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Evaluation of quality changes of differently formulated cloudy mixed juices during refrigerated storage after high pressure processing

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A R T I C L E I N F O

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A B S T R A C T

Cloudy fruit and vegetable mixed juice (MJ) pasteurized by high pressure processing (HPP) showed an increasing market demand. However, browning, sedimentation, and flavor changes of HPP juice during storage have been a great challenge for the beverage industry. The aim of this work was to investigate quality changes of HPP MJs during storage and to explore the potential to create the shelf-stable MJs with fresh-like organoleptic quality through HPP. In the work, commercial MJ1 (orange, mango, and kiwifruit) and MJ2 (carrot and pineapple) were formulated and their quality changes during storage were investigated. The results indicated no visible color changes and sedimentation were observed in MJ1 and MJ2 during refrigerated storage (90 days). However, sucrose decreased as glucose and fructose increased; a large number of aldehydes and alcohols decreased but some terpenoids increased during storage. In general, blending proper fruit and vegetable to produce MJs combing with HPP could maintain high cloud and color stability, but sugars and volatiles clearly changed during storage.

1. Introduction

The market demand and value of cloudy fruit and vegetable mixed juice (MJ) are high because of the mouthfeel sensation and health benefits (Wellala et al., 2020a). As known, the MJs are rich sources of vitamins, dietary fiber, mineral compounds, carotenoids, and polyphenols for human nutrition (Wellala et al., 2019). Besides, blending proper fruit and vegetable to produce MJ has more benefits. For example, the undesirable flavor of some vegetable juices (e.g., broccoli juice) can be greatly improved by blending fruit juices that are rich in fruity aroma and sugar content (Houka et al., 2006). On the other hand, cloud or pulp particles contribute to tactile properties, thereby enhancing the mouthfeel sensation of cloudy MJ (Will et al., 2008). In a convenience-conscious society, cloudy MJs are constantly in demand as an alternative to fresh fruits and vegetables for a healthy daily diet.

High pressure processing (HPP), a non-thermal processing technology used commercially in the beverage and juice sectors, is considered successful in responding to these demands (Yi et al., 2018a). The most attractive properties of HPP juices for consumers are the fresh-like organoleptic quality and high retention of bioactive compounds, which are sensitive to high temperature (Bhattacharjee et al., 2019; Liu et al., 2016). Furthermore, HPP juices are popular with manufacturers and retailers because of their premium prices and high demands. Among juice products, HPP MJs, particularly fruit and vegetable MJ, which are perceived as more natural and healthier because of their lower sugar content than other beverages, are expected to increase in share going forward (Koutchma et al., 2016). However, the quality changes of HPP MJs during storage have been scarcely studied and reported.

The work aimed to design shelf-stable MJs using HPP and different formulations. The specific objectives of this study were to (1) compare the quality stability of differently formulated MJs pasteurized by HPP and (2) investigate their quality evolution during chilled shelf life, including cloud stability, color changes, and flavor changes.

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2. Materials and methods

2.1. Sample preparation and processing

In the present work, two commercially formulated MJs including fruit-based MJ (MJ1; orange, mango, and kiwifruit) and fruit-and-vegetable-based MJ (MJ2; carrot and pineapple) were investigated. The formulations and processing procedures of the MJs were provided and optimized by a local juice production company (Yunnan Inja U-fresh Supply Chain Co., Ltd). According to consumer preliminary sensory assessments on color, taste, and aroma (data not shown), the two MJs with the highest scores were selected as the research objectives. All fruits and vegetables were purchased from a local market in Kunming, China. The details of MJ production are as follows.

MJ1 (orange, mango, and kiwifruit-based fruit juice): Navel oranges (*Citrus sinensis*) were first peeled and juiced using a laboratory-scale juicer (Joyoung Juicer JYZ-E3, China). SunGold kiwifruit (*Actinidia chinensis*) and Tainong mango (*Mangifera indica*) were peeled and deseeded. Then, kiwifruit and mango flesh were blended using a mixer (Joyoung JYL-C051, China) to obtain a uniform puree. Finally, MJ1 was obtained by combining orange juice, mango puree, and kiwifruit puree with a volume ratio of 10:3:2.

MJ2 (carrot and pineapple-based fruit/vegetable juice): Fresh Hongsen carrots (*Daucus carota*) were crushed and blended with water at a ratio of 1:1 (w:v) using a laboratory-scale mixer. The juice was filtered using two layers of 200-mesh cloths and kept in a cooling room. Cloudy Phulae pineapple (*Ananas comosus*) juice was collected using the laboratory-scale juicer. The same volumes of carrot and pineapple juices were mixed to obtain MJ2.

The formulated MJs were first homogenized at 20 MPa by a high pressure homogenizer (GJJ-0.06/70 MPa; Shanghai Noni Light Industrial Machinery, Shanghai, China). All MJs were filled into polyethylene bottles after homogenization. Then, the samples were pasteurized under 550 MPa for 5 min at room temperature using a HHP equipment (HHP-600; BaoTou KeFa High Pressure Technology Co., Ltd., Baotou, China). The processing temperature of samples were below 30 °C during HPP. Besides, under the HPP condition the results of preliminary study found the counts of total aerobic bacteria were less than 100 cfu/mL and yeast and molds of MJs were below detection limit in the end of the 90-day refrigerated storage (data not shown). Therefore, all samples in the study were stored in a cooling room at 4 °C for 90 days and sampled at different time periods (0, 7, 14, 21, 28, 35, 42, 60, and 90 days). Rheological properties, turbidity, and color were immediately analyzed after sampling. Other samples were transferred to 15 and 50 mL polypropylene tubes, frozen, and stored at −40 °C for other quality attributes analysis (e.g. vitamin C, pigments, sugar, and volatile compounds).

2.2. Quality properties

2.2.1. pH, TSS, and TA

The pH value of each sample was analyzed using a pH meter (FE28-Standard, Mettler Toledo, Zurich) at room temperature. TSS was measured using a refractometer (TD-45, Jinkelida, China) at 20 °C. The results are expressed in degrees °Brix, and all assays were conducted in triplicate. TA was analyzed using an automatic potentiometric titrator (907 GPD Titrino, Metrohm, Switzerland) according to the following equation (Liu et al., 2016):

$$TA(\%) = \frac{C \times V_2 \times K}{V_1} \times \frac{V_0}{W} \times 100 \quad (1)$$

where C is the NaOH concentration (0.1 mol/L), W is the total sample weight (g), V_2 is the NaOH volume used (mL), V_1 is the sample volume used (mL), V_0 is the total sample volume (mL), and K is the citric acid conversion factor (0.064).

