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Comparative study of quality of cloudy pomegranate juice treated by high hydrostatic pressure and high temperature short time

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ABSTRACT

Inactivation of microorganisms and its kinetic model of high hydrostatic pressure (HHP) processing of cloudy pomegranate juice at different pressures (300 and 400 MPa) and different treatment times (2.5, 5, 10, 15, 20, and 25 min) were studied. Besides, HHP (400 MPa/5 min) and high temperature short time (HTST) (110 °C/8.6 s) treatment were comparatively evaluated by examining their impacts on microorganisms, pH, total soluble solids (TSS), titratable acidity (TA), color, total phenols, anthocyanins, antioxidant capacity and shelf-life characteristics of 90 days at 4 °C.

The inactivation effect of microorganisms by HHP fitted Weibull model well and HHP at 400 MPa/5 min inactivated microorganisms effectively. The microbial safety was ensured in HHP-treated and HTST-treated sample. A greater retention of the original color, anthocyanins and antioxidant capacity and increased total phenols were observed in HHP-treated samples immediately after processing. During storage, color changed and anthocyanins content, total phenols and the antioxidant activity decreased, where the changes depended on the applied treatments. The pH, TSS and TA did not show significant change immediately after HHP or HTST treatment and during storage.

Industrial Relevance: Cloudy pomegranate juice is one of the most popular fruit juice and requires strict processing and storage conditions to keep the safety and quality. Our research presents a fair comparison between HHP and HTST treatment. The available data shows the different impacts on cloudy pomegranate juice of HHP and HTST treatment and the changes of quality during storage. This study would provide technical support for commercial application, evaluation and the criteria establishment for commercial production of HHP and HTST treatment in juice industry, and also provide a non-thermal treatment to meet the growing demand from consumers for healthier food products.

1. Introduction

Pomegranate (*Punica granatum* L.), one of the important fruits grown in Turkey, Iran, USA, Middle East, Mediterranean and Arabic countries, is originated from South-East Asia and has two thousand years of cultivation history in China (Maskan, 2006). Pomegranate is consumed as fresh fruit and juice, also used in desserts and salad (Al-Maiman & Ahmad, 2002). The richly colored grains in pomegranate contain considerable amount of acids, sugars, Vitamin A, Vitamin E, polysaccharides, important minerals and phenolic compounds such as catechins, ellagic tannins and anthocyanins such as 3-glucosides and 3,5-diglucosides of delphinidin, cyanidin, and pelargonidin and give a delicious and nutritional juice (López-Rubira, Conesa, Allende, & Artés, 2005). Several studies have highlighted the nutritional and bioactive compounds and the antioxidant activity of pomegranate juice. For instance, Gil, Tomas-Barberan, Hess-Pierce, Holcroft, and Kader (2000) and Seeram et al. (2008) have reported the pomegranate juice contained much more antioxidant compounds than other fruit juices and beverages and had the greatest antioxidant activity among the commonly consumed polyphenol-rich beverages in the US market. Braga et al. (2005) and Malik and Mukhtar (2006) found that attributed to its phenolic fraction, pomegranate juice had anticarcinogenic, antimicrobial, antioxidant, antiviral and anti-atherogenic effects. Some clinical research studies suggested that pomegranate juice changed the blood parameters, increased the prostate specific antigen, improved sperm quality and was helpful against heart disease, Alzheimer's disease and cancer (Khan, Afaq, Kweon, Kim, & Mukhtar, 2007; Turk et al., 2008). As reported, a glass of pomegranate juice contained about 40% of the Recommended Daily Allowance (RDA) of Vitamin C and pomegranate juice was sometimes regarded as a functional food (Du, Wang, & Francis, 1975).

Owing to its nutrition and taste, the demand for pomegranate juice has increased. Pomegranate has a brief harvest season and fresh

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pomegranate juice is susceptible to spoilage, therefore further processing is desirable to extend shelf-life. Thermal processing is the most commonly used preservation technique. However, many reactions such as pigment degradation, especially carotenoids or anthocyanins, and browning reactions such as the Maillard reaction, enzymatic browning and oxidation of ascorbic acid take place during thermal processing, which induce adverse effects on sensory and nutritional values (Ibarz, Pagan, & Garza, 1999; Suh, Noh, Kang, Kim, & Lee, 2003). It has been reported that thermal processing caused the loss of nutritional components and changes of color in strawberry juice (Patras, Brunton, Da Pieve, & Butler, 2009). It also has been found that the more severe the heat treatment was, the more destruction of carotenoids occured (Chen, Peng, & Chen, 1995). Dede, Alpas, and Bayindirli (2007) found that heat treatments at 60 °C for 5–15 min and 80 °C significantly ($p < 0.05$) reduced the ascorbic acid content and the free-radical scavenging activity of carrot juice. Patras et al. (2009) found that anthocyanins readily degraded and color quality lost in anthocyanins containing juices due to thermal process. Therefore, in order to preserve the sensory and nutritional quality,there is a real need to find a novel method to destroy undesirable microorganisms and minimize the degradation of the functional molecules.

Non-thermal processes, including high hydrostatic pressure (HHP), high pressure carbon dioxide, high-intensity pulsed electric fields, oscillating magnetic fields, light pulses, and irradiation, can meet the demand of consumers for natural, fresh-like and safe food (Barbosa-Canovas, Pothakamury, Palou, & Swanson, 1998). HHP, one of the most promising methods, uses water as pressure transmitting medium to subject foods to 100–1000 MPa at room or mild process temperatures (<60 °C) and instantaneously transmits isostatic pressure to the product, independent of size, shape and food composition yielding highly homogeneous products (Bala, Farkas, & Turek, 2008; Oey, Lille, Van Loey, & Hendrickx, 2008). Structure of high-molecular-weight molecules such as proteins and carbohydrates are altered, harmful pathogens and vegetative spoilage microorganisms are inactivated by HHP treatment. However, owing to the limited impacts of HHP treatment on the covalent bonds of low molecular-mass compounds, smaller molecules such as volatile compounds, pigments, vitamins, and other compounds connected with the sensory, nutritional, and health promoting are unaffected or affected very minimally (Bala et al., 2008; Oey et al., 2008; Zabetakis, Leclerc, & Kajda, 2000).

