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### Quality assurance in pepper and orange juice blend treated by high pressure processing and high temperature short time

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#### ABSTRACT

The impact of mixing ratio of pepper juice/orange juice ( $\nu/\nu$ ) at 1:5, 1:2, 1:1, 1:0.5 and 1:0.2 on sensory attributes of pepper and orange juice blend (POJB) was studied, and the ratio of 1:0.5 achieved the highest ratings in mouthfeel and overall acceptability and was chosen for the following study. Effects of high pressure processing (HPP, 550 MPa, 5 min, ambient temperature) and high temperature short time (HTST, 110 °C, 8.6 s) on quality attributes of the POJBs were compared. Reduction of total aerobic bacteria (TAB) and molds and yeasts (M&Y) in the POJBs was >4 log cycles after HPP and HTST, and the two POJBs were microbiologically safe during the whole refrigerated storage. The color, flavor, appearance, mouthfeel and overall acceptability of HPP-treated POJB were closer to untreated POJB than the HTST-treated one. After 25 days, 77.3% and 75.3% of total phenols content, 90.8% and 90.7% of ascorbic acid, and more than 80% of antioxidant capacity in two POJBs were retained, respectively. Particle size distribution (PSD) of HPP-treated POJB was consistent with untreated POJB (1~76 µm, 3 peaks at 4.2, 17, 52 µm), while HTST changed the PSD (1 to 33 µm, 2 peaks at 2.4 and 17 µm). Higher level of sedimentation in HPP-treated POJB during storage was interpreted by higher residual PME activity (67.0%) and larger and more unstable pulp particles. The POIBs behaved as Newtonian fluids, their viscosity right after processing were ranked as HPP > untreated > HTST, and the values were slightly reduced during storage. Industrial Relevance: This study was intended to develop yellow sweet pepper and orange juice blend (POJB), which are not available on the market. Further this study was also intended to explore the application of high pressure processing (HPP) and high temperature short time (HTST) on quality assurance of the POJB. This study would provide technical support for commercialization of juice blend products treated by high pressure. © 2015 Elsevier Ltd. All rights reserved.

#### 1. Introduction

In a health-awareness society, consumption of fresh vegetable juices with less sweetness and lower calorie, like kale, cabbage, lettuce, celery, pepper juices, etc., is springing up (Simsek, El, Kancabas Kilinc, & Karakaya, 2014; Song et al., 2007), but these juices are not widely acceptable due to their unpleasant flavor and taste. Blending with fresh fruit juices becomes a solution for this problem. Juice blending is also one of the best methods to improve the nutritional quality of the juice (Rathod, Shakya, & Ade, 2014). However, the development of this kind of juice blend is limited due to the short shelf life (Kim & Rhee, 2015; Song et al., 2007). Preservation studies of juice blend are more challenging considering higher pH value and more unstable quality characters of blend. Additionally, consumption of freshly unpasteurized juice blends might raise the risk of various foodborne illness, thus FDA has required

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http://dx.doi.org/10.1016/j.ifset.2015.08.001 1466-8564/© 2015 Elsevier Ltd. All rights reserved. processors to achieve a 5 log reduction of the most resistant pathogens in the finished products (Nguyen-The, 2012; Tiwari, O'Donnell, & Cullen, 2009). The demand for safe juice blends possessing great quality, such as sensory freshness characteristics and biological properties has led researchers and manufacturers to develop new processing and conservation technologies (Hernández-Carrión, Hernando, & Quiles, 2014).

In recent years, there has been considerable interest in food preservation by non-thermal technologies, which are effective at ambient or sub-lethal temperatures to minimise negative thermal effects on food nutritional and quality parameters (Knorr, 2003; Rawson et al., 2011; Tiwari et al., 2009). High pressure processing (HPP) (Jiang et al., 2009; Zhao et al., 2013), high-intensity pulsed electric field (Sánchez-Vega, Elez-Martínez, & Martín-Belloso, 2014; Zhong et al., 2005), high pressure carbon dioxide (Li, Zhao, Wu, Zhang, & Liao, 2012; Zhou, Wang, Hu, Wu, & Liao, 2009) and radiation sterilization processing (Kumar et al., 2012; Song et al., 2007) have been applied to ensure product safety and quality of juices and blends. HPP, which subjects foods to 100~1000 MPa using water as pressure transmitting medium at room or mild process temperatures, is one of the most promising alternatives of preservation technology, providing food with fresh-like quality (Oey,

Lille, Van Loey, & Hendrickx, 2008). HPP has become a commercially implemented technology, spreading from its origins in Japan, followed by USA and Europe (Pereira & Vicente, 2010), this technology has also been commercialized in China in the very recent 5 years.

Sweet peppers belong to the species Capsicum annuum, which are an excellent source of essential nutrients such as carbohydrates, vitamins and minerals (Faustino, Barroca, & Guine, 2007). According to National Nutrient Database for Standard Reference from the United States Department of Agriculture (Release 27:2014), the nutritional compositions of sweet peppers in raw state (per 100 g of edible portion) are 93.89 g of water, 0.86 g of protein, 0.17 g total lipids, 4.64 g of carbohydrate, 2.40 g total sugars and 1.70 g total dietary fiber, corresponding to an energy of 20 kJ. Sweet peppers have attracted the attention of researchers owing to their high content of bioactive compounds, such as fiber, phenols, flavonoids and carotenoids, which possess antioxidant and anti-inflammatory activity, consumption of fresh sweet peppers appears to improve scar formation, prevent atherosclerosis and haemorrhages, reduce blood cholesterol levels and improve stamina (Faustino et al., 2007). However, consumers did not accept the taste of sweet pepper juice, since it was less sweet and slightly astringent.

