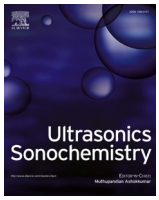
Ultrasonics Sonochemistry 117 (2025) 107338

Contents lists available at ScienceDirect

Ultrasonics Sonochemistry

journal homepage: www.elsevier.com/locate/ultson



Preparation high quality camellia oil by combining ultrasound pre-treatment and microwave as drying method: Interactive effect on drying kinetics, metabolite profile and antioxidant ability

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ARTICLE INFO

Keywords:
Drying methods
Camellia oilseeds
Microwave
Ultrasonic
Antioxidant capacity

ABSTRACT

This study systematically investigates the effects of different drying methods—Hot Air Drying (HAD), Microwave Drying (MWD), and Ultrasound-Microwave Combined Drying (UMWD)—on the drying kinetics, total phenolic content (TPC), antioxidant activity, and metabolome of Camellia oils (COs). UMWD significantly reduced drying time and increased TPC by 102.20 % and 395.94 % compared to MWD and HAD, respectively. The antioxidant capacity, as measured by FRAP, DPPH, and ABTS assays, was enhanced to 8.51, 11.35, and 37.68 μg VC/mL under UMWD conditions, showing marked improvements over MWD and HAD. Metabolomic analysis identified 1,350 metabolites, with 447 differential metabolites specific to UMWD. A total of 47 antioxidant-related metabolites (ACCMs) were identified, most of which exhibited up to a 10-fold increase in UMWD/HAD comparisons. These findings demonstrate that UMWD effectively enhances both the bioactive components and antioxidant capacity of COs, making a significant contribution to the preparation of high-quality camellia oil. Additionally, the study offers new insights into how ultrasound-assisted drying methods can enhance the bioactive components of food products.

1. Introduction

Oilseeds are a vital source of dietary energy and essential nutrients, playing a significant role in human health and nutrition. The drying process is crucial for the commercialization of oilseeds, especially for post-harvest oil extraction, as it directly impacts the retention and bioavailability of bioactive compounds in the oil. While conventional hot air drying (HAD) is widely used, it poses several challenges, including high energy consumption, low efficiency, and insufficient preservation of heat-sensitive bioactive compounds [1]. This has led to the exploration of alternative, more sustainable drying technologies, with microwave drying (MWD) emerging as a promising option. Numerous studies have shown that MWD is more effective in retaining bioactive components (such as TPC, anthocyanins) compared to HAD, especially in *Ocimum basilicum* L. postharvest residues [2], *Lycium ruthenicum* Murr. [3], and *Lycopersicon esculentum* [4]. Additionally, MWD has been linked to enhanced retention of phytochemical compounds and improved antioxidant activity [3,5,6]. For example, MWD can disrupt cell structures, aiding in the release of bound phenolic acids [5]. However, other research suggests that HAD may better preserve certain unstable bioactive substances, such as adenosine, polysaccharides, and triterpenes, which are found in higher concentrations (1.15–1.22 times) in HAD-treated samples compared to MWD-treated ones. At the same time, the bioavailability of polysaccharides (419.91 %) is significantly higher in the MWD group compared to other drying methods (68.74 %–78.92 %) [6]. These findings highlight that different drying methods can have distinct activation effects on various foods and bioactive components.

It is well-known that the drying process also often results in the loss of antioxidants, nutrients, flavor, color, and other sensory properties. Exposure to heat, light, and humidity during drying accelerates oxidative processes, activating enzymes and leading to the degradation of

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https://doi.org/10.1016/j.ultsonch.2025.107338
Received 26 January 2025; Received in revised form 20 March 2025; Accepted 31 March 2025
Available online 3 April 2025

antioxidant capacity [7]. To mitigate these quality losses, pre-treatment techniques, such as ultrasound (US), are being explored to maintain or reduce food quality deterioration during drying. Hybrid drying systems that combine US with other drying methods are being investigated as a means to improve the retention of bioactive compounds and nutrients [8]. For instance, US-assisted drying has been shown to improve the retention of bioactive compounds in mushrooms, with increases of 24–27 % in electron carrier (EC) activity and 15–41 % in total phenolic content (TPC) compared to those without US treatment [1]. Wang et al. demonstrated that ultrasonic pre-treatment (USP) could enhance the drying process while preserving high levels of phenols [9]. Similar improvements have been observed in other foods, where USP has led to notable increases in *Inula viscosa* L. [10]. Moreover, researchers have explored the combined effects of USP and MWD. USP has been shown to significantly improve drying efficiency and physical properties during microwave drying, promoting the formation of porous structures in dried samples [11]. Additionally, USP facilitates protein hydrolysis, increasing the free amino acid content by 3.69 %–11.40 %. However, some studies suggest that the combined use of USP and drying could lead to a decrease in TPC, total flavonoid content (TFC), anthocyanins, and antioxidant activity in some foods [11]. Recent reviews have noted that the combination of USP with MWD or other drying methods does not always result in a significant enhancement of bioactive compounds and may even have negative effects on food quality [1]. These discrepancies highlight the need for more tailored research, emphasizing the importance of understanding the individual and interactive effects of ultrasound-assisted drying on food bioactive components [1].

Camellia oil (CO), derived from the seeds of *Camellia oleifera*, is a high-quality edible oil renowned for its rich nutritional profile and health benefits, particularly its antioxidant properties, which are crucial in preventing oxidative stress-related diseases [12,13]. Post-harvest drying is a key step in the preparation of CO and other plant-based oils. Recent studies have shown that HAD of oilseeds prior to extraction can significantly enhance the concentration of bioactive compounds in the oil [14]. However, to the best of our knowledge, the effects of different drying methods on the composition and antioxidant properties of CO have not been thoroughly investigated. This gap in knowledge limits the development of innovative drying technologies that could improve CO quality. Based on existing literature, we hypothesize that MAD, compared to traditional HAD, can more effectively release active compounds from camellia oilseeds, thereby enhancing the oil's quality and antioxidant activity. Moreover, we propose that combining USP with microwave drying (UMWD) could further amplify these effects. This study aims to test this hypothesis by employing metabolomics and *in vitro* antioxidant assays to identify and quantify the metabolic changes associated with various drying methods and their relationship to enhanced antioxidant activity. Through this research, we aim to contribute to the development of more efficient and sustainable methods for oilseed processing, benefiting both the food industry and consumer health.

2. Materials and methods

2.1. Chemicals and reagents

HPLC-grade methanol and acetonitrile were obtained from Merck (Hangzhou, China), while formic acid came from Aladdin (Shanghai, China). DPPH and ABTS standards were purchased from Shanghai Yuanye Bio-Technology Co., Ltd. Analytical-grade solvents were sourced from Shanghai GuoYao Chemical Reagents.

2.2. Preparation of camellia oil samples

Camellia oilseeds used in this study were sourced from Hunan Province and belonged to the Xianglin series. Approximately 20 kg of camellia fruits, uniformly matured and exhibiting consistent cracking, were randomly collected from the site. The hulls were manually removed, and the seeds were subsequently dried using three drying methods until their moisture content reached 6–8 %.

The specific drying protocols were as follows:

HAD: Seeds were dried in an electric thermostatic hot-air oven (XMA-2000, Germany) at 40 °C.

MWD: A microwave oven (NN-GM33HB, China, 2450 MHz, 23 L capacity) was used for drying seeds. Approximately 80 g of samples were weighed and placed on a glass drying tray with a diameter of 15 cm. The microwave power was set to 400 W, with a drying temperature of 25 ± 1 ℃. The specific power applied was 5 W/g. The drying process was completed in approximately 10 min.

UMWD: Ultrasound pretreatment was performed in an ultrasonic bath (SB-5200DTD, China, 10 L, 40 kHz frequency, 300 W power). The seed-to-water ratio was adjusted to 1:10. The seeds were sonicated for 30 min and the temperature of the sonication process was ensured not to exceed 25 °C. The subsequent microwave processing followed the same procedure as described for MWD.

