

Effects of ultra-high pressure on biochemical and physical modification of lychee (*Litchi chinensis* Sonn.)

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Abstract

To evaluate the possibility of using high pressure as an alternative to canning for lychee preservation, fresh lychees and samples preserved in syrup were subjected to various pressures (200–600 MPa), temperatures (20–60 °C) and times (10 or 20 min) and subsequently analysed for physical attributes, peroxidase (POD) and polyphenol oxidase (PPO) activities. Pressure treatment caused less loss of visual quality in both fresh and syrup processed lychee than thermal processing. The optimal pH for lychee POD and PPO were 5.0–8.0 and 7.0, respectively. Pressure treatment at 200 MPa caused a marked increase of POD activity and this effect was greater at 40 °C than at 20 and 60 °C. Pressure treatment at 400 and 600 MPa, and temperatures of 20–40 °C, did not affect the activity of POD, but some inactivation at 60 °C was observed. The combined effect of pressure and temperature on PPO activity were more marked at the longer treatment time (20 min) and under the more severe treatments. A pressure 600 MPa, at 60 °C for 20 min caused extensive inactivation of POD and PPO in fresh lychee, over 50% and 90% respectively but for those processed in syrup, the effects were less marked, presumably due to baroprotection by the syrup. Overall lychee POD was more pressure resistant than PPO.

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1. Introduction

Lychee (*Litchi chinensis* Sonn.) has a white, juicy aril which is surrounded by a reddish prickly leather-like skin and contains a shiny brown, usually large seed. The major lychee production areas in the world are China, Taiwan, Vietnam, Thailand, India, South Africa and the Malagasy Republic (Menzel, Watson, & Simpson, 1988). Availability of the fresh fruit is limited because of its short production period and shelf life. The most common process to preserve lychees is canning. As with

many other fruits and vegetables, a pink discoloration occurs in canned lychee (Wu & Chen, 1999). This phenomena is not only of sensory importance but also leads to nutritional losses. Increasing consumer demand for safe, high-quality, freshlike products that are shelf-stable, minimally processed and additive free stimulated the interest of the food manufacturing industries in such novel processing techniques as high-pressure (Weemaes, Ludikhuyze, Van den Broeck, & Hendrickx, 1999). This process has good potential for the development of new processes for food preservation or product modifications (Cano, Hernández, & De Ancos, 1997). For the development of high pressure processed fruits and vegetables, it is essential to know the influence of high pressure on the activities of such enzymes as polyphenol oxidase (PPO), peroxidase (POD) and lipoxygenase.

Abbreviations: POD, peroxidase; PPO, polyphenol oxidase.

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PPO catalyzes the oxidative reaction associated with undesirable browning of damaged tissues in fresh fruits and vegetables (Fujita et al., 1995). POD is associated with off-flavors and off-colors in raw and unblanched vegetables (López et al., 1994). Because of their relatively high thermal stability, POD activity is usually used to evaluate the efficiency of thermal processing (Quaglia, Gravina, Paperi, & Paoletti, 1996). In a buffer system, Seyderhelm, Boguslawski, Michaelis, and Knorr (1996) found that the pressure induced inactivation of enzymes were in the following decreasing order: lipoxygenase, lactoperoxidase, pectinesterase, lipase, phosphatase, catalase, PPO and POD. Since PPO and POD are so pressure stable when considering the commercial exploitation of pressure processed fruits and vegetables, they have received most attention.

Effects of high-pressure treatments on enzymes can be either reversible or irreversible and inactivation relates to conformational changes in the protein structure. The effects found depend on the type of enzyme, the nature of the substrate, pressure, temperature and processing time. Enzymatic reactions may be enhanced or inhibited by pressure, depending on whether the volume change associated with the reaction is positive or negative. Pressure-induced changes in the catalytic rate may be due to changes in the enzyme–substrate interaction, changes in the reaction mechanism, the effect on a particular rate-limiting step or the overall catalytic rate (Ludikhuyze & Hendrickx, 2001).

This study aimed to investigate the effects of combined ultra-high pressure/temperature on POD and PPO activities in lychees and some quality parameters of interest.

