Heliyon 10 (2024) e24512

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Effects of different drying methods on *Rubus chingii* Hu fruit during processing

Can Qian [a], Hongfa Li [b], Zhuoni Hou [a,*], Zongsuo Liang [a,**]

[a] *Key Laboratory of Plant Secondary Metabolism and Regulation of Zhejiang Province, College of Life Sciences and Medicine, Zhejiang Sci-Tech University, Hangzhou, 310018, China*
[b] *Hanguang Primary Processing Co., Ltd, Hangzhou, 311700, China*

ARTICLE INFO

Keywords:
Rubus chingii Hu
Drying method
HS-SPME-GC-MS
Physical property
Chemical property

ABSTRACT

In this study, the dried fruits of *Rubus chingii* Hu (Chinese name: Fu-Pen-Zi; FPZ) were processed and dried by three methods—in the shade, the sun, and the oven. The composition regarding the standard ingredient, color, and antioxidant capacities were investigated pro- and post-processing. The technique of headspace–solid-phase-microextraction–gas-chromatography–mass spectrometry (HS-SPME-GC-MS) and flavoromics were used to analyze the flavor-conferring metabolites of FPZ. The results obtained revealed that the highest use value and antioxidant capacities were detected in the FPZ fruits processed and dried in the shade. A total of 358 metabolites were detected from them mainly consisting of terpenoids, heterocyclic compounds, and esters. In differential analysis, the down-regulation of the metabolites was much greater than their up-regulation after all three drying methods. In an evaluation of the characteristic compounds and flavors produced after the three methods, there were variations mainly regarding the green and fruity odors. Therefore, considerable insights may be obtained for the development of novel agricultural methods and applications in the pharmaceutical and cosmetic industries by analyzing and comparing the variations in the chemical composition detected pre- and post-processing of the FPZ fruits. This paper provides a scientific basis for quality control in fruits and their clinical applications.

1. Introduction

Rubus chingii Hu is a perennial woody plant in the Rosaceae family, which is distributed in Japan and the Zhejiang, Jiangxi, and Anhui provinces of China [1]. The Chinese name "Fu-Pen-Zi" (FPZ) refers to the dried fruits of *R. chingii* Hu, which are used as traditional Chinese medicine (TCM) and food [2]. The dried, green fruits of *R. chingii* Hu are used as TCM to treat spermatorrhea, dysuria, impotence, premature ejaculation, and the dimming of the eyes [3]. The red fruits of *R. chingii* Hu can be consumed raw or used to prepare juices, jams, and wine [3]. These fruits are widely used in food [4], cosmetics, agriculture [5,6], and other industries owing to their antioxidant [7], antibacterial, anti-inflammatory [3, 7], anti-aging [8], and other effects. Products containing FPZ extract are sold in health care markets in various countries (Fig. 1A,B,C).

Chinese herbs are affected by the conditions occurring during the concoction process [9], among which the method of drying has a

* Corresponding author. College of Life Sciences and Medicine, Zhejiang Sci-Tech University, Hangzhou, 310018, China.
** Corresponding author. College of Life Sciences and Medicine, Zhejiang Sci-Tech University, Hangzhou, 310018, China.
E-mail addresses: houzhuoni@zstu.edu.cn (Z. Hou), liangzs@ms.iswc.ac.cn (Z. Liang).

https://doi.org/10.1016/j.heliyon.2024.e24512
Received 18 July 2023; Received in revised form 4 January 2024; Accepted 10 January 2024
Available online 14 January 2024

significant impact [10,11]. The drying methods currently used in the processing of Chinese herbs are natural, hot air, infrared, and infrared combined with hot air, vacuum freeze, microwave vacuum, vacuum pulsation, and radio frequency vacuum drying [11,12]. Drying can affect the microstructure [13], color [14], contents of total phenols and flavonoids, antioxidant capacity, and the levels of other bioactive ingredients [15,16].

