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

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) for 48 h. Subsequently, the dried mulberry fruits were ground and passed through a 60-mesh screen. All drying treatments were performed in triplicate. Comparatively, 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).
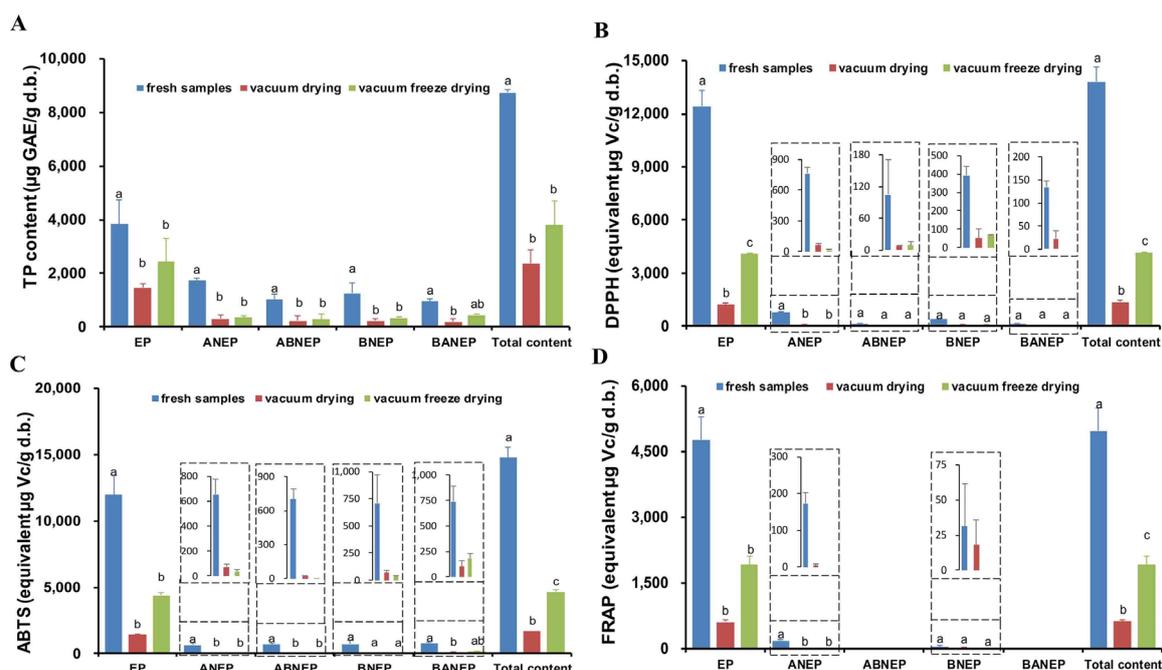
Figure 1. The effects of drying process on polyphenol content (A) and antioxidant activity (B-D) of mulberry fruit. TP: total polyphenols; EP: extractable polyphenols; ANEP: non-extractable polyphenols extracted after acid hydrolysis; ABNEP: non-extractable polyphenols extracted after acid-alkali sequential hydrolysis; BNEP: non-extractable polyphenols extracted after alkaline hydrolysis; and BANEP: non-extractable polyphenols extracted after alkali-acid sequential hydrolysis. Different letters indicate significant difference among different groups as determined by ANOVA and Tukey's *post-hoc* test ($p < 0.05$).

