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Phytochemical Profiles of Different Mulberry (*Morus* sp.) Species from China

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Mulberry is rich in alkaloids, polyphenols, flavonoids, and anthocyanins, which have been suggested to be responsible for health benefits. The concentrations of 1-deoxynojirimycin (DNJ), resveratrol, oxyresveratrol, cyanidin-3-*O*-β-glucoside (Cy-3-glu), cyanidin-3-*O*-β-rutinoside (Cy-3-rut), and rutin in mulberry juice, fruits, and leaves of 8 species grown in China were examined. It is the first time content determination of DNJ in mulberry juice and oxyresveratrol in mulberry fruits and leaves has been reported. Among the varieties tested, *Da 10* (*Morus atropurpurea* Roxb.) was the most valuable cultivar considering its high content of functional components. Besides, *Hetianbaisang* (*M. alba* Linn.), *Taiwanguosang* (*M. atropurpurea* Roxb.), *Fujian 2 hao* (*M. alba* Linn.), *Gaozhoujisang* (*M. australis* Poir.), and *Shanxiguosang* (*M. nigra* Linn.) were rich in DNJ, resveratrol, oxyresveratrol, anthocyanins, and flavonoids, respectively. The high contents of functional compounds in mulberry juice, fruits, and leaves implied that they might be potential resources for the development of functional drinks and food.

KEYWORDS: Mulberry (*Morus* sp.); DNJ (1-deoxynojirimycin); resveratrol; oxyresveratrol; anthocyanin; flavonoid

INTRODUCTION

Mulberry (*Morus*, *Moraceae*) plants are distributed widely in China. Mulberry fruits are nutritional foodstuff, and mulberry leaves have been historically used as feed for silkworms. In traditional Chinese medicine, dried mulberry fruits have been used as a tonic, while the mulberry leaves have been used as medicinal materials. They were recommended as having antioxidant, antimicrobial, and anti-inflammatory properties (*1*).

Recent investigations revealed that the fruits and leaves of mulberry plants contained many bioactive components, such as alkaloids, anthocyanins, and flavonoids (*2*, *3*). Mulberry leaves are rich in alkaloid components including 1-deoxynojirimycin (DNJ), which is known as one of the most potent glycosidase inhibitors that decreases blood sugar levels (*3*−*6*). Some methods have been established to detect DNJ in mulberry leaves (*3*, *7*, *8*).

Resveratrol (trans-3,4′,5-trihydroxystilbene) and oxyresveratrol (*trans*-2,3′,4,5′-tetrahydroxystilbene) are hydroxystilbenes found in many plant species including grapes, peanuts, and mulberries (*9*). Resveratrol recently received much attention based on its potential neuroprotectant (*10*, *11*) and cardioprotective effects (*12*). Oxyresveratrol has an inhibitory effect on tyrosinase to limit melanin biosynthesis and is used as cosmetic materials and medical agents for hyperpigmentation disorders (*13*, *14*). Resveratrol was analyzed in mulberry fruits (*15*), but no reports about the quantification of oxyresveratrol in mulberry fruits or leaves are available.

Anthocyanins are a group of natural phenolic compounds responsible for the coloring of plant leaves, flowers, and fruits. Twenty anthocyanins were identified in plants, but only six of them can be used as food additives (*2*). Anthocyanins also drew attention because of their potential health benefits as antioxidant and anti-inflammatory compounds (*2*). For example, anthocyanins had high inhibitory ability on lipid oxidation (*16*), and mulberry anthocyanin extract had antimetastasis activity to inhibit the migration of B16−F1 cells (*17*). A pH-differential method using an UV−vis spectrophotometer has been used for the quantification of total anthocyanins in mulberry, and high performance liquid chromatography (HPLC) methods have been used for component analysis of anthocyanins in mulberry plants (*18*, *19*).

Flavonoids exist widely in the plant kingdom. Mulberry leaves were shown to contain at least four flavonoids, including rutin (*20*). Flavonoids have long been recognized to possess anti-inflammatory, antioxidant, antiallergic, hepatoprotective, antithrombotic, antiviral, and anticarcinogenic activities. A colorimetric method using aluminum chloride was used to determine the total flavonoid content in mulberry leaves, and RP-HPLC was used for the separation and determination of rutin and quercetin in the extracts of mulberry leaves and fruits (*20*, *21*).

The production and consumption of mulberry juices, fruits, and leaves are increasing rapidly because of their good taste and nutritional value. Moreover, there are at least 15 species of

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mulberry known in China (*22*), but only a few species were analyzed for their phytochemical profiles and developed for food or food additives. In our study, a compositional comparison between different species and cultivars was undertaken aiming at exploiting the nutrient profiles of mulberry fruits and leaves and promoting the further development of the rich mulberry resources.

MATERIALS AND METHODS

Chemicals. DNJ was purchased from Shanghai Shunbo Bioengineering Co., Ltd., Shanghai, China. FMOC-OSu was purchased from Shanghai Puredu Bioengineering Co., Ltd., Shanghai, China. Cyanidin-3-*O*-β-glucoside (Cy-3-glu) and Cyanidin-3-*O*-β-rutinoside (Cy-3-rut) was purchased from Tianjin Yifang Technical, Ltd., Tianjin, China. Rutin was purchased from Tianjin Jianfeng Natural Products, Ltd., Tianjin, China. Resveratrol and oxyresveratrol were isolated and purified in our lab with individual purity no less than 98% (HPLC assay, UV detection). All other chemicals were of analytical or HPLC grade.

Samples. Fruits of 38 cultivars of mulberry belonging to 8 species were harvested all around China at the commercial ripe stage. The other three samples of *Da 10* (*M. atropurpurea* Roxb.) were harvested from different areas. Thirty-three cultivars of mulberry leaves were picked at the same time (**Table 1**) as the fruits.

Preparation of Samples. *Juice Extraction*. One hundred grams of fruit of each cultivar was randomly selected and ground by hand with a mortar and pestle, and the juice was forced through three layers of gauze (0.9 mm × 0.9 mm) under pressure. The fruit juice was centrifuged at 6700*g* for 10 min, stored at 4 °C, and then analyzed immediately.

Fruit and Fruit Marc Lyophilization. One hundred grams of fruit or 20 g of fruit marc (residue after juice extraction) of each cultivar was frozen at −80 °C for 24 h, lyophilized at −53 °C, milled into powder, and oven-dried at 50 °C until constant weight.

Leaf Dryness. Two hundred grams of mulberry leaves of each cultivar was air-dried, comminuted, and oven-dried at 50 °C until constant weight.

Extraction and Derivatization of DNJ. The fruit juice was centrifuged (6700*g* for 10 min) before the derivatization procedure. Then, 0.50 g of the fruit and marc powder or 1.00 g of the leaf powder was mixed with 25 mL of water and boiled for 2 h. The supernatant was saved and diluted to 25 mL with water and then used for subsequent derivatization.

DNJ lacks a chromophore in its molecule and is therefore difficult to detect by high-performance liquid chromatography with diode array detection (HPLC-DAD) analysis. A sample pretreatment with 9-fluorenylmethyl succinimidyl carbonate (FMOC-OSu) was necessary for HPLC-DAD detection (*8*).

