Qingyang *et al.*
Food Production, Processing and Nutrition (2024) 6:41
https://doi.org/10.1186/s43014-023-00216-2

Food Production, Processing and Nutrition

RESEARCH

Open Access

Comparative investigation on the phenolic compounds and antioxidant capacity of walnut kernel from different drying methods

Li Qingyang[1†], Wang Shuting[2†], Wang Ruohui[1], Shen Danyu[1], Mo Runhong[1], Tang Fubin[1] and Liu Yihua[1*]

Abstract

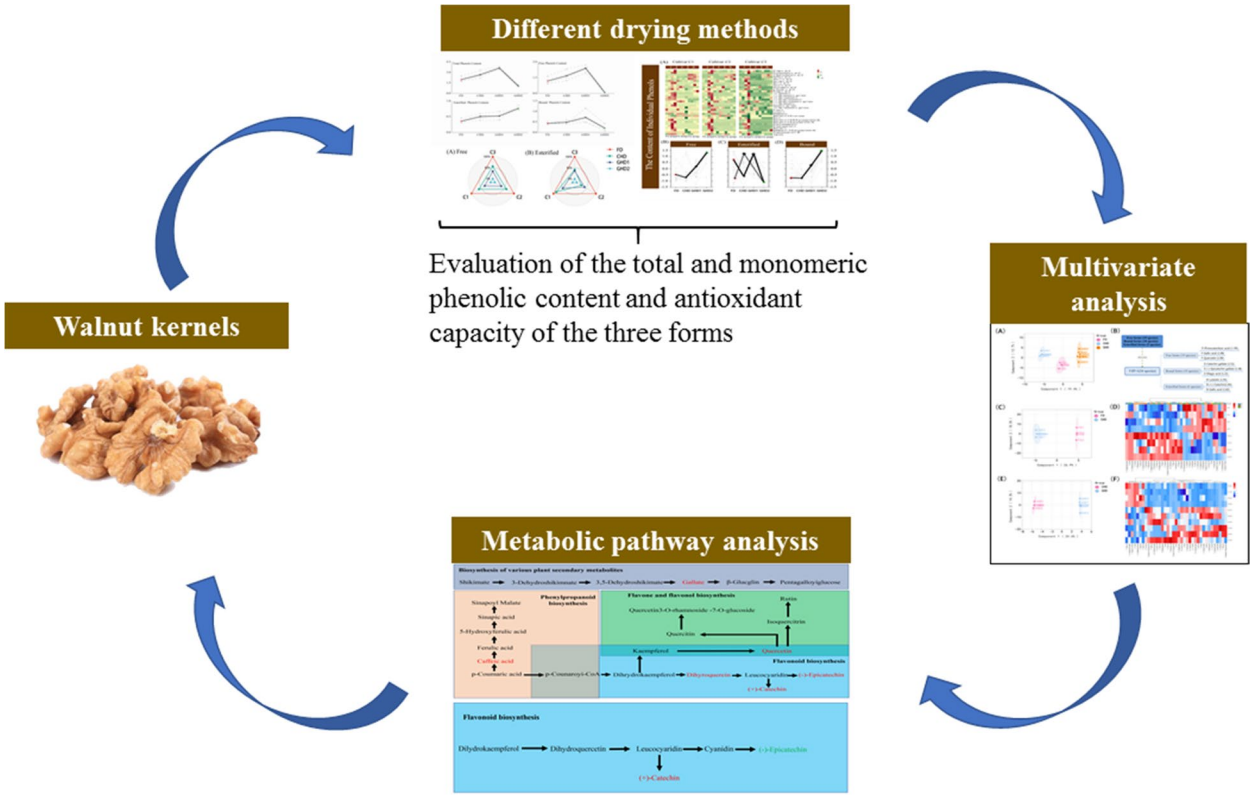
Drying techniques are being used more and more to extend the shelf life of industrial products. Drying could influence the content of phenolics in food and their antioxidant activity. This study estimated the effects of different drying methods (freeze drying (FD), gradient hot air drying (GHD), and constant hot air drying (CHD)) on phenolic profiles and antioxidant activities in walnut kernels. With a maximum content of 3.61 mg g^{-1}, GHD was found to be the most effective in preserving total phenols, while CHD and FD had maximum contents of 2.66 mg g^{-1} and 1.96 mg g^{-1}, respectively. The concentration of most monomeric phenols detected in the kernels increased with temperature, particularly in the free and bound forms. Gallic acid (free form) levels in GHD2 (194.54 μg g^{-1}) were 55.77 and 60.08 times higher, respectively, than in FD and CHD. GHD dried walnuts had higher antioxidant activity than FD and CHD dried walnuts. Furthermore, bioinformatics analysis revealed three key metabolic pathways associated with the mechanisms underlying drying changes. The GHD technique, according to these findings, is a better choice for drying walnut in order to preserve its phenolics and antioxidant activity.

Keywords Walnut, Drying methods, Phenolic, Antioxidant

[†]Li Qingyang and Wang Shuting contributed to this article equally and are co-first authors.

*Correspondence:
Liu Yihua
liuyh@caf.ac.cn
Full list of author information is available at the end of the article

Graphical Abstract



Introduction

Walnut contains phenolic compounds, which are known for their antioxidant activity and ability to lower the risk of cardiovascular and degenerative diseases (Zheng et al. 2020). Fresh walnut is easily deteriorated or infected by toxins due to its high moisture content and seamed hard shell. As a result, drying is essential for walnut postharvest. The drying process is a popular food preservation method (Onwude et al. 2022). Water is removed from foods in order to slow or stop microorganism growth or chemical reactions. This process extends the shelf life of food; however, important quality features such as odor, color, taste and phenolic compounds should be preserved as much as possible to increase consumer preference (Adak et al. 2017; Guclu et al. 2021).

However, drying food frequently results in phytochemical degradation (Nemzer et al. 2018). Hot air drying (HD) is a popular method for reducing moisture while maintaining food quality. Most studies discovered that providing constant heat for batch drying processes not only increases energy costs but also promotes the degradation of phenolic acids, flavonoids, and anthocyanins in different varieties of rice (Lang et al. 2019), turmeric (Chumroenphat et al. 2021), and apricot kernels (Aljuhaimi et al. 2017). In addition, drying at high temperatures can cause phenolic changes in the foods' phenolic content. The total isoflavone content of soybean is reduced at high drying temperatures of 130℃ and 150℃ when compared to 50℃ and 70℃. However, high drying temperature conditions promote the release of aglycone isoflavone (Ferreira et al. 2019).

Freeze drying (FD) is an effective method for retaining total phenolic content in fruits like blueberries (Nemzer et al. 2018), quinces (Szychowski et al. 2018) and tomatoes (Tan et al. 2021). When compared to hot air drying, freeze drying increased the total phenolic content of blueberries by more than 30% (Nemzer et al. 2018). Nonetheless, previous research has shown that it is unwise to choose a drying method for a specific plant based solely on data from other plants, because different drying processes are well known to be suitable for different plants (Tan et al. 2021). Gradient hot air drying (GHD) has surpassed constant hot air drying (CHD) in popularity in recent years due to its higher work efficiency from high

temperature rapid dehydration followed by constant temperature drying.