Yellow sweet pepper juice is attractive, and the yellowness of sweet pepper depends on the ratio of total chlorophyll and carotenoids content (Selahle, Sivakumar, Jifon, & Soundy, 2015). Orange juice is one of the most popular beverages due to its attractive color and pleasant taste in the world (Wibowo, Grauwet, et al., 2015; Wibowo, Vervoort, et al., 2015). The bright yellow/orange color of orange juice was also determined by the composition and concentration of carotenoids (Meléndez-Martínez, Gómez-Robledo, Melgosa, Vicario, & Heredia, 2011).

Orange juice and yellow sweet pepper juice showed similar color, and the widely accepted flavor of orange juice could ameliorate the flavor of yellow sweet pepper juice. The deliverance of a good impression through color will determine consumers' acceptability and their purchase decision (Wibowo, Grauwet, et al., 2015; Wibowo, Vervoort, et al., 2015), so this study was intended to develop a yellow sweet pepper and orange juice blend (POJB), which are not available on the market. HPP and high temperature short time (HTST) were applied to assure the quality of POJB during storage.

#### 2. Materials and methods

#### 2.1. Preparation of sweet pepper and orange juice blend

Fresh yellow sweet peppers (Hebei province, China) and navel oranges (Jiangxi province, China) were purchased from a local market (Beijing, China) in March 2014. Peppers were cleaned with tap water and cut into pieces after removing the seeds, and then they were pressed with a mechanical juice extractor (Joyong Electric Appliance Co., Shandong, China). Navel oranges were washed, peeled, sliced into pieces, then pressed with the same extractor. Two juices were filtered using a four-layer gauze to get original cloudy juices, respectively. Sweet pepper cloudy juice (pH 4.82, 6.4 °Bx, titratable acidity (TA) = 0.18%) and navel orange could juice (pH 3.97, 12.9 °Bx, TA = 0.48%) were then mixed into POJBs by using five ratios of 1:5, 1:2, 1:1, 1:0.5 and 1:0.2 (pepper juice/orange juice, v/v) for optimizing the mixing ratio. The POJBs were analyzed based on sensory evaluations as described later. The POJB with the highest ratings in sensory evaluation was used for further HPP and HTST processing and storage study.

#### 2.2. High pressure processing and high temperature short time treatment

#### *2.2.1. High pressure processing*

For the HPP group, 100 mL polyethylene terephthalate bottles with screw-cup closures were filled with POJB. 550 MPa and 5 min HPP treatment were carried out using a hydrostatic pressurization unit (HHP-700, Baotou Kefa Co., Ltd., Inner Mongolia, China) with a

capacity of 7.0 L at ambient temperature ( $\approx$  25 °C). Distilled water was the pressure-transmitting fluid. The pressurization rate was about 120 MPa/min and the depressurization was immediate (less than three seconds). The treatment time reported in this study did not include the pressure increase or release time.

#### 2.2.2. High temperature short time

For the HTST treatment, the POJB was pasteurized at 110 °C for 8.6 s in a pilot scale pasteurizer with a tubular heat exchanger (Armfield FT74, HTST/UHT Processing Unit, Hampshire, England) according to previous studies (Huang et al., 2013; Wang et al., 2012). After pasteurization, the POJB was aseptically filling into the identical polyethylene terephthalate bottles used in HPP after cooling to 20 °C.

#### 2.3. Storage study

Both POJBs after HPP and HTST were stored at 4 °C in darkness, quality analysis was run at Day 1, 3, 6, 9, 15, 20 and 25 of refrigerated storage. Experiments were executed in triplicate for each group.

#### 2.4. Sensory evaluation

The procedure performed for sensory evaluation was described (Wang et al., 2014). A panel of 20 members participated in the sensory tests. Panelists were graduate students from the College of Food Science and Nutritional Engineering at China Agricultural University, and they were trained at least once previously for sensory test. The panelists were asked to rate the samples by their preference for color, appearance, flavor, mouth-feel and overall acceptability by marking on the score sheet standard as described (Wang et al., 2014) with a little modification (Table 1). A nine point hedonic scale was used here, where higher numbers represented the stronger preference for particular attributes (one = dislike extremely; two = dislike very much; five = neither like nor dislike; eight = like very much; nine = like extremely). Two groups of the POJBs were served in randomly numbered scentless paper cups on a tray. A cup containing potable water and a piece of non-salted cracker were also provided to the panelists to eliminate the residual taste between samples. A score of five was taken as the lower limit of acceptability.

#### 2.5. Microbial analysis

Total plate count method was used to measure viable cells of natural microorganisms. Twenty milliliters of the POJB was serially diluted with 0.85% sterile NaCl solution to 250 mL, and 1.0 mL of diluted samples, which were filled into duplicated plates of appropriate agar. The plate count agar incubated at  $36 \pm 1$  °C for  $24 \pm 2$  h was for detecting the viable cells of total aerobic bacteria (TAB) and the Rose Bengal agar incubated at  $28 \pm 1$  °C for  $72 \pm 2$  h was for detecting the viable cells of molds & yeasts (M&Y).