2.2.2. Turbidity

Turbidity was analyzed based on the procedure reported by Bhat and Goh (2017). First, 10 mL of juice was centrifuged at 4200×*g* for 10 min at 25 °C. The collected supernatant was analyzed under 660 nm by a spectrophotometer (TU-19, PERSEE, China). The blank sample was distilled water. Turbidity was calculated according to Equations (2) and (3):

$$Transmittance = 100 \times 10^{-Abs} \quad (2)$$

$$Turbidity = 100 - Transmittance \quad (3)$$

2.2.3. Rheological properties

Rheological properties of MJs at different storage moments were measured using a modular compact rheometer (MCR 102, Anton Paar, Austria) fitted with a Couette-geometry sensor (concentric cylinder, Anton Paar CC27) with a cup and bob radius ratio of 1.085 (bob radius = 26.658 mm). The temperature was set constant at 25 °C. The apparent viscosity was measured by a logarithmically increasing stepwise protocol (1–100 s^{-1}). Steady-state flow properties were modelled by Herschel-Bulkley Model (Equation (4)) and apparent viscosities at shear rate of 10 s^{-1} was reported. All analysis was performed in six replicates.

$$\eta = \eta_0 + k\,(y)^{n-1} \quad (4)$$

Where k is consistency index, n is flow behavior properties, and η_0 is yield stress.

2.2.4. Color

Color (L^*, a^*, and b^* values) was analyzed using a hand-held colorimeter (CR-400, Minolta, Japan). Total color difference (ΔE^*) was calculated based on Equation (5).

$$\Delta E^* = \sqrt{[(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2]} \quad (5)$$

where the variables with subscript “0” are the initial values of juice colors immediately after HPP. All measurements were conducted in six replicates.

2.2.5. Vitamin C content

Total vitamin C, ascorbic acid (AA), and dehydroascorbic acid (DHAA) were measured according to our previous method (Yi et al., 2018a). Extraction was conducted for AA and total vitamin C analyses. DHAA concentration was calculated by subtracting AA from total vitamin C. Quantification was conducted on a high-performance liquid chromatography (HPLC) system equipped with a UV detector (1260 Series, Agilent Technologies, USA) and TC-C18 column (250 mm × 4.6 mm, 5 μm particle size; Agilent Technologies, USA). The isocratic elution buffers, Na_2EDTA (1 mmol/L) and CH_3COONH_4 (10 mmol/L), were used at a flow rate of 0.8 mL/min under pH 3.0. Injection volume was 20 μL, and UV detection was conducted at 245 nm at 20 °C. An external standard solution of AA (99%; Acros Organics, Aladdin, China) was used. Chromatographic analyses were carried out in triplicate.

2.2.6. Total carotenoids

Carotenoids were extracted and measured following the reported methods (Xie et al., 2019) with minor modification. Samples were ultrasonically extracted with acetone solution (containing 0.1 g/L butylated hydroxytoluene) for 15 min. Centrifugation was performed at 4000×*g* for 10 min at 4 °C, and the obtained supernatant was collected. The extraction procedures were repeated several times until the residue became colorless. The extraction solution was added to the final supernatant at a total volume of 25 mL. The total carotenoid was calculated according to Equations (6)–(8), respectively:

$$C_a = 11.75A_{662} - 2.35A_{645} \quad (6)$$

$$C_b = 18.61A_{645} - 396A_{662} \quad (7)$$

$$C_{xc} = \frac{1000A_{470} - 2.27C_a - 81.4C_b}{227} \quad (8)$$

where C_a, C_b and C_{xc} are the contents of chlorophyll *a*, chlorophyll *b* and total carotenoid, respectively (μg/mL); A_{662}, A_{645}, and A_{470} indicate the absorbance at 662, 645, and 470 nm, respectively. The total carotenoid contents of MJI and MJ2 were measured. The extraction and analyses of each sample were performed in triplicate.

2.2.7. Sugar profile

Sugar profile was analyzed according to our previous study with slight modification (Yi et al., 2016). Juice (10 mL) was mixed with the extraction buffer (1 mL; 500 μL of 150 g/L $K_4[Fe(CN)_6]$ and 500 μL of 300 g/L $ZnSO_4$) and rested for 30 min at room temperature. Each mixture was centrifuged at 15,000×*g* for 20 min at 4 °C and the obtained supernatant was filtrated using a 0.45 μm syringe filter. Sugar profile was analyzed using the HPLC system coupled with an evaporative light scattering detector (1260 Series, Agilent Technologies, USA). Separation was conducted on a column (Asahipak NH2P-50 4E, Shodex, Japan) coupled with a guard cartridge using an isocratic elution (75% v/v acetonitrile/water) at 30 °C. The flow rate was 1 mL/min and the injection volume was 5 μL. External standards (glucose, fructose, and sucrose) were used for identification and quantification. The extraction and analyses of each sample were performed in triplicate.

2.2.8. Volatile fraction

Volatile profile was analyzed using untargeted headspace–solid-phase microextraction–gas chromatography–mass spectrometry (HS-SPME-GC-MS) instrument (QP2010, Shimadzu, Japan) according to our method with minor modification (Yi et al., 2017). Each juice (5 mL) was mixed and homogenized with NaCl (1.8 g) in an amber glass vial. The vials were incubated at 40 °C for 15 min with shaking at 500 r/min. Then, volatiles were extracted using divinylbenzene/carboxen/polydimethylsiloxane SPME fiber (Zhenzheng, China) at 40 °C for 10 min. In the next step, the compounds were thermally desorbed at 230 °C for 5 min. The injection mode was splitless, and the column was an HP-5 column (30 m × 0.32 mm × 0.25 μm; Agilent Technologies, USA). The carrier gas was helium (flow rate of 1.5 mL/min). Column oven temperature was initially set at 40 °C for 2 min, then increased to 120 °C at 4 °C/min, further ramped to 200 °C at 7 °C/min, finally raised to 250 °C at 50 °C/min, held for 2 min at 250 °C, and cooled back to the initial temperature. Electron ionization mode was used at 70 eV with a scanning range and rate of 5–400 m/z and 3.0 scans/s, respectively. The ion source and quadrupole temperatures for MS were 230 and 250 °C, respectively. Volatile analyses at each sampling moment were repeated six times. Volatile compounds were identified through the comparison of their mass spectra with spectral library data in the National Institute of Standards and Technology 14 database. Retention index (RI) was calculated relative to the mixtures of *n*-alkanes (C3–C25) and compared with reference values.

