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Physical and biochemical qualities of pressurized and pasteurized longan juices upon storage

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Abstract

The physical and biochemical properties of pressurized longan juice at 500 MPa; 25°C for 30 min and pasteurized juice at 90°C for 2 min were investigated during storage at 4°C for 4 weeks. Color parameters indicated that although enzymatic degradation in pressurized longan juice was the primary cause of lower *L* parameter (lightness) during storage, its magnitude was apparently lesser than browning caused by Maillard reaction in pasteurized juices reflected by *b** (yellowness) and BI (browning index) parameters. The results of residual enzymes showed that during storage, polyphenol-oxidase had greater rate of degradation and was more resistant to pressure than peroxidase. The bioactive components like ascorbic acid in pressurized juice exhibited higher reduction during storage than that of pasteurized juice. However, residual ascorbic acid in pressurized juice was still higher than that thermal treated juice throughout the storage period. The other bioactive components in pressurized juice were also gradually degraded during storage by 71%, 66%, 28% and 37%, while in pasteurized products substantially reduced by 60%, 69%, 23% and 35% for gallic acid, ellagic acid, total phenols and antioxidant activity (DPPH assay), respectively.

Key words: Bioactive components, Enzyme activities, Longan juice, Pressurization, Pasteurization

Introduction

Longan (*Dimocarpus longan* Lour.) is a succulent, sweet and white aril fruit, grown commercially in many countries, especially Thailand. Its juice contains significant amounts of bioactive compounds such as ascorbic, gallic and ellagic acids (Chaikham and Apichartsrangkoon, 2012a, b). The ascorbic acid is well known for its strong antioxidant activity (Zaouay et al., 2014), whist gallic and ellagic acids have proven pharmacological properties such as antityrosinase, antiglycated, antifungal and anticancer (Yang et al., 2011; Rangkadilok et al., 2012). To preserve longan juice, conventional pasteurization has been used for decades however, thermal processes could degrade its nutritional, color, flavor and antioxidant qualities. In this context, non-thermal process such as high hydrostatic pressure is an emerging technique. The benefits of this technology have been revealed by several researchers. For instance, Dajanta et al. (2012) reported that pressurized lychee at 600 MPa with 30°C or 50°C for 20 min displayed lower *a** (redness) and *b** (yellowness) parameters than that of pasteurized lychee. Similarly, Barba et al. (2013) found high retention of ascorbic acid, total phenolics, anthocyanins and total antioxidant capacity in pressurized blueberry juices at 200-600 MPa; 42°C for 5-15 min. Our previous study showed that pressurized pennywort juice at 400 MPa; 30°C for 20 min was an appropriate condition to preserve bioactive compounds such as asiaticoside, madecassoside, β-carotene, ascorbic acid, total phenols and antioxidant capacities (FRAP assay) (Apichartsrangkoon et al., 2012). Earlier, Polydera et al. (2005) supported that pressurized orange juice at 600 MPa; 40°C for 4 min brought better ascorbic acid retention during post processing and storage as compared to pasteurization at 80°C for 1 min.

Enzymes are the key problem for high pressure processing, as the effects of high-pressure can be either reversible or irreversible due to the extent of

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enzyme inactivity. In addition, the efficiency of inactivating enzyme relates to the degree of conformational changes in the protein structure (Ludikhuyze et al., 2003), therefore, a proper condition for pressurization of individual food commodity needs to be established. Previous researches illustrated that the ability of pressure to inactivate enzymes depended on many factors such as the nature of enzymes and their substrates, properties of the food matrix (pH and moisture content), type of test matrix (whole fruit, fruit piece, puree or juice), the levels of pressure, temperature and holding time applied (Rastogi et al., 2007). Generally, enzymes associated with color degradation are polyphenol-oxidase (PPO) and peroxidase (POD). Earlier, Phunchaisri and Apichartsrangkoon (2005) found that pressure 600 MPa at 60°C for 20 min caused extensive inactivation of PPO and POD in fresh lychee by 90 and 50% respectively but in processed syrup, the effect was less pronounced due to baroprotection by the syrup. Likewise, Chaikham and Apichartsrangkoon (2012a) reported that PPO was completely inactivated in pasteurized longan juices at 90°C for 2 min, whereas pressurized juices at 300 and 500 MPa for 30 min at 25°C, the activity of this enzyme remained 135% and 95% respectively. Previously, Guerrero-Beltrán and Barbosa-Cánovas (2004) and Polydera et al. (2005) observed that residual enzymatic activity in pressurized purée was the major cause of browning development during storage. To evaluate the impact of high pressure on the quality of longan juice, this study aimed to apply pressure 500 MPa at 25°C for 30 min to process longan juice after which its physicochemical properties particularly enzyme activities were determined during storage at 4°C for 4 weeks in comparison with those of pasteurized longan juice.