After drying, camellia oil was extracted from the seeds using a ZYJ-420 screw oil press (Hubei Yijiaoyi Machinery Equipment Group Co., Ltd, Hubei, China). The extracted oil underwent natural precipitation under light-avoidance conditions (4℃) for more than 20 h, followed by centrifugation. The upper oil layer from each sample was separated and refrigerated prior to further analyses.

2.3. Drying kinetics

An empirical model was employed to determine all samples' dry matter moisture ratio (MR) (Eq. (1)).

$$MR = \frac{M - M_e}{M_0 - M_e} \tag{1}$$

Where M = moisture content at every time intervals, M_e = equilibrium water content and M_0 = initial water content.

Eq. (2) was used to determine the DR of all samples at a given point.

$$DR = \frac{M_{t1} - M_{t2}}{t_1 - t_2} \tag{2}$$

where t_1 and t_2 denote the drying times (min), and M_{t1} and M_{t2} represent the samples' moisture contents (kg water/kg dry matter).

2.4. Determination of TPC

To determine the total phenolic content, 5 g of CO was extracted with 50 mL of an 80 % methanol–water solution in a cold-water bath. The extraction process was repeated three times, and the combined liquid extracts were concentrated by photoevaporation to remove methanol. The pH of the resulting extracts was adjusted to 2.0 using 2 M HCl, followed by three rounds of liquid–liquid extraction with ethyl acetate. The supernatants were evaporated to dryness and re-dissolved in MS-grade methanol to obtain the phenolic extracts.

The total phenolic content was assessed using the Folin–Ciocalteu method. Briefly, 1 mL of the phenolic extract was transferred to a colorimetric tube, followed by the sequential addition of 5 mL of distilled water, 1 mL of Folin reagent, and 3 mL of 75 g/L sodium carbonate solution (Na_2CO_3). The mixture was allowed to react in the dark for 2 h at room temperature. The absorbance was measured at 765 nm using a spectrophotometer (Perkin Elmer, USA).

2.5. Determination of antioxidant activity

The ferric reducing antioxidant power FRAP, DPPH, and ABTS methods were performed according to the procedures outlined by She et al., 2024 [13]. Further details can be found in the supplementary materials.

2.6. Widely-targeted metabolomic analyses

2.6.1. Sample extraction

Samples were retrieved from the −80°C freezer and allowed to thaw before thorough mixing by vortexing for 30 s. A 500 μL aliquot of each sample was transferred into a new tube, followed by the addition of 1000 μL of a 70 % methanol internal standard extraction solution. The mixture was vortexed for 3 min to ensure complete integration and then stored overnight at 4°C.

The following day, the mixture was vortexed again for 3 min and briefly sonicated for 30 s to remove any foam. The solution was then centrifuged at 12,000 rpm and 4°C for 3 min to separate the phases. The upper 800 μL of the organic phase was carefully extracted. The collected solution was filtered through a 0.22 μm microporous membrane and stored in an injection vial for UPLC-MS/MS analysis.

2.6.2. Instrumental analysis conditions

The analytical setup for data acquisition is anchored by an Ultra Performance Liquid Chromatography (UPLC) system (ExionLC™ AD) and a Tandem mass spectrometer. Detailed instrument parameters are provided in the supplementary materials.

2.6.3. Metabolite analysis and quantification

Metabolite identification was carried out utilizing secondary spectral data from our proprietary database, MWDB. Quantification of metabolites was achieved through the multiple reaction monitoring (MRM) mode on a triple quadrupole mass spectrometer.

2.7. Data analysis

Statistical significance of the data and linear regression modeling were determined by SPSS 22.0. A metabolite co-expression network was constructed utilizing the weighted gene co-expression network analysis (WGCNA) R package (v 4.2.2). To visualize group segregation and identify significantly altered metabolites, Orthogonal Partial Least-Squares Discriminant Analysis (OPLS-DA) was applied, with differential metabolic markers identified based on VIP > 1. Data visualization was carried out using Metware Cloud (https://cloud.metware.cn) and Origin2019.

3. Results and discussions

3.1. Effect of treatments on drying kinetics of camellia oilseeds

The drying behavior of camellia oilseeds can be comprehensively analyzed through two key graphical representations: the drying curve and the drying rate curve. The drying curve elucidates the relationship between the moisture ratio (MR) and drying time, while the drying rate curve delineates the variation in drying rate (DR) as a function of drying time. These curves provide critical insights into the drying kinetics of the material, as illustrated in Fig. 1. The time required for camellia oilseeds to reach the target moisture content varies significantly depending on the drying method employed. Notably, MWD and UMWD substantially reduce the drying time compared to HAD (Fig. 1A, C, and E). Specifically, the integration of USP before microwave drying markedly accelerates the reduction in moisture content. This enhanced efficiency is attributed to the cavitation effect generated by ultrasonic waves. During USP, cavitation bubbles form within the cellular structure of the oilseeds, disrupting cell walls and enhancing water diffusion [15]. The disruption of the cellular matrix increases the permeability of the tissue, allowing for more efficient water migration from the interior to the surface of the product. Consequently, this facilitates more rapid and