Ellagic acid (EA) and kaempferol-3-*O*-rutinoside (KR) are index components of FPZ. According to the Pharmacopoeia of the People's Republic of China, the quality standard of EA is not less than 0.20 %, and KR is not less than 0.03 %.EA demonstrates free radical scavenging, hepatoprotective, and immune regulating activities [17]. However, the content of EA in FPZ herbs was usually found to be <0.2 % in preliminary research. The physical properties (e.g., color and flavor) and chemical properties (e.g., bioactive components and antioxidant activity) of FPZ are altered after processing, and studies concerning their effects on quality are not adequate. Various drying methods can have different effects on the flavor-conferring components of herbs or foods, and the levels of certain volatile organic compounds (VOCs) can be reduced and transformed during the process of drying [18,19]. Metabolomics has been widely used in the fields of animal- and plant-environmental interactions, analysis of the composition of medicinal plants, food processing, food identification, etc [20,21]. Among them, the headspace (HS) technique is a convenient and rapid extraction method that in combination with GC-MS plays an important role in the study of flavor-producing compounds in drugs, tea, fermentation products, and meat [22,23]. HS can avoid a tedious pre-treatment step and more effectively reduce the interference generated by organic solvents and analyze the composition, content and odor of volatile substances. Therefore, the HS method was employed to analyze the volatile metabolites during the processing of FPZ. This study provides fundamental insights into the processing of FPZ through the comprehensive analysis of the effects of processing on the color, standard ingredient, antioxidant activity, and flavor of FPZ.

2. Materials and methods

2.1. Plant materials

FPZ was harvested in 2022 at the planting base located in Chun'an County, Zhejiang, China. Plant samples were dried in oven after picking and stored in cool and ventilated place by the Hanguang Primary Processing Company, Chun'an County, Zhejiang, China.

The insect and pest-free FPZ fruit samples (Original) of similar size were exposed to the following treatments: (1) the FPZ samples were piled with distilled water, the water amount is 20 %–25 % of the sample, subject to full infiltration; (2) heap wetting and absorption for 3 h; (3) the oven "Huirun" process: after fully wetting the samples, they were placed in the oven and rewetted in the hot wet state for 45 h. The temperature was adjusted to 40 °C–60 °C. (4) the set drying method: the rewetted samples were dried to a

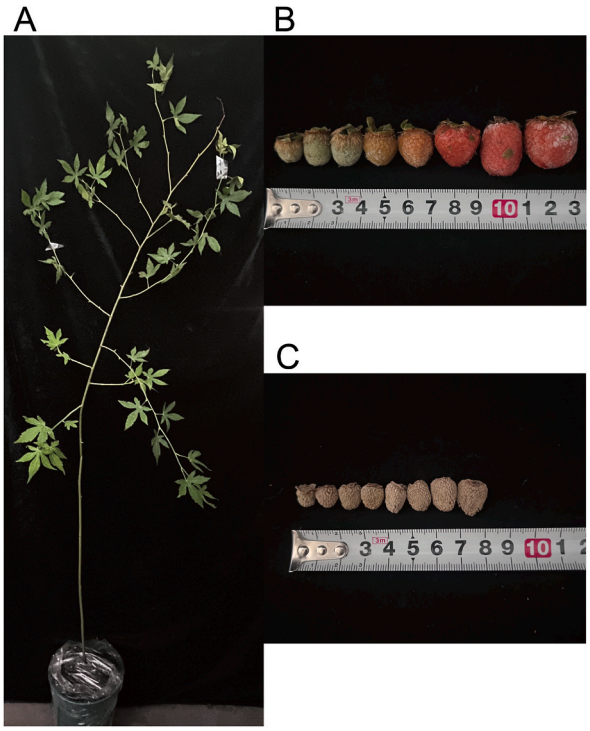

Fig. 1. The plant of *Rubus chingii* Hu (A). The fruits of *Rubus chingii* Hu (B). The dried fruits of *Rubus chingii* Hu (C). Note: Rubus chingii Hu in Journ. Arn. Arb. 6:141. 1925"R. chungii"; Hu et Chun in Icon. Pl. Sin. 2:23. pl. 73. 1929. ——*R. palmatus* (non Thunb.) Hemsl. in Journ. Linn. Soc. 23:234. 1887. p. p. quoad speim. e Ningpo; Ohwi, Fl. Jap. 748. 1978 (new. ed.) — *R. officinalis* Koidz. in Bot Mag. Tokyo. 44:105. 1930. — *Flora of China*.