HHP has been applied to fermented foods such as cheeses, ham and yogurt, and some fruit and vegetable products such as tomato puree (Rodrigo, Van Loey, & Hendrickx, 2007), apple juice (Baron, Dénes, & Durier, 2006; Valdramidis et al., 2009), mango pulp (Ahmed, Ramaswamy, & Hiremath, 2005), orange juice (Katsaros, Tsevdou, Panagiotou, & Taoukis, 2010), and strawberry puree (Cao et al., 2011), some of which are currently available on market. However, there has not been pomegranate juice processed by HHP on the market. Only did Varela-Santos et al. (2012) and Ferrari, Maresca, and Ciccarone (2010) study the effect of HHP on pomegranate juice. Ferrari et al. (2010) has studied the application of HHP for the stabilization of clear pomegranate juice and the different effects of processing variables (pressure, temperature and time) on the microbiological stability and properties. Varela-Santos et al. (2012) has studied the effect of HHP processing (350~550 MPa for 30, 90 and 150 s) on microorganisms, physicochemical properties, bioactive compounds of pomegranate juice and its shelf-life during 35 days of storage at 4 °C. However, there has been no comparative study on the microorganisms, quality attributes and shelf-life of cloudy pomegranate juice (CPJ) treated by HHP and thermal treatment, especially high temperature short time (HTST).

Therefore, the study was undertaken to investigate: (1) the effectiveness and kinetic model about microorganisms inactivation of HHP processing on CPJ; (2) the comparison of microorganisms, color, pH, TSS, TA, total phenols, anthocyanins, and antioxidant capacity between HHP-treated and HTST-treated CPJ; (3) the comparison of the microbiological safety and the quality characteristics between HHP-treated and HTST-treated CPJ during storage of 90 days at 4 °C.

2. Materials and methods

2.1. Preparation of CPJ

Pomegranates (Pyaman, Hetian, Xinjiang, China) were purchased from the wholesale market in Urumqi (Xinjiang, China) and stored at about 8 °C in the market. The pomegranates were transported to Beijing by air and stored at an average temperature of 4 °C. The pomegranates were washed with tap water and manually separated into sacs. The juice in the sacs was manually pressed and extracted, then filtered with 400 mesh filter cloth, centrifuged in 9000 rpm, mixed to ensure its uniformity. The CPJ was stored at the cold warehouse about 4 °C until the experiment started.

2.2. HHP process system

HHP treatment was carried out by a hydrostatic pressurization unit with a 7 L capacity (HHP-650, Baotou Kefa Co., Ltd., Inner Mongolia, China). The pressure-transmitting fluid was distilled water. The pressurization rate was about 120 MPa/min and the depressurization was immediate (<3 s). The treatment time reported in this study did not include the pressure increase and release time. A pressure transducer (PPM-T229A, Changsha Taihe Electronic Equipment, Co., Ltd, Fujian, China) was attached to the vessel and used to measure the pressure in the vessel. The pressure level and treatment time were continuously recorded during the pressurization cycle. The initial temperature in the processing vessel was nearly 20 °C and when 300 MPa and 400 MPa were applied, the temperature reached approximately 29 °C and 32 °C due to the adiabatic compression, which was calculated as 3 °C/100 MPa, since there was no temperature monitoring and recording system (Bala et al., 2008). When the pressurization was finished, the temperature quickly dropped to its initial temperature due to heat transfer from the samples to the stainless steel of the vessel (Chen & Hoover, 2003).

2.3. HHP processing

Freshly squeezed CPJ was filled into the bottles identical to the ones used for the control samples, and then placed into the vessel for HHP treatment. Samples were subjected to pressures of 300, 400 MPa for 2.5, 5, 10, 15, 20, and 25 min at ambient temperature. Based on microbiological analysis results of these treatments, 400 MPa/5 min was selected as the sterilized condition for shelf-life study of CPJ.

2.4. HTST processing

A tubular heat exchanger unit (Armfield FT74, HTST/UHT Processing Unit, Hampshire, England) was used for high temperature short time (HTST) pasteurization (110 °C/8.6 s). The juice entered and exited the heat exchanger at ambient temperature, and then was transferred into packages identical to the ones used before.

2.5. Storage conditions

The treated samples were stored at 4 ± 2 °C in the dark. Sample analysis was carried out after 0, 10, 20, 30, 45, 60, 75 and 90 days storage. Before each measurement, samples were equilibrated at an ambient temperature (20 ± 1 °C).

2.6. Microbiological enumeration

To detect the viable natural microorganisms in CPJ, the total plate count method was used (National Food Hygienic Standard of China

4789.2-2010). Untreated or treated samples were serially diluted with sterile 0.85% NaCl solution, and 1.0 mL of each dilution was plated into duplicate plates of appropriate agar. Nutrient agar (Beijing Land Bridging Technology Co. Ltd., Beijing, China) was used for counting the viable total aerobic bacteria (TAB) after incubation at 37 °C for 48 ± 2 h. Rose Bengal agar (Beijing Land Bridging Technology Co. Ltd., Beijing, China) was used for counting the viable yeasts and molds (Y&M) after incubation at 27 °C for 72 ± 2 h.

After incubation, the colonies were enumerated. Inactivation effect was expressed as $\log_{10}N$, where N represented the number of microorganisms colonies of the untreated or treated samples.

2.7. Kinetic study of HHP microbial inactivation

The data that reflected the effect of microbial inactivation was fitted to the modified Weibull equation (Chen & Hoover, 2004; Pilavtepe-Celik, Buzrul, Alpas, & Bozoğlu, 2009)

$$\log_{10}S = -bt^n$$

Where: $\log_{10} S = \log_{10} \frac{N}{N0} N_0$ was the number of initial count of microorganisms in control, and N was the corresponding viable count after HHP, b, n and t were the scale factors, shape factors and treatment time (min), respectively (Peleg & Cole, 1998). The Weibull distribution corresponds to a concave upward survival curve if $n < 1$, concave downward if $n > 1$, and linear if $n = 1$.

2.8. Measurements of quality parameters

2.8.1. Determination of color

Color assessment was conducted at an ambient temperature (20 ± 1 °C) using a Color Difference Meter (HunterLab ColorQuest XE, Hunter Associates Laboratory, Inc, Virginia, USA) in the reflectance mode. The chromo-meter was calibrated with a white color standard. Color was expressed in L^* for lightness, a^* for redness and b^* for yellowness. Three measurements were performed and results were averaged. In addition, total color difference (ΔE) was calculated using the following equations, where L^*_0, a^*_0 and b^*_0 were the control values for untreated CPJ.

$$\Delta E = \left[(L^*-L^*_0)^2 + (a^*-a^*_0)^2 + (b^*-b^*_0)^2\right]^{1/2}.$$

2.8.2. Determination of pH

The pH was measured at an ambient temperature (20 ± 1 °C) using digital Thermo Orion 555A pH meter (Thermo Fisher Scientific Inc, MA, USA). The meter was calibrated with commercial buffer solutions at pH 6.8 and 4.0. 10 mL CPJ was inserted with a pH electrode (Thermo Orion Ross 9103BN, MA, USA) and pH was recorded after stabilization.

2.8.3. Determination of total soluble solids (TSS)

The TSS was determined as °Brix at an ambient temperature (20 ± 1 °C) by WAY-2S digital Abbe Refraction meter (Shanghai Precision and Scientific Instrument Co., Shanghai, China).