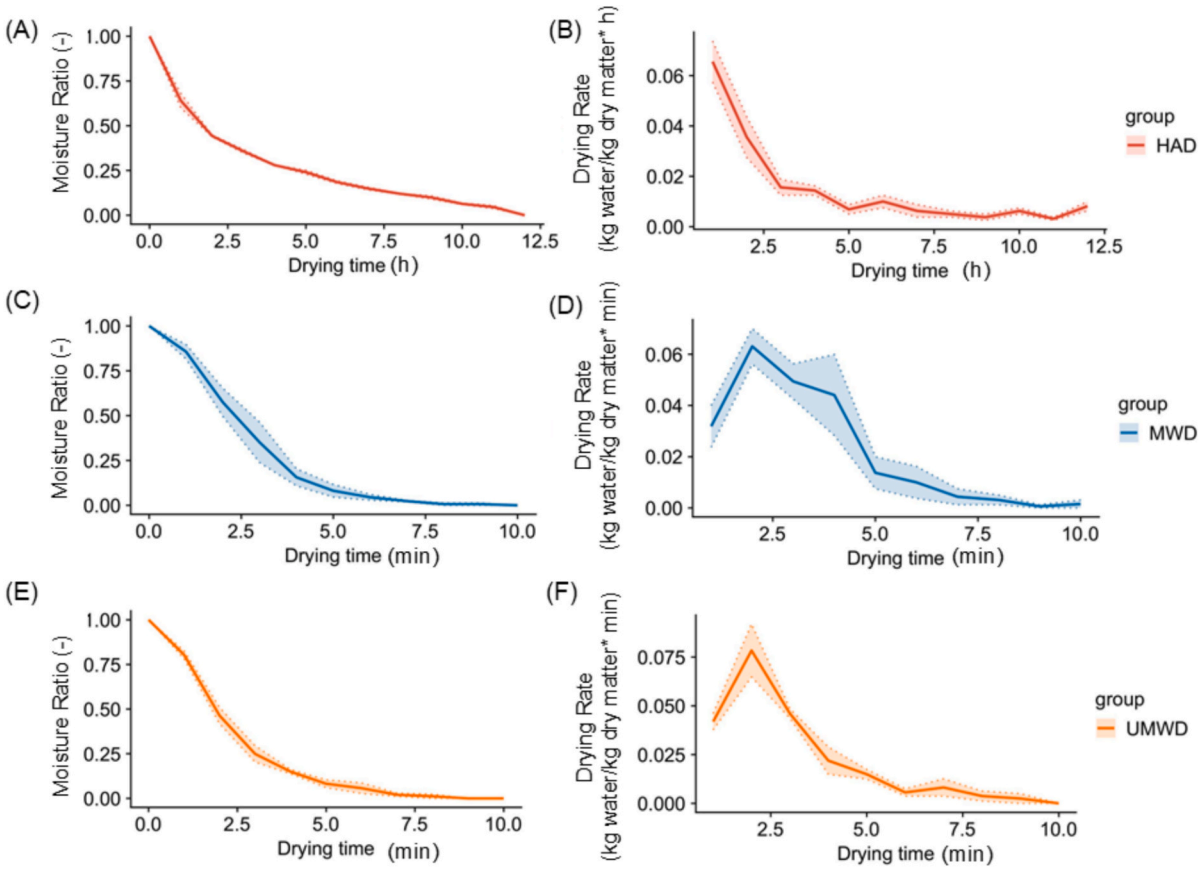

Fig. 1. (A) Drying curve of camellia oilseeds under HAD. (B) Drying rate curve of camellia oilseeds under HAD. (C) Drying curve of camellia oilseeds under MWD. (D) Drying rate curve of camellia oilseeds under MWD. (E) Drying curve of camellia oilseeds under UMWD. (F) Drying rate curve of camellia oilseeds under UMWD.

effective water removal, thereby accelerating the drying process.

To further illustrate the advantages of USP, we analyzed the drying rate curves. In the case of HAD for camellia oilseeds, drying occurs entirely in the deceleration phase, where the drying rate gradually decreases as drying time increases (Fig. 1B). This slow and steady decline in drying rate limits the overall efficiency of the process. In contrast, MWD and UMWD exhibit a distinct pattern: the drying rate initially increases, reaches a peak, and then gradually declines (Fig. 1D and 1F). Importantly, the maximum drying rate achieved with UMWD is notably higher than that of MWD, underscoring the significant contribution of ultrasonic pre-treatment to the drying kinetics. The enhanced initial drying rate observed in UMWD indicates that USP effectively prepares the oilseeds for more efficient microwave drying, leading to faster and more uniform moisture reduction.

3.2. Effect of treatments on TPC and antioxidant activity

Phenolic compounds play a crucial role in the sensory and nutritional qualities of foods; however, they are highly sensitive to drying methods due to their reactivity and heat sensitivity [15]. In this study, the TPC of COs subjected to three different drying methods showed significant differences ($P < 0.05$). The TPC values were 10.58 mg/g for HAD, 25.95 mg/g for MWD, and 52.47 mg/g for UMWD (Fig. 2A). Compared to HAD, MWD significantly increased the TPC of COs by an impressive 145.27 %. This finding is consistent with previous studies, where MWD has been shown to enhance the TPC of various fruits, such as apricots (by 24.81 %) [16] and tomato by-products (by 35.21 %–80.77 %) [4]. The substantial increase in TPC observed with MWD can be attributed to its ability to rapidly remove moisture, thereby minimizing thermal degradation and preserving phenolic compounds. The most remarkable enhancement in TPC was achieved with UMWD, which resulted in a dramatic increase of 395.94 % compared to HAD and 102.20 % compared to MWD. This significant boost in TPC can be attributed to the cavitation effect of ultrasound, which disrupts cell walls and facilitates the release of phenolic compounds bound within the cells [1]. This phenomenon aligns with studies showing that the TPC of pears increases with rising microwave power at lower temperatures [15], suggesting that the combination of USP and MWD creates an optimal environment for phenolic extraction.

In addition to enhancing TPC, MWD has been shown to improve the oxidative stability of tiger nuts compared to HAD [17]. Given that the health properties of COs are largely derived from its antioxidant potential [18], we further evaluated its antioxidant capacity of COs using three common assays (Fig. 2B, C, D). The results consistently ranked the antioxidant capacity across the three drying methods as follows: UMWD (FRAP: 8.51 μg VC/mL; DPPH: 11.35 μg VC/mL; ABTS: 37.68 μg VC/mL) > MWD (FRAP: 2.50 μg VC/mL; DPPH: 8.23 μg VC/mL; ABTS: 17.27 μg VC/mL) > HAD (FRAP: 0.40 μg VC/mL; DPPH: 4.49 μg VC/mL; ABTS: 8.84 μg VC/mL). These findings are consistent with previous studies on goldenberries [19], which demonstrated that MWD and USP significantly enhance the antioxidant capacity of COs. The superior antioxidant activity observed in UMWD-treated samples can be attributed to the synergistic effects of USP and MWD, which not only preserve but also concentrate phenolic compounds, leading to enhanced antioxidant properties.

3.3. Effect of treatments on metabolic profile of COs

Numerous studies have highlighted the nutritional value of COs and identified key bioactive components, including fatty acids, phenolic compounds, terpenes, and phytosterols [18]. Our previous research has also reported on the lipid composition and phenolic metabolome of COs [13,20]. In this study, LC-MS/MS was further utilized to explore the overall metabolic composition of COs and to assess how drying methods influence it. A total of 1350 metabolites were identified across the HAD, MWD, and MWD samples. Regardless of the drying methods applied, the metabolic profile of COs predominantly consisted of flavonoids (19.78 %), lipids (12.59 %), amino acids and derivatives (10.96 %), and phenolic acids (8.22 %) (Fig. 3A). OPLS-DA analysis revealed a clear separation between the three groups based on the first two principal components (Fig. 3B), confirming that the metabolite composition of COs varies with drying methods. A total of 627, 799, and 692 differential

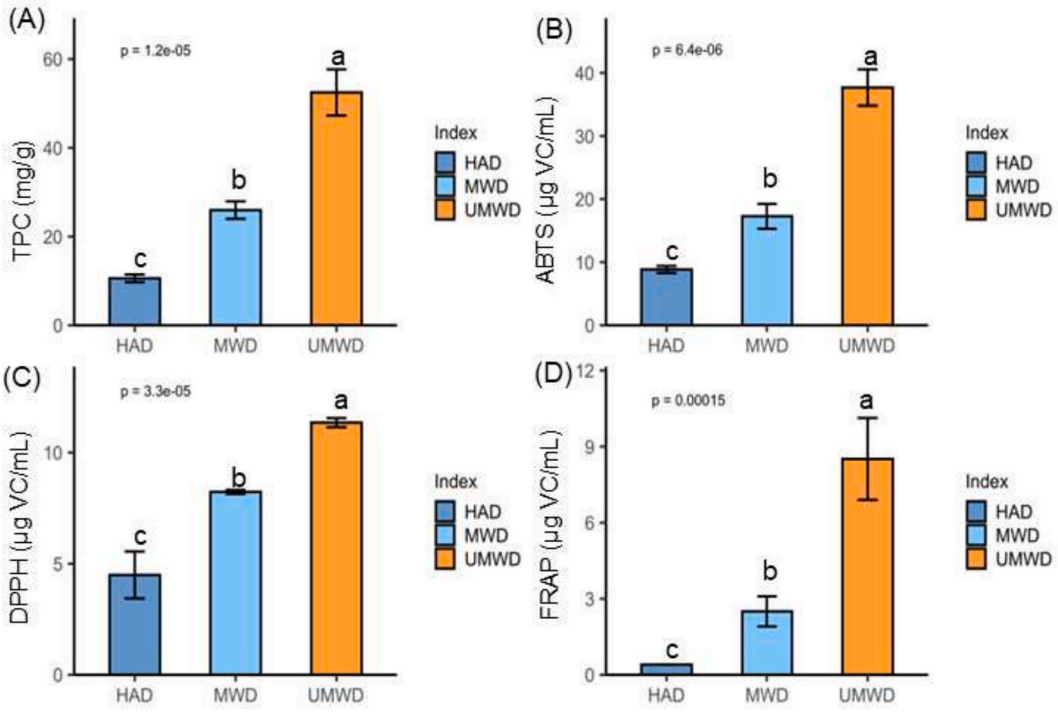

Fig. 2. The content of TPC and anti-oxidant capacity of COs under different drying methods. Note: Different lowercase letters show significant difference ($P < 0.05$) in different drying methods. HAD: Hot-air drying; MWD: microwave drying; UMWD: Ultrasonic pretreatment combined with microwave drying.