2. Materials and methods

2.1. Raw materials

Lychees (*Litchi chinensis* Sonn.) were purchased from a commercial orchard in Chiangmai province of Thailand and stored at 2 °C for 2–3 days prior to high pressure and canning processing. Some lychees were kept at –18 °C up to 2 months for enzyme activity measurements.

2.2. Canning process

The lychees were canned following the commercial process of the Royal Agriculture Company Ltd. (Chiangmai, Thailand). The lychees were peeled, destoned and soaked in a solution of 1% CaCl₂ and 0.1% citric acid for 15 min. The soaked lychees were washed 3 times in deionised water, 120 g of lychees were filled in a plain can and 175 g of syrup, which consisted of 300 g sucrose, 1.3 g citric acid and 700 g deionised water

were added. The filled cans were exhausted in steam for 10 min at 80–85 °C, then sealed, sterilized in boiling water for 18 min and cooled to 45 °C. All canned lychees were stored at room temperature for 2 weeks prior to analysis for pH, water activity (a_w), soluble solid contents and color and 2 months prior to analysis for enzymatic activity.

2.3. High Pressure processing

The lychees were peeled, destoned and sealed in polyethylene bags (Cryovac Ltd., UK) taking care to exclude as much air as possible. Each bag contained three lychees so that the total weight was about 40–45 g. The bags were processed at pressures of 200, 400 and 600 MPa at 20, 40 and 60 °C for 10 or 20 min in a prototype Stansted 'Food-Lab' model 900 high pressure rig (Stansted Fluid Power Ltd., Stansted, UK), untreated samples served as controls.

Further sets of three lychees were mixed with the syrup used in the canning experiment in the ratio of fruit to syrup of 1:1 and were subjected to the pressure/temperature regimes described above.

The pressure treated samples (and controls) were stored at 2 °C for 2 weeks and at –18 °C for 2 months.

After 2 weeks at 2 °C the lychee samples were taken and analysed for pH, a_w , soluble solid contents and color, whereas those stored at –18 °C were analysed for enzymatic activity. Previous works had shown that storage at –4 and –20 °C for up to 18 months has no effect on the total enzyme activity (Vámos-Vigyázó, 1981).

2.4. Assay for POD activity

Ten grams of lychee were homogenized at 4 °C with a mixture of 40 ml of 50 mM potassium phosphate, 1 M KCl, 2% polyvinylpyrrolidone (PVPP), pH 6.2. The homogenate were centrifuged at 16,000 × g for 30 min at 4 °C (Sorvall® RC5 C Plus) and filtered through Whatman No 41 paper, the filtrate were used for the enzyme assays (modified from Huang, Hart, Lee, & Wicker, 1990). A 0.1 ml aliquot of crude enzyme extracted was added to 2.15 ml of 0.01 M sodium acetate buffer, pH 6.0 containing 0.5% guaiacol, 0.25 ml of 0.1% hydrogen peroxide and subsequently its absorbance at 470 nm was followed for at least 5 min with a Perkin Elmer UV/VIS Spectrophotometer Lambda 20. One unit of POD activity was defined as an increase of 0.1 unit of absorbance per min at 470 nm (Flurkey & Jen, 1978).

The pH optima for POD was determined using the above incubation mixture by adjusting the pH from 2.0 to 11.0 (0.5 pH unit intervals) with 1% NaOH or 1% acetic acid and assayed as described above.

2.5. Assay for PPO activity

Extraction of the enzyme and measurement of its activity was carried out according to the procedure described by Huang et al. (1990) and Flurkey and Jen (1978). A 0.05 ml aliquot of crude enzyme extracted was added to a mixture of 2.2 ml of 0.1 M potassium phosphate buffer, pH 6.5 and 0.25 ml of 0.2 M catechol, and its absorbance at 420 nm followed for at least 5 min. One unit of PPO activity was defined as an increase of 0.1 unit of absorbance per min at 420 nm. The pH optimum was determined using the above incubation mixture by adjusting the pH values from 3.0 to 9.0 (0.5 pH unit intervals) with 1% NaOH or 1% H₃PO₄ and assayed as described above.

