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Comparison of high hydrostatic pressure and thermal processing on physicochemical and antioxidant properties of Maoberry (*Antidesma thwaitesianum* Müell. Arg.) juice

Chaikham, P.

Division of Food Science and Technology, Faculty of Science and Technology, Phranakhon Si Ayutthaya Rajabhat University, Phranakhon Si Ayutthaya 13000, Thailand

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Abstract

The alteration of microbiological, physicochemical and antioxidant properties of Maoberry (*Antidesma thwaitesianum* Müell. Arg.) juices after pressurization (400 or 600 MPa/25°C/10 min) and pasteurization (90°C/1 min), and their storage stability at 4°C for 4 weeks were determined. It was found that pressurization and pasteurization were satisfactory eliminated the general microbes in Maoberry juice, also both processed juices exhibited a better microbiological stability throughout the refrigerated storage period. A significant change of pH in low pressure treated juice (400 MPa) during storage could be due primarily to the increase of general microbes. Both processing had no effect on the dynamic viscosity of Maoberry juice, but the reductions of the viscosity in all processed juices were observed after storage for 4 weeks. Considering the color parameters, the reddish (a^*) of pressurized juices were similar to the fresh juice, suggesting that high pressure could preserve the anthocyanins (red-purple pigments) in the juice better than thermal treatment. The polyphenol oxidase (PPO) activity was completely destroyed after pasteurization, whilst this enzyme still remained in pressurized juices at 400 and 600 MPa up to 79% and 53% respectively. Upon the storage, all pressurized juices showed a gradually decrease in the residual PPO activity when the storage was extended. This study also displayed that the retentions of ascorbic acid, phenols and anthocyanins as well as antioxidant capacity (DHHP and FRAP assays) in pressurized juices were significantly higher than that the pasteurized juice during the entire storage.

Introduction

High pressure processing has been demonstrated as a non-thermal technique to eliminate pathogenic and spoilage bacteria as well as inhibit several enzymes bring about to enhance safety and shelf-life extension for foods (Wang *et al*., 2012). Furthermore, this technique can be also preserved color, flavor and nutritional values, including anti-oxidative components and properties of fruit and vegetable products (Apichartsrangkoon *et al*., 2009, 2012; Chaikham and Apichartsrangkoon, 2012a, b). Barba *et al.* (2013) exhibited that pressurization at 200, 400 or 600 MPa for 5, 9 or 15 min resulted in more than 92% ascorbic acid retention in blueberry juice, while the anthocyanins were similar to the fresh juice. Additionally, Apichartsrangkoon *et al.* (2009) observed that high pressure processing could be preserved some volatile compounds including acyclic alcohols, aldehydes and terpenoids in pennywort juice better than thermal processing.

Maoberry or Mao (*Antidesma thwaitesianum* Müell. Arg.) juice, commercially available for human health benefits, have become more popular in Thailand. Its fruit is classified in the family *Stilaginaceae*, genus *Antidesma* and commonly grown in the warm climate of tropical Asia, Africa and Australia (Puangpronpitag *et al.*, 2011). Maoberry fruits contain high amounts of several bioactive components such as ascorbic acid, polyphenolic compounds, anthocyanins and flavonoids (Butkhup and Samappito, 2011a), and have been recognized to possess a numeral of health-promoting effects including antioxidant, anti-carcinogenic, anti-apoptotic and anti-inflammatory activities (Puangpronpitag *et al.*, 2011). Furthermore, Butkhup and Samappito (2011b) found that Mao seeds and skin-pulp residues contained high amounts of phenolic acids and high antioxidant activity, also the methanolic extracts showed inhibitory activities against some pathogenic and spoilage bacteria.

The polyphenols and anthocyanins in fruit juices can be degraded by some oxidase enzymes, i.e. polyphenol oxidase (PPO) and peroxidase (POD), during non-thermal processing and storage (Tiwari *et al.*, 2009). Since PPO is high pressure resistance enzyme, thus it is interesting to study the effects of

*Corresponding author.
Email: *pittaya.chaikham@gmail.com*

high pressure processing and storage time on the alteration of PPO activity. PPO (E.C. 1.14.18.1) is a copper-containing enzyme that catalyzes the o-hydroxylation of monophenols to o-diphenols and the consequent oxidation of o-diphenols to o-quinones (Jang and Moon, 2011). Ferrari *et al.* (2010) revealed that enzyme PPO can be interrelated to the loss of anthocyanins of fruit products after pressurization and storage, since the residual activity of the enzymes could bring about the debasement of the anthocyanins.

The objectives of this work were carried out to determine the effects of high pressure (400 or 600 MPa/25°C/10 min) and thermal (90°C/1 min) processing on general microbiological, physicochemical and antioxidant properties of Maoberry juice and to evaluate the stability of all processed juices during refrigerated storage at 4°C for 4 weeks.