2.8.4. Determination of titratable acidity (TA)

10 mL CPJ was titrated using standardized 0.02 mol/L NaOH to the phenolphthalein end point (pH = 8.2 ± 0.1). The volume of NaOH was converted to mg citric acid per L juice (Rodrigo et al., 2003).

2.8.5. Determination of total anthocyanins

The total anthocyanins measurement was based on the spectrophotometric pH differential method reported by Giusti and Wrolstad (2001) with small modifications. The CPJ was centrifuged at 12,000 rpm for 15 min at an ambient temperature (20 ± 1 °C). Then the supernatant was collected for further analysis.

The supernatant was diluted to 0.5:4.5 using 0.025 M potassium chloride solution and 0.4 M sodium acetate solution adjusted to pH 1.0 and 4.5 with HCl, respectively, then equilibrized for 15 min in the dark. The absorbance of each dilution was measured at 510 nm and 700 nm using a spectrophotometer (UV-726, Shimadzu, Shanghai, China), with a distilled water as blank. Anthocyanins content was calculated by the following equation.

$$\text{Anthocyanins content}(\text{mg/L}) = (\text{A} \times \text{MW} \times \text{DF} \times 1000)/(\varepsilon \times 1)$$

Where A = (A510–A700) pH1.0 − (A510–A700) pH4.5, MW is the molecular weight of cyanidin-3-glucosde (449.2 g/mol), ε, the molar absorptivity (26, 900 L/cm/mg), DF = 10.

2.8.6. Determination of total phenols

The total phenols were determined using the Folin–Ciocalteau method described by Cao et al. (2011) with some modifications. 2.5 g CPJ was mixed with 30 mL methanol, the mixture was kept in the 90 w, 55 °C ultrasonic cleaner for 30 min, then centrifuged at 9000 rpm for 10 min at 4 °C. The supernatant was collected and made to 50 mL with absolute methanol. 400 μL of the sample was mixed with 2 mL of Folin–Ciocalteau reagent (previously diluted 10-fold with distilled water) and set for 1 h in the dark at room temperature. Then 1.8 mL sodium carbonate (7.5%) was added to the mixture and reacted for 15 min, and the mixture was immediately measured at 765 nm by a spectrophotometer (UV-726, Shimadzu, Shanghai, China). Results were expressed as mg of gallic acid equivalent (GAE) per 100 mL of sample (mg GAE/100 mL).

2.8.7. Determination of antioxidant capacity

2.8.7.1. Antioxidant capacity determined by stable radical method (DPPH). Antioxidant capacity was determined using the DPPH assay (Hegedues et al., 2011; Suarez-Jacobo et al., 2011) with some modifications. The CPJ was centrifuged at 12,000 rpm for 15 min at 4 °C. Then the supernatant was collected for further analysis. 0.2 mL of 10-fold diluted supernatant was mixed with 4.0 mL of a methanolic solution of DPPH (0.14 mmol/L). The mixture was set in the dark for 70 min, and then the absorbance was measured at 517 nm with a spectrophotometer (UV-726, T6, PG General, Beijing, China). A new Trolox calibration curve was made for each assay. The results were expressed as Trolox equivalents (TE) where one TE equals the net protection produced by 1 μm Trolox.

2.8.7.2. Antioxidant capacity determined by ferric reducing antioxidant power (FRAP). Antioxidant capacity was determined using the FRAP assay (Stracke, Rufer, Weibel, Bub, & Watzl, 2009) with some modifications. The freshly prepared FRAP solution contained 25 mL 0.3 mol/L acetate buffer (pH 3.6), 2.5 mL 10 mmol/L TPTZ (dissolved in 40 mmol/L HCl) and 2.5 mL 20 mmol/L ferric chloride. The CPJ was centrifuged at 12,000 rpm for 15 min at 4 °C. Then the supernatant was collected for further analysis. 0.2 mL of 10-fold diluted supernatant was mixed with 4.0 mL of FRAP solution (0.14 mmol/L) and reacted for 10 min at 37 °C. The ferric reducing ability was measured by monitoring the absorbance at 593 nm using a spectrophotometer (UV-726, Shimadzu, Shanghai, China) and the FRAP solution was used as blank. Trolox was used as a control to obtain the standard curve. FRAP value was calculated relevant to the activity of Trolox and expressed as Trolox equivalents (TE).

2.9. Statistical analysis

The experiment was performed in triplicate. The data were analyzed using the Statistical Program for Social Sciences (SPSS17.0, Chicago, IL,

USA) software for analysis of variance and Duncan's test. The significance was established at $P < 0.05$. Relationships between variables were examined using Pearson correlation coefficients.

3. Results and discussion

3.1. Inactivation of natural microorganisms and the shelf-life extension of CPJ

3.1.1. Inactivation of natural microorganisms in CPJ by HHP at 300 MPa and 400 MPa

The inactivation of the TAB and Y&M in CPJ by HHP is illustrated in Table 1. The initial counts of TAB and Y&M in untreated (control) CPJ were 6.05 $\log_{10}$ CFU/mL and 3.69 $\log_{10}$ CFU/mL, respectively. After HHP treatment, the TAB count and the Y&M count were significantly reduced. The treatment at 300 MPa/2.5 min resulted in 3.17 log cycles reduction in the TAB count and 1.85 log cycles reduction in the Y&M count. The population of TAB and Y&M decreased as the treatment time extended from 2.5 min to 25 min. The treatment at 300 MPa/25 min made the TAB count and Y&M count reduced to 2.53 $\log_{10}$ CFU/mL and 1.51 $\log_{10}$ CFU/mL, respectively, but not to reach the national hygienic standard for fruit and vegetable juice (GB19297-2003, ≤100 CFU/mL and ≤20 CFU/mL, respectively). When the sample was treated at the pressure of 400 MPa for 2.5 min, the TAB count reduced to 2.04 $\log_{10}$ CFU/mL and the Y&M count was not detected. Instead, at 400 MPa for 5 min, 10 min, 15 min, the TAB counts reduced to 1.52 $\log_{10}$ CFU/mL, 1.33 $\log_{10}$ CFU/mL and 1.22 $\log_{10}$ CFU/mL, respectively and the Y&M counts were not detected, all reaching the national hygienic standard for fruit and vegetable juice. When the treatment time reached to 20 min and 25 min, the TAB counts and the Y&M counts were not detected. Therefore, at the pressure of 400 MPa for 5 min, the optimal sterilization effect was attained. This may be due to the low pH (<4.6) of CPJ. Mackey, Forestiere, and Isaacs (1995) reported that low pH and pressure had a synergistic effect on microorganism inactivation. This result highlighted the efficacy of HHP processing pomegranate juice as the experiments performed by Ferrari et al. (2010) and Varela-Santos et al. (2012) before. However, there always were differences among different experiments, although the materials were the same fruit. It has been known that the composition of food had strong protective effect on microorganisms (Cheftel, 1995). Besides, pressure resistance varies considerably among species and among strains within a species (Alpas et al., 1999). Different species of pomegranate have different sugar concentration, pH and TSS, and have different strains of microorganisms. These reasons explained for the variation of inactivation effect among our research and other researches about pomegranate juice by HHP.

