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Alteration of Bioactive Compounds and Antioxidative Properties In Thermal, Ultra-High Pressure and Ultrasound Treated Maoberry (*Antidesma Bunius* L.) Juice during Refrigerated Storage

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Abstract

Maoberry (*Antidesma bunius* L.) is a tropical fruit locally referred to as "*Mao-Luang*" in Thailand. The fruit contains high amounts of ascorbic acid and phenolic compounds with antioxidative potential, which has demonstrated medicinal value in terms of anti-cancer and anti-diabetic effects. In this term, this research purposed to determine the changes of predominant bioactive phytochemicals, antioxidant capacity and microbiological quality of pasteurized (85ºC/1 min), pressurized (500 and 600 MPa/30ºC/30 min), and ultra-sonicated (20 kHz/60% and 80% amplitude/30 min) maoberry juices during storageat 4°C for 30 days. The results displayed that ascorbic acid, phenolic acids (gallic and vanillic acids), anthocyanins (cyanidin 3-o-glucoside and cyanidin 3-rutinoside), flavonoids [(+)-catechin and (-)-epicatechin), 2,2-diphenyl-1-picryl hydrazyl hydrate (DPPH) radical inhibition and ferric reducing antioxidant power (FRAP) value in pressurized and ultra-sonicated juices displayed higher reduction rate during storage than those in pasteurized juice. Nevertheless, at the final stage of storage, both juicesstill contained higher levels of antioxidant compounds and properties than inthermally treated juice.All the treated samples were shown to reduce initial microbial load of fresh maoberry juice to a non-detectable amount, while maintaining their quality during prolonged refrigerated storage.

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Antioxidants;
Pasteurization;
Pressurization;
Maoberry;
Ultra-sonication.

Introduction

As the presence and varietyof natural nutraceuticals and novel functional food products continue to grow, maoberry juice is widely recognized as a nutrient-rich and healthy beverage optionin today's market. Antioxidant compounds in fruits, for

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example, polyphenolic compounds and ascorbic acid (vitamin C), are believed to contribute to the prevention of oxidative stress related diseases,viz. coronary artery disease, cancers, diabetes, and aging-related disorders.[1-2] Maoberry fruits (*Antidesma bunius* L.)are vastly grown in the northern and northeastern regions of Thailand, and are commonly used to make jam, jelly, red wine, ready-to-drink juice, and juice concentrates.[3-5] They comprise of significant amounts of bioactive phytochemicals, viz. ascorbic acid, phenolic acids, anthocyanins, and flavonoids,thus contain pharmacological properties.[6-7]

There is strong research evidence regarding the health benefits of maoberry fruit extracts, such as pathogen inhibition and prevention of human breast epithelial cell apoptosis, contributed by their antioxidant constituents.[8-9] While maoberry juice is widely consumed for health-related purposes, traditional methods used in fruit juice thermal processing, such as pasteurization and sterilization, were shown to induce detrimental effects characterized by high levels of reduction in such beneficial compounds.[5, 10-11] In terms of microbiological stability,previous studies have indicated significant reductions in microbial counts following ultra-sonication of mulberry and blueberry juices, with correspondingly greater effects observed as sonication intensity increased.[12-13] Similarly, ultra-high hydrostatic pressure (HHP) and ultra-sonic (US) techniques were shown to maintain various bioactive compounds and to inactivate microbial growth in grapefruit, pomegranate, jabuticaba, and aronia juices.[14-17] Only few studies to date have observed the effectiveness of applyingthese advanced technologies to maoberry juice preservation. Therefore, the objective of this research was to investigate the influence of pasteurization (85ºC/1 min), pressurization (500 and 600 MPa/30ºC/30 min) and ultra-sonication (20 kHz/60% and 80% amplitude/30 min) on antioxidative properties and microbiological quality of maoberry juice. The storage stability of each treated juices at 4ºC for 30 dayswas also monitored.

Materials and Methods

Chemicals

Folin–Ciocalteu phenol reagent,2,2-diphenyl-1-picryl hydrazyl hydrate (DPPH), 2,4,6-tripyridyls-triazine (TPTZ),ferric chloride hexahydrate, (+)-catechin, (-)-epicatechin, gallic acid, vanillic acid, cyanidin-3-o-glucoside and cyanidin 3-rutinoside were obtained from Sigma–Aldrich (St. Louis, MO, USA). High performance liquid chromatography (HPLC)grade methanol, absolute ethanol, acetonitrile, acetic acid, sulphuric acid and phosphoric acid were purchased from Merck (Darmstadt, Germany). Plate Count Agar (PCA), Potato Dextrose Agar (PDA) and Violet Red Bile Lactose Agar (VRBL) were supplied from Hi-Media (Mumbai, India). Deionized water and castor oil were provided from Chemical & Lab Supplies (Bangkok, Thailand).