maximum final moisture level of 12 %. Considering the production and processing conditions, the chosen drying methods were natural drying in the shade (YD) or the sun (SD) and hot-air oven drying (HD) (Fig. 2).

2.2. Reagents and instruments used

The reagents used were: methanol, acetonitrile, and formic acid (chromatographic purity; Fisher Scientific, NH, USA); L-2-chlorophenylalanine (Hengchuang Bio, Shanghai, China); sodium chloride (analytical purity; Sinopharm, Beijing, China); n-hexane (chromatographic purity; Merck, NJ, USA); standards (chromatographic purity; BioBioPha Co. Ltd., Kunming, China or Sigma-Aldrich, MO, USA).

The instruments used were: an MA98 multi-angle spectrophotometer (X-Rite® Inc., MI, USA); an ACQUITY™ ultra-high performance liquid chromatograph [UPLC] (Waters™, Antwerp, Belgium); an ACQUITY™ UPLC BEH C18 liquid chromatography column [2.1 × 100 mm, 1.7 μm] (Waters™, Antwerp, Belgium); a 120 μm divinylbenzene/carboxen/polydimethylsiloxane extraction head (Agilent, CA, USA); a solid-phase microextraction (SPME) device: SPME Arrow (CTC Analytics AG, Zwingen, Switzerland); an 7890B-7000D GC-MS/MS (Agilent, CA, USA); a DB-5MS GC-MS/MS [30 m × 0.25 mm × 0.25 μm] column (Agilent, CA, USA); an aging unit (Fiber Conditioning Station, CTC Analytics AG, Zwingen, Switzerland); a sample heating chamber (Agitator, CTC Analytics AG, Zwingen, Switzerland); a BT25S electronic balance (Sartorius AG, Göttingen, Germany); an F-060SD ultrasonic cleaning machine (Shenzhen Fuyang Technology Group Co. Ltd., Guangdong, China); a TYXH-I vortex oscillator (Shanghai Hannuo Instrument Co. Ltd., Shanghai, China); a TGL-16MS desktop, high-speed, refrigerated centrifuge (Shanghai Luxianyi Centrifuge Instrument Co. Ltd., Shanghai, China); and an MM400 ball mill (Retsch, Düsseldorf, Germany).

2.3. Index determination

The determination of the standard ingredients EA and KR was performed by setting the following parameters in UPLC – column temperature: 35 °C; mobile phases: 0.1 % acetic acid (A), acetonitrile (B); flow rate: 0.4 mL/min; injection volume: 5 μL; detection wavelength: 254 nm, 344 nm; gradient elution: from min 0–2, 94 %→94 % of A; from min 2–3, 94 % →84 % of A; from min 3–8, 84 %→75 % of A; finally from min 8–10, 75 %→94 % of A.

The determination of the color was carried out at the Engineering Research Center of Eco-dyeing and Finishing Technology, Zhejiang University of Technology, Zhejiang, China using a small-aperture model with a light source of D65/10°.

The antioxidant capacity was measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and ferric ion reducing antioxidant potential (FRAP). The antioxidant capacities of the Original, YD, SD, and HD FPZ were measured by using the ABTS, DPPH, and FRAP kits (Kemin Biotechnology Co. Ltd.).