2.6. Quality determinations

The soluble solid contents of all samples were determined using a digital refractometer (Atago, Japan). Results are reported as °Brix at 20 °C. For water activity (a_w), the samples were macerated and placed in a cylindrical cup, 4.0 cm diameter × 1.0 cm high, filled approximately 2/3 full. The a_w was measured at 25 °C with a Novasine AWC meter (AWC 200, Switzerland). For color, samples were assessed using a Hunterlab Color Quest Spectrophotometer or Perkin Elmer UV/VIS Spectrophotometer Lambda 20 in the reflection mode. Results were expressed as Hunter L (brightness), a (green/red) and b (yellow/blue) values.

2.7. Statistical analysis

All data were analyzed using SPSS v 11.5 (SPSS Inc., Chicago, USA). After an analysis of variance (ANOVA), significant difference ($p < 0.05$) among means were determined by Duncan's multiple range test.

3. Results and discussions

3.1. Physical characteristics of the processed lychees

All processes decreased the pH of the lychees (originally 4.72) by between 0.12 pH units for those lychees processed under pressure to 0.60 pH units for those canned in syrup under commercial conditions (Table 1). Not unexpectedly all the lychees processed in syrup (containing citric acid and sugar) had lower pH values and higher soluble solid contents than the fresh lychees. There was also a marked increase in the soluble solid contents and concomitant decrease in water activity in the pressure treated samples indicating some loss of water during processing and/or solubilisation of previously insoluble material. A small amount of fluid was seen in the pressure treated, non-syruped samples.

Table 1

General characteristics of fresh lychee and lychees processed by various means

Treatments	pH	Soluble solid contents (°Brix)	Water activity (a_w)
Fresh lychee	4.72	11.2	0.993
Pressurised fresh lychee ^a	4.60 ± 0.13	16.3 ± 1.79	0.967 ± 0.009
Lychee in syrup	4.22	22.6	0.965
Pressurised syrup lychee ^a	4.25 ± 0.10	22.8 ± 0.47	0.961 ± 0.003
Canned lychee	4.12	23.9	0.966

^a The values quoted are means ± SD of all the different pressure/temperature regimes (18) since there was no significant difference between treatments.

With respect to color it is interesting to note that when applied at 20 °C for 20 min pressure, even up to 600 MPa had little effect. The L value increased slightly from a value of $53.9 ± 1.12$ in the untreated sample to $58.2 ± 4.56$ in those treated at 600 MPa. At 60 °C the L value increased significantly even at 200 MPa (to $62.2 ± 3.72$) but then changed little on further increasing the pressure (to $68.0 ± 4.75$) (Table 2). There was no significant difference between treatment times of 10 and 20 min.

Increasing pressure, at both 20 and 60 °C caused a decrease in the a value indicating that pressure may be an effective means of minimising the pink discoloration sometimes seen in lychees, since a decreased a value indicates less redness (Cheng & Hwang, 1984). The b value in the pressurised samples changed little when treated at mild temperature (20–60 °C) and high pressure (200–600 MPa) for 20 min although treatment at 60 °C increased the value. The results are summarised in Table 2, the 10 min treated samples gave very similar results to those treated for 20 min and are thus not reported. Similar trends were seen in those samples treated in syrup with increasing pressure at 60 °C increasing the L value, although the effects on the a value were less marked (Table 3). Interestingly the samples canned under commercial conditions had lower a and higher b values than all the pressurised samples agreeing with Yen and Lin (1996) who reported that heating can cause an increase in L and b values in guava puree.

In conclusion it can be seen that pressure treatment causes less loss of visual quality in both fresh and syrup processed lychees than thermal processing.

