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Effect of combined high pressure and thermal treatment on kiwifruit peroxidase

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Abstract

The effects of high pressure and heat treatments on peroxidase (POD) activity in kiwifruit were investigated. Pressure levels ranging from 200 to 600 MPa and temperatures varying from 10 to 50 °C were applied for up to 30 min. Assays were carried out on crude peroxidase in kiwifruit juice and on partially purified peroxidase in a model system. Pressures higher than 400 MPa could be combined with mild heat (⩽50 °C) to accelerate enzyme inactivation. Prolongation of the exposure time had no great effect after the first 15 min. The slope of POD in kiwifruit juice at 30 °C was slightly decreased compared with that in a model system. Furthermore, the optimum pH for POD was 6.0–8.5. The presence of POD isoenzymes and their difference in resistance to pressure were thought to be responsible for the final residual activity observed in this study.

Keywords: Kiwifruit; Peroxidase; High pressure

1. Introduction

Enzymes, such as peroxidase (POD) and polyphenoloxidase (PPO), can induce very negative changes in the color and flavor of vegetables during storage. To prevent these undesirable reactions, heat treatment causing enzyme inactivation is usually employed for the preservation of many foods (Williams, Lim, Chen, Pangborn, & Whitaker, 1986). However, the application of heat treatment is limited by the alteration of sensory characteristics and loss of nutrients caused by this process (Bomber, Dietrich, Hudson, Hamilton, & Farkas, 1975). Consumer demand for fresh-like food products with minimal degradation of nutritional and organoleptic properties has stimulated research on new non-thermal treatments in the food industry (Denys, Van Loey, & Hendrickx, 2000). Therefore, to overcome these limitations, a new technology, high pressure processing, has been introduced in the food industry as an alternative to thermal treatments, including pasteurization (Knorr, 1993). Currently this method is successfully applied on a commercial scale for pasteurization of a whole range of food products, including fruit juices, guacamole, oysters and ham.

High pressure (HP) treatment of food is already a real alternative to the conventional time-temperature processing in terms of pasteurization. High pressure is able to inactivate enzymes or microorganisms and achieve the pasteurization of food. Moreover, this technology allows the preservation of precious natural food properties like vitamins or natural aroma following treatment (Hendrickx & Knorr, 2002). Concerning the inactivation of enzymes, instead of using pressures higher than 600 MPa at room temperature, the use of lower pressures at increased temperatures is possible during HP treatment. Nevertheless, processing temperature must be low to minimize undesirable color and flavor changes.

Kiwifruit is native to China and has become a popular fruit because of its bright green flesh, high content of ascorbic acid, and protease (Préstamo, 1989). However, its consumption is limited because of being a seasonal, sensitive and perishable fruit. The newly collected fruit perishes

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easily and only keeps fresh for one or two weeks under normal conditions (Beever & Hopkirk, 1990; Heatherbell, 1975).

Peroxidase (EC 1.11.1.7) is a ubiquitous enzyme in plant cells. It is related to food quality in processing, and can contribute to adverse changes in the flavor and color of both raw fruits and processed products (López et al. (1994). Miesle, Proctor and Lagrimini (1991) pointed out that peroxidase promotes lipid oxidation with consequent off-flavor formation. In fact, phenolic oxidation mediated by peroxidase is believed to be associated with deterioration in flavor, color, texture and nutritional qualities of processed foods (Burnette, 1977). Préstamo, Arabas, Fonberg-Broczek, and Arroyo (2001), as well as Grison and Pilet (1985) reported that the inactivation of POD leads to the inactivation of PPO. Since the enzyme is pressure stable when considering the commercial exploitation of pressure processed fruits and vegetables, it had received most attention. The interest in POD is dictated by its high activity in kiwifruit and its contribution to the quality of kiwifruit. Furthermore, it is now well established that peroxidase activity in plants is associated with the occurrence of a substantial number of distinct POD isoenzymes. Quaglia, Gravina, Paperi, and Paoletti (1996) indicated the presence of numerous peroxidase isoenzymes, which react in different ways against pressure.