2.3. Data analysis

All data were analyzed by principal component analysis (PCA) and partial least squares (PLS) regression via the chemometric Solo software (8.8.1 version, Eigenvector Research, USA). The inputs included: (1) the combination of both MJs, (2) MJ1 only and (3) MJ2 only. First, mean centering and variance scaling were carried out as pre-processing steps. Then, PCA was conducted to detect outliers. Third, PLS was performed to investigate the quality evolution of the MJs during storage. *X* variables were quality attributes, and *Y* variables were storage times. The bi-plots, combined scores, and correlation loading plots were illustrated using OriginPro software (version 8, Origin Lab Corporation, USA). Lastly, variable identification (VID) coefficient was calculated to identify the relation between quality parameter and storage time (Yi et al., 2018a). In the present study, variables with an absolute VID value above 0.900 were selected as markers.

The ANOVA and Tukey's honest significant difference test ($P < 0.05$) were performed in SPSS 20.0 statistics software (IBM, Armonk, USA). The analyses of the quality attributes of each sample were repeated at least six times.

3. Results and discussion

3.1. Multivariate analysis of quality changes of mixed juice during storage

PLS Bi-plots of the first two latent variables (i.e., LV1 and LV2) for the combination of both MJs and for individual MJs (MJ1 and MJ2) were shown in Fig. 1. As shown in Fig. 1A, a similar horizontal projection of MJs described by LV1 (*Y* variance, 82%) is illustrated on the bi-plot, which indicates that obvious quality changes occurred of MJs during storage. Besides, two clearly separated groups of MJ1 (yellow symbols) and MJ2 (orange symbols) were found, showing clear difference of quality attributes between MJ1 and MJ2. In order to gain a closer insight into the specific quality evolution of each MJ, individual bi-plots for MJ1 and MJ2 were constructed in Fig. 1B and C respectively. As shown, sucrose, turbidity, and most volatile compounds are close at the early stage of storage, whereas fructose, glucose, and ΔE^* are close at the end stage of storage. The result indicates that most of the quality parameters changed, especially volatile fraction. The VID coefficient of each quality parameter was calculated, and the parameters with an absolute VID value over 0.900 were chosen as discriminant attributes and are marked in Fig. 1D–E. According to the VID analysis, far more discriminant parameters were acquired for MJ1 compared to MJ2, most of which are volatile components. Furthermore, the discriminant volatiles with negative VID values could be grouped under acids, aldehydes, ketones, alcohols, terpenes, and esters. The finding demonstrates that concentrations of the volatiles clearly decreased during storage. Only a few aromatic compounds with positive VID values were selected, including one ketone (geranyl acetone), three terpenoids, two oxidative compounds, and one furan compound, indicating they increased during storage. In addition to volatiles, sucrose, turbidity, and vitamin C had highly negative VID values showing decreasing trends, whereas fructose and color coordinates (L^*, a^*, b^*, and ΔE^* values) exhibited positive VID values with increased trends during storage.

In general, of all quality attributes, aroma and taste were the main quality parameters changing during storage. In order to understand how the quality parameters changed and possible reaction pathways, more results and discussion on cloud stability, color changes, and flavor fraction of different MJs were conducted in the following sections.

3.2. Cloud stability of mixed juice during storage

One of the main challenges in MJ production is to enhance the cloud stability of the complex system. In addition to visual observation (Fig. 2), the turbidity of MJs during storage was investigated in this study (**Section 3.2.1**). According to our previous finding, viscosity is one of the main factors affecting cloud stability of juice (Yi et al., 2018a). Therefore, a comparison of the rheological properties of the MJs was also performed (**Section 3.2.2**).

3.2.1. Turbidity

As shown in Table 1, cloudy MJ1 and MJ2 were turbid. The result agreed with the visual observation, where no clear sedimentation appeared in MJ1 and MJ2 during 90 days of storage. Furthermore, a decrease trend of the turbidity was observed in both MJs. The turbidity of MJ1 and MJ2 decreased by 14.48% and 32.21%, respectively. A similar decreased turbidity was reported in a HPP fruit and vegetable smoothie mixing with apple, carrot, zucchini, pumpkin and leek, stored for 28 days at 4 °C (Hurtado et al., 2019). Cloudy fruit and vegetable MJs

