002. The effects of manothermosonication on tomato pectic enzymes and tomato paste rheological properties. Journal of Food Engineering 53: 273-278.

Vieira, M.C., Teixeira, A.A. and Silva, C.L.M. 2000. Mathematical modeling of the thermal degradation kinetics of vitamin C in cupuaçu (*Theobroma grandiflorum*) nectar. Journal of Food Engineering 43: 1−7.

Walkling-Ribeiro, M., Noci, F., Riener, J., Cronin, D.A., Lyng, J.G. and Morgan, D.J. 2007. The Impact of thermosonication and pulsed electric fields on *Staphylococcus aureus* inactivation and selected quality parameters in orange juice. Food and Bioprocess Technology DOI: 10.1007/s11947-007-0045-7.

Wilson, C.W. 1980. Guava, In: Nagy S, Shaw PE (Eds), Tropical and Subtropical Fruits. Composition, Properties and Uses. Westport CT: AVI Publishing. p. 279–299.

Yemenicioğlu, A., Özkan, M., Velioğlu, S. and Cemeroğlu, B. 1998. Thermal inactivation kinetics of peroxidase and lipoxygenase from fresh pinto beans (*Phaseolus vulgaris*). Z. Lebensmitteluntersuchung und Forschung A, 206(4): 294–296.