The aim of this work was to study the effects of high pressure combined with heat treatment on kiwifruit peroxidase in both food and model systems. The presence of POD isoenzymes and their impact on kiwifruit were also investigated.

2. Materials and methods

2.1. Materials

Kiwifruits (*Actinidia chinensis* Planch) were purchased at commercial maturity from a local store. Insoluble polyvinylpolypyrrolidone (PVPP) was obtained from Fluka Chemicals Co. (Buchs, Switzerland). Electrophoresis reagents were bought from Bio-Rad Laboratories Inc. (Nanjing, China). All other chemicals were of analytical grade.

2.2. Methods

2.2.1. Extraction and partial purification procedure

Extraction of the enzyme from kiwifruit was carried out using the method of Rastogi, Eshtiaghi, and Knorr (1999) as well as that of Phunchaisri and Apichartsrangkoon (2005) with some modification. Kiwifruits were cleaned with detergent and rinsed twice with distilled water. Then, they were peeled with a surgical blade to prevent damage of the outer pericarp tissue and cut into halves along their major axis. Peeled kiwifruits (100 g) were suspended in 200 ml of 50 mM sodium phosphate buffer (pH 7.5), with 1 M NaCl and 2% PVPP, and mixed for 15 s in a Waring commercial blender (Philips, Holland). The resulting homogenate was centrifuged for 20 min (4 °C; 16,000*g*) in a Beckman J2-HS centrifuge and the supernatant was filtered through four layers of cheese cloth.

Part of the resulting filtrate was adjusted to 7.5 to minimize the difference of pH (Anese, Nicola, Dall'Aglio, & Lerici, 1995). The obtained juice served as sample for further experiments to determine the total POD activity.

The other part of the resulting filtrate was subjected to ammonium sulphate precipitation at 0 °C. The fraction precipitating between 25% and 85% saturation was redissolved in the same buffer and dialysed for 24 h (cellulose membrane, Medicell International Ltd., 6-27/32, London, UK) to remove excess of ammonium sulphate ions. The dialyzed fraction was kept in tubes at −80 °C and was used as the source of POD for further analyses.

2.2.2. POD activity assay

POD activity was assayed using a modification of the spectrophotometric method of Rastogi et al. (1999) and Préstamo (1989). The sample cuvette contained 0.1 ml enzyme extract and a mixture composed of 2.5 ml sodium phosphate buffer (10 mM, pH 6.5) with 0.2 ml (1%, w/v) *p*-phenylenediamine as H-donor and 0.2 ml (3%, v/v) hydrogen peroxidase as oxidant. An increase in absorbance at 430 nm was recorded automatically for 3 min at 25 °C (Ultrospec 2100, Unico Co., USA). The enzyme extract was replaced by 0.1 ml sodium phosphate buffer (10 mM, pH 6.5) in the blank sample. Enzyme activity was calculated from the linear part of the curve obtained by plotting absorbance against incubation time. One POD unit was defined as an increase of 0.1 in absorbance per min (Flurkey & Jen, 1978). Values reported were means of three replications, with respective standard deviations.

2.2.3. Optimum pH

The POD activity as a function of pH was determined using different buffers at pH ranging from 3.0 to 11.0 as follows: 50 mM sodium acetate buffer (pH 3.0–5.0), phosphate buffer (pH 6.0–8.0), carbonate buffer (pH 9.0–10.0) and NaOH (pH 12.0). The enzyme activity was measured after 10 min of reaction time. The experiments were done in triplicate.

2.2.4. Combined high pressure and thermal stability of partially purified POD in kiwifruit

Experiments on isothermal–isobaric inactivation were carried out in mono-vessel high pressure equipment (UHPF-800 MPa-3 L, Baotou Kefa Co., China) according to Wang, Zhou, and Chen (2008) with a maximum pressurization capacity of 800 MPa. An oil mixture, bis(2-ethylhexyl)sebacate (Li-Dong Precision Machinery Co., Shenzhen, China), was used as the pressure medium. The pressure chamber (3 l, 12 cm inner diameter), surrounded by a thermostatic jacket connected to a water bath, could be heated or cooled to the desired temperature before pressurization.