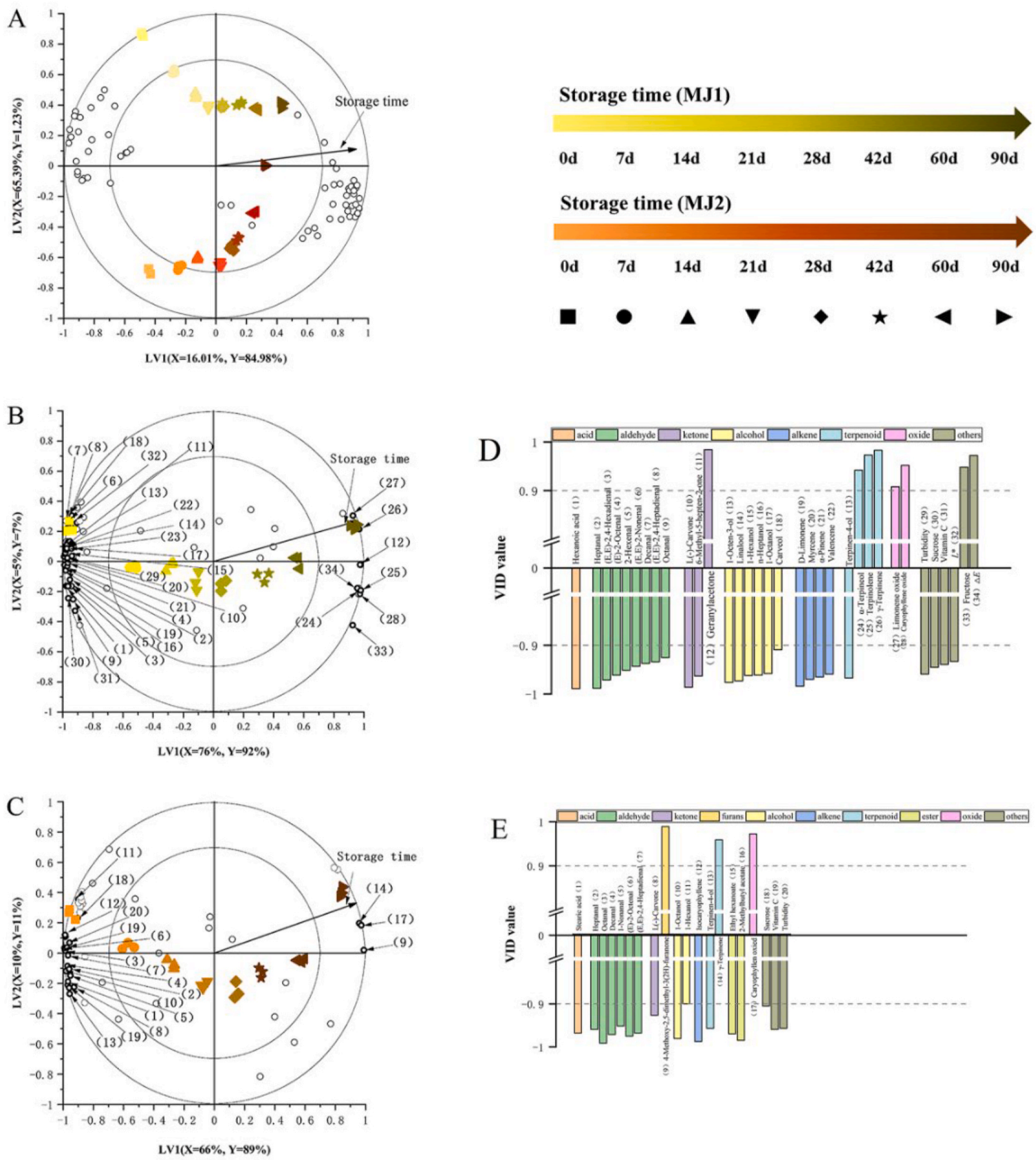

Fig. 1. Bi-plots on quality changes during storage of both MJs **(A)**, MJ1 **(B)** and MJ2 **(C)** and discriminant volatiles of MJ1 **(D)** and MJ2 **(E)**.

Fig. 2. Visual appearance of HPP MJs during storage (90 days, 4 °C).

Table 1
Physicochemical properties of HPP MJs during storage (4 °C, 90 days).

Storage time (days)	Turbidity	L^*	a^*	b^*	ΔE^*[a]	AA (mg/100 mL)	DHAA (mg/100 mL)	Vitamin C (mg/100 mL)	Total carotenoids (μg/100 mL)
MJ1									
0	90.27±0.94^{e}	38.99±0.01^{c}	0.43±0.03^{a}	23.62±0.04^{ab}	–[b]	39.83±2.54^{c}	14.02±3.00^{b}	53.85±0.71^{e}	306.66±2.53^{a}
7	89.17±0.84^{e}	38.93±0.03^{c}	0.45±0.02^{a}	23.60±0.06^{ab}	0.09±0.02^{a}	38.39±2.04^{c}	13.92±3.52^{b}	52.31±1.51^{e}	306.45±3.42^{a}
14	85.72±0.53^{d}	38.93±0.05^{c}	0.45±0.02^{a}	23.77±0.44^{b}	0.39±0.05^{b}	38.94±0.91^{c}	13.31±2.02^{b}	48.58±0.70^{d}	307.35±4.73^{a}
21	83.31±0.73^{c}	38.70±0.17^{c}	0.43±0.02^{a}	23.55±0.24^{ab}	0.39±0.03^{b}	35.95±1.07^{c}	13.19±1.24^{b}	52.13±0.85^{e}	306.97±2.40^{a}
28	82.43±0.08^{bc}	38.63±0.31^{c}	0.42±0.02^{a}	23.11±0.08^{a}	0.68±0.07^{c}	35.27±2.31^{c}	12.73±0.26^{b}	48.68±0.81^{d}	307.73±1.38^{a}
42	82.01±0.11^{bc}	38.22±0.15^{b}	0.46±0.02^{a}	23.15±0.02^{ab}	0.91±0.13^{d}	28.76±1.01^{b}	12.13±1.00^{b}	40.89±0.77^{c}	305.27±2.81^{a}
60	81.25±0.32^{b}	37.50±0.13^{a}	0.43±0.02^{a}	23.18±0.02^{ab}	1.55±0.12^{e}	24.54±1.38^{ab}	9.71±0.67^{ab}	34.25±0.70^{b}	306.88±0.91^{a}
90	77.20±0.24^{a}	37.20±0.02^{a}	0.46±0.03^{a}	23.42±0.35^{ab}	1.82±0.05^{f}	22.39±0.89^{a}	6.65±0.64^{a}	31.18±0.84^{a}	307.25±1.07^{a}
MJ2									
0	97.57±0.78^{f}	34.27±0.39^{ab}	15.66±0.24^{a}	21.57±0.33^{a}	–	17.55±0.34^{b}	4.73±0.92^{c}	22.28±0.58^{d}	236.97±1.88^{b}
7	93.2±0.76^{e}	34.20±0.10^{a}	15.27±0.14^{a}	21.45±0.38^{a}	0.53±0.09^{a}	17.48±0.20^{b}	4.34±0.31^{bc}	21.83±0.49^{d}	230.48±4.73^{ab}
14	82.45±0.69^{d}	34.71±0.05^{ab}	15.61±0.33^{a}	21.67±0.10^{a}	0.53±0.04^{a}	17.31±0.49^{b}	3.96±0.98^{bc}	20.76±0.07^{c}	223.85±3.46^{a}
21	81.99±1.42^{d}	34.90±0.10^{b}	15.23±0.16^{a}	21.50±0.21^{a}	0.78±0.08^{a}	16.80±0.55^{ab}	4.12±0.36^{bc}	20.48±0.03^{c}	223.21±2.06^{a}
28	73.55±0.75^{b}	34.74±0.15^{ab}	15.72±0.16^{a}	21.50±0.43^{a}	0.59±0.24^{a}	16.77±0.82^{ab}	2.87±0.66^{ab}	20.17±0.17^{c}	220.87±2.01^{a}
42	77.92±1.08^{c}	34.39±0.14^{ab}	15.48±0.11^{a}	21.16±0.13^{a}	0.52±0.11^{a}	16.36±0.34^{ab}	1.74±0.83^{a}	18.51±0.04^{b}	224.45±4.23^{a}
60	74.09±2.91^{b}	34.90±0.04^{b}	15.66±0.14^{a}	21.61±0.24^{a}	0.67±0.05^{a}	15.66±0.49^{a}	1.68±0.66^{a}	17.34±0.17^{a}	225.43±4.00^{a}
90	66.14±0.60^{a}	34.54±0.44^{ab}	15.54±0.16^{a}	21.62±0.18^{a}	0.50±0.10^{a}	15.51±0.32^{a}	1.72±0.48^{a}	17.23±0.17^{a}	224.21±3.86^{a}

Values with the different letters within one column are significantly different ($P < 0.05$).
[a] The juice immediately after HPP is used as a reference to calculate ΔE^*.
[b] Not Detectable.

are considered as a colloidal dispersion of electrically charged particles in a complex serum of pectin, sugars, organic acids, and salts (Genovese and Lozano, 2006). Shortly after juice extraction, coarse particles settle out immediately by gravity, while fine particles remaining in suspension (Genovese and Lozano, 2006). However, numerous chemical and biochemical changes occurring during shelf-life lead to cloud loss of MJs, such as possibly molecular polymerization, molecular interactions, and pectin cross-links (Hurtado et al., 2019; Zeng et al., 2019).