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,
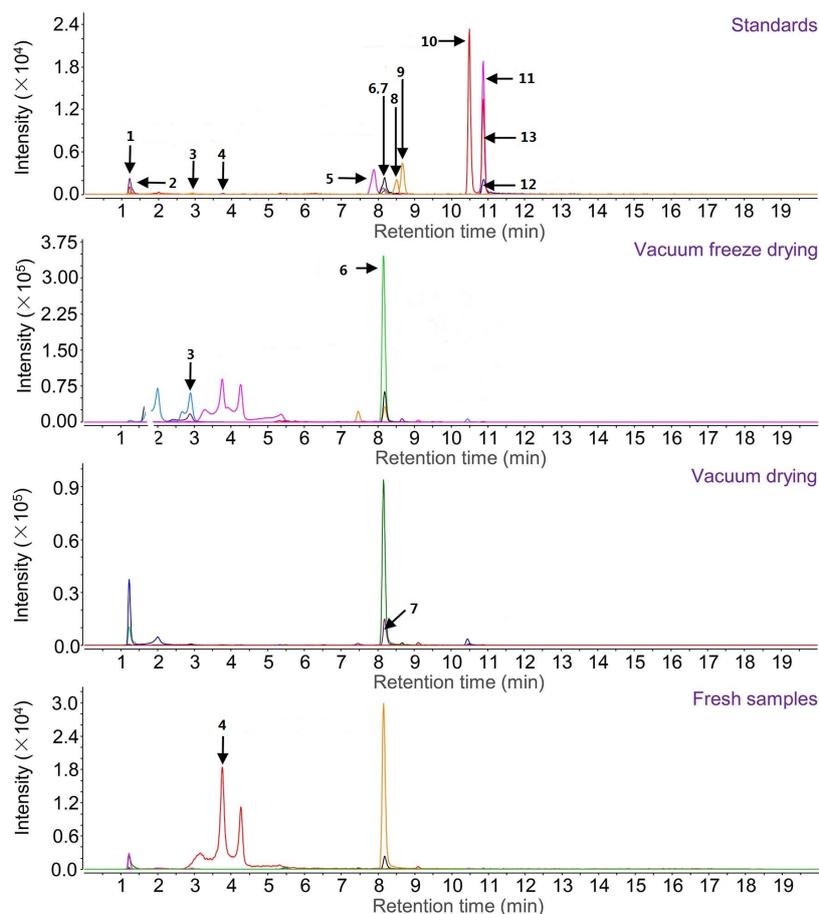

Figure 2. UHPLC-QqQ-MS/MS chromatogram of extractable polyphenols in fresh and dried mulberry fruits. 1: Chlorogenic acid; 2: Protocatechuic acid; 3: Cryptochlorogenic acid; 4: Cyanidin-3-*O*-glucoside; 5: Narirutin; 6: Rutin; 7: Quercetin glucoside; 8: Resveratrol; 9: Phloridzin; 10: Naringenin; 11: Phloretin; 12: Luteolin; and 13: Quercetin. The mass spectrograms of these compounds are given in Table 2.

Table 1. Content of extractable and non-extractable phenolics in mulberry fruit.

Compound	EP (mg/g DW)			ANEP (mg/g DW)			ABNEP (mg/g DW)			BNEP (mg/g DW)			BANEP (mg/g DW)		
	Fresh sample	VD	VFD	Fresh sample	VD	VFD	Fresh sample	VD	VFD	Fresh sample	VD	VFD	Fresh sample	VD	VFD
Chlorogenic acid	30.77 ± 2.16[b]	130.78 ± 20.73[a]	33.10 ± 11.52[b]	6.52 ± 0.81[a]	0.51 ± 0.18[b]	0.39 ± 0.06[b]	0.46 ± 0.14[a]	0.28 ± 0.12[a]	0.22 ± 0.10[a]	0.80 ± 0.06[a]	0.18 ± 0.021[b]	0.26 ± 0.02[b]	nd	nd	nd
Protocatechuic acid	20.85 ± 1.11[a]	14.66 ± 3.95[b]	19.70 ± 4.39[a]	74.62 ± 9.81[a]	12.36 ± 2.72[b]	5.80 ± 1.48[c]	22.60 ± 0.04[a]	7.98 ± 0.89[b]	7.81 ± 0.52[b]	68.60 ± 7.80[a]	10.71 ± 0.09[b]	11.12 ± 0.8[b]	8.81 ± 0.72[a]	2.22 ± 0.03[b]	1.76 ± 0.24[c]
Cryptochlorogenic acid	14.68 ± 1.89[b]	26.71 ± 3.56[a]	16.03 ± 3.29[b]	9.86 ± 2.70[a]	2.51 ± 0.02[b]	2.34 ± 0.02[b]	nd	nd	nd	nd	nd	nd	nd	nd	nd
Cyanidin-3-*O*-glucoside	55.48 ± 5.27[a]	0.20 ± 0.07[c]	22.18 ± 2.99[b]	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Narirutin	0.14 ± 0.02[a]	0.08 ± 0.00[b]	0.05 ± 0.00[b]	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Rutin	198.76 ± 17.19[a]	94.24 ± 7.33[b]	71.88 ± 4.98[c]	1.36 ± 0.06[a]	0.61 ± 0.04[b]	0.34 ± 0.02[c]	0.52 ± 0.04[a]	0.04 ± 0.00[b]	0.04 ± 0.00[b]	2.72 ± 0.32[a]	0.31 ± 0.09[b]	0.50 ± 0.16[b]	0.78 ± 0.12[a]	0.24 ± 0.01[c]	0.40 ± 0.06[b]
Quercetin glucoside	36.99 ± 1.02[a]	33.19 ± 0.71[b]	19.58 ± 2.17[c]	nd	nd	nd	nd	nd	nd	0.54 ± 0.00[a]	nd	0.26 ± 0.04[b]	nd	nd	nd
Resveratrol	1.46 ± 0.34[a]	0.60 ± 0.08[c]	0.92 ± 0.10[b]	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Phloridzin	0.65 ± 0.13[a]	0.71 ± 0.01[a]	0.82 ± 0.16[a]	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Naringenin	1.56 ± 0.34[a]	0.43 ± 0.01[c]	0.55 ± 0.00[b]	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Phloretin	1.22 ± 0.35[a]	0.08 ± 0.00[c]	0.25 ± 0.06[b]	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Luteolin	0.43 ± 0.07[a]	0.23 ± 0.03[b]	0.26 ± 0.00[b]	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Quercetin	2.47 ± 0.19[c]	3.95 ± 0.17[a]	3.03 ± 0.30[b]	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