Materials and Methods

Maoberry juice preparation

Maoberry fruits were harvested from Phupan Vallay, Sakon Nakhon, Thailand, during August-September of the 2012. The fruits were washed and extracted with distill water at a ratio of 1:1 (w/w). Subsequently, the solids were separated by centrifuging at 1,200 rpm through a sterile filter cloth. The filtrate was adjusted to the total soluble solids of 10°Brix with sucrose for standardization. One hundred milliliters of extracted juice were packed in a laminated bag (nylon plus polyethylene; SiamPack, Bangkok, Thailand) and subjected to pressure 400 or 600 MPa at 25°C for 10 min. The high pressure vessel was a 'Food Lab' model 900 high pressure rig (Stansted Fluid Power; Stansted, Essex, UK). The rate of pressure increase was about 300 MPa/min. During this high pressure treatment an adiabatic increase in temperature was occurred. At ambient temperature (~25°C), the monitored cell temperature increased by about 8°C up to 600 MPa but decreased to the set equilibrium value in less than 2.5 min. The pressure transmitting medium was a mixture of castor oil (Chemical & Lab Supplies, Bangkok, Thailand) and 98% ethanol (Chemical & Lab Supplies) at a ratio of 20:80 (v/v) (Chaikham and Apichartsrangkoon, 2012a, b). For pasteurization, 250 ml juice was packed in a retort pouch (SiamPack), heated in boiling water until the inside core of the package reached 90±5°C for 1 min. Subsequently, all processed juices were stored at 4°C and continuously analyzed once a week for 4 weeks.

General microbiological assessments

The assessments of total plate count and yeasts-mounds in fresh, pressurized and pasteurized Maoberry juices were investigated followed the Bacteriological Analytical Manual (BAM) as described by the US Food and Drug Administration (2001).

Determination of dynamic viscosity

A control stress AR 2000 rheometer (TA Instruments, Inc., New Castle, DE) was used with commercial computer software (Rheology Advantage Analysis software Version 4.1). A concentric cylinder geometry (stator inner radius 15 mm, rotor outer radius 14 mm, cylinder immersed height 42 mm, gap 5920 μm) was used to determine the dynamic viscosity of fresh and processed Maoberry juices. A 19.6-ml aliquot of the juice was poured into the stationary cup and allowed to equilibrate to 25±2°C, which controlled by a circulating water system. Viscosity was calculated from the average of five points of the flow curves obtained in the shear rate range between 1 and 10 s^{-1}.

Color parameter measurements

A colorimeter (Minolta Chroma Meter CR-300, Kyoto, Japan) was used to measure the color parameters of fresh and processed samples. Analytical data were expressed as L^* (lightness), a^* (greenness/redness) and b^* (yellowness/blueness) parameters. In addition, chroma value (C^*) and total different colors (ΔE) were calculated using the following equations (Cao *et al.*, 2012);

$$\text{Chroma value } (C^*) = [(a^*)^2 + (b^*)^2]^{1/2}$$
$$\text{Total different colors } (\Delta E) = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}.$$

pH measurement

The pH of fresh and processed Maoberry juices was measured using a Sartorius PB-20 pH meter (Gottingen, Germany). The pH meter was calibrated using pH 4 and pH 10 standard stock solutions (Fisher Scientific Co., IL) prior to analysis.

Determination of polyphenol oxidase activity

PPO activity was carried out following the procedures described by Chaikham and Apichartsrangkoon (2012a, b). Twenty milliliters of Maoberry juice were extracted using 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. The mixed solution was stirred at ~120 rpm

for 20 min and centrifuged at 4,000 rpm for 10 min. A 0.1-ml aliquot of supernatant of crude enzyme extract was added to a mixture of 2 ml of 0.2 M (pH 7) potassium phosphate (Ajax) and 0.4 ml of 0.5 M pyrocatechol (Fluka, Buchs, Switzerland) and the absorbance measured using a Perkin Elmer UV WINLAB spectrophotometer (Waltham, MA) at a λ_{max} 420 nm for 5 min. One unit of PPO activity was defined as an increase of 0.001 unit of absorbance per min (Unit/ml crude enzyme extract). Residual polyphenol oxidase activities (%) were calculated based on the PPO activity of fresh juice (100%).

Determination of ascorbic acid

The concentrations of ascorbic acid in fresh and processed Maoberry juices were determined following the method as described by Chaikham and Apicartsrangkoon (2012a,b) with some modifications. Two milliliters of the juice were mixed with 18 ml of diluted sulphuric acid (pH 2.2; Merck, Munich, Germany), stirred at 150 rpm for 20 min and then centrifuged at 4,000 rpm at 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 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 to a λ_{max} 250 nm. Chromatographic separation was performed with a C18 column (YMC-Pack ODS-AM, 5 µm, 4.6 mm ID × 250 mm; YMC, Kyoto Japan). The isocratic system used 0.1 M acetic acid (Merck) in deionized water (RCI Lab-Scan, Bangkok, Thailand) as a mobile phase with a flow rate of 1.5 ml/min at 30°C. A 20-µl filtrate was injected into the column. The peak area of each component was determined and converted to concentration.

