Quality changes in high hydrostatic pressure and thermal pasteurized grapefruit juice during cold storage

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Abstract This study evaluated the effects of high hydrostatic pressure (HHP) and thermal pasteurization (TP) on microbial counts, physicochemical properties, antioxidant characteristics, naringin and naringenin contents, and naringinase activity of grapefruit juice during 21 days cold storage period. Results showed that HHP and TP significantly decreased the total microbial, coliform, and yeast counts. No significant differences between HHP-treated grapefruit juice (600 MPa/5 min) and untreated fruit juice with respect to physicochemical properties such as total titratable acidity, pH, and total soluble solids was observed after 21 days of storage. Although HHP affected the colour and antioxidant characteristics of grapefruit juice, the extent of effect was significantly lower than that for TP-treated fruit juice. This demonstrated that HHP could better maintain the original flavour and quality of grapefruit juice compared to TP. In addition, 92% naringinase activity was maintained in HHP-600 group on Day 21, which increased the degradation of bitter naringin into non-bitter naringenin during the cold storage of grapefruit juice. In summary, HHP can simultaneously maintain the microbiological safety of grapefruit juice along with its original quality characteristics. HHP can effectively extend the storage

period and safety during cold chain transport, and hence highly applicable in the grapefruit juice industry.

Keywords Grapefruit juice · High pressure · Naringinase · Pasteurization

Introduction

Grapefruit (*Citrus paradisi* Macfad) is one of the economic citrus species grown in the Asian countries and also in America. Grapefruits have recently attracted attention due to their beneficial health effects, including antioxidant, anti-hyperlipidaemia, anti-cancer, anti-obesity, and anti-diabetic activities, mostly attributed to their intrinsic bioactive components, including vitamin C, flavonoids, and limonin (Ding et al. 2013; KunduSen et al. 2011; Mäkynen et al. 2013; Tsai et al. 2007). However, citrus fruits also contain bitter flavanone glycosides, such as naringin, limonin, and nomilin. Naringin lends a bitter and unpleasant taste to grapefruit juice, thereby posing an important economic challenge in commercial production of the juice. Among the few commercial de-bittering processes available, enzymatic de-bittering technology is regarded as the most promising method owing to its high specificity and efficiency. In addition, it is easy to remove the bitter taste during large-scale commercial production (Yadav et al. 2010). Naringinase and β -D-glucosidase have many practical applications in the fruit juice industry since they can degrade the bitter naringin into non-naringin substances, thereby improving the taste of orange juice (Ni et al. 2012). Real et al. (2007) had reported that treatment with high hydrostatic pressure (HHP) of 160 MPa can promote naringin hydrolysis in orange juice.

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High pressure processing (HHP) is a new foodstuff processing technology. It can inhibit pathogenic microorganisms in foodstuffs at room temperature, increase shelf life, and avoid the destruction of heat-sensitive components. It often utilises water as a pressure transmitting medium, with a pressure of 600 MPa being used often in commercial foodstuff production (Wang et al. 2016). Increase in the demand for foods with organoleptic properties has resulted in extensive research on non-thermal storage technologies, which have the least influence on the sensory characteristics of fruit and vegetable products (Oms-Oliu et al. 2012). The Food and Drug Administration of the USA has approved HHP as a non-thermal processing technique to replace the conventional pasteurisation method. HHP has already been performed on a few commercial products sold in the market (including fruit juice, mandarin oranges, grapefruits, apples, oranges, and carrot juice) (Guerrero-Beltrán et al. 2005). Bayindirli et al. (2006) found that treatment with 300 MPa could inactivate even pressure-resistant pathogens, such as *Staphylococcus aureus*, *E. coli* O157: H7, and *Salmonella* species. Conversely, HHP of 400 MPa at mild temperature (< 50 °C) inactivated PPO activity. Varela-Santos et al. (2012) found that HHP at 350 MPa for 2.5 min is sufficient to decrease the naturally occurring microorganisms, responsible for decay, to undetectable levels in pomegranate juice, and that microbiological storage period could be extended to more than 35 days when juices were stored in 4 °C-cold storage. Fernández-García et al. (2001) also reported that orange, lemon, and carrot juice treated by HHP and cold storage for 21 days resulted in no evident difference in anti-oxidative capacities, vitamin C levels, or carotene content. Compared to conventional pasteurisation methods, HHP could maintain antioxidant activity in orange juice.