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 ($p < 0.05$).

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

Number	Name	RT (min)	Fragmentor (V)	CE (V)	MS [M-H]-	MS/MS (*m/z*)
1	Chlorogenic acid	1.210	90	15	353	190.9
2	Protocatechuic acid	1.225	90	10	153	109
3	Cryptochlorogenic acid	2.952	100	20	353	173, 179
4	Cyanidin-3-*O*-glucoside	3.761	160	20	447	284.9, 298.9
5	Narirutin	7.865	160	20	579	271, 295
6	Rutin	8.149	160	40	609	299.9
7	Quercetin glucoside	8.174	150	20	462.9	300.8, 342.9
8	Resveratrol	8.474	130	25	227	143, 185
9	Phloridzin	8.646	150	20	434.9	272.9, 167
10	Quercetin	10.422	130	25	300.9	150.9, 107
11	Naringenin	10.478	100	25	271	119, 150.8
12	Luteolin	10.860	140	30	284.9	132.9
13	Phloretin	10.861	120	20	273	167, 123

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

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 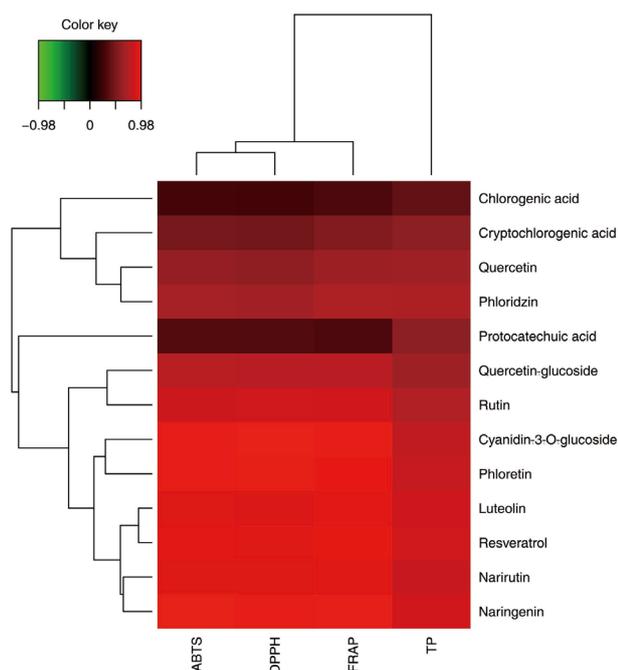Figure 3, the levels of most flavonoids were positively correlated with antioxidant activity. In particular, cyanidin-3-*O*-glucoside and phloretin significantly promoted antioxidant capacity and total polyphenols. Cyanidin-3-*O*-glucoside was previously reported to have a strong bioactivity. For example, 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

Figure 3. The correlation of phenolic compounds and antioxidant activity in mulberry fruits.

mulberry fruits.

References

Acosta-Estrada, B. A., Gutiérrez-Uribe, J. A. and Serna-Saldívar, S. O. 2014. Bound phenolics in foods, a review. Food Chemistry 152: 46-55.

Behzad, S., Sureda, A., Barreca, D., Nabavi, S. F., Rastrelli, L. and Nabavi, S. M. 2017. Health effects of phloretin: from chemistry to medicine. Phytochemistry Reviews 16(3): 527-533.