Materials and Methods

Sample preparation

Fresh Longans (*Dimocarpus longan*Lour.) collected from an orchard in Chiang Mai, Thailand after peeling and stone removal were chopped and blended with drinking water 1:1 (w/w) and centrifuged at a speed 1,000×*g* to separate the juice from the pulp. For standardization, the fresh longan juice was adjusted to 15% total soluble solids (°Brix) with sucrose. Afterwards, 100 mL of juice was then packed in a laminated bag (nylon plus polyethylene) and subjected to pressure 500 MPa at 25°C for 30 min (Chaikham and Apichartsrangkoon, 2012a, b). The high pressure vessel was a 'Food Lab' model 900 (Stansted Fluid Power, Essex, UK). The rate of pressure increase was about 330 MPa/min. The pressure transmitting medium was a mixture of castor oil and 98% ethanol (Chemical & Lab Supplies, Bangkok, Thailand) in 20:80 (v/v), respectively. For pasteurization, 250 mL longan juice was packed in a retort pouch and heated in boiling water until the inside core of the pouch reached 90°C for 2 min (Chaikham and Apichartsrangkoon, 2012a, b). Both processed juices were then stored at 4°C and analyzed continuously once a week for 4 weeks.

Color measurements

A colorimeter, model Color Quest XE (Hunter Lab, Reston, VA) was used to determine the color, L, a^* and b^* parameters, of processed longan juices. Browning Index (BI) was calculated from the following equations (Ferrari *et al*., 2010).

$$BI = [100(x - 0.31)]/0.172, \text{ where } x = (a^* + 1.75L)/(5.645L + a^* - 3.012b^*)$$

Determination of enzyme activities

Crude enzyme extraction

The enzymes were extracted following the procedures described by Toralles et al. (2005) and Phunchaisri and Apichartsrangkoon (2005) with some modifications. Accordingly, 20 mL of longan juice was extracted by adding 50 mL of 0.05 M (pH 6.2) sodium hydrogen phosphate buffer (Ajax, Sydney, Australia) combined with 0.1 M sodium chloride (Ajax). All solutions were cooled to 4°C, stirred at 200×*g* for 30 min and then centrifuged at 4,500×*g* at 4°C for 30 min.

Activity of polyphenol-oxidase

For the purpose, 0.1 mL supernatant of extracted crude enzyme was added to a mixture of 2 mL 0.2 M (pH 7) potassium phosphate (Ajax) plus 0.4 mL of 0.5 M pyrocatechol (Fluka, Buchs, Switzerland). Afterwards, the mixed solution was measured for absorbance by a spectrophotometer (Perkin Elmer UV WINLAB, Perkin Elmer, Waltham, MA) at a λ_{max} 420 nm for 5 min. One unit of PPO activity was defined as an increase of 0.1 unit of absorbance per minute (Unit/mL). Percentage of residual PPO activities was also calculated based on the PPO activity of untreated sample (100%) (Chaikham and Apichartsrangkoon, 2012a).

Activity of peroxidase

In this context, 0.1 mL aliquot of crude enzyme extract was added to a mixture of 2.15 mL of 0.01 M (pH 6) sodium acetate buffer (Ajax) and 5 mM guaiacol (Sigma-Aldrich, Munich, Germany), plus 0.25 mL of 1 mM hydrogen peroxide (Merck,

Darmstadt, Germany). The mixed solution was then measured for an absorbance using a Perkin Elmer UV WINLAB spectrophotometer at a λ_{max}470 nm for 5 min. One unit of POD activity was defined as an increase of 0.1 unit of absorbance per min (Unit/mL). Percentage of residual POD activities was also calculated based on the POD activity of untreated sample (100%) (Apichartsrangkoon et al., 2013).