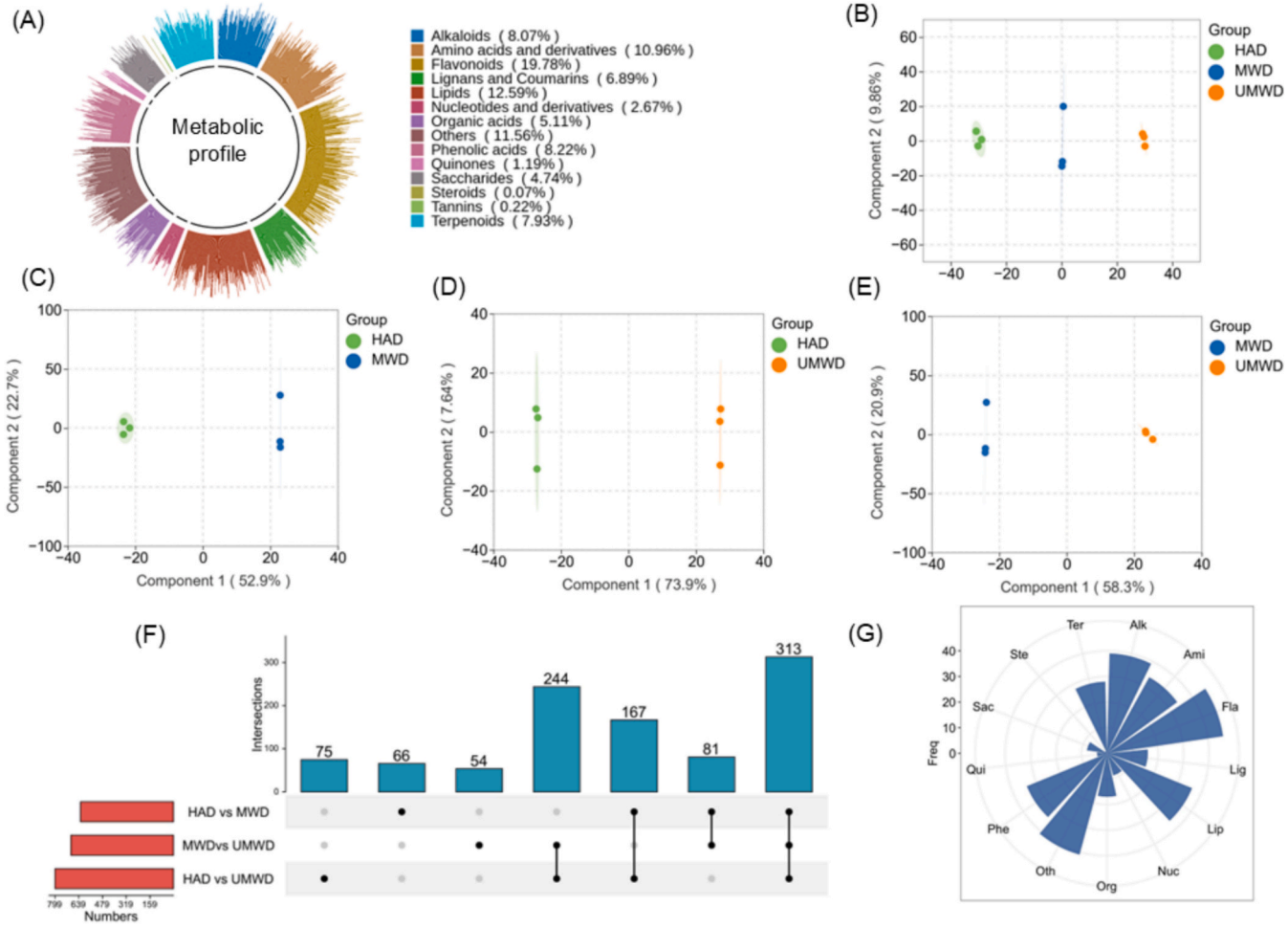

Fig. 3. (A) Metabolic profile of COs. (B) OPLS-DA plot of metabolomics between COs under three drying methods. (C) OPLS-DA plot of metabolomics between COs under HAD and MWD groups. (D) OPLS-DA plot of metabolomics between COs under HAD and UMWD groups. (E) OPLS-DA plot of metabolomics between COs under MWD and UMWD groups. (F) The Venn-UpSet diagram of DEMs among three drying groups. (G) Categories distribution of common DEMs among the three drying groups. Note: Alk: Alkaloids, Ami: Amino acids and derivatives, Fla: Flavonoids, Lig: Lignans and Coumarins, Lip: Lipids, Nuc: Nucleotides and derivatives, Org: Organic acids, Oth: Others, Phe: Phenolic acids, Qui: Quinones, Ter: Terpenoids, Sac: Saccharides.

metabolites (DEMs) with a Variable Importance in Projection (VIP) score greater than 1.0 were identified in the comparisons of HAD vs. MWD (Fig. 3C), HAD vs. UMWD (Fig. 3D), and MWD vs. UMWD (Fig. 3E), respectively. The Venn-UpSet diagram (Fig. 3F) indicated that 313 DEMs were shared among the three comparison groups. These common DEMs primarily included 46 flavonoids, 39 alkaloids, 34 amino acids and derivatives lipids, and 34 phenolic acids (Fig. 3G).

3.4. Identification of dry-sensitive metabolites (DSMs)

Drying-sensitive metabolites (DSMs) were identified based on stringent criteria: $P \leq 0.05$, Fold Change (FC) ≥ 2 or ≤ 0.5, and VIP ≥ 1. Using these conditions, the number of DSMs identified in each comparison group was as follows: 205 DSMs for HAD vs. MWD (Fig. 4A), 538 DSMs for HAD vs. UMWD (Fig. 4B), and 341 DSMs for MWD vs. UMWD (Fig. 4C). The distribution of DSM categories across the different comparison groups is shown in Fig. 4D. Among these categories, flavonoids were the most prominent DSMs, accounting for over 19 % in all groups.

MWD significantly enhanced the retention of flavanone-like compounds in tomato, particularly naringenin and lycopene, as reported in previous studies [4]. Similarly, our study found that MWD increased naringin content by 1.83-fold compared to HAD. Furthermore, specific flavonoids such as cosmosiin and luteolin-4′-O-glucoside exhibited a remarkable increase, with levels elevated by over 100-fold under MWD conditions. While earlier studies on tomatoes [4], apricot [16], and *L. ruthenicum* [3] have generally indicated microwave drying enhances phenolic compound content, our findings reveal a more complex pattern. In our study, although MWD increased the levels of certain up-regulated flavonoids, it also resulted in a higher number of down-regulated flavonoids, with 21 flavonoids showing significant reduction. Although MWD elevated certain up-regulated flavonoids, it also led to a significant reduction in 21 flavonoids, highlighting the dual effects of MWD on the flavonoid profile. In addition, USP showed a unique effect on metabolite preservation and transformation. Consistent with previous findings [9], USP positively affected the retention of phenolic compounds (e.g., lignans, coumarins, and phenolic acids), with more than 90 % of these metabolite showing increased abundance.