#### 2.6. Physicochemical characters

pH, total soluble solid (TSS) and TA were determined by Orion 868 pH meter (Thermo Orion, USA), WAY-2S digital Abbe Refraction meter (Shanghai Precision and Scientific Instrument Co., Ltd, China) and 851 GPD automatic titrator (Metrohm Co. Ltd., Switzerland) at  $20 \pm 1$  °C, respectively. TSS was reported as degrees Brix and TA was expressed as the percentage of citric acid content.

#### 2.7. Assessment of bioactive compounds and antioxidant capacity

#### 2.7.1. Total phenols content

Total phenols content (TPC) was determined using the Folin– Ciocalteu method described (Cao et al., 2012) with a little modification of sample quantity, which was 0.4 mL of the POJB mixed with 2 mL 10-

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#### Table 1

Standard score sheet for sensory evaluation of the POJBs.

Scores	Color	Flavor	Mouthfeel	Appearance	Overall acceptability
9 8 7	Uniform color, bright yellow	Appropriate proportion of yellow pepper and orange flavor, pure aroma, no objectionable odor	Good mouthfeel. Appropriate consistency, smooth and exquisite	No delamination or flocculation, good fluidity and stable cloud appearance	Excellent
6 5 4	Basically uniform color, yellow	Generally appropriate proportion of yellow pepper and orange flavor, acceptable odor	General mouthfeel. Relatively homogeneous texture	Slight delamination or flocculation, good fluidity and basically stable cloud appearance	General
3 2 1	Non-uniform color, anomalous color	Excessive sweetness or sourness. Astringency or other off-flavor	Bad mouthfeel. Inappropriate consistency, non-homogeneous and granular	Distinct delamination or flocculation, poor fluidity,	Unacceptable

fold diluted Folin–Ciocalteu reagent and set for following quantification. Results were expressed as µg gallic acid/100 mL of the POJB.

#### 2.7.2. Ascorbic acid

Ascorbic acid (AA) test was carried as following: 20 mL of the POJB was mixed with 100 mL 2.5% metaphosphoric acid, then incubated at 4 °C for 2 h. After incubation, the mixture was centrifuged using 5157  $\times$ g for 15 min at 4 °C, then the supernatant was filtered through 0.45 µm two-layer cheese-cloths. Lastly, the supernatant was collected for HPLC, and results were expressed as mg AA/L of the POJB (Cao et al., 2012).

#### 2.7.3. Antioxidant capacity

Antioxidant capacity (AC) was studied through the two classical free-radical scavenging-effect on 2,2-diphenyl-1-picrylhydrazyl (DPPH\*) and ferric reducing/antioxidant power (FRAP) according to the method used before with modifications (Cao et al., 2012). For DPPH\* assay, the reaction started by adding 100 µL of 10-fold diluted POJB to the cuvette containing 4 mL of methanol solution of the free radical (DPPH\*, 0.14 mmol/L). The mixture was set away from light for 45 min, and then the absorbance was measured at 517 nm with a spectrophotometer (UV-726, T6, PG General, Beijing, China). For FRAP method, freshly prepared FRAP working solution contained 25 mL of acetate buffer (0.3 mol/L, pH 3.6) plus 2.5 mL of 2,4,6-tripyridyl-striazine (TPTZ, 10 mmol/L, dissolved in 40 mmol/L HCl) and 2.5 mL of ferric chloride (FeCl<sub>3</sub>·6H<sub>2</sub>O, 20 mmol/L). Four milliliters of FRAP solution at 37 °C was mixed with 100 µL 10-fold diluted POJB. Ten minutes later, the ferric reducing ability of strawberry juice was measured by monitoring the increase of absorbance at 593 nm with a spectrophotometer (UV-726, T6, PG General, Beijing, China) and the FRAP solution was used as blank.

#### 2.8. Color profile

The ColorQuest XE Color Difference Meter (Hunter Associates Laboratory Inc., Virginia, USA) was used to measure color parameters *L*, *a* and *b* using reflection mode, illuminant D65, 10° Observer. Total color difference ( $\Delta E$ ) was calculated based on *L*, *a* and *b* values and using the equation as described before (Wang et al., 2014),

$$\Delta \mathbf{E} = \sqrt{L_t - L_0^2 + a_t - a_0^2 + b_t - b_0^2} \tag{1}$$

where  $L_t$ ,  $a_t$  and  $b_t$  stand for the *L*, *a* and *b* values, respectively, of the POJB stored at 4 °C after 1, 3, 6, 9, 15, 20 and 25 days,  $L_0$  (41.07  $\pm$  0.01),  $a_0$  (0.68  $\pm$  0.02) and  $b_0$  (23.02  $\pm$  0.06) were the *L*, *a* and *b* values of the untreated POJB.