Derivatization. One hundred microliters of DNJ standard solution, juice, fruit extract, fruit marc extract, leaf extract, or water was mixed with 100 μL of 0.5 M sodium borate buffer (pH 9.0) in a 1.5 mL microtube. Two hundred microliters of 0.01 mol/L FMOC-OSu in CH_3CN was added and reacted at 30 °C for 1 h in a water bath. One hundred microliters of 0.1 M glycine was added to terminate the reaction. The mixture was then mixed with 100 μL of 1% (v/v) aqueous acetic acid to stabilize DNJ-FMOC, and filtered through 0.45 μm membrane filter before being injected into the HPLC system.

Extraction of Resveratrol and Oxyresveratrol. Lyophilized fruit (0.50 g) or fruit marc powder (0.50 g) and 1.00 g of leaf powder of each cultivar were ultrasonically treated for 1 h at 25 °C with 25 mL of methanol and adjusted to 25 mL in a volumetric flask. Juice was diluted with water (1:2, v/v). All prepared sample solutions were filtered through 0.45 μm membrane filters before being injected into the HPLC system.

Extraction of Anthocyanins and Flavonoids. Lyophilized fruit (0.50 g) powder, 0.80 g of lyophilized fruit marc powder, or 1.00 g of dried leaf powder of each cultivar was ultrasonically treated for 1 h at 25 °C with 50 mL of methanol/0.1% HCl (1:1, v/v). The juice of each mulberry cultivar was diluted with extract solution (1:2, v/v). All prepared sample solutions were filtered through 0.45 μm membrane filters and diluted for appropriate multiples with extraction solution before being injected into the HPLC system.

Preparation of Standards. Standard DNJ was dissolved with water. Cy-3-glu and Cy-3-rut were dissolved with 50% methanol containing 0.05% HCl. Resveratrol, oxyresveratrol, and rutin were dissolved with methanol. All standard samples were adjusted to 10 mL in a volumetric flask to obtain a 900 mg/L DNJ, 43 mg/L resveratrol, 70 mg/L oxyresveratrol, 800 mg/L Cy-3-glu, 300 mg/L Cy-3-rut, and 400 mg/L rutin stock solutions. Stock solutions of each standard were diluted with their solvent and adjusted to 10 mL in a volumetric flask for making calibration curves.

Table 1. Species, Harvest Area, and Time of Mulberry Sampling

no.	Latin name	sample name	harvest area	harvest time
1	*M. atropurpurea* Roxb.	Da 10 (Guangdong)[a]	Guangdong	April, 2008
2		Da 10 (Zhejiang)[a]	Zhejiang	May, 2008
3		Da 10 (Shanghai)	Shanghai	May, 2008
4		Da 10 (Jiangsu)[a]	Jiangsu	May, 2008
5		Tang 10[a]	Guangdong	April, 2008
6		Zhongsang 5801[a]	Jiangsu	May, 2008
7		Taiwanguosang[a]	Jiangsu	May, 2008
8	*M. multicaulis* Perr.	Huangjiguan[a]	Zhejiang	May,2008
9		Shengxianqingsang[a]	Zhejiang	May, 2008
10		Hongmanao[a]	Zhejiang	May, 2008
11		Husang 208[a]	Zhejiang	May,2008
12		Husang 7 hao[a]	Jiangsu	May, 2008
13		Husang 32 hao[a]	Jiangsu	May, 2008
14		Lu-8[a]	Jiangsu	May, 2008
15		Hongyahaisang[a]	Jiangsu	May, 2008
16		Hongguo 2 hao[a]	Zhejiang	May, 2008
17	*M. nigra* Linn.	Shanxiguosang[a]	Shanxi	May, 2008
18		Luoyu 1 hao	Xinjiang	May, 2008
19		Yaosang	Xinjiang	May, 2008
20	*M. cathayana* Hemsl.	Dejiang 15 hao[a]	Jiangsu	May, 2008
21		Yaan 3 hao[a]	Jiangsu	May, 2008
22		Huai 30−2[a]	Jiangsu	May, 2008
23	*M. alba* Linn.	Shishengsang[a]	Hangzhou	May, 2008
24		Dahuasang[a]	Hangzhou	May, 2008
25		Heigehu[a]	Suzhou	May, 2008
26		Fujian 1 hao[a]	Fujian	May, 2008
27		Fujian 2 hao[a]	Fujian	May, 2008
28		Tianquan 1 hao[a]	Jiangsu	May, 2008
29		Heizaoshenzi	Beijing	June, 2008
30		Dabaishen[a]	Jiangsu	May, 2008
31		Lushenzi[a]	Jiangsu	May, 2008
32		Dahongpao[a]	Jiangsu	May, 2008
33		Hetianbaisang	Xinjiang	May, 2008
34		Dabaie	Beijing	June, 2008
35	*M. laevigata* Wall.	Sinan 2 hao[a]	Jiangsu	May, 2008
36	*M. australis* Poir.	Gaozhoujisang[a]	Jiangsu	May, 2008
37	*M.* Cross Bred.	Nongsang 12 hao[a]	Zhejiang	May, 2008
38		Xinsang 1 hao[a]	Zhejiang	May, 2008
39		Nongsang 8 hao[a]	Jiangsu	May, 2008
40		Nongsang 14 hao[a]	Jiangsu	May, 2008
41		Xinyizhilai[a]	Jiangsu	May, 2008

[a] Cultivars of which both fruits and leaves were collected.

Analysis of DNJ by HPLC. *HPLC Conditions*. Quantitative analysis was performed on an Agilent HPLC system 1100 series (degasser G1322A, quaternary pump G1311A, autosampler G1313A, column heater G1316A, and diode array detector G1315B). Samples were separated on Agilent ZORBAX Eclipse SB-C_{18} (Φ 4.6 × 250 mm, 5 μm) at 30 °C. Flow rate: 1 mL/min. Detection wavelength: 254 nm. Solvent A, methanol; solvent B, water. The gradient elution program was performed as follows: initial 16 min run of 55% A (v/v), followed by a 3 min linear gradient to 90% A, and holding for 9 min. The injection volume was 10 μL.

Analysis of Resveratrol and Oxyresveratrol by HPLC. HPLC analysis was performed on the same system as DNJ. Samples were separated on Agilent ZORBAX Eclipse SB-C_{18} (Φ 4.6 × 250 mm, 5 μm) at 30 °C. Flow rate: 1 mL/min. Detection wavelength: 303 nm. Solvent A, methanol; solvent B, water. The elution program was performed as 39% A for 25 min. The injection volume was 20 μL.