Determination of total anthocyanins

Total anthocyanins in fresh and processed juices were analyzed by pH-differential method as described by Giusti and Wrolstad (2001). In brief, 10 ml juice was separately mixed with 20 ml of 0.25 M potassium chloride buffer (pH 1; Ajax) and 20 ml of 0.4 M sodium acetate buffer (pH 4.5; Ajax). The mixture was filtered using a Whatman® filter paper no. 4 and the absorbance of the filtrate was measured at the maximum absorbance wave length and 700 nm using a Perkin Elmer UV WINLAB spectrophotometer. The concentration of total anthocyanins was calculated as follows;

Total anthocyanin (mg/L) = A(MW)(DF)×1,000/εL, when MW is molecular weight of anthocyanin which is 449.2 g/mol for cyanidin-3-glucoside, DF is dilution factor, L is path length (1.0 cm), ε is extinction coefficient for cyanidin-3-glucoside (26,900) and A is absorbance of sample which was calculated as follows;

$$A = (A_{\lambda max} - A_{700})_{pH\ 1} - (A_{\lambda max} - A_{700})_{pH\ 4.5}$$

Determination of total phenols

Total phenols were determined using the Folin-Ciocalteu reagent (Zainol *et al*., 2003). Two milliliters of the sample were stirred with 8 ml of 100% ethanol for 15 min, and centrifuged at 4,000 rpm at room temperature for 10 min. A 0.5-ml aliquot of supernatant was added to 2.5 ml of 10% Folin-Ciocalteu reagent (Sigma-Aldrich, St. Louis, MO) and allowed to react for 5 min. Subsequently, 2 ml of saturated sodium carbonate solution (Ajax) were added to the mixture and held for 2 h at room temperature. The apparent blue complex was determined at a λ_{max} 765 nm. Total phenolic contents were expressed as mg gallic acid equivalent per 100 ml sample (mg GAE/100 ml).

Determination of 2,2-diphenyl-1-picrylhydrazyl radical-scavenging activity

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity was determined following a modified method as described by Chaikham and Apicartsrangkoon (2012a, b). Two milliliters of Maoberry juice was mixed with 8 ml of 100% methanol for 10 min and centrifuged at 4,000 rpm for 15 min. Subsequently, 1.6 ml supernatant was mixed with 0.4 ml of 1.5 µM DPPH radical (Fluka) methanol solution. The mixture was shaken and allowed to stand for 30 min at room temperature. Absorbance of the solution was measured at a λ_{max} 517 nm. A control was prepared using 1.6 ml methanol. Percentage inhibition of DPPH radicals was calculated:

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

Ferric-reducing antioxidant power assay

The ability to reduce ferric ions was measured using a method described by Benzie and Stain (1996) with some modifications. An aliquot (l ml) of the juice was added to 10 ml of deionized water and 3 ml of ferric-reducing antioxidant power (FRAP) reagent (10:1:1 of 300 mM sodium acetate buffer at pH 3.6, 10 mM 2,4,6-tripyridyls-triazine solution and 20 mM $FeCl_3.6H_2O$ solution) and the mixture was incubated in a water bath at 37°C for 30 min. Absorbance was measured at a λ_{max} 593 nm. The antioxidant capacity based on the ability to reduce ferric ions of the extract

was expressed as mmol Fe (II) per mililiter of the sample (mM $FeSO_4$/ml).

Statistical data analysis

All data were the means of six replications with standard deviations (means±SD). Analysis of variance (ANOVA) was carried out using SPSS Version 15.0 (SPSS Inc., Chicago, IL), and determination of significant differences among treatment means was done by Duncan's multiple range tests ($P \leq 0.05$).

Results and Discussion

Microbiological qualities and pH value

As shown in Table 1, the general microbes in fresh Maoberry juice had proliferated rapidly after storage for 1 week. This result demonstrated that this juice could not be kept more than a week at 4°C. Thus the changes of the qualities of fresh Maoberry juice were displayed only the initial state of storage. After thermal and pressure treatments, total plate counts in all processed juices were completely eliminated. Upon the storage, total plate counts in pressurized juice at 400 MPa were detected after storage for 4 weeks, while the other processed batches were not found the growth of these microbes throughout the storage period. In addition, yeasts and moulds were also not detected after processing and storage. Similarly, Timmermans *et al.* (2011) exhibited that total aerobic bacteria and yeasts-moulds in pasteurized (72°C/20 s) and pressurized (600 MPa/17°C/1 min) orange juices were less than 3 and 1 log CFU/g respectively, and kept below the detection limit during 8 weeks of storage at 4°C. In overall, the microbiological qualities of all stored Maoberry juices still complied with the limits of the Thai Community Product Standard for Mao or Maoberry juice (TCPS No. 486/2004; Thai Industrial Standard Institute, 2004). Hence, the results in this study displayed that both pressurized and pasteurized Maoberry juices showed a better microbiological stability.