To further explore the cumulative effects of MWD and USP on DSMs, we visualized the changes using line plots (Fig. 4E). These plots revealed several key trends. First, USP enhanced the number of flavonoid-related DSMs in the HAD vs. MWD comparison, both for up-regulated and down-regulated flavonoids, suggesting a synergistic effect. In contrast, for amino acid-related DSMs, USP appeared to buffer the impact of microwave drying, reducing the total number of DSMs influenced by MWD. For specific metabolite categories, USP had divergent effects on up- and down-regulated DSMs. For example, compared to HAD, the number of up-regulated lipids in MWD continued to increase after USP, while the number of down-regulated lipids in MWD significantly

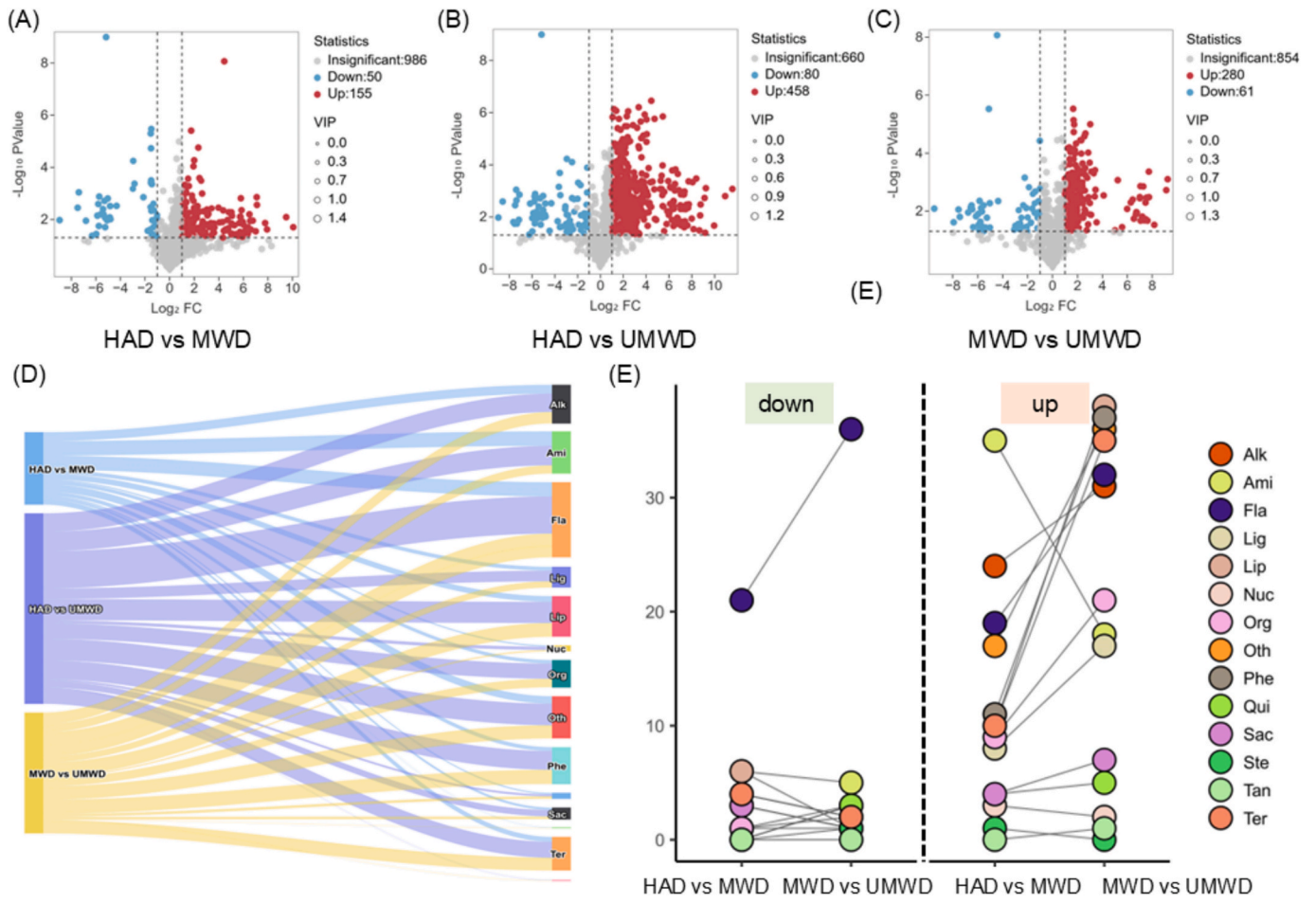

Fig. 4. (A) Volcano diagram of the DSM of COs under HAD and MWD groups. (B) Volcano diagram of the DSM of COs under HAD and UMWD groups. (C) Volcano diagram of the DSM of COs under MWD and UMWD groups. (D) Distribution of categories of DSMs resulting from different comparison groups. (E) The line graph of the number of DSMs between different drying methods. Note: Alk: Alkaloids, Ami: Amino acids and derivatives, Fla: Flavonoids, Lig: Lignans and Coumarins, Lip: Lipids, Nuc: Nucleotides and derivatives, Org: Organic acids, Oth: Others, Phe: Phenolic acids, Qui: Quinones, Ter: Terpenoids, Sac: Saccharides.

decreased following USP. These findings suggest that the combination of USP and MWD not only alters the abundance of DSMs but also modulates the balance between up- and down-regulated metabolites within specific categories.

3.5. Comparative analysis of metabolites response to MWD and USP

The identified 621 DSMs were classified into six clusters using the K-means clustering algorithm (Fig. 5A). Clusters 1 and 3, which contained 15 and 71 DSMs respectively, were classified as MWD-specific DSMs. The protective effect of MWD on phenolic compounds was most notable in flavonoids, which accounted for 63.64 % of the MWD-specific phenolic substances (Fig. 5B). Notably, ten flavonoids, including luteolin-4′-O-glucoside, cosmosiin, and trifolin, showed more than a 20-fold upregulation. This finding further supports previous studies [21], which demonstrated that MWD enhances the preservation of phenolic compounds in COs. In addition to phenolic compounds, the current study revealed that MWD also promotes the upregulation of other non-phenolic substances, particularly amino acids and their derivatives, lipids, and organic acids.

Clusters 2 and 4 were identified as UMWD-specific DSMs, which exhibited a linear response to the synergistic effect of microwave and ultrasound treatment. A total of 447 DSMs were significantly upregulated in the UMWD-specific metabolite group, suggesting that USP can better preserve the bioactive components in COs. Similar findings regarding the preservation effects of USP have been reported in previous studies on yarrow [22], *Inula viscosa* L. [10], and sweet potatoes [23]. Among the UMWD-specific phenolic metabolites, flavonoids, phenolic acids, lignans and coumarins, and tannins accounted for 54, 49, 28, and 1 compound (s), respectively. Most of these phenolic compounds exhibited a 2- to 10-fold upregulation. USP further enhanced the levels of non-phenolic DSMs, particularly lipids, terpenoids, organic acids, alkaloids, and amino acids and their derivatives, with each category showing more than 40 upregulated metabolites (Fig. 5B).

In conclusion, these findings highlight the potential of MWD for preserving bioactive components. Furthermore, the synergistic effect of ultrasound and microwave treatments not only enhanced this preservation effect but also expanded it to a broader range of non-phenolic metabolites.