Analysis of Cy-3-glu, Cy-3-rut, and Rutin by HPLC. HPLC analysis was performed on the same system as DNJ. Samples were separated on Agilent ZORBAX Eclipse SB-C_{18} (Φ 4.6 × 250 mm,

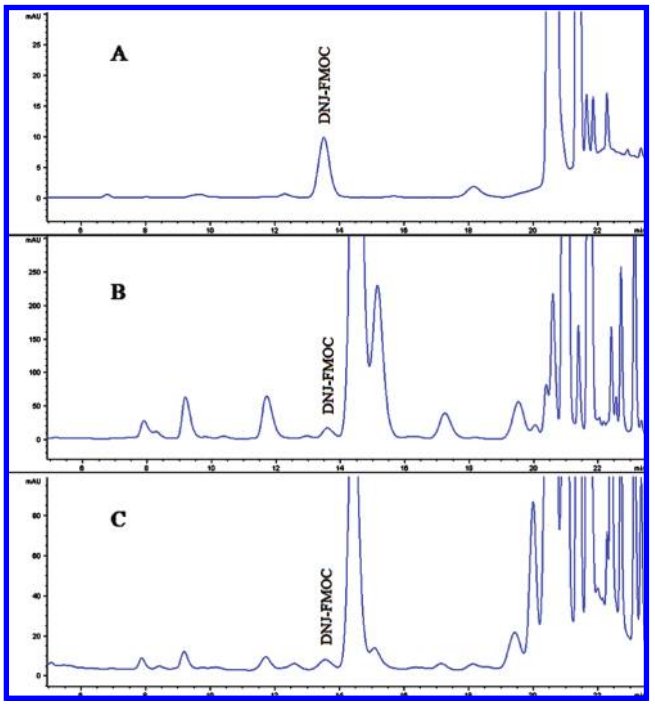

Figure 1. Typical chromatographic profiles obtained at 254 nm for the DNJ standard (**A**), juice of *Tang 10* (*M. atropurpurea* Roxb.) (**B**), and leaves of *Da 10* (*Guangdong*) (*M. atropurpurea* Roxb.) (**C**).

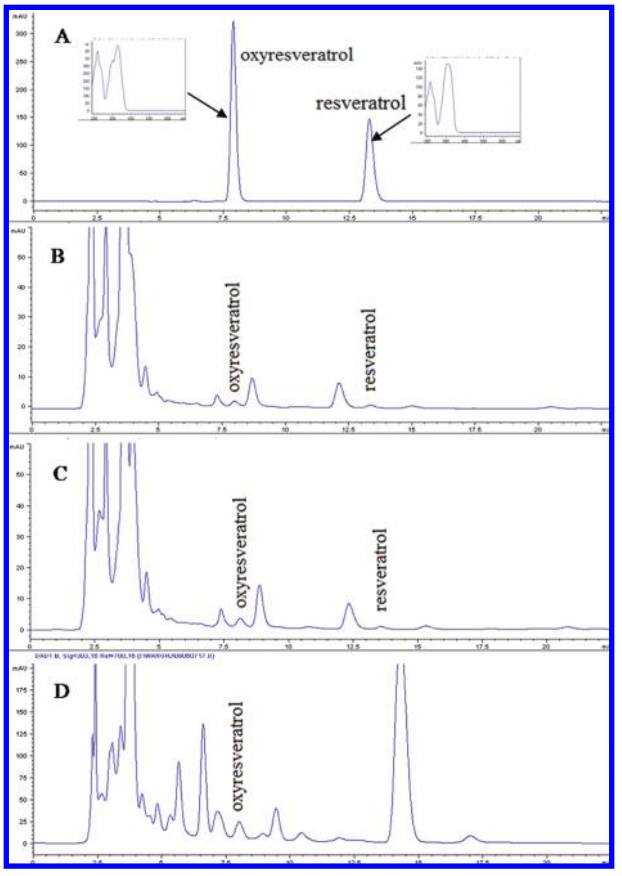

Figure 2. Typical chromatographic profiles obtained at 303 nm for resveratrol and oxyresveratrol standards (**A**), fruits of *Huangjiguan* (*M. multicaulis* Perr.) (**B**), fruit marc of *Da 10* (*Zhejiang*) (*M. atropurpurea* Roxb.) (**C**), and leaves of *Taiwanguosang* (*M. atropurpurea* Roxb.) (**D**).

5 μm) at 30 °C. Flow rate: 1 mL/min. Detection wavelengths: 520 nm for anthocyanins and 254 nm for flavonoids. Solvent A, methanol; solvent B, 0.1% HCl. The gradient elution program was performed as follows: a linear gradient of 20 to 35% A within the first 15 min, followed by a 5 min linear gradient to 50% A, a 3 min linear gradient to 70% A, and holding for 7 min. The injection volume was 10 μL.

Total anthocyanin content was calculated as Cy-3-glu equivalents according to the area % of Cy-3-glu in the total peak areas of anthocyanins. The flavonoids were identified according to the UV scanning spectrograms, and total flavonoid was calculated as rutin equivalents according to area % of rutin in the total peak areas of flavonoids.

RESULTS AND DISCUSSION

Analysis of Method Validation. Mulberry juice, fruit extract, fruit marc extract, and leaf extract were analyzed by HPLC. Peak purity was checked through DAD spectrum scanning to help verifying separation conditions and identifying DNJ, resveratrol, oxyresveratrol, Cy-3-glu, Cy-3-rut, and rutin peaks in addition to comparing with peaks and retention time of standards (**Figures 1**, **2**, **3**, and **4**).

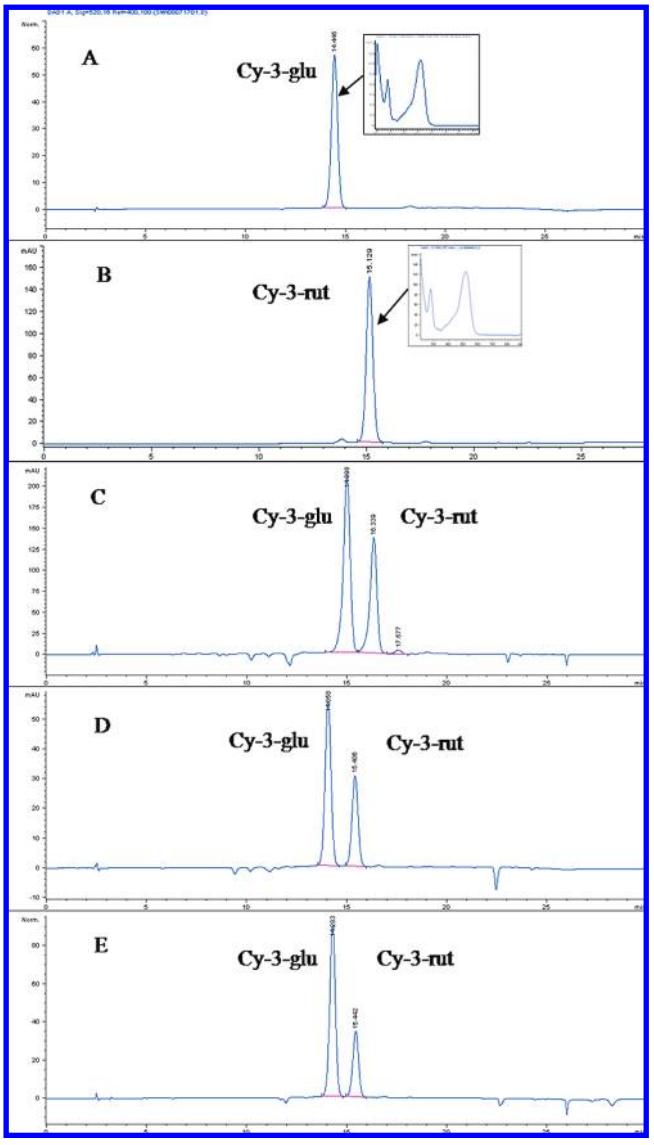

Figure 3. Typical chromatographic profiles obtained at 520 nm for the Cy-3-glu standard (**A**), Cy-3-rut standard (**B**), juice of *Gaozhoujisang* (*M. australis* Poir.) (**C**), fruits of *Hongguo 2 hao* (*M. atropurpurea* Roxb.) (**D**), and fruit marc of *Tang 10* (*M. atropurpurea* Roxb.) (**E**).