Chen, P.-N., Chu, S.-C., Chiou, H.-L., Kuo, W.-H., Chiang, C.-L. and Hsieh, Y.-S. 2006. Mulberry anthocyanins, cyanidin 3-rutinoside and cyanidin 3-glucoside, exhibited an inhibitory effect on the migration and invasion of a human lung cancer cell line. Cancer Letters 235(2): 248-259.

Domínguez-Rodríguez, G., Marina, M. L. and Plaza, M. 2017. Strategies for the extraction and analysis of non-extractable polyphenols from plants. Journal of Chromatography A 1514: 1-15.

Donno, D., Mellano, M. G., Raimondo, E., Cerutti, A. K., Prgomet, Z. and Beccaro, G. L. 2016. Influence of applied drying methods on phytochemical composition in fresh and dried goji fruits by HPLC fingerprint. European Food Research and Technology 242(11): 1961-1974.

Gilgun-Sherki, Y., Rosenbaum, Z., Melamed, E. and Offen, D. 2002. Antioxidant therapy in acute central nervous system injury: current state. Pharmacological Reviews 54(2): 271-284.

Gümüşay, O. A., Borazan, A. A., Ercal, N. and Demirkol, O. 2015. Drying effects on the antioxidant properties of tomatoes and ginger. Food Chemistry 173: 156-162.

Horszwald, A., Julien, H. and Andlauer, W. 2013. Characterisation of Aronia powders obtained by different drying processes. Food Chemistry 141(3): 2858-2863.

Jin, W., Mujumdar, A. S., Zhang, M. and Shi, W. 2018. Novel drying techniques for spices and herbs: a review. Food Engineering Reviews 10(1): 34-45.

Khanal, R. C., Howard, L. R. and Prior, R. L. 2010. Effect of heating on the stability of grape and blueberry pomace procyanidins and total anthocyanins. Food Research International 43(5): 1464-1469.

Khattak, M. K., Hanif, M., Khan, M., Ramzan, M. and Abdurab. 2019. Comparison of drying process and preservatives on drying kinetics, texture and antioxidants retention in mulberry fruits. Journal of Animal and Plant Sciences 29(3):803-808.

Kim, A.-J. and Park, S. 2006. Mulberry extract supplements ameliorate the inflammation-related hematological parameters in carrageenan-induced arthritic rats. Journal of Medicinal Food 9(3): 431-435.

Kim, H. G., Ju, M. S., Shim, J. S., Kim, M. C., Lee, S.-H., Huh, Y., … and Oh, M. S. 2010. Mulberry fruit protects dopaminergic neurons in toxin-induced Parkinson's disease models. British Journal of Nutrition 104(1): 8-16.

Lachowicz, S., Michalska, A., Lech, K., Majerska, J., Oszmianski, J. and Figiel, A. 2019. Comparison of the effect of four drying methods on polyphenols in saskatoon berry. LWT 111: 727-736.

Li, W., Wang, X., Zhang, J., Zhao, X., Wu, Y., Tan, S., … and Gao, X. 2019. Multivariate analysis illuminates the effects of vacuum drying on the extractable and nonextractable polyphenols profile of loquat fruit. Journal of Food Science 84(4): 726-737.

Mahmood, T., Anwar, F., Abbas, M. and Saari, N. 2012. Effect of maturity on phenolics (phenolic acids and flavonoids) profile of strawberry cultivars and mulberry species from Pakistan. International Journal of Molecular Sciences 13(4): 4591-4607.

Michalska, A., Wojdyło, A., Honke, J., Ciska, E. and Andlauer, W. 2018. Drying-induced physico-chemical changes in cranberry products. Food Chemistry 240: 448-455.

Michalska, A., Wojdyło, A., Lech, K., Łysiak, G. P. and Figiel, A. 2016. Physicochemical properties of whole fruit plum powders obtained using different drying technologies. Food Chemistry 207: 223-232.

Natić, M. M., Dabić, D. Č., Papetti, A., Fotirić Akšić, M. M., Ognjanov, V., Ljubojević, M. and Tešić, Ž. 2015. Analysis and characterisation of phytochemicals in mulberry (Morus alba L.) fruits grown in Vojvodina, North Serbia. Food Chemistry 171: 128-136.

Nunes, J. C., Lago, M. G., Castelo-Branco, V. N., Oliveira, F. R., Torres, A. G., Perrone, D. and Monteiro, M. 2016. Effect of drying method on volatile compounds, phenolic profile and antioxidant capacity of guava powders. Food Chemistry 197(Part A): 881-890.