3.6. Identification of antioxidant contribution differential metabolites (ACMs) using WGCNA

To investigate the metabolic components of COs associated with antioxidant activity, we employed Weighted Gene Co-expression Network Analysis (WGCNA). This advanced analytical approach allows for the identification of metabolite groups that co-vary with antioxidant capacity, thereby elucidating the underlying biochemical pathways responsible for the observed antioxidant effects. Using 621 DSMs, we constructed a co-expression network and categorized the metabolites into three distinct modules (Fig. 6A). The turquoise module showed a significant positive correlation with the TPC, DPPH, ABTS, and

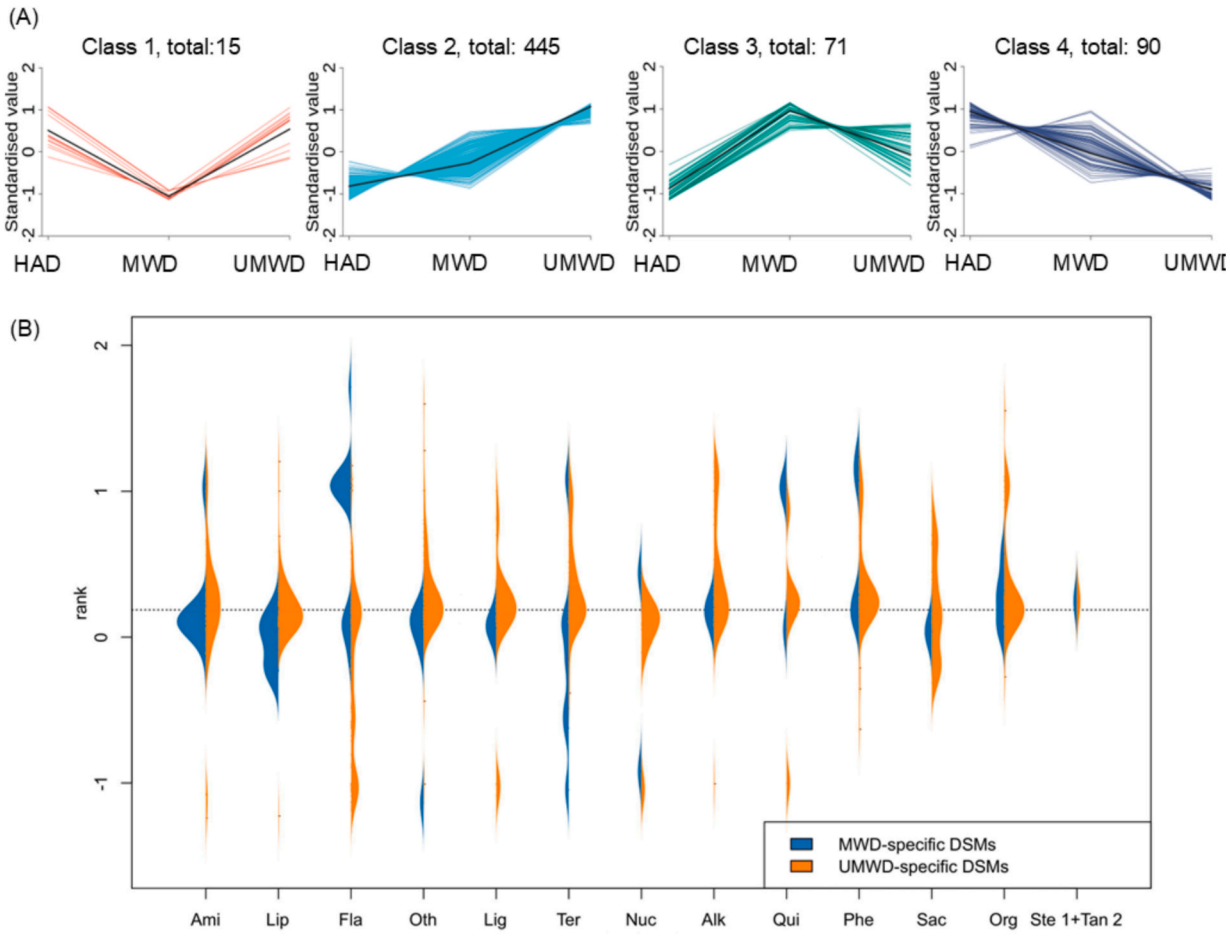

Fig. 5. (A) The K-means cluster analysis diagram for DSMs. (B) The pod plots of MWD-specific and UMWD-specific DSMs for multiplicative display on different categories. Note: Alk: Alkaloids, Ami: Amino acids and derivatives, Fla: Flavonoids, Lig: Lignans and Coumarins, Lip: Lipids, Nuc: Nucleotides and derivatives, Org: Organic acids, Oth: Others, Phe: Phenolic acids, Qui: Quinones, Ter: Terpenoids, Sac: Saccharides.

FRAP results, with correlation coefficients exceeding 0.9 ($P \leq 0.05$) (Fig. 6B). Further analysis, combining metabolite significance (MS) with module membership (MM) criteria (|MS.trait| > 0.9, |MM.turquoise. corr| > 0.9, and Kwithin > 200), revealed that 215, 276, and 157 metabolites from the turquoise module were significantly associated with FRAP, ABTS, and DPPH, respectively (Fig. 6C). Interestingly, all of these ACMs belonged to UMWD-specific DSMs, and all were up-regulated. These ACMs were predominantly alkaloids (13.77 %), phenolic acids (13.11 %), flavonoids (11.80 %), and terpenoids (10.82 %) across the various antioxidant assays (Fig. 6D). A total of 110 ACMs were common to all antioxidant assay groups (Fig. 6E), including 19 phenolic acids, 14 alkaloids, 14 terpenoids, and 12 flavonoids.

3.7. Identification for antioxidant-related metabolites and their activation effects by MWD and USP

To further investigate the specific correlation between metabolites and antioxidant capacity under different drying treatments, we analyzed 110 common ACMs with significant contributions to antioxidant capacity, along with 155 and 280 upregulated DSMs from HAD vs. MWD and MWD vs. UMWD, respectively (Fig. S1). These analyses yielded a novel library of antioxidant capacity-contributing metabolites (ACCMs) (Table 1). This library consists of 47 ACCM compounds, primarily including 13 alkaloids, 10 amino acids and derivatives, and 6 phenolic acids. Notably, more than 65 % of the ACCMs are secondary metabolites, highlighting their predominant role in antioxidant capacity, while primary metabolites, such as amino acids and derivatives, made a lesser contribution.

Interestingly, although phenolic acids have traditionally been regarded as the main contributors to antioxidant capacity [24], our findings further confirm the significant role of other secondary metabolites, particularly alkaloids, in enhancing antioxidant potential. This aligns with recent studies suggesting that various classes of secondary metabolites, beyond phenolic acids, play crucial role in antioxidant mechanisms [25].

All 47 ACCMs identified in this study were unique to UMWD-specific DSMs. The fold changes of these compounds in the MWD/HAD comparisons generally ranged from 4 to 90, while the UMWD/MWD fold changes for nearly all ACCM compounds were between 2 and 4. Furthermore, except for 3-ethyl-4-methylpyrrole-2,5-dione, amides, and lycopene-1, the UMWD/MWD fold changes of all ACCM compounds were consistently higher than their respective MWD/HAD fold changes. This suggests that MWD has a stronger stimulatory effect on the production or release of certain ACCMs, particularly 11-keto-ursolic acid, genipin, and selina-3,7(11)-diene-8-one.

To further validate the functional significance of these metabolites, we conducted a literature survey using PubChem (https://pubchem.ncbi.nlm.nih.gov/) and relevant references (https://webofscience.com/WOS) (Table 1, Table S1). Our findings indicate that most identified ACCMs are bioactive compounds with reported health benefits. For instance, ganoderic acid X, methyl orsellinate, and genipin exhibit strong anti-inflammatory and antimicrobial activities [26–28]. Notably, ganoderic acid X has been shown to inhibit HepG2 and HuH6 cell proliferation and migration in a dose-dependent manner while inducing

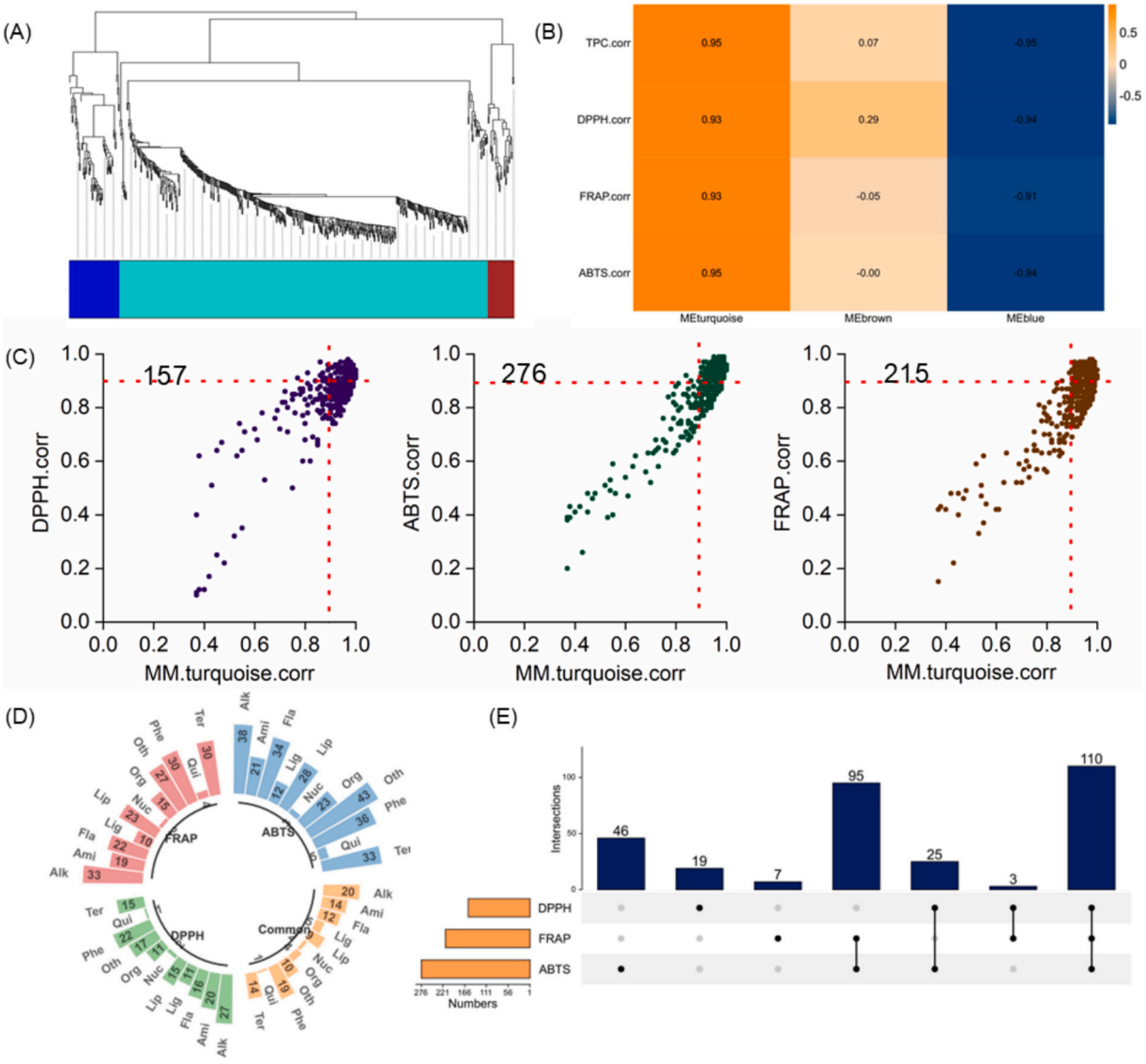

Fig. 6. (A) Clustering dendrogram of the average network adjacency for the identification of metabolite co-expression modules. (B) Heat map of different color modules in relation to antioxidant capacity and TPC based on WGCNA results. (C) The scatter plots of ACMs based on |MS. trait| > 0.9, |MM.turquoise. corr| > 0.9, and Kwithin > 200 screening conditions. (D) The number of metabolite categories in FRAP-specific, ABTS-specific, and DPPH specific ACMs and Common ACMs. (E) The Venn-UpSet diagram of ACMs. Note: Alk: Alkaloids, Ami: Amino acids and derivatives, Fla: Flavonoids, Lig: Lignans and Coumarins, Lip: Lipids, Nuc: Nucleotides and derivatives, Org: Organic acids, Oth: Others, Phe: Phenolic acids, Qui: Quinones, Ter: Terpenoids, Sac: Saccharides.

apoptosis. In vivo studies further demonstrated its potential in hepatoblastoma treatment by significantly reducing tumor volume and mass, as supported by proteomics-based investigations [29].

However, we acknowledge that some identified compounds, such as 5-hydroxymethylfurfural (5-HMF) and nicotinic acid methyl ester, have been linked to potential adverse effects. 5-HMF has been associated with cytotoxicity, mutagenicity, and potential carcinogenicity [30]. Similarly, nicotinic acid methyl ester has been reported to cause skin inflammation but also enhances the anticancer activity of ruthenium complexes [31,32].

Numerous studies have observed a significant increase in HMF levels in various foods following heat treatment [30,33]. 5-HMF is primarily formed via the Maillard reaction and acid-catalyzed caramelization of sugars, with high processing temperatures being a key factor influencing its content in foods [33]. For instance, heating Atemoya at 85°C resulted in an over 120-fold increase in HMF concentration [34], a value significantly higher than our findings, where the highest fold change observed in the UMWD/HAD group was 41.07.

On the other hand, while direct correlations between drying temperature and increased HMF formation have been reported in various foods [35], no toxic effects of HMF have been observed at daily doses ranging from 80 to 100 mg/kg body weight [36]. This suggests that although HMF formation is influenced by processing conditions, its health risks may depend on both concentration and exposure levels.

Moreover, USP prior to MWD led to a more pronounced increase in the levels of these bioactive compounds. For most ACCM compounds, the UMWD/HAD fold changes exceeded 10. For example, tropic acid, 7,8-dihydroxy-4-methylcoumarin, and hydroxy coumestrol exhibited significant increases in abundance under UMWD conditions compared to HAD. Specifically, tropic acid, which displayed a 24.86-fold change in MWD/HAD comparisons, exhibited a fold change exceeding 100 in UMWD/HAD comparisons after USP. Tropic acid, a key component of *Physochlainae Radix*, is traditionally used in treating cough and asthma [37]. These results confirm that USP further enhances the accumulation of bioactive compounds, providing strong evidence for our hypothesis that MWD facilitates the activation and release of metabolites,

Table 1
The information about the library of antioxidant capacity contributing metabolites.