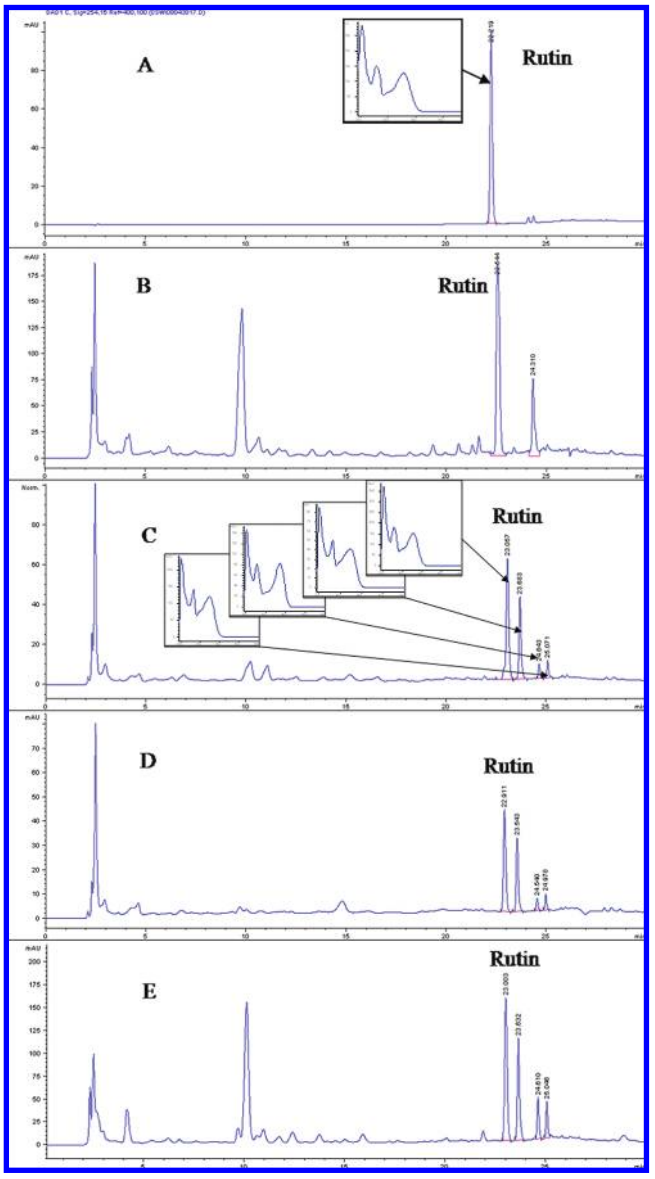

Figure 4. Typical chromatographic profiles obtained at 254 nm for the rutin standard (**A**), juice of *Da 10* (*Guangdong*) (*M. atropurpurea* Roxb.) (**B**), fruits of *Husang 7 hao* (*M. multicaulis* Perr.) (**C**), fruit marc of *Husang 7 hao* (*M. multicaulis* Perr.) (**D**), and leaves of *Shanxiguosang* (*M. nigra* Linn.) (**E**).

Table 2. Calibration Curves and Detection Limits of the Analytes ($n = 5$)[a]

analyte	linear regression (mg/L)	R^2	linear range (mg/L)	LOD (ng)
DNJ in juice	$y = 500.49x - 3.432$	0.9994	25−450	0.25
DNJ in leaves	$y = 427.87x - 4.8282$	0.9997	45−900	0.45
resveratrol	$y = 6686.2x - 0.0908$	0.9999	0.022−1.376	0.11
oxyresveratrol	$y = 4191.1x + 0.7911$	0.9999	0.035−14.00	0.17
Cy-3-glu	$y = 894.1x - 8.70$	1.0000	0.4−800	0.20
Cy-3-rut	$y = 2178.4x + 33.16$	0.9999	0.3−300	0.15
rutin	$y = 1648.6x + 4.44$	0.9999	0.4−400	0.20

[a] y, peak area; x, the concentration of each reference compound (mg/L); R^2, correlation coefficient of regression equations; LOD, limit of detection (S/N = 3).

Calibration Curves. A set of calibration curves are summarized in **Table 2**. All calibration curves showed good linear regressions ($R^2 \geq 0.9994$) within testing ranges.

Recovery Test. DNJ standard (2.5 mg) was added into 1.0 g mulberry leaf powder of *Da 10* (*Morus atropurpurea* Roxb., Guangdong) and extracted and detected together. Fifteen microliters of 172 μg/mL resveratrol and 175 μL 70 μg/mL oxyresveratrol standards were added into 0.5 g of lyophilized fruit powder of *Da 10* (*Morus atropurpurea* Roxb., Zhejiang) and extracted and detected together. Cy-3-glu (32.5 mg), 6.0 mg of Cy-3-rut, and 1.0 mg of rutin standards were added into 0.5 g of lyophilized fruit powder of *Da 10* (*Morus atropurpurea* Roxb., Shanghai) and extracted and detected together. The recovery % was calculated using the following formula:

$$\text{recovery (\%)} = 100 \times (\text{amount found} - \text{original amount}) / \text{amount spiked}$$

The recoveries of DNJ, resveratrol, oxyresveratrol, Cy-3-glu, Cy-3-rut, and rutin were 95, 95, 106, 102, 103, and 96% ($n = 3$), respectively, and the relative standard deviations (RSDs) were less than 3%.

Repeatability Test. A repeatability test was done with six replicates, and the RSDs for the six compounds were from 1.9% to 2.8% (**Table 3**).

Precision Test. Intraday and interday variations were utilized to determine the precision of the developed assays. The intraday variation was determined by analyzing the six replicate samples (extract of leaves or fruits of *Da 10* (Guangdong), *Morus atropurpurea* Roxb.) within one day, and interday variation was determined within three consecutive days. The results indicated that the RSD values of the overall intraday and interday variations were less than 2.7% for the compounds tested (**Table 3**).

Therefore, this HPLC-DAD method was considered as accurate and sensitive enough for quantitative evaluation of the six active compounds in mulberry plants.

Quantitative Analysis. The complete summary of the results of DNJ, resveratrol, oxyresveratrol, Cy-3-glu, Cy-3-rut, and rutin, total anthocyanins and total flavonoids in mulberry fruit juice, lyophilized fruit, and lyophilized fruit marc of 38 cultivars and dried leaves of 33 cultivars from Guangdong, Zhejiang, Jiangsu, Fujian, Shanghai, Shanxi, Beijing, Shandong, Hebei, and Xinjiang provinces in China is shown in **Tables 4**−**7**.

Most species of mulberry that we studied have not been examined for their phytochemical properties before. These results are, therefore, meaningful for the exploitation of mulberry fruits and leaves.