The inactivation of microorganisms by heat and other processing methods was used to be considered to follow first-order kinetics (Schaffner & Labuza, 1997). However, significant deviations from linearity have been reported frequently in the literature and microbial inactivation under HHP treatment exhibited non-linear kinetics in many cases (Buzrul, Alpas, Largeteau, & Demazeau, 2008; Chen & Hoover, 2004; Peleg & Cole, 1998). The isobaric survival curves exhibited a tail and could be described by nonlinear models. Over the years, a number of models, such as the Cerf, modified Gompertz, log-logistic, Baranyi, and Weibull models have been proposed to describe these non-linear survival curves (Chen & Hoover, 2004; Pilavtepe-Celik et al., 2009). Among them, the Weibull model has been gaining popularity due to its simplicity and flexibility. It has been successfully used to model inactivation of *L. monocytogenes* by simultaneous application of pressure and mild heat, thermal inactivation of *Salmonella enterica serovar Typhimurium*, *Bacillus cereus*, and *Clostridium botulinum* (Chen & Hoover, 2004). In this treatment, Weibull model could describe the majority of microbial death process.

Fig. 1 shows that kinetic curves of microbial inactivation fitted with Weibull model. As shown in Table 2, R^2 of treatments under 300 MPa and 400 MPa are both more than 0.95, declaring that the microbial inactivation by HHP fitted the Weibull model well. The value of b at 400 MPa was higher than that at 300 MPa, stating that the effect of microbial inactivation at 400 MPa was better than that at 300 MPa for the same holding time, which was consistent with our experiment results. The values of n under 400 MPa and 300 MPa were both less than 1, demonstrating that the decreasing trend of the TAB count became slower with extension of holding time. It showed up as the strengthened tailing of the curve and the phenomenon just as shown in the Fig. 1 that the effect of microbial inactivation was not improved with the extension of holding time.

3.1.2. The shelf-life extension of CPJ treated by HHP and HTST

In order to study the stability of the CPJ and ensure the optimal storage effect, 400 MPa for 5 min of HHP and 110 °C for 8.6 s of HTST were selected as sterilizing conditions. Then the microbiological safety was compared between HHP-treated and HTST-treated samples during storage at 4 °C.

Fig. 2-A describes that, the TAB count of the CPJ treated by HHP was 25 CFU/mL at the beginning of storage and decreased to 6 CFU/mL after 10 days. It was probably because microorganisms were not completely inactivated by the 400 MPa/5 min treatment, but pressure-tolerance bacteria was injured and did not survive in the low pH and low temperature during the storage. Noma, Tomita, Shimoda, and Hayakawa (2004) reported that HHP caused cell damage and decreased tolerance to pH. Koseki and Yamamoto (2006) reported that the recovery of bacteria depended greatly on the storage temperature. Peñas, Frias, Gomez, and Vidal-Valverde (2010) found that injured cells stored in milk at 4 °C may not be able to completely repair physiological damage and therefore these still-injured cells experience slow death during continued storage. Then the TAB count increased as time extended due

Table 1
Microbial inactivation in cloudy pomegranate juice.

Treatments conditions	Aerobic bacteria ($\log_{10}$ N)		Yeasts and molds ($\log_{10}$ N)	
	300 MPa	400 MPa	300 MPa	400 Mpa
0 min	6.05 ± 0.20	6.05 ± 0.20	3.69 ± 0.17	3.69 ± 0.17
2.5 min	2.88 ± 0.18	2.04 ± 0.17	1.84 ± 0.15	ND
5 min	2.82 ± 0.15	1.52 ± 0.04	1.82 ± 0.17	ND
10 min	2.70 ± 0.20	1.33 ± 0.09	1.71 ± 0.17	ND
15 min	2.64 ± 0.18	1.22 ± 0.17	1.64 ± 0.14	ND
20 min	2.60 ± 0.13	ND	1.57 ± 0.16	ND
25 min	2.53 ± 0.16	ND	1.51 ± 0.12	ND

All data were the means ± SD, $n = 3$, ND = not detected (detection limit < 1 CFU/mL).

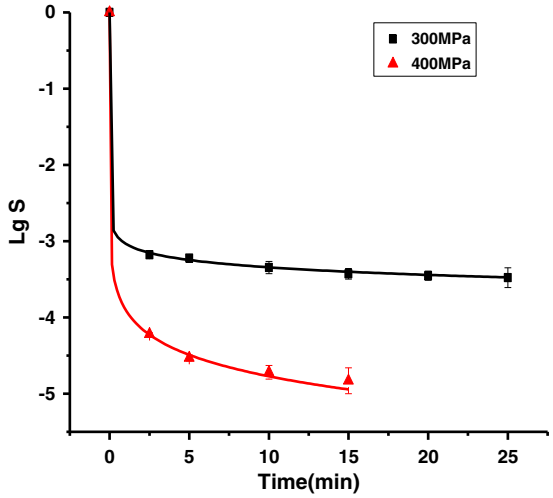

Fig. 1. The Weibull model of microbial inactivation of cloudy pomegranate juice treated by HHP.

Table 2
The Weibull model kinetic parameters of Microbial inactivation in cloudy pomegranate juice treated by HHP.

Pressure/ Mpa	*b*	*n*	R^2
300	3.033	0.042	0.99994
400	3.900	0.088	0.99996

to the survivor microorganisms. Then the TAB count was 28 CFU/mL at the sixtieth day and was 47 CFU/mL at the ninetieth day, up to the national hygienic standard for fruit and vegetable juice. The TAB count of the CPJ treated by HTST increased as time extended during all the storage time. After 20 days, the TAB count of the HTST-treated CPJ was larger than that of HHP-treated CPJ, and the difference was significant ($p < 0.05$). At the ninetieth day, the TAB count of the HTST-treated CPJ was 54 CFU/mL, still reaching the national hygienic standard for fruit and vegetable juice. Therefore, both shelf-lives at 4 °C of HHP-treated and HTST-treated CPJ could attain 90 days.

Fig. 2-B shows that, the variation of the Y&M count in CPJ was related to the processing method. Y&M was inactivated and detected occasionally in HHP-treated sample during storage. However, Y&M in HTST-treated sample increased during storage, reaching 15.8 CFU/mL at the ninetieth day, but still meeting the national hygienic standard for fruit and vegetable juice.