Preparation of Fresh and Processed Maoberry Juices

Organic maoberry fruits were purchased from a local market in Pupan district, Sakon Nakhon province, Thailand. The fresh fruits were washed twice prior to extraction with a juice extractor before packing and processes. The extracted juice (200 mL) was filled in aretort pouch bag (P.P. Pack, Mahasarakham, Thailand) before pasteurizationat 85ºC for 1 minute using a thermostatic water bath (LAUDA, Lauda-Königshofen, Germany). Pressurized maoberry juice was prepared using a'Food Lab' high-pressure rig (Stansted Fluid Power, Stansted, Essex, UK). The pressure transmitting medium was a mixture of castor oil and absolute ethanol at a ratio of 1:4 (v/v).The juice (200 mL) was filled in a nylon-polyethylene bag (P.P. Pack, Mahasarakham, Thailand), followed by pressure levels of 500 and 600 MPa at 30ºC for 30 minutes. Besides, maoberry juice (200 mL) was filled into a 300 mLsterile beaker, then ultra-sonicated at a frequency of 20 kHz and 60-80% amplitudes (VCX 130 PB 130 W, Sonics & Materials Inc., Newtown, CT) in an aseptic area. After processing for 30 minutes, the core temperature of ultra-sonicated juices increased from the baseline of 18.63 ± 0.45ºC to 55.02 ± 2.14ºC and 70.36 ± 1.83ºC at wave amplitudes of 60% and 80%, respectively. Subsequently, those pasteurized and ultra-sonicated juices were immersed in an ice-water bath for cooling down to ambient temperature. All the processed samples were then refrigerated at 4ºC for 30 days. During storage, sampling was carried outat 5 day-intervals to determination of bioactive compounds, antioxidative properties and microbiological quality.

Determination of Bioactive Phytochemicals
Ascorbic Acid

To extract ascorbic acid, fresh and processed maoberry juices (10 mL) were mixed with 40mL of diluted sulphuric acid (pH 2.2)for 10 minusing a sonicator bath before filtering through a 0.20-µm nylon membrane (Millex Syringe Filter; Sigma–Aldrich, St. Louis, MO, USA). A20-µLfiltrate was injected into the HPLC system, which consisted of aYMC-Pack ODS-AM C18 column (5 µm, 4.6 mm ID × 250 mm).4The detector was adjusted to wavelength of λmax250 nm. Acetic acid solution (0.1 M) was used as a mobile phasewith a flow rate of 1.5 mL/min and column temperature was adjusted to 30°C.7 Ascorbic acid identification was carried out by comparing its retention time and spectra with the standard using a calibration curve. Peak area was identified and converted to the concentration of ascorbic acid.

Predominant Phenolic Compounds

All predominant phenolic compounds,viz. gallic acid, vanillic acid, cyanidin 3-o-glucoside, cyanidin 3-rutinoside, (+)-catechin and (-)-epicatechin, in fresh, processed and stored sampleswere determined according to themodified protocol of Jorjong *et al*.[6] To extract those phenolic compounds, the juice (20 mL) was well-mixed with 30mL of methanol using a sonicator bath for 10 min. After that, each mixture was filtered through a 0.20-µm nylon membrane and 20 µL filtrate were injected to the HPLC system.[4] Acetonitrile (eluent A) and diluted phosphoric acid (pH 2.58, eluent B) was used as a mobile phase with a flow rate of 0.8 mL/min for separation both gallic and vanillic acids.The gradient system of the mobile phase commenced from 0 min (100% A) to 15 min (91% A), 22 min (89% A), 38 min (82% A), 43 min (77% A), 45 min (70% A), 55 min (20% A), and 60 min (95% A). The detector was adjusted to wave length of λmax 280 nm. For determination of cyanidin 3-o-glucoside and cyanidin 3-rutinoside concentrations, 4% (v/v) phosphoric acid (eluent A) and acetonitrile (eluent B) was used as a mobile phase with a flow rate of 1 mL/min and column temperature of 40°C. The UV–Vis detection was at λmax520 nm. The linear gradient started with 94% B at 0 min, 75% B at 55 min, 75% B at 65 min at isocratic elution for 70 min.6Besides, the mobile phase for (+)-catechin and (-)-epicatechin was acetonitrile/deionized water (2/97.8, v/v) plus 0.2% phosphoric acid (eluent A) and acetonitrile/deionized water (97.8/2, v/v) plus 0.2% phosphoric acid (eluent B). The flow rate of mobile phase and column temperature were set at 0.6 mL/min and at 40°C, respectively. The UV–Vis detection was at λmax 520 nm. Gradient elution started with 20% B, 50% B at 30 min, 60% B at 35 min, 20% B at 40 min at isocratic elution for 55 min.[3] Quantification was carried out by comparing the retention times and absorbance along the spectra, as well as by the use of reference standards. The concentrations ofphenolic compounds were calculated using a corresponding external standard.