The quantitative analysis results revealed a great diversity in components and contents of DNJ, resveratrol, oxyresveratrol, Cy-3-glu, Cy-3-rut, total anthocyanins, rutin, and total flavonoids in different species of mulberry. In mulberry fruit juice of 38 cultivars (**Table 4**), DNJ varied between 33.95−346.36 mg/L, Cy-3-glu between 13.94−2091.27 mg/L, Cy-3-rut between 6.01−473.86 mg/L, total anthocyanins calculated as Cy-3-glu equivalents between 22.19−3300.31 mg/L, rutin between 3.35−133.04 mg/L, and total flavonoids calculated as rutin equivalents between 8.66−191.30 mg/L. In lyophilized mulberry fruit of 38 cultivars (**Table 5**), oxyresveratrol varied between 0.0024−0.0295 mg/g, resveratrol between 0.0021−0.0053 mg/g, Cy-3-glu between 0.57−62.93 mg/g, Cy-3-rut between 0.05−12.70 mg/g, total anthocyanins calculated as Cy-3-glu equivalents between 0.87−96.08 mg/g, rutin between 0.045−1.957 mg/g, and total flavonoids calculated as rutin equivalents between 0.178−2.485 mg/g. In lyophilized mulberry fruit marc of 38 cultivars (**Table 6**), oxyresveratrol varied between 0.0030−0.0373 mg/g, resveratrol between 0.0010−0.0068 mg/g, Cy-3-glu between 0.02−81.43 mg/g, Cy-3-rut between 0.01−12.93 mg/g, total anthocyanins calculated as Cy-3-glu equivalents between 0.03−115.76 mg/g, rutin between 0.118−2.427 mg/g, and total flavonoids calculated as rutin equivalents between 0.190−2.662 mg/g. In dried mulberry leaves of 33 cultivars (**Table 7**), DNJ varied between 1.389−3.483 mg/g, oxyresveratrol between

Table 3. Analytical Results of Repeatability, Intra- and Interday Variabilities for the Analytes in Mulberry ($n = 6$)[a]

	intraday		interday		repeatability	
analyte	content (mg/g)	RSD (%)	content (mg/g)	RSD (%)	content (mg/g)	RSD (%)
DNJ in leaves	0.2511	1.7	0.2502	2.2	0.2507	2.6
resveratrol in fruit	0.0033	1.4	0.0032	2.1	0.0035	2.3
oxyresveratrol in fruit	0.0168	1.1	0.0166	1.7	0.0168	2.4
Cy-3-glu in fruit	32.37	1.6	32.17	2.5	32.32	2.5
Cy-3-rut in fruit	7.15	1.8	7.02	2.0	7.10	2.8
rutin in leaves	2.244	2.1	2.225	2.7	2.240	1.9

[a] RSD: relative standard deviation.

Table 4. Juice Rate and Contents of Analyzed Components of Mulberry Juice ($n = 3$)[a]

no.	cultivars	juice rate	A	B	C	D	E	F	G	H
1	Da 10 (Guangdong)	78.95	130.24	ND	ND	1214.25	473.86	2417.13	133.04	191.30
2	Da 10 (Zhejiang)	57.21	130.96	ND	ND	1777.55	341.56	3208.69	15.72	28.56
3	Da 10 (Shanghai)	66.99	75.49	ND	ND	1305.84	404.10	2673.05	62.99	85.39
4	Da 10 (Jiangsu)	76.14	74.98	ND	ND	1226.07	447.54	2375.37	49.98	75.43
5	Tang 10	78.18	119.46	ND	ND	665.67	273.01	1366.14	42.32	50.97
6	Zhongsang 5801	45.59	59.75	ND	ND	653.76	153.41	1153.05	40.58	47.57
7	Taiwanguosang	50.97	59.83	ND	ND	1367.28	383.10	2594.56	63.21	79.51
8	Huangjiguan	31.41	53.44	ND	ND	28.60	8.62	36.44	10.04	25.03
9	Shengxianqingsang	47.03	88.97	ND	ND	795.72	192.49	1127.96	20.25	31.60
10	Hongmanao	38.86	74.18	ND	ND	77.23	22.10	121.05	13.78	40.13
11	Husang 208	39.02	74.26	ND	ND	230.43	116.74	312.60	7.82	23.53
12	Husang 7 hao	36.23	50.31	ND	ND	21.62	41.72	24.57	6.93	38.77
13	Husang 32 hao	50.46	109.23	ND	ND	135.27	40.68	188.24	7.11	29.13
14	Lu-8	51.38	113.06	ND	ND	253.61	66.16	374.58	16.45	62.33
15	Hongyahaisang	42.38	152.94	ND	ND	293.41	117.41	493.02	7.99	38.80
16	Hongguo 2 hao	47.39	79.65	ND	ND	513.99	351.24	1090.03	48.98	64.97
17	Shanxiguosang	67.16	132.79	ND	ND	1131.51	351.60	2309.34	86.84	123.67
18	Luoyu 1 hao	46.82	264.84	ND	ND	153.10	94.70	261.12	23.82	42.34
19	Yaosang	44.19	315.01	ND	ND	13.94	6.010	22.19	11.75	37.46
20	Dejiang 15 hao	42.65	91.36	ND	ND	55.42	304.50	386.48	13.26	22.82
21	Yaan 3 hao	33.33	172.40	ND	ND	28.80	72.80	82.76	3.49	8.66
22	Huai 30−2	54.97	144.27	ND	ND	163.12	246.84	970.54	16.29	53.09
23	Shishengsang	31.58	110.66	ND	ND	238.57	50.36	324.41	12.13	26.71
24	Dahuasang	47.37	114.90	ND	ND	852.78	353.28	1442.20	10.17	19.75
25	Heigehu	50.24	35.80	ND	ND	28.38	15.16	43.15	4.60	28.18
26	Fujian 1 hao	53.10	96.88	ND	ND	54.72	32.17	79.40	5.21	85.39
27	Fujian 2 hao	30.33	33.95	ND	ND	76.55	59.85	118.78	3.70	17.48
28	Tianquan 1 hao	41.86	46.93	ND	ND	57.46	8.85	65.38	7.79	39.69
29	Heizaoshenzi	43.05	107.07	ND	ND	1714.91	159.30	2493.90	51.57	101.39
30	Dabaishen	40.21	100.63	ND	ND	ND	ND	ND	3.35	12.10
31	Lushenzi	47.06	72.94	ND	ND	ND	ND	ND	4.04	11.45
32	Dahongpao	37.88	121.85	ND	ND	ND	ND	ND	5.18	15.63
33	Hetianbaisang	50.22	346.36	ND	ND	ND	ND	ND	8.93	58.87
34	Dabaie	44.95	179.42	ND	ND	ND	ND	ND	14.66	34.23
35	Sinan 2 hao	42.50	72.42	ND	ND	36.34	52.64	61.98	6.08	15.40
36	Gaozhoujisang	58.67	131.28	ND	ND	2091.27	363.32	3300.31	16.29	49.84
37	Nongsang 12 hao	50.25	62.63	ND	ND	243.42	61.17	351.61	13.42	30.53
38	Xinsang 1 hao	48.17	115.46	ND	ND	606.98	260.56	1034.34	12.21	21.92
39	Nongsang 8 hao	57.97	134.17	ND	ND	56.08	36.46	105.56	16.66	47.79
40	Nongsang 14 hao	49.77	174.63	ND	ND	288.66	116.36	428.27	5.99	33.81
41	Xinyizhilai	39.13	106.24	ND	ND	53.44	8.37	63.42	5.41	28.84

[a] Juice rate (%, mL/g); A, DNJ (mg/L); B, oxyresveratrol (mg/L); C, resveratrol (mg/L); D, Cy-3-glu (mg/L); E, Cy-3-rut (mg/L); F, total anthocyanin (mg/L, calculated as Cy-3-glu); G, rutin (mg/L); H, total flavonoid (mg/L, calculated as rutin). ND, not detected.