3.2. pH Optima

The pH optimum for POD was 5.0–8.0 with maximal activity at 6.0 (Fig. 1), which is similar to the values found by Nagle and Haard (1975), Baardseth and Slinde (1980) and Fujita et al. (1995) for POD from banana, carrot, swede, broccoli and cabbage. The broad pH optima observed is probably due to the presence of

Table 2
Effect of high pressure and mild temperature on the color characteristics of fresh lychees

Treatments			Color characteristics		
Temperature (°C)	Pressure (MPa)	Time (min)	L value	a value	b value
<i>Untreated^a</i>			53.9 ± 1.12	−0.15 ± 0.31	−3.71 ± 0.40
20	200	10	56.0 ± 3.50	−0.31 ± 0.39	−2.47 ± 1.13
20	400	10	60.4 ± 2.21	−0.42 ± 0.37	−3.93 ± 2.02
20	600	10	58.5 ± 3.18	−0.76 ± 0.80	−0.91 ± 0.62
20	200	20	ND	ND	ND
20	400	20	57.7 ± 4.35	−0.82 ± 0.42	−3.77 ± 0.94
20	600	20	58.2 ± 4.56	−0.55 ± 0.55	−3.18 ± 1.14
<i>Untreated^b</i>			50.2 ± 4.69	0.43 ± 0.74	2.05 ± 1.79
60	200	10	66.8 ± 4.98	0.37 ± 1.10	3.63 ± 2.92
60	400	10	68.3 ± 5.59	−0.61 ± 1.02	3.92 ± 2.26
60	600	10	67.5 ± 3.37	−1.24 ± 0.67	4.38 ± 2.49
60	200	20	62.2 ± 3.72	−0.06 ± 0.64	2.21 ± 1.73
60	400	20	67.0 ± 3.64	−0.43 ± 1.54	3.72 ± 1.52
60	600	20	68.0 ± 4.75	−1.84 ± 0.78	2.97 ± 1.91

All values are the mean ± SD of 12 determinations.

ND = not detected.

^a Color measurement at 20 °C using Perkin Elmer UV/VIS Spectrophotometer Lambda 20.

^b Color measurement at 60 °C using Hunterlab Color Quest Spectrophotometer because the Perkin Elmer UV/VIS Spectrophotometer was unavailable.

Table 3
Effect of high pressure and mild temperature on the color characteristics of lychees processed in syrup

Treatments			Color characteristics		
Temperature (°C)	Pressure (MPa)	Time (min)	L value	a value	b value
<i>Untreated^a</i>			55.0 ± 3.04	−0.61 ± 0.25	−4.34 ± 0.56
20	200	10	54.7 ± 1.24	−0.48 ± 0.24	−4.31 ± 0.59
20	400	10	56.5 ± 1.27	−0.61 ± 0.17	−4.81 ± 0.60
20	600	10	56.7 ± 1.52	−0.78 ± 0.27	−4.64 ± 0.64
20	200	20	54.6 ± 2.65	−0.59 ± 0.14	−3.70 ± 0.52
20	400	20	56.0 ± 1.95	−0.74 ± 0.54	−4.19 ± 0.72
20	600	20	57.9 ± 1.38	−0.75 ± 0.18	−4.36 ± 0.78
<i>Canned lychee^b</i>			66.3 ± 3.99	−1.61 ± 1.52	6.21 ± 3.99
60	200	10	61.6 ± 2.95	−1.42 ± 0.66	1.68 ± 1.89
60	400	10	61.9 ± 4.69	−1.08 ± 0.52	1.85 ± 1.53
60	600	10	62.3 ± 4.06	−1.19 ± 0.66	1.74 ± 2.49
60	200	20	64.2 ± 4.80	−1.01 ± 1.10	1.93 ± 1.47
60	400	20	64.8 ± 3.63	−1.29 ± 0.45	2.73 ± 1.45
60	600	20	68.5 ± 5.64	−1.32 ± 0.51	4.20 ± 3.29

All values are the mean ± SD of 12 determinations.

^a Color measurement at 20 °C by using Perkin Elmer UV/VIS Spectrophotometer Lambda 20.

^b Color measurement at 60 °C by using Hunterlab Color Quest Spectrophotometer because the Perkin Elmer UV/VIS Spectrophotometer was unavailable.

isoenzymes of different pH optima. The loss of activity observed on acidification is attributed to the change in the protein from the native state to a reversible denatured state, brought about by detachment of the heme from the protein (Vámos-Vigyázó, 1981).