Determination of ascorbic acid

Ascorbic acid was determined following the procedure described by Rodriguez-Comesaña et al. (2002) with slight modifications. To perform the experiment, 2 mL of longan juice was mixed with 8 mL of diluted sulfuric acid (pH 2.2) (Merck), stirred for 30 min and then centrifuged at 2,000×*g* by maintaining temperature 4°C for 15 min. The supernatant was filtered through a 0.20-µm nylon membrane (Vertical, Bangkok, Thailand) and the filtrate was used for High Performance Liquid Chromatography (HPLC) assay. The HPLC system (Shimadzu LC-10AD, Shimadzu, Kyoto, Japan) consisted of a low-pressure pump and a photodiode array detector (SPD-M20A, Shimadzu) adjusted for a λ_{max} 250 nm. Chromatographic separation was performed with C_{18} column (YMC-Pack ODS-AM, 5 µm, 4.6 mm ID×250 mm; YMC, Kyoto, Japan). The mobile phase of isocratic elution was 0.1 M acetic acid (Merck) in deionized water (HPLC grade; RCI Lab-Scan, Bangkok, Thailand) with flow rate of 1.5 mL/min at 30°C. Further, 20 µL sample was injected into the column. The peak area of each component was determined and converted to concentration with a standard calibration curve. The chromatograms of ascorbic acid in processed longan juices stored for week 0, 2 and 4 are shown in Figure 1.

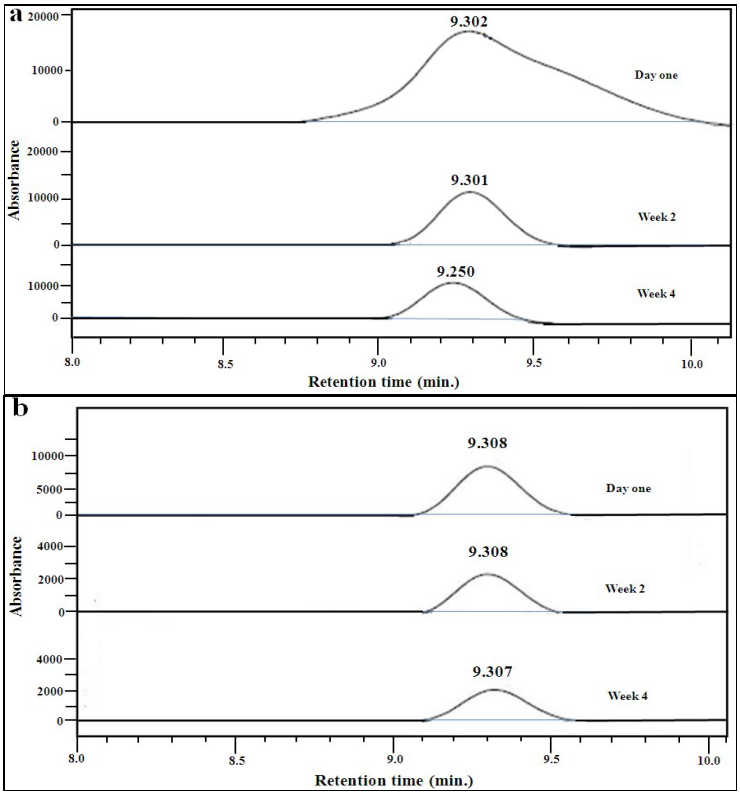

Figure 1. Chromatograms of ascorbic acid in processed longan juices storing at initial states, week 2 and week 4; (a) pressurized juice and (b) pasteurized juice.

Determination of gallic and ellagic acids

Gallic and ellagic acids were estimated through HPLC following the procedure of Rangkadilok et al. (2005). In this reference, 5 mL of longan juice was mixed with 5 mL of 100% methanol (RCI Lab-Scan) and stirred for 30 min, then centrifuged at 2,000×*g* for 15 min. The supernatant was filtered through a 0.20-µm nylon filter (Vertical) and the filtrate was used for HPLC assay. The mobile phase for HPLC analysis was a mixture of 0.4% formic acid (solvent A) (Merck) and 100% methanol (solvent B) (RCI Lab-Scan) with a flow rate of 1 mL/min. The gradient system of the mobile phase commenced from 0 (100% A) to 4 min (95% A:5% B), 10 min (70% A:30% B), 16 min (66% A:34% B), 22 min (45% A:55% B), 28 min (55% A:45% B) and 34 min (100% A), and maintained at this state to 40 min. The temperature of the column was adjusted to 25°C and UV detection was at a λ_{max} 270 nm with an injection volume of 20 µL. Peak areas were determined and converted to concentration with their standard calibration curves. The chromatograms of gallic and ellagic acids in the pressurized longan juice stored from week 0 to week 4 are shown in Figure 2.

