Effects of two drying methods on the stability and antioxidant activity of phenolic compounds in mulberry fruits

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Abstract
Mulberry is a health-promoting fruit with abundant phenolic compounds. The effects of vacuum drying (VD) and vacuum freeze-drying (VFD) on oxidation resistance and polyphenols, including extractable and non-extractable phenols, were compared in the present work. The total polyphenols and antioxidant capacity of mulberry fruits treated with VFD were higher than those treated with VD. Thirteen phenolic compounds in fresh and dried mulberry fruits were identified and quantified by UPLC-QqQ/MS. The content of extractable phenol was significantly higher than that of non-extractable phenol. In addition, correlation analysis showed that flavonoids cyanidin-3-O-glucoside and phloretin were closely related to the total polyphenols and antioxidant activity. These results provide the basis for further development of dried mulberry products containing high contents of total polyphenols and antioxidant activity.

Introduction
Mulberry (Morus alba L.) is an edible fruit, and frequently used in Chinese traditional medicine. Mulberry fruits are rich in beneficial bioactive substances, especially phenolic compounds including phenolic acids, flavonoids, and anthocyanins (Natić et al., 2015). It exhibits a variety of biological activities such as anti-tumor (Qian et al., 2015), antioxidant (Shih et al., 2010), anti-inflammatory (Kim and Park, 2006), and neuroprotective (Kim et al., 2010). Antioxidant activity plays a key role in reducing the risks of cancers, heart diseases, stroke, and cardiovascular diseases (Gilgun-Sherki et al., 2002). Antioxidants are also used as preservatives in the food industry (Winkler et al., 2006). Recently, mulberry has become more popular among consumers due to its health benefits.

Fresh mulberry fruits are not generally available due to their short harvest season and the difficulty in storing these delicate fruits. In order to improve the consumption and availability of mulberry fruits, it is necessary to choose suitable processing methods to keep the mulberry fruits fresh. Drying is a classic technique for fruit preservation because many biochemical reactions are inhibited when moisture content is significantly reduced (Jin et al., 2018). Drying methods have been developed from traditional natural drying and hot air drying to modern vacuum drying (VD) and vacuum freeze-drying (VFD). Hot air drying is the most commonly used drying method, but the nutrients in fruits can be easily degraded by heating and oxidation. Due to limited oxygen, VD reduces oxidative damage, but it is relatively time-consuming (Papoutsis et al., 2017). The VFD process involves removing water from a frozen matrix by sublimation, so it can effectively preserve most of the phytochemical characteristics of fresh fruits (Donno et al., 2016). However, VFD is also a slow drying process, and its processing cost is typically much higher than that of VD. When compared with VD, VFD can better preserve the proanthocyanins and antioxidant activity of Aronia powder (Horszwald et al., 2013). However, the total polyphenols and antioxidant capacity were higher in lemon pomace samples dried by VD than those dried by VFD (Papoutsis et al., 2017), and the polyphenols were higher in plum powder dried by VD than those dried by VFD (Michalska et al., 2016). Therefore, different drying processes may lead to degradation and loss of nutritional values in different fruits.

Previous studies have investigated the drying kinetics, colour, texture, and total phenolic contents of mulberry fruits dried by sun, microwave, hot air, and vacuum drying methods (Khattak et al., 2019; Suna and Özkan-Karabacak, 2019). However, to the best of our knowledge, the effects of currently used VD and VFD methods on the total polyphenols and antioxidant activity...
activities of dried mulberry fruits have not yet been compared. In the present work, UPLC-QqQ-MS/MS was used for the first time to compare the effects of these two drying processes on the polyphenol profile of mulberry fruits. Non-extractable polyphenols (NEPs), an important phenolic fraction, have never been investigated in mulberry fruits. NEPs may make up a large proportion of the total polyphenols in fruits and vegetables. For example, the content of NEPs in beans can be as high as 68.3% (Acosta-Estrada et al., 2014; Peng et al., 2017). The NEPs in fruits are highly sensitive to drying (Nunes et al., 2016). Therefore, it is necessary to compare the effects of VD and VFD on the extractable and non-extractable polyphenol profiles and antioxidant activities of mulberry fruits.

Materials and methods

Materials

In mid-May, fresh mulberry fruits at full ripening stage were purchased from a major supermarket chain in Fuling, Chongqing, China. The fruits without mechanical damage were selected for drying.

