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Selection of drying technology based on dynamic effects on physicochemical properties and flavours of mulberry

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Abstract: Mulberry is gaining attention for its potential health benefits. However, post-harvest deterioration of quality necessitates drying to prolong storage. This study aimed to investigate the effects of natural drying (ND), hot air drying (HAD), and vacuum freeze drying (VFD) on bioactive compounds and volatile organic compounds of fresh mulberries. The results showed that VFD had the highest retention of total phenols (5 553.87 ± 744.97 μg GAE·g^{-1}) (GAE – gallic acid equivalent), total flavonoids (3 575.51 ± 465.98 μg rutin·g^{-1}), and total anthocyanins (64.98 ± 13.15 μg C3C·g^{-1}) after prolonged drying. Additionally, flavour was identified as the most important indicator influencing consumer preference for dried mulberries, and VFD was found to be effective in retaining the natural flavours of mulberries. Although thermal treatment leads to a significant loss of organic compounds, HAD showed good retention of active substances and lower energy consumption in a shorter processing time.

Keywords: drying methods; fruit; gas chromatography-mass spectrometry (GC-MS); volatile compounds

Mulberry is a traditional fruit with medicinal properties that belongs to the genus Morus in the Moraceae family. It grows mainly in tropical and subtropical regions and has various bioactive functions, such as anti-inflammatory, anti-oxidation ability, anti-atherosclerosis activity and immunomodulatory activity (Liu and Lin 2012; Abouzed et al. 2020; Wang et al. 2021). However, its thin skin and high moisture content make it susceptible to spoilage and require pretreatment before shipping. Drying is a common preservation method to preserve fruit by removing moisture and reducing enzyme reactions. There are many drying methods including natural drying (ND), hot air drying (HAD), relative humidity drying, variable temperature expansion drying, spray drying, and vacuum freeze drying (VFD) (Suna and Ozkan-Karabacak 2019).

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ND using the sun in the open areas and HAD using heat pumps to generate hot air to dry moisture are two traditional drying methods. They are simple to operate and have low equipment costs; however, the quality of the dried product is mediocre, with a high loss of active substances. In addition, VFD is a slow process of drying by sublimating the water in frozen material, it can effectively preserve the nutritional and organoleptic properties of fresh fruits (Xu et al. 2021). Based on the above literature, different drying methods have different characteristics. It is crucial to find a suitable drying method that can produce dried mulberry products with excellent quality and energy consumption savings.

At present, there are fewer studies on the dynamic changes in mulberry substances and flavours during the drying process. Mulberry flavour is one of the important quality indicators of dried products, which is produced by the combined synergy of volatile organic compounds (VOCs). Esters and aldehydes are the primary aromatic substances in mulberry flavour, generally formed by the reaction of precursors such as lipids and amino acids. However, under the drying treatment, these VOCs are thermally decomposed, oxidised, and rearranged by groups to form different substances again, eventually leading to changes in flavour.

Therefore, the aim of this study was to investigate the effects of ND, HAD, and VFD on physical properties (morphology, moisture, total acids, reducing sugars) and bioactive compounds [total phenols (TP), total flavonoids (TF), and total anthocyanins (TA)], as well as to reveal the evolution of mulberry flavours under these drying methods.

MATERIAL AND METHODS

Drying methods. Mulberries were picked at the planting base (Yibin, Sichuan Province, China) in mid-April at the ripening stage. Fresh mulberries with intact flesh were selected with three techniques for drying. The dried samples were removed at set times (2, 4, 6, 8, 12, 16, 20, and 24 h) and stored at –20 °C for further analysis. Each drying process was repeated three times. The process is shown in Figure 1. For the HAD, samples were placed equally on disks on two gauze layers and in a hot air dryer (DHG-9053A; Ningbo Xinzhi Biotechnology Co., China). The drying conditions were set with a temperature of 60 °C and a wind speed of 2 m·s^{-1}. The setting of the drying temperature is based on the preliminary experiments conducted by our research group to improve drying efficiency and maintain product quality. For the VFD, samples were laid flat on trays and pre-frozen in a constant temperature refrigerator at –40 °C for 24 h, then placed in a vacuum freeze drier (SCIENTZ-10N; Shanghai Yiheng Scientific instrument Co., China) and dried under the conditions: the cold trap temperature at –60 °C, and the vacuum less than 10 MPa.