Determination of total phenolic compounds

Total phenols were determined by Folin-Ciocalteu reagent method (Zainol et al., 2003). Accordingly, 2 mL of longan juice was stirred with 8 mL of 100% cooled ethanol (Chemical & Lab Supplies) for 15 min, and centrifuged at 2,000×*g* for 15 min. Later, 0.5 mL of supernatant was added to 2.5 ml of 10% Folin-Ciocalteu reagent (Sigma-Aldrich) and allowed to react for 5 min. Subsequently, 2 mL of saturated sodium carbonate solution (Ajax) was added to the mixture and held for 2 h at room temperature. The apparent blue complex was measured at a λ_{max} 765 nm by a Perkin Elmer UV WINLAB spectrophotometer. Total phenols were expressed as milligram gallic acid equivalent per 100 mL sample (mg GAE/100 mL).

Determination of antioxidant capacity

The 2,2-Diphenyl-1-picrylthydrazyl (DPPH) radical-scavenging activity was assessed following the modified protocol of Tangkanakul et al. (2006). Purposely, 2 mL of longan juice was mixed with 8 mL of 100% methanol (RCI Lab-Scan) for 15 min and centrifuged at 2,000×*g* for 15 min. Afterwards, 1.6 mL supernatant was added to 0.4 mL of 1.5 µM DPPH radical (Fluka, Buchs SG., Switzerland) methanol solution. The mixture was thoroughly shaken and allowed to stand for 30 min at room temperature. The absorbance of the solution was measured at a λ_{max} 517 nm using spectrophotometer (Perkin Elmer UV WINLAB). The percentage inhibition of DPPH radicals was calculated by equation below:

$$\%\text{DPPH radical scavenging activity} = [1 - (Abs_{samples}/Abs_{control})] \times 100$$

where Abs is the absorbance.

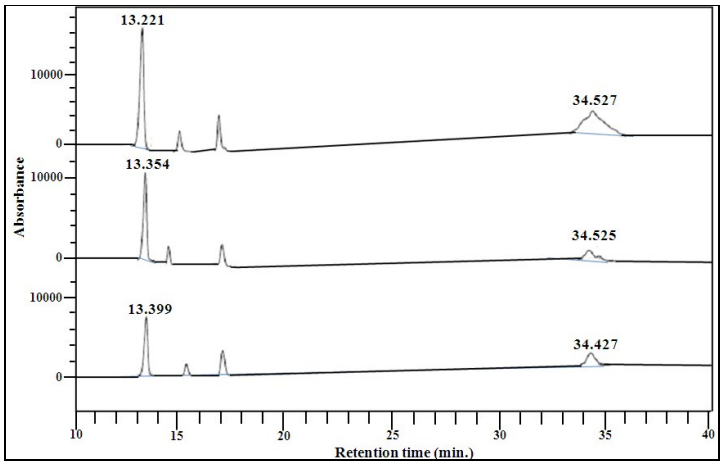

Figure 2. Chromatograms of gallic acid (~ 13.3 min) and ellagic acid (~ 34.5 min) in pressurized longan juice storing at initial states, week 2 and week 4.

Microbiological assessments

The assessments of standard plate counts, yeasts and molds in processed longan juice samples were carried out following the BAM method of US Food and Drug Administration (2001).

Data analysis

All data were the means of triplicate determinations with individual duplication (n=6). The analysis of variance (ANOVA) and linear regression to correlate various parameters with storage time were performed using SPSS version 15.0 (SPSS Inc., Chicago, USA). The determination of significant differences among treatment means was done by Duncan's multiple range tests ($P<0.05$).

Results and Discussion

Color stabilities

Color parameters of pressurized and pasteurized longan juices during storage are shown in Table 1. L parameter of pressurized longan juice displayed significantly higher ($P<0.05$) than pasteurized juice but was equivalent to untreated sample. These indicated that pressure could efficiently preserve the lightness of this fruit juice. When considering the stability of L parameters on storage, both processed juices were significantly decreased ($P<0.05$) their lightness with the increase of storage time. The declining rate of pressurized juice was greater than that of pasteurized juice indicated by the slopes of their trend-lines, which could be due to the impact of enzymatic degradation by residual PPO and POD (Table 1). A study by Barba et al. (2012) who pressurized blueberry juice at 600 MPa at 42°C for 5 min stored at 4°C for 56 days. They found L parameters significantly decreasing with an increase of storage time. Keenan et al. (2010) and Daoudi et al. (2002) also found similar results with pressurized fruit smoothies at 450 MPa for 1-5 min and pressurized white grape juice at 400 MPa at 40°C for 10 min, respectively.

The $-a^*$ for greenness of pressurized longan juice showed the same trends as untreated sample, whereas pasteurized juice had shifted to a^* in the red zone. Upon storage both a^* parameters significantly increased ($P<0.05$) with the increase of storage time. In addition, the $-a^*$ of pressurized juice was shifted to a^* from week three onward. Nevertheless, both a^* parameters of processed juices showed similar rate of reduction on storage indicated by the slopes of their trend-lines. A report by Keenan et al. (2012) revealed that pressurized fruit smoothies at 600 MPa for 10 min, its a^* parameters significantly increased on storage at 4°C for 10 h.