Indeed, depending on their association with the food matrix, phenolic compounds exist in a variety of forms, including free, esterified and bound (Wu et al. 2021). The bioavailability of phenols is directly related to their various forms (Chen et al. 2015), which in turn affects their active functions in the organism, such as antioxidant activity (Wu et al. 2021). Heating energy can boost phenolic bioavailability by causing them to bind to the plant matrix, which results in increased antioxidant activity (Ribas-Agusti et al. 2018). Most reports on the phenolics and antioxidant properties of food drying processes are currently based primarily on soluble free phenolics, resulting in an underestimation of the phenolic and antioxidant properties of foods because the bound phenolic content and activities are not considered (Chumroenphat et al. 2021; Dibanda et al. 2020). A recent study found that freeze drying strawberries could release bound phenolics in flavonoids (64.4 → 90.8%) and anthocyanins (47.2 → 83.4%) (Kamiloglu, 2019). Bamboo shoots dried by hot air drying, on the other hand, had higher TPC and stronger antioxidant activities than freeze-drying in insoluble-bound phenolics (Li et al. 2021).

The phenolics and antioxidant activity of walnuts are affected by drying methods. However, to the best of our knowledge, no research has been conducted on the effects of the postharvest drying process on the phenolic compounds and antioxidant capacities of walnuts dried using various methods. As a result, the antioxidant and phenolic compounds (forms and quantities) of freeze, gradient hot air, and constant hot air-dried walnuts were evaluated in this study. Evaluating changes in these biochemical parameters after harvest will be a useful way to indicate the difference in fruit quality and behavior between walnuts drying methods. Furthermore, the purpose of this study is to provide valuable evidence that improves our understanding of the mechanisms involved in phenol metabolism during the drying process of walnuts.

Materials and methods

Chemicals and reagents

All targeted phenolic compounds (purity > 98%), which were purchased from Sigma-Aldrich, Shanghai, China. HPLC grade methanol (MeOH), ethanol (EtOH), acetonitrile (ACN) and formic acid (FA) were purchased from Merck China, Hangzhou.

Sample collection

The walnut samples were collected in China's main producing area (Shanxi province). All samples (3 kg) were collected in 2020 from 10- to 15-year-old trees in a local orchard during the commercial mature stage. At each collection site, 5–10 walnuts were collected evenly from the upper and lower parts of each tree, as well as from all four directions (east, south, west, and north). The walnut samples were then thoroughly mixed to produce a complete and representative sample. The green skin was mechanically removed, and the walnut shell was manually cracked. Finally, the walnut kernels were kept at −20℃ until they were analyzed. The detailed sampling method can be found in Wu et al. (Wu et al. 2021).

Drying methods

Each method was used to dry 100 g walnut kernels, and each method achieved a moisture content of less than 8%. Here is a more detailed explanation of each drying method: (1) Freeze drying (FD): The walnut kernels were evenly spread on trays in a single layer. A lyophilizer (FD-1D-80, China) was set to 30 Pa absolute pressure and −50℃ condenser temperature. (2) Constant hot air-dried (CHD): For this method, an electric thermo-static drying hot-air oven (XMA-2000, Germany) set to 40℃ was used. (3) Gradient hot air-dried 1 (GHD1): The walnut kernels were first dried at 80℃for 4 h before being transferred to 40℃ for further drying. (4) Gradient hot air-dried 2 (GHD2): The walnut kernels were dried at 105℃ for 2 h before being transferred to 40℃ for additional drying.

Extraction of free, esterified and bound form phenolic compounds

The details for the extraction procedure could be seen in our previous work (Wu et al. 2020). The dried walnut samples were ground and thoroughly mixed in a grinder. The mixed samples (1.0 g) was extracted three times with 50 mL of 70% acetone. Then removed the acetone in supernatants by evaporating, then reduced the pH to 2.0 using 2 M HCl. Next, the free phenolic compounds were extracted (three times) by ethyl acetate, the ethyl acetate fractions were flash evaporated, redissolved in MS grade methanol. The lower aqueous solution and solid residue were treated for 1 h with a 2 mol L^{-1} sodium hydroxide solution. After that, the pH was adjusted to 2.0 with 2 mol L^{-1} hydrochloric acid. Three ethyl acetate extractions were performed on the mixture. The supernatant that resulted was combined and evaporated to dryness. Finally, 10 mL of methanol reagent was added to obtain separate extracts of esterified phenols and bound phenols.

Determination of total phenolic content

The TPC of the different forms of phenol was determined by the Folin-Ciocalteu method (Wang et al. 2022). Pipette 1 mL of free, esterified and bound phenol extracts in a colorimetric tube, followed by 5 mL of water, 1 mL

of folin reagent and 3 mL of 75 g L^{-1} Na_2CO_3 were added sequentially. The reaction solution was allowed to stand for 2 h at room temperature protected from light and then its absorbance value was measured at 765 nm in a spectrophotometer (Perkin Elmer, USA).

Qualification by UPLC-MS/MS

The ultra-performance liquid chromatography coupled with a triple quadrupole mass spectrometer (UPLC-MS/MS) consisted of an ultra-high performance liquid chromatography (Agilent 1290 Infinity II, Agilent Technologies Inc., California, USA) with Poroshell 120 EC-C18 column (100×2.1 mm, 1.9 μm), hyphenated to a triple quadrupole mass spectrometer (6460 C QQQ, Agilent Technologies Inc., California, USA) equipped with electrospray ionization (ESI). The method and instrument parameter setting followed from previous work (Shen et al. 2021).

Antioxidant activity

Radical scavenging activities of 1,1-diphenyl-2-picrylhydrazylradical scavenging activity (DPPH) assay were measured as the protocol of Mohd Hazli et al. (2019). The 2 mL of the gradient sample solution was mixed separately with 2 mL of DPPH solution (1×10^{-4} mol L^{-1}). The mixture was allowed to react for 30 min at a temperature of 37℃. After the reaction, the absorbance values were measured at 517 nm. The DPPH scavenging rate was determined and expressed as the half inhibitory concentration (IC_{50}). The antioxidant activity was quantified and expressed as VC equivalent.

Statistical analysis

The orthogonal partial least-squares discriminant analysis (OPLS-DA) was used to discriminate phenolics profiles between different drying groups. The variable importance in the projection (VIP) and absolute Log2FC (foldchange) were used to determine the phenolics that were significantly regulated among the groups. The VIP values were extracted from the OPLS-DA results, and the data was visualized using R software.

Results and discussion

Effect of drying methods on TPC in walnut kernel

Previous research on different post-treatment temperatures in food has revealed that FD is better suited to maximize polyphenol retention (as measured by TPC). However, most studies in this field focused on foods with low oil content, such as blueberries (Nemzer et al. 2018) and tomatoes (Tan et al. 2021), with a lack of studies on foods with high oil content. In this study, the TPCs of walnut samples were determined under various drying methods, and the results are shown in Fig. 1. The TPC of walnuts in the FD group is clearly lower than that of walnuts in the CHD and GHD1 groups. FD has a TPC of 1.96 mg g^{-1}, while CHD and GHD1 have TPCs of 2.66 and 3.61 mg g^{-1}, respectively. This implies that FD may not be the best drying method for foods with a high oil