Drying process

For VD, fresh mulberry fruits were placed in a vacuum chamber (DZF-1B, Shanghai Yuejin Medical Instrument Co., Ltd., Shanghai, China) at 80°C for 48 h. Before VFD, mulberry fruits were frozen in an ultra-cold storage freezer (DW-86L828W, Haier Group Co., Ltd., Shandong, China) at -60°C for 12 h, and then were dried in a vacuum lyophiliser (Scientz-30ND, Ningbo Xinzhi Freeze Drying Equipment Co., Ltd., Zhejiang, China) at -60°C for 12 h, and then were dried in a vacuum lyophiliser (Scientz-30ND, Ningbo Xinzhi Freeze Drying Equipment Co., Ltd., Zhejiang, China) for 48 h. Subsequently, the dried mulberry fruits were ground and passed through a 60-mesh screen. All drying treatments were performed in triplicate. Comparative- ly, VFD costs at least 16 times as much as VD.

Preparation of extractable phenolics (EPs) and non-extractable phenolics (NEPs)

The EPs and NEPs were extracted from mulberry fruits as previously described (Li et al., 2019). The binding phenolics obtained by acid hydrolysis and acid-alkali sequential hydrolysis were called ANEP and ABNEP, respectively. Similarly, the binding phenolics obtained by alkaline and alkali-acid sequential hydrolysis were called BNEP and BANEP, respectively. All extracts were freeze-dried into powder in vacuum, and then dissolved into ethanol for further analyses of total polyphenols, antioxidant activities, and phenolic compositions.

Total phenolic content and antioxidant activity assays

Total phenolic contents were determined using the Folin-Ciocalteau assay as previously described (Mahmood et al., 2012; Sarker et al., 2020a), and all samples were measured in triplicate. The results were expressed in milligrams of gallic acid equivalent per gram of drying weight (mg GAE/g DW). The antioxidant activities were measured by DPPH (Sarker and Oba, 2020), ABTS (Sarker et al., 2020b), and FRAP (Li et al., 2019) assays, and all samples were evaluated in triplicate. The results were expressed in micrograms vitamin C equivalents per gram of mulberry fruits (equivalent µg Vc/g DW).

Polyphenol profile determined by UPLC-QqQ-MS/MS

The phenolic composition in EP, ANEP, ABNEP, BNEP, and BANEP was analysed as previously described (Li et al., 2019). Briefly, analysis was performed by ultra-HPLC and triple quadruple mass spectrometry (6460QqQ-MS/MS; Agilent) equipped with an electrospray ionisation source and ZORBAX Eclipse Plus C_{18} column (100 × 2.1 mm i.d., 1.8 µm; Agilent, Waldbronn, Germany), with 0.1% aqueous formic acid (A) and acetonitrile (B) as the mobile phase. The gradient elution procedure was as follows: 0 to 11.5 min from 80% to 10% A, 11.5 to 12.5 min with 10% A, and 12.5 to 15 min from 10% A to 80% A. The injection volume was 5 µL, and the column temperature was maintained at 30°C.

Statistical analysis

All values were expressed as mean values ± standard deviations. One-way analysis of variance (ANOVA) and Duncan’s multiple-range tests were used to determine the significant difference (p < 0.05). Additionally, the correlation between polyphenols and antioxidant activity of mulberry fruits was analysed by a multivariate linear sparse partial least-squares analysis (sPLS) in mixOmics package of R (×64.3.4.3) (Rohart et al., 2017).

Results and discussion

Total polyphenols and antioxidant capacity

The EPs were measured in fresh mulberry fruits, vacuum-dried mulberry fruits, and vacuum freeze-dried mulberry fruits as 3.829, 1.452, and 2.416 µg GAE/g, respectively (Figure 1A). When compared with fresh samples, both VD and VFD significantly decreased the EP levels. These results are consistent with previous reports that EP decreased after drying of fruits and vegetables (Sun et al., 2015; Papoutsis et al., 2017).
The NEPs are linked to macromolecules through ester and glycosidic bonds, preventing them from being extracted by water or organic solvents. NEPs can be released by acid, alkaline, or enzymatic hydrolysis (Domínguez-Rodríguez et al., 2017). To the best of our knowledge, this is the first investigation of NEPs in mulberry fruits. Peng et al. (2017) believed that NEPs accounted for 9.94 to 95.6% of the total phenolic compounds in fruits and vegetables. In the present work, the NEPs accounted for approximately 50, 39, and 36% of the total polyphenols in fresh, vacuum-dried, and freeze-dried mulberry fruits, respectively. As shown in Figure 1A, both VD and VFD resulted in the losses of ANEP, ABNEP, BNEP, and BANEP. The total polyphenols significantly decreased after both VD and VFD. The loss of phenolic compounds may reflect the destruction of cell structure caused by ice crystal formation and degradation at high temperature (Nunes et al., 2016). Generally, VFD better preserved polyphenols than VD, which agrees with the result of a previous study in which freeze-dried tomato and ginger had higher contents of phenolic compounds than those preserved by VD (Gümüşay et al., 2015). The greater loss after VD may reflect the accelerated degradation due to increased heat.