In the research, the CPJ was fresh squeezed juice without any preservatives. Even so, the two treatments ensured the microbiological safety and the 90-day shelf-life at storage of 4 °C.

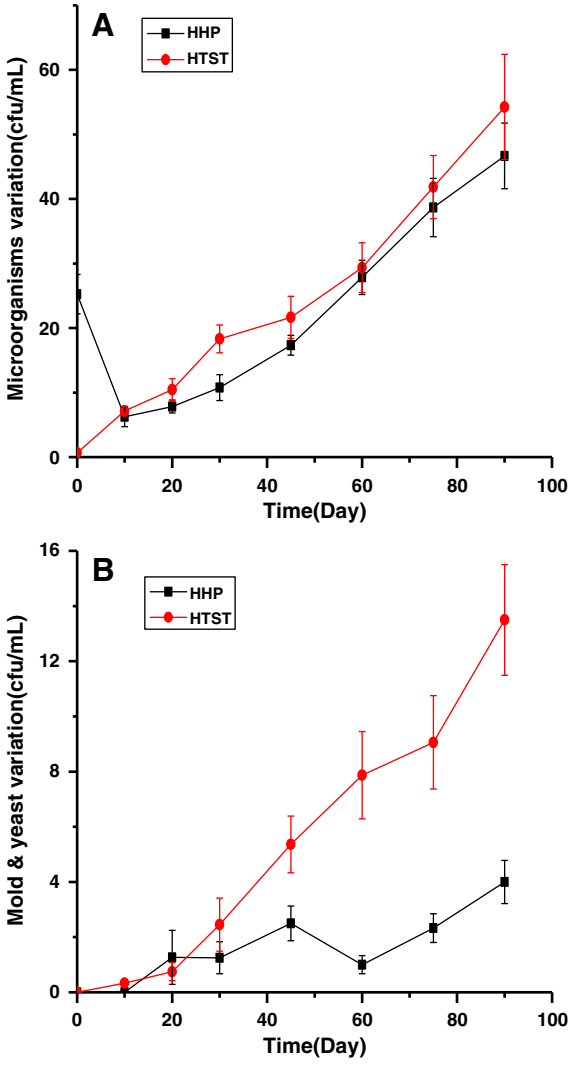

Fig. 2. TAB variation (A) and Y&M variation (B) of cloudy pomegranate juice during storage.

3.2. Color analysis

Effects of HHP and HTST processing on the color are shown in Table 3. Compared with the freshly squeezed juice, the L^* value of the HHP-treated CPJ exhibited a slight decrease ($p > 0.05$), while the HTST-treated CPJ exhibited a significant increase ($p < 0.05$), indicating that the HTST-treated sample became brighter. The a^* values, representing the variation between red and green color, of the two samples showed no alteration after treatment, indicating that HHP and HTST did not exert significant influence on the redness. The b^* value represented the variation between yellow and blue. HHP treatment increased the b^* value significantly ($p < 0.05$), showing that the blueness decreased and the yellowness increased. HTST treatment decreased the b^* value significantly ($p < 0.05$), showing that the blueness increased. The ΔE values, which indicated the total color difference, showed the color alteration of samples was significant or not. Francis and Clydesdale (1975) reported that a ΔE of 2 would be a noticeable difference in the visual perception of many products. ΔE of HHP-treated sample was less than 2, while ΔE of HTST-treated sample was more than 2. This may be because HHP treatment had less effect than HTST on the pigments, such as chlorophylls, lycopene and anthocyanins (Qiu, Jiang, Wang, & Gao, 2006; Mclnerney, Seccafien, Stewart, & Bird, 2007).

Fig. 3 describes the variation of the L^*, a^*, and b^* values during the storage at 4 °C. As shown in Fig. 3-A, regardless of the processing methods, the values of L^* decreased with the time extension. The L^* value of HHP-treated sample decreased fast at beginning of the storage and the trend gradually slowed down after the thirtieth day. The L^* value of HTST-treated sample decreased gradually during the storage. The result stated that there was a gradual reduction in brightness of the treated samples. Besides, the reduction of L^* value of HHP-treated sample was greater than that of HTST-treated sample at the same storage condition, indicating that the CPJ treated by HTST was brighter than that treated by HHP.

The Fig. 3-B reflects the variation of a^* value during the storage. The a^* value of the HTST-treated sample was more stable than that of the HHP-treated sample. The a^* value of the HHP-treated sample decreased sharply at the beginning of the storage, indicating that the redness became lighter significantly ($p < 0.5$) and the content of the free molecules responsible for the redness may change. At the twentieth day the variation of a^* value trended to level off, representing that the redness did not change much. The red color of CPJ was primarily associated with anthocyanin pigment. Anthocyanins were very sensitive to various agents, including enzymes, pH, temperature, oxygen and microorganisms and caused redness fading. At the beginning of storage, small amounts of oxygen existed in the bottle and the microbial inactivation effect of the HHP treatment was less powerful than that of HTST treatment. With the impacts of enzymes, oxygen and microorganisms, anthocyanins degraded and the red color became lighter.

However, the variation of b^* value was relatively complicated. From the Fig. 3-C, the b^* value of the HHP-treated CPJ increased significantly ($p < 0.05$) at the beginning, then decreased slowly from the tenth day to the thirtieth day, and then increased slowly. The b^* value of the HTST-treated CPJ went down at first and then up. The b^* value of HHP-treated or HTST-treated sample had no significant difference between each test point.

Overall, in both the HHP-treated and HTST-treated CPJ during the storage, color changed, but the HTST-treated sample preserved the color better. High pressure has been found to induce discoloration in mushrooms and onions because of the activity of polyphenoloxidase,

Table 3
Effects of HHP and HTST on the color, PH, TSS, TA, total phenols, anthocyanins and antioxidant activity in cloudy pomegranate juice.

Treatments	Color parameters				pH	TSS	TA	Total phenols (mg/100 g)	Anthocyanins (mg/100 g)	Antioxidant activity (mM)	
	L^*	a^*	b^*	ΔE						FRAP	DPPH
CK	32.60 ± 0.00b	6.68 ± 0.10a	(−1.82) ±0.05b	0.00	3.77 ± 0.005a	16.2 ± 0.1a	0.42 ± 0.001a	120.48 ± 1.75b	68.54 ± 0.68a	6.49 ± 0.04a	6.45 ± 0.14a
HHP	31.76 ± 1.28b	6.49 ± 0.23a	(−1.27) ±0.14a	0.78	3.77 ± 0.005a	16.2 ± 0.1a	0.42 ± 0.003a	124.63 ± 1.09a	61.11 ± 0.69b	6.27 ± 0.06b	6.12 ± 0.12b
HTST	34.29 ± 0.72a	7.31 ± 0.74a	(−2.36) ±0.09c	2.36	3.78 ± 0.03a	16.1 ± 0.15a	0.41 ± 0.004b	111.44 ± 1.46c	59.51 ± 0.48c	6.10 ± 0.05c	5.79 ± 0.07c

All data were the means ± SD, $n = 3$. Values with different letters within one column are significantly different ($p < 0.05$).