The pH of thermally processed juice was significantly lower ($P \leq 0.05$) than those fresh and pressurized juices, which might be due to the increase of hydrogen ion concentration by hydrolysis reaction during thermal processing (Queiroz *et al*., 2010). A significant reduction of pH value ($P \leq 0.05$) of pressurized Maoberry juice at 400 MPa was observed after the fourth week of storage, whereas the pHs of other juices were not significantly different ($P > 0.05$) throughout the entire storage (Table 1). The changes of pH of lower pressure treated juice might be associated with the increase of general microbes. Jacobo-Velázquez and Hernández-Brenes (2010) found that pH of pressurized avocado paste (600 MPa/23°C/3 min) apparently declined from 6.4 to 5.8 during storage at 4°C for 45 days.

Dynamic viscosity

Table 1 depicts the processing had no effect on the dynamic viscosity of Maoberry juice ($P > 0.05$). The viscosity of all processed juices apparently decreased ($P \leq 0.05$) by 1.79-5.66%, 5.66-14.29%, 11.54-25.00% and 17.31-28.57%, corresponding to 1, 2, 3 and 4 weeks of storage. Cao *et al.* (2012) pressurized cloudy strawberry juices at 600 MPa for 4 min and stored at 4°C, and illustrated that the viscosity of the juice markedly decreased by 32.52% after storing for 1 month. The reduction of the dynamic viscosity in the juices might be due primarily to the sedimentation of pulps and degradation of pectin by pectin methyl esterase and acid hydrolysis reactions (Cao *et al.*, 2012).

PPO activity

The PPO activity was absolutely inhibited after pasteurization, whilst this enzyme still remained in pressurized juices at 400 and 600 MPa up to 79% and 53% respectively (Table 1). Earlier, our study demonstrated that PPO was completely inactivated in pasteurized longan juice at 90°C for 2 min, but in pressurized batches at 500 MPa and 25°C for 30 min, the residual PPO activities were more than 95% (Chaikham and Apichartsrangkoon, 2012a). Considering the storage period, this enzyme activity significantly decreased ($P \leq 0.05$) with increasing storage time, at week 4 of storage PPO activity declined by 20% and 15% for the juices processed at pressure 400 and 600 MPa, respectively. Keenan *et al.* (2012) reported that PPO enzyme activity in pressurized smoothies (450 or 600 MPa/20°C/5 or 10 min) significantly decreased by approximately 50% after storage at 4°C for 10 h. Likewise, Gerrero-Beltrán *et al.* (2006) illustrated that the PPO activity of pressurized mango puree (552 MPa/25°C/5 min) tremendously reduced by more than 200% during the entire storage at 3°C for 27 days. The reduction of PPO activity might be ascribable to enzyme forming complex with some phenolic substances in the products capacitating loss of nutritional values (Skrede *et al.*, 2000).

Color parameters

Color parameters of fresh and processed juices during storage are presented in Table 2. The L^* parameters (lightness) of pressurized juices displayed significantly lower ($P \leq 0.05$) than fresh and pasteurized juices. No significant difference of

Table 1. Changes of general microbiological qualities and physicochemical properties of fresh and processed Maoberry juices during refrigerated storage at 4°C