Determination of Antioxidant Capacity
DPPH Assay

Briefly, the sample (2 mL) waswell-mixed with 8 mL of 100% methanol using an orbital shaker for 10 min before centrifugation at 4,500 rpm for 10 min. Subsequently, the supernatant (1.6 mL) was poured into 0.4 mL of 1.5 µM DPPH radical in methanol, mixed, and left to stand for 30 min in the dark. The absorbance of the mixture was thenmeasured at wavelength of λmax 517 nmusing a UV–Visible spectrophotometer (UV1800; Shimadzu, Tokyo, Japan). DPPH radical inhibition was calculated, where A_0 is absorbance of the control (1.6 mL of methanol), and A_1 is absorbance of the sample.[18]

$$\text{DPPH radicalinhibition (\%)} = [1 - (A_1/A_0)] \times 100$$

FRAP Assay

FRAP value was determined according to the modified method as described by Benzie and Stain.[19] One mL juice was poured into 9 mL deionized water and well-mixed for 20 min beforefiltering through a 0.20-µm nylon membrane. Afterwards, 3mL FRAP reagent (10:1:1 of 300 mM sodium acetate buffer at pH 3.6, 10 mM TPTZ and 20 mM ferric chloride hexahydrate reagent) was added to the filtrate, mixed and incubated at 37°C for 30 min in the dark room. The absorbance of the mixture was then measured at wavelength of λmax 593 nm. The results were expressed as mM offerrous sulfateper 100 mLsample (mM FeSO4/100 mL).

Assessments of Microbiological Quality

The indicator microbes, viz. total bacteria, yeast-mold and coliformcounts,in the sample were carried out using the PCA, PDA and VRBL media, respectively. The media were poured into the Petri dish and agitated gently to homogenize with the

diluted sample. After formed a gel, all plates were incubated at 37°C for 24 h before counting the colonies. Those microbes were calculated as colony forming units per milliliter sample (CFU/mL).[20]

Statistical Analysis

All data were existed as mean ± standard deviation (S.D.) of six replications (n = 6). One-way analysis of variance (ANOVA) was carried out using SPSS software (SPSS Inc., Chicago, IL) at a significance level of 95%. Duncan's New Multiple Range Test (DMRT) was used to differentiate the means.

Results and Discussion

Bioactive Compounds and Antioxidative Properties of Maoberry Juices

Ascorbic acid is a strong reducing agent with high antioxidant potential. However, it is also susceptible to oxidation or degradation by light, oxygen, heat, peroxide and enzymes.[18, 21] In this study, the change in ascorbic acid concentrations in maoberry juice affected by thermal, ultra-high pressure, and ultra-sonic treatments were determined, as shown in Table 1. The amount of ascorbic acid in all processed samples was significantly lower (P≤0.05) than thatoffresh juice, with the lowest contents in pasteurized juice. In pressurized maoberry juices, there was no significant difference on ascorbic acid concentrations (P>0.05), regardless of different levels of pressure treatment. On the other hand, the increased amplitude of ultra-sonication was found to significantly correlate with the reduced levels of the compound (P≤0.05).The internal temperature of the sample increased from 22.19±2.31ºC to 74.12±1.45ºC after ultra-sonication at 80% amplitude. Pressurization and ultra-sonication treatments tended to degrade ascorbic acid in maoberry juice,which is similar in effect of heat treatment (i.e. pasteurization).[22] Landl *et al*. also revealed that ascorbic acid can be degraded to dehydroascorbic acid, and further irreversibly converted into 2,3-diketogulonic acid.[23] Overall, our results showed that ultra-high pressure and ultra-sonic processing could retain ascorbic acid concentration in maoberry juice by 85.49–86.66%, and 64.15–69.78%, respectively, and are shown to have greater conservative effects compared to pasteurization. Similarly, the findings of Vega-Galvez *et al*.[24] and Khandpur and Gogate,[25] reported that pasteurization processing had led to significantly increased degradation of ascorbic acid in fruit and vegetable juices when compared to pressurization and ultra-sonication.