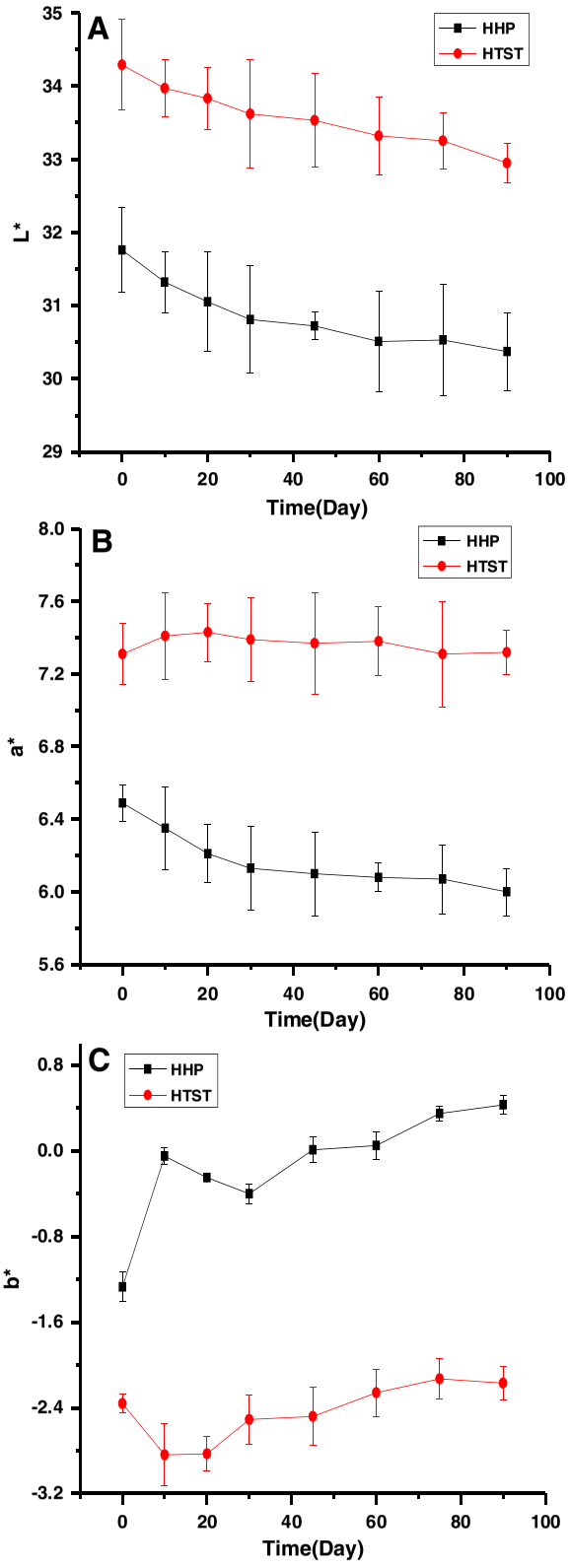

Fig. 3. L^*, a^* and b^* value variation of cloudy pomegranate juice during storage.

responsible for browning (Butz, Koller, Tauscher, & Wolf, 1994). The alteration of color had relation with the incomplete inactivation of the enzymes and the small amounts of oxygen in the bottle that could promote the degradation of anthocyanins (Wesche-Ebeling & Montgomery, 1990). The changes induced by thermal processing were possibly due

to changes in pigment (Lee & Coates, 2003), since a more complete inactivation of polyphenoloxidase and peroxidase.

3.3. pH, TSS and TA analysis

As shown in Table 3, HHP did not cause any change in pH, TSS and TA. HTST insignificantly increased pH ($p > 0.05$) and decreased TSS ($p > 0.05$), but decreased TA significantly ($p < 0.05$).

Table 4 shows the variation of pH, TSS and TA during the storage. TSS and pH of the HHP-treated and HTST-treated CPJ had a slight fluctuation ($p > 0.05$). TA of the HHP-treated sample decreased after the sixth day and TA of the HTST-treated sample showed a gradual downward trend. The decrease of TA could be attributed to the variation of pH and a chain degradation reaction of alpha hydroxy acid during the storage. The differences between our research and some other researches may be due to different cultivars, different growing seasons or small variations in the determination assays.

3.4. Anthocyanins

The changes of anthocyanins are shown in Table 3. There were significant differences ($p < 0.05$) in the content of anthocyanins among freshly squeezed, HHP-treated and HTST-treated CPJ. The HHP treatment decreased the content of anthocyanins, but the impact was smaller than that of HTST. It has been reported that anthocyanins were unstable at high temperature during processing or storage (Patras, Brunton, Tiwari, & Butler, 2011).

The content variations of anthocyanins during storage are exhibited in Fig. 4-A. During the storage at 4 °C, the content of anthocyanins in HHP-treated and HTST-treated CPJ decreased with the time extension. The decrease was at a faster pace in the first 30 days and then slowed down. The initial anthocyanins content of HHP-treated sample was higher than that of HTST-treated sample. However, at the twentieth day the content of anthocyanins of HHP-treated sample was 54.98 ± 0.74 mg/100 mL, a little lower ($p > 0.05$) than the 55.26 ± 0.69 mg/100 mL content of HTST-treated sample. In the later storage time, the content of anthocyanins of HHP-treated sample was lower than that of HTST-treated sample. At the end of storage, the content of anthocyanins of HHP-treated sample was 46.23 ± 0.82 mg/100 mL and that of HTST-treated sample was 48.65 ± 0.79 mg/100 mL, the difference was significant ($p < 0.05$).