Next, DPPH, ABTS\(^+\), and FRAP assays were applied to assess the primary antioxidant capacity of fresh and dried mulberry fruits. As shown in Figures 1B - 1D, EPs showed stronger antioxidant activity than NEPs in both fresh and dried mulberry fruits. However, in terms of both EPs and NEPs, fresh samples showed the highest DPPH, ABTS\(^+\), and FRAP activities, followed by freeze-dried samples and vacuum-dried samples (Figures 1B - 1D). The variation of antioxidant activity in differently treated mulberry fruits was consistent with the measurements of total polyphenols, suggesting that total polyphenols played an important role in antioxidant activity. Previous studies have indicated that freeze-dried fruits and vegetables showed higher antioxidant activity than those subjected to VD, and antioxidant activity was closely associated with the content of total polyphenols (Jin et al., 2018; Lachowicz et al., 2019). Due to a higher number of total polyphenols and higher antioxidant activities, VFD seems to be a better choice for drying mulberry fruits.

**Polyphenol profile**

To further determine the effects of drying methods on the polyphenol profiles of mulberry fruits, UPLC-QqQ-MS/MS was used to investigate the polyphenols in EPs and NEPs. Thirteen polyphenols (Table 1) were identified by comparing the retention time, molecular weight, characteristic
fragments, and transitions with those of standards 
(Figure 2, Table 2). The results showed that both fresh and dried mulberry mainly contained chlorogenic acid, protocatechuic acid, cryptochlorogenic acid, cyanidin-3-O-glucoside, rutin, and quercetin glucoside, which are consistent with the findings by Zhang et al. (2008) of nine polyphenols detected by HPLC-DAD-ESI-MS/MS, with chlorogenic acid, protocatechuic acid, and rutin identified being the main phenolics in mulberry fruits (Zhang et al., 2008). The analysis of total polyphenols indicated NEPs were an important component in mulberry fruits. However, as shown in Table 1, most flavonoids were not detected in the NEPs. This result differs from the result of a study of loquat which showed that non-extractable flavonoids were the main flavonoids (Li et al., 2019). When compared with EP, polyphenols in NEPs were significantly reduced. Except for a small amount of rutin and quercetin glucoside, polyphenols in the NEPs were mostly phenolic acids. Notably, there was a significantly higher amount of protocatechuic acid in ANEP and BNEP fractions of fresh mulberry fruits than in EP, indicating the release of protocatechuic acid after acid or alkaline hydrolysis.