Qualities	Maoberry juices	Storage periods (weeks)				
		Initial state	1	2	3	4
Total plate counts (CFU/ml)	Fresh juice	$8.32\pm2.05^{Ab}\times10^{4}$	$6.47\pm1.34\times10^{8Aa}$	-	-	-
	Pasteurized juice	nd^{Ba}	nd^{Ba}	nd^{Aa}	nd^{Ba}	nd^{Ba}
	Pressurized juice at 400 MPa	nd^{Bb}	nd^{Bb}	nd^{Ab}	nd^{Ab}	$2.58\pm0.45^{Aa}\times10^{2}$
	Pressurized juice at 600 MPa	nd^{Bb}	nd^{Bb}	nd^{Ab}	nd^{Ba}	nd^{Ba}
Yeasts & moulds (CFU/ml)	Fresh juice	$3.68\pm0.82^{Ab}\times10^{2}$	$2.78\pm0.90\times10^{5Aa}$	-	-	-
	Pasteurized juice	nd^{Ba}	nd^{Ba}	nd^{Aa}	nd^{Aa}	nd^{Aa}
	Pressurized juice at 400 MPa	nd^{Ba}	nd^{Ba}	nd^{Aa}	nd^{Aa}	nd^{Aa}
	Pressurized juice at 600 MPa	nd^{Ba}	nd^{Ba}	nd^{Aa}	nd^{Aa}	nd^{Aa}
pH	Fresh juice	3.45 ± 0.02^{A}	-	-	-	-
	Pasteurized juice	3.32 ± 0.01^{Ba}	3.35 ± 0.02^{Ba}	3.33 ± 0.01^{Ba}	3.34 ± 0.01^{Ba}	3.32 ± 0.02^{Ba}
	Pressurized juice at 400 MPa	3.46 ± 0.02^{Aa}	3.44 ± 0.03^{Aa}	3.45 ± 0.01^{Aa}	3.42 ± 0.02^{Aa}	3.35 ± 0.02^{Bb}
	Pressurized juice at 600 MPa	3.44 ± 0.03^{Aa}	3.44 ± 0.01^{Aa}	3.43 ± 0.02^{Aa}	3.43 ± 0.01^{Aa}	3.43 ± 0.02^{Aa}
Viscosity (Pa.s)	Fresh juice	0.0054 ± 0.0002^{Aa}	-	-	-	-
	Pasteurized juice	0.0053 ± 0.0005^{Aa}	0.0050 ± 0.0002^{Aa}	0.0050 ± 0.0004^{Aa}	0.0044 ± 0.0005^{Aab}	0.0041 ± 0.0003^{Ab}
	Pressurized juice at 400 MPa	0.0052 ± 0.0003^{Aa}	0.0051 ± 0.0003^{Aa}	0.0048 ± 0.0002^{Aa}	0.0046 ± 0.0002^{Aab}	0.0043 ± 0.0001^{Ab}
	Pressurized juice at 600 MPa	0.0056 ± 0.0002^{Aa}	0.0055 ± 0.0004^{Aa}	0.0048 ± 0.0005^{Aab}	0.0042 ± 0.0004^{Abc}	0.0040 ± 0.0002^{Ac}
PPO activity (Unit/ml)	Fresh juice	79.64 ± 3.48^{A}	-	-	-	-
	Pasteurized juice	nd^{Da}	nd^{Ca}	nd^{Ca}	nd^{Ca}	nd^{Ca}
	Pressurized juice at 400 MPa	63.25 ± 1.68^{Ba}	62.80 ± 0.90^{Aa}	60.16 ± 1.16^{Aa}	57.92 ± 0.85^{Ab}	50.65 ± 2.37^{Ac}
	Pressurized juice at 600 MPa	42.83 ± 2.05^{Ca}	42.75 ± 1.02^{Ba}	40.83 ± 0.92^{Ba}	38.19 ± 1.08^{Bb}	36.09 ± 1.45^{Bb}
Residual PPO activity (%)	Fresh juice	100^{A}	-	-	-	-
	Pasteurized juice	nd^{Da}	nd^{Ca}	nd^{Ca}	nd^{Ca}	nd^{Ca}
	Pressurized juice at 400 MPa	79.42 ± 2.45^{Ba}	78.85 ± 1.73^{Aa}	76.54 ± 1.59^{Aa}	72.73 ± 1.24^{Ab}	63.60 ± 3.18^{Ac}
	Pressurized juice at 600 MPa	53.78 ± 3.12^{Ca}	53.68 ± 1.80^{Ba}	52.27 ± 1.46^{Ba}	47.95 ± 2.03^{Bb}	45.32 ± 2.35^{Bb}

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 ($n = 6$). nd = not detected

L^* parameter ($P > 0.05$) in both pressurized products with both pressure levels was observed. Upon the storage, all processed juices were significantly increased ($P \leq 0.05$) their lightness as the storage time increased. Barba *et al*. (2013) found that the L^* values of pressurized blueberry juices at different pressure levels (200, 400 and 600 MPa) and holding times (5, 9 and 15 min) significantly decreased as compared to the untreated juice. In addition, Zhao *et al.* (2013) observed that the L^* values of pressurized (400 or 500 MPa/25°C/2 or 4 min) and pasteurized (85°C/15 s) cucumber juices increased significantly after 15 days of refrigerated storage.

The redness intensities (a^* parameter) of pressurized juices were similar to the fresh juice ($P > 0.05$), whereas pasteurized juice showed the lowest trend ($P \leq 0.05$) (Table 2). Red color of Maoberry juice is mainly due to the presence of anthocyanin pigments. It was praiseworthy to note that the amounts of anthocyanins in pasteurized juice were significantly lower ($P \leq 0.05$) than those in the others (Table 3). Patras *et al*. (2009) illustrated that thermal treatment (70°C/2 min) caused a significant reduction of reddish (a^*) of strawberry and blackberry purées when compared to untreated batch, whereas Barba *et al.* (2012) encountered that the a^* value of pressurized blueberry juice was not significantly different from fresh samples. Regarding the refrigerated storage period, the a^* parameters of all processed juices apparently decreased ($P \leq 0.05$) with the increase of storage time, which corresponded with a gradual degradation of anthocyanins as observed in Table 3. The decreases in reddish were also the characteristic changes in high pressure treated strawberry (Cao *et al*., 2012) and Chinese bayberry (Yu *et al*., 2013) juices during storage at 4°C for 6 months and 25 days, respectively.

Color b^* parameter (yellowish) of Maoberry juice was relatively stable ($P > 0.05$) after thermal processing, while the significant decreases ($P \leq 0.05$) of yellowish in pressurized juices at both pressure levels were observed. The yellowish of blueberry juice also significantly diminished after pressurization at 400 MPa for 15 min (Barba *et al*., 2013). Analogous to a^* parameter, the b^* parameter of all processed juices trended to decline during the entire storage ($P \leq 0.05$). Similar observations were exhibited by Cao *et al.* (2012) and Landl *et al.* (2010) with pressurized strawberry juices and apple purées after refrigerated storage for 6 months and 21 days, respectively.