Similar with a^* parameter, b^* for yellowish of pressurized longan juice was much lower than that of pasteurized juice, though it was significantly different ($P<0.05$) from the untreated sample. Additionally, the b^* parameters of pressurized juice were relatively stable upon storage, while those of pasteurized juice significantly increased ($P<0.05$) or higher increasing rate indicated by the slopes of their trend-lines. A study of Keenan et al. (2011) showed that pressurized apple puree at 500 MPa for 1.5 min had b^* parameter increasing on storage at 4°C for 30 days.

Browning is a major factor reflecting the quality of fruit juice during storage. The intensity of the total color changes (L, a^* and b^*) could be estimated by Browning Index (BI). Table 1 illustrates that pressurized and untreated juices displayed much lower BI than that pasteurized juice. In addition, the BI of both processed juices significantly increased with the increase of storage time for which pasteurized juice showed higher rate of changing BI than another juice (slopes of the trend-lines). High BI of pasteurized juice might be a reflection of high b^* and low L parameters, which could primarily be due to an influence of Maillard condensation (Ibarz et al., 2000). Other researchers, for instance, Polydera et al. (2005) pasteurized (80°C/1 min) and pressurized (600 MPa/40°C/4 min) orange juices stored at 0-30°C for 60 days. They observed that the rate of browning in both processed juices gradually increased with the increase of storage time. Similar results were obtained by Cao et al. (2012) who pressurized strawberry juice at 600 MPa for 4 min and storing at 4°C for 6 months.

To sum up, although enzymatic browning in pressurized longan juice was the prime cause of lower L parameter during storage, its magnitude was apparently lesser than browning caused by Maillard reaction in pasteurized juices reflected by b^* and BI parameters.

Table 1. Storage stability of color parameters and enzyme activities of processed longan juices.

Parameters of	Longan juices	Storage periods (weeks)					Slopes
		Initial states	1	2	3	4	
*L** (lightness)	Untreated	46.52 ± 0.31^{A}					
	Pressurized	46.76 ± 0.15^{Aa}	45.96 ± 0.11^{b}	45.37 ± 0.01^{c}	44.72 ± 0.06^{d}	43.61 ± 0.05^{e}	-0.75 (R^2=0.99)
	Pasteurized	41.09 ± 0.03^{Ba}	40.86 ± 0.18^{a}	40.62 ± 0.03^{b}	40.52 ± 0.08^{c}	40.68 ± 0.05^{c}	-0.12 (R^2=0.66)
*-a** (greenness)	Untreated	-0.26 ± 0.04^{B}					
	Pressurized	-1.14 ± 0.33^{Ce}	-0.90 ± 0.01^{d}	-0.61 ± 0.03^{c}	0.14 ± 0.01^{b}	0.48 ± 0.06^{a}	0.43 (R^2=0.96)
	Pasteurized	0.80 ± 0.05^{Ad}	1.04 ± 0.17^{c}	2.14 ± 0.02^{b}	2.09 ± 0.02^{b}	2.34 ± 0.02^{a}	0.41 (R^2=0.85)
*b** (yellowish)	Untreated	3.32 ± 0.13^{C}					
	Pressurized	3.84 ± 0.03^{Bb}	3.96 ± 0.01^{a}	3.93 ± 0.02^{a}	3.99 ± 0.02^{a}	3.97 ± 0.01^{a}	0.03 (R^2=0.61)
	Pasteurized	6.89 ± 0.05^{Ac}	7.50 ± 0.12^{b}	7.58 ± 0.01^{b}	7.61 ± 0.02^{b}	7.99 ± 0.02^{a}	0.23 (R^2=0.85)
Browning Index, BI	Untreated	5.11 ± 0.23^{C}					
	Pressurized	6.48 ± 0.58^{Be}	7.24 ± 0.04^{d}	7.76 ± 0.08^{c}	9.24 ± 0.03^{b}	10.02 ± 0.11^{a}	0.91 (R^2=0.98)
	Pasteurized	19.18 ± 0.16^{Ae}	21.47 ± 0.43^{d}	23.90 ± 0.02^{b}	23.03 ± 0.05^{c}	25.38 ± 0.08^{a}	1.40 (R^2=0.86)
PPO activity (Unit/mL)	Untreated	300.62 ± 5.48^{A}					
	Pressurized	281.50 ± 3.10^{Ba}	235.62 ± 2.53^{b}	162.66 ± 3.18^{c}	135.37 ± 1.96^{d}	88.56 ± 2.43^{e}	-48.6 (R^2=0.98)
Residual PPO (%)	Untreated	100.00^{A}					
	Pressurized	93.64 ± 4.52^{Ba}	80.38 ± 2.81^{b}	55.49 ± 5.02^{c}	46.18 ± 2.55^{d}	30.21 ± 3.24^{e}	-16.11 (R^2=0.98)
POD activity (Unit/mL)	Untreated	89.56 ± 4.85^{A}					
	Pressurized	44.78 ± 1.35^{Ba}	40.96 ± 1.62^{b}	20.75 ± 2.03^{c}	8.53 ± 0.25^{d}	5.70 ± 0.19^{e}	-11.1 (R^2=0.94)
Residual POD (%)	Untreated	100.00^{A}					
	Pressurized	49.53 ± 3.65^{Ba}	45.31 ± 2.44^{b}	22.95 ± 4.50^{c}	9.43 ± 0.98^{d}	6.31 ± 0.53^{e}	-12.23 (R^2=0.94)