When compared with fresh mulberry fruits, VD and VFD induced changes in both EP and NEPs (Table 1). This result was similar to that of a previous study that both oven drying and freeze-drying processes reduced EPs and NEPs in guava fruits (Nunes et al., 2016). VD and VFD reduced all the flavonoids. For example, both VD and VFD significantly reduced the content of rutin in EP (Table 1). In contrast, the content of chlorogenic acid significantly increased after VD, possibly due to the transformation of the caffeoyl-hexoside precursor (Villegas and Kojima, 1986). A previous study reported that the content of chlorogenic acid in vacuum-dried cranberry was significantly higher than that in vacuum freeze-dried cranberry, and the higher the VD temperature, the lower the ratio of caffeoyl-hexoside to chlorogenic acid (Michalska et al., 2018). The level of caffeoyl-hexoside was not determined in the present work, and future work should investigate a potential increase of chlorogenic acid by VD. The amount of cyanidin-3-O-glucoside,
Table 1. Content of extractable and non-extractable phenolics in mulberry fruit.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Fresh sample EP (mg/g DW)</th>
<th>VD</th>
<th>VFD</th>
<th>Fresh sample ANEP (mg/g DW)</th>
<th>VD</th>
<th>VFD</th>
<th>Fresh sample ABNEP (mg/g DW)</th>
<th>VD</th>
<th>VFD</th>
<th>Fresh sample BNEP (mg/g DW)</th>
<th>VD</th>
<th>VFD</th>
<th>Fresh sample BANEP (mg/g DW)</th>
<th>VD</th>
<th>VFD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorogenic acid</td>
<td>30.77 ± 130.78 ±</td>
<td>6.52 ± 0.51 ±</td>
<td>0.46 ± 0.28 ±</td>
<td>0.80 ± 0.18 ±</td>
<td>0.26 ±</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
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<tr>
<td>Protocatechuic acid</td>
<td>20.85 ± 14.66 ±</td>
<td>74.62 ± 12.36 ±</td>
<td>22.60 ± 7.98 ±</td>
<td>68.60 ± 10.71 ±</td>
<td>11.12 ±</td>
<td>8.18 ± 2.22 ±</td>
<td>1.76 ±</td>
<td>8.81 ± 2.22 ±</td>
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<td></td>
</tr>
<tr>
<td>Cryptochlorogenic acid</td>
<td>14.68 ± 26.71 ±</td>
<td>9.86 ± 2.51 ±</td>
<td>3.49 ±</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Cyanidin-3-O-glucoside</td>
<td>55.48 ± 5.27 ±</td>
<td>22.18 ±</td>
<td>0.04 ±</td>
<td>0.12 ±</td>
<td>0.04</td>
<td>0.02 ±</td>
<td>0.02 ±</td>
<td>0.02 ±</td>
<td>0.03 ±</td>
<td>0.24 ±</td>
<td></td>
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</tr>
<tr>
<td>Narirutin</td>
<td>0.14 ± 0.08 ±</td>
<td>0.05 ±</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
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<tr>
<td>Rutin</td>
<td>198.76 ± 74.24 ±</td>
<td>1.36 ±</td>
<td>0.34 ±</td>
<td>0.52 ± 0.04 ±</td>
<td>0.04 ±</td>
<td>2.72 ± 0.31 ±</td>
<td>0.50 ±</td>
<td>0.78 ± 0.24 ±</td>
<td>0.40 ±</td>
<td>8.18 ± 2.22 ±</td>
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</tr>
<tr>
<td>Quercetin glucoside</td>
<td>36.99 ± 1.02 ±</td>
<td>0.71 ±</td>
<td>2.17 ±</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>0.54 ±</td>
<td>0.26 ±</td>
<td>nd</td>
<td>nd</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resveratrol</td>
<td>1.46 ± 0.60 ±</td>
<td>0.92 ±</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
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<td>nd</td>
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<tr>
<td>Phloridzin</td>
<td>0.65 ± 0.71 ±</td>
<td>0.82 ±</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
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</tr>
<tr>
<td>Naringenin</td>
<td>1.56 ± 0.43 ±</td>
<td>0.55 ±</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
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</tr>
<tr>
<td>Phloretin</td>
<td>1.22 ± 0.08 ±</td>
<td>0.25 ±</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
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<td>nd</td>
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</tr>
<tr>
<td>Luteolin</td>
<td>0.43 ± 0.23 ±</td>
<td>0.26 ±</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Quercetin</td>
<td>2.47 ± 0.43 ±</td>
<td>3.03 ±</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
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<td>nd</td>
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</tr>
</tbody>
</table>

EP: extractable polyphenols; ANEP: non-extractable polyphenols by acid hydrolysis; ABNEP: non-extractable polyphenols by acid-alkali sequential hydrolysis; BNEP: non-extractable polyphenols by alkaline hydrolysis; BANEP: non-extractable polyphenols by alkali-acid sequential hydrolysis; DW: dry weight; nd: not detected. Different letters mean in the same extracts indicate significant differences among means of treatments (p < 0.05).
a major anthocyanin in mulberry fruits, dramatically decreased after VD (Wen et al., 2019), suggesting that it could be degraded by heating. This result is similar to those reported for grape and blueberry, with heating leading to a significant decrease in anthocyanins (Khanal et al., 2010). Similarly, ultra-high pressure homogenisation processing could promote the degradation of cyanidin-3-O-glucoside in mulberry juice (Yu et al., 2014).