#### 2.9. Particle size distribution assessment

LS 230 particle size analyzer (Beckman Coulter, Inc., Florida, USA) was utilized and particle size distribution (PSD) was calculated by the Fraunhofer model as described (Zhou et al., 2009). First, distilled

water was pumped as the speed of 8 L/min to full the sample cell, then the POJB was added to the cell using a pipette and was mixed well. Measurement began when obscuration percentage increased from zero to 8%. The PSD was represented by parameters volume mean diameter D [4, 3] and surface mean diameter D [3, 2].

#### 2.10. Pectin methylesterase determination

Pectin methylesterase (PME) activity was determined as the method in a previous study (Bi, Wu, Zhang, Xu, & Liao, 2011). Sixty milliliters of a substrate solution (1% apple pectin) with 0.1 M NaCl was incubated at 30 °C and adjusted to pH = 7.5 using 0.05 M NaOH by 851 GPD automatic titrator (Metrohm Co. Ltd., Switzerland). Five milliliters of the POJB was added to the substrate, then automatically readjusted the pH to 7.5 with 0.05 M NaOH. After that, the volumes of 0.05 M NaOH consumed by titration were recorded every 30 s for 10 min. The reaction curve for consumption of NaOH vs time was drew, and the slope of the initial linear portion was used to calculate PME activity (units/mL) as formula (2),

$$PME activity = \frac{Slope \times C_{NaOH}}{V_{sample}}$$
(2)

PME residual activity was estimated with formula (3),

$$PME \text{ residual activity} = \frac{PME \text{ activity}_{treated}}{PME \text{ activity}_{untreated}} \times 100\%$$
(3)

#### 2.11. Rheological characters

Rheological measurements were performed immediately after treatments using a controlled shear rate AR 550 rheometer (TA Instrument, Waters Co., Ltd., Surrey, UK) as previously reported (Li, Wang, Wang, & Liao, 2014). The POJB was placed between parallel plates (40 mm diameter) using a gap size set at 1 mm. For the steady-flow studies, approximately 3 mL of the POJB was placed between the plates and the measurements were made at 25 °C with the shear rate exponentially increased from one to 200 s<sup>-1</sup>. The obtained data was analyzed using the LS v3.29 software.

#### 3. Statistical analysis

Experiments were carried out in triplicate. All the obtained data was collected and summarized by Microsoft Office 2013 Excel (Redmond, USA). An analysis of variance (ANOVA) was performed with Origin 8.0 (OriginLab Corporation, Northampton, MA), and significance was established at P<0.05. Curve fittings and plotting drawings were completed by the Origin Pro 8.0 platform (Massachusetts, USA).

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#### 4. Results and discussion

#### 4.1. Selecting mixing ratios of pepper juice and orange juice

The sensory evaluation and cloudy appearance of the POJBs were carried as a function of mixing pepper juice and orange juice. The sensory attributes, including color, appearance, flavor, mouthfeel and overall acceptability of the POJBs with different ratios are shown in Fig. 1A. The POJBs with more orange juice presented better flavor and mouthfeel, and decreased acceptability of appearance. Especially, obvious precipitation in the POJBs appeared at mixing ratios of 1:5 and 1:2 (Fig. 1B). Generally, a customer expects that the visual appearance of a cloudy drink will have neither a sediment nor a cream phase (Beveridge, 2002). The POJB at the ratio of 1:0.5 achieved the highest ratings in mouthfeel and overall evaluation categories, and the POJBs at the ratios of 1:1 and 1:0.2 did not offer good overall acceptability despite of great appearance. No visible color difference was identified among all five groups. Therefore, the POJB with the ratio of 1:0.5 was opted throughout this study. The values of pH, TSS and TA in this optimized POJB were 4.33  $\pm$  0.01, 9.43  $\pm$  0.23 °Bx and 0.40  $\pm$  0.04%.

#### 4.2. Effects of HPP and HTST on microorganisms, sensory attributes of POJB

Initial counts of TAB and M&Y in the untreated POJB are 4.90  $log_{10}$  CFU/mL and 4.18  $log_{10}$  CFU/mL, respectively. TAB in the POJBs is undetectable right after HPP or HTST, and less than the detection limit of 1.00  $log_{10}$  CFU/mL during the first three days, then it slightly increased to 1.98 and 1.85  $log_{10}$  CFU/mL at Day 25 during refrigerated storage (Table 2). According to the microbial criteria mandated by the Chinese National Hygienic Standard for Fruit and Vegetable Juice (GB 19297-2003), the acceptable TAB, molds, and yeasts in vegetable and fruit juices are less than two, 1.30 and 1.30  $log_{10}$  CFU/mL, respectively. The



**Fig. 1.** Sensory evaluation (A) and cloud appearance (B) of the POJBs. (POJBs were prepared with different ratios of pepper juice/orange juice = v/v).

TAB was less than  $2.00 \log_{10}$  CFU/mL and the M&Y was undetectable in both HPP and HTST groups during 25 days of refrigerated storage. These microbial data suggested that both HPP- and HTST-treated POJBs are microbiologically safe during 25 days of refrigerated storage in this work.