Therefore, determination of the effect of HHP on microbial counts and physicochemical characteristics of grapefruit juice would be required to evaluate the commercial applicability of HHP in grapefruit juice-related beverages. Our previous studies had demonstrated that HHP treatments at 100 MPa, 200 MPa, 300 MPa, and 400 MPa significantly reduced the concentration of naringin in grapefruit juice from 851.09 mg/l (control) to 475.64 mg/l, 468.77 mg/l, 539.43 mg/l, and 458.34 mg/l, respectively (Wang, 2015). The current study aimed to compare the effects of HHP and thermal processing (TP) on microbial counts, physicochemical characteristics, antioxidant activity, naringin and naringenin contents, and naringinase activity in grapefruit juice after processing, treatment, and storage at 4 °C.

Materials and methods

High pressure and thermal pasteurization of grapefruit juice

Fresh grapefruits were purchased from a local market in Taipei City, Taiwan. A juicer was used to prepare grapefruit juice, which was then vacuum-packed in polyethylene bags (50 ml) and placed in a 6.2-l pressure container (Baotou Kefa Inc., Baotou City, China). The packages were compressed for 5 min at 300 and 600 MPa. Deionised water was used as transmit medium at an initial temperature of 10 °C, and pressure was increased at a rate of 300 MPa/min with an error of ± 10 MPa to the target pressure. The pressurised samples were stored at 4 °C. For TP, grapefruit juice was vacuum-packed in polyethylene bags (50 ml) and immersed in an 85 ± 1 °C water bath for 45 s. After achieving the necessary processing conditions, the packages were moved to ice bath immediately. Unprocessed grapefruit juice was used as control sample for comparison with TP- and HHP-treated juice.

Physicochemical analysis

A refractometer (ATAGO Co., Ltd.) was used to quantitate soluble solids (°Brix) at 25 °C. A pH meter was used to measure the pH of fruit juice samples (at 20 ± 1 °C) using glass electrodes (Mettler Toledo, Columbia, OH). Titratable acidity was determined based on the procedure described by AOAC (1990). A HunterLab LabScan spectrophotometer (CR-200; Minolta) was used for all colour measurements. A colorimeter (HunterLab Co., VA) was used for colour analysis at 25 ± 2 °C using the reflective mode. Colour was expressed as L^* , a^* , and b^* values. In addition, the following formula was applied to calculate the colour difference: $\Delta E = ((\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2)^{1/2}$.

Naringin and naringenin contents and naringinase activity measurements

Naringin and naringenin contents were analysed using high-performance liquid chromatography (HPLC), based on the method described by Ribeiro et al. (2010). The HPLC system was composed of a high-pressure solvent transfer pump, a variable wavelength UV–Vis detector (model no. L-7400), and a 20- μ l injectable sample. Separation was carried out on an RP-18 analysis column, at a temperature of 35 °C and detection wavelength of 280 nm. The mobile phase [11.4% methanol, 26.6% acetonitrile, and 62% deionised water (v/v)] helped the juice to flow with isocratic elution at 0.4 ml/min for 28 min. Naringinase activity, representing the ability to degrade naringin

into naringenin, was expressed as the amount of enzyme required to produce 1 μmol naringenin per min per unit.

Antioxidant analysis

The concentration of phenolic compounds was measured using the method described by Taga et al. (1984), taking gallic acid as the standard. A 0.1-ml sample was mixed with 2.0 ml of 0.02 g/ml Na_2CO_3 . After 2 min, 0.1 ml 50% Folin–Ciocalteu reagent was added to the mixture and the latter allowed to stand for 30 min. A spectrophotometer was used to measure its absorbance at 750 nm. Spectrophotometry was employed to quantitate the levels of flavonoids based on the method described by Chang et al. (2002). Approximately 0.1 ml of grapefruit juice, 0.2 ml of deionised water, and 30 μl of NaNO_2 were mixed together. After 5 min, 30 μl of 5% $\text{Al}(\text{NO}_3)_3$ was added to the mixture and left to stand for 6 min. Absorbance (at 510 nm) of the mixture was measured immediately after the addition of 0.2 ml 1 M NaOH and 0.44 ml deionised water. Quercetin (QE) was used as a standard. A six-point standard curve (0–500 $\mu\text{g}/\text{ml}$) was used to measure the total flavonoid content in grapefruit juice. Data was expressed as mg of QE/ml grapefruit juice.