3.2.2. Rheological properties

Rheological properties of MJs were investigated as shown in Fig. 3 and Table 2. All HPP MJs were non-Newtonian pseudoplastic fluid with shear thinning behaviors, which could be well fitted by Herschel–Bulkley modellings ($R^2 \geq 0.99$). It was in accordance with the results found in peach-carrot-apple MJ (Wellala et al., 2020b). According to the estimated parameters (Table 2), MJ1 has a significantly higher consistency index (≥656.18 mPa.s^n), apparent viscosity (≥139.22 mPa s^{-1}, shear rate = 10 s^{-1}), and dynamic yield stress (≥12.88 mPa) compared with that of MJ2 (≤44.13 mPa.s^n; ≤13.42 mPa s^{-1}, shear rate = 10 s^{-1}; ≤6.18 mPa, respectively). As known, the consistency index also corresponded to the apparent viscosity (Peng et al., 2016). Table 2 shows that the apparent viscosity of both MJs remained stable during storage. It agreed with our previous work found in HPP apple-kiwifruit MJ during storage (Yi et al., 2018b).

According to the Stokes's law, juice viscosity and particle size are mainly responsible for cloud stabilization (Beveridge, 2002). In the work, a standard high pressure homogenization (HPH) with an upstream pressure of 20 MPa was used to modulate particle size distribution and rheological properties of pulp-enriched cloudy MJs. In the way, part of the suspended pulp is converted into colloidal pulp by size reduction, leading to a slower sedimentation (Yi et al., 2018b). Meanwhile, an increased apparent viscosity could further improve the cloud stability of MJs during storage (Yi et al., 2018a, 2018b), which might explain why the MJ1 with higher apparent viscosity showed more stable cloud and higher turbidity than MJ2.

3.3. Color changes of mixed juice during storage

Color stability of both MJs during storage was visually observed (Fig. 2) and instrumentally analyzed (Table 1). Changes in the color coordinates (L^*, a^*, b^*, and ΔE^* values) and total carotenoids of MJs during storage was investigated in **Sections 3.3.1** and **3.3.2**, respectively. In addition, AA and vitamin C are natural anti-browning agents that are strongly associated with enzymatic browning (Yi et al., 2018a). Therefore, contents of AA and DHAA in MJs were quantified and analyzed (**Section 3.3.3**).

3.3.1. Color

Table 1 shows that no significant changes were observed in the colorimetric parameters (L^*, a^*, and b^* value) of MJ2 during storage ($P > 0.05$). As for MJ1, L^* value decreased from 38.99 to 37.20 during storage, indicating it slightly turned dark. While a^* and b^* value of MJ1 remained stable during storage ($P > 0.05$). Total color difference (ΔE^* value) is a parameter that evaluates the overall color changes of juice during storage. The difference of color could be visible for consumers when the ΔE^* value exceeds 3.0 (Buvé et al., 2018). During the whole storage, ΔE^* value of MJ1 and MJ2 were below 2.0, particularly for MJ2 (around 0.6). The result can be confirmed by the visual observation

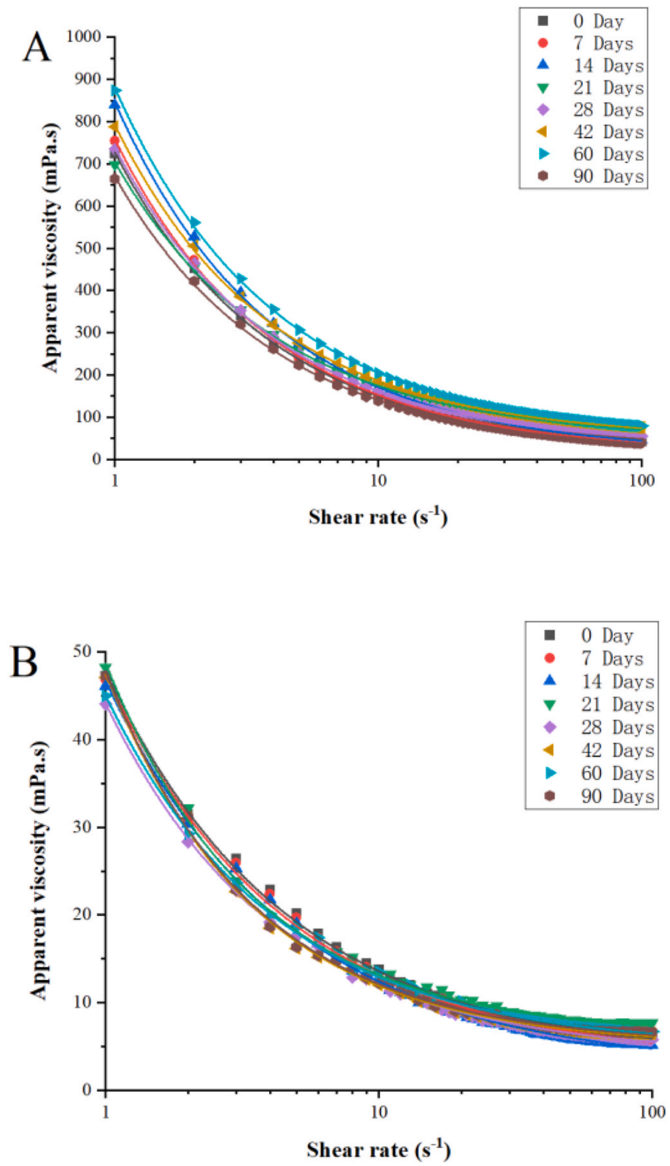

Fig. 3. The rheological properties of HPP MJ1 **(A)** and MJ2 **(B)** during storage (90 days, 4 °C). The full lines represent the fitted values by the Herschel-Bulkey kinetic modelling and the different symbols represent the experimental data.

shown in Fig. 2, where MJ1 and MJ2 had good color stability throughout storage period. In addition, no significant changes were observed in the ΔE^* value of MJ2 during storage ($P > 0.05$), but an increase of ΔE^* was found in MJ1, demonstrating that MJ2 was more stable on color than MJ1. It seems that orange, mango, kiwifruit, carrot, and pineapple were color stable ingredients for juice production for HPP and short time refrigerated storage. Similar results were also reported in kiwifruit puree (Yi et al., 2018a), orange juice (Bull et al., 2004), and carrot juice (Zhang et al., 2016).