Sensory attributes of HPP- and HTST-treated POJBs, as well as untreated juice are shown in Fig. 2A. The untreated POJB achieved higher ratings in color, appearance, mouthfeel and overall acceptability. The HPP-treated POJB exhibited great color, flavor and appearance, which could be barely distinguished from the untreated one by the panelists, and its mouthfeel and overall acceptability were closer to that of the untreated sample. The HTST group presented great color and appearance, however, its mouthfeel, flavor and overall acceptability achieved the lowest ratings. Especially, a score of five was taken as the lower limit of acceptability in this work, and the flavor score of the HTST-treated POJB was only 5.73, suggesting that HTST totally changed the raw flavor of the POJB. More positive results about sensory evaluation were also found in other HPP pasteurized fruit juices, such as navel orange juice (Baxter, Easton, Schneebeli, & Whitfield, 2005), yellow passion fruit juice (Laboissière et al., 2007), and citrus juices (Hartyáni et al., 2011). However, few publications about vegetable and fruit juice blend have been reported. The indistinguishable color of both HPP and HTST treatments in this work was due to pressure-stable (García, Butz, Bognàr, & Tauscher, 2001) and heat-stable capabilities of carotenoids (Sanchez-Moreno, Plaza, de Ancos, & Cano, 2006). Similarly, total carotenoids content in HPP- and HTST-processed mango nectar exhibited no significant change (Liu, Wang, Li, Bi, & Liao, 2014b). For the flavor character, small molecular flavor compounds are usually not directly affected by HPP, and it has been observed by means of both chemical and sensory analyses (Oey, Lille, et al., 2008). It has been demonstrated that HPP had less influence on the volatile profiles of mango nectar than HTST using headspace fingerprinting, and the change was attributed to an indirect effect of HPP, which disturbed the flavor by enhancing and retarding enzymatic and chemical reactions, but they did not execute a sensory evaluation of the flavor balance (Liu, Grauwet, et al., 2014). Juice is a complex mixture of polysaccharides, proteins and lower molecular weight compounds that are responsible for the appearance and mouthfeel of the juice (Beveridge, 2002; Galant, Widmer, Luzio, & Cameron, 2014; Oey, Lille, et al., 2008; Oey, Van der Plancken, Van Loey, & Hendrickx, 2008). Based on the overall acceptability, the results highlighted that HPP imparted less destruction on the sensory attributes and maintained the intrinsic character of the POJBs than HTST did.

### 4.3. Effect of HPP and HTST on physicochemical and bioactive characters, and antioxidant capacity of POJB

pH and TA values showed no significant difference and TSS exhibited a slight reduction after both treatments, and these characters in the HPP- and HTST-treated POJBs were stable during 25 days of refrigerated storage (data not shown).

HPP did not cause significant loss of AA and TPC, while HTST resulted in a considerable reduction of AA and had no effect on TPC (data not shown). AA and TPC in the HPP- and HTST-treated POJBs during refrigerated storage are shown in Fig. 3A, remarkable decrease of AA is observed in both groups, percentage of surplus AA and TPC is 77.3% and 90.8% in HPP group, and 75.3% and 90.7% in HTST group, respectively. Loss of AC agrees with the loss of TPC and AA, in which the AC in the HPP- and HTST-processed POJBs using DPPH\* and FRAP methods decreases with the increase of storage days, but about 80% of AC is retained at the end of the storage (Fig. 3B). These data indicated that the POJBs exhibited high retention of TPC, AA and AC after HPP, as well as HTST in this study. Stability of AA was dependent on the molar ratio of AA and oxygen concentrations (Taoukis et al., 1998). Oxygen played an important role in AA degradation both at atmospheric pressure and at elevated pressure (Oey, Van der Plancken, et al., 2008). The similar loss of AA in the HPP- and HTST-processed POJBs during storage might be

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#### Table 2

Microbiological and color characters of the POJBs treated by HPP and HTST during storage at 4 °C.

Time (Day)	TAB (log <sub>10</sub> CFU/g)		M&Y (log <sub>10</sub> CFU/g)		L		а		b		ΔΕ	
	HPP	HTST	HPP	HTST	HPP	HTST	HPP	HTST	HPP	HTST	HPP	HTST
Untreated	4.90	4.90	4.18	4.18	$41.07\pm0.01^{a}$	$41.07\pm0.01^{\text{b}}$	$0.68\pm0.02^{a}$	$0.68\pm0.02^{a}$	$23.02\pm0.06^a$	$23.02\pm0.06^{\text{b}}$	0	0
1	ND	ND	ND	ND	$42.11\pm0.03^{\text{a}}$	$45.08\pm0.06^{a}$	$0.49\pm0.04^{\rm b}$	$0.53\pm0.04^{\rm b}$	$24.18\pm0.06^{a}$	$28.64\pm0.06^a$	$1.62\pm0.08^{\rm b}$	$6.95\pm0.06^{\rm b}$
3	0.95	0.90	ND	ND	$41.49\pm0.14^{\text{a}}$	$45.20\pm0.12^{a}$	$0.39\pm0.05^{\rm d}$	$0.66\pm0.03^{a}$	$23.22\pm0.17^{a}$	$28.45\pm0.32^{a}$	$0.60\pm0.14^{\rm c}$	$6.86\pm0.34^{b}$
6	1.18	1.08	ND	ND	$40.69\pm0.37^a$	$44.77\pm0.05^a$	$0.27\pm0.06^{\rm e}$	$0.59\pm0.04^{\rm b}$	$22.26\pm0.11^{ab}$	$27.26\pm0.87^a$	$0.92\pm0.09^{\rm b}$	$5.69 \pm 0.83^{\circ}$
9	1.32	1.23	ND	ND	$40.23\pm0.03^a$	$45.36\pm0.07^a$	$0.43\pm0.06^{\rm c}$	$0.73\pm0.04^a$	$22.04 \pm 0.11^{ab}$	$28.95\pm0.06^a$	$1.28 \pm 0.15^{b}$	$7.37\pm0.07^{\rm b}$
15	1.71	1.62	ND	ND	$39.54 \pm 0.05^{ab}$	$45.37\pm0.13^a$	$0.27\pm0.05^{e}$	$0.71\pm0.02^a$	$19.76 \pm 0.19^{ m b}$	$\textbf{27.48} \pm \textbf{1.07}^{a}$	$3.58\pm0.17^a$	$6.24\pm0.75^{\rm b}$
20	1.88	1.75	ND	ND	$38.89\pm0.37^{\rm b}$	$45.30\pm0.08^{a}$	$0.38\pm0.03^{\rm d}$	$0.75\pm0.04^a$	$19.97 \pm 0.15^{\rm b}$	$28.33 \pm 0.61^a$	$3.72\pm0.17^a$	$6.83\pm0.60^{\rm b}$
25	1.98	1.85	ND	ND	$39.36 \pm 0.57^{ab}$	$45.95\pm0.21^{a}$	$0.37\pm0.02^{\mathrm{d}}$	$0.72\pm0.02^{a}$	$20.37\pm0.37^{\rm b}$	$29.34\pm0.75^a$	$3.14\pm0.23^{\text{a}}$	$8.03\pm0.74^{a}$