For PPO, maximal enzyme activity was at pH 7.0 (Fig. 2). The pH corresponding to maximal activity is in agreement with the values found for avocado, pear and plum PPO as reported by Weemaes, Ludikhuyze, Van den Broeck, Hendrickx, and Tobback (1998). On raising the pH from 7.0 to 8.0, a sharp drop in enzyme activity was noticed. Moreover, the enzyme showed very

low activity at or below pH 4.0. A sharp drop in PPO activity at pH values above 7.0 was also noted by Weemaes et al. (1998) for apple, grape, pear, avocado and plum.

3.3. Effect of combined UHP/temperature treatment on POD activity

The results of treatment for both 10 and 20 min are summarised in Figs. 3 and 4. It is seen that to a large extent the results obtained for treatment times of 10 and 20 min are similar and perhaps the most marked effect is

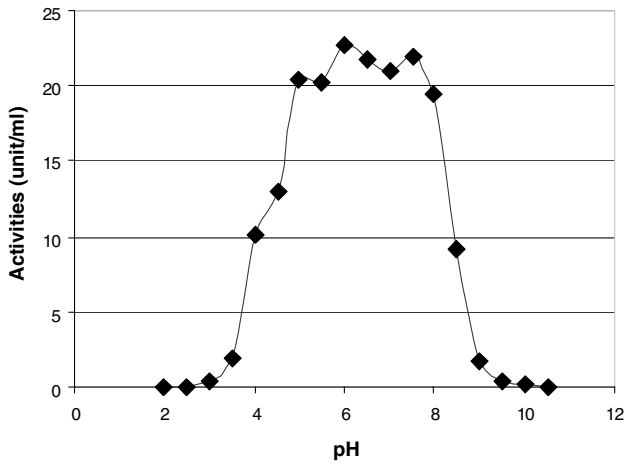


Fig. 1. The variation of lychee peroxidase activity as a function of pH.

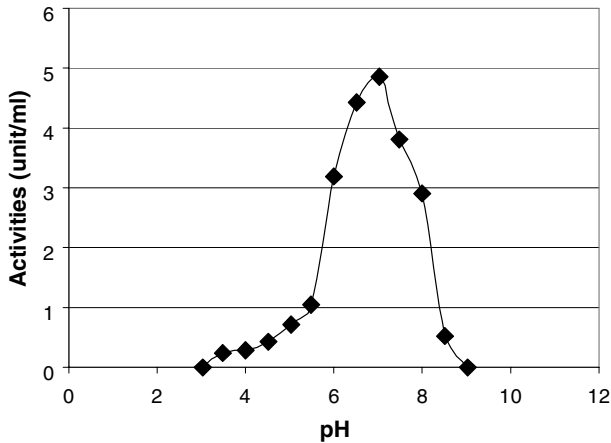


Fig. 2. The variation of lychee polyphenol oxidase activity as a function of pH.

the apparent increase in activity seen after treatment at 200 MPa in the non-syruped lychees, the effect appeared more marked at 40 °C compared with both 20 and 60 °C. This type of effect has also been noted in tomato puree (Hernández & Cano, 1998) and for PPO in mushroom (Gomes & Ledward, 1996). Because of the activation phenomena, probably related to better availability of the substrate (Gomes & Ledward, 1996) the only marked inactivation of POD in lychee was at 600 MPa and 60 °C (Figs. 3 and 4). Ogawa (1991) observed that POD from citrus juice retained 70% of its activity and Quaglia et al. (1996) found that fresh pea POD retained 50% of its activity after treatment at 600 MPa and 60 °C for 10 min.

When processed in syrup (Figs. 5 and 6) the effects are for less marked and although there is still some evidence of activation at moderate pressure all the differences are small.