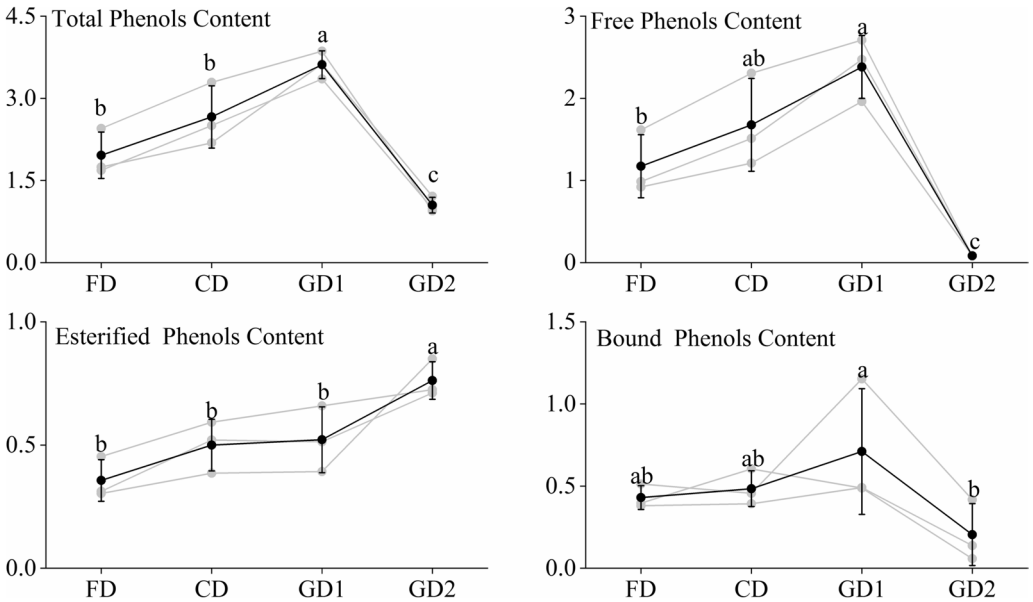


Fig. 1 The content of TPC in walnut kernels under different drying methods. **A** Total phenols Content. **B** Free Phenols Content. **C** Esterified Phenols Content. **D** Bound Phenols Content. Note: FD: Freeze drying; CHD: Constant hot air-dried; GHD1: Gradient hot air-dried 1; GHD2: Gradient hot air-dried 2. Different letters in each subplot represent significant differences ($p < 0.05$) between among drying methods

content. In addition, previous studies rarely investigated total phenol content in various forms by drying methods, especially esterified phenol. According to the findings of this study, both free and bound phenols increased and then decreased as temperature increased, with the highest values found in GHD1(80℃→40℃) with contents of 2.38 mg g^{-1} (free) and 0.71 mg g^{-1} (bound). A similar phenomenon has been observed in black rice, with higher levels of bound phenolics observed at drying temperatures ranging from 60℃ to 80℃ (Lang et al. 2019). Thermal degradation of phenolic compounds is the most likely cause of the decrease in total free and bound phenols content (Lang et al. 2019). The persistent increase in total esterified phenols as temperature increased was particularly intriguing. GHD2 had the least amount of free (0.08 mg g^{-1}) and bound (0.20 mg g^{-1}) phenol content, but the most esterified phenol (0.76 mg g^{-1}). These findings can be explained by claiming that high temperatures disrupt the cell structure (Dibanda et al. 2020; You et al. 2021), promoting the release of esterified phenols and thus increasing contents.

Effect of drying methods on phenolic compounds in walnut kernel

In walnut kernels dried under four different conditions, 32 phenolic compounds were identified and quantified, including phenolic acids (11), flavanoids (20), and quinone (1) (Fig. 2A). Most of the detected monomeric phenols in kernels increased in concentration as temperature increased, most notably protocatechuic acid, gallic acid, and 4-hydroxybenzoic acid. This effect is most visible for free and bound phenols (Fig. 2B and D). Protocatechuic acid levels have been found to rise with increasing drying temperature in studies on black rice (Lang et al. 2019) and turmeric (Chumroenphat et al. 2021). In this study, protocatechuic acid (free from) content in GHD2 was especially high, reaching 524.09 μg g^{-1}, while FD, CHD and GHD1 contained only 15.36, 10.33 and 183.01 μg g^{-1}. The level of gallic acid (free form) level found in GHD2 (194.54 μg g^{-1}) was 55.77 and 60.08 times higher than in FD and CHD, respectively. Previous studies on soybeans (Ferreira et al. 2019) found that drying temperature had a significant effect on the bound forms of syringic acid, ferulic acid, epicatechin, coumaric acid and hydroxybenzoic acid. The coefficients of variation for these phenols in walnuts, however, were all less than 30%, making them far less temperature sensitive than gallic acid and (+)-catechin, which had coefficients of variation above 100%. Interestingly, most free phenols were found to decrease with increasing temperature in a study of black rice (Lang et al. 2019), which contradicts our findings. This is because different foods have different substrates, enzymatic activity, and cell structure, resulting in different monomeric phenols degradation patterns (Lachowicz et al. 2019; Tan et al. 2021). However, it is possible that the GHD drying method inactivates the relevant phenol degradation enzymes, allowing for better preservation of the unstable phenols in walnuts. Few studies have been conducted on the temperature response of esterified phenols. Individual phenols in the esterified form showed a clear trend of increasing and then decreasing with temperature (Fig. 2C), with the lowest contents of individual phenols, such as ellagic acid (0.89 μg g^{-1}) and kaempferol (0.88 μg g^{-1}), occurring at GHD2. A previous study (Dibanda et al. 2020), found that when phenols are esterified, the link is broken during heating, resulting in an increase in their content. This is consistent with current findings that high temperatures break the esterified phenols link in walnuts, increasing the free phenol content.

Effect of drying methods on anti-oxidant capacity

DPPH assays were used to compare the effects of four different drying methods on the antioxidant activity of walnut kernels. In all four drying conditions, the free phenol had the highest antioxidant capacity of the three phenolic forms. With a focus on GHD2, the IC_{50} value for free phenols stood out with its remarkable low value of 9.08 μg mL^{-1}. In contrast, esterified phenols exhibited a moderately higher value of 108.30 μg mL^{-1}, while bound phenols exceeded 500 μg mL^{-1} (Table 1). This finding not only supports the previous discovery that free phenols in walnuts are the primary contributors to total antioxidant capacity (Wu et al. 2021), but also indicates that free phenols play a crucial role in maintaining the antioxidant capacity during post-treatment drying. Radar plots (Fig. 3A and B) depicts phenol's antioxidant capacity under various drying conditions. When combined with Table 1, the ranking of antioxidant capacity is clear: GHD2 > GHD1 > CHD > FD. In terms of free phenols, HD (GHD2: 9.08 μg mL^{-1}) has up to 14 times the antioxidant capacity of FD (124.31 μg mL^{-1}). This suggests that HD can boost walnut antioxidant capacity. The findings are consistent with previous research on other types of nuts, such as pistachios (Rodriguez-Bencomo et al. 2015) and cashews (Chandrasekara & Shahidi, 2011). Because several phenolics and antioxidant capacity have been shown to be highly correlated (Sawicki et al. 2021; Zhang et al. 2019), on the one hand, the antioxidant levels of nuts are partially restored by the development of antioxidant-rich compounds. Changes in nutrient macromolecules and the walnut matrix, on the other hand, result in the production and accumulation of Maillard-derived melanoidins as well as the efficient release of antioxidants (Dibanda et al. 2020; Wang et al. 2021). Notably, this phenomenon has been found in non-oil-containing fruits such as turmeric and myrtle (Alkaltham et al. 2021). As