Trolox equivalent antioxidant capacity (TEAC) was calculated to quantitate the total antioxidant activity of grapefruit juice based on the method described by Miller et al. (1993), with slight modifications. TEAC is based on the antioxidant removal of blue-green $\text{ABTS}^{\cdot+}$ (free radical cations) relative to that of $\text{ABTS}^{\cdot+}$, by water-soluble vitamin E analogue and Trolox. $\text{ABTS}^{\cdot+}$ is produced by the interaction between ABTS (100 μM), hydrogen peroxide (50 μM), and peroxidase (4.4 units/ml). For antioxidant activity, 0.25 ml of grapefruit juice, equivalent volumes of ABTS, hydrogen peroxide, peroxidase, and 1.5 ml of deionised water were mixed completely. After 10 min of mixing, absorbance at 734 nm was recorded. The TEAC value was calculated based on the reduction in absorbance at 734 nm after addition of the reactants. A dose–response curve of Trolox was drawn and antioxidant capacity was shown as TEAC. Higher the TEAC value of a sample, stronger is its antioxidant activity.

Microbiological counts

Ten millilitres of grapefruit juice were mixed with 90 ml of buffered peptone water (BPW). The mixture was serially diluted in BPW. A 1-ml sample of each diluted solution was inoculated on a 3 M PetrifilmTM plate for enumeration of aerobic bacteria, *E. coli*, yeast, and mold. Enumeration of aerobic bacteria (aerobic plate count; APC) was performed after incubation at 35 °C for 48 h, whereas that of *E. coli* and yeast and mold (Y&M) was carried out

after incubation at 25 °C for 120 h. The detection limit was 10 colony forming units (CFU)/ml (AOAC 2011). The grapefruit juice was stored at 4 °C for 28 days. Microbiological counts were performed on days 0, 7, 14, 21, and 28 for each HHP-treated, TP-treated, and control juice samples.

Statistical analysis

Results were expressed as the mean values of triplicate experimental data. Data were expressed as means $\pm$ standard deviations, and a statistical analysis system (SAS Inc., NC) was used for analysis. One-way analysis of variance was performed. Duncan's multiple range test was used to compare the significant differences between the mean values. A difference of $p < 0.05$ was considered statistically significant.

Results and discussion

Effects of HHP and TP on physicochemical characteristics

Table 1 shows the effects of HHP (300 or 600 MPa for 5 min) and TP (85 °C for 45 s) on total soluble solids ($^{\circ}\text{Brix}$), total titratable acidity (%), pH, colour changes (ΔE), and browning index after 21 days of storage of grapefruit juice at 4 °C. Neither HHP nor TP had any significant effect on the total soluble content in grapefruit juice. Total titratable acidity of the control and HHP-600 samples gradually increased, after storage for 21 days, from 0.04 and 0.04%, respectively, on Day 0 to 0.93% and 0.39%, respectively, on Day 21. In contrast, pH values of the HHP-300 and HHP-600 samples decreased from 5.06 and 5.8, respectively, on Day 0 to 3.40 and 4.7, respectively, on Day 21 in storage. Microbial counts revealed an increasing trend in the control and HHP-300 samples during the storage period. Since microorganisms metabolise the sugar in fruit juice to produce organic acids, thereby decreasing pH, it might explain why total titratable acidity increased and pH decreased in the results of our present study. In the HHP-300 and HHP-600 juices, titratable acidity was maintained within 0.39–0.59, which was not significantly different from that observed on Day 21 in TP group (0.31). pH in HHP-300 group decreased to 3.4 on Day 21 while APC and Y&M counts were significantly increased on Day 21, hence implying that the microorganisms that survived the HHP treatment began to proliferate on Day 21, accelerating the pH reduction. No notable pH change was observed in either HHP-600 or TP sample after storage for 14 days. We also analysed colour changes in the untreated grapefruit juice, from different