0.0053−0.1799 mg/g, rutin between 0.440−3.446 mg/g, and total flavonoids calculated as rutin equivalents between 0.821−10.231 mg/g.

DNJ is one of the main polyhydroxylated piperidine alkaloids in mulberry. In our study, the content of DNJ in mulberry leaves was 1.389−3.483 mg/g (**Table 7**), which was in the same range as

Table 5. Contents of Analyzed Components of Mulberry Fruits ($n = 3$)[a]

no.	cultivars	B	C	D	E	F	G	H
1	Da 10 (Guangdong)	0.0168	ND	32.37	7.15	50.93	1.064	1.118
2	Da 10 (Zhejiang)	0.0245	0.0053	15.38	4.65	27.78	1.113	1.212
3	Da 10 (Shanghai)	0.0252	0.0042	62.93	12.70	96.08	1.957	2.052
4	Da 10 (Jiangsu)	0.0199	0.0046	28.23	5.52	43.10	1.289	1.397
5	Tang 10	0.0160	ND	21.04	5.02	34.29	0.873	0.913
6	Zhongsang 5801	0.0243	ND	23.82	4.34	35.06	1.038	1.690
7	Taiwanguosang	0.0163	ND	32.45	7.22	51.99	1.556	1.718
8	Huangjiguan	0.0185	ND	6.15	0.89	8.54	0.815	0.815
9	Shengxianqingsang	0.0156	0.0041	10.31	1.42	14.14	0.470	0.760
10	Hongmanao	0.0208	0.0034	7.22	1.09	10.12	0.329	0.377
11	Husang 208	0.0167	ND	6.40	1.01	9.08	0.228	0.357
12	Husang 7 hao	0.0121	ND	0.69	0.05	0.87	0.862	1.533
13	Husang 32 hao	0.0148	ND	6.84	0.57	8.47	0.491	1.087
14	Lu-8	0.0123	ND	0.96	0.78	1.21	0.690	1.165
15	Hongyahaisang	0.0131	0.0042	10.25	1.53	14.39	0.731	1.550
16	Hongguo 2 hao	0.0175	0.0040	14.73	3.09	22.91	0.515	0.605
17	Shanxiguosang	0.0067	ND	31.66	8.49	54.25	1.714	2.485
18	Luoyu 1 hao	0.0194	ND	6.48	1.00	9.44	0.354	0.559
19	Yaosang	0.0139	ND	0.88	0.14	1.12	0.179	0.445
20	Dejiang 15 hao	0.0212	ND	19.94	3.94	30.85	0.869	0.984
21	Yaan 3 hao	0.0196	ND	1.40	1.04	4.50	0.288	0.527
22	Huai 30−2	0.0268	ND	0.72	3.28	5.16	0.089	0.394
23	Shishengsang	0.0251	0.0026	2.55	0.48	3.92	0.345	0.556
24	Dahuasang	0.0165	0.0029	12.12	1.56	16.35	0.897	1.232
25	Heigehu	0.0110	ND	8.28	0.99	11.12	0.466	0.888
26	Fujian 1 hao	0.0236	0.0043	2.59	0.24	3.47	0.432	1.410
27	Fujian 2 hao	0.0295	0.0034	6.42	0.96	9.33	0.570	1.470
28	Tianquan 1 hao	0.0149	ND	13.00	2.91	20.95	0.773	1.405
29	Heizaoshenzi	0.0183	0.0021	23.88	3.03	32.99	0.330	0.471
30	Dabaishen	0.0029	ND	ND	ND	ND	0.103	0.327
31	Lushenzi	0.0048	ND	ND	ND	ND	0.089	0.293
32	Dahongpao	0.0102	ND	ND	ND	ND	0.188	0.473
33	Hetianbaisang	0.0121	ND	ND	ND	ND	0.251	0.609
34	Dabaie	0.0133	ND	ND	ND	ND	0.185	0.461
35	Sinan 2 hao	0.0109	ND	0.57	1.23	2.33	0.045	0.178
36	Gaozhoujisang	0.0243	0.0021	35.97	6.16	52.60	1.143	1.287
37	Nongsang 12 hao	0.0090	ND	7.23	1.18	10.34	0.447	0.863
38	Xinsang 1 hao	0.0175	ND	12.34	1.76	16.91	0.806	1.459
39	Nongsang 8 hao	0.0024	ND	4.14	0.54	5.88	0.372	0.600
40	Nongsang 14 hao	0.0123	ND	5.94	0.66	7.96	0.407	0.600
41	Xinyizhilai	0.0099	ND	2.23	0.14	2.80	0.270	0.488

[a] B, oxyresveratrol (mg/g); C, resveratrol (mg/g); D, Cy-3-glu (mg/g); E, Cy-3-rut (mg/g); F, total anthocyanins (mg/g, calculated as Cy-3-glu); G, rutin (mg/g); H, total flavonoid (mg/g, calculated as rutin). ND: not detected.

Table 6. Contents of Analyzed Components of Mulberry Fruit Marc ($n = 3$)[a]