All treated group data were mean  $\pm$  SD, degrees of freedom = 6; Different superscripted letters represented a significant difference within the same column (P < 0.05); ND was not detected.

limited by restricted oxygen content in the POBJ system. On the other hand, enzymatic browning of vegetable and fruit products usually started with the initial enzymatic oxidation of phenols to quinones by polyphenol oxidase in the presence of oxygen (Özoğlu & Bayındırlı, 2002). AA is commonly used to prevent enzymatic discoloration by reducing the colorless quinones to diphenols before they can undergo further reaction to form pigments (McEvily, Iyengar, & Otwell, 1992), which suggested that AA can protect phenols from enzymatic degradation. Higher retention of TPC at the end of storage agreed with this interpretation. The degradation of AC during refrigerated storage agreed with previous studies of strawberry juice (Cao et al., 2011), tomato puree (FernandezGarcia, Butz, & Tauscher, 2001), purple sweet potato nectar (Wang et al., 2012) and mango nectar (Liu, Wang, Li, Bi, & Liao, 2014a).



**Fig. 2.** Sensory evaluation (A) of the POJBs after HPP and HTST and the cloud appearance (B) of the POJBs during refrigerated storage. (The POJBs were prepared with the ratio of 1:0.5 of pepper juice and orange juice (*v*/*v*)).

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AA-HPP

TPC-HPP

Day 15

AA-HTST

TPC-HTST

T

Day 25

DPPH\* - HPP -O--- DPPH\* - HTST

- FRAP-HPP

-▽-… FRAP-HTST

vellowness discrepancy of the untreated or HPP-treated POIB from the HTST-treated POJB was explained by different isomers of carotenoids. *b*-cryptoxanthin or lutein has been reported to be the major carotenoids in pasteurized orange juice (Lee & Coates, 2003; Timmermans et al., 2011). 5,6-epoxidecarotenoids (*cis*-violaxanthin and antheraxanthin) often serves as the predominant carotenoids in fresh orange juice or juice without heat treatment, isomerizations of the main 5,6-epoxide carotenoids to 5,8-epoxides was probably responsible for the color changes of HTST-processed (90 °C, 30 s) juice (Lee & Coates, 2003). Study on the color of carotenoid-rich fruit juices demonstrated similar results of insignificant changes after HPP,  $\Delta E$  values were higher in HTST-treated apricot nectars (Huang et al., 2013) and mango nectar (Liu, Wang, et al., 2014a) than in HPP-treated samples. Highly unsaturated structure in carotenoids (Wibowo, Grauwet, et al., 2015; Wibowo, Vervoort, et al., 2015) also led to the decrease of yellowness by further oxidation reactions, and HPP could not fully inactivate enzymes compared with HTST, therefore, sigificant color loss in HPP group during storage was attributed to the isomerization or oxidation reactions of carotenoids. Still, the lower  $\Delta E$  value in the HPP-treated POJB during the whole storage suggested that HPP retains the freshlike color and appearance of the POJB than HTST.

#### 4.5. Effect of HPP and HTST on PSD and cloud stability in POIB

PSD in the HPP-treated POIB was consistent with the untreated POIB (data not shown). As shown in Fig 4A, the PSD in the HPP-treated POJB at Day 1 exhibits a distribution from 1 to 76 µm and three peaks at 4.2, 17, 52 µm, respectively. However, the PSD in the HTST-treated POJB at Day 1 shows a distinct distribution from 1 to 33 µm and two peaks at 2.4 and 17 µm (Fig. 4B). Similarly, apple sauce pasteurized at 85 °C for 10 min showed a high amount of smaller particles, while non-



Fig. 4. PSD of the POJBs treated by HPP (A) and HTST (B) during refrigerated storage.