Table 1 Physicochemical properties of grapefruit juice during 21 days of storage at 4 °C

Quality attributes	Treatment	Storage time (days)			
		0	7	14	21
Total soluble solids (°Brix)	Untreated	10.2 ± 0.1Aa	10.1 ± 0.1Aa	10.2 ± 0.1Aa	10.1 ± 0.1Aa
	HHP-300	10.2 ± 0.1Aa	10.1 ± 0.1Aa	10.2 ± 0.1Aa	10.0 ± 0.1Aa
	HHP-600	10.2 ± 0.1Aa	10.1 ± 0.1Aa	10.3 ± 0.1Aa	10.2 ± 0.1Aa
	TP	10.3 ± 0.1Aa	10.2 ± 0.1Aa	10.3 ± 0.1Aa	10.2 ± 0.1Aa
Total titratable acidity (%)	Untreated	0.04 ± 0.01Ad	0.16 ± 0.09Ac	0.46 ± 0.07Ab	0.93 ± 0.08Aa
	HHP-300	0.04 ± 0.01Ad	0.12 ± 0.03Bc	0.33 ± 0.08Bb	0.59 ± 0.05Ba
	HHP-600	0.04 ± 0.01Ac	0.07 ± 0.02Cb	0.09 ± 0.09Cb	0.39 ± 0.05Ba
	TP	0.04 ± 0.01Ac	0.05 ± 0.01Cc	0.08 ± 0.05Cb	0.31 ± 0.06Ba
pH	Untreated	5.8 ± 0.1Aa	4.1 ± 0.3Db	3.7 ± 0.3Bb	3.3 ± 0.4Bc
	HHP-300	5.8 ± 0.1Aa	4.7 ± 0.2Cb	3.9 ± 0.3Bc	3.4 ± 0.2Bd
	HHP-600	5.8 ± 0.1Aa	5.5 ± 0.1Ba	5.3 ± 0.2Aa	4.7 ± 0.2Ab
	TP	5.8 ± 0.1Aa	5.6 ± 0.1Aa	5.5 ± 0.1Aa	5.2 ± 0.1Ab
ΔE	Untreated	–eD	1.11 ± 0.03Db	1.75 ± 0.11Ba	1.83 ± 0.05Ba
	HHP-300	1.17 ± 0.08Cc	1.32 ± 0.06Cb	1.65 ± 0.09Ba	1.72 ± 0.03Ba
	HHP-600	1.52 ± 0.13Bb	1.62 ± 0.03Bb	1.74 ± 0.02Ba	1.70 ± 0.01Ba
	TP	2.71 ± 0.16Aa	2.90 ± 0.05Aa	2.87 ± 0.02Aa	2.97 ± 0.02Aa

All data are shown as the means ± standard deviations from three replicates. A, B, C, and D: different letters in the same column indicate statistically significant differences; a, b, c, and d: different letters in the same row indicate statistically significant differences ($p < 0.05$)

groups, on Day 0 as a reference. Analysis showed that as the processing pressure increased, extent of colour change also increased. On Day 0, the ΔE values of HHP-300 and HHP-600 samples were 1.17 and 1.52, respectively, whereas that of TP sample was higher (2.71). In addition, ΔE values did not significantly change with prolonged storage. Similar changes in ΔE were observed in all groups after storage for 21 days.