Means in the same column or row followed by the same capital or lowercase letters respectively are not significantly different (P>0.05). Means were the determination of six replications.

Slopes are the slopes of linear trend-lines from the correlation of parameters *VS* storage periods.

Stability of PPO and POD activities

Since both PPO and POD activities in pasteurized juice were completely inactivated, only residual enzymes in pressurized juice shown in Table 1. Table 1 illustrates that activities of PPO and POD remained in pressurized juice up to 94% and 50% respectively, suggesting that PPO was more resistant to pressure than POD. In addition, these enzyme activities significantly decreased (P<0.05) with the increase of storage time. However, higher slope of a trend-line of PPO activities than that of POD activities could be observed, suggesting that PPO showed greater rate of degradation upon storage than POD. It was likely that the increase of Cu^{2+} in PPO matrix was more rapid than the loss of heme group from POD protein under acidic condition (Gray and Montgomery, 2003). Previous studies revealed that residual PPO activity remained unchanging during storage and caused further color degradation (Krebbers et al., 2002). In this study at week four of storage period, PPO and POD activities reduced by 64% and 44 %, respectively. Generally, PPO and POD could oxidize phenolic compounds and subsequently formed brown melanins or brown pigments in fruit and vegetable products (Artés et al., 2007). Effects of pressure on PPO and POD were also found by Keenan et al. (2012) who pressurized fruit smoothies with 450 MPa at 20°C for 5 min and noticed that PPO activity was reduced by 35%, while PPO in pasteurized samples (70°C/>10 min) was completely inactivated. Phunchaisri and Apichartsrangkoon (2005) pressurized fresh lychee at 600 MPa at 60°C for 20 min and found that PPO and POD were inactivated by 50% and 90%, respectively. Apichartsrangkoon et al. (2013) pressurized green-chili paste at 400-500 MPa at 25°C for 20-40 min and observed that the activities of residual PPO and POD retained around 90-110% and 80-90%, respectively.

Table 2. Storage stability of bioactive components of processed longan juices.