20 25 Storage time (Day) Fig. 3. Total phenols content, ascorbic acid and antioxidant capacity of the POJBs treated by HPP and HTST during refrigerated storage. (A: Total phenols content (TPC) and ascorbic acid (AA); B: Antioxidant capacity evaluated by DPPH\* and FRAP methods).

### 4.4. Effect of HPP and HTST on color profiles of POJB

5

10

15

6

А

1.00

0.95

0.90

0.85 C/C

0.80

0.75

0.70

1.00

0.95

0.90

0.85 A/A

0.80

0.75

0.70 0

В

Day 1

Day 9

As shown in Table 2, HTST significantly raises the lightness (L) and yellowness (b) values of the POJBs and HPP shows no effect on the two parameters, while both treatments slightly reduce redness (*a*) values. During refrigerated storage of 25 days, L and b values in the HTST-treated POJB did not change, but they showed significant decrease in HPP-treated POJB. The a values of the two groups shifted toward adverse directions with the increase of storage days. Overall, calculated  $\Delta E$ in the HTST-treated POJB ranges from 5.69 to 7.37 during storage, while it is between 0.60~1.28 during the first nine days, and rises above three in the remaining ten days in the HPP group (Table 2).  $\Delta E$  is considered as 'not noticeable', 'slightly noticeable', 'noticeable', 'well visible' and 'great' when it crosses the corresponding lower limit of 0, 0.5, 1.5, 3 and 6, respectively (Cserhalmi, Sass-Kiss, Tóth-Markus, & Lechner, 2006). The  $\Delta E$ value of the POJBs was  $1.62 \pm 0.08$  after HPP and  $6.95 \pm 0.06$  after HTST, indicating that HPP maintained the original color of the POJB compared with HTST, color variances of the HTST-treated sample during storage, as well as the HPP-treated POJB after 15-day strorage were perceptible in this study.

The changes of *L* and *b* in the HTST-treated POJB might be related with the alteration of particle distribution and isomerization of cartenoids after thermal processing. All individual carotenoids as well as the total content significantly correlated with *b* values in carotenoid-rich fruit juices (Cortes, Esteve, Rodrigo, Torregrosa, & Frigola, 2006; Meléndez-Martínez et al., 2011), therefore, the

thermally processed samples had larger particles due to the weaker cell wall adhesion of the heated tissue (Athiphunamphai, Bar, Cooley, & Padilla-Zakour, 2014). Size distribution studies of sweet pepper juice have not been reported in literature, however, relevant information regarding orange juices has been well reviewed (Beveridge, 2002). Particles of cloudy orange juice after centrifugation at  $360 \times g$  for 10 min were detected by light and electron microscopy as chromoplastids (colored, smooth surface, 1 µm), pulp (rag-like, 2~10 µm) or cell wall fragments, spherical oil droplets (~1 µm) and needle-like crystals of hesperidin (0.5 to 3 µm long, 0.05 to 0.2 µm thick) (Merin & Shomer, 1984; Mizrahi & Berk, 1970). The larger PSD range in the POJBs in this study might be due to the filtration pre-treatment through a fourlayer gauze without centrifugation, which did not block the cell debris (>10 µm) generated by physical damage, consequently, more pulps, also indicating more dietary fiber, were retained. As shown in Fig. 4, PSD results are further verified by smaller values of D<sub>[4,3]</sub> = 5.12  $\pm$ 0.13  $\mu m$  and larger values of  $D_{[3,2]}$  = 4.16  $\pm$  0.02  $\mu m$  in the HTST group compared with the HPP sample,  $D_{[4,3]}=6.12\pm0.70~\mu m$  and  $D_{[3,2]} = 3.91 \pm 0.03 \ \mu\text{m}$ .  $D_{[4,3]}$  was more influenced by larger particles, whereas D<sub>[3,2]</sub> was greatly impacted by smaller particles (Augusto, Ibarz, & Cristianini, 2012). During storage, the PSD are stable in the two groups for the very first 9 days. The volume of small particles (<10 µm) ranged between 83.3%~83.1% in the HPP group and 90.9%~90.6% in the HTST group, respectively. The range of the PSD expands from 0.4 to 110 µm after 15 days, and it seems that larger particles show up, but the volume percent of small particles increases to 88.6% in the HPP-treated POJB, which adversely decreases to 87.8% in the HTST group. Distingushed PSD curves in the HPP-treated POJB (0.4~33 µm, 3 peaks at 0.9, 4.2 and 22 µm) and HTST-treated POJB (0.4~27 µm, 2 peaks at 1.0 and 4.0  $\mu$ m) are demonstrated at the end of the storage (Fig. 4).

The system of cloudy juice was well known to be destabilized by the activity of PME and usually through thermal treatment to inactivate this enzyme and preserve cloud stability (Cameron, Baker, & Grohmann, 1997). PME was totally inactivated after HTST, and 67.0% residual PME activity in the HPP-treated POJB was retained. No recovering PME activity was found in both groups after 25 days of storage (data not shown).