These results correspond to observations made in other studies. Nayak et al. (2016) had reported that HHP could preserve physicochemical properties, such as pH, sugar content, total acidity, viscosity, and apple juice colour. They also found that the aroma and flavour characteristics of apple juice remained unaffected in HHP-treated fruit juice samples that were stored at 4 °C. Yi et al. (2017) observed that HHP was associated with better natural colour retention in apple juice than TP. However, TP demonstrated advantages in enzyme inactivation by completely inactivating PPO and (Peroxidase) POD; PPO and POD seemed to be resistant to HHP. Both HHP and TP could maintain stable sugar and organic acid contents. Xu et al. (2015) compared HHP at 550 MPa for 5 min with HTST at 110 °C for 8.6 s for microbiological safety and colour of mixed pepper and orange juice. On Day 25 of storage, HHP was found to be better than HTST, since it could preserve natural flavour of the fresh fruit juice. HHP involves room temperature pasteurisation of foodstuffs, thereby avoiding adverse effects. Based on the above

results, we conclude that the use of effective pasteurisation methods to kill microorganisms in foodstuffs would help to preserve the quality of vegetable and fruit products during cold storage period. HHP can effectively maintain the microbiological safety of foodstuffs with less negative effects on relevant quality parameters of the products.

Quantitation of naringin and naringenin contents and naringinase activity

Table 2 shows the effects of HHP and TP on naringin and naringenin contents and naringinase activity in grapefruit juice. On Day 0, grapefruit juice from HHP-300 and HHP-600 groups showed a significant decrease in naringin content from 712.2 (control) to 514.6 and 428.3 μg/ml, respectively. Conversely, no significant difference in naringin content was observed in TP samples. As storage duration increased, naringin content in the various groups showed a gradual decreasing trend. After 7-day storage, the naringenin content of fruit juices that underwent different treatment strategies were significantly different. Compared to other groups, HHP-600 group had the highest naringenin content. On Day 21, the naringenin content of HHP-300, HHP-600, and TP groups were 417.6, 449.2, and 263.4 μg/ml, respectively. Analysis of naringenin content showed that it significantly increased in the control as well as HHP groups on Day 21 of storage. Compared to the values observed on Day 0, naringinase activity on Day 21 in TP

Table 2 Naringin and naringenin contents and naringinase activity of grapefruit juice during 21 days of storage at 4 °C

Quality attributes	Treatment	Storage time (days)			
		0	7	14	21
Naringin (µg/ml)	Untreated	712.2 ± 11.6Aa	705.2 ± 19.1Aa	695.6 ± 16.8Aa	689.3 ± 15.4Aa
	HHP-300	514.6 ± 17.2Ba	515.6 ± 11.5Ba	513.7 ± 10.6Ba	501.3 ± 15.7Ba
	HHP-600	428.3 ± 13.9Ba	399.8 ± 12.6Cb	303.4 ± 18.3Bb	292.5 ± 18.1Bb
	TP	508.4 ± 10.4Aa	503.8 ± 9.5Aa	505.7 ± 11.6Aa	501.5 ± 13.2Aa
Naringenin (µg/ml)	Untreated	261.3 ± 8.5Cb	288.2 ± 5.2Ca	285.1 ± 4.7Ba	291.2 ± 3.9Ca
	HHP-300	394.6 ± 9.2Bb	402.4 ± 6.5Bb	412.3 ± 7.1Aa	417.6 ± 5.3Ba
	HHP-600	418.4 ± 14.3Ab	431.2 ± 4.3Aa	436.2 ± 4.2Aa	449.2 ± 6.1Aa
	TP	245.5 ± 7.9Dc	251.2 ± 3.6Db	259.2 ± 5.3Ca	263.4 ± 5.2 Da
Aringinase (%)	Untreated	100.0 ± 1.6Aa	93.4 ± 5.2Ab	91.1 ± 7.9Ab	92.1 ± 4.6Ab
	HHP-300	97.5 ± 3.3Aa	94.3 ± 3.7Aa	95.3 ± 2.1Aa	93.5 ± 5.3Aa
	HHP-600	98.2 ± 1.5Aa	90.2 ± 4.9Ab	93.2 ± 3.8Ab	91.6 ± 3.3Ab
	TP	67.2 ± 3.9Ba	59.3 ± 3.4Bb	61.2 ± 5.4Bb	56.2 ± 6.5Bc