**Correlation analysis**

To illustrate the relationship between individual polyphenols and antioxidant activity, multivariate linear sparse partial least-squares analysis (sPLS) was performed. As shown in Figure 3, the levels of most flavonoids were positively correlated with antioxidant activity. In particular, cyanidin-3-O-glucoside and phloretin significantly promoted antioxidant capacity and total polyphenols. Cyanidin-3-O-glucoside was previously reported to have a strong bioactivity. For example, the protection of cyanidin-3-O-glucoside against endothelial dysfunction might be attributable to its high antioxidant activity (Serraino et al., 2003). Cyanidin-3-O-glucoside also inhibited the migration and invasion of A549 human lung carcinoma cells (Chen et al., 2006), suggesting its potential therapeutic value for cancer. In addition, phloretin, a naturally occurring dihydrochalcone identified in apple, kumquat, and vegetables exhibited high antioxidant activity (Behzad et al., 2017). Overall, the results of the present work suggest that flavonoids, rather than phenolic acids, contribute to the antioxidant activity of mulberry fruits.

**Conclusion**

To the best of our knowledge, this is the first study of the effects of VD and VFD on the extractable and non-extractable polyphenols of mulberry fruits. The results clearly showed that VFD was better than VD for drying mulberry fruits, with stronger antioxidant activity, higher total polyphenols, and more cyanidin-3-O-glucoside. However, VD was superior to VFD in maintaining a high level of chlorogenic acid. Few studies have examined the effects of innovative drying processes, such as air-impingement jet drying and heat pump drying on the nutrients and bioactive compounds of mulberry fruits. In addition, the degradation kinetics of anthocyanins in mulberry fruits during drying is rarely reported. Our next steps are therefore to determine the effects of innovative and combined drying processes on the polyphenol profiles of mulberry fruits.

**Table 2. Identification of phenolic compounds using UHPLC-QqQ-MS/MS.**

<table>
<thead>
<tr>
<th>Number</th>
<th>Name</th>
<th>RT (min)</th>
<th>Fragmentor (V)</th>
<th>CE (V)</th>
<th>MS [M-H]- (m/z)</th>
<th>MS/MS (m/z)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Chlorogenic acid</td>
<td>1.210</td>
<td>90</td>
<td>15</td>
<td>353</td>
<td>190.9</td>
</tr>
<tr>
<td>2</td>
<td>Protocatechuic acid</td>
<td>1.225</td>
<td>90</td>
<td>10</td>
<td>153</td>
<td>109</td>
</tr>
<tr>
<td>3</td>
<td>Cryptochlorogenic acid</td>
<td>2.952</td>
<td>100</td>
<td>20</td>
<td>353</td>
<td>173, 179</td>
</tr>
<tr>
<td>4</td>
<td>Cyanidin-3-O-glucoside</td>
<td>3.761</td>
<td>160</td>
<td>20</td>
<td>447</td>
<td>284.9, 298.9</td>
</tr>
<tr>
<td>5</td>
<td>Naringtin</td>
<td>4.085</td>
<td>160</td>
<td>40</td>
<td>609</td>
<td>299.9</td>
</tr>
<tr>
<td>6</td>
<td>Rutin</td>
<td>8.149</td>
<td>160</td>
<td>20</td>
<td>579</td>
<td>271, 295</td>
</tr>
<tr>
<td>7</td>
<td>Quercetin glucoside</td>
<td>10.142</td>
<td>130</td>
<td>25</td>
<td>227</td>
<td>143, 185</td>
</tr>
<tr>
<td>8</td>
<td>Resveratrol</td>
<td>8.474</td>
<td>150</td>
<td>20</td>
<td>462.9</td>
<td>300.8, 342.9</td>
</tr>
<tr>
<td>9</td>
<td>Phloridzin</td>
<td>8.646</td>
<td>150</td>
<td>20</td>
<td>343.9</td>
<td>272.9, 167</td>
</tr>
<tr>
<td>10</td>
<td>Quercetin</td>
<td>10.422</td>
<td>130</td>
<td>25</td>
<td>300.9</td>
<td>150.9, 107</td>
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<tr>
<td>11</td>
<td>Naringenin</td>
<td>10.478</td>
<td>130</td>
<td>25</td>
<td>271</td>
<td>119, 150.8</td>
</tr>
<tr>
<td>12</td>
<td>Luteolin</td>
<td>10.860</td>
<td>140</td>
<td>30</td>
<td>284.9</td>
<td>132.9</td>
</tr>
<tr>
<td>13</td>
<td>Phloretin</td>
<td>10.861</td>
<td>120</td>
<td>20</td>
<td>273</td>
<td>167, 123</td>
</tr>
</tbody>
</table>

V: volt; RT: retention time (min); and CE: collision energy.

![Figure 3](image-url)
mulberry fruits.

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References