2.4. GC-MS analyses

The samples were ground to a fine powder and 500 mg of the powder was transferred to a 20 mL HS vial (Agilent, CA, USA), containing 2 mL saturated NaCl solution, to reveal more aroma components. The vials were sealed using crimp-top caps with TFE-silicone HS septa (Agilent, CA, USA). For SPME analysis, each vial was incubated at 60 °C for 5 min, then a 120 μm DVB/CAR/PDMS fiber was exposed to the HS of the sample at 60 °C for 15 min.

After sampling, desorption of the VOCs from the fiber coating was carried out in the injection port of the GC apparatus at 250 °C for 5 min in the splitless mode. The identification and quantification of VOCs were carried out using a GC-MS equipped with a 30 m × 0.25 mm × 0.25 μm DB-5MS (5 % phenyl-polymethylsiloxane) capillary column. Helium was used as the carrier gas at a linear velocity of 1.2 mL/min. The injector temperature was maintained at 250 °C and the detector at 280 °C. The temperature of the oven was programmed to maintain at 40 °C for 3.5 min, increase at 10 °C/min up to 100 °C, then at 7 °C/min up to 180 °C, then at 25 °C/min up to 280 °C, and hold for 5 min. The mass spectra were recorded in the electron impact (EI) ionization mode at 70 eV. The quadrupole mass

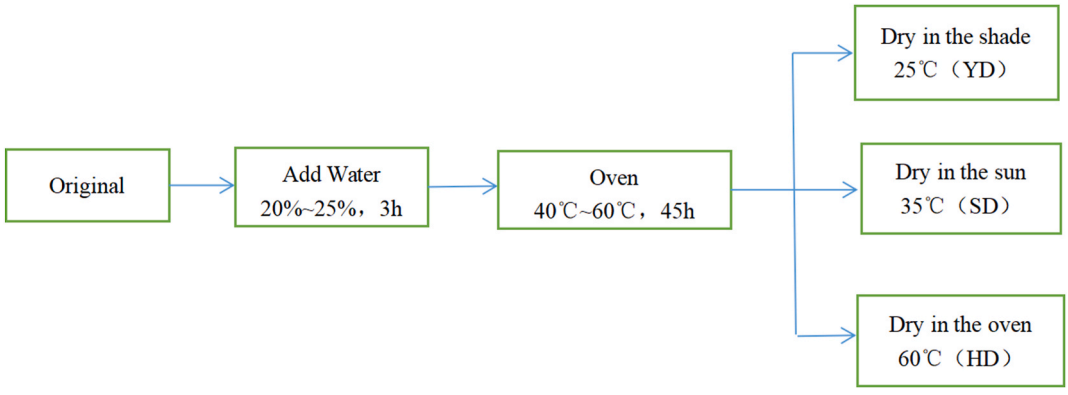

Fig. 2. Three kinds of processing and drying processing of FPZ.