All data are shown as the means ± standard deviations from three replicates. A, B, C, and D: different letters in the same column indicate statistically significant differences; a, b, and c: different letters in the same row indicate statistically significant differences ($p < 0.05$). –, not determined (levels of sucrose content were below the detection limit of the corresponding treatment). ND, not determined (levels of enzyme activity were below the limit of detection at the corresponding treatment)

group decreased, whereas 92%, 93%, and 91% of naringinase activity was retained in the control, HHP-300, and HHP-600 groups, respectively. Reduction in naringin content in the control, HHP-300, and HHP-600 groups corresponded to increasing storage duration. Compared to the data monitored on Day 0, the control, HHP-300, HHP-600, and TP samples showed a reduction of 3.2%, 2.5%, 31.7%, and 1.3%, respectively, on Day 21. Changes in naringin content were opposite to those in naringenin content of the samples. These results were related to the naringinase activity measured in the present study. Flavonoid enzymes are crucial for the conversion of naringin to naringenin (Real et al. 2007). Our results showed that no treatment significantly affected naringinase activity in grapefruit juice. After storage for 21 days, the control and HHP groups maintained approximately $\geq 90\%$ of naringinase activity. However, TP showed the highest inhibitory effect on enzyme activity. After HHP treatment, naringinase activity was retained, which promoted naringin conversion, thereby increasing naringenin content. Conversely, TP inhibited enzyme activity, due to which, the highest naringin content was maintained.

Bitter components contained in citrus fruits pose a problem in commercial fruit juice production. The main contributors to the bitter taste are naringin and limonin. Even though existing high-pressure applications can inhibit enzyme activity, evidence shows that high pressure can also induce stability and activation of some enzymes (Eisenmenger and Reyes-De-Corcuera 2009). Pedro et al. (2007) designed an immobilised enzyme system for high-

pressure naringin hydrolysis. Pressure of naringin-immobilised Ca-alginate beads (2%) for naringin hydrolysis was shown to result in higher residual activity when reaction rate increased by 35–70% from atmospheric pressure (0.1 MPa) to high pressure (160 MPa). Ribeiro et al. (2010) also reported a higher naringin conversion rate (81%) when naringinase Ca-alginate beads were used for naringin hydrolysis during 30 min of processing at 205 MPa and 60 °C. Marques et al. (2007) demonstrated high pressure–temperature effects on naringin hydrolysis by response surface methodology. They predicted that the maximum naringinase activity at 41 °C and 158 MPa was 0.13 mM/min and the maximum reducing sugar concentration at 38 °C and 168 MPa was 8 mM. Interaction of temperature and pressure exhibited significant effects on naringinase activity and decreased sugar accumulation after 1 h. In that study, high pressure promoted naringinase hydrolysis of naringin to naringenin to reduce the bitterness of grapefruit juice. HHP-induced naringinase activation enables it to be suitable for processing grapefruit juice, since modification of conditions can optimise naringinase activity, simultaneously inactivating unwanted pressure-unstable microorganisms.

Antioxidant characterizations

Table 3 describes the effects of HHP and TP on antioxidant properties of grapefruit juice after 21 days of cold storage. The phenol contents on Day 1 were 4.17 mg/ml, 4.52 mg/ml, and 4.29 mg/ml in the control, HHP-300, and HHP-600

Table 3 Antioxidant properties of grapefruit juice during 21 days of storage at 4 °C