Physical and chemical analyses. The moisture content (MC) was determined by oven drying method with a slightly modified (Xu et al. 2021). The reducing sugars (RS) of mulberry was measured according to the 3,5-dinitrosalicylic acid (DNS method) as reported by Toma and Leung (Toma and Leung 1987). The titratable total acid (TTA) of mulberry was determined by the method expressed as MAANTE-KULJUS (Maante-Kuljus

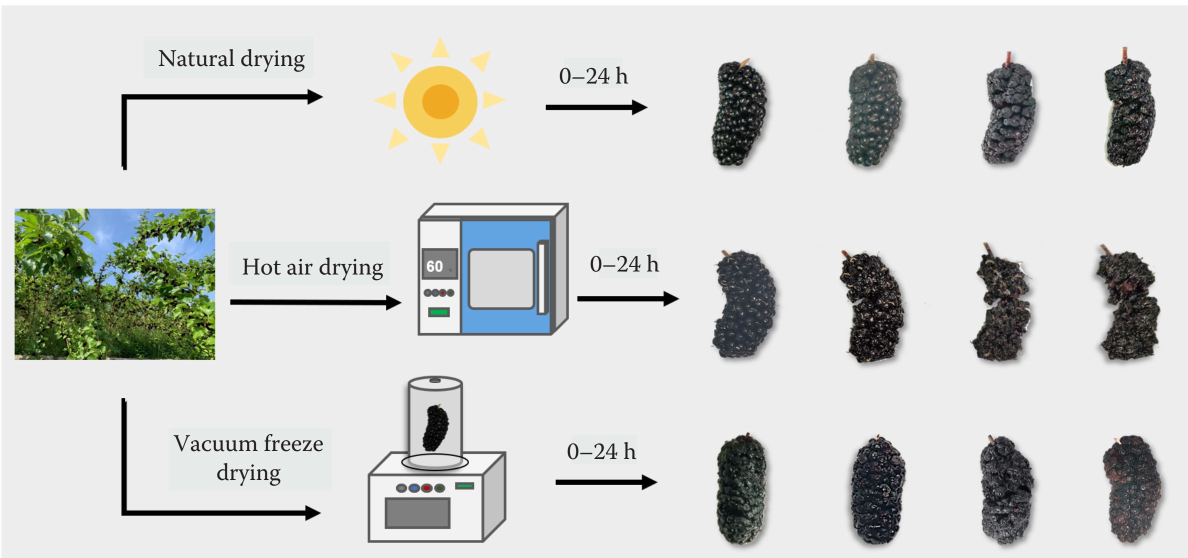

Figure 1. Diagram of the drying process of mulberry

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et al. 2019). Briefly, the content was determined by titrating the NaOH solution to pH 8.2 as the endpoint. Total phenols (TP), total flavonoids (TF), and anthocyanins (TA) were determined by the Folin-Ciocalteu colourimetric method (Zou et al. 2016), colourimetric method (Yang et al. 2020), and pH difference method (Nguyen et al. 2022).

Volatile organic compounds. Volatile organic compounds (VOCs) were measured by a gas chromatography-mass spectrometry (GC-MS) system (model 7890A-5975; Agilent) using FID and DB-WAX column (30 m × 0.32 mm, 0.25 µm; Agilent). 5 g of dried mulberry was placed in a 20 mL headspace vial with 2 g NaCl and 10 µL 2-octanol (2% mL·mL^{-1}), sealed and equilibrated at 50 °C for 5 min, and then extracted for 45 min. The initial temperature was 50 °C and held for 2 min, then ramped up to 230 °C at a rate of 4 °C·min^{-1} and held for 5 min. After gas chromatography, the samples obtained mass spectra were compared using the NIST05a.L standard library (provided by Agilent, USA), substances with a match greater than or equal to 80% (maximum 100%) were selected for qualitative analysis, and their correctness was confirmed by combining retention time and relevant mass spectral information. The relative content of VOCs is analysed semi-quantitatively based on the ratio of the peak area of the internal standard to the peak area of the substance being detected.