Gallic and vanillic acids are predominant phenolic acids found in maoberry fruits. According to Jorjong *et al*., there are 15 cultivars of maoberry fruits grown in Northeastern Thailand, containing, on average, around 159.47 and 179.75 mg/100 g (dry basis) of gallic and vanillic acids, respectively.6Gallic acid is commonly regarded as a bioactive phenol compound with antioxidative, anti-carcinogenic, antibacterial, antifungal and antiviral properties.[26] Vanillic acid wasalso found to be associated with a variety of pharmacologic activities,viz. inhibiting carcinogenesis, apoptosis and inflammation, and anti-hypertensive, and anti-colitis effects.27-29From the results presented in Table 1, both phenolic acids have portrayed greater sensitivity to heat treatments. Phenolic acid concentrations in thermally and ultra-sonic treated juices were notably lower (P≤0.05) than those in other juices.While there was no significant effect of varied pressure levels on these components as compared to those in fresh juice, both phenolic acid contents showed a declining trend when the amplitude level increased. Keenan *et al*. stated that pressure could affect the structure of large polymers by keeping small molecules, such as phenolic acids, intact.[30] Similarly, in the study of Chaikham and Apichartsrangkoon, phenolic acids (i.e. gallic and ellagic acids) in longan juice were not affected by pressurization conditions.[31] This might suggest that gallic and vanillic acids tend to remain stableduring pressure treatment.According to the study of Chaikham*et al*.about ultra-sonification effects on maoberry juice, an increase in total phenolic content was observed following treatment at 80% amplitude.[18] Similar trends were observed in ultra-sonication of grapefruit juice[10] and purple cactus pear juice.[32] Higher wave amplitudes during ultra-sonication could facilitate the release of phenolic compounds from plant-cell walls, which may contribute to the overall effect.[22] Contrarily, the study of Kwaw *et al*., a significant increase in phenolic concentrations, including gallic and vanillic acids, was observed on ultra-sonication of fermented mulberry (Morus nigra) juice.[5] Although little is known about the effect of ultra-sonification on polyphenolic content of foods, it was suggested that combining ultra-sonication with pulsed-light treatment may facilitate greater release of antioxidants via phytonutrient degradation into phenolic compounds.[5]

Table 1 : Antioxidative properties and microbiological quality of fresh and treated maoberry juices

Properties	Fresh juice	Pasteurization	Pressurization		Ultra-sonication	
			500 MPa	600 MPa	60% amplitude	80% amplitude
Bioactive components (mg/100 mL)						
Ascorbic acid	29.15 ± 1.03^{a}	10.63 ± 0.82^{e}	24.92 ± 1.47^{b}	25.26 ± 2.01^{b}	20.34 ± 1.77^{c}	18.70 ± 1.35^{d}
Gallic acid	20.04 ± 1.84^{a}	12.29 ± 1.12^{d}	21.18 ± 2.16^{ab}	21.33 ± 1.40^{a}	19.13 ± 0.52^{b}	15.03 ± 1.05^{c}
Vanillic acid	21.98 ± 1.25^{a}	11.08 ± 0.73^{d}	21.50 ± 1.23^{a}	22.02 ± 0.95^{a}	17.09 ± 1.10^{b}	14.65 ± 0.87^{c}
Cyanidin 3-O-glucoside	5.38 ± 0.11^{b}	3.03 ± 0.20^{d}	5.74 ± 0.18^{ab}	5.90 ± 0.10^{a}	5.53 ± 0.07^{b}	5.19 ± 0.13^{c}
Cyanidin 3-rutinoside	3.37 ± 0.24^{b}	2.54 ± 0.13^{d}	3.83 ± 0.11^{a}	3.85 ± 0.09^{a}	3.40 ± 0.15^{b}	3.02 ± 0.04^{c}
(+)-Catechin	18.65 ± 1.10^{b}	10.04 ± 0.97^{d}	20.10 ± 2.07^{ab}	22.54 ± 1.82^{a}	18.63 ± 1.74^{b}	16.40 ± 0.68^{c}
(-)-Epicatechin	49.30 ± 2.13^{b}	38.24 ± 2.39^{d}	54.02 ± 1.14^{a}	52.28 ± 2.56^{ab}	48.50 ± 1.90^{b}	45.11 ± 1.00^{c}
Antioxidant activities						
DPPH inhibition (%)	45.07 ± 1.50^{a}	25.85 ± 1.17^{e}	40.02 ± 0.71^{b}	41.34 ± 1.09^{b}	38.12 ± 1.63^{c}	30.09 ± 0.36^{d}
FRAP value (mM Fe(II)/100 mL)	18.34 ± 0.33^{a}	12.03 ± 0.12^{d}	16.20 ± 0.43^{b}	15.95 ± 0.30^{b}	14.62 ± 0.24^{c}	13.41 ± 0.31^{cd}
Microbiological qualities						
Total plate counts (log CFU/mL)	6.14 ± 0.30^{a}	nd ^{b}	nd ^{b}	nd ^{b}	nd ^{b}	nd ^{b}
Yeasts and molds (log CFU/mL)	2.05 ± 0.12^{a}	nd ^{b}	nd ^{b}	nd ^{b}	nd ^{b}	nd ^{b}
Fecal coliforms (log CFU/mL)	1.60 ± 0.16^{a}	nd ^{b}	nd ^{b}	nd ^{b}	nd ^{b}	nd ^{b}

Means in the rows with different lower case letters show significant difference within treatments (P≤0.05).