Parameters of	Longan juice	Storage periods (weeks)					Slopes
		Initial states	1	2	3	4	
Ascorbic acid (mg/100 mL)	Untreated	6.28±0.59^{A} (100.00%)					
	Pressurized	5.83±1.12^{Aa} (92.83%)	4.50±0.30^{a} (71.66%)	3.28±0.16^{b} (52.23%)	2.71±0.54^{bc} (43.15%)	2.88±0.03^{c} (45.86%)	-0.77 (R^2=0.86)
	Pasteurized	2.78±0.48^{Ba} (44.27%)	0.99±0.08^{b} (15.76%)	0.52±0.01^{d} (8.28%)	0.61±0.20^{c} (9.71%)	0.48±0.03^{d} (7.64%)	-0.50 (R^2=0.65)
Gallic acid (mg/100 mL)	Untreated	4.70±1.24^{A} (100.00%)					
	Pressurized	4.84±0.60^{Aa} (102.98%)	2.96±0.44^{b} (62.98%)	2.42±0.90^{bc} (51.49%)	1.91±0.20^{c} (40.64%)	1.51±0.55^{c} (32.13%)	-0.77 (R^2=0.87)
	Pasteurized	4.47±0.65^{Aa} (95.11%)	3.46±0.20^{b} (73.62%)	2.55±0.06^{c} (54.26%)	1.81±0.58^{d} (38.51%)	1.63±0.25^{d} (34.68%)	-0.73 (R^2=0.95)
Ellagic acid (mg/100 mL)	Untreated	1.92±0.57^{A} (100.00%)					
	Pressurized	1.89±0.25^{Aa} (98.44%)	1.78±0.06^{a} (92.71%)	1.51±0.18^{b} (78.65%)	0.77±0.07^{c} (40.10%)	0.62±0.08^{c} (32.29%)	-0.36 (R^2=0.92)
	Pasteurized	1.73±0.31^{Aa} (90.10%)	1.63±0.25^{ab} (84.90%)	1.35±0.09^{b} (70.31%)	0.59±0.12^{c} (30.73%)	0.40±0.06^{d} (20.83%)	-0.37 (R^2=0.93)
Total phenols (mg/100 mL)	Untreated	275.96±5.73^{A} (100.00%)					
	Pressurized	278.30±11.56^{Aa} (100.85%)	258.96±3.06^{b} (93.84%)	236.69±1.63^{c} (85.77%)	240.10±17.58^{c} (87.01%)	201.92±6.67^{d} (73.17%)	-17.16 (R^2=0.91)
	Pasteurized	185.79±6.88^{Ba} (67.32%)	176.38±1.59^{b} (63.92%)	157.08±1.47^{c} (56.92%)	137.35±3.35^{d} (49.77%)	122.24±0.35^{e} (44.30%)	-16.61 (R^2=0.99)
Antioxidant capacity (% DPPH radical inhibition)	Untreated	13.56±0.42^{A} (100.00%)					
	Pressurized	10.71±0.13^{Ba} (78.98%)	8.21±0.31^{b} (60.55%)	8.07±0.30^{b} (59.51%)	5.56±0.26^{c} (41.00%)	5.65±0.32^{c} (41.67%)	-1.28 (R^2=0.90)
	Pasteurized	8.88±0.21^{Ca} (65.49%)	7.92±0.16^{b} (58.41%)	6.88±0.58^{c} (50.74%)	5.49±0.12^{d} (40.49%)	4.14±0.25^{e} (30.53%)	-1.19 (R^2=0.99)

Means in the same column or row followed by the same capital or lowercase letters respectively are not significantly different (P>0.05). Means were the determination of six replications. Slopes are the slopes of linear trend-lines from the correlation of parameters *VS* storage periods.

Stability of bioactive components

Ascorbic acid is believed to be a highly reducing agent however; it is also susceptible to oxidation by various factors such as light, oxygen, heat, peroxide and enzymes. Table 2 shows that ascorbic acid in both processed longan juices significantly decreased (P<0.05) with the increase of storage time. At the fourth week, ascorbic acid in pressurized juice reduced from 92.8% to 45.8% accounting for around 50% reduction, while those in pasteurized juice reduced from 44.3% to 7.6% accounting for 83% reduction. Although the reducing rate of ascorbic acid in pressurized juice was slightly greater than that in pasteurized juice shown by the slopes of their trend-lines, pressurized juice still retained higher amount of this vitamin than thermal treated juice throughout the storage period. Ascorbic acid might degrade to dehydroascorbic acid and further irreversibly converted into 2,3-diketogulonic acid (Landl et al., 2010). Apichartsrangkoon et al. (2012) also found similar results with pressurized pennywort juice at 400 MPa at 30°C for 20 min stored for 4 months. Landl et al. (2010) noticed that ascorbic acid in pasteurized (75°C/5 min) and pressurized (400 or 600 MPa/20°C/5 min) acidified apple purées significantly decreased after storing at 5°C for 3 weeks. Similar results were obtained by Vega-Galvez et al. (2011) who pressurized Aloe vera gel (*Aloe barbadensis* Miller) at 300-500 MPa at 20°C for 3 min stored at 4°C for 35 days. Cao et al. (2012) also found a reduction of ascorbic acid in pressurized (600 MPa/4 min) clear and cloudy strawberry juices by 48.91% and 39.61% respectively after storing for six months at 4°C.

Table 3. Changes of pH and microbiological qualities in processed longan juices.