no.	cultivars	B	C	D	E	F	G	H
1	Da 10 (Guangdong)	0.0322	0.0051	34.52	3.84	44.82	1.180	1.517
2	Da 10 (Zhejiang)	0.0373	0.0064	25.55	5.86	41.09	1.030	1.260
3	Da 10 (Shanghai)	0.0356	0.0055	81.43	12.93	115.76	2.427	2.662
4	Da 10 (Jiangsu)	0.0294	0.0065	51.52	7.77	71.15	1.890	2.142
5	Tang 10	0.0262	0.0053	25.93	3.81	35.96	0.778	1.129
6	Zhongsang 5801	0.0248	0.0018	13.73	1.66	18.12	0.808	1.268
7	Taiwanguosang	0.0319	0.0068	39.62	6.38	56.39	1.203	1.340
8	Huangjiguan	0.0193	ND	5.13	0.63	6.95	0.804	1.582
9	Shengxianqingsang	0.0209	0.0042	15.85	2.95	23.68	0.767	1.053
10	Hongmanao	0.0187	ND	6.12	0.78	8.30	0.681	1.271
11	Husang 208	0.0183	ND	2.35	0.32	3.24	0.525	0.854
12	Husang 7 hao	0.0140	ND	1.35	0.05	1.56	0.473	0.870
13	Husang 32 hao	0.0166	ND	10.72	0.77	12.89	0.311	0.507
14	Lu-8	0.0155	ND	12.61	0.72	14.78	0.555	0.948
15	Hongyahaisang	0.0119	0.0033	21.59	2.69	28.80	0.724	1.119
16	Hongguo 2 hao	0.0196	0.0028	13.28	1.66	17.68	0.534	0.675
17	Shanxiguosang	0.0119	ND	28.15	5.55	42.80	2.268	2.525
18	Luoyu 1 hao	0.0265	ND	4.91	0.74	6.89	0.718	1.679
19	Yaosang	0.0130	ND	0.02	0.01	0.03	0.148	0.402
20	Dejiang 15 hao	0.0149	0.0053	1.75	0.18	2.22	0.118	0.190
21	Yaan 3 hao	0.0243	0.0040	2.05	0.30	2.83	0.129	0.197
22	Huai 30−2	0.0315	0.0045	3.71	0.63	5.34	0.170	0.273
23	Shishengsang	0.0219	ND	5.85	2.44	8.18	0.184	0.352
24	Dahuasang	0.0279	ND	12.26	1.31	15.91	0.422	0.764
25	Heigehu	0.0033	ND	6.81	0.60	8.57	0.316	0.647
26	Fujian 1 hao	0.0294	0.0028	1.14	0.06	1.36	0.162	0.298
27	Fujian 2 hao	0.0363	0.0048	1.86	0.17	2.37	0.296	0.674
28	Tianquan 1 hao	0.0189	0.0015	2.68	0.49	3.97	0.286	0.526
29	Heizaoshenzi	0.0196	0.0017	23.85	2.15	29.73	0.413	0.709
30	Dabaishen	ND	ND	ND	ND	ND	0.208	0.810
31	Lushenzi	0.0139	ND	ND	ND	ND	0.577	1.349
32	Dahongpao	0.0121	ND	ND	ND	ND	0.540	1.412
33	Hetianbaisang	0.0143	ND	ND	ND	ND	0.146	0.357
34	Dabaie	0.0099	0.0010	ND	ND	ND	0.299	0.970
35	Sinan 2 hao	0.0125	ND	0.87	0.12	1.22	0.154	0.297
36	Gaozhoujisang	0.0264	0.0035	40.10	5.13	53.87	1.328	1.592
37	Nongsang 12 hao	0.0121	ND	12.15	1.67	16.58	0.332	0.388
38	Xinsang 1 hao	0.0201	ND	15.18	2.41	21.46	1.204	1.307
39	Nongsang 8 hao	0.0030	ND	7.07	0.64	8.93	0.422	0.728
40	Nongsang 14 hao	0.0139	ND	8.40	0.59	10.12	0.366	0.628
41	Xinyizhilai	0.0109	ND	2.67	0.11	3.20	0.252	0.447

[a] B, oxyresveratrol (mg/g); C, resveratrol (mg/g); D, Cy-3-glu (mg/g); E, Cy-3-rut (mg/g); F, total anthocyanins (mg/g, calculated as Cy-3-glu); G, rutin (mg/g); H, total flavonoid (mg/g, calculated as rutin). ND: not detected.

the values found in mulberry leaves by other authors from other countries or regions (*23*, *3*). It is the first time to quantitatively determine DNJ in mulberry juice by derivatization with *N*-(9-fluorenylmethoxycarbonyloxy) succinimide (FMOC-OSu) followed by RP-HPLC. The juice of *Hetianbaisang* (*M. alba* Linn.), *Luoyu 1 hao* (*M. nigra* Linn.), and *Yaosang* (*M. nigra* Linn.), which are all cultivated in Xinjiang province, contained more DNJ. For mulberry leaves, the variety *Taiwanguosang* (*M. atropurpurea* Roxb.) and *Shanxiguosang* (*M. nigra* Linn.) were among the highest in DNJ (**Table 4**). In our study, DNJ was not detected in mulberry fruit or marc because the current detect method for DNJ analysis in our experiment may not be sensitive enough for mulberry fruits, and a further study should be conducted. DNJ content of mulberry species differed significantly in mulberry juice and leaves of various species or from different planting regions. The DNJ content of the same cultivar *Da 10* (*M. atropurpurea* Roxb.) gathered from four regions was different, which means that the planting regions could affect the content. Mulberry gathered from Xinjiang contained more DNJ in the juice, which suggested that fruits of high DNJ content can be obtained by harvesting in Xinjiang. Different species from the same region also showed a great difference in DNJ content. For example, *Gaozhoujisang* (*M.australis* Poir.) contained more DNJ in juice than *Sinan 2 hao* (*M.laevigata* Wall.), while both of them were gathered in Zhenjiang, Jingsu (**Table 4**). Comparing different species, we found that the *M. nigra* Linn. contained more DNJ than other species, such as the juice of *Luoyu 1 hao* (*M. nigra* Linn.) and *Yaosang* (*M. nigra* Linn.), and leaves of

Table 7. Contents of Analyzed Components of Mulberry Leaves ($n = 3$)[a]

no.	cultivars	A	B	C	D	E	F	G	H
1	Da 10 (Guangdong)	2.511	0.1799	ND	ND	ND	ND	2.244	2.959
2	Da 10 (Zhejiang)	1.576	0.0359	ND	ND	ND	ND	2.979	3.861
3	Da 10 (Jiangsu)	2.619	0.0845	ND	ND	ND	ND	0.961	1.281
4	Tang 10	1.389	0.0800	ND	ND	ND	ND	0.601	0.821
5	Zhongsang 5801	3.029	0.0414	ND	ND	ND	ND	1.256	3.674
6	Taiwanguosang	3.483	0.0237	ND	ND	ND	ND	3.397	4.382
7	Huangjiguan	2.076	0.0782	ND	ND	ND	ND	2.112	5.949
8	Shengxianqingsang	2.034	0.0488	ND	ND	ND	ND	2.718	8.109
9	Hongmanao	1.838	0.0076	ND	ND	ND	ND	2.354	7.454
10	Husang 208	2.316	0.0137	ND	ND	ND	ND	1.460	5.500
11	Husang 7 hao	2.646	0.0224	ND	ND	ND	ND	2.618	7.871
12	Husang 32 hao	2.561	0.0522	ND	ND	ND	ND	1.803	5.598
13	Lu-8	1.881	0.0169	ND	ND	ND	ND	3.057	10.231
14	Hongyahaisang	2.055	0.0476	ND	ND	ND	ND	3.036	9.606
15	Hongguo 2 hao	1.583	0.0251	ND	ND	ND	ND	3.088	4.404
16	Shanxiguosang	3.250	0.0323	ND	ND	ND	ND	2.148	4.357
17	Dejiang 15 hao	2.726	0.0131	ND	ND	ND	ND	1.248	5.473
18	Yaan 3 hao	2.977	0.0231	ND	ND	ND	ND	0.440	3.378
19	Huai 30−2	1.864	0.0676	ND	ND	ND	ND	0.660	6.101
20	Shishengsang	3.024	0.0168	ND	ND	ND	ND	1.529	5.320
21	Dahuasang	1.964	0.0284	ND	ND	ND	ND	2.524	8.489
22	Heigehu	3.073	0.0385	ND	ND	ND	ND	1.167	4.669
23	Fujian 1 hao	1.438	0.0147	ND	ND	ND	ND	1.716	5.592
24	Fujian 2 hao	2.374	0.0095	ND	ND	ND	ND	1.022	3.869
25	Tianquan 1 hao	2.924	0.0129	ND	ND	ND	ND	1.576	4.810
26	Dabaishen	2.519	0.0776	ND	ND	ND	ND	1.964	6.624
27	Lushenzi	2.653	0.0528	ND	ND	ND	ND	1.278	4.367
28	Dahongpao	2.102	0.0423	ND	ND	ND	ND	2.374	8.346
29	Sinan 2 hao	2.595	0.0230	ND	ND	ND	ND	0.483	1.868
30	Gaozhoujisang	2.889	0.0400	ND	ND	ND	ND	3.446	6.524
31	Nongsang 12 hao	2.419	0.0053	ND	ND	ND	ND	1.756	4.233
32	Xinsang 1 hao	3.326	0.0189	ND	ND	ND	ND	1.626	4.195
33	Nongsang 8 hao	3.208	0.0081	ND	ND	ND	ND	1.270	4.311
34	Nongsang 14 hao	3.338	0.0201	ND	ND	ND	ND	2.299	7.683
35	Xinyizhilai	3.282	0.1065	ND	ND	ND	ND	1.720	6.716