Besides, major anthocyanins (cyanidin 3-o-glucoside and cyanidin 3-rutinoside) and flavonoids [(+)-catechin and(-)-epicatechin] in fresh and processed maoberry juices were also investigated (Table 1). It was found that the levels of cyanidin 3-o-glucoside, cyanidin 3-rutinoside, (+)-catechin and (-)-epicatechin in maoberry juice were noticeably enhanced after pressurization, increasing by roughly 6.69-9.66%, 13.65-14.24%, 7.77-20.86%, and 6.04-9.57%, respectively, while such components in ultra-sonicatedmaoberry juice at 60% amplitude were similar in concentration tothose of unprocessed juice. The increase in levels of anthocyanins and flavonoids may be related to the increased extractability as a result of processing. Contrarily, it was observed that ultrasonication at 80% amplitude resulted in significant reduction of all anthocyanins and flavonoids in maoberry juice, as compared to fresh juice. Overall, the pasteurized samples contained the lowest contents of compounds. According to the results, it could be derived that harsh thermal processing, including pasteurization and ultra-sonication with high frequency amplitude, may have caused the higher levels of predominant anthocyanin and flavonoid degradation in maoberry juice.

DPPHradical inhibition has been used to determine the antioxidant activity of maoberry juice, as indicated by the ability ofantioxidants to donate a hydrogen atom or electron in radical stabilization. FRAP assay isgenerally used to measure the capacity of a substance inreducing TPTZ–Fe(III) complex to TPTZ–Fe(II) complex.[1, 31, 33] From the results in Table 1, DPPH radical inhibition and FRAP valuesofall processed maoberry juice samples were lower than that of fresh juice. Significantly lower levels (P≤0.05) were observed inpasteurized juice and juice ultra-sonicatedat 80% amplitude for 30 minutes. Lowest values were found in pasteurized juice, similar to that observed for ascorbic acid, phenolic acids, anthocyanins and flavonoids. However, in this study, only high correlation coefficients of ascorbic acid versus antioxidant activities were derived (R2 = 8.85-9.05). In the study of Chaikham and Baipong, it was elucidated that high hydrostatic pressure processing demonstrated significantly greater effectiveness in preserving bioactive components, i.e. ascorbic acid, total anthocyanins, total phenols, and antioxidant activities (DPPH and FRAP assays)of maoberry juice compared to thermal processing.[7]

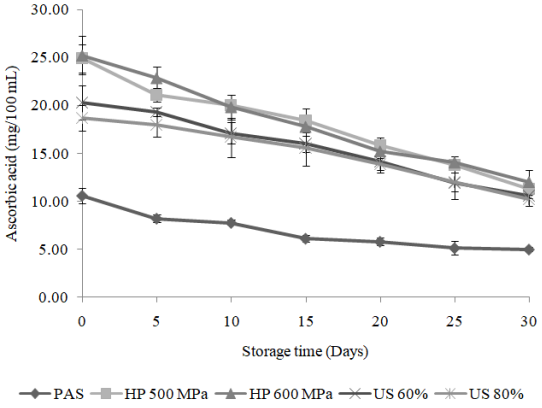

Fig.1 : Changes of ascorbic acid in thermally, high pressure and ultra-sonic treated maoberry juices during storage at 4ºC for 30 days. PAS is pasteurization, HP is pressurization and US is ultra-sonication

Storage Stability of Bioactive Compounds and Antioxidant Activities of Processed Maoberry Juices

Figure 1 illustrates significant decrease in levels of ascorbic acid in all processed maoberry juices (P≤0.05) with increasing storage time. At the final stage of storage, ascorbic acid concentrations in all samples had reduced by around 50%from their initials. The highest rate of reduction was observed in pressurized juices, following by ultra-sonicated and pasteurized juices, respectively. These findings were confirmed by the slopes of linear trend-lines (linear equations) from the correlation of ascorbic acid content versusstorage time (Table 2). However,as observed in Figure 1, the pressurized juices still retained higher amounts of ascorbic acid thanthat of ultra-sonicated juices, followed by thermally treated juice throughout the entire storage duration.