Statistical analysis. All data were expressed as mean ± standard deviation (SD) of triplicate determinations. One-way ANOVA was performed using IBM SPSS statistics 26.0. Differences were considered statistically significant at $P < 0.05$. Visualisation of correlation network plots was performed by Cytoscape software. Cluster analysis, correlation analysis, partial least squares discriminant analysis and their visualisation were implemented through R language (R, version 4.2.2).

Code availability. R 4.2.2 software was used for data processing and visualisation in this study (software and its software packages from https://www.r-project.org).

RESULTS AND DISCUSSION

Moisture content. Moisture is the main factor affecting the quality of dried products, and Figure 2 shows the drying curves of the three methods. The figure shows that HAD had the fastest decrease in MR, followed by VFD, indicating the highest efficiency of HAD. Specifically, ND, HAD, and VFD were in an accelerated drying period during the first 8 h, and the water in the samples evaporated quickly. As the free water evaporated, the drying process entered a deceleration phase. The drying rate of ND and VFD slowed down and reached a stable phase at 16 and 12 h, respectively. The comparison shows that HAD generated an effective thermal gradient with significantly higher efficiency than ND and VFD (Zhao et al. 2020; Mugodo and Workneh 2021).

Effect on biological composition of mulberry by drying technology. The nutritional value of mulberry is mainly attributed to its rich active substances, as can be clearly seen in Table S1 in the Electronic Supplementary Material (ESM), from the effect of different drying treatments on the TP, TF, and TA of the samples. Specifically, the TP content of fresh mulberry fruits (9 165.07 ± 106.55 µg GAE·g^{-1} DW) (GAE – gallic acid equivalent) was significantly decreased, and after drying treatment ND (3 978.23 ± 169.68 µg GAE·g^{-1} DW), HAD (1 358.12 ± 310.33 µg GAE·g^{-1} DW), and VFD (5 553.87 ± 744.97 µg GAE·g^{-1} DW) had retention rates of 43.41, 14.82, and 60.60%, respectively. Meanwhile, the TA retention of ND, HAD, and VFD were 33.05, 11.30, and 57.18%, respectively, consistent with previous reports by Macura (Macura et al. 2019).

TA is an active polyphenolic substance that is quickly decomposed by oxidation when subjected to heat. HAD and ND caused rapid degradation of TA and TP in fresh fruits. VFD also reduced TA and TP, but this may be because the ice crystals formed during freezing disrupted the cellular tissue and exposed the phenolics to low oxygen conditions. Interestingly, the range of active substances in HAD and ND increased slightly during drying, possibly because higher temperatures broke the cell structure and released bound phenolics or because the Maillard reaction converted some precursors into new phenolic molecules through non-enzymatic browning (Francisco and Resurreccion 2009).

After 12 h of drying, the content of active substances in HAD decreased significantly ($P < 0.05$). Currently, the sample undergoes tissue rupture due to water loss, increasing the contact area with oxygen, while the cell wall destruction releases oxidase and hydrolase. Although some enzymes are inactivated at higher temperatures (Lu et al. 2021), peroxidase POD is a heat-stable enzyme with many isozymes assisting it in enzymatic oxidation reactions. VFD is recognized for its effectiveness in retaining active substances due to low vacuum oxygen and low-temperature drying. In this study, VFD again showed the highest retention of active substances. The specific physical index data are shown in Table S1.