Anthocyanins were responsible for the nutrition properties and the bright red color, one of the most important sensory characteristics of pomegranate juice that affected consumer sensory acceptance. Anthocyanins were very sensitive to various agents, as mentioned in the paper. Ochoa, Kesseler, Vullioud, and Lozano (1999) reported that most of the anthocyanins were polymerized rather than being lost during storage. It also was reported that oxidation and condensation of anthocyanin pigments with phenolic compounds, such as ferulic and syringic acids, would cause the loss of anthocyanins, but condensation products were unstable and further degraded to colorless compounds

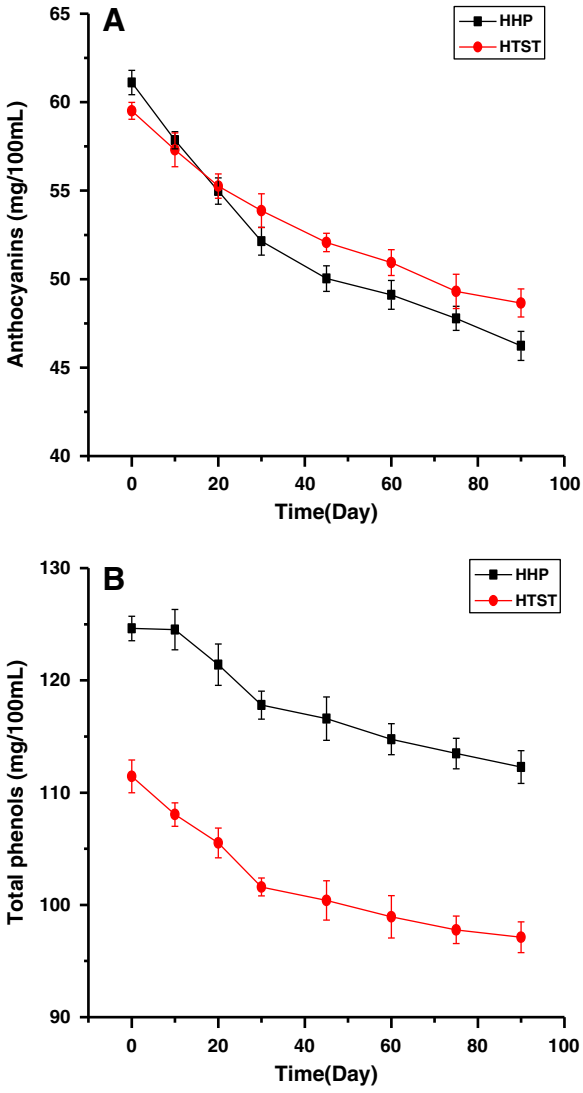

Fig. 4. Anthocyanins (A) and total phenols (B) variation of cloudy pomegranate juice during storage.

(Castañeda-Ovando, De Pacheco-Hernández, De Páez-Hernández, Rodríguez, & Galán-Vidal, 2009). Cao et al. (2012) found a loss of anthocyanins in cloudy juices, which was possibly due to higher concentrations of oxygen absorbed in pulp particles promoting the degradation of anthocyanins. The stability of anthocyanins was also influenced by other fruit components, for instance byproducts of degradation of ascorbic acid and monosaccharides may accelerated anthocyanins

Table 4
The changes of pomegranate juice on some main physico-chemical characteristics during storage.

Storage time (day)	pH		TSS		TA	
	HHP	HTST	HHP	HTST	HHP	HTST
0	3.77 ± 0.005a	3.78 ± 0.03a	16.2 ± 0.1a	16.1 ± 0.15a	0.42 ± 0.003a	0.41 ± 0.004a
10	3.77 ± 0.01a	3.78 ± 0.03a	16.2 ± 0.1a	16.1 ± 0.1a	0.42 ± 0.001a	0.41 ± 0.001a
20	3.77 ± 0.005a	3.79 ± 0.05a	16.1 ± 0.15a	16.0 ± 0.2a	0.42 ± 0.002a	0.40 ± 0.003b
30	3.76 ± 0.004a	3.78 ± 0.03a	16.2 ± 0.1a	16.1 ± 0.15a	0.42 ± 0.001a	0.40 ± 0.001b
45	3.77 ± 0.005a	3.80 ± 0.04a	16.1 ± 0.2a	16.1 ± 0.15a	0.42 ± 0.002a	0.38 ± 0.005c
60	3.76 ± 0.01a	3.79 ± 0.03a	16.2 ± 0.1a	16.1 ± 0.2a	0.41 ± 0.001b	0.40 ± 0.001b
75	3.77 ± 0.005a	3.80 ± 0.01a	16.1 ± 0.15a	16.0 ± 0.1a	0.42 ± 0.002a	0.38 ± 0.004c
90	3.78 ± 0.015a	3.81 ± 0.03a	16.0 ± 0.1a	16.0 ± 0.2a	0.41 ± 0.003b	0.37 ± 0.006d

All data were the means ± SD, $n = 3$. Values with different letters within one column are significantly different ($p < 0.05$).

degradation during storage (García-Viguera & Bridle, 1999; Es-Safi, Cheynier, & Moutounet, 2002). The degradation of anthocyanins could also be caused by the residual activity of the enzymes along with the dissolved oxygen during the storage (Zabetakis et al., 2000; Suthanthangjai, Kajda, & Zabetakis, 2005). The anthocyanins of the CPJ degraded at a relatively high rate at the beginning of the storage, due to the oxygen in the bottle. At later storage, small amounts of enzyme, the variation of light and the variation of sugar and acid content resulted in the loss of anthocyanins. Enzyme activity decreased when the temperature increased. The inactivation effect of HTST on enzyme was stronger than that of HHP. So the dropping rate of anthocyanins content was lower and anthocyanins were better preserved in HTST-treated sample than that in HHP-treated sample, which was consistent with the color variation in the latter storage.

3.5. Total phenols

The changes of total phenols of CPJ are described in Table 3. Compared with the freshly squeezed CPJ, the total phenols of the HHP-treated sample increased significantly ($p < 0.05$) and the total phenols of the HTST-treated sample decreased significantly ($p < 0.05$). Keenan et al. (2010) reported that total phenolic content in smoothies by HHP of 450 MPa for 5 min was higher than thermal processing samples. Some studies reported the HHP increased the total phenols of red fruits (Corramles, Toepfl, Butz, Knorr, & Tausche, 2008; Terefe, Matthies, Simons, & Versteeg, 2009). Ferrari et al. (2010) reported that a pressure of 400 MPa increased the total phenols in clear pomegranate juice. Barba, Esteve, and Frigola (2013) reported that the total phenols in blueberry juice significantly increased after 400 MPa for 15 min. As Le Chatelier's theory illustrates, during the pressure promoting period, the volume of system tends to be reduced, the extracting solvent comes into cells to integrate with bioactive components and the pressurized cells show increased permeability (Chen et al., 2005; Xi et al., 2009). The increase of total phenols in HHP-treated sample may be related to plant cell disruption and an increased extractability of some antioxidant components (Queiroz et al., 2010).

The changes of total phenols of CPJ during storage are shown in Fig. 4-B. Total phenols in the HHP-treated and HTST-treated CPJ decreased with the extension of the storage time. Total phenols in the HHP-treated sample did not change significantly ($p > 0.05$) in the first ten days, then decreased rapidly between the tenth day and thirtieth day, then decreased gradually in the later storage time. In the HHP-treated sample, total phenols were 124.63 ± 1.09 mg/100 mL at 0 day, 117.80 ± 1.25 mg/100 mL at the thirtieth day, 112.28 ±1.45 mg/100 mL at the ninetieth day, the differences were significant ($p < 0.05$). Total phenols of the HTST-treated sample decreased rapidly in the first thirty days and the decline rate was higher than that in the HPP-treatment sample, and then decreased gradually in the later storage time. Total phenols in the HTST-treated sample were 111.44 ± 1.46 mg/100 mL at 0 day, 101.60 ± 0.79 mg/100 mL at the thirtieth day and 97.12 ± 1.37 mg/100 mL at the ninetieth day. No matter the HPP-treatment or HTST-treated sample, there was no significant difference in the total phenols among each detected points from the thirtieth day to ninetieth day during the storage.