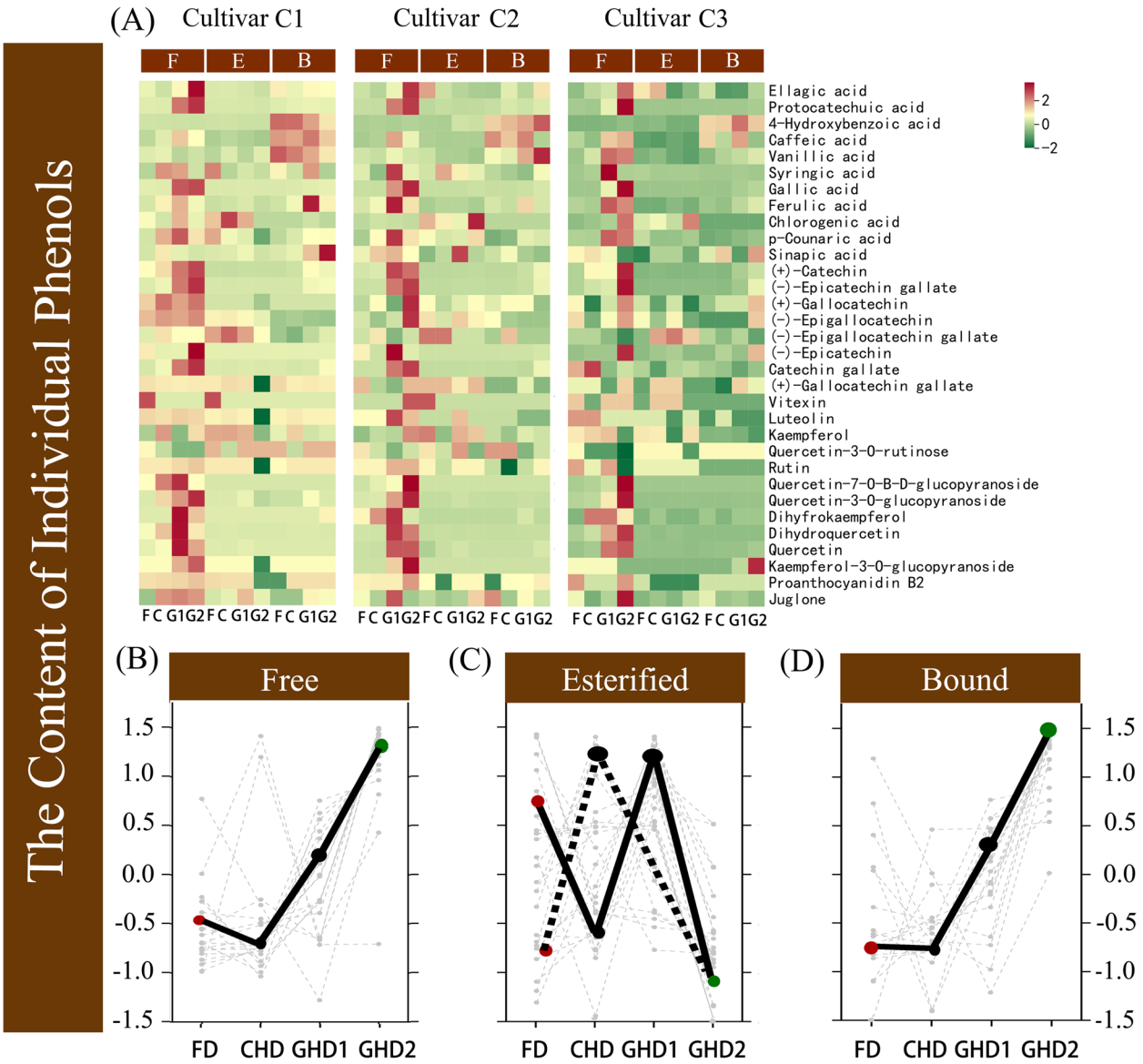


Fig. 2 The content of phenolic compounds in walnut kernels under different drying methods. **A** Heat map of phenolic compounds under different drying methods. **B** Trend plot of free phenol under different drying methods. **C** Trend plot of esterified phenol under different drying methods. **D** Trend plot of bound phenol under different drying methods.Note: FD: Freeze drying; CHD: Constant hot air-dried; GHD1: Gradient hot air-dried 1; GHD2: Gradient hot air-dried 2

Table 1 Antioxidant capacity of walnuts under different drying methods

	IC_{50} (μg mL^{-1})				Relative antioxidant capacity			
	FD	CHD	GHD1	GHD2	FD	CHD	GHD1	GHD2
Free	124.31a	78.01ab	46.39bc	9.08c	1.00	1.59	2.68	13.69
Esterified	513.27a	287.45ab	281.21ab	108.30b	1.00	1.79	1.83	4.74
Bound	< 500	< 500	> 500	> 500	/	/	/	/

The IC50 value represents the inhibitor concentration after removing half of the DPPH radical; the higher the IC50 value, the lower the antioxidant activity. Significant differences are represented by different letters in the same row ($p < 0.05$). Relative antioxidant capacity means the multiplication of the antioxidant capacity of walnuts relative to FD under the other three conditions; The "$IC_{50} > 500$" means that the inhibitor concentration has reached 500 μg mL^{-1}, but the free radical scavenging has not yet reached half, so the relative antioxidant capacity cannot be calculated and is expressed as " / "

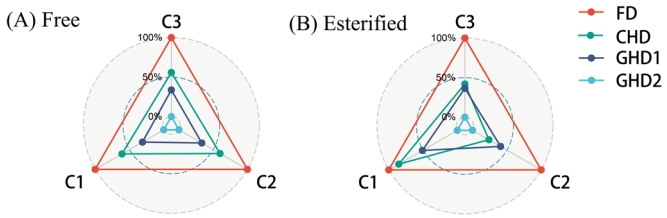


Fig. 3 Antioxidant activities evaluated by DPPH assays under different drying methods. **A** Free Phenols. **C** Esterified Phenols. Note: Radar plot of anti-oxidant capacity on four drying methods assessed. The closer to the center of the radar plot, the higher the antioxidant capacity. FD: Freeze drying; CHD: Constant hot air-dried; GHD1: Gradient hot air-dried 1; GHD2: Gradient hot air-dried

a result, our findings in this study suggested that HD can boost the antioxidant activity of most kinds of fruits.

Primary different metabolites between walnut kernels under different drying methods

The OPLS-DA analysis was used to identify significant phenolic differences in walnuts dried at different temperatures (FD, CHD and GHD groups). The results clearly demonstrate that the first two components can distinguish between walnut samples treated at various temperatures (Fig. 4). Some phenols are considered to contribute significantly to drying condition variation (VIP > 1), including 19 free, 10 bound, and 5 esterified phenols. As shown in Fig. 4C and Table S1, among the monomeric phenols that contributed significantly (VIP > 1) to the separation of FD and CHD, 11 monomeric phenols were up-regulated and 9 were down-regulated. The CHD group had 10.27 μg g^{-1} more protocatechuic acid than the FD group, which contributed significantly to the CHD group's higher overall monomeric phenol content. In a study on tomatoes, FD was found to be more effective than HD in preserving almost all of the monomeric phenols (Tan et al. 2021), and in quinces, FD preserved primarily the higher levels of monomeric phenols such as procyanidin B2 and (-)-epicatechin (Szychowski et al. 2018). However, in this study, when compared to the heating treatment group, the FD treatment group reduced most of the higher levels of monomeric phenols, despite the fact that the phenols retained by FD were mostly at low concentrations (< 1 μg g^{-1}). This phenomenon also explains why, as previously stated (in Sect. 3.3), the antioxidant activity of the phenol extracts obtained after the FD treatment was significantly lower than that of the other heating treatment groups. When our findings are compared to those of previous studies, it is clear that choosing a drying method for a specific food based solely on data from other foods is risky. The reported differences between walnuts and other foods may be due to the formation of ice crystals during the FD process, which may disrupt the composition of the walnut matrix (Ribas-Agusti et al. 2018), resulting in changes in the reaction of the monomeric phenols with the relevant enzymes, or it may be due to an effect on the sensitivity of the monomeric phenols to antioxidant activity. Further investigation is still required.