3.3.2. *Total carotenoids*

The yellow, orange, and red color of juice is mainly because of carotenoids (Chandra et al., 2021). Changes in total carotenoids of MJs were shown in Table 1. There was no significant difference in total carotenoids of MJ1 (305–308 μg/100 mL) and MJ2 (220–236 μg/100 mL) ($P > 0.05$). It could be confirmed by the visual observation shown in Fig. 2, where MJ1 and MJ2 had good main color stability (yellow and orange) during storage period. Although carotenoids are susceptible to oxidation and isomerization during processing and storage (Liu et al., 2019), carotenoids have been proven to be highly stable to HPP, even combined with moderate to high temperatures (Sánchez et al., 2014). Andrés et al. (2016) reported that the carotenoids was stable in the mixed fruit smoothie treated by HPP. Besides, stable carotenoids in HPP orange juice were observed during storage (Plaza et al., 2011).

Table 2
Rheological properties of MJs during storage (90 days at 4 °C).

Storage time (days)	Viscosity (10 s^{-1} mPa.s)	Herschel Bulkey model parameters: Consistency index (k) (mPa.sn)	Flow behavior index (n) (−)	Yield stress (η_0) (mPa)	R^2
MJ1					
0	148.39±9.89 ^{ab}	710.92 ± 0.97 ^{b}	0.73 ± 0.01 ^{a}	16.30 ± 0.32 ^{c}	0.99
7	151.99±9.63 ^{ab}	743.17 ± 1.09 ^{c}	0.73 ± 0.01 ^{a}	15.17 ± 0.36 ^{b}	0.99
14	169.09±11.00 ^{bc}	830.75 ± 1.26 ^{d}	0.72 ± 0.01 ^{a}	14.61 ± 0.43 ^{b}	0.99
21	173.22±9.46 ^{bc}	657.18 ± 1.18 ^{a}	0.71 ± 0.01 ^{a}	46.88 ± 0.42 ^{e}	0.99
28	160.39±9.89 ^{abc}	712.92 ± 0.93 ^{b}	0.73 ± 0.01 ^{a}	28.30 ± 0.31 ^{d}	0.99
42	185.99±9.63 ^{cd}	745.17 ± 1.10 ^{c}	0.73 ± 0.01 ^{a}	49.17 ± 0.36 ^{f}	0.99
60	203.94±11.00 ^{d}	829.75 ± 1.23 ^{d}	0.72 ± 0.01 ^{a}	48.61 ± 0.43 ^{f}	0.99
90	139.22±9.46 ^{a}	656.18 ± 1.12 ^{a}	0.71 ± 0.01 ^{a}	12.88 ± 0.42 ^{a}	0.99
MJ2					
0	13.61±0.98 ^{a}	44.13 ± 0.24 ^{c}	0.65 ± 0.01 ^{a}	3.73 ± 0.11 ^{c}	0.99
7	13.14±0.98 ^{a}	44.13 ± 0.25 ^{c}	0.65 ± 0.01 ^{a}	3.26 ± 0.11 ^{b}	0.99
14	12.45±0.98 ^{a}	44.13 ± 0.25 ^{c}	0.65 ± 0.01 ^{a}	2.57 ± 0.11 ^{a}	0.99
21	13.42±0.34 ^{a}	42.52 ± 0.26 ^{b}	0.79 ± 0.01 ^{c}	6.18 ± 0.08 ^{f}	0.99
28	12.18±0.76 ^{a}	40.27 ± 0.18 ^{a}	0.70 ± 0.01 ^{b}	3.87± 0.07 ^{c}	0.99
42	12.23±0.36 ^{a}	42.52 ± 0.26 ^{b}	0.79 ± 0.01 ^{c}	4.98± 0.08 ^{d}	0.99
60	13.10±0.76 ^{a}	40.27 ± 0.18 ^{a}	0.70± 0.01 ^{b}	4.79 ± 0.08 ^{de}	0.99
90	12.42±0.36 ^{a}	42.52 ± 0.26 ^{b}	0.79 ± 0.01 ^{c}	5.17± 0.08 ^{e}	0.99

Values with the different letters within one column are significantly different ($P < 0.05$).

3.3.3. *Vitamin C*

Orange and kiwifruit are natural sources of AA (Bull et al., 2004; Wellala et al., 2019). The initial AA concentration of MJ1 and MJ2 were 39.83 mg/100 mL and 17.45 mg/100 mL, respectively. Besides, the AA contents significantly decreased in both MJs during storage, particularly in MJ1 ($P < 0.05$). Meanwhile, the total vitamin C and DHAA of MJs also gradually decreased during storage. As HPP cannot fully inactivate peroxidase (POD) and polyphenol oxidase (PPO) in MJs, which was reported with high activity in kiwifruit, mango, and orange juices (Dars et al., 2019; Liu et al., 2019; Wan et al., 2020), the residual enzyme might induce the oxidation of AA. The AA could be rapidly oxidized to DHAA and that was further transformed to 2,3-diketogulonic acid and other breakdown products (Cánovas et al., 2020). The degradation of both AA and DHAA could be related to the enzymatic browning (Yi et al., 2018a). Besides, sucrose was hydrolyzed to fructose and glucose in MJ1 and MJ2 (Table 3). The reducing sugars could also participate in non-enzymatic browning reactions of MJs (Buvé et al., 2018).

In general, MJ2 showed the good color stability compared to MJ1. Although there was no clear browning observed in MJs during 90 days' storage, an enzymatic browning during prolonged storage time might be occurred when AA was completely consumed. In addition to enzymatic

Table 3
Sugars and acidity of MJs during storage at 4 °C for 90 days.

Storage time (days)	TSS (°Brix)	Fructose (mg/mL)	Glucose (mg/mL)	Sucrose (mg/mL)	pH	TA (%)
MJ1						
0	14.03 ± 0.06^{a}	24.41 ± 1.84^{a}	18.24 ± 0.59^{a}	28.72 ± 1.55^{e}	3.84 ± 0.04^{a}	0.31 ± 0.02^{a}
7	14.33 ± 0.23^{a}	24.61 ± 0.47^{a}	19.81 ± 0.53^{ab}	22.19 ± 0.57^{d}	3.89 ± 0.06^{a}	0.31 ± 0.01^{a}
14	14.13 ± 0.23^{a}	27.17 ± 1.54^{ab}	20.54 ± 0.92^{ab}	19.16 ± 0.39^{c}	3.85 ± 0.04^{a}	0.31 ± 0.02^{a}
21	14.33 ± 0.23^{a}	29.98 ± 1.36^{bc}	22.65 ± 0.90^{b}	17.37 ± 0.58^{bc}	4.01 ± 0.15^{b}	0.30 ± 0.03^{a}
28	14.30 ± 0.52^{a}	33.21 ± 0.44^{cd}	22.21 ± 2.29^{bc}	16.26 ± 1.10^{b}	3.83 ± 0.02^{a}	0.31 ± 0.01^{a}
42	14.73 ± 0.12^{a}	34.49 ± 2.03^{d}	26.19 ± 1.49^{cd}	11.20 ± 0.16^{a}	3.83 ± 0.05^{a}	0.31 ± 0.01^{a}
60	14.53 ± 0.31^{a}	38.53 ± 0.37^{e}	25.92 ± 1.38^{d}	9.88 ± 0.01^{a}	4.00 ± 0.07^{b}	0.31 ± 0.01^{a}
90	14.17 ± 0.13^{a}	51.62 ± 0.39^{f}	37.22 ± 0.46^{e}	9.72 ± 0.01^{a}	3.87 ± 0.08^{a}	0.30 ± 0.00^{a}
MJ2						
0	9.26 ± 0.46^{ab}	11.09 ± 0.06^{a}	12.41 ± 0.07^{a}	33.75 ± 0.18^{a}	4.04 ± 0.05^{b}	0.24 ± 0.00^{a}
7	9.03 ± 0.06^{ab}	11.15 ± 0.08^{a}	12.57 ± 0.09^{a}	31.95 ± 0.74^{a}	3.96 ± 0.45^{a}	0.23 ± 0.01^{a}
14	9.23 ± 0.32^{ab}	11.46 ± 0.13^{a}	12.79 ± 0.14^{a}	30.46 ± 1.29^{a}	3.96 ± 0.02^{a}	0.23 ± 0.00^{a}
21	9.03 ± 0.06^{a}	11.50 ± 0.20^{a}	12.75 ± 0.27^{a}	27.57 ± 1.07^{a}	4.06 ± 0.01^{c}	0.25 ± 0.01^{a}
28	9.60 ± 0.35^{ab}	11.67 ± 0.06^{a}	12.78 ± 0.01^{a}	26.03 ± 1.09^{a}	3.97 ± 0.01^{ab}	0.24 ± 0.01^{a}
42	9.80 ± 0.17^{b}	12.57 ± 0.53^{a}	13.69 ± 0.47^{a}	27.40 ± 0.82^{a}	3.96 ± 0.01^{a}	0.24 ± 0.02^{a}
60	9.10 ± 0.10^{ab}	13.75 ± 0.70^{a}	14.95 ± 1.02^{a}	26.77 ± 0.04^{a}	3.95 ± 0.01^{a}	0.24 ± 0.02^{a}
90	9.20 ± 0.20^{ab}	16.61 ± 0.74^{a}	17.86 ± 0.61^{a}	25.46 ± 1.09^{a}	3.92 ± 0.02^{a}	0.24 ± 0.02^{a}

Values with the different letters within one column are significantly different ($P < 0.05$).

browning, more expansion study would be worthwhile to investigate some non-enzymatic browning reaction of MJs during storage.

3.4. *Flavor changes of mixed juice during storage*

Changes in pH, TA, TSS, and sugar profile are discussed in **Section 3.4.1**, and changes in aromatic attributes (HS-SPME-GC-MS volatile fingerprinting) are discussed in **Section 3.4.2**.

3.4.1. *pH, TA, TSS, and sugar profile*

High acidity (0.30%–0.31%) and TSS (14.03–14.17 °Brix) were found in MJ1, followed by MJ2 (acidity, 0.23%–0.25%; TSS, 9.03–9.80 °Brix) as shown in Table 3. The pH, TA, and TSS values of both MJs did not significantly change during refrigerated storage ($P > 0.05$). The result was in agreement with the study reported by Juarez-Enriquez et al. (2015), who reported that no changes occurred in the pH, TA, and TSS of HPP apple juice during storage for 34 days.

However, the sugar profiles of MJs in our study varied and showed different change trends during storage (Table 3). Sucrose, fructose, and glucose were the main sugars in MJ1 (28.72 mg/mL, 24.41 mg/mL, and 18.24 mg/mL, respectively) and MJ2 (33.75 mg/mL, 11.09 mg/mL, and 12.41 mg/mL, respectively). The sucrose concentrations of MJ1 and MJ2 decreased, whereas their glucose and fructose contents increased during storage. A comparable trend was found in cloudy apple juice (Wibowo et al., 2015). The conversion of sucrose to glucose or fructose during storage could be related to acid and soluble invertase-catalyzed sucrose hydrolysis (Wibowo et al., 2015).

3.4.2. *Volatile fraction*

The representative total ion chromatograms of volatile components of fresh MJs are depicted in Fig. 4. Similar numbers (n = 32–34) of volatile compounds were detected in two MJs. However, the abundance of volatile compounds in MJ1 was obviously higher than that in MJ2. The main volatile compounds are numbered in Fig. 4, and their RIs, chemical group, and odor description are listed in Table 4.

As shown, D-limonene was the most abundant volatile component in MJ1, which imparts the strong sweet citrus odor provided by orange (Mastello et al., 2018). Besides, some alkenes (α-pinene, β-myrcene, and [E]-β-ocimene), terpenoids (terpinen-4-ol), aldehydes ([E,E]-2,4-heptadienal), and esters (ethyl 3-hydroxyhexanoate) also play a fundamental role in MJ1, which represent sweet and tropical fruit odors (Bai et al., 2016). It seems that the aroma from orange was more dominant than that from kiwifruit and mango in MJ1. As for MJ2, esters (methyl hexanoate and ethyl hexanoate), ketones (6-methyl-5-hepten-2-one and L [-]-carvone), alkenes (α-caryophyllene), and terpenoids (γ-terpinene

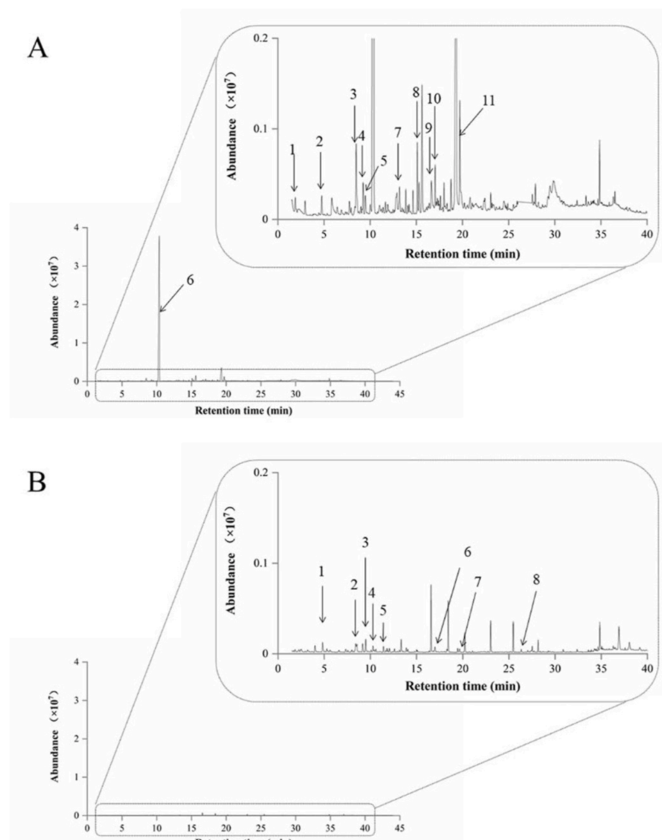

Fig. 4. Total ion chromatogram of the headspace of MJ1 **(A)** and MJ2 **(B)** in the beginning of storage (day 0), obtained by HS-SPME-GC-MS. The main volatile compounds are identified with number as indicated in Table 4.

and terpinen-4-ol) were the key aromatic compounds. Among these compounds, methyl hexanoate, 6-methyl-5-hepten-2-one, ethyl hexanoate, γ-terpinene, and terpinen-4-ol are found in pineapple (Steingass et al., 2014), and L(−)-carvone and caryophyllene are contributed by carrot (Keser et al., 2020).

According to the VID, most of the volatile compounds of the MJs, including esters (2-methylbutyl acetate, isoamyl acetate, and methyl hexanoate), alkenes (D-limonene, myrcene, α-pinene, and β-pinene), alcohols (linalool, carveol, eucalyptol, [E]-6-nonenol, and [E,Z]-3,6-

Table 4
Main volatile components identified in MJs pasteurized by HPP.

Peak number	Components [a]	RI [b]	Chemical group	Odor description [c]	Identification [d]
MJ1					
1	(E,E)-2,4-Hexadienal	911	aldehyde	sweet, green, citrus, kiwifruit	MS, RI
2	α-Pinene	938	alkene	woody, fresh herbal, citrus	MS, RI
3	β-Myrcene	981	alkene	vegetative, citrus, fruity with a tropical mango	MS, RI
4	(E,E)-2,4-Heptadienal	1012	aldehyde	sweet,creamy, fatty, citrus peel	MS, RI
5	(E)-β-Ocimene	1039	alkene	green, tropical, woody with floral, mango	MS, RI
6	D-Limonene	1044	alkene	citrus, orange, fresh, sweet	MS, RI
7	Linalool	1104	alcohol	citrus, orange, lemon, floral, waxy, woody	MS, RI
8	Ethyl 3-hydroxyhexanoate	1136	ester	sweet, fruity, citrus	MS, RI
9	Terpinen-4-ol	1177	terpenoid	peppery, woody, earthy, musty, sweet	MS, RI
10	α-Terpineol	1190	terpenoid	pine, floral, lilac	MS, RI
11	L(−)-Carvone	1231	ketone	sweet, minty, spearmint, caraway	MS, RI
MJ2					
1	Methyl hexanoate	924	ester	fruity, fatty, pineapple	MS, RI
2	6-Methyl-5-hepten-2-one	987	ketone	fruity, apple, musty, pineapple	MS, RI
3	Ethyl Hexanoate	1002	ester	sweet, pineapple, fruity, waxy, banana	MS, RI
4	γ-Terpinene	1030	terpenoid	citrus, pineapple, oily, green with a tropical fruity nuance	MS, RI
5	4-Methoxy-2,5-dimethyl-3(2H)-furanone	1065	ketone	moldy, earthy, vegetable, potato	MS, RI
6	Terpinen-4-ol	1177	terpenoid	peppery, woody, earthy, musty sweet	MS, RI
7	L(−)-Carvone	1231	ketone	sweet, minty, spearmint, caraway	MS, RI
8	α-Caryophyllene	1428	alkene	woody, Oceanic-watery, Spicy-clove	MS, RI

[a] The reliability of the identification proposal is carried out: mass spectrum and retention index agreed with database or literature.
[b] Calculated retention index (RI) on HP-5 column.
[c] Odor description were obtained from literature data (http://www.thegoodscentscompany.com).
[d] Identification methods: MS, mass spectrometry; RI, retention indices.

nonadienol), aldehydes (heptanal, decanal, [E,Z]-2,6-nonadienal, [E,E]-2,4-hexadienal, [E]-2-nonenal, and β-cyclocitral), ketones (L[-]-carvone, 6-methyl-5-hepten-2-one, and [Z]-jasmone), and acids (stearic acid and oleic acid), clearly decreased during storage (Fig. 1). Few volatile compounds increased, which were mostly terpenoids (terpineol, α-terpineol, and terpinen-4-ol) and oxides (limonene oxide and caryophyllene oxide). Acid-catalyzed hydrolysis and oxidation might be the main reasons for the degradation and formation of compounds. For example, a decrease in linalool corresponded to an increase in some terpenoids (terpineol, α-terpineol, and terpinen-4-ol) during storage and could be linked to acid-catalyzed degradation (Bacigalupi et al., 2013). Limonene oxide and caryophyllene oxide are the oxidation products of limonene and caryophyllene, respectively (Choi and Sawamura, 2002).

Generally, sucrose hydrolysis and volatile changes would cause the loss of fresh-like flavor of MJs during storage. In other words, the loss of sweet taste and fruity aroma of HPP MJs during storage would be a big challenge for the industry and restrict the development of HPP application in MJ production.

4. Conclusions

The research explored the potential creation of natural, fresh, and shelf-stable HPP juices from the combination of appropriate ingredients. Among the different formulated MJs, MJ2 (carrot and pineapple) showed the higher quality stability during chilled storage than MJ1 (orange, mango, and kiwifruit). During 90 days refrigerated storage, color and cloud stability of MJs were stable, while sucrose hydrolysis and key aromatic compounds degradation were the main quality changes. Therefore, in the future, flavor changes during storage should be paid more attention for HPP MJ processing. In general, the outcome of the work provides an efficient protocol in designing HPP MJs with high shelf-life quality stable. Apart from formulations of MJ1 and MJ2 in the study, other combination of fruit and vegetable with similar characteristics could also be used to blend for MJs.

CRediT authorship contribution statement

Minbo Li: Investigation, Data curation, Writing – original draft. **Qihui Liu:** Data curation, Writing – original draft. **Wanzhen Zhang:** Methodology, Writing – review & editing. **Litao Zhang:** Funding acquisition. **Linyan Zhou:** Investigation. **Shengbao Cai:** Conceptualization. **Xiaosong Hu:** Supervision. **Junjie Yi:** Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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