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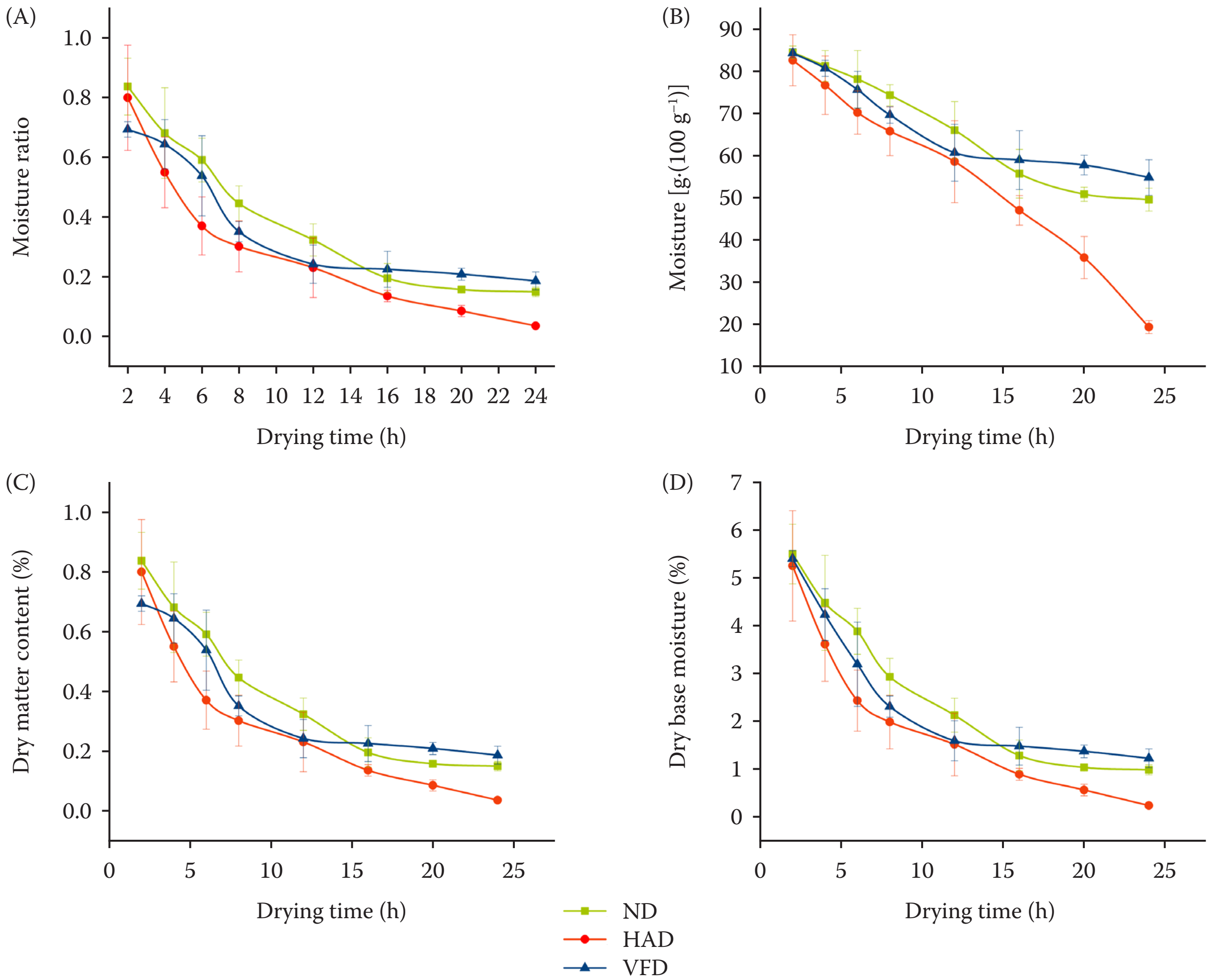

Figure 2. Dynamic moisture changes of mulberry under different drying methods

ND – natural drying; HAD – hot air drying; VFD – vacuum freeze drying

Principal component analysis. Figure 3A shows significant correlations between TP, TF, and TA and both moisture and total acid. These physicochemical factors may act synergistically to degrade the active substances, as previously reported (Sun et al. 2020). They also correlate strongly with water, suggesting that the stability of these compounds depends on multiple factors that should be considered holistically during the drying and processing of samples. Using principal component analysis (PCA) analysis on samples with different drying treatments to reflect differences between other groups. From Figure 3B, it can be noticed that on the first principal component, the three drying treatments are separated in time from the positive to the negative axis and have approximately the same trend. After 24 h of treatment, ND, HAD, and VFD reached the farthest distance from the fresh sample. Among the dynamic changes, ND-8 h and HAD-6 h appeared on the negative half-axis of the first principal component, separated from fresh and VFD, indicating that some characteristics of ND and HAD started to change. However, in the first principal component, VFD-24 h remains on the same negative axis as the fresh sample, separate from ND-24 h and HAD-24 h.