In addition, the groups CHD and GHD were clearly distinguished (Fig. 4D), with 26 monomeric phenols (21 up-regulated, 5 down-regulated) identified as having significant contributions (VIP > 1) (Table S2). Notably, the majority of the monomeric phenols that were up-regulated in the CHD vs. FD comparison continued to rise in GHD, most notably protocatechuic acid (+)-catechin and (-)-epicatechin gallate. Furthermore, gallic acid and protocatechuic acid had the greatest differences among the lipids that made a significant contribution, with the GHD group having 162.81 μg g^{-1} and 353.55 μg g^{-1}, respectively, 48.42 and 34.22 times more than the CHD group. This is due to the fact that CHD drying is a slow process that causes the degradation of some key compounds (Ma et al. 2019). GHD drying, on the other hand, shortens the drying time and allows the product to be dried to the desired moisture content within the production time frame. The high temperature phase, on the other hand, causes enzyme inactivation (Chumroenphat et al. 2021), which results in better phenolic compound preservation in walnuts. Previous studies have shown that GHD is an effective drying strategy for preserving the physical properties of foods such as Ganoderma lucidum (Chin & Law, 2010) and bananas (Chua et al. 2000). Our findings also indicate that GHD has a high potential for industrial use in optimizing the content of beneficial chemical components in walnuts during the post-harvest process.

Differential expression of phenolics metabolism pathways during different drying methods

Drying after harvest is an important step in the fruit processing process. Understanding the changes in phenolic content during drying and improving quality traits require characterizing the phenol accumulation pattern during drying. Significant differential metabolites were further identified in this study using the more stringent criteria of FC (Fold change) > 1.5 and VIP ≥ 1. A total of six metabolites showed differential expression in the FD and CHD groups, with five showing an increase and one

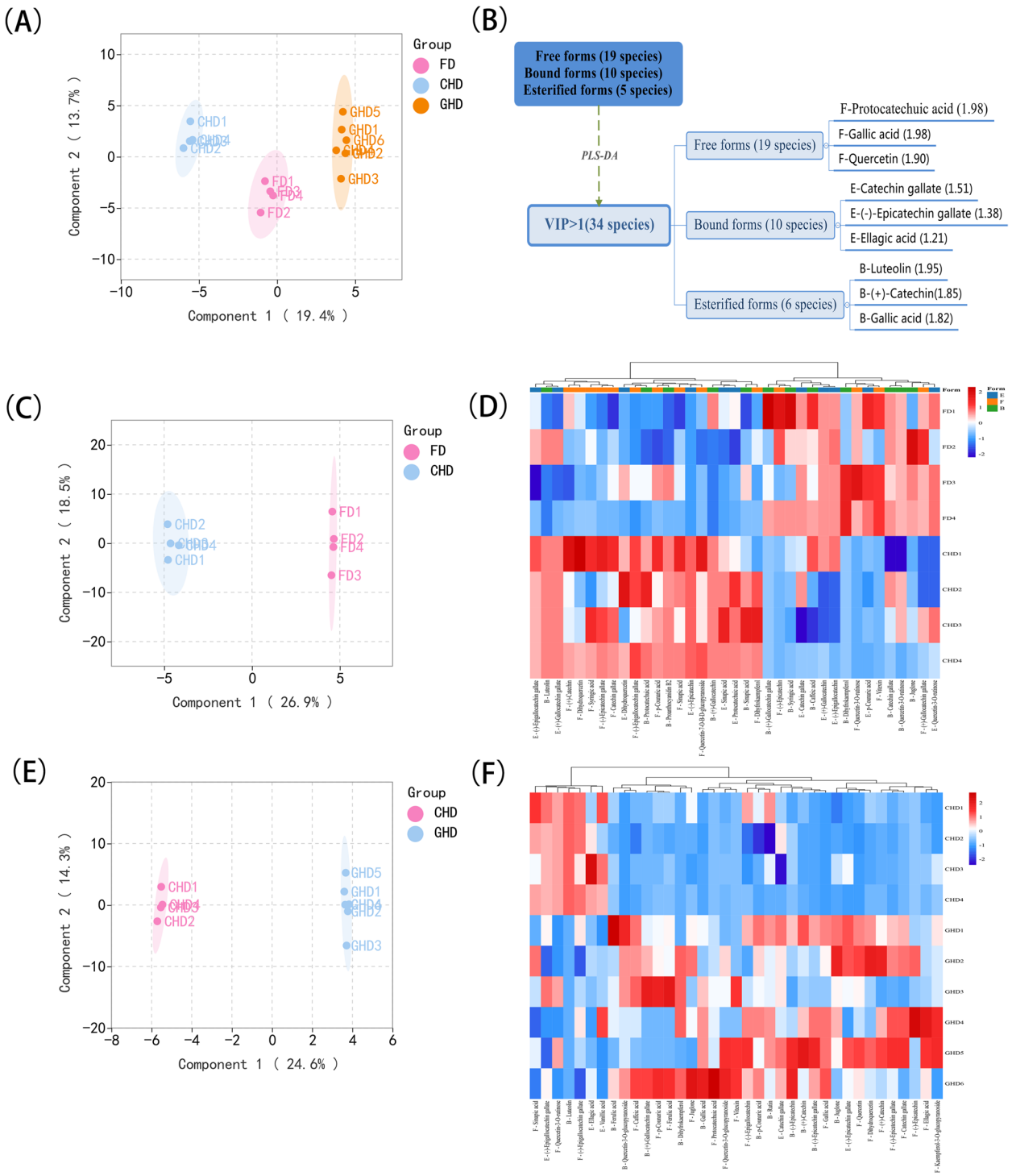


Fig. 4 Multivariate analysis of phenolics distinguishes walnuts under different drying methods. **A** OPLS-DA scores plot based on FD, CHD and GHD. **B** VIP scores of individual phenolics in OPLS-DA. **C** OPLS-DA scores plot based on FD and CHD. **D** Heat map of phenol differences between group FD and CHD. **E** OPLS-DA scores plot based on GHD and CHD. **F** Heat map of phenol differences between group GHD and CHD

showing a decrease (Fig. 5A). Six differential metabolites were enriched in three pathways using the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment: flavonoid biosynthesis, biosynthesis of secondary metabolites and metabolic pathways (Fig. 5B). Our findings indicate that the upregulation of (+)-catechin and downregulation of (-)-epicatechin in CHD is primarily associated with the flavonoid biosynthesis