Enzyme samples (1.0 ml) were enclosed in polyethylene bags, taking care to exclude as much air as possible. The

temperature in the insulated vessels was first equilibrated using an external cryostat. Then, the bags were placed in the pressure vessel, which was already equilibrated at 10, 30 and 50 °C. Pressure was built up slowly (about 100 MPa/min) to minimize temperature increase due to adiabatic heating (Ludikhuyze, Indrawati, Van den Broeck, Weemaes, & Hendrickx, 1998). In this study, the pressure was controlled at 0.1, 200, 400 and 600 MPa. To count for the effects of adiabatic heating, a blank sample was taken after pressure build-up, cooled in an ice bath and the initial residual POD activity measured (A_0). The other samples were then pressurized under isothermal–isobaric conditions for exactly 0, 5, 10, 15, 20, 25, 30 min (after pressure build-up). The pressure come-up time and depressurization were not included in the pressure hold-time. The pressure was released and samples were immediately cooled in an ice bath and the final residual POD activity was measured within 60 min of storage time in ice bath (A_t). The experiments were done in triplicate.

2.2.5. Native polyacrylamide gel electrophoresis (Native-PAGE) and activity staining

Native polyacrylamide gel electrophoresis (Native-PAGE) and activity detection were carried out to investigate about POD isoenzymes according to Laemmli (1970) with a 10% acrylamide separating gel and a 4% acrylamide stacking gel, except that SDS was excluded. After electrophoretic separation through protein migration, the gels were stained for POD activity by the riboflavin–nitroblue tetrazolium method (Beauchamp & Fridovich, 1971) with little modification. The gels were incubated at 37 °C in a light shelter. Then they were stained using 50 ml sodium phosphate buffer (50 mM, pH 6.0), 10 ml methanol (10%, v/v) and 2.5 ml *o*-dianisidine in methanol (1%, w/v). After 5 min, 8 ml hydrogen peroxide (3%, v/v) were added to induce color. The stained gels were washed with distilled water and fixed with a solution containing distilled water, methanol and acetic acid (5:5:1).

2.2.6. Statistical analysis

Analysis of variance (ANOVA) of the effect of pressure, temperature and process time as well as their interactions on residual POD activity were performed using SAS software (SAS System for Windows Version 8.1) to analyze the experimental results.

3. Results and discussions

3.1. POD optimal pH

The measurement of POD activity as a function of pH indicated that the pH optimum for POD varies from 6.0 to 8.5 (Fig. 1) and the activity dropped rapidly on both acidic and alkaline sides. These results are different from those reported by Fúster, Préstamo, and Cano (1994), but correlate relatively well with results from Phunchaisri and Apichartsrangkoon (2005), Nagle and Haard (1975), as well as Baardseth and Slinde (1980) on POD from lychee, banana, carrot, swede and Brussels sprouts, respectively. The broad range of pH optima observed was probably due to the presence of isoenzymes having different pH optima (Phunchaisri and Apichartsrangkoon 2005).

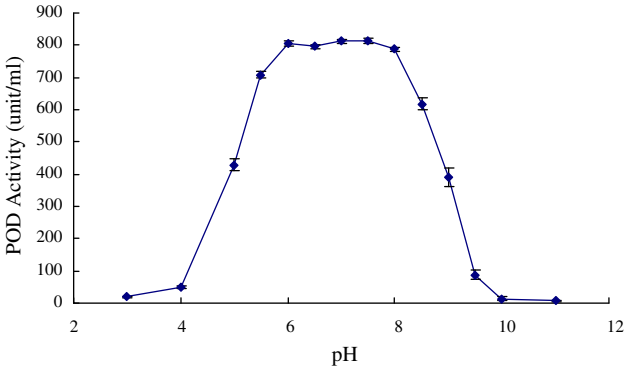

Fig. 1. Variation of kiwifruit POD activity as function of pH. Values are means of three replications with corresponding sstandard deviations.

3.2. Effect of combined high pressure and thermal treatment on POD activity

The residual activity of partially purified POD after combined high pressure and temperature treatment are shown in Figs. 2–4. Results revealed that, at each temperature, an increase in pressure level results in a decrease of enzyme activity.