Table 3 illustrates the chroma intensities (C^*) of pressurized juices were significant higher ($P \leq 0.05$) than that of pasteurized and fresh juices. All processed juices were significantly decreased ($P \leq 0.05$) in the C^* parameter after storing at 4°C for 4 weeks. Gironés-Vilaplana *et al*. (2012) reported that the C^* values of lemon juice tended to decline during

Table 2. Changes of instrument color parameters of fresh and processed Maoberry juices during refrigerated storage at 4°C

Color parameters	Maoberry juices	Storage periods (weeks)				
		Initial state	1	2	3	4
*L** (lightness/darkness)	Fresh juice	19.79 ± 0.08^{A}	-	-	-	-
	Pasteurized juice	19.80 ± 0.12^{Ae}	20.02 ± 0.03^{Ad}	20.55 ± 0.10^{Ac}	21.38 ± 0.05^{Ab}	22.42 ± 0.09^{Ca}
	Pressurized juice at 400 MPa	18.49 ± 0.05^{Be}	19.64 ± 0.06^{Bd}	20.04 ± 0.09^{Bc}	20.96 ± 0.11^{Bb}	23.68 ± 0.03^{Aa}
	Pressurized juice at 600 MPa	18.45 ± 0.07^{Be}	19.82 ± 0.05^{Bd}	20.13 ± 0.04^{Bc}	20.83 ± 0.08^{Bb}	22.90 ± 0.05^{Ba}
*a** (redness/greenness)	Fresh juice	1.82 ± 0.03^{A}	-	-	-	-
	Pasteurized juice	1.75 ± 0.02^{Ba}	1.73 ± 0.03^{Ba}	1.74 ± 0.04^{Ba}	1.70 ± 0.04^{Bab}	1.68 ± 0.01^{Ab}
	Pressurized juice at 400 MPa	1.86 ± 0.02^{Aa}	1.82 ± 0.02^{Aab}	1.82 ± 0.01^{Ab}	1.79 ± 0.03^{ABb}	1.72 ± 0.02^{Ac}
	Pressurized juice at 600 MPa	1.88 ± 0.04^{Aa}	1.85 ± 0.02^{Aa}	1.83 ± 0.05^{Aab}	1.80 ± 0.03^{Ab}	1.70 ± 0.04^{Ac}
*b** (yellowness/blueness)	Fresh juice	0.91 ± 0.04^{A}	-	-	-	-
	Pasteurized juice	0.97 ± 0.03^{Aa}	0.93 ± 0.05^{Aab}	0.90 ± 0.03^{Ab}	0.82 ± 0.01^{Ac}	0.78 ± 0.01^{Ad}
	Pressurized juice at 400 MPa	0.83 ± 0.02^{Ba}	0.82 ± 0.02^{Ba}	0.81 ± 0.03^{Ba}	0.73 ± 0.02^{Bb}	0.69 ± 0.02^{Bb}
	Pressurized juice at 600 MPa	0.80 ± 0.02^{Ba}	0.81 ± 0.02^{Ba}	0.78 ± 0.02^{Ba}	0.73 ± 0.01^{Bb}	0.65 ± 0.02^{Bc}
*C** (chroma value)	Fresh juice	2.02 ± 0.01^{B}	-	-	-	-
	Pasteurized juice	2.00 ± 0.02^{Ba}	1.96 ± 0.01^{Bb}	1.96 ± 0.01^{Bb}	1.89 ± 0.03^{Ac}	1.84 ± 0.02^{Ac}
	Pressurized juice at 400 MPa	2.06 ± 0.01^{Aa}	2.00 ± 0.02^{Ab}	1.99 ± 0.01^{Ab}	1.93 ± 0.02^{Ac}	1.85 ± 0.01^{Ad}
	Pressurized juice at 600 MPa	2.08 ± 0.03^{Aa}	2.02 ± 0.01^{Ab}	1.97 ± 0.02^{ABc}	1.94 ± 0.02^{Ac}	1.82 ± 0.03^{Ad}
?*E* (total different colors)	Fresh juice	-	-	-	-	-
	Pasteurized juice	0.09 ± 0.01^{Be}	0.25 ± 0.03^{Ad}	0.76 ± 0.02^{Ac}	1.60 ± 0.01^{Ab}	2.64 ± 0.02^{Ca}
	Pressurized juice at 400 MPa	1.30 ± 0.04^{Ad}	0.17 ± 0.02^{Be}	0.27 ± 0.02^{Cc}	1.18 ± 0.03^{Bb}	3.90 ± 0.01^{Aa}
	Pressurized juice at 600 MPa	1.35 ± 0.02^{Ad}	0.11 ± 0.01^{Ce}	0.36 ± 0.01^{Bc}	1.06 ± 0.01^{Cb}	3.12 ± 0.02^{Ba}

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 ($n = 6$).

storage at 4 and 25°C for 70 days. Similar results were displayed by Guerrero-Beltrán and Barbosa-Cánovas (2004) with pressurized peach purees after storage at 3°C for 13 days.