	Compounds	Class I	Class II	MWD/HAD	UMWD/MWD	UMWD/HAD	DPPH.cor	FRAP.cor	ABTS.cor
1	Ganoderic Acid X	Terpenoids	Triterpene	1066.83	2.83	3022.88	0.95	0.94	0.97
2	11-Keto-ursolic acid	Terpenoids	Triterpene	94.04	2.71	255.02	0.94	0.9	0.97
3	Genipin	Terpenoids	Monoterpenoids	83.51	4.02	335.51	0.93	0.96	0.98
4	Selina-3,7(11)-dien-8-one	Terpenoids	Monoterpenoids	70.96	2.19	155.38	0.96	0.94	0.95
5	5-formyl-2,6-dihydroxy-1,7-dimethyl-9,10-dihydrophenanthrene	Terpenoids	Ditepenoids	50.8	2.17	110.17	0.94	0.91	0.96
6	Rheic Acid	Quinones	Anthraquinone	33.76	3.47	117.05	0.94	0.96	0.98
7	4-Hydroxybenzoylmalic acid	Phenolic acids	Phenolic acids	33.19	4.94	164.01	0.91	0.97	0.98
8	Methyl Orsellinate	Phenolic acids	Phenolic acids	33.03	2.09	68.93	0.97	0.92	0.96
9	2',4'-Dimethoxyacetophenone	Phenolic acids	Phenolic acids	28.25	2.99	84.52	0.95	0.94	0.99
10	2,4,5-Trimethoxybenzoic acid	Phenolic acids	Phenolic acids	27.32	2.86	78.14	0.95	0.95	0.98
11	Dihydrocaffeic acid	Phenolic acids	Phenolic acids	26.1	2.23	58.26	0.96	0.91	0.95
12	Tropic acid	Phenolic acids	Phenolic acids	24.86	4.4	109.45	0.92	0.97	0.99
13	Phloroglucinol	Others	Others	23.47	3.01	70.59	0.95	0.96	0.98
14	4-Methyl-5-thiazoleethanol	Others	Others	20.22	4.53	91.6	0.91	0.95	0.98
15	5-hydroxymaltol	Others	Ketone compounds	15.73	2.61	41.12	0.96	0.95	0.99
16	5-Hydroxymethylfurfural	Others	Aldehyde compounds	12.61	3.26	41.07	0.94	0.97	0.97
17	5-Methoxyfurfural	Others	Aldehyde compounds	12.1	3.59	43.48	0.93	0.97	0.98
18	5-Aminovaleric acid	Organic acids	Organic acids	10.71	3.99	42.75	0.92	0.95	0.98
19	6-Hydroxy-7-methoxycoumarin	Lignans and Coumarins	Coumarins	10.21	4.32	44.1	0.92	0.97	0.98
20	7,8-Dihydroxy-4-methylcoumarin	Lignans and Coumarins	Coumarins	8.44	5.21	43.93	0.9	0.97	0.99
21	Hydroxy coumestrol	Lignans and Coumarins	Coumarins	8.25	4.71	38.82	0.91	0.97	0.99
22	7,8-Dihydroxy-4-phenylcoumarin	Lignans and Coumarins	Coumarins	8.11	3.23	26.23	0.93	0.95	0.98
23	Kaempferol	Flavonoids	Flavonols	7.48	2.93	21.9	0.94	0.96	0.99
24	Pinostrobin Chalcone	Flavonoids	Chalcones	6.86	2.56	17.61	0.95	0.96	0.97
25	N-Ethylmaleimide	Amino acids and derivatives	Amino acids and derivatives	6.77	2.07	14	0.98	0.95	0.97
26	N-Ethylphenylalanine	Amino acids and derivatives	Amino acids and derivatives	6.46	2.53	16.32	0.96	0.94	0.97
27	3-(Pyrazol-1-yl)-L-alanine	Amino acids and derivatives	Amino acids and derivatives	6.45	2.11	13.59	0.97	0.94	0.97
28	Cyclo(L-Ala-L-Pro)	Amino acids and derivatives	Amino acids and derivatives	6.23	2.09	13.05	0.97	0.96	0.97
29	Cyclo(D-Leu-L-Pro)	Amino acids and derivatives	Amino acids and derivatives	5.38	2.79	15.04	0.95	0.98	0.99
30	Methyl L-pyroglutamate	Amino acids and derivatives	Amino acids and derivatives	5.06	2.43	12.29	0.96	0.96	0.99
31	Cyclo(Pro-Leu)	Amino acids and derivatives	Amino acids and derivatives	4.89	2.54	12.4	0.95	0.96	0.98
32	Cyclo(Pro-Val)	Amino acids and derivatives	Amino acids and derivatives	4.82	2.69	12.97	0.95	0.97	0.97
33	Cyclo(D-Val-L-Pro)	Amino acids and derivatives	Amino acids and derivatives	4.72	3.49	16.45	0.93	0.98	0.98
34	Cyclo(Val-Ala)	Amino acids and derivatives	Amino acids and derivatives	4.63	2.23	10.35	0.94	0.9	0.97
35	3-quinolinecarboxylic acid	Alkaloids	Quinoline alkaloids	4.49	2.69	12.07	0.95	0.96	0.99
36	1-(Hydroxymethyl)hexahydro-1h-pyrrolizin-2-ol	Alkaloids	Pyrrole alkaloids	4.13	2.46	10.19	0.96	0.97	0.98
37	Gentianamine	Alkaloids	Pyridine alkaloids	4.05	2.58	10.47	0.95	0.96	0.98
38	3-Succinoylpyridine	Alkaloids	Pyridine alkaloids	3.93	3.42	13.45	0.92	0.95	0.99
39	Nicotinic Acid Methyl Ester	Alkaloids	Pyridine alkaloids	3.72	2.43	9.05	0.95	0.98	0.96
40	3-ethyl-4-methyl-pyrrole-2,5-dione	Alkaloids	Pyridine alkaloids	3.09	3.72	11.5	0.9	0.97	0.96
41	Indole-3-acetic acid	Alkaloids	Plumerane	2.97	2.57	7.64	0.95	0.96	0.98
42	3-Chloro-4-hydroxypiperidin-2-one	Alkaloids	Piperidine alkaloids	2.97	2.28	6.78	0.94	0.91	0.96
43	Corypalline	Alkaloids	Isoquinoline alkaloids	2.84	2.71	7.69	0.93	0.96	0.97
44	Pilosine	Alkaloids	Alkaloids	2.57	2.03	5.23	0.96	0.95	0.97
45	Mandelamide	Alkaloids	Alkaloids	2.52	3.69	9.29	0.91	0.97	0.98
46	Deoxymutaaspergillic acid	Alkaloids	Alkaloids	2.32	2.3	5.34	0.94	0.95	0.98
47	Lycoperodine-1	Alkaloids	Alkaloids	2.23	2.76	6.15	0.91	0.98	0.97

Note: The base color yellow signifies a beneficial effect, while blue indicates a potentially adverse effect. Green represents a two-sided effect. Unlabeled colors indicate that no relevant reports have been made or that there is no relevant activity in the concentration range tested.

ultimately improving the antioxidant activity of camellia oil.

4. Conclusion

This study introduces an innovative approach that combines ultrasonic pretreatment with drying methods to enhance camellia oilseed processing. For the first time, the synergistic effects of drying kinetics, metabolic profile, and antioxidant capacity were comprehensively investigated. The results demonstrate that UMWD, through the synergistic interaction of ultrasonic cavitation and rapid microwave drying, significantly accelerated water migration, resulting in a 150 % improvement in TPC and antioxidant capacity compared to traditional HAD. Additionally, a widely-targeted metabolomics analysis identified 1,350 metabolites in camellia oils, while WGCNA linked the elevated ABTS, DPPH, and FRAP values in UMWD samples to the upregulation of 215 ACMs. These ACMs were primarily alkaloids (13.77 %), phenolic acids (13.11 %), flavonoids (11.80 %), and terpenoids (10.82 %). Remarkably, many ACCMs showed fold changes exceeding 10 in UMWD/HAD comparisons. These findings underscore the significant enhancement of functional components in camellia oil through UMWD, providing novel insights and strong scientific evidence for more efficient and sustainable oilseed processing methods. This work offers valuable guidance for the development of functional, high-quality oils and promotes advancements in both the food industry and consumer health.

CRediT authorship contribution statement

Qingyang Li: Writing – original draft, Visualization, Investigation, Data curation, Conceptualization. **Maokai Cui:** Visualization, Software, Formal analysis, Data curation. **Jiarong She:** Visualization, Validation, Methodology, Investigation. **Shiman Sun:** Visualization, Investigation, Formal analysis. **Lingyuan Zhou:** Validation, Methodology, Investigation. **Fubin Tang:** Methodology, Investigation, Conceptualization. **Yirong Guo:** Validation, Methodology, Conceptualization. **Yihua Liu:** Writing – review & editing, Visualization, Software, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This study was supported by the “Pioneer” and “Leading Goose” R&D Program of Zhejiang (2023C02045) and the Hunan Forestry Science and Technology Key Research & Innovation Project (XLKJ202302).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ultsonch.2025.107338.

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