These results are undoubtedly due to the baroprotective effect of the syrup as has been found by other work-

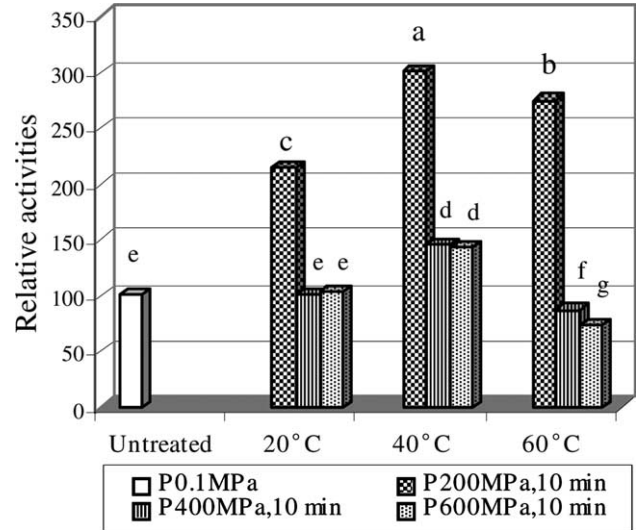


Fig. 3. Effect of combined UHP/temperature at 10 min on POD activity of fresh lychee. (All values are the means of duplicate determinations on three samples.) Bars with different superscript were significantly different ($p < 0.05$).

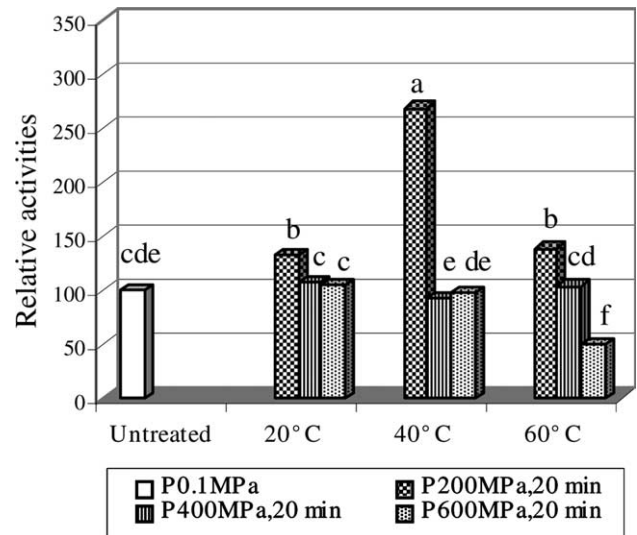


Fig. 4. Effect of combined UHP/temperature at 20 min on PPO activity of fresh lychee. (All values are the means of duplicate determinations on three samples.) Bars with different superscript were significantly different ($p < 0.05$).

ers including Seyderhelm et al. (1996), who demonstrated that the inactivation of pectinesterase in orange juice containing 30% sucrose (60 °Brix) was much lower than in orange juice of 11 °Brix.

3.4. Effect of combined UHP/temperature treatment on PPO activity

The results are shown in Figs. 7 and 8 and it is seen that, although after 10 min treatment the results are non-uniform, after 20 min a clear pattern emerges

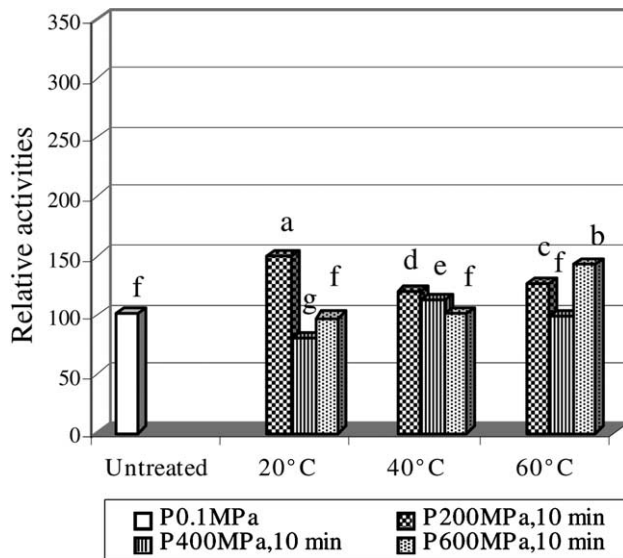


Fig. 5. Effect of combined UHP/temperature at 10 min on POD activity of syrup lychee. (All values are the means of duplicate determinations on three samples.) Bars with different superscript were significantly different ($p < 0.05$).