Evolution of volatile organic compounds. Hierarchical cluster analysis (Figure 4) and PCA (Figure 5) were used to visualise the data. In the hierarchical cluster analysis, the colours visualise that the VOCs in the four samples vary widely, especially for HAD, where higher temperatures inactivate or passivate the relevant enzymes, leading to the loss of important VOCs such as esters and aldehydes.

The changes in flavour substances in the samples come from the various reactions that occur during the drying process, which is due to the different principles of drying methods (Liu et al. 2021). During drying,

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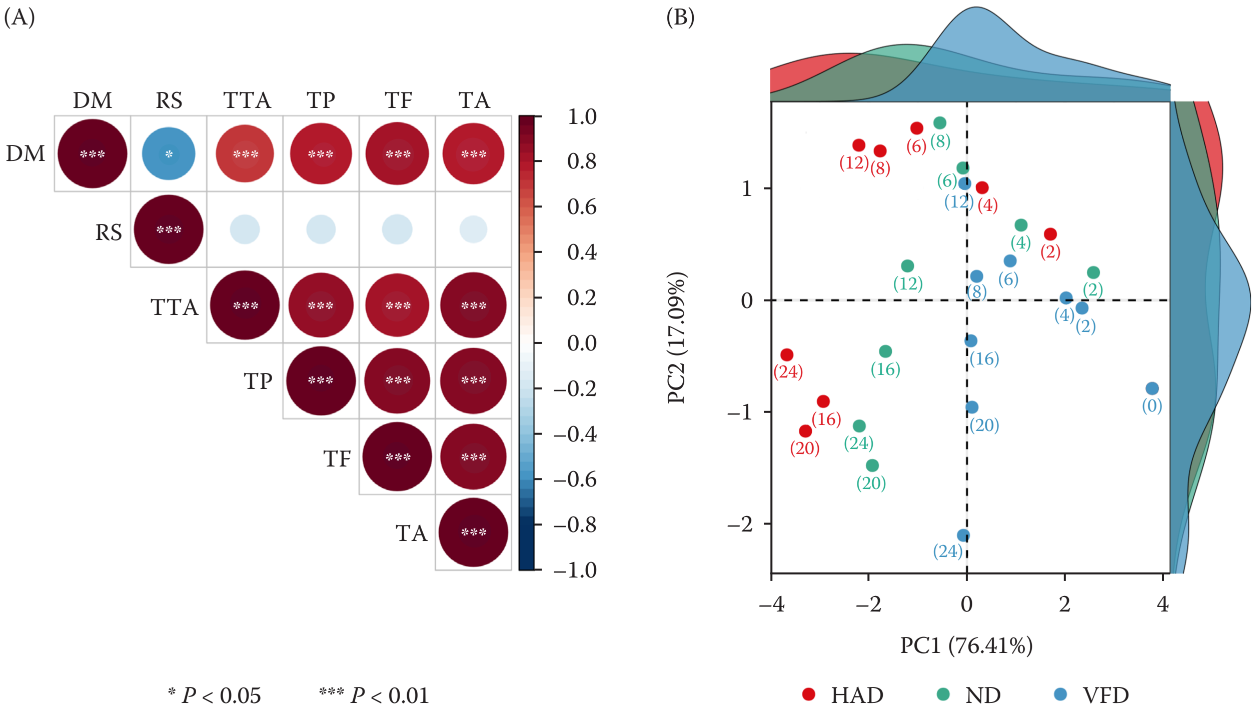

Figure 3. (A) Correlation analysis of physicochemical properties; (B) principal component analysis

DM – dry matter; RS – reducing sugar; TTA – titratable total acid; TP – total phenols; TF – total flavonoids; TA – total anthocyanins; PC – principal component; HAD – hot air drying; ND – natural drying; VFD – vacuum freeze drying

some glycosides belonging to the aromatic compounds are hydrolysed by β-glucosidase to alcohols, providing a specific plant aroma and sweetness (Wu et al. 2019). Firstly, the ethanol content detected from each sample was relatively high, and ethanol also affects the taste of the samples by generating ethyl ester through esterification with acetyl coenzyme (CoA) (Tietel et al. 2011). Only three alcohols were jointly detected in the four samples, and all decreased in content. Some substances with grassy and citrus aromas, such as 2-heptanol, and decanol, were detected only in fresh samples, indicating that some compounds are unstable and easily degraded. The VFD showed good retention of pentanol, cyclobutanol, butanediol and other alcohols, being very similar to fresh samples.