In this study, concentrations of predominant phenolic acids, anthocyanins and flavonoids in treated maoberry juices were directly affected by prolonged storage time(Figures 2-4). The amounts of gallic acid, vanillic acid, cyanidin 3-o-glucoside, cyanidin 3-rutinoside, (+)-catechin, and (-)-epicatechin had notablydeclined (P≤0.05) during refrigerated storage for 30 days. The degradation rates of these phytochemical compounds, as indicated by the slopes from the linear equations (Table 2), were greater in pressurized juices, followed by ultrasonicated and pasteurized juices, respectively, with exception to the slope of linear trend-line of cyanidin 3-rutinoside in pasteurized juice being

higher than that observed for ultra-sonicated juices. However, on the 30th day of storage, the concentrations of these components were significantly higher in pressurized juicescompared to ultra-sonicated juices, followed by pasteurized juice, with exceptions to cyanidin 3-o-glucoside, cyanidin 3-rutinoside, and (+)-catechin concentrations in ultra-sonicated juices.Results from Figures 3 and 4 have observed no significant difference (P>0.05) between pressurized and ultra-sonicated juices in terms of levels of those phenolic acids. According to the report of da Silveira *et al*., it was found that high pressure effect was more pronounced at preserving the thermal sensitivity bioactive compounds (i.e. anthocyanins, non-anthocyanin, phenolic compounds, and tocopherols) and antioxidant capacity (peroxyl radical scavenging, hypochlorous acid scavenging, and hydrogen peroxide scavenging assays) in açaí juice, than the thermal processing.[34]

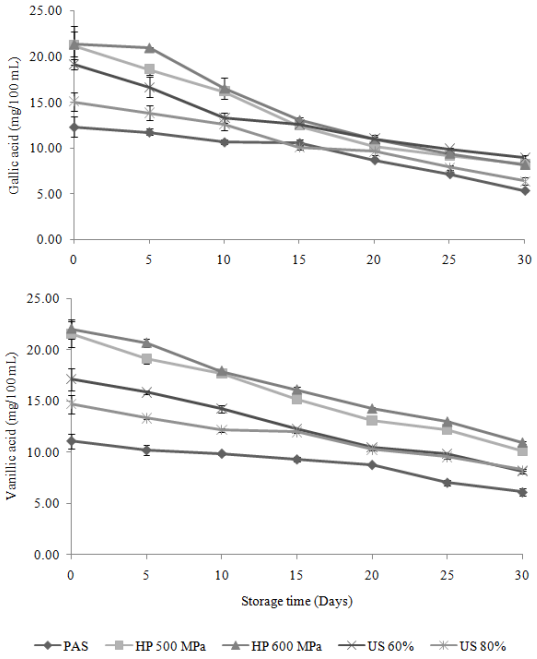

Fig. 2: Changes of predominant phenolic acids in thermally, high pressure and ultra-sonic treated maoberry juices during storage at 4ºC for 30 days. PAS is pasteurization, HP is pressurization and US is ultra-sonication

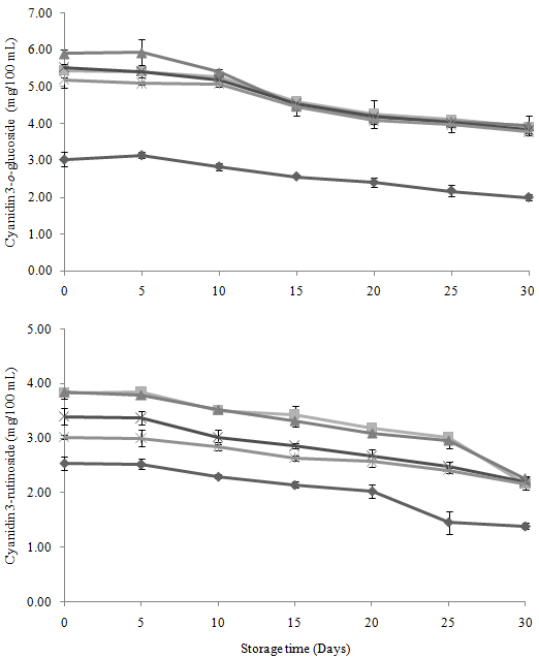

Fig. 3: Changes of predominant anthocyanins in thermally, high pressure and ultra-sonic treated maoberry juices during storage at 4ºC for 30 days. PAS is pasteurization, HP is pressurization and US is ultra-sonication

Table 2: Linear equations and R-square values of linear trend-lines from the correlation of antioxidant properties (y) versus storage period (x)

Antioxidant properties	Treatments	Linear equations	R-square values (R2)
Ascorbic acid	Pasteurization	y = -0.178x + 9.650	0.899
	Pressurization at 500 MPa	y = -0.425x + 24.31	0.985
	Pressurization at 600 MPa	y = -0.443x + 24.80	0.988
	Ultra-sonication at 60% amplitude	y = -0.334x + 20.67	0.993
	Ultra-sonication at 80% amplitude	y = -0.287x + 19.32	0.981