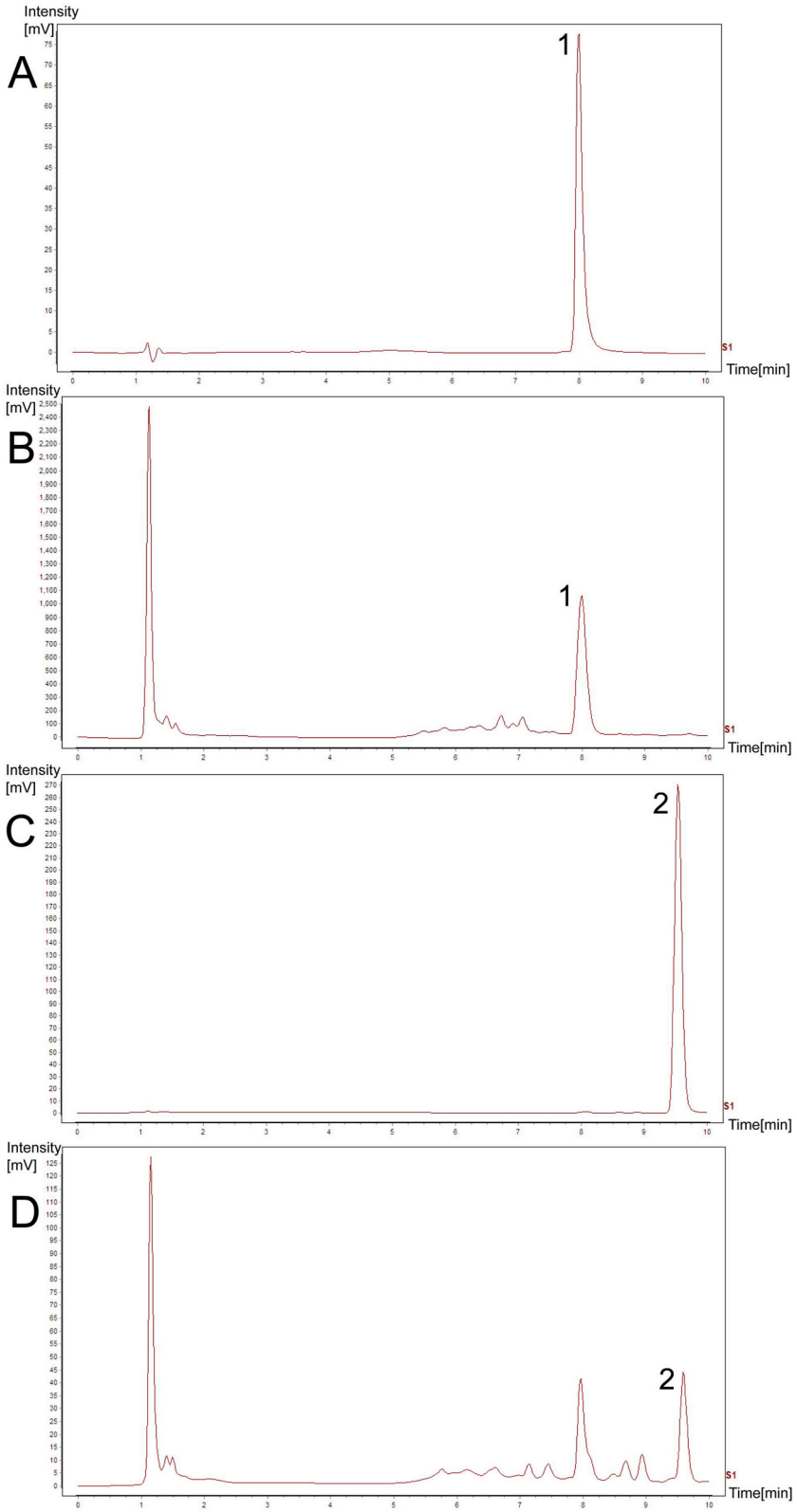

Fig. 3. The EA standard chromatogramand (A). The EA of FPZ chromatogramand (B). The KR standard chromatogramand (C). The KR of FPZ chromatogramand (D). Note: 1: EA, 2: KR.