Esters are the main characteristic flavour substances of fruits, strongly influenced by drying and primarily produced by the dehydration condensation of higher fatty acids and ethanol. A total of 25 esters were detected in four samples, including ethyl acetate, ethyl caproate, ethyl butyrate and ethyl octanoate, all of which were basic aromas of mulberries (Zhu et al. 2018). ND had the highest number of esters, followed by fresh and VFD. Interestingly, ND also had much less ethanol than the other three groups, possibly because of the contamination by some environmental microorganisms in the open and dry environment of ND. These microorganisms used ethanol to produce various esters, increasing the complexity of flavour substances in the samples. In terms of ester content, the fresh sample undoubtedly possessed the most, but next instead, HAD retained more than ND and VFD, especially in ethyl acetate.

Aldehydes are the most abundant aromatic compounds in mulberry, mainly derived from the oxidation of unsaturated fatty acids. The aldehyde content in fresh, ND, and VFD was similar, and they all contained hexanal, nonanal and benzaldehyde. Hexanal and nonanal have a grassy and fatty flavour, and benzaldehyde, produced by phenylalanine degradation, has a bitter almond flavour. These aldehydes are widely present in mulberry and constitute the basic flavour of mulberry (Chen et al. 2021).

HAD had much lower aldehydes than the other groups, contrary to other reports that drying increases the diversity of aldehydes (Pranata et al. 2021). This may be due to changes in the cell structure of the samples during prolonged processing, which may enhance the release of alcohol dehydrogenase and lipoxygenase, which reduce these C6 aldehydes to C6 alcohols, and the volatilisation of some organic matter, leading to a significant decrease in aldehydes (Campestre

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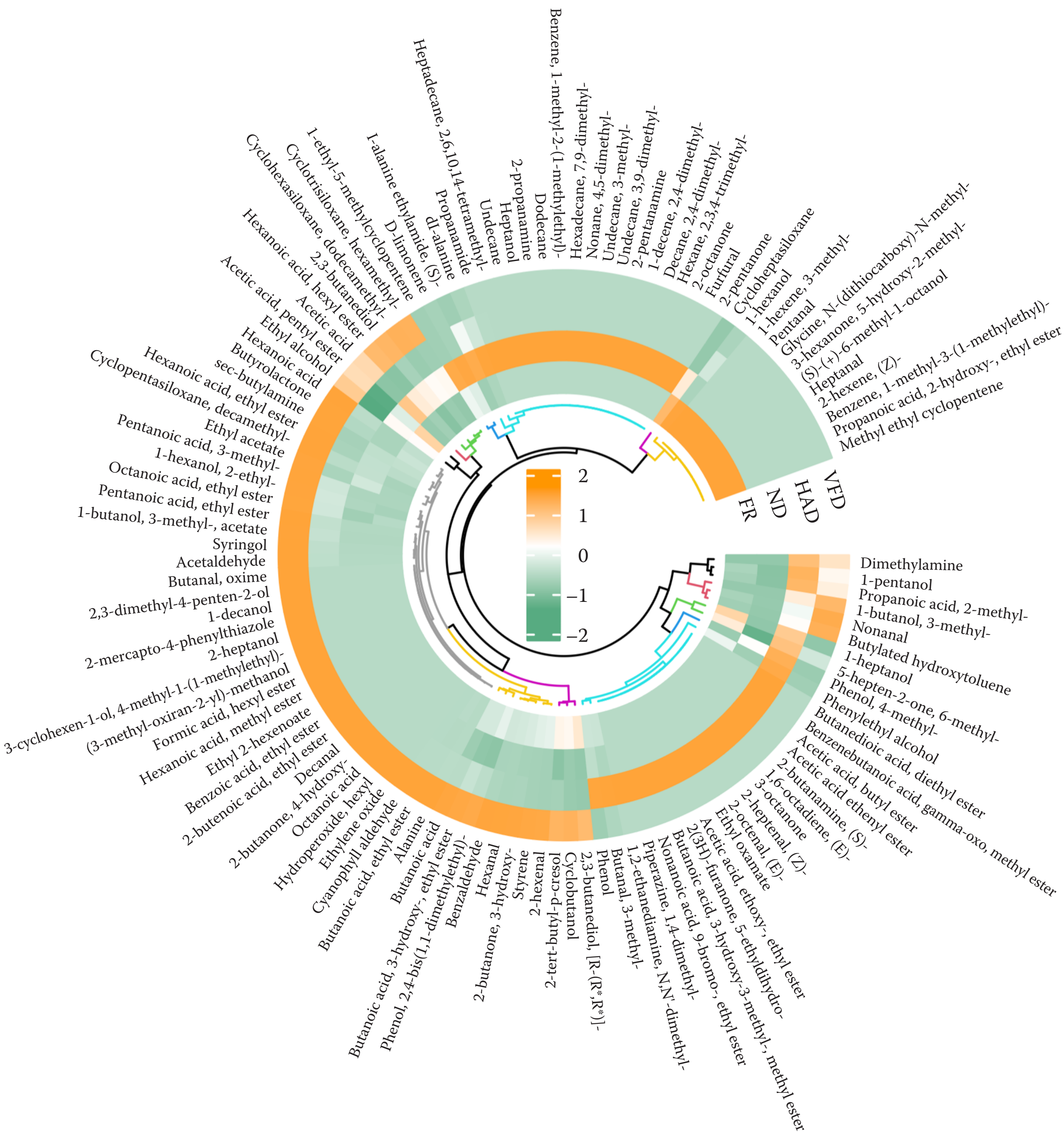