The stability of the cloudy appearance of the POJBs during refrigerated storage is shown in Fig. 2B, the untreated POJB spoiled due to growth of naturally-occurring microorganisms after 24 h, so it was absent in the following timepoint pictures. Obvious phase separation and an unwanted appearance in the HPP-treated POJB after 15 days are illustrated, which was attributed to residual PME activity, but HTST provides better stability of the cloudy appearance in the POJB (Fig. 2B). The higher level of sedimentation in the HPP-treated POJB after 15 days was interpreted by higher residual PME activity, as well as larger and more unstable pulp particles.

#### 4.6. Effect of HPP and HTST on rheological characters in POJB

Shear stress/viscosity vs shear rate in the HPP- and HTST-treated POJBs are presented in Fig. 5, the flow behaviors are well retained regardless of treatments or storage time. The viscosity of all the juices shows no alteration in the shear rate range between 6 and  $100 \text{ s}^{-1}$ , and the POJBs behave as Newtonian fluids (Fig. 5C and D). The viscosity values of the POIBs right after processing are ranked as HPP > untreated > HTST, and the values slightly reduce with the increase of the storage time in both groups (Fig. 5C and D). The decrease rate of the viscosity in the HPP-treated POJB was slightly faster than in the HTST-treated sample during refrigerated storage. The viscosity decrease in the HPP-treated POJB during refrigerated storage was in agreement with an earlier study on HPP-processed cloudy strawberry juices (Cao et al., 2012). Literature also reported some juices exhibiting the non-Newtonian behavior, such as peach juice (Zhou, Wang, Liu, Bi, & Liao, 2014), sourcherry juice and concentrate (Belibağli & Dalgic, 2007) and pineapple juice (Shamsudin, Daud, Takrif, Hassan, & Ilicali, 2009). Rheological behavior study of orange juices with different water content (0.34~0.73%) at a wide range of temperature (0.5~62 °C) was also reported as non-Newtonian fluids, and well represented by the Herschel-Bulkley model (Telis-Romeroa, Telis, & Yamashita, 1999), nevertheless, to our knowledge, data for sweet pepper juice were not available. The pasteurized juice (100 °C, 20 min) showed a pseudoplastic behavior and was well fitted to all models (Bingham, Casson, Ostwald-De-Waele, Herschel-Bulkley and Mizhari-Berk models) except the



Fig. 5. Rheological characters of the POIBs treated by HPP and HTST during refrigerated storage (A and C: HPP; B and D: HTST).

Newtonian while the untreated carrot juice was well described by the Newtonian model (Vandresen, Quadri, Souza, & Hotza, 2009). Additionally, traditional pasteurization (100 °C, 20 min) in that work raised the juice viscosity, while HTST here reduced it. Generally speaking, the colloidal materials contribute to the increase of the juice viscosity, as the 'swelling' of the particles and the penetration of water between the cellulose chains during heating induced the high viscosity of the pasteurized juice (Vandresen et al., 2009). The opposite results in the HTST-treated POJB here might be attributed to the discrepancy of short processing time of 8.6 s in this work. On the other hand, larger and irregular particles contributed to a high viscosity, thereby causing a higher hindrance to the flow than the finer and regular particles (Espinosa-Muñoz, Renard, Symoneaux, Biau, & Cuvelier, 2013), therefore higher viscosity right after HPP could be attributed to a wider range of the PSD.

Loss of the viscosity in cloudy juices during storage was possibly attributed to the precipitation of pulps and degradation of pectin (Cao et al., 2012). Pectin degradation had been reviewed through enzymatic and non-enzymatic pathways (Sila et al., 2009). Usually, pectin was very stable around pH 3.5, its pKa value, nonetheless, a number of reactions were proposed for its non-enzymatic degradation, and the basecatalyzed splitting of pectin chains via the  $\beta$ -elimination reaction occurs even when pectin was at a weakly acidic pH (Keijbets, Pilnik, & Vaal, 1977). This could explain the viscosity decrease in HTST samples during storage, since the pH value of the POJB was 4.33, a weakly acidic system. Differently, 50% PME activity was directly linked with pectin enzymatic degradation and viscosity decreases in the HPP-treated POJB during storage. Furthermore, higher rate of viscosity decrease in the HPP group might be related to the involvement of both enzymatic and non-enzymatic degradation of pectin, while PME was totally inactivated in the HTST group. Lastly, these results agreed with the cloudy appearance of the juices in both treatments.

#### 5. Conclusions

Juice blend by mixing sweet pepper juice and orange juice at the ratio of 1:0.5 was used in this study. Both HPP (550 MPa, 5 min) and HTST (110 °C, 8.6 s) were sufficient to provide microbial safety and acceptable color for POJB with 25 days shelf-life, HPP was superior to HTST in the fresh-like quality assurance of the juice blend, especially on original flavor. Subsequently, the greatest challenge of preserving the POJB was maintaining the cloudy stability in this work. A juice extractor with soft extraction setting adjustment might improve its cloudy stability, and cause soft extraction providing more stable juice than hard extraction (Cameron, Baker, Buslig, & Grohmann, 1999). Additionally, blanching pre-treatment combined with HPP could be considered for enhancing the inactivation of PME, at the same time, the sensory assessment, especially for the fresh-like flavor, could be taken into account for further combination research. There is also a small part of merchants that supplied their consumers with the layered HPP-processed juice, and claims that this kind of product indicated freshness and additive free, nevertheless, altering consumer's attention and nurturing consumption habits are really long-standing tasks.

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