[a] A, DNJ (mg/g); B, oxyresveratrol (mg/g); C, resveratrol (mg/g); D, Cy-3-glu (mg/g); E, Cy-3-rut (mg/g); F, total anthocyanins (mg/g, calculated as Cy-3-glu); G, rutin (mg/g); H, total flavonoid (mg/g, calculated as rutin). ND: not detected.

Shanxiguosang (*M. nigra* Linn.). The DNJ contents of different cultivars from the same planting regions and of the same species also exhibited significant differences. For example, the juice of *Yaan 3 hao* (*M. cathayana* Hemsl.) contained more DNJ than that of *Dejiang 15 hao* (*M. cathayana* Hemsl.) and *Huai 30−2* (*M. cathayana* Hemsl.), which were all gathered from Zhenjiang, Jiangsu (**Table 4**). It was thus clear that the planting regions, species, and cultivars were all influencing factors of DNJ content in mulberry, while there were other factors such as different field positions of the tree (*3*) and harvesting season (*24*).

Resveratrol and oxyresveratrol have recently received much attention. Oxyresveratrol has been isolated from mulberry wood (*13*), but no data were found about the quantitative determination of oxyresveratrol in mulberry fruits or leaves. In our study, mulberry fruits and leaves contain more oxyresveratrol than resveratrol (**Table 5**). Resveratrol was not detected in many fruits, marc, or leaves probably because of low content. The content of those two compounds differed significantly in various mulberry species or from different planting regions. We found that the variety *Da 10* (*M. atropurpurea* Roxb.) from four different provinces in China and the variety *Taiwanguosang* (*M. atropurpurea* Roxb.) had higher content of oxyresveratrol and resveratrol, which meant that they were potential resources for new variety development. Among the different species that we gathered, *M. atropurpurea* Roxb. had higher content than others. White mulberry contains very small amounts of oxyresveratrol and resveratrol, compared to that in red ones. The concentrations of oxyresveratrol and resveratrol in fruit were less than those in marc, and they were not detected in the juice (**Tables 4**−**7**); therefore, obviously, the main content was in marc. Resveratrol is a naturally occurring phytoalexin produced in mulberry in response to injury by some spermatophytes or ultraviolet irradiation (*25*). Therefore, while the mulberry was injured by microbial infection or mechanical damage, the content of resveratrol in plant tissue may be higher than that of normal tissue. The marc contained more resveratrol may be because the mulberry plant was probably stimulated to synthesize and secrete it in mulberry skin by the injury caused by picking. The exact reason and whether oxyresvetatrol has a synthetic mechanism similar to that of resveratrol need to be studied further.

Mulberry juice of some cultivars, such as *Da 10* (*M. atropurpurea* Roxb., Guangdong) (1214.25 mg/L of Cy-3-glu, 473.86 mg/L of Cy-3-rut), contained more Cy-3-glu and Cy-3-rut compared to some other juices, such as chokeberry (Cy-3-glu, 74.30 mg/L), elderberry (Cy-3-rut, 111.70 mg/L), blackberry (Cy-3-glu, 743.30 mg/L; Cy-3-rut, 10.80 mg/L), and sour cherry (Cy-3-glu, 743.30 mg/L; Cy-3-rut, 93.00 mg/L) (*26*). Mulberry fruits of some cultivars also had high amounts of anthocyanin, such as *Shanxiguosang* (*M. nigra* Linn.) (31.66 mg/g of Cy-3-glu, 8.49 mg/L of Cy-3-rut and 54.25 mg/g of total anthocyanin) as compared to muscadine grapes (less than 2.8 mg/g dry weight of anthocyanin in cyanidin equivalents) (*27*).

However, the contents of anthocyanins in different species varied widely. Contents of anthocyanins in juice, fruits, and fruit marc (**Tables 4**−**6**) in species *M. multicaulis* Perr. were higher than those in other species. Different cultivars of the same species also had various contents of anthocyanins. For example, in *M. alba* Linn., contents of Cy-3-glu ranged from 0.69 mg/g to 14.73 mg/g in fruits of different cultivars (**Table 5**). Mulberry of *Da10* (*M. atropurpurea* Roxb.) contained higher amounts of anthocyanin than other cultivars, but the fruits harvested from different areas had variable amounts of anthocyanins. This highlighted that not only the cultivars but also the plant growing environment could affect anthocyanin content.

The top five varieties *Gaozhoujisang* (*M. australis* Poir.), *Da10* (*M. atropurpurea* Roxb.) from four areas, *Shanxiguosang* (*M. nigra* Linn.), *Taiwanguosang* (*M. atropurpurea* Roxb.), and *Heizaoshenzi* (*M. alba* Linn.) which contained high contents of anthocyanin in juice and fruit (**Tables 4** and **5**) are potential resources for plants with high amounts of anthocyanin and may serve as the genetic resource for further improvement in their nutrient composition.

In mulberry juice, fruits, fruit marc, and leaves of most species examined, the main flavonoid was rutin, and the other three flavonoids were identified according to their UV scanning spectrograms (**Figure 4**). Mulberry juice was rich in flavonoid, and the highest content of 191.30 mg/L was found for *Da10* (Guangdong) (*M. atropurpurea* Roxb.). In previously published results, total flavonoid content of mulberry fruit was 2.5 mg/g fresh matter as estimated by the aluminum chloride colorimetric method. This was higher than strawberry, oriental plum, loquat, and some other vegetables with the same preparation method (*28*). It was higher than the results presented here probably because of the different detection methods. In our study, the highest content of total flavonoids in mulberry fruit was 2.485 mg/g, which was

higher than that in the fruits of mulberry plants in Korea (0.654 mg/g) (*16*).

In this study, mulberry fruits of 38 cultivars and mulberry leaves of 33 cultivars were examined for their DNJ, resveratrol, oxyresveratrol, anthocyanin, and flavonoid content. The results suggest significant differences in the concentration of these compounds in different mulberry species and cultivars. Mulberry fruit is predominately used for food and drinks; therefore, the cultivars with higher contents of DNJ, resveratrol, oxyresveratrol, anthocyanins, and flavonoids are particularly suitable for commercial planting, development, and industrial beverage, and food applications.

Our study is the first quantification of DNJ in mulberry juice and of oxyresveratrol in mulberry fruits and leaves. The data presented here indicate that mulberry has the potential to be further developed into a nutritionally interesting raw material for food and beverage applications because of its compositional richness and also because mulberry fruits contain higher amounts of anthocyanins than other well-known fruits such as elderberry and black berry.

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