Figure 4. Hierarchical cluster analysis of six main VOC types (alcohols, esters, ketones, acids, aldehydes, and other flavours)

VOC – volatile organic compound; VFD – vacuum freeze drying; HAD – hot air drying; ND – natural drying; FR – fresh samples

et al. 2017). Furfural, a product of the Merad reaction, is increasingly appearing as a feature of HAD. ND detected 2-hexenal and (E)-2-octenal with a fruity-floral taste, also associated with microbial spoilage, indicating a high microbial activity of ND.

Ketones and acids are less diverse and abundant than aldehydes, esters, and alcohols, but they are also important precursors and flavour substances of mulberries. Ketones can be produced by triglyceride oxidation and Steck amino acid degradation. 3-hydroxy-2-butanone or 2,3-butanedione was detected in all four groups, which are associated with microbial spoilage and deterioration, and they can also be reduced to 2,3-butanediol, with an irritating odour. (Kritikos et al. 2020). a few acids were detected in all groups, such as hexanoic acid and acetic acid, which are essential organic substances in mulberry. Hexanoic acid has a sour taste, probably derived from the conversion of acetaldehyde

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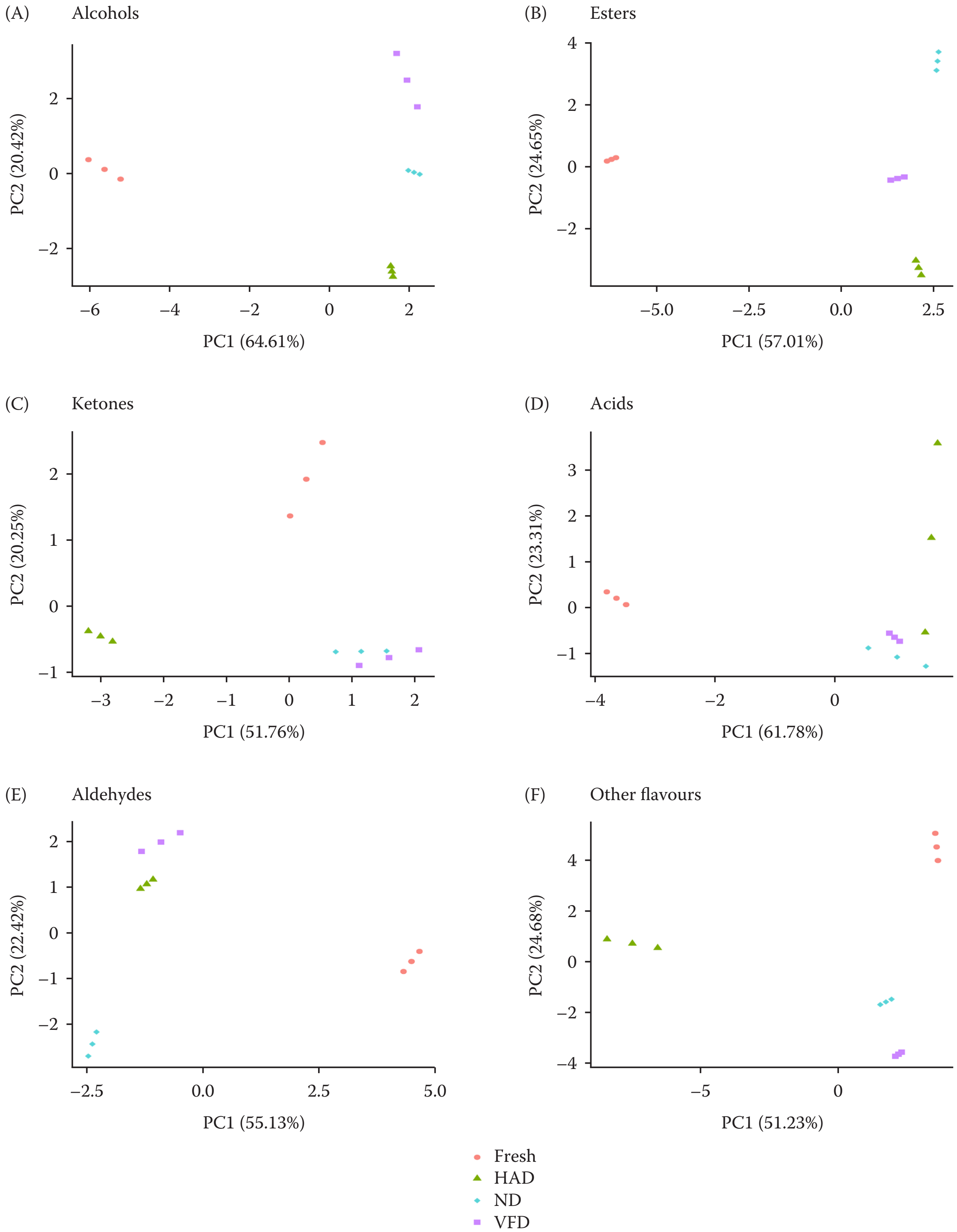

Figure 5. Principal component analysis of six main VOC types: (A) alcohols, (B) esters, (C) ketones, (D) acids, (E) aldehydes, and (F) other flavours

VOC – volatile organic compound; PC – principal component; fresh – fresh samples; HAD – hot air drying; ND – natural drying; VFD – vacuum freeze drying

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during fermentation. Acetic acid is also formed from hemicellulose in heat-treated plant material to further form furfural and other aldehydes.

CONCLUSION

This study investigated the effects of three drying methods (ND, HAD, and VFD) on the quality of mulberry fruit products. The results showed that the different drying methods had significant effects on the physicochemical properties, active factors, flavours, and appearances of the dried mulberry products. Among the three methods, VFD was the best method for preserving the morphology, active substances, and volatile organic compounds (VOCs) related to the flavours of mulberry. However, VFD has some disadvantages such as high equipment cost, long drying time, and low energy efficiency. Therefore, if there is no requirement for the shelf life of dried mulberries, HAD for a short period of time can be considered as an alternative method. The results of the study provide useful information for choosing suitable drying methods to process mulberry fruits and improve their quality and value. Nonetheless, this study has some limitations, including the small-scale drying technique and the limited analysis of VOCs. Future studies can explore the possibility of enhancing the activity of the dried products by applying post-treatment methods such as rehydration.

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