Compared with control, POD activity at 10 °C showed a slight increase ($P > 0.05$) under treatment at 200 MPa from 10 to 20 min (Fig. 2), whereas at 400 and 600 MPa, the activity decreased with regard to that observed at 200 MPa. As shown in Fig. 3, POD activity at 30 °C was higher after treatment at 200 MPa for 15 min and the activity decreased following longer exposure time intervals. Cano, Hernandez, and De Ancos (1997) observed that high

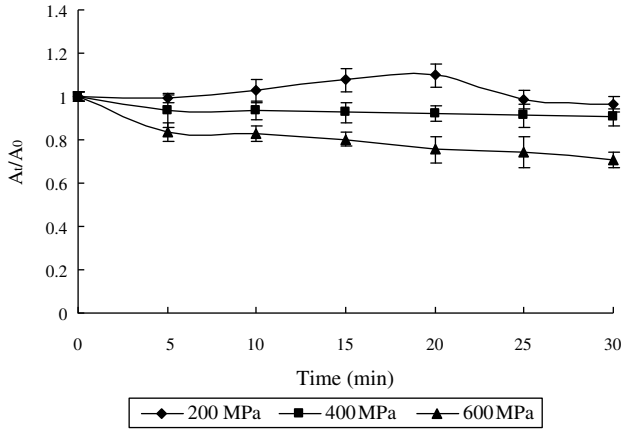

Fig. 2. Effect of high pressure treatment on partially purified kiwifruit POD activity for different exposure time intervals at 10 °C. A_t/A_0 indicates the relative residual POD activity. Values are means of three replications with corresponding standard deviations.

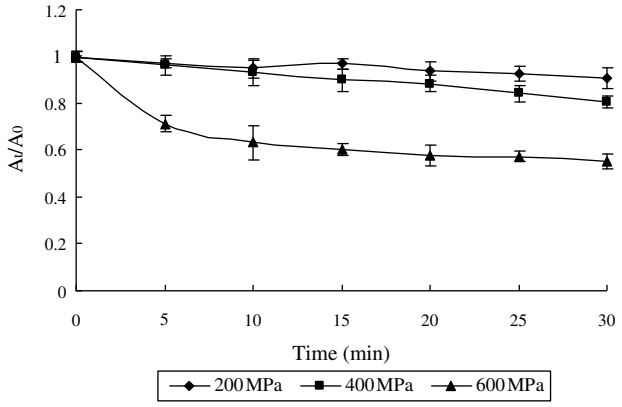

Fig. 3. Effect of high pressure treatment on partially purified kiwifruit POD activity for different exposure time intervals at 30 °C. A_t/A_0 indicates the relative residual POD activity. Values are means of three replications with corresponding standard deviations.

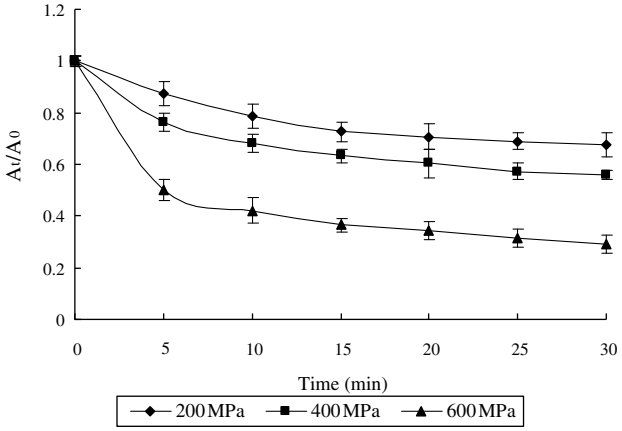

Fig. 4. Effect of high pressure treatment on partially purified kiwifruit POD activity for different exposure time intervals at 50 °C. A_t/A_0 indicates the relative residual POD activity. Values are means of three replications with corresponding standard deviations.

pressure treatment at 32–60 °C adversely increased POD activity in orange juice. Furthermore, POD in strawberry puree was increasingly inactivated by treatments up to 300 MPa at 20 °C for 15 min, while above 300 MPa, POD activity increased slightly. This phenomenon may be due to the positive conformational changes in the protein structure at 200 MPa (Cheftel, 1992).