Qualities	Processed longan juice	Storage periods (weeks)					Slopes
		Initial states	1	2	3	4	
pH	Untreated	6.89 ± 0.02^{A}					
	Pressurized	6.92 ± 0.01^{Aa}	6.96 ± 0.04^{a}	6.78 ± 0.02^{b}	6.75 ± 0.01^{b}	6.75 ± 0.02^{b}	-0.06 (R^2=0.75)
	Pasteurized	6.68 ± 0.03^{Ba}	6.66 ± 0.02^{a}	6.47 ± 0.04^{b}	6.45 ± 0.04^{bc}	6.41 ± 0.01^{c}	-0.08 (R^2=0.88)
TPC (CFU/mL)	Untreated	$8.5\pm1.4\times10^{3}$					
	Pressurized	Not found	Not found	Not found	Not found	Not found	-
	Pasteurized	Not found	Not found	Not found	$1.3\pm0.6\times10$	$6.5\pm1.3\times10^{2}$	-
Yeasts & Moulds (CFU/mL)	Untreated	$6.5\pm0.8\times10$					
	Pressurized	Not found	Not found	Not found	Not found	Not found	-
	Pasteurized	Not found	Not found	Not found	Not found	Not found	-

Means in the same column or row followed by the same capital or lowercase letters respectively are not significantly different (P>0.05). Means were the determination of six replications. Slopes are the slopes of linear trend-lines of parameters *VS* storage periods. (TPC=total plate count).

Gallic acid is a bioactive phenol that performs antioxidative, anticarcinogenic, antifungal and antiviral properties shown *in vitro* experiment (Rangkadilok et al., 2005). Ellagic acid has antiproliferative and antioxidant properties due to its ability to directly inhibit the DNA binding of certain carcinogens including nitrosamines and polycyclic aromatic hydrocarbons (Aiyer et al., 2008; Mishra and Vinayak, 2011). Table 2 elucidates that storage time directly affected the quality of these bioactive compounds and antioxidant activity in pressurized juice, since the concentration of gallic acid, ellagic acid, total phenols and antioxidant activity (DPPH assay) significantly decreased (P<0.05) after storing for four weeks or reduced by 71%, 66%, 28% and 37%, respectively. The bioactive components in pasteurized juice were also degraded substantially by 60%, 69%, 23% and 35% for gallic acid, ellagic acid, total phenols and antioxidant activity (DPPH assay), respectively. Additionally, both processed juices displayed parallel rate of reduction upon storage indicated by the slopes of their trend-lines. However, the reason of these reductions might be due partially to the residual PPO and POD involving in the oxidative degradation of phenols (Tiwari et al., 2009). The studies by Keenan et al. (2010) revealed that ascorbic acid, total phenols and antioxidant activity (DPPH and FRAP assays) in pressurized (450 MPa/25°C/1-5 min) and pasteurized (70°C/10 min) fruit smoothies significantly diminished after storing at 4°C for 30 days. Similar results were obtained by Vega-Galvez et al. (2011, 2012) who pressurized Aloe vera gel at 300-500 MPa at 20°C for 3 min and kept at 4°C for 35 or 60 days.

pH and general microbiological quality

Table 3 shows that pH of both processed juices significantly decreased (P<0.05) after the second week of storage. The pH of both juices showed similar reducing rate indicated by the slopes of their trend-lines. A study by Jacobo-Velázquez and Hernández-Brenes (2010) showed that pH of pressurized (600 MPa/23°C/3 min) avocado paste declined from 6.5 to 5.8 during storage at 4°C for 45 days. The change of product pH might be associated with the increase of viable microbes, particularly, pasteurized samples after storing for three weeks. Nevertheless, these microbiological qualities still complied with the general standard for fruit juices and nectars (CODEX STAN 247-2005) (FAO/WHO Food Standards, 2005). In addition, the number of yeasts and moulds in all products were satisfactorily eliminated and had not been detected throughout the storage period. Similar results were obtained by Lavinas et al. (2008) who pressurized cashew apple juice at 400 MPa for 3 min and kept at 4°C for 8 weeks, while Varela-Santos et al. (2012) pressurized pomegranate juice at 450-550 MPa for 30-150 s, and stored at 4°C for 35 days.

Conclusions

The color parameters indicated that although enzymatic degradation in pressurized longan juice during storage was the primary cause of lower *L* parameter however; its magnitude was apparently lesser than browning by Maillard reaction in pasteurized juice as reflected by *b** and BI parameters. Results of residual enzymes showed that during storage, PPO showed greater rate of degradation and was more resistant to pressure than

POD. During storage, the bioactive components like ascorbic acid in pressurized juice showed greater reducing rate than that of pasteurized juice. Conclusively, residual ascorbic acid in pressurized juice was still higher than that thermal treated juice in the entire storage study. Likewise, other bioactive components in pressurized juice were also gradually degraded by 71%, 66%, 28% and 37%, while in pasteurized products substantially reduced by 60%, 69%, 23% and 35% for gallic acid, ellagic acid, total phenols and antioxidant activity (DPPH assay), respectively.

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