Gallic acid	Pasteurization	y = -1.142x + 14.04	0.944
	Pressurization at 500 MPa	y = -2.285x + 22.81	0.961
	Pressurization at 600 MPa	y = -2.437x + 24.07	0.959
	Ultra-sonication at 60% amplitude	y = -1.645x + 19.66	0.944
	Ultra-sonication at 80% amplitude	y = -1.460x + 16.59	0.987
Vanillic acid	Pasteurization	y = -0.798x + 12.09	0.947
	Pressurization at 500 MPa	y = -1.876x + 23.02	0.990
	Pressurization at 600 MPa	y = -1.871x + 23.87	0.993
	Ultra-sonication at 60% amplitude	y = -1.521x + 18.61	0.990
	Ultra-sonication at 80% amplitude	y = -1.019x + 15.52	0.984
Cyanidin 3-o-glucoside	Pasteurization	y = -0.195x + 3.377	0.952
	Pressurization at 500 MPa	y = -0.292x + 5.887	0.939
	Pressurization at 600 MPa	y = -0.386x + 6.384	0.907
	Ultra-sonication at 60% amplitude	y = -0.316x + 5.950	0.958
	Ultra-sonication at 80% amplitude	y = -0.267x + 5.594	0.934
Cyanidin 3 -rutinoside	Pasteurization	y = -0.209x + 2.894	0.924
	Pressurization at 500 MPa	y = -0.249x + 4.282	0.863
	Pressurization at 600 MPa	y = -0.246x + 4.241	0.921
	Ultra-sonication at 60% amplitude	y = -0.204x + 3.675	0.982
	Ultra-sonication at 80% amplitude	y = -0.143x + 3.238	0.969
(+)-Catechin	Pasteurization	y = -0.600x + 10.48	0.989
	Pressurization at 500 MPa	y = -1.658x + 22.30	0.979
	Pressurization at 600 MPa	y = -2.224x + 25.42	0.981
	Ultra-sonication at 60% amplitude	y = -1.718x + 21.29	0.951
	Ultra-sonication at 80% amplitude	y = -1.026x + 18.03	0.942
(-)-Epicatechin	Pasteurization	y = -1.523x + 39.72	0.987
	Pressurization at 500 MPa	y = -2.166x + 54.81	0.958
	Pressurization at 600 MPa	y = -2.049x + 53.69	0.969
	Ultra-sonication at 60% amplitude	y = -2.037x + 50.18	0.976
	Ultra-sonication at 80% amplitude	y = -1.737x + 47.72	0.976
DPPH inhibition	Pasteurization	y = -2.516x + 28.08	0.996
	Pressurization at 500 MPa	y = -2.548x + 42.31	0.971
	Pressurization at 600 MPa	y = -2.651x + 43.39	0.977
	Ultra-sonication at 60% amplitude	y = -2.880x + 41.26	0.982
	Ultra-sonication at 80% amplitude	y = -2.147x + 31.87	0.997
FRAP value	Pasteurization	y = -0.937x + 12.44	0.961
	Pressurization at 500 MPa	y = -1.349x + 16.47	0.928
	Pressurization at 600 MPa	y = -1.373x + 16.77	0.978
	Ultra-sonication at 60% amplitude	y = -1.192x + 15.30	0.977
	Ultra-sonication at 80% amplitude	y = -1.018x + 14.18	0.994

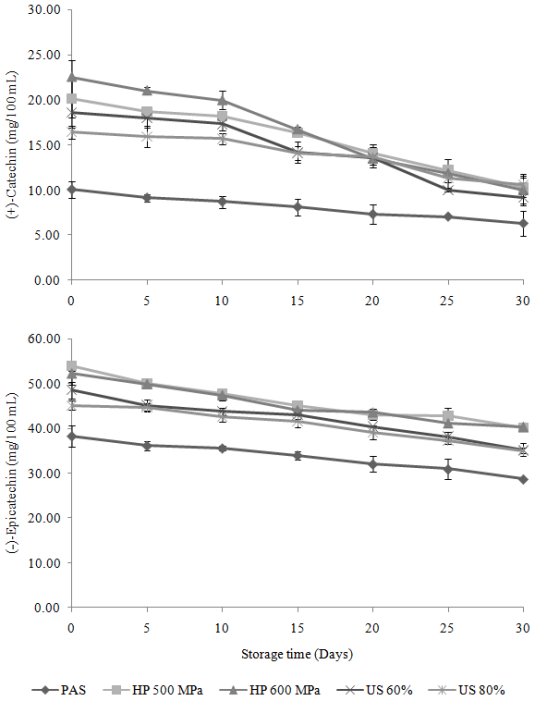

Fig. 4: Changes of predominant flavonoids in thermally, high pressure and ultra-sonic treated maoberry juices during storage at 4ºC for 30 days. PAS is pasteurization, HP is pressurization and US is ultra-sonication