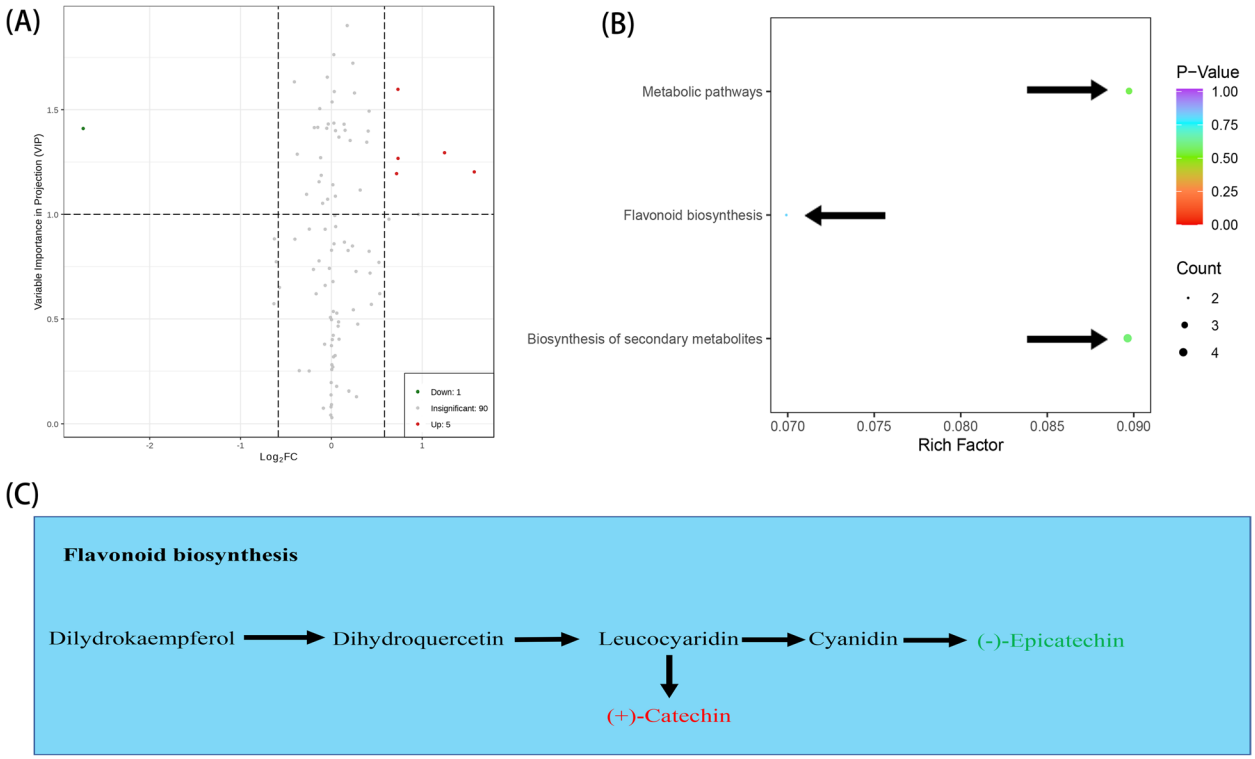


Fig. 5 The differential metabolic analysis in response to drying methods (FD vs. CHD). **A** Volcano diagram of the differential metabolites of walnuts under FD and CHD. **B** Metabolic pathway diagram for differences between group FD and CHD. **C** Polyphenol synthesis pathways and content changes under FD. For image A, red and green dots represent up-regulated and down-regulated significant difference metabolites, respectively. For image B, the bubble size represents the number of different metabolites involved in this pathway, and the bubble color represents the p-value of this metabolic pathway. The important metabolic pathways are pointed out by arrows. For image B, the same panel represents a metabolic pathway, where red font indicates significant up-regulation and green font indicates significant down-regulation. The same below

metabolic pathway. 17 species (15 up and 2 down) were designated as significantly differentiated in the CHD vs. GHD comparison group (Fig. 6A). Six of the most important pathways were chosen: biosynthesis of various plant secondary metabolites, metabolic pathways, biosynthesis of secondary metabolites, phenylpropanoid biosynthesis, flavonoid biosynthesis, and flavone and flavonol biosynthesis (Fig. 6B). Among them, metabolic pathways, flavonoid biosynthesis, and biosynthesis of secondary metabolites were all the same as in the CHD vs. FD comparison group (Figs. 5C and 6C), indicating that they are important phenolic metabolic pathways in the drying process of walnuts. Previous research has shown that the phenylalanine pathway and flavonoid biosynthesis result in the biosynthesis of coumarins, flavonoids, isoflavones, and flavanols, all of which are important phenolics of plant stress resistance (Ai et al. 2021). The flavonoid biosynthetic pathway, in particular, has been shown to be induced by drought stress and high temperature treatments in response to stress (Chao et al. 2017; Yu et al. 2022). This study also discovered that quercetin, (+)-catechin and (-)-epicatechin in the flavonoid biosynthesis pathway were up-regulated in the flavonoid biosynthesis pathway in response to heat stress. Furthermore, the flavonoid biosynthesis pathway comes before the flavone and flavonol biosynthesis pathway. This also has an impact on the biosynthesis of important individual phenols found in walnuts, such as quercetin3-O-rhamnoside −7-O-glucoside and rutin. Besides, the upregulation of gallate in the GHD is associated with pathway of biosynthesis of various plant secondary metabolites. Overall, the obtained findings were especially intriguing because they provided direct evidence that the phenolic metabolic pathways affected by GHD are better preserved for phenolics.

Conclusion

This study compares the effects of various post-harvest drying methods on phenols and antioxidant capacity in walnuts in order to choose the optimal drying method for walnuts. The findings of this study challenge the findings of numerous previous studies that suggested

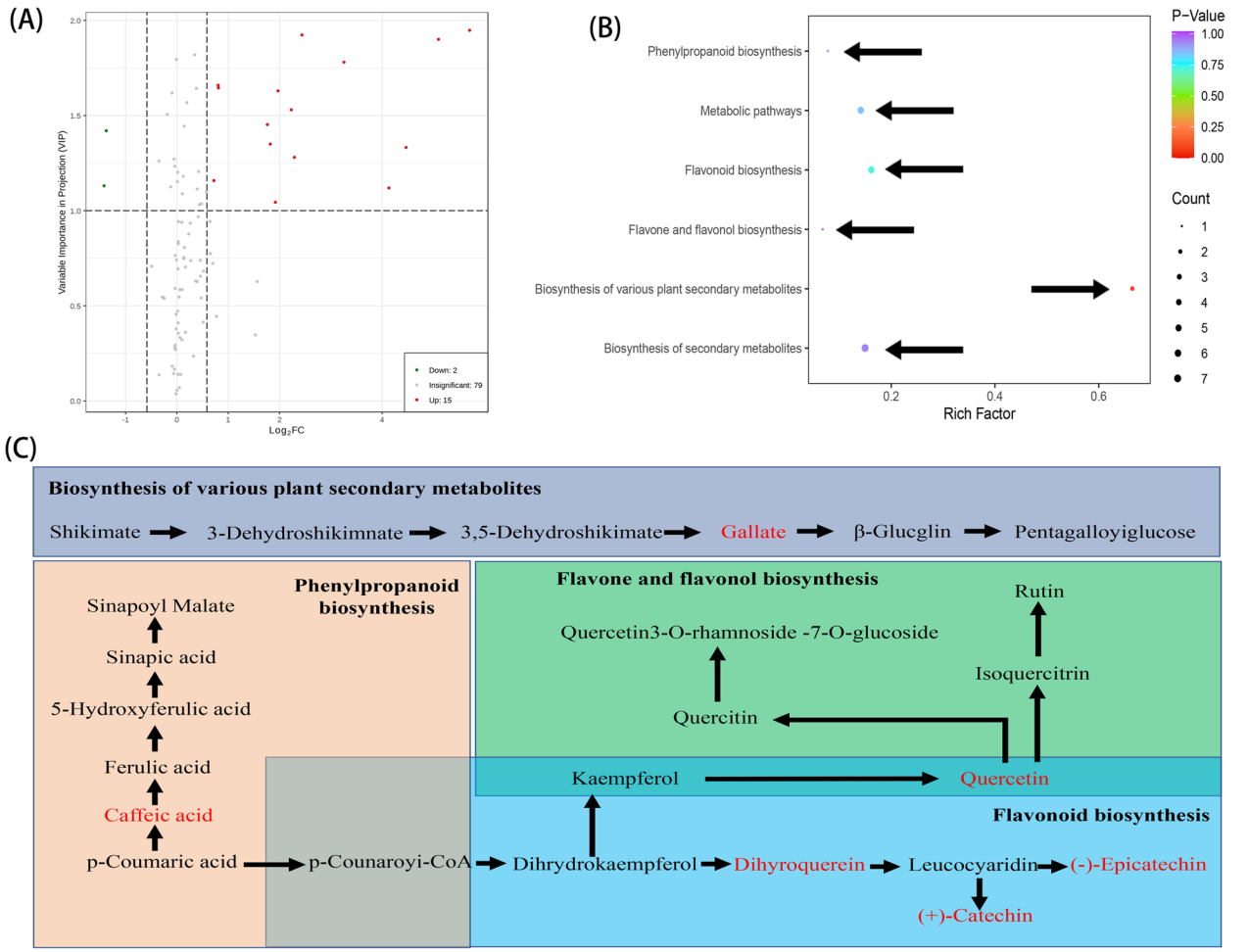


Fig. 6 The differential metabolic analysis in response to drying methods (CHD vs. GHD). **A** Volcano diagram of the differential metabolites of walnuts under GHD and CHD. **B** Metabolic pathway diagram for differences between group GHD and CHD. **C** Polyphenol synthesis pathways and content changes under GHD