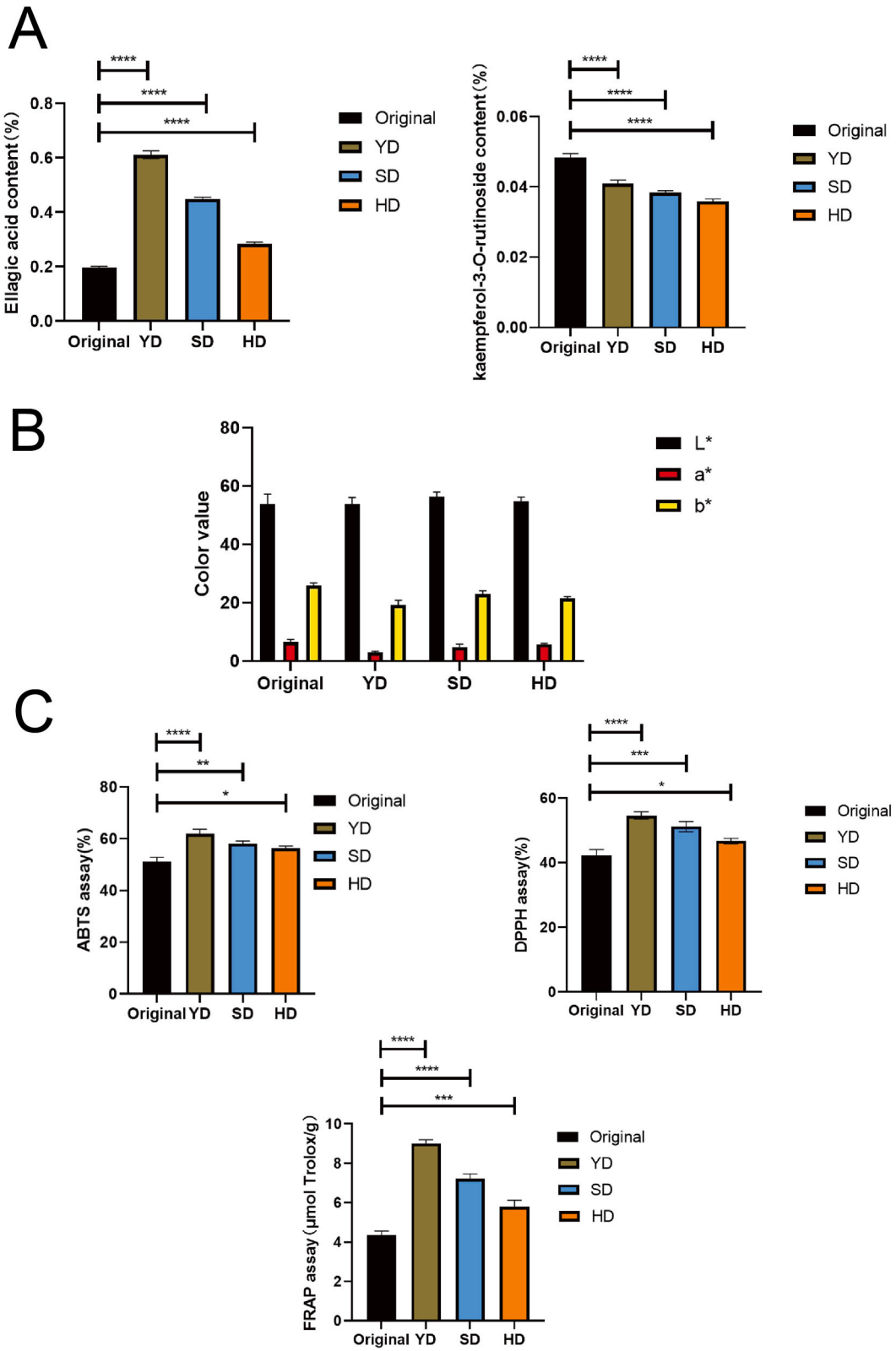

Fig. 4. The EA and KR content of FPZ (A). The color values of FPZ (B). The antioxidant capacity of FPZ(C). Note:*, **, ***and**** indicate significant at $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$ and $p \leq 0.0001$.

detector, ion source, and transfer line temperatures were set at 150, 230, and 280 °C respectively. The MS was set at the selected ion monitoring (SIM) mode and was used for the identification and quantification of the analytes. Identification of volatile compounds was achieved by comparing the mass spectra with the data system library (MWGC V1.2 or NIST20) and linear retention index (Metware Biotechnology Co., Ltd., Wuhan, China).