Quality attributes	Treatment	Storage time (days)			
		0	7	14	21
Total phenolics (mg/ml)	Untreated	4.17 ± 0.11Ab	4.72 ± 0.10Ba	4.77 ± 0.08Ba	4.29 ± 0.12Ab
	HHP-300	4.52 ± 0.14Ab	5.11 ± 0.13Aa	4.92 ± 0.17Ba	4.59 ± 0.09Ab
	HHP-600	4.39 ± 0.16Ac	5.19 ± 0.09Aa	5.10 ± 0.11Aa	4.61 ± 0.23Ab
	TP	4.51 ± 0.18Ab	4.90 ± 0.12Ba	4.74 ± 0.09Ba	4.01 ± 0.31Ab
Total flavonoids (mg/ml)	Untreated	0.42 ± 0.02Aa	0.34 ± 0.03Ab	0.28 ± 0.01Ac	0.19 ± 0.01Ad
	HHP-300	0.43 ± 0.0Aa	0.36 ± 0.02Ab	0.26 ± 0.02Ac	0.17 ± 0.01Ad
	HHP-600	0.39 ± 0.02Aa	0.31 ± 0.03Ab	0.29 ± 0.01Ac	0.16 ± 0.01Ad
	TP	0.40 ± 0.05Aa	0.34 ± 0.02Ab	0.19 ± 0.01Bc	0.10 ± 0.01Bd
TEAC (mM trolox)	Untreated	7.39 ± 0.21Aa	7.32 ± 0.11Aa	6.89 ± 0.18Aa	6.31 ± 0.18Ab
	HHP-300	7.55 ± 0.15Aa	6.91 ± 0.21Bb	6.90 ± 0.25Ab	6.29 ± 0.23Ac
	HHP-600	7.64 ± 0.17Aa	6.79 ± 0.17Bb	6.81 ± 0.14Ab	6.51 ± 0.18Ac
	TP	7.18 ± 0.11Ba	6.74 ± 0.16Bb	6.18 ± 0.17Bc	5.27 ± 0.19Bd

All data are shown as the means ± standard deviations of three replicates. A and B: different letters in the same column indicate statistically significant differences; a, b, c, and d: different letters in the same row indicate statistically significant differences ($p < 0.05$)

groups, respectively. Since HHP resulted in tissue softening, increase in total phenol content of HHP-treated tissues may be attributed to the destruction of plant cells and tissues, resulting in substance extraction. The processing conditions used in the present study should not cause significant destruction of total phenol content; however, we observed a small decrease in total phenol content of grapefruit juice as the storage duration increased. This decline was significantly observed in the TP sample (approximately 11% on Day 21). A previous study had reported theories on oxidative cleavage of phenolic substances and their polymerisation reactions with proteins during storage. These theories might help in understanding the reduction in total phenol content observed here (Cao et al. 2011). We also observed that total flavonoid content decreased with increase of storage duration, similar to the trend of total phenolic substances. TP or HHP did not have any significant effect on total flavonoid content. On day 0, HHP significantly increased TEAC in grapefruit juice from 7.39 mM Trolox in the control group to 7.55 and 7.64 mM Trolox in HHP-300 and HHP-600 samples, respectively. Correlation between antioxidant levels resulted in significant increase in TEAC values, considering the effect of HHP on total phenol content on Day 0. However, prolonging storage duration resulted in decrease of TEAC from 7.55, 7.64, and 7.18 mM in the HHP-300, HHP-600, and TP groups, respectively, on Day 0 to 6.29, 6.51, and 5.27 mM, respectively, on Day 21. Among these groups, TP group showed the most significant decline, retaining only 73.3% of TEAC.

The present study showed that HHP has mild destructive effects on the bioactive components of grapefruit juice,

thereby retaining more natural plant nutrients and relatively high natural antioxidant ability in fruits and vegetables. A study by Wang et al. (2016) showed that HHP treatment at 500 MPa for 10 min can retain total phenol, total flavonoid, and resveratrol contents in mulberry juice at levels significantly higher than that of fruit juice samples treated with TP. Chen et al. (2015) reported that HHP treatment at 400 and 600 MPa resulted in asparagus juice having similar microbiological safety as that treated with TP. However, compared to TP, HHP treatment maintained significantly higher ascorbic acid, rutin, total phenolic contents, and total antioxidant activity. Alexandrakis et al. (2014) observed that HHP did not have any significant effect on the antioxidant activity of Sea Buckthorn Juice; however, even mild treatment at 60 °C for 1 min could decrease its antioxidant activity. In summary, these results show that HHP promotes the extraction of antioxidants, preservation of more functional components, and reduction of natural nutrient loss. All these advantages directly suggest HHP technology as a suitable substitute for TP technology.