Except for treatment at 200 MPa and 10–30 °C, the slope of inactivation curve was decreased by all other process conditions after 15 min (Figs. 2–4). Quaglia et al. (1996) indicated that a further increase of exposure time seems to have no particular effect on POD inactivation.

Regarding temperature, POD activity followed a decreasing potential curve when pressure levels of 200, 400 and 600 MPa were applied at higher temperatures (Fig. 2–4). In other words, results showed that POD activity decreased when the temperature increased. The synergistic effect of temperature and pressure on POD deactivation was enhanced by the elevation of temperature. At 50 °C, a significant inactivation ($P < 0.05$) of POD activity was observed at all pressure levels after various exposure times (Fig. 4).

The residual POD activity in kiwifruit after treatment at 600 MPa and 50 °C for 30 min was found to be 29.40 ± 3.51%. These results showed that pressure level of 600 MPa at 50 °C for 30 min was not enough to inactivate completely POD. This observation is similar to that from Cano et al. (1997) for POD inactivation in strawberry puree and orange juice, where strawberry POD could be successfully inactivated using a combination of pressures up to 280 MPa and temperatures up to 45 °C and a good POD inactivation (50%) could be obtained in orange juice with a combination of 400 MPa and 32 °C. The relatively high residual activity could be due to the presence of numerous POD isoenzymes, which react in different ways toward pressure (Quaglia et al., 1996) and heat (Luna, Garrote, & Bressan, 1986).

Compared with the inactivation curve of partially purified POD at the same temperature, the slope of POD in kiwifruit juice at 30 °C was slightly decreased (Fig. 5), but the difference was not statistically significant ($P > 0.05$). Seyderhelm, Boguslawski, Michaelis, and Knorr (1996) indicated that real food systems show a protective effect of food ingredients at pressures applied for POD inactivation.

3.3. Effect of high pressure on POD isoenzymes

Polyacrylamide gel electrophoresis (Native PAGE) and activity detection were carried out on the partially purified enzyme before and after treatment under pressure ranging from 200 to 600 MPa at 30 °C for 15 min (Fig. 6).

At the beginning, two staining bands were observed on the untreated enzyme. This suggested that there may be two isoenzymes, POD A and B. After 15 min of treatment at 200 MPa at 30 °C, the intensity of POD A decreased while that of POD B slightly faded, and a new band (POD C) was observed. POD A and B disappeared as pressure increased at 600 MPa. From this observation, there may be several isoenzymes that have different resistance to high pressures.

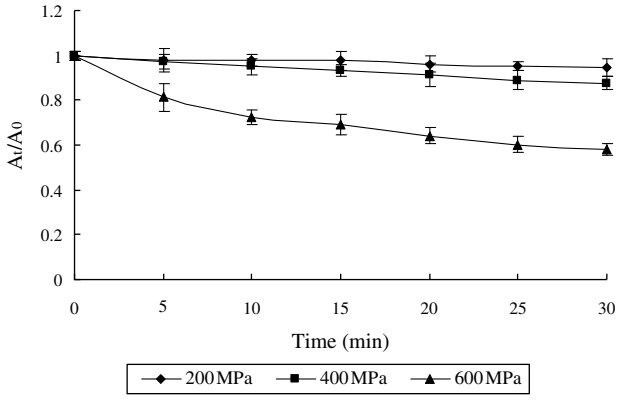

Fig. 5. Effect of high pressure treatment on POD activity in kiwifruit juice for different exposure time intervals at 30 °C. A_t/A_0 indicates the relative residual POD activity. Values are means of three replications with corresponding standard deviations.