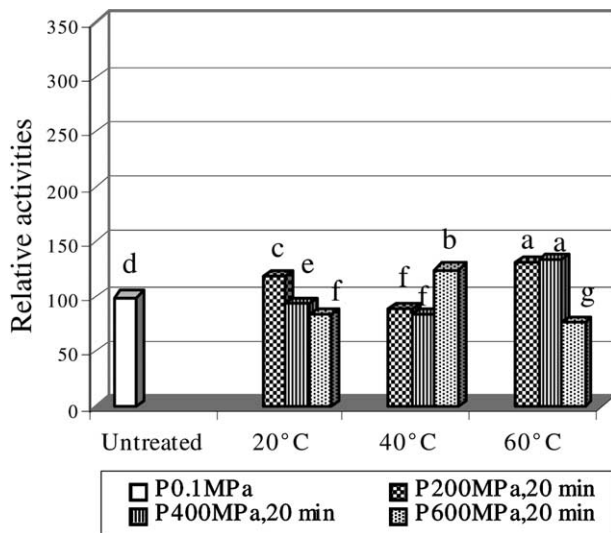


Fig. 6. Effect of combined UHP/temperature at 20 min on POD activity of syrup lychee. (All values are the means of duplicate determinations on three samples.) Bars with different superscript were significantly different ($p < 0.05$).

demonstrating that at mild temperatures (20 and 40 °C) the degree of inactivation of PPO decreases with treatment time (10 or 20 min) for the three pressure levels. Increasing temperature brings about significant decreases in activity, with over 90% loss of activity at 600 MPa and 60 °C for both treatment times (10 and 20 min). These results are in good agreement with those of Castellari, Matricardi, Arfelli, Rovere, and Amati (1997) on grape must PPO and Gomes and Ledward (1996) on potato PPO. There is no evidence of the pres-

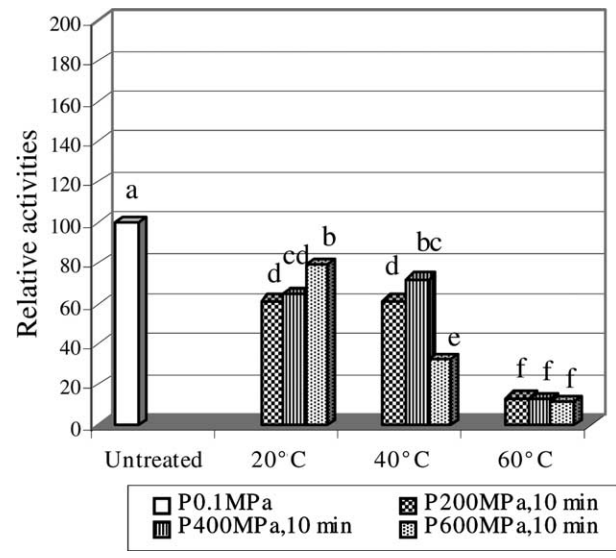


Fig. 7. Effect of combined UHP/temperature at 10 min on PPO activity of fresh lychee. (All values are the means of duplicate determinations on three samples.) Bars with different superscript were significantly different ($p < 0.05$).

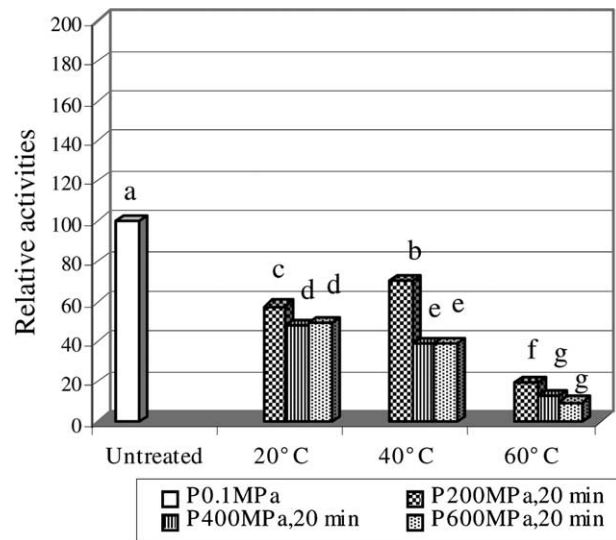


Fig. 8. Effect of combined UHP/temperature at 20 min on PPO activity of fresh lychee. (All values are the means of duplicate determinations on three samples.) Bars with different superscript were significantly different ($p < 0.05$).

sure activation seen by Gomes and Ledward (1996) in mushrooms.