Pressurized Maoberry juices showed much higher total different colors (ΔE) than that pasteurized juice ($P \leq 0.05$). The ΔE parameters noticeably decreased ($P \leq 0.05$) in the first week of storage and then significantly rose ($P \leq 0.05$) after 3 weeks onward. At the final state of storage, the ΔE values of all processed juices were apparently higher than that the initial state ($P \leq 0.05$). Cao *et al*. (2012) depicted that ΔE values of pressurized cloudy and clear strawberry juices apparently increased with increasing storage time, and the values were ranged of 5.36-7.80 at 4°C and 14.33-18.03 at 25°C for cloudy and clear juices in order. The alteration of color parameters primarily correlated with the stability of color pigments in the product. Keenan *et al.* (2010) stated that the degradation of anthocyanin pigments, responsible for the appearing red-purple color of Maoberry fruits, may have been catalyzed by the presence of oxidase enzymes including PPO during processing and storage.

Bioactive compounds and antioxidant capacity

Table 3 shows the amounts of ascorbic acid in all processed juices were significantly lower ($P \leq 0.05$) than that fresh juice, particularly in pasteurized juice showed lowest content. The pressure levels had no effect on the degradation of ascorbic acid. Formerly, our results discovered that ascorbic acid in longan juice was relatively sensitive to thermal processing, since it tremendously diminished on pasteurization (90°C/2 min) when compared with fresh and pressurized (300 or 500 MPa/25°C/30 min) juices (Chaikham and Apichartsrangkoon, 2012a). Barba *et al.* (2010) elucidated that pressurized (100-400 MPa/30°C/2-9 min) and pasteurized (98°C/21 s) vegetable beverages retained ascorbic acid around 91% and 89%, respectively.

Total phenols and total anthocyanins were relatively stable under high pressure processing ($P > 0.05$), whilst a significant decrease of these compounds was found in pasteurized sample ($P \leq 0.05$). Cao *et al*. (2011) observed that total phenols and anthocyanins of strawberry purées were no significant change after high pressure processing at 400-600 MPa for 5-25 min. On the other hand, Patras *et al.* (2009) reported anthocyanins in pasteurized strawberry purée at 70°C for 2 min dramatically diminished by 22%, as compared to untreated sample. The anthocyanin contents were closely correlated to the a* parameter of Maoberry juices ($r = 0.901$), thus the degradation of anthocyanins could be mainly led to the degradation of the reddish in the products (Table 2). In overall, total anthocyanins and phenols showed to be pressure patient constituents.

Processing had effect on antioxidant capacity (DPPH and FRAP assays) in Maoberry juice, however pressurized products still more remained this property than pasteurized batch ($P \leq 0.05$). The antioxidant capacity is associated to the amounts of various bioactive components including ascorbic acid, phenols or anthocyanins. In this study, high correlation coefficient of total anthocyanins and

Table 3. Storage stability of bioactive components and antioxidant capacity of fresh and processed Maoberry juices during refrigerated storage at 4°C