freeze drying (FD) as a superior method for preserving phenolic and antioxidant capacity in a variety of foods. HD, particularly GHD, is a superior postharvest drying technique for walnuts. GHD dried walnuts have a maximum TPC of 1.84 times and a maximum antioxidant capacity of 9.21 times that of FD dried walnuts. The concentrations of most of the detected monomeric phenols in kernels increased with temperature, most notably protocatechuic acid, gallic acid, and 4-hydroxybenzoic acid. The three key important metabolic pathways in walnuts that respond to heat stress (metabolic pathways, flavonoid biosynthesis and biosynthesis of secondary metabolites) have been identified. These findings contribute to an understanding of the changes in phenolics caused by different drying methods, as well as useful information for the application of key metabolites in walnut drying process.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43014-023-00216-2.

Additional file 1: Table S1. Monomeric phenols that contributed significantly (VIP > 1) to the separation of FD and CHD. **Table S2.** Monomeric phenols that contributed significantly (VIP > 1) to the separation of CHD and GHD.

Acknowledgements
The authors acknowledged the support of the Fundamental Research Funds of CAF.

Authors' contributions
All authors contributed to the study conception and design. The detailed work is as following: Qingyang Li, Writing, Conceptualization, Visualization, Investigation, Data Curation; Shuting Wang, Writing Conceptualization, proof reading the original draft, and editing; Ruohui Wang, Investigation; Validation, Methodology, Formal analysis; Danyu Shen, Methodology, Validation; Runhong Mo, Investigation; Fubin Tang, Investigation; Yihua Liu, Writing, Review & Editing; Conceptualization; Software; visualization.

Funding
This study was supported by the Fundamental Research Funds of CAF (CAFYBB2019QD002).

Availability of data and materials
The data regarding this study is available and can be produced on proper request to the corresponding author.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Research Institute of Subtropical Forestry, Chinese Academy of Forestry, Fuyang 311400, P.R. of China. [2]Hangzhou Center for Disease Control and Prevention, Hangzhou, Zhejiang 310021, P.R. of China.

Received: 14 August 2023 Accepted: 2 December 2023
Published online: 04 May 2024

References

Adak, N., Heybeli, N., & Ertekin, C. (2017). Infrared drying of strawberry. *Food Chemistry,219*, 109–116. https://doi.org/10.1016/j.foodchem.2016.09.103

Ai, Z., Zhang, Y., Li, X., Sun, W., & Liu, Y. (2021). Widely targeted metabolomics analysis to reveal transformation mechanism of cistanche deserticola active compounds during steaming and drying processes. *Frontiers in Nutrition,8*, 742511. https://doi.org/10.3389/fnut.2021.742511

Aljuhaimi, F., Musa Özcan, M., Ghafoor, K., & Babiker, E. E. (2017). The effect of microwave roasting on bioactive compounds, antioxidant activity and fatty acid composition of apricot kernel and oils. *Food Chemistry,243*, 414–419. https://doi.org/10.1016/j.foodchem.2017.09.100

Alkaltham, M. S., Salamatullah, A. M., Özcan, M. M., Uslu, N., Hayat, K., & Mohamed Ahmed, I. A. (2021). Influence of different drying methods on antioxidant activity, total phenol, and phenolic compounds of myrtle (Myrtus communis L.) fruits. *Journal of Food Processing and Preservation,45*(4), e15308. https://doi.org/10.1111/jfpp.15308

Chandrasekara, N., & Shahidi, F. (2011). Effect of roasting on phenolic content and antioxidant activities of whole cashew nuts, kernels, and testa. *Journal of Agricultural and Food Chemistry,59*(9), 5006–5014. https://doi.org/10.1021/jf2000772

Chao, J., Dai, Y., Cheng, H. Y., Lam, W., Cheng, Y. C., Li, K., & Lee, M. S. (2017). Improving the concentrations of the active components in the herbal tea ingredient, uraria crinita: the effect of post-harvest oven-drying processing. *Science Reports,7*, 38763. https://doi.org/10.1038/srep38763

Chen, P. X., Dupuis, J. H., Marcone, M. F., Pauls, P. K., Liu, R., Liu, Q., & Tsao, R. (2015). Physicochemical properties and in vitro digestibility of cooked regular and nondarkening cranberry beans (Phaseolus vulgaris L.) and their effects on bioaccessibility, phenolic composition, and antioxidant activity. *Journal of Agriculture and Food Chemistry,63*(48), 10448–10458. https://doi.org/10.1021/acs.jafc.5b04005

Chin, S. K., & Law, C. L. (2010). Product quality and drying characteristics of intermittent heat pump drying ofGanoderma tsugaeMurrill. *Drying Technology,28*(12), 1457–1465. https://doi.org/10.1080/07373937.2010.482707

Chua, K. J., Mujumdar, A. S., Chou, S. K., Hawlader, M. N. A., & Ho, J. C. (2000). Convective drying of banana, guava and potato pieces: effect of cyclical variations of air temperature on drying kinetics and color change. *Drying Technology,18*(4–5), 907–936. https://doi.org/10.1080/07373930008917744

Chumroenphat, T., Somboonwatthanakul, I., Saensouk, S., & Siriamornpun, S. (2021). Changes in curcuminoids and chemical components of turmeric (Curcuma longa L.) under freeze-drying and low-temperature drying methods. *Food Chemistry,339*, 128121. https://doi.org/10.1016/j.foodchem.2020.128121

Dibanda, R. F., Akdowa, E. P., & Tongwa, Q. M. (2020). Effect of microwave blanching on antioxidant activity, phenolic compounds and browning behaviour of some fruit peelings. *Food Chemistry,302*, 125308. https://doi.org/10.1016/j.foodchem.2019.125308

Ferreira, C. D., Ziegler, V., Schwanz Goebel, J. T., Hoffmann, J. F., Carvalho, I. R., Chaves, F. C., & de Oliveira, M. (2019). Changes in phenolic acid and isoflavone contents during soybean drying and storage. *Journal of Agricultural and Food Chemistry,67*(4), 1146–1155. https://doi.org/10.1021/acs.jafc.8b06808

Guclu, G., Keser, D., Kelebek, H., Keskin, M., Sekerli, Y. E., Soysal, Y., & Selli, S. (2021). Impact of production and drying methods on the volatile and phenolic characteristics of fresh and powdered sweet red peppers. *Food Chemistry,338*, 128129. https://doi.org/10.1016/j.foodchem.2020.128129

Kamiloglu, S. (2019). Effect of different freezing methods on the bioaccessibility of strawberry polyphenols. *International Journal of Food Science & Technology,54*(8), 2652–2660. https://doi.org/10.1111/ijfs.14249