2.5. Data analyses

MassHunter software version B.08.00 (Agilent) was used to process mass spectrum data. Statistical analyses of all the samples were performed in triplicates using SPSS software version 24.0 (SPSS Inc., Chicago, IL, USA), and image processing was performed using GraphPad Prism software version 9.5 (graphpad.com). Metabolomics data were processed using multivariate statistical analysis methods, including principal component analysis (PCA), hierarchical cluster analysis (HCA), and orthogonal partial least squares discriminant analysis (OPLS-DA). Crucial metabolites identified during the FPZ processing and drying were analyzed by using the variable important in projection (VIP), foldchange (FC), and p-values from the *t*-test were used for the differentially-detected metabolite (DDMs) analysis and the resolution of flavor.

3. Results and discussion

3.1. Standard ingredient determination

Chromatograms of FPZ standard ingredients and samples were obtained in UPLC (Fig. 3A,B,C,D). The average of EA content of the Original FPZ samples was 0.196 %, while they were 0.612 %, 0.449 %, and 0.284 % after the YD, SD, and HD treatments, respectively. Meanwhile, the average of KR content of the Original FPZ samples was 0.048 %, while they were 0.041 %, 0.038 %, and 0.036 % after the YD, SD, and HD treatments, respectively. The EA content significantly improved after processing, which solved the problem of the EA content not meeting the required standard, but the KR content decreased slightly (Fig. 4A).

EA is a dimeric derivative of gallic acid and occurs as highly soluble ellagitannins (ETs) in a variety of nuts and fruits, including raspberries, euphorbia, digitonin, strawberries, and pomegranates [24]. In plants, the ester bonds in the ETs are hydrolyzed to form hexahydroxydiphenol (HHDP) under either acidic or alkaline conditions and then HHDP is lactonized to the EA which is highly insoluble in water [25,26]. The hydrolysis of ETs after the processing of FPZ resulting in the production of EA may be responsible for its increased content.

Flavonol glycosides are the prevalent forms of flavonoids occurring in FPZ, mainly kaempferol and quercetin, and their derivative side-groups which are generally monosaccharides and minute amounts of disaccharides, such as brassinose. Flavonols are unstable and the breakage of the glycosidic bonds may be responsible for the decrease in the contents of KR during the processing and drying steps [27].

3.2. Color determination

The color values L*, a*, and b* pre- and post-processing were measured based on the CIELAB color space. The L* value of the Original FPZ was 53.87, and the* values of the YD, SD, and HD after processing were 53.93, 56.36, and 54.71, respectively. The b* value of the Original FPZ was 25.98, and was 19.29, 22.97, and 21.55 after the YD, SD, and HD processing, respectively (Fig. 4B). It was observed that after the processing of FPZ, the L* value increased, the a* and b* values decreased, the L*, a*, and b* values were different among the three drying methods, and there were certain differences in the color of each group. It can improve the basis of color as a simple line standard for selecting FPZ.

Processing can produce color changes in barley grass [28], sweet pepper purees [29], and tea [30]. The changes in the color of FPZ during processing may be due to alterations in humidity, temperature, and light. The rise and fall of temperature can cause fading and volatilization; the herbs with a very high moisture content can heat up internally, which is conducive to the propagation of microorganisms. The compounds in the herbs can undergo oxidation, decomposition, polymerization, and other such chemical processes under light; especially the pigments can be damaged.

3.3. Antioxidant capacity

The radical scavenging capacity of ABTS was 51.07 %, 61.94 %, 58.11 %, and 56.38 % in the Original, YD, SD, and HD, respectively. For DPPH, it was 42.24 %, 54.64 %, 51.21 %, and 46.70 % in the Original, YD, SD, and HD, respectively. The FRAP yielded similar results. For FRAP, it was 4.35, 9.00, 7.23, and 5.79 μmol Trolox/g in the Original, YD, SD, and HD, respectively. Thus it was observed that processing could improve the antioxidant capacity of FPZ; the efficiencies of the three drying methods were in the order of YD > SD > HD (Fig. 4C).

The content of ETs in FPZ was much higher than those of the other phenolics, which contributed to the antioxidant capacity of FPZ. The two lactone and four hydroxyl groups unique to EA conferred the enhanced free radical scavenging ability of EA [31]. The increased antioