

# Comparative metabolomics analysis in the clean label ingredient of NFC spine grape juice processed by mild heating vs high pressure processing

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## Abstract

Not from concentrate (NFC) fruit juice is the crucial clean label ingredient for new-style tea-making due to its pleasant color and fresh aroma. Here, we compared the effects of mild heating (MH) and high pressure processing (HPP) on physicochemical characters and phytochemicals in NFC spine grape juice based on metabolomics analysis. Similar compound profiles were observed between HPP-treated and fresh juices. The richer phytochemical compounds comprised malvidin-3-O-glucoside, malvidin-3,5-di-O-glucoside, quercetin-3-O-rhamnoside, quercetin-3-O-glucuronide, catechin, caffeic acid, ferulic acid, procyanidin B1, procyanidin B2 were obtained after MH treatment. Nine marker phenolics and two marker tripeptides (i.e., Glu-Val-Phe and Leu-Leu-Tyr) were identified to differentiate MH from HPP treatment, of which higher contents occurred in the MH group. Storage time experiments showed that the Glu-Val-Phe could serve as potential markers for monitoring storage of spine grape juice. These results provide new insights into the effects of processing on individual phytochemical changes and the guide for commercial application of production of spine grape NFC juice.

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## Introduction

Tea has gained increasing popularity among consumers due to its taste, flavor, aroma and health-promoting effects. New style tea drinks have emerged as favorites for young people, which mix tea with other components, such as fruits juice and fresh milk<sup>[1]</sup>. Over 80% of consumers buy 5–14 cups tea monthly, with 72% of customers spending USD 29 monthly<sup>[2]</sup>.

Not from concentrate (NFC) juice is considered to be a clean label ingredient for new-style tea drinks as it harnesses the natural flavour and nutrition of the original fruit and therefore can be consumed directly after blending mixing with tea<sup>[3]</sup>. Bright color berries including grape, strawberry and mulberry, as well as rich flavored tropical fruits such as mangos and coconuts, are the most commonly used juice ingredients, which endow fruit tea with interesting organoleptic properties (pleasant color, fresh flavour and diverse taste) and nutritional value.

Thermal processing is the most common and traditional technique used for food ingredient processing to extend shelf life. Adverse effects on organoleptic properties of foods caused by thermal processing have been reported. Therefore, various milder processing technology has come to light. High pressure processing (HPP) is a non-thermal pasteurization technique, which has been suggested to have superior retention of nutritional quality compared to traditional thermal processing methods. The effect of thermal processing and HPP on fruit,

juice, puree, or pulp product has been widely investigated, and there is abundant information about traditional 'targeted' index such as microorganism, color, total phenols and total anthocyanins<sup>[4,5]</sup>. However, HPP may not always be a better alternative to thermal processing for fruit and its products processing depending on food matrix and processing condition<sup>[6]</sup>.

Spine grape (*Vitis davidii* Foex) is a valuable and prevalent wild grape species in China, belongs to the East Asian *Vitis* spp.. High level of phenolics, flavonoids, and anthocyanins content of spine grape is the cause of strong color<sup>[7]</sup>, and it has distinctive aroma descriptors such as wild rose, violets and wild strawberries<sup>[8]</sup>. The aim of this investigation is to reveal the physicochemical characteristics together with phytochemical compound profiles of spine grape juice processed by mild heating (MH) and HPP on the basis of targeted and untargeted approaches. From a practical perspective, this investigation contributes sufficient dataset outcomes for additional exploitation of spine grapes as a potential food ingredient.

## Materials and methods

### Sample preparation

NFC juice are pasteurized or frozen and packed immediately after processing, then frozen NFC juice ingredients are delivered into new-style tea stores, these frozen juice ingredients are thawed and temporarily stored in the refrigerator at 4 °C for later use. Therefore, in this work, the juice after treatment, as

well as the thawed juice with a total of 21 d refrigerated storage, were carried out in step. All these samples were collected for further analysis. The process is shown schematically in [Supplemental Fig. S1](#).

Two spine grape varieties (Hunan province, China), namely ZiQiu (ZQ) and TianCi (TC), were used here. Grapes were washed, then pressed with a juicer (SJ 600, Dongah Co., Ltd., Korea) that automatically separates the pomace from the juice. The obtained spine grape juice was poured into polyethylene terephthalate bottles for further MH and HPP processing. The processing condition was selected based on a previous study with little modification<sup>[4]</sup>: for the MH group, the juice samples were blanched at 85 °C for 5 min; for HPP group, 500 MPa and 10 min HPP treatment was applied using a hydrostatic pressurization (CQC30L-600®, Beijing Suyuan Zhongtian technology™, China). The counts of total number of microorganisms in the two groups was less than 1.00 log CFU/mL. The samples of two groups were divided into two parts: one for physicochemical characters and phytochemical compound analysis, and one for -20 °C frozen storage. The thawed juices with a total of 21 d refrigerated storage were also analyzed.

### TSS, pH, and color

The total soluble-solid content (TSS) was determined by WAY-2S digital Abbe® Refraction meter (Shanghai Precision Instrument™, China). The pH was determined through standard pH meter (ThermoScientific, Waltham, MA, USA). Color parameters *L*, *a* and *b* were measured by a colorimeter (HunterLab, Germany). Three replicates were performed for each sample.

### Sugar and organic acid content

An ion chromatography (ICS 3000+, ThermoFisher Scientific, USA) equipped with an automated sample injector (Dionex AS autosampler) was used for determining sugar and organic acid content. The spine grape juice samples were diluted to desired concentration and filtered through a 0.22- $\mu$ m membrane, before chromatographic analysis. The analysis of sugars was conducted using ion chromatography with HPAEC-PAD on a gold working electrode (Dionex Germany). The PA20 anion analysis column (3 mm  $\times$  150 mm), and PA20 guard column (4 mm  $\times$  50 mm) was used for chromatographic separation. The eluant procedure was carried out in accordance with the method of Wang & Xu<sup>[9]</sup>. The determination of organic acids was conducted by ion chromatography with suppressed conductivity detection (Dionex Germany). A Dionex IonPac™ AS11-HC (4 mm  $\times$  250 mm) column was used for chromatographic separation. The gradient elution conditions with A-water, B-200 mmol NaOH and the procedure was as follows (in minutes): 0–45, 6% B; 45.01–50, 6%–35% B; 50.01–59, 35% B; 59.01–60, 35%–6% B. Injection volume, flow rate, and column temperature were 25  $\mu$ L, 1.0 mL/min and 35 °C, accordingly. Three replicates were performed for each sample.

### Total phenolic content and total anthocyanin content

Total phenolic content (TPC) and total anthocyanin content (TAC) were measured directly in the supernatant of NFC spine grape juice after centrifugation. TPC was determined using Folin-Ciocalteu method by measuring the absorbance of the solution at 765 nm. The TPC in the samples was calculated by applying gallic acid as standard curve. TAC analysis was carried out through pH differential protocol<sup>[7]</sup>. The samples were diluted in potassium chloride buffer (pH 1) and sodium acetate

buffer (pH 4.5), and then stabilized for 20 min at room temperature before measuring the absorbance at 520 and 700 nm. The content of TAC in the samples was expressed as malvidin-3-glucoside mg/mL.

### LC-MS/MS acquisition and data analysis

#### LC/MS acquisition

The data acquisition was conducted through LC-ESI-QTOF MS, with methodology based on Xu et al. with some modifications<sup>[3]</sup>. Grape juice was filtered through a 0.22- $\mu$ m membrane, and a volume of 2  $\mu$ L was injected. Six replicates were carried out per sample group. Chromatographic separation was performed by LC system (SCIEX, USA) with a flow rate of 0.3 mL/min using C18 column (ZORBAX RRHD Eclipse Plus® C18, 1.8  $\mu$ m, 2.1 mm  $\times$  100 mm, Aligent™, USA) kept at 40 °C. Mobile phases A was 0.2% formic aqueous solution and B was acetonitrile. Gradient elution is performed from 5%–30% B (v/v) within 11.50 min, to 100% B at 11.51 min, and held at 100% B to 15.00 min. Afterwards, the B decreased to 5% in 0.1 min, and keep at 5% from 15.01–18 min. A QTOF Mass Spectrometer (TripleTOF 6600®, SCIEX™, USA) was connected to UPLC system, operating with a DuoSpray ion source in negative mode. The information dependent acquisition (IDA) mode was selected, comprising a TOF-MS scan (accumulation time of 50 ms; CE of 10 eV) together with 15 MS2 scans of product ions (accumulation time of 30 ms each; CE of 35 eV) with dynamic background subtraction. The cycle time of IDA was 545 ms. Mass acquisition range was set as m/z 50–1,000. A standard quality control (QC) strategy was used to assess instrument stability and analyte reproducibility.

#### Targeted phytochemical compounds analysis

A total of 26 known phytochemical compounds in grape reported previously<sup>[10,11]</sup>, were selected as the target compounds ([Supplemental Table S1](#)), including seven anthocyanins, six flavonols, five flavan-3-ols, six phenolic acids and two stilbenes. The accurate mass and fragment ions information from documented reference were initially established, and then the targeted list data was imported to MasterView® (SCIEX™, USA) to build the in-house library. Raw sample data was also imported for statistical analysis. Both accurate mass of the parent and the MS/MS information are used to identify and then confirm a metabolite<sup>[12]</sup>. The mass tolerance was established to 10 ppm, MS2 tolerances to 15 ppm. Save the acquired data as a MQ file and then imported in MultiQuant Software (SCIEX, Redwood City, CA, USA) as a 'quantitation method from text' for further quantitative analysis. Finally, the peak area of matched phytochemical compounds in samples were exported.

#### Untargeted metabolomics analysis

The untargeted metabolomics analysis was conducted referring to the method of Xu et al. with some modifications<sup>[3]</sup>. The raw data files were processed by MS-DIAL 4.60 (RIKEN Center, Japan), and then further analyzed through Microsoft Office Excel 2016® (Microsoft Corporation™, USA). MS1 features whose detection rate (DR) was below 80% or relative standard deviations (RSD) over 30% within the QC group were removed. The peak list of filtered data was further processed for multivariate analysis. The identification of compounds was conducted by matching MS/MS information of the filtered compounds with databases within a deviation of 10 ppm for MS and 15 ppm for MS/MS, accordingly.

## Statistical analysis

Results were expressed as the mean  $\pm$  standard deviation. Data analysis was carried out using one-way ANOVA. Significant differences among mean values were established at  $P < 0.05$  and determined by the Duncan test using SPSS (version 19.0, SPSS Inc., USA). Curves fittings and plotting drawings were completed by GraphPad Prism (GraphPad Software program Inc., La Jolla, CA, USA). The principal component analysis (PCA) score plot and heatmap diagrams were drawn using the online platform ([www.metaboanalyst.ca](http://www.metaboanalyst.ca)), and the OPLS-DA diagram was drawn by SIMCA® (Version 14.1, Umetrics™, Sweden).

## Results

### Physicochemical analysis

The TSS contents presented no significant difference between the MH and HPP group, ranging from 14.27% to 14.60% (Table 1). Glucose and fructose were detected in all samples, and these values were not significantly different between groups. The pH values ranged from 3.45 to 3.84. The HPP-treated juice had significantly higher pH values compared with fresh juice and MH-treated juice. Three organic acids including tartaric acid, malic acid and citric acid were detected in spine grape juice (Table 1). The citric acid contents were no significantly different among the MH, HPP and fresh groups. No detectable changes were found for the tartaric acid and malic acid between the MH and the fresh juice, but a 38% decrease of tartaric acid and a 25% decrease of malic acid were found in HPP-treated samples. The color parameters are presented in Table 1. The MH had the highest lightness ( $L$ ) and redness ( $a$ ) values, which were significantly greater compared with HPP and fresh groups, indicating the MH-treated juice had lighter and redder color.

### The targeted phytochemical compounds of MH- and HPP- processed NFC spine grape

Grape as a prevalent and global consumed fruits contains multiple phytochemicals, such as flavonoids, phenolic acids, and stilbenes, all of which are strong antioxidants<sup>[10]</sup>. A total of 26 phytochemical compounds were identified in NFC spine grape juice and the detailed information is shown in Supplemental Table S1. The rates of change in the 26 phytochemical compounds are shown in Fig. 1a–e.

Anthocyanin is a water-soluble pigment, which has a pivotal role for grape juice color. The TAC in spine grape juice after

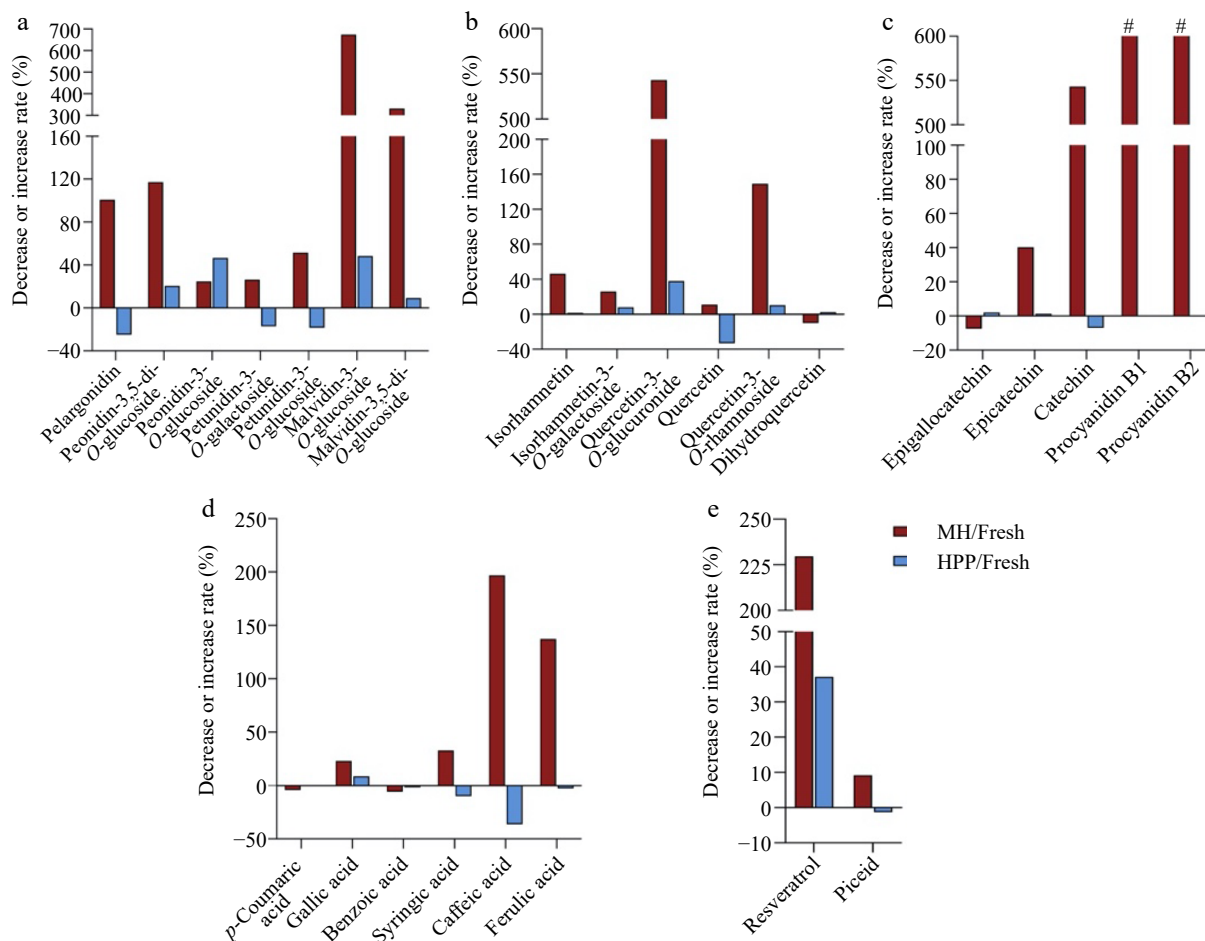
different treatments is shown in Table 1, ranging from 115.74 to 299.77 mg/L. Compared with fresh juice, the TAC in MH and HPP spine grape juice increased by about 100% and 15%, respectively. The peak areas of individual anthocyanins were registered and the percent of area variation of anthocyanins in MH- and HPP-treated juice compared with the fresh juice are shown in Fig. 1a. The change of individual anthocyanins content after HPP treatment was different. The contents of peonidin and malvidin glycosides showed an increase of 9%–48%, while pelargonidin and petunidin derivatives decreased by 17%–24% compared with the fresh juice. In the MH group, the relative contents of all identified individual anthocyanins were increased, especially for malvidin-3-*O*-glucoside, malvidin-3,5-di-*O*-glucoside, and peonidin-3,5-di-*O*-glucoside, increasing by 120%–600%.

In addition to being a good source of anthocyanins, grapes and their products are also rich in other polyphenols<sup>[13]</sup>. The TPC of the spine grape juice that underwent MH processing increased by 50%, while that of the HPP juice increased by 16% (Table 1). Based on the targeted LC-MS metabolite analysis, six kinds of flavonols, five kinds of flavan-3-ols, six kinds of phenolic acids, resveratrol and piceid were detected in spine grape juice. Flavonols and Flavan-3-ol are the major flavonoid subgroups in grape, possessing multi-beneficial health effects in humans<sup>[14]</sup>. The six kinds of flavonols including quercetin, isorhamnetin and its derivatives were detected in all samples (Fig. 1b). The contents of isorhamnetin, isorhamnetin-3-*O*-galactoside, quercetin-3-*O*-glucuronide, and quercetin-3-*O*-rhamnoside in MH and HPP groups were higher than that of the fresh group. The contents of quercetin-3-*O*-glucuronide and quercetin-3-*O*-rhamnoside were enhanced by more than 200% after MH processing. The quercetin in HPP juice and dihydroquercetin in MH juices showed 33% and 10% decreases, respectively. Flavan-3-ols mainly existed in grape seed and peel<sup>[15]</sup>. The five kinds of flavan-3-ols, including three monomers (epigallocatechin, epicatechin, and catechin) and two dimers (procyanidin B1 and procyanidin B2) were identified. The flava-3-ols content was stable after HPP treatment, whereas MH-treated juice had notable higher catechin, procyanidin B1 and procyanidin B2 contents.

Phenolic acids are predominant phenolic substances in grape juice. They are involved in the browning reactions, served as precursors of volatile phenols, and have antimicrobial and antioxidant activity<sup>[7]</sup>. As shown in Fig. 1d, both MH- and

**Table 1.** The traditional index of MH- and HPP-processed NFC spine grape juice.

Variety	TC			ZQ			
	Processing method	Fresh	MH	HPP	Fresh	MH	HPP
pH		3.45 $\pm$ 0.02a	3.45 $\pm$ 0.04a	3.60 $\pm$ 0.03b	3.71 $\pm$ 0.03b	3.57 $\pm$ 0.04c	3.84 $\pm$ 0.03a
TSS (%)		14.60 $\pm$ 0.10a	14.43 $\pm$ 0.06a	14.50 $\pm$ 0.10a	14.27 $\pm$ 0.38a	14.57 $\pm$ 0.15a	14.27 $\pm$ 0.12a
Glucose (g/L)		71.59 $\pm$ 2.03a	70.43 $\pm$ 4.76a	68.14 $\pm$ 2.83a	71.66 $\pm$ 1.69a	68.85 $\pm$ 2.32a	68.86 $\pm$ 1.71a
Fructose (g/L)		72.79 $\pm$ 2.36a	71.07 $\pm$ 3.23a	71.90 $\pm$ 2.56a	74.17 $\pm$ 2.93a	70.07 $\pm$ 2.53a	70.21 $\pm$ 1.87a
Tartaric acid (mg/L)		4,822.08 $\pm$ 285.58a	4,925.37 $\pm$ 380.44a	2,810.97 $\pm$ 50.57b	4,262.18 $\pm$ 141.90a	4,446.3 $\pm$ 438.02a	2,791.82 $\pm$ 231.73b
Malic acid (mg/L)		472.13 $\pm$ 20.71a	456.06 $\pm$ 5.99a	343.91 $\pm$ 30.30b	668.52 $\pm$ 12.53a	536.71 $\pm$ 31.26b	498.85 $\pm$ 19.84c
Citric acid (mg/L)		128.07 $\pm$ 2.72a	125.07 $\pm$ 2.29a	126.05 $\pm$ 2.07a	212.59 $\pm$ 2.76a	217.04 $\pm$ 3.51a	211.67 $\pm$ 2.04a
TPC (mg/L)		593.42 $\pm$ 29.22c	983.12 $\pm$ 58.29a	671.82 $\pm$ 14.00b	611.68 $\pm$ 25.68c	1031.53 $\pm$ 58.94a	726.75 $\pm$ 24.23b
TAC (mg/L)		156.87 $\pm$ 4.91b	299.77 $\pm$ 4.59a	179.92 $\pm$ 6.01b	115.74 $\pm$ 10.81b	269.08 $\pm$ 6.63a	134.40 $\pm$ 5.01b
$L$		25.64 $\pm$ 0.6c	28.75 $\pm$ 0.66a	26.59 $\pm$ 0.46b	24.36 $\pm$ 0.21c	29.27 $\pm$ 1.02a	27.56 $\pm$ 0.61b
$a$		6.03 $\pm$ 0.23b	9.57 $\pm$ 0.42a	6.34 $\pm$ 0.75b	3.98 $\pm$ 0.14c	8.28 $\pm$ 0.18b	4.24 $\pm$ 0.27b
$b$		-2.46 $\pm$ 0.17b	-2.30 $\pm$ 0.14b	-1.94 $\pm$ 0.08a	-2.47 $\pm$ 0.09a	-2.34 $\pm$ 0.13a	-2.28 $\pm$ 0.13a



**Fig. 1** The increase or decrease rates of phytochemical compounds (compared with fresh group) in MH-and HPP-processed NFC spine grape juice. (a) Anthocyanins, (b) Flavonols, (c) Flavan-3-ols, (d) Phenolic acids, (e) Stilbenes. # indicate compounds only detected in the MH group.

HPP-processed caused a slight reduction of *p*-coumaric acid and benzoic acid, together with an increase of gallic acid. The contents of syringic acid, ferulic acid and caffeic acid showed a distinct variation with the different processing method. Compared with fresh juice, the amounts of ferulic acid and caffeic acid in the MH group increased up to 100%, whereas decreased by 3% and 36% in the HPP group.

Grapes and its products are the main sources of bioactive stilbenes in diet<sup>[10]</sup>. The resveratrol and piceid were detected in spine grape juice (Fig. 1e). The contents of resveratrol in MH and HPP groups respectively enhanced by 229% and 37% compared with fresh juice.

### Untargeted metabolomics of MH- and HPP- processed NFC spine grape juice

#### An overview of metabolomics data

The total ion chromatograms for QC samples demonstrated dataset collection was stable (Supplemental Fig. S2), consequently serving in further investigations. Overall, 1,655 raw MS1 features were collected after filtering data according to the value of DR (> 80) and RSD (< 30). Three groups shared 1,255 compounds. The 388 compounds were only found in MH (Fig. 2a). Principal component analysis (PCA) was conducted to identify variations across all three groups (MH, HPP, and fresh), as shown in Fig. 2b. The PC1 and PC2 explained 42% and 28%

of the total variance, respectively. The MH group was separated, while the fresh and HPP groups gathered together. The content level of metabolites in three groups was visualized by heatmap (Fig. 2c). MH-treated samples branch off in the dendrogram. The second group was the HPP-treated samples together with fresh samples.

#### Putative annotation of differential compounds in MH and HPP

To find differential compounds between MH-treated and HPP-treated spine grape juice, an OPLS-DA model was built (Fig. 2d). Clear separation of MH and HPP groups was observed in the horizontal direction. Features having VIP > 1 were screened. Meanwhile, the fold change between the two groups of samples and the *p*-value were carried out. The differential compounds were finally selected depending upon VIP > 1, fold change > 2, and *p* < 0.05. Specifically, 11 differential compounds were annotated, including nine phenolic compounds and two peptides (Table 2).

As shown in Fig. 3a–i, the contents of malvidin-3-O-glucoside, malvidin-3,5-di-O-glucoside, quercitrin-3-O-rhamnoside, quercetin-3-O-glucuronide, catechin, caffeic acid, ferulic acid, procyanidin B1 and procyanidin B2 in MH-treated grape juice were significantly higher than that of HPP-treated juice, which were consistent with the results of targeted phytochemical compounds analysis. Unexpectedly, there are two tripeptides were identified (Fig. 3j–k). The contents of Leu-



**Table 2.** The putative annotated markers of MH- and HPP-processed NFC spine grape juice.

ID	Metabolites	Formula	Adduct type	Rt (min)	Average m/z	Reference m/z	ppm	p-value	log <sub>2</sub> Fold change	Being identified in targeted analysis	log <sub>2</sub> Fold change (in storage)
14109	Malvidin-3,5-O-diglucoside	C <sub>29</sub> H <sub>34</sub> O <sub>17</sub>	[M+H] <sup>+</sup>	3.798	655.1869	655.1869	0.00	0.012	1.8	√	>1.0
10725	Malvidin-3-O-glucoside	C <sub>23</sub> H <sub>25</sub> O <sub>12</sub>	[M] <sup>+</sup>	5.766	493.1338	493.1335	0.57	0.012	2.6	√	>1.0
10254	Quercetin-3-O-glucuronide	C <sub>21</sub> H <sub>18</sub> O <sub>13</sub>	[M+H] <sup>+</sup>	6.829	479.0808	479.082	-2.54	0.00	2.2	√	<1.0
9283	Quercitrin-3-O-rhamnoside	C <sub>21</sub> H <sub>20</sub> O <sub>11</sub>	[M+H] <sup>+</sup>	7.707	449.1088	449.10779	2.15	0.00	1.2	√	>1.0
1216	Caffeic acid	C <sub>9</sub> H <sub>8</sub> O <sub>4</sub>	[M+H] <sup>+</sup>	3.373	181.0494	181.0495	-0.61	0.00	2.0	√	>1.0
1560	Ferulic acid	C <sub>10</sub> H <sub>10</sub> O <sub>4</sub>	[M+H] <sup>+</sup>	4.708	195.06523	195.0643	4.76	0.00	1.3	√	<1.0
4046	Catechin	C <sub>15</sub> H <sub>14</sub> O <sub>6</sub>	[M+H] <sup>+</sup>	4.061	291.086	291.086	-0.10	0.00	3.9	√	>1.0
12875	Procyanidin B1	C <sub>30</sub> H <sub>26</sub> O <sub>12</sub>	[M+H] <sup>+</sup>	3.530	579.159	579.1497	0.38	-	-	√	>1.0
12873	Procyanidin B2	C <sub>30</sub> H <sub>26</sub> O <sub>12</sub>	[M+H] <sup>+</sup>	4.515	579.1506	579.1497	0.16	-	-	√	>1.0
7503	Glu-Val-Phe	C <sub>19</sub> H <sub>27</sub> N <sub>3</sub> O <sub>6</sub>	[M+H] <sup>+</sup>	5.265	394.1969	394.1971	-0.61	0.015	3.1	×	>1.0
7954	Leu-Leu-Tyr	C <sub>21</sub> H <sub>33</sub> N <sub>3</sub> O <sub>5</sub>	[M+H] <sup>+</sup>	6.293	408.2488	408.2493	-1.35	-	-	×	>1.0

Procyanidin B1, procyanidin B2 and Leu-Leu-Tyr only detected in the MH group.

Glu-Val-Phe were greater than 60% regardless of variety and treatment, indicating that the Glu-Val-Phe has the potential to be a monitoring marker for quality change of spine grape juice during storage.

## Discussion

### Influence of MH on spine grape juice

In this study, the TAC and TPC in the MH group were significantly higher than that of the fresh group suggesting that MH was favorable for promoting the release of phenolics in NFC spine grape juices. Similar effects of MH on fruit juices were also reported<sup>[6]</sup>. Genova et al. found that pasteurization (78 °C for 30 min) significantly increased the concentrations of anthocyanins and polyphenol in grape (*Vitis vinifera* L.) juice<sup>[17]</sup>. The TAC in blueberry juice after thermal treatment was observed to be twice as high as that of the control<sup>[18]</sup>. The possible reason for this result is that the heating process helps to disrupt the cell wall, promotes mass transfer efficiencies, and improves solubility of substances, thus increasing the extraction rate of phenolics and anthocyanins<sup>[19,20]</sup>. Some previous research suggested that traditional thermal processing always causes undesirable degradation of some compounds, especially for heat-sensitive bioactive phytochemicals<sup>[21]</sup>. For example, the TAC of pomegranate juice after heat treatment at 85 °C for 10 min was reduced by 18.8%<sup>[22]</sup>. Gil-Izquierdo et al. observed that the pasteurization treatments (70 °C for 30 s and 92–95 °C for 30 s) reduced flavonone content and phenolic acid content in orange juice<sup>[23]</sup>. The contradictory results might depend on food matrix and processing parameters. A large number of solid particles in NFC spine grape juice may be an important factor for the positive effect of MH<sup>[5]</sup>. More soluble and insoluble-bound compounds can be extracted from solid particles from grape seed and skin during heating processing<sup>[24,25]</sup>. Additionally, the mild and/or short-time treatment condition in our experiment could reduce the negative effects of thermal processing.

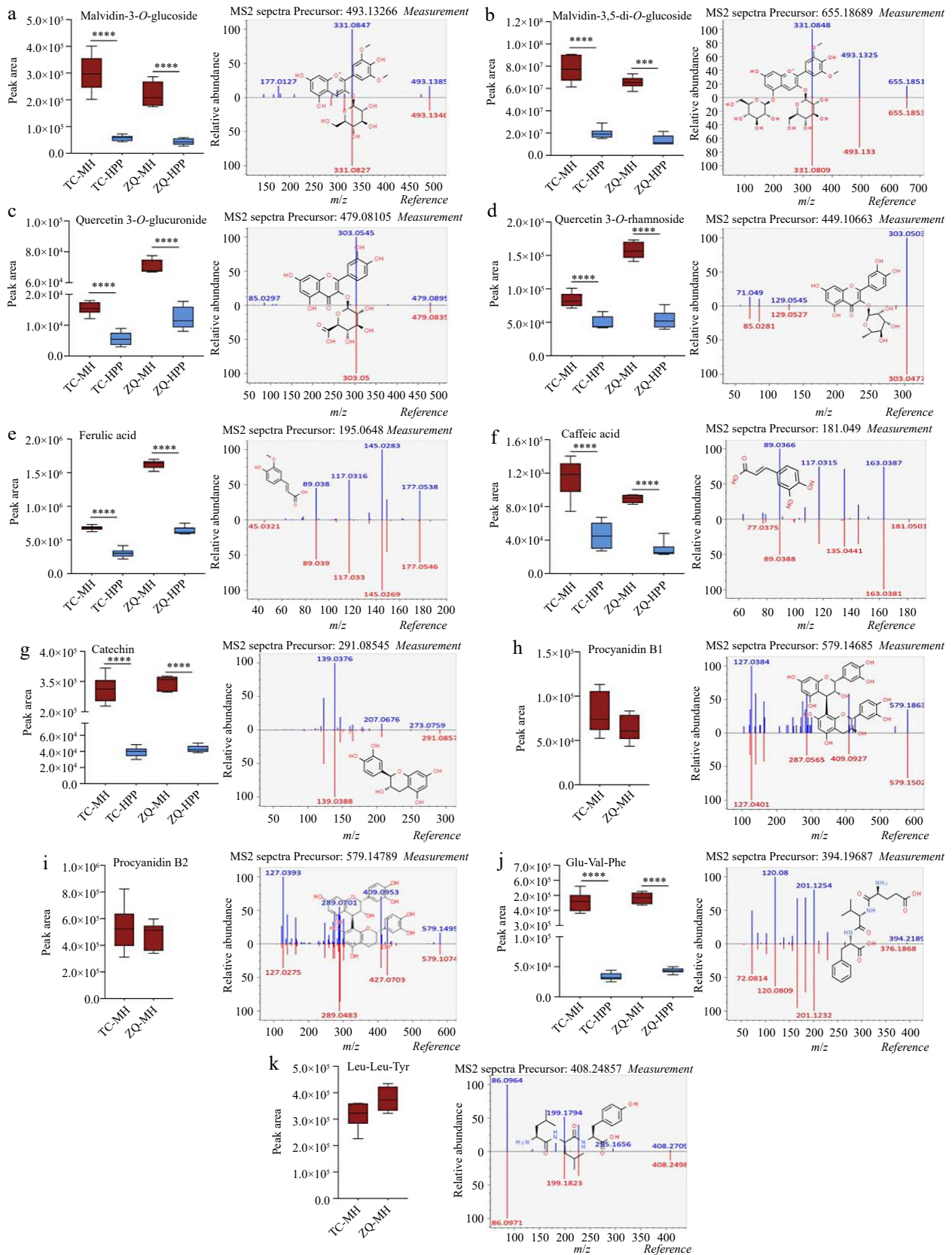
The results for the change of targeted phytochemical compounds contents were the same as the TPC and TAC. All identified individual anthocyanins were increased after MH

treatment, although the change rate was different. The stability of anthocyanins was influenced by the modification of glycosylation, methylation and acylation. Malvidin-type anthocyanins are more stable because of fewer OH-group and more methoxyl group. The same results was observed by Liu et al., who found that the content of malvidin derivative increased after heating, which was different from the change of other anthocyanins<sup>[26]</sup>. The disaccharide and trisaccharide anthocyanins were confirmed to be generally more stable than the mono-glycosylated anthocyanins<sup>[27]</sup>, which could explain the significant increase in malvidin-3,5-di-O-glucoside and peonidin-3,5-di-O-glucoside.

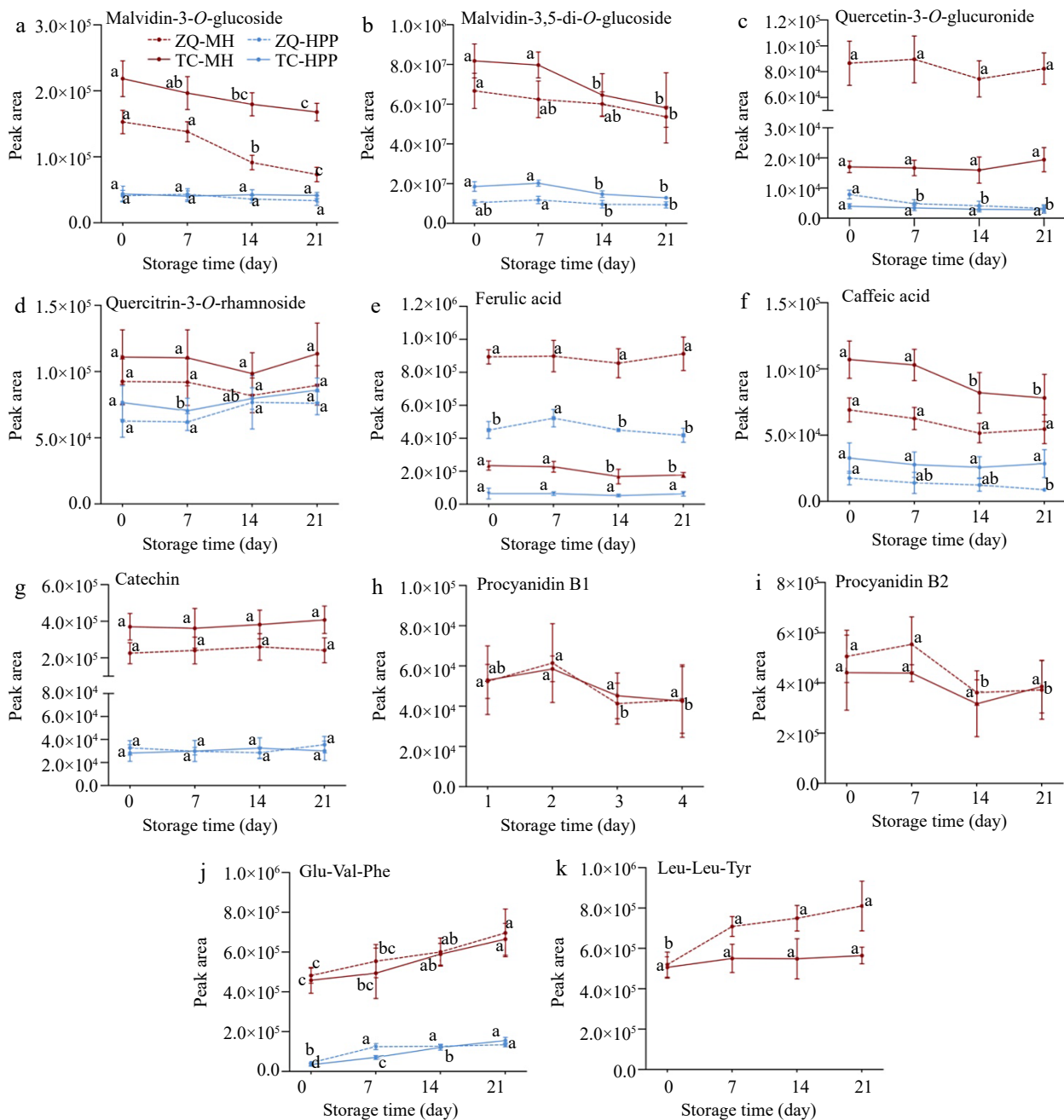
The content of catechin, procyanidin B1 and procyanidin B2 increased dramatically after MH treatment. In general, Flavan-3-ols are mainly located in both grape skin and seed<sup>[28]</sup>. During heat processing, more flavan-3-ols were leached from seed and peel particles residue in juice, similar to the effects of thermomaceration<sup>[29]</sup>. The reactions occurring during processing, such as degradation and depolymerization and polymerization of flavan-3-ols, also influence the final content of individual flavan-3-ols in grape juice<sup>[30]</sup>. The specific evolving process between monomers and dimers in grape juice processing needed to be further investigated. Compared with fresh juices, the higher yield of ferulic acid and caffeic acid was also obtained in MH, probably because the cleaving of ester- and glycoside-bound phenolic acids to release free phenolic acids during heating<sup>[31]</sup>. Similarly, Su et al. reported a 125% increase in ferulic acids of Huaizhi lychee juice after heat treatment at 121 °C<sup>[32]</sup>.

### Influence of HPP on spine grape juice

The TAC and TPC of spine grape juice treated with HPP were only slightly increased. HPP has been widely demonstrated as an excellent non-thermal technology for the processing of fruit juices because of its negligible influence upon sensory and nutritional properties, unaffected small molecule structural formations, including bioactive compounds<sup>[33]</sup>. Similar to our results, Barba et al. found a 15.5% and 23% increase in TAC and TPC for blueberry juice after treatment at 500 MPa/15 min<sup>[34]</sup>. Yuan et al. found HPP induced a 3%–13% increase in TPC and a 6%–17% increase in TAC across varying conditions compared



**Fig. 3** The putative annotated markers between MH- and HPP-processed NFC spine grape juice. The left are the box-plots, and the right are the chemical structures of individual biomarkers. (a) Malvidin-3-*O*-glucoside, (b) Malvidin-3,5-di-*O*-glucoside, (c) Quercetin-3-*O*-glucuronide, (d) Quercitrin-3-*O*-rhamnoside, (e) Ferulic acid, (f) Caffeic acid, (g) Catechin, (h) Procyanidin B1, (i) Procyanidin B2, (j) Glu-Val-Phe, (k) Leu-Leu-Tyr. Procyanidin B1, procyanidin B2 and Leu-Leu-Tyr only detected in the MH group.



**Fig. 4** Changes of annotated markers during storage. (a) Malvidin-3-O-glucoside, (b) Malvidin-3,5-di-O-glucoside, (c) Quercetin-3-O-glucuronide, (d) Quercitrin-3-O-rhamnoside, (e) Ferulic acid, (f) Caffeic acid, (g) Catechin, (h) Procyanidin B1, (i) Procyanidin B2, (j) Glu-Val-Phe, (k) Leu-Leu-Tyr. The solid and dotted lines represent different grape varieties. Different letters indicated significant difference between storage day.

with untreated aronia purée<sup>[35]</sup>. The increases of TPC and TAC are often attributed to the disruption of cell wall, plasma membrane, and organelles in plant matrices under high pressure condition<sup>[36]</sup>.

The change of phytochemical compounds contents after HPP treatment showed different trends of variation (Fig. 1). The different rate of change might be explained by its structure. On the one hand, the selective extraction of HPP has been reported. For example, malvidin was extracted in larger quantity from grapes than peonidin > petunidin > delphinidin > cyanidin<sup>[37]</sup>, which is in accordance with our results. On the

other hand, the activation of oxidase enzymes (such as polyphenol oxidases) during HPP<sup>[38]</sup>, may be the reason for the loss of the easily-oxidized compound including pelargonidin, petunidin derivatives and quercetin. As for the decrease of ferulic acid and caffeic acid, we presumed that the interactions between phenolic acids and other components are reinforced, leading to lower phenolic acids in HPP-treated spine grape juices<sup>[39]</sup>. The esterification of tartaric acid and ferulic acid/caffeic acid has been reported previously<sup>[40]</sup>, which might be also related to the significantly lower tartaric acid content in HPP-treated spine grape juice (Table 1).



### Putative annotation of differential compounds in MH and HPP

Untargeted metabolomics analysis revealed that the spine grape juice processed by MH and HPP were clearly distinguished by metabolite profiles (Fig. 2). Eleven differential compounds were annotated, and most of the annotated potential markers were phenolic compounds. Phenolic compounds, as the contributor to antioxidant capacity and color of grape juice, play an important role in consumers' preference. All of the annotated phenolic markers show higher contents in MH-treated samples (Fig. 3a–i). Previous studies showed that HPP was better to preserve bioactive components and overall nutritional quality of food compared to conventional thermal processing methods. But the evidence is not always consistent in relation to changes of bioactive phytochemicals. Similar to our results, Zhang et al. found a significantly higher content of malvidin glycoside in blueberry puree after thermal processing when compared with HPP treatment<sup>[41]</sup>. Talcott et al. compared HPP and thermal-treated muscadine grape juice and found the higher anthocyanins in pasteurized muscadine grape juice compared to HPP-treated juice<sup>[42]</sup>. The possible cause of the poor HPP performance was the higher oxidase enzymes activity during HPP processing than thermal processing. Additionally, since the NFC spine grape juice in this experiment contained a mass of particles from peel or seed, the MH could lead to a higher extractability of phenolic compounds than HPP.

Owing largely to its low abundance in most plant organs, identifying peptide library in plant-derived food stuffs presents great challenges<sup>[43]</sup>. The Glu-Val-Phe and Leu-Leu-Tyr were first identified in grape. The peptide content in the MH was significantly higher compared with that in the HPP. The possible reason for the result is that the heating process accelerates protein degradation<sup>[44]</sup>, producing more peptides. Previously, Xu et al. also identified seven peptides as differential markers, whose concentration in multi-heat processed juices was higher than that of mild heat treatment juices<sup>[3]</sup>.

### Change of the annotated markers during storage

A relatively irregular fluctuation was observed for different phenolic compounds during storage. In general, there may be a balance between the release and degradation of phenolics. Bound phenolics were gradually liberated, meanwhile, free phenolics degraded due to oxidation and enzyme activity. Within a certain period, the released amounts of phenolics exceeded that of the degraded ones, leading to an increase in phenolics<sup>[45]</sup>. The increase phenomenon was more commonly observed in the HPP than in the MH. We hypothesized that the reinforced interaction and the enhanced absorption of phenolics by cell wall components induced by HPP provided effective protection for phenolic compounds with a sustained release<sup>[46]</sup>. Take anthocyanins for example, it was reported that 13–18% of anthocyanins bind to cellulose or pectin when they first come into contact with the plant cell wall<sup>[47]</sup>, and the interaction was affected by the processing method. Hou et al. found that the binding rate between the major constituents of plant cell and cyanidin-3-O-glucoside was raised by 32.8% after treatment (500 MPa/15 min)<sup>[48]</sup>. At the later stages of storage, the dropping of anthocyanins might be related to the detected microorganisms (data not shown) in HPP-treated juice. The available scientific data on individual phenolics degradation in complex juices systems after different processing method is

limited. The influence of the food matrix and compound structure must be considered and assessed.

### Conclusions

The treatments of MH and HPP showed different effects on the physicochemical characters and phytochemical compound profiles of NFC spine grape juice based on metabolomics. The metabolites profile of HPP-processed juice was similar to the fresh juice, whereas MH-processed juice promoted more phytochemical compounds release. Eleven annotated markers differed significantly between MH and HPP groups, including nine kinds of phenolics and two kinds of peptides, and these components were significantly higher after MH treatment. An upward trend of malvidin-3,5-di-O-glucosid and ferulic acid was observed at earlier storage, following a decrease. The continuous rising content of Glu-Val-Phe indicated the potential to serve as juice quality monitoring markers during storage. The outcome of this study could provide new insights into the effects of processing on individual phytochemical changes and act as a guide for commercial application in the production of spine grape NFC juice as a color-rich ingredient to make new style tea drinks. However, the fate of compounds in juices and tea mixtures, such as the effects of bound and free anthocyanins on contribution to color and bioavailability need further exploration.

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### Conflict of interest

The authors declare that they have no conflict of interest.

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### References

1. AP News. 2020. *Nayuki and 36Kr Release 2019 New-style Tea Beverage Consumption Whitepaper*. <https://apnews.com/article/technology-business-china-b1ac7bdad0b6f5dbb981f354c0333fa8>.
2. Bolton D. 2020. *New Style Tea Beverages Reshape Asian Tea Market*. [www.worldteanews.com/Insights/new-style-tea-beverages-reshape-asian-tea-market](http://www.worldteanews.com/Insights/new-style-tea-beverages-reshape-asian-tea-market).
3. Xu L, Xu Z, Kelly S, Liao X. 2020. Integrating untargeted metabolomics and targeted analysis for not from concentrate and from concentrate orange juices discrimination and authentication. *Food Chemistry* 329:127130
4. Xu Z, Wang Y, Ren P, Ni Y, Liao X. 2016. Quality of banana puree during storage: a comparison of high pressure processing and thermal pasteurization methods. *Food and Bioprocess Technology* 9:407–20
5. Aaby K, Grimsbo IH, Hovda MB, Rode TM. 2018. Effect of high pressure and thermal processing on shelf life and quality of strawberry purée and juice. *Food Chemistry* 260:115–23

6. Huang Y, Di X, Burton-Freeman BM, Edirisinghe I. 2016. Chemical changes of bioactive phytochemicals during thermal processing. *Reference Module in Food Science*. <https://doi.org/10.1016/B978-0-08-100596-5.03055-9>
7. Meng J, Fang Y, Qin M, Zhuang X, Zhang Z. 2012. Varietal differences among the phenolic profiles and antioxidant properties of four cultivars of spine grape (*Vitis davidii* Foex) in Chongyi County (China). *Food Chemistry* 134:2049–56
8. Meng JF, Xu TF, Song CZ, Li XL, Yue TX, et al. 2013. Characteristic free aromatic components of nine clones of spine grape (*Vitis davidii* Foex) from Zhongfang County (China). *Food Research International* 54:1795–800
9. Wang K, Xu Z. 2022. Comparison of freshly squeezed, non-thermally and thermally processed orange juice based on traditional quality characters, untargeted metabolomics, and volatile overview. *Food Chemistry* 373:131430
10. Zhu L, Zhang Y, Lu J. 2012. Phenolic contents and compositions in skins of red wine grape cultivars among various genetic backgrounds and originations. *International Journal of Molecular Science* 13:3492–510
11. Myrssi ED, Koulocheri SD, Iliopoulos V, Haroutounian SA. 2021. High-throughput quantification of 32 bioactive antioxidant phenolic compounds in grapes, wines and vinification byproducts by LC-MS/MS. *Antioxidants* 10:1174
12. Yi L, Dong N, Yun Y, Deng B, Ren D, et al. 2016. Chemometric methods in data processing of mass spectrometry-based metabolomics: A review. *Analytica Chimica Acta* 914:17–34
13. Blumberg JB, Vita JA, Chen CYO. 2015. Concord grape juice polyphenols and cardiovascular risk factors: dose-response relationships. *Nutrients* 7:10032–52
14. Yilmaz Y, Toledo RT. 2004. Major flavonoids in grape seeds and skins: Antioxidant capacity of catechin, epicatechin, and gallic acid. *Journal of Agricultural and Food Chemistry* 52:255–60
15. Padilla-González GF, Grosskopf E, Sadgrove NJ, Simmonds MSJ. 2022. Chemical diversity of flavan-3-ols in grape seeds: Modulating factors and quality requirements. *Plants* 11:809
16. Guo R, Xu X, Carole B, Li X, Gao M, et al. 2013. Genome-wide identification, evolutionary and expression analysis of the aspartic protease gene superfamily in grape. *BMC Genomics* 14:554
17. Genova G, Tosetti R, Tonutti P. 2016. Berry ripening, pre-processing and thermal treatments affect the phenolic composition and antioxidant capacity of grape (*Vitis vinifera* L.) juice. *Journal of the Science of Food and Agriculture* 96:664–71
18. Rossi M, Giussani E, Morelli R, Lo Scalzo R, Nani RC, et al. 2003. Effect of fruit blanching on phenolics and radical scavenging activity of highbush blueberry juice. *Food Research International* 36:999–1005
19. El Darra N, Grimi N, Maroun RG, Louka N, Vorobiev E. 2013. Pulsed electric field, ultrasound, and thermal pretreatments for better phenolic extraction during red fermentation. *European Food Research and Technology* 236:47–56
20. Lambri M, Torchio F, Colangelo D, Segade SR, Giacosa S, et al. 2015. Influence of different berry thermal treatment conditions, grape anthocyanin profile, and skin hardness on the extraction of anthocyanin compounds in the colored grape juice production. *Food Research International* 77:584–90
21. Buckow R, Kastell A, Terefe NS, Versteeg C. 2010. Pressure and temperature effects on degradation kinetics and storage stability of total anthocyanins in blueberry Juice. *Journal of Agricultural and Food Chemistry* 58:10076–84
22. Subasi BG, Alpas H. 2017. Effect of high hydrostatic pressure processing and squeezing pressure on some quality properties of pomegranate juice against thermal treatment. *High Pressure Research* 37:78–92
23. Gil-Izquierdo A, Gil MI, Ferreres F, Tomás-Barberán FA. 2001. In vitro availability of flavonoids and other phenolics in orange juice. *Journal of Agricultural and Food Chemistry* 49:1035–41
24. Szalóki-Dorkó L, Végvári G, Ladányi M, Ficzek G, Stéger-Máté M. 2015. Degradation of anthocyanin content in sour cherry juice during heat treatment. *Food Technology and Biotechnology* 53:354–60
25. Lo Scalzo R, Iannocari T, Summa C, Morelli R, Rapisarda P. 2004. Effect of thermal treatments on antioxidant and antiradical activity of blood orange juice. *Food Chemistry* 85:41–47
26. Liu Y, Liu Y, Tao C, Liu M, Pan Y, et al. 2018. Effect of temperature and pH on stability of anthocyanin obtained from blueberry. *Journal of Food Measurement and Characterization* 12:1744–53
27. Zhao C, Chen Z, Bai X, Ding C, Long T, et al. 2014. Structure-activity relationships of anthocyanidin glycosylation. *Molecular Diversity* 18:687–700
28. González-Manzano S, Rivas-Gonzalo JC, Santos-Buelga C. 2004. Extraction of flavan-3-ols from grape seed and skin into wine using simulated maceration. *Analytica Chimica Acta* 513:283–89
29. Aguilar T, Loyola C, de Bruijn J, Bustamante L, Vergara C, et al. 2016. Effect of thermomaceration and enzymatic maceration on phenolic compounds of grape must enriched by grape pomace, vine leaves and canes. *European Food Research and Technology* 242:1149–58
30. Fuleki T, Ricardo-Da-Silva JM. 2003. Effects of cultivar and processing method on the contents of catechins and procyanidins in grape juice. *Journal of Agricultural and Food Chemistry* 51:640–46
31. Xu G, Ye X, Chen J, Liu D. 2007. Effect of heat treatment on the phenolic compounds and antioxidant capacity of citrus peel extract. *Journal of Agricultural and Food Chemistry* 55:330–35
32. Su DX, Wang ZN, Dong LH, Huang F, Zhang RF, et al. 2019. Impact of thermal processing and storage temperature on the phenolic profile and antioxidant activity of different varieties of lychee juice. *LWT* 116:108578
33. Rosenthal A, Pokhrel PR, da Rocha Ferreira EH, Tiburski JH, Barbosa-Cánovas GV, et al. 2018. High pressure processing of fruit products. In *Fruit Preservation: Novel and Conventional Technologies*, eds. Rosenthal A, Deliza R, Welti-Chanes J, Barbosa-Cánovas CV. New York: Springer. pp. 351–98. [https://doi.org/10.1007/978-1-4939-3311-2\\_13](https://doi.org/10.1007/978-1-4939-3311-2_13)
34. Barba FJ, Esteve MJ, Frigola A. 2013. Physicochemical and nutritional characteristics of blueberry juice after high pressure processing. *Food Research International* 50:545–49
35. Yuan B, Danao MGC, Stratton JE, Weier SA, Weller CL, Lu M. 2018. High pressure processing (HPP) of aronia berry purée: Effects on physicochemical properties, microbial counts, bioactive compounds, and antioxidant capacities. *Innovative Food Science & Emerging Technologies* 47:249–55
36. Zhang L, Wang X, Manickavasagan A, Lim LT. 2022. Extraction and physicochemical characteristics of high pressure-assisted cold brew coffee. *Future Foods* 5:100113
37. Corrales M, García AF, Butz P, Tauscher B. 2009. Extraction of anthocyanins from grape skins assisted by high hydrostatic pressure. *Journal of Food Engineering* 90:415–21
38. Roobab U, Abida A, Afzal R, Madni GM, Zeng XA, et al. 2022. Impact of high-pressure treatments on enzyme activity of fruit-based beverages: an overview. *International Journal of Food Science & Technology* 57:801–15
39. He Y, Wen LK, Yu HS, Zheng F, Wang ZT, et al. 2018. Effects of high hydrostatic pressure-assisted organic acids on the copigmentation of *Vitis amurensis* Rupr anthocyanins. *Food Chemistry* 268:15–26
40. Sullivan ML. 2014. Perennial peanut (*Arachis glabrata* Benth.) leaves contain hydroxycinnamoyl-CoA:tartaric acid hydroxycinnamoyl transferase activity and accumulate hydroxycinnamoyl-tartaric acid esters. *Planta* 239:1091–100
41. Zhang W, Shen Y, Li Z, Xie X, Gong ES, et al. 2021. Effects of high hydrostatic pressure and thermal processing on anthocyanin content, polyphenol oxidase and beta-glucosidase activities, color, and antioxidant activities of blueberry (*Vaccinium* Spp.) puree. *Food Chemistry* 342:128564

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42. Talcott ST, Brenes CH, Pires DM, Del Pozo-Insfran D. 2003. Phytochemical stability and color retention of copigmented and processed muscadine grape juice. *Journal of Agricultural and Food Chemistry* 51:957–63
43. Piovesana S, Capriotti AL, Cavaliere C, La Barbera G, Montone CM, et al. 2018. Recent trends and analytical challenges in plant bioactive peptide separation, identification and validation. *Analytical and Bioanalytical Chemistry* 410:3425–44
44. Sirtori E, Isak I, Resta D, Boschini G, Arnoldi A. 2012. Mechanical and thermal processing effects on protein integrity and peptide fingerprint of pea protein isolate. *Food Chemistry* 134:113–21
45. Stübler AS, Böhmker L, Juadjur A, Heinz V, Rauh C, et al. 2021. Matrix- and technology-dependent stability and bioaccessibility of strawberry anthocyanins during Storage. *Antioxidants* 10:30
46. Zou H, Ma Y, Xu Z, Liao X, Chen A, et al. 2018. Isolation of strawberry anthocyanins using high-speed counter-current chromatography and the copigmentation with catechin or epicatechin by high pressure processing. *Food Chemistry* 247:81–88
47. Padayachee A, Netzel G, Netzel M, Day L, Zabaras D, et al. 2012. Binding of polyphenols to plant cell wall analogues - Part 1: Anthocyanins. *Food Chem.* 134:155–61
48. Hou ZQ, Luan LQ, Hu XX, Chen SG, Ye XQ. 2022. Beneficial effects of high pressure processing on the interaction between RG-I pectin and cyanidin-3-glucoside. *Food Chemistry* 383:132373



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