When treated in syrup the baroprotective effect of the mixture is again seen, although there is still significant inactivation under the more extreme conditions (Figs. 9 and 10).

In conclusion, this initial work has demonstrated that the use of high pressures and moderate temperatures may be an effective means of extending the shelf life of lychees with minimal effect on the quality, although

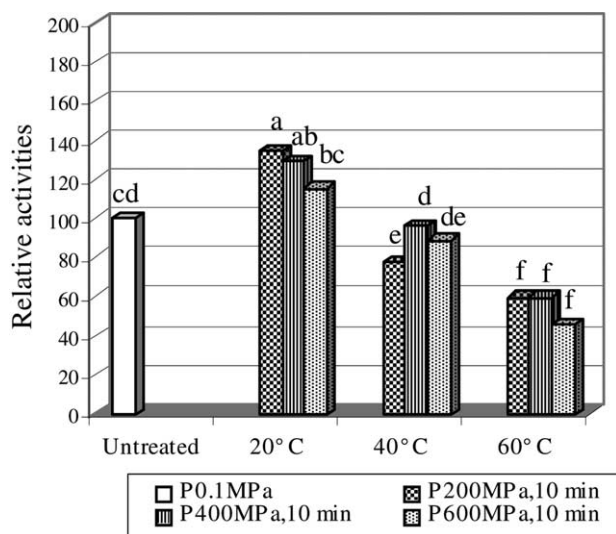


Fig. 9. Effect of combined UHP/temperature at 10 min on PPO activity of syrup lychee. (All values are the means of duplicate determinations on three samples.) Bars with different superscript were significantly different ($p < 0.05$).

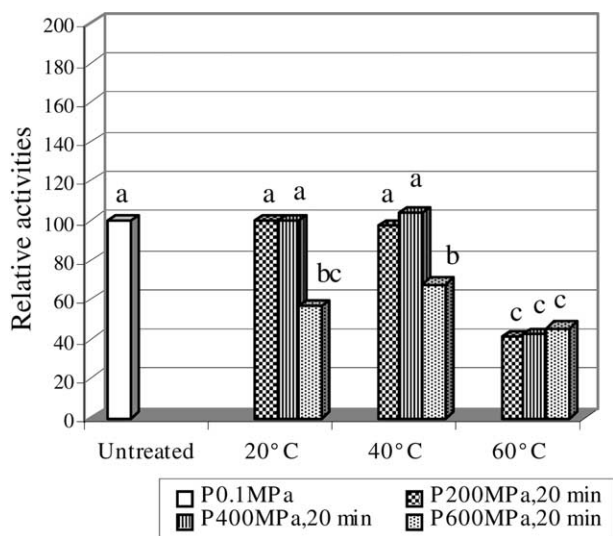


Fig. 10. Effect of combined UHP/temperature at 20 min on PPO activity of syrup lychee. (All values are the means of duplicate determinations on three samples.) Bars with different superscript were significantly different ($p < 0.05$).

the high residual activity of POD may lead to problems as may lipoxygenase, an enzyme not studied to date this work.

4. Conclusions

The optimum pH for lychee POD and PPO activities was 5.0–8.0 and 7.0, respectively. The overall effects of combined ultra-high pressure/temperature led to less loss of visual quality in both fresh and syrup processed

lychee than in those thermal processed. Combination of higher pressure (600 MPa) and temperature (60 °C) causes extensive inactivation of both enzymes in fresh lychee, but for those processed in syrup, the effects were less due to baroprotection by the syrup. Lychee POD is more pressure resistant than PPO.

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