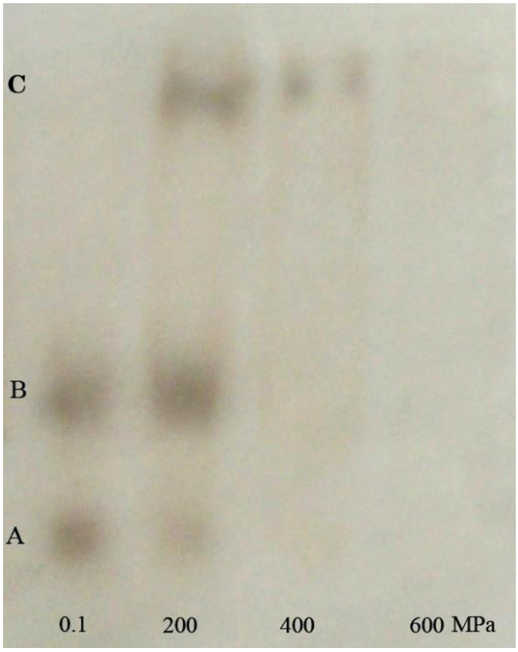

Fig. 6. Effect of high pressure treatment on POD isoenzymes in kiwifruit.

Bayindirli, Alpas, Bozoğlu, and Hizal (2006) suggested that isoenzymes resistant to pressure are responsible for the final residual activity.

4. Conclusions

Kiwifruit POD was found to have a broad optimum pH (6.0–8.5), which is probably due to the presence of several isoenzymes with different pH optima. High pressure treatment constitutes an effective technology to inactivate deleterious enzymes in kiwifruit. Pressures higher than 400 MPa can be combined with mild heat ($\leqslant$50 °C) to accelerate enzyme inactivation. The slope of POD in kiwifruit juice at 30 °C was lower than that in a model system. There are several POD isoenzymes in kiwifruit and those resistant to pressure may be responsible for the activity still remaining after treatment.

A further research is needed to study the modification of food properties due to treatment by combined temperature and pressure. Overall, the influence of POD isoenzymes needs more evidence. This would certainly enhance the understanding of high pressure processing and facilitate the application of high pressure technology in the food industry.

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References

Anese, M., Nicola, M. C., Dall'Aglio, G., & Lerici, C. R. (1995). Effect of high pressure treatments on peroxidase and polyphenoloxidase activities. *Journal of Food Biochemistry, 18*, 285–293.

Baardseth, P., & Slinde, E. (1980). Heat inactivation and pH optima of peroxidase and catalase in carrot, swede and Brussels sprouts. *Food Chemistry, 5*, 169–174.

Bayindirli, M., Alpas, H., Bozoğlu, F., & Hizal, M. (2006). Efficiency of high pressure treatment on inactivation of pathogenic microorganisms and enzymes in apple, orange, apricot and sour cherry juices. *Food Control, 17*, 52–58.

Beever, D. J., & Hopkirk, G. (1990). Fruit development and fruit physiology. In I. J. Warrington & G. C. Weston (Eds.), *Kiwifruit: Science and management* (pp. 97–126). New Zealand: Ray Richards Publisher.

Beauchamp, C., & Fridovich, I. (1971). Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. *Analysis Biochemistry, 44*, 276–287.

Bomber, J. L., Dietrich, W. C., Hudson, J. S., Hamilton, H. K., & Farkas, O. F. (1975). Yields and solid loss in steam blanching, cooling and freezing vegetables. *Journal of Food Science, 40*, 660–664.

Burnette, F. S. (1977). Peroxidase and its relationship to food flavor and quality: A review. *Journal of Food Science, 42*, 1–6.

Cano, M. P., Hernandez, A., & De Ancos, B. (1997). High pressure and temperature effects on enzyme inactivation in strawberry and orange products. *Journal of Food Science, 62*, 85–88.

Cheftel, J. C. (1992). Effects of high hydrostatic pressure on food constituents: An overview. In C. Balny, R. Hayashi, K. Heremans, & P. Masson (Eds.), *High pressure and biotechnology, Colloque INSERM, Vol, 224*, (pp. 195–209). Montrouge: John Libbey Eurotext.

Denys, S., Van Loey, A. M., & Hendrickx, M. E. (2000). A modeling approach for evaluating process uniformity during batch high hydrostatic pressure processing: Combination of a numerical heat transfer model and enzyme inactivation kinetics. *Innovative Food Science & Emerging Technologies, 1*, 5–19.

Flurkey, W. H., & Jen, J. J. (1978). Peroxidase and polyphenol oxidase activities in developing peaches. *Journal of Food Science, 43*, 1826–1828, 1831.