3.6. Antioxidant activity

As shown in Table 3, the antioxidant activities of fresh squeezed, HHP-treated and HTST-treated CPJ measured by DPPH method and FRAP method reduced sequentially, the differences between each other were significant ($p < 0.05$). Dede et al. (2007) and Indrawati, Van Loey, and Hendrickx (2004) also reported HHP treatment resulted in the decrease of antioxidant activity in tomato, carrot and orange juice. The result stated that HHP treatment had an adverse effect on the antioxidant activity, but the adverse effect was less than that of HTST treatment. The antioxidant activity had a positive correlation with the content of anthocyanins and total phenols. Fang et al. (2009) reported that anthocyanins and total phenolic acids contributed to the antioxidant capacity and low antioxidant activity in some bayberry juices was probably due to the low total phenolic acids and absence of anthocyanins. Previous studies demonstrated that phenolic compounds were responsible for antioxidant capacity in fruits, and the higher was total phenols, the stronger was the antioxidant capacity (Silva, Escribano-Bailón, Alonso, Rivas-Gonzalo, & Santos-Buela, 2007; Patras et al., 2009). Du, Li, Ma, and Liang (2009) also reported that antioxidant capacity measured by FRAP and DPPH methods was highly correlated to total phenols. The content of anthocyanins decreased by 10.8% and the total phenols increased in the HHP-treated sample, while the content of anthocyanins decreased by 13.2%, similar to 10.8%, and the total phenols decreased in the HTST-treated sample. So HHP treatment kept the antioxidant activity better than HTST treatment.

The changes of the DPPH antioxidant activity and the FRAP antioxidant activity are shown in Fig. 5. Both of the DPPH antioxidant activity and the FRAP antioxidant activity decreased with the extension of storage time. The loss of DPPH and FRAP antioxidant capacity was more associated with the loss of total anthocyanins and total phenols during storage at 4 °C. Kalt, Forney, Martin, and Prior (1999) reported that the antioxidant capacity of fresh strawberries, raspberries and

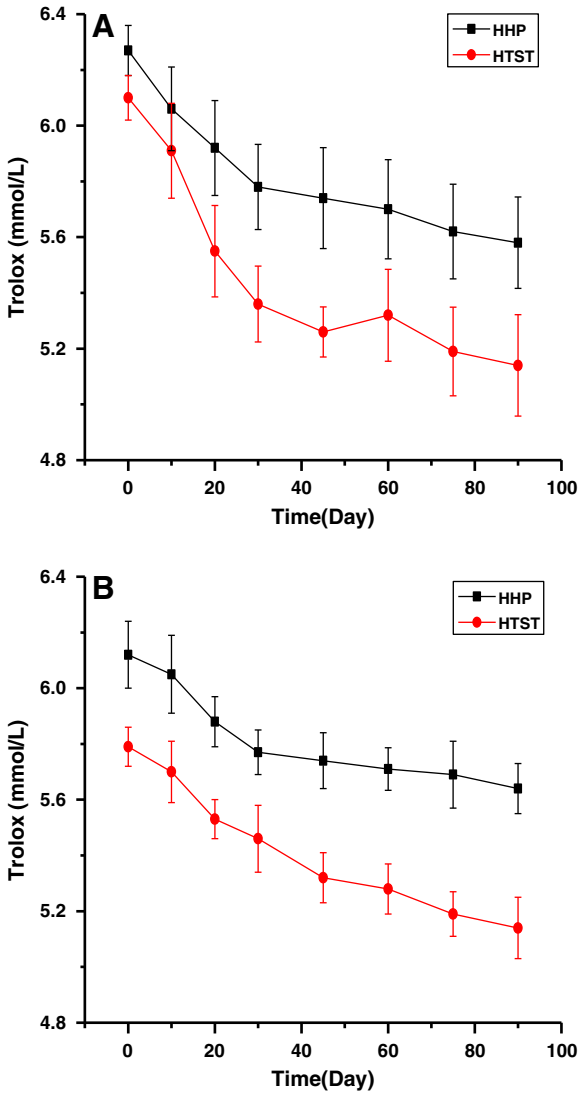

Fig. 5. FRAP antioxidant activity (A) and DPPH antioxidant activity (B) variation of cloudy pomegranate juice during storage.

blueberries during storage was related to phenols and anthocyanins. The DPPH and FRAP antioxidant activity in the HHP-treated CPJ decreased faster in the first thirty days and then decreased gradually. The falling rate of the FRAP antioxidant activity in HTST-treated sample was higher than that in the HHP-treated sample in the first thirty days. The DPPH and FRAP antioxidant activity in the HHP-treated sample decreased by 7.24% and 11%, respectively. The DPPH antioxidant activity in the HTST-treated sample fell from 5.79 ± 0.07 mM to 5.14 ± 0.11 mM and the fall rate was balanced during the storage. The FRAP antioxidant activity in the HTST-treated sample decreased by 15.74%, from 6.10 ± 0.05 mM to 5.14 ± 0.18 mM. Both of the DPPH antioxidant activity and FRAP antioxidant activity in HHP-treated sample were higher than that of the HTST-treated sample and the difference was significant ($p < 0.05$).

4. Conclusions

The inactivation of microorganisms by HHP treatment in CPJ fitted Weibull model and had a positive correlation with pressure and holding time. The Y&M was completely inactivated by HHP at or above 400 MPa and the TAB was reduced to meet national hygienic standard for fruit and vegetable juice by HHP at 400 MPa for 5 min.

400 MPa for 5 min of HHP and 110 °C for 8.6 s of HTST were selected as sterilizing conditions for comparative study. HHP-treated sample had greater retention of color, anthocyanins and antioxidant activity, and elevated total phenols immediately after processing. During the storage at 4 °C, both HHP and HTST treatment guaranteed the microbiological safety and the brightness became lower and the red color became lighter in both samples, but the HTST-treated sample had better color than the HHP-treated sample. Besides, the content of anthocyanins, total phenols and the antioxidant activity in both samples decreased, but in general, HHP treatment preserved the nutrient composition better. Both of HHP and HTST treatment did not have significant effect on pH, TSS and TA.

We can conclude that HHP has a good prospect for use in the food industry as an alternative to thermal pasteurization. The applied conditions require further optimization to assure the inactivation of microbial and optimal flavor during storage.

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