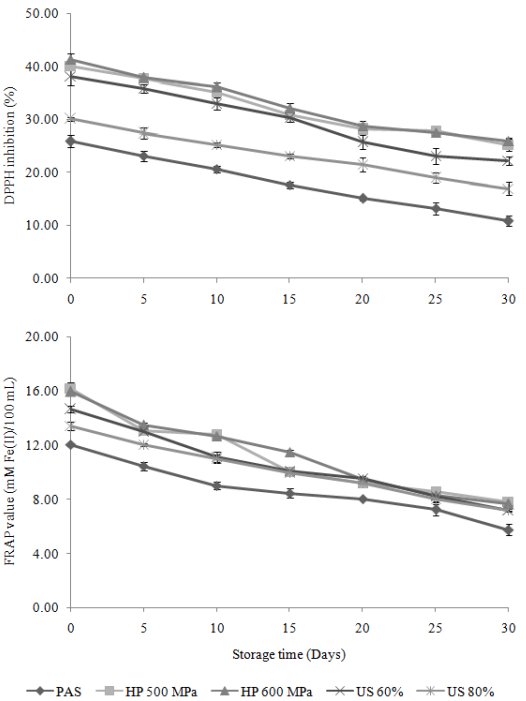

Fig. 5: Changes of antioxidant activities in thermally, high pressure and ultra-sonic treated maoberry juices during storage at 4ºC for 30 days. PAS is pasteurization, HP is pressurization and US is ultra-sonication

Further more, the antioxidant activities (DPPH and FRAP assays) of all treated maoberry juice samples were also determined. In thiscase, DPPH radical inhibition and FRAP values of maoberry juice substantially decreased with prolonged storage duration (Figure 5). Moreover, similar reduction rates of both values in the processed juices were observed with increased storage time, as indicated by the slopes of their linear trend-lines from the equationsin Table 2. Similarly, Morales-de la Peña *et al*. reported a depletion of ascorbic acid content and antioxidant capacity in fruit juice-soy milk beverages with prolonged chilled storage.[35] A study on cold storage of blueberry juice demonstrated stability in antioxidant capacity for up to 10 days of refrigeration at 4°C, yet a significant loss of 83% anthocyanins was observed.[36] This could be possibly be due to reactions with other phenolic compounds in the juice.

Microbiological Changesin Maoberry Juice after Processing and During Storage

From Table 1, the initial amounts of total plate counts, yeasts-molds and fecal coliforms in fresh maoberry juice were 6.14, 2.05, and 1.60 log CFU/mL, respectively. In this term, it was found that all indicator microorganisms were effectivelyeliminated via applied processing methods. The microbial quality of the treated samples complied with the standard for ready-to-drink maoberry juice.[37] Many scientific findings have demonstrated the inhibitory effects of high pressure treatment on the survival of microorganisms, resulting from changes in cell morphology and inhibition of metabolic reactions essential for cell maintenance and genetic mechanisms. The ultra-sonication process enhances formation of biocides, includingfree radicals and hydrogen peroxide, through cavitation, thus preventing the growth of microbial vegetative cells.[22]

Following storage conditions, the microbiological quality of processed juice samples remained within the general standard ofready-to-drink maoberry juice.[37] During microbiological testing, none of the indicator microbial species were detected throughout the entire storage period (data not shown). Similarly, Wu *et al*. reported that thermal and high hydrostatic pressure processing was able to effectively reduce microbial count of pineapple fruit juice to a satisfactory level.[38] According to a study by Donsì *et al*., pulsed high pressure processing was shown to reduce initial microbial count of Annurca apple and orange juices, while maintaining low levels of microbial proliferation following 21 days of chilled storage, which complied with the (EC) Regulation no. 2073/2005 on the microbiological criteria for foodstuffs.39 Furthermore, significantly lower levels of microbial load and increased shelf-stability was observed following pasteurization, highpressure treatment and refrigerated storage of acidic appleand sugarcane juices.40-41

Conclusion

In summary, different processing methods were evaluated based on their effectiveness in preserving maoberry juice. Pressurization and ultra-sonication of maoberry juice were shown to retain higher ascorbic acid compared to conventional method. Phenolic content, on the other hand, noticeably reduced following thermal processing methods of pasteurization and high-amplitude ultra-sonication. With prolonged refrigerated storage, the antioxidant capacity of all maoberry juices declined. All processing methods were shown to reduce initial microbial load of fresh maoberry juice to a non-detectable amount, while maintaining their microbial quality during prolonged refrigerated storage. Although pressurization has demonstrated the ability to preserve bioactive compounds and enhance storage stability, few studies have observed the effects of novel processing adaptations on maoberry juice. Further research would be necessary to fully determine thecapacity and effectiveness of alternative preservation methods.

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Conflict of Interest

The authors declare no conflict of interest.

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