Changes of microbial counts in juice

Figure 1 shows the effects of HHP on APC, coliform, and Y&M levels in grapefruit juice after storage at 4 °C for 21 days. The average initial microbial counts for aerobic microorganisms, coliforms, and Y&M in the control (unprocessed) grapefruit juice were 2.76, 1.52, and 2.04 log CFU/ml, respectively. Our results showed that HHP remarkably reduced AP, coliforms, and Y&M counts ($p < 0.05$). HHP at 300 MPa showed a decrease of 0.7 log

Fig. 1 Growth curve of **a** aerobic plate count, **b** coliforms, and **c** yeasts and molds in untreated, TP-, and HHP-treated grapefruit juice during storage at 4 °C for 21 days. Data are expressed as the mean $\pm$ SD ($n = 3$)

CFU/ml in Y&M counts, although APC and coliform counts did not show any significant decrease. Conversely, HHP at 600 MPa resulted in a significant decrease in APC, by more than 1.5 log CFU/ml, along with decrease in coliform and Y&C counts, below detectable levels (< 1.0 log). Exerting more than 600 MPa of pressure for more than 5 min may result in similar reduction in microorganism counts as seen in TP (the common processing method used in food industry). There was no significant difference in microbial counts in grapefruit juice between HHP-300 and control samples. APC and coliform counts in the control samples reached peaks of 5.2 and 3.6 log CFU/ml, respectively, on Day 14. On Day 21, these values gradually

decreased to 4.6 and 3.2 log CFU/ml, respectively. Similarly, Y&M counts reached 3.9 log CFU/ml on Day 14, although they did not significantly decrease as storage prolonged, and were maintained at 3.8 log CFU/ml. After 4 °C-storage for 21 days, HHP-600 group showed a significant decrease in microbial counts (1.1 log CFU/ml vs 4.6 log CFU/ml) and a significantly lower APC count than the control group. The coliform and Y&M counts of HHP-600 group decreased below the detection limit (1.0 log CFU/ml). Compared to the control group on Day 21, TP and 600 MPa-treated grapefruit juice (considered to be effective against all microorganisms) resulted in a decrease in APC burden below 3.3 and 3.5 log CFU/ml, respectively, similar to the trends shown for coliform and Y&M counts. These results showed that HHP at 600 MPa can remarkably reduce APC, coliforms, and Y&M in grapefruit juice and significantly extend shelf life upon 4 °C-storage.

A summary of the mechanism of inactivation of microorganisms by HHP would be that high pressure causes a series of physiological changes in microbial cells, including changes in microbial permeability or rupture of cell structure, irreversible damage to cell membrane, and functional defects in osmoregulation, resulting in the loss of normal metabolic functions (Huang et al. 2014). Previous studies had shown that HHP can extend the shelf life of plant-based foodstuffs. Nayak et al. (2016) showed that HHP decreased microbial counts in apple juice. After 60 days of cold storage, total bacterial and Y&M counts were both maintained below 1 log CFU/ml. Wang et al. (2016) found that HHP treatment of mulberry juice at 500 MPa for 10 min achieved similar microbiological safety as TP at 85 °C for 15 min, and could be used as a substitute for conventional TP in the production of high-quality mulberry juice. Kultur et al. (2018) found that treatment at 400 MPa for 15 min inhibited aerobic mesophilic bacteria and total yeasts and molds in vegetable-based infant foods. All these studies showed that HHP inhibits the growth of microorganisms in fruit and vegetable products during the cold storage period. Therefore, this technology can aid in improving microbiological quality and safety and extend the shelf life of food products.

Conclusion

HHP at 600 MPa for 5 min can ensure microbiological safety of grapefruit juice when stored at 4 °C for 21 days. With regard to the physicochemical properties of grapefruit juice, although HHP caused significant changes in colour, it was lower than that observed in TP-treated juices. In addition, HHP-treated fruit juices preserved more antioxidants and higher antioxidant capacity than TP-treated juices. In addition, HHP increased naringinase activity,

thereby increasing the degradation of bitter naringin into non-bitter naringenin during storage of grapefruit juice. Therefore, HHP might be an effective method for extending the shelf life of grapefruit juice in long-term cold storage. Compared to TP, HHP demonstrated better retention of quality and sensory characteristics as well as natural nutritional compounds, beneficial to human health, in grapefruit juice.

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