Bioactive compounds	Maoberry juices	Storage periods (weeks)				
		Initial state	1	2	3	4
Ascorbic acid	Fresh juice	19.05 ± 2.42^{A}	-	-	-	-
(mg/100 ml)	Pasteurized juice	10.94 ± 1.09^{Ca}	10.80 ± 1.96^{Ba}	10.38 ± 0.74^{Ba}	8.64 ± 0.56^{Bb}	5.49 ± 1.07^{Bc}
	Pressurized juice at 400 MPa	14.59 ± 2.05^{Ba}	13.92 ± 1.08^{Aa}	13.09 ± 0.90^{Aa}	10.35 ± 1.15^{Ab}	8.88 ± 0.99^{Ac}
	Pressurized juice at 600 MPa	15.24 ± 1.63^{Ba}	13.54 ± 0.85^{Aab}	12.90 ± 1.02^{ABb}	10.30 ± 1.33^{Ac}	8.67 ± 0.92^{Ad}
Total anthocyanins	Fresh juice	59.46 ± 3.18^{A}	-	-	-	-
(mg/100 ml)	Pasteurized juice	52.04 ± 2.03^{Ba}	52.34 ± 1.52^{Ba}	50.18 ± 2.05^{Bab}	48.47 ± 0.64^{Bb}	45.03 ± 1.11^{Bc}
	Pressurized juice at 400 MPa	61.02 ± 1.78^{Aa}	60.55 ± 1.06^{Aab}	60.17 ± 1.09^{Aab}	58.32 ± 1.24^{Ab}	53.24 ± 2.45^{Ac}
	Pressurized juice at 600 MPa	63.58 ± 2.44^{Aa}	60.49 ± 2.98^{Aab}	60.95 ± 0.93^{Aab}	59.08 ± 1.59^{Ab}	53.09 ± 1.90^{Ac}
Total phenols	Fresh juice	390.67 ± 9.51^{A}	-	-	-	-
(mg/100 ml)	Pasteurized juice	352.14 ± 11.43^{Ba}	350.62 ± 6.48^{Bab}	348.12 ± 5.47^{Bb}	340.75 ± 4.21^{Bb}	335.19 ± 6.04^{Bb}
	Pressurized juice at 400 MPa	395.45 ± 8.39^{Aa}	387.54 ± 10.02^{Aab}	380.71 ± 6.79^{Ab}	366.38 ± 5.08^{Ac}	358.40 ± 12.15^{Ac}
	Pressurized juice at 600 MPa	390.80 ± 5.64^{Aa}	385.75 ± 8.65^{Aab}	381.43 ± 9.07^{Ab}	370.42 ± 10.12^{Abc}	360.97 ± 9.26^{Ac}
DPPH radical-scavenging activity	Fresh juice	67.33 ± 1.25^{A}	-	-	-	-
(% inhibition)	Pasteurized juice	50.49 ± 2.01^{Ca}	48.70 ± 1.05^{Ba}	48.56 ± 0.85^{Ba}	45.10 ± 2.15^{Bb}	43.78 ± 1.98^{Bb}
	Pressurized juice at 400 MPa	62.82 ± 1.57^{Ba}	60.45 ± 1.14^{Aa}	57.19 ± 0.70^{Ab}	52.85 ± 1.26^{Ac}	50.60 ± 2.08^{Ac}
	Pressurized juice at 600 MPa	63.56 ± 1.18^{Ba}	61.08 ± 0.94^{Aa}	58.90 ± 1.09^{Ab}	51.91 ± 1.40^{Ac}	52.11 ± 2.15^{Ac}
FRAP value	Fresh juice	10.04 ± 0.40^{A}	-	-	-	-
(mM $FeSO_4$/ml)	Pasteurized juice	6.32 ± 0.45^{Ca}	6.24 ± 0.19^{Ba}	6.18 ± 0.38^{Bab}	5.63 ± 0.17^{Bb}	5.08 ± 0.20^{Bc}
	Pressurized juice at 400 MPa	8.85 ± 0.32^{Ba}	8.71 ± 0.40^{Aab}	8.52 ± 0.30^{Aab}	8.09 ± 0.42^{Abc}	7.51 ± 0.22^{Ac}
	Pressurized juice at 600 MPa	8.90 ± 0.23^{Ba}	8.75 ± 0.35^{Aab}	8.44 ± 0.26^{Aab}	8.12 ± 0.31^{Ab}	7.46 ± 0.18^{Ac}

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 ($n = 6$).

phenols versus antioxidant capacity was found (data not shown). Indrawati *et al*. (2004) displayed that antioxidant capacity in carrot juice slightly decreased after pressurization. Keenan *et al.* (2012) depicted that pressurization led to higher antioxidant activity than that pasteurization for fruit smoothies.

Considering the storage period, the levels of ascorbic acid, anthocyanins and total phenols as well as antioxidant capacity (DPPH and FRAP assays) significantly declined ($P \leq 0.05$) with increasing the storage time. Nevertheless, most of these bioactive components in the pressurized juices still remained in higher quantities than those in the pasteurized sample throughout the storage period. Previously, Apichartsrangkoon *et al*. (2012) discovered the significant losses of ascorbic acid in pressurized (400 MPa/< 30°C/20 min) and pasteurized (90°C/3 min) pennywort juices during the entire storage at 4°C for 4 months. They also reported that an amount of ascorbic acid retained in pressurized sample was significantly higher than those thermally treated batches in every respect. Similar findings were obtained by Cao *et al.* (2012) and Keenan *et al*. (2010) with strawberry juices and fruit smoothies, respectively. Ascorbic acid might degrade to dehydroascorbic acid and further irreversibly converted into 2,3-diketogulonic acid (Landl *et al*., 2010). Besides ascorbic acid, Castañeda-Ovando *et al*. (2009) stated that the loss of anthocyanins and phenols during storage could due mainly to oxidation or/and condensation reactions. In addition, the reduction of phenols in pressurized products during storage might be due to the residual PPO and POD involving in the oxidative degradation of phenols (Tiwari *et al.*, 2009). The loss of antioxidant capacity (DPPH and FRAP assays) during storage was also related to the reduction of ascorbic acid, total phenols and anthocyanins.

Conclusions

In summary, high pressure and thermal processing were satisfactory eliminated the general microbes in Maoberry juice; in addition, both processed juices exhibited a better microbiological stability throughout the refrigerated storage period. The change of pH of low pressure treated juice (400 MPa) during storage could be due primarily to the increase of general microbes. The PPO activity was absolutely inhibited after pasteurization, but this enzyme still remained in pressurized juices at 400 and 600 MPa up to 79% and 53% respectively. In addition, upon the storage all pressurized juices showed a significant decrease in the residual PPO activity when the storage increased. Considering the color parameters, the reddish of pressurized juices were similar to the fresh juice, suggesting that high pressure could preserve the anthocyanins (red-purple pigments) in the juice better than thermal treatment. The retentions of ascorbic acid, phenols and anthocyanins as well as antioxidant capacity (DHHP and FRAP assays) in pressurized juices were significantly higher than that the pasteurized juice throughout the entire storage.

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