Lachowicz, S., Michalska, A., Lech, K., Majerska, J., Oszmianski, J., & Figiel, A. (2019). Comparison of the effect of four drying methods on polyphenols in saskatoon berry. *LWT-Food Science and Technology,111*, 727–736. https://doi.org/10.1016/j.lwt.2019.05.054

Lang, G. H., da Silva Lindemann, I., Ferreira, C. D., Hoffmann, J. F., Vanier, N. L., & de Oliveira, M. (2019). Effects of drying temperature and long-term storage conditions on black rice phenolic compounds. *Food Chemistry,287*, 197–204. https://doi.org/10.1016/j.foodchem.2019.02.028

Li, J., Li, W., Deng, Z., Li, H., Yu, Y., & Zhang, B. (2021). Comparison of free, conjugated, and insoluble-bound phenolics and their antioxidant activities in oven-drying and freeze-drying bamboo (Phyllostachys edulis) shoot tips. *Journal of Food Science,86*(9), 4223–4243. https://doi.org/10.1111/1750-3841.15881

Ma, X., Moilanen, J., Laaksonen, O., Yang, W., Tenhu, E., & Yang, B. (2019). Phenolic compounds and antioxidant activities of tea-type infusions processed from sea buckthorn (Hippophae rhamnoides) leaves. *Food Chemistry,272*, 1–11. https://doi.org/10.1016/j.foodchem.2018.08.006

Mohd Hazli, U. H. A., Abdul-Aziz, A., Mat-Junit, S., Chee, C. F., & Kong, K. W. (2019). Solid-liquid extraction of bioactive compounds with antioxidant potential from Alternanthera Sesillis (red) and identification of the polyphenols using UHPLC-QqQ-MS/MS. *Food Research International,115*, 241–250. https://doi.org/10.1016/j.foodres.2018.08.094

Nemzer, B., Vargas, L., Xia, X., Sintara, M., & Feng, H. (2018). Phytochemical and physical properties of blueberries, tart cherries, strawberries, and cranberries as affected by different drying methods. *Food Chemistry,262*, 242–250. https://doi.org/10.1016/j.foodchem.2018.04.047

Onwude, D. I., Iranshahi, K., Rubinetti, D., Schudel, S., Schemminger, J., Martynenko, A., & Defraeye, T. (2022). How much do process parameters affect the residual quality attributes of dried fruits and vegetables for convective drying? *Food and Bioproducts Processing,131*, 176–190. https://doi.org/10.1016/j.fbp.2021.11.005

Ribas-Agusti, A., Martin-Belloso, O., Soliva-Fortuny, R., & Elez-Martinez, P. (2018). Food processing strategies to enhance phenolic compounds bioaccessibility and bioavailability in plant-based foods. *Critical Reviews in food Science and Nutrition,58*(15), 2531–2548. https://doi.org/10.1080/10408398.2017.1331200

Rodriguez-Bencomo, J. J., Kelebek, H., Sonmezdag, A. S., Rodriguez-Alcala, L. M., Fontecha, J., & Selli, S. (2015). Characterization of the aroma-active, phenolic, and lipid profiles of the pistachio (Pistacia vera L.) nut as affected by the single and double roasting process. *Journal of Agricultural and Food Chemistry,63*(35), 7830–7839. https://doi.org/10.1021/acs.jafc.5b02576

Sawicki, T., Wiczkowski, W., Hrynkiewicz, M., Baczek, N., Hornowski, A., Honke, J., & Topolska, J. (2021). Characterization of the phenolic acid profile andin vitrobioactive properties of white beetroot products. *International Journal of Food Science and Technology,56*(2), 629–638. https://doi.org/10.1111/ijfs.14710

Shen, D., Yuan, X., Zhao, Z., Wu, S., Liao, L., Tang, F., & Liu, Y. (2021). Determination of phenolic compounds in walnut kernel and its pellicle by Ultra-high-performance liquid chromatography-tandem mass spectrometry. *Food Analytical Methods,14*(11), 2408–2419. https://doi.org/10.1007/s12161-021-02069-2

Szychowski, P. J., Lech, K., Sendra-Nadal, E., Hernández, F., Figiel, A., Wojdyło, A., & Carbonell-Barrachina, Á. A. (2018). Kinetics, biocompounds, antioxidant activity, and sensory attributes of quinces as affected by drying method.

Food Chemistry,255, 157–164. https://doi.org/10.1016/j.foodchem.2018.02.075

Tan, S., Ke, Z., Chai, D., Miao, Y., Luo, K., & Li, W. (2021). Lycopene, polyphenols and antioxidant activities of three characteristic tomato cultivars subjected to two drying methods. *Food Chemistry,338*, 128062. https://doi.org/10.1016/j.foodchem.2020.128062

Wang, H., Fang, X. M., Sutar, P. P., Meng, J. S., Wang, J., Yu, X. L., & Xiao, H. W. (2021). Effects of vacuum-steam pulsed blanching on drying kinetics, colour, phytochemical contents, antioxidant capacity of carrot and the mechanism of carrot quality changes revealed by texture, microstructure and ultrastructure. *Food Chemistry,338*, 127799. https://doi.org/10.1016/j.foodchem.2020.127799

Wang, R., Tian, X., Li, Q., Liao, L., Wu, S., Tang, F., & Liu, Y. (2022). Walnut pellicle color affects its phenolic composition: Free, esterified and bound phenolic compounds in various colored-pellicle walnuts. *Journal of Food Composition and Analysis,109*, 104470. https://doi.org/10.1016/j.jfca.2022.104470

Wu, S., Ni, Z., Wang, R., Zhao, B., Han, Y., Zheng, Y., & Liu, Y. (2020). The effects of cultivar and climate zone on phytochemical components of walnut (Juglans regia L). *Food and Energy Security, 9*(2), e196. https://doi.org/10.1002/fes3.196

Wu, S., Shen, D., Wang, R., Li, Q., Mo, R., Zheng, Y., & Liu, Y. (2021). Phenolic profiles and antioxidant activities of free, esterified and bound phenolic compounds in walnut kernel. *Food Chemistry,350*, 129217. https://doi.org/10.1016/j.foodchem.2021.129217

You, B., Yang, S., Yu, J., Xian, W., Deng, Y., Huang, W.,. .. Yang, R. (2021). Effect of thermal and dry salt-curing processing on free and bound phenolics and antioxidant activity in Prunus mume fruits together with the phenolic bioaccessibility. *LWT-Food Science and Technology*, *145*, 111355. https://doi.org/10.1016/j.lwt.2021.111355.

Yu, W., Liu, H., Luo, J., Zhang, S., Xiang, P., Wang, W.,. .. Lu, Y. (2022). Partial root-zone simulated drought induces greater flavonoid accumulation than full root-zone simulated water deficiency in the leaves of Ginkgo biloba. *Environmental and Experimental Botany*, *201*, 104998. https://doi.org/10.1016/j.envexpbot.2022.104998.

Zhang, M., Zheng, C., Yang, M., Zhou, Q., Li, W., Liu, C., & Huang, F. (2019). Primary metabolites and polyphenols in rapeseed (Brassica napus L.) cultivars in China. *Journal of the American Oil Chemists Society,96*(3), 303–317. https://doi.org/10.1002/aocs.12179

Zheng, Y., Wu, S., Wang, R., Wu, Y., Zhang, W., Han, Y., & Liu, Y. (2020). Analysis and correlationship of chemical components of various walnut (Juglans regia L.) cultivars. *Journal of Food Measurement and Characterization,14*(6), 3605–3614. https://doi.org/10.1007/s11694-020-00603-0

Publisher's Note