Papoutsis, K., Pristijono, P., Golding, J. B., Stathopoulos, C. E., Bowyer, M. C., Scarlett, C. J. and Vuong, Q. V. 2017. Effect of vacuum-drying, hot air-drying and freeze-drying on polyphenols and antioxidant capacity of lemon (*Citrus limon*) pomace aqueous extracts. International Journal of Food Science and Technology 52(4): 880-887.

Peng, H., Li, W., Li, H., Deng, Z. and Zhang, B. 2017. Extractable and non-extractable bound phenolic compositions and their antioxidant properties in seed coat and cotyledon of black soybean (*Glycine max* (L.) Merr). Journal of Functional Foods 32: 296-312.

Qian, Z., Wu, Z., Huang, L., Qiu, H., Wang, L., Li, L., … and Gou, D. 2015. Mulberry fruit prevents LPS-induced NF-κB/pERK/MAPK signals in macrophages and suppresses acute colitis and colorectal tumorigenesis in mice. Scientific Reports 5: article ID 17348.

Rohart, F., Gautier, B., Singh, A. and Lê Cao, K. A. 2017. mixOmics: an R package for 'omics feature selection and multiple data integration. PLoS Computational Biology 13(11): article ID e1005752.

Sarker, U. and Oba, S. 2020. Nutrients, minerals, pigments, phytochemicals, and radical scavenging activity in *Amaranthus blitum* leafy vegetables. Scientific Reports 10: article ID 3868.

Sarker, U., Hossain, M. M. and Oba, S. 2020a. Nutritional and antioxidant components and antioxidant capacity in green morph *Amaranthus* leafy vegetable. Scientific Reports 10: article ID 1336.

Sarker, U., Oba, S. and Daramy, M. A. 2020b. Nutrients, minerals, antioxidant pigments and phytochemicals, and antioxidant capacity of the leaves of stem amaranth. Scientific Reports 10: article ID 3892.

Serraino, I., Dugo, L., Dugo, P., Mondello, L., Mazzon, E., Dugo, G., … and Cuzzocrea, S. 2003. Protective effects of cyanidin-3-*O*-glucoside from blackberry extract against peroxynitrite-induced endothelial dysfunction and vascular failure. Life Sciences 73(9): 1097-1114.

Shih, P.-H., Chan, Y.-C., Liao, J.-W., Wang, M.-F.

and Yen, G.-C. 2010. Antioxidant and cognitive promotion effects of anthocyanin-rich mulberry (*Morus atropurpurea* L.) on senescence-accelerated mice and prevention of Alzheimer's disease. Journal of Nutritional Biochemistry 21(7): 598-605.

Sun, Y., Shen, Y., Liu, D. and Ye, X. 2015. Effects of drying methods on phytochemical compounds and antioxidant activity of physiologically dropped un-matured citrus fruits. LWT - Food Science and Technology 60(2): 1269-1275.

Suna, S. and Özkan-Karabacak, A. 2019. Investigation of drying kinetics and physicochemical properties of mulberry leather (pestil) dried with different methods. Journal of Food Processing and Preservation 43(8): article ID e14051.

Villegas, R. J. and Kojima, M. 1986. Purification and characterization of hydroxycinnamoyl D-glucose. Quinate hydroxycinnamoyl transferase in the root of sweet potato, *Ipomoea batatas* Lam. Journal of Biological Chemistry 261(19): 8729-8733.

Wen, P., Hu, T.-G., Linhardt, R. J., Liao, S.-T., Wu, H. and Zou, Y.-X. 2019. Mulberry: a review of bioactive compounds and advanced processing technology. Trends in Food Science and Technology 83: 138-158.

Winkler, C., Frick, B., Schroecksnadel, K., Schennach, H. and Fuchs, D. 2006. Food preservatives sodium sulfite and sorbic acid suppress mitogen-stimulated peripheral blood mononuclear cells. Food and Chemical Toxicology 44(12): 2003-2007.

Yu, Y., Xu, Y., Wu, J., Xiao, G., Fu, M. and Zhang, Y. 2014. Effect of ultra-high pressure homogenisation processing on phenolic compounds, antioxidant capacity and anti-glucosidase of mulberry juice. Food Chemistry 153: 114-120.

Zhang, W., Han, F., He, J. and Duan, C. 2008. HPLC-DAD-ESI-MS/MS analysis and antioxidant activities of nonanthocyanin phenolics in mulberry (*Morus alba* L.). Journal of Food Science 73(6): C512-C518.