Fúster, C., Préstamo, G., & Cano, M. P. (1994). Drip loss, peroxidase and sensory changes in kiwi fruit slices during frozen storage. *Journal of the Science of Food and Agriculture, 64*, 23–29.

Grison, R., & Pilet, P. E. (1985). Maize root peroxidase relationship with polyphenol oxidase. *Phytochemistry, 24*, 2519–2521.

Heatherbell, D. A. (1975). Identification and quantitative analysis of sugars and non-volatile organic acids in Chinese gooseberry fruit (*Actinidia chinensis* planch). *Journal of the Science of Food and Agriculture, 26*, 815–820.

Hendrickx, M. E. G, & Knorr, D. (2002). *Ultra high pressure treatments of foods*. New York: Kluwer Academic/Plenum Publishers.

Knorr, D. (1993). Effects of high-hydrostatic pressure processes on food safety and quality. *Food Technology, 47*, 156–161.

Laemmli, U. K. (1970). Cleavage of the structural proteins during the assembly of the head of bacteriophage T4. *Nature, 227*, 680–685.

López, P., Sala, F. J., de la Fuente, J. L., Condón, S., Raso, J., & Burgos, J. (1994). Inactivation of peroxidase, lipoxygenase and polyphenol oxidase by manothermosonication. *Journal of Agricultural and Food Chemistry, 42*, 252–256.

Ludikhuyze, L., Indrawati Van den Broeck, I., Weemaes, C., & Hendrickx, M. (1998). Effect of combined pressure and temperature on soybean lipoxygenase 1. Influence of extrinsic and intrinsic factors on isobaric–isothermal inactivation kinetics. *Journal of Agricultural and Food Chemistry, 46*, 4074–4080.

Luna, J. A., Garrote, R. L., & Bressan, J. A. (1986). Thermo-kinetic modeling of peroxidase inactivation during blanching, cooling of corn on the cob. *Journal of Food Science, 51*, 141–145.

Miesle, T. J., Proctor, A., & Lagrimini, L. M. (1991). Peroxidase activity, isoenzymes, and tissue localization in developing high-bush blueberry fruits. *Journal of the American Society for Horticultural Science, 116*(5), 827–830.

Nagle, N. E., & Haard, N. F. (1975). Fractionation and characterization of peroxidase from ripe banana fruit. *Journal of Food Science, 40*, 576–579.

Préstamo, G. (1989). Peroxidase of Kiwifruit. *Journal of Food Science, 54*, 760, 762.

Préstamo, G., Arabas, J., Fonberg-Broczek, M., & Arroyo, G. (2001). Reaction of B. *Reaction of B. cereus* bacteria and peroxidase enzymes under pressures >400 MPa. Journal of Agricultural and Food Chemistry, *49*, 2830–2834.

Phunchaisri, C., & Apichartsrangkoon, A. (2005). Effects of ultra-high pressure on biochemical and physical modification of lychee (*Litchi chinensis* Sonn). *Food Chemistry, 93*, 57–64.

Quaglia, G. B., Gravina, R., Paperi, R., & Paoletti, F. (1996). Effect of high pressure treatments on peroxidase activity, ascorbic acid content and texture in green peas. *Lebensmittel Wissenschaft und Technologie – Food Science and Technology, 29*, 552–555.

Rastogi, N. K., Eshtiaghi, M. N., & Knorr, D. (1999). Effect of combined high pressure and heat treatment on the reduction of peroxidase and polyphenoloxidase activity in red grapes. *Food Biotechnology, 13*, 195–208.

Seyderhelm, I., Boguslawski, S., Michaelis, G., & Knorr, D. (1996). Pressure induced inactivation of selected food enzymes. *Journal of Food Science, 61*, 308–310.

Wang, R., Zhou, X., & Chen, Z. (2008). High pressure inactivation of lipoxygenase in soy milk and crude soybean extract. *Food Chemistry, 106*, 603–611.

Williams, D. C., Lim, M. H., Chen, A. O., Pangborn, R. M., & Whitaker, J. R. (1986). Blanching of vegetables for freezing – which indicator enzyme to choose. *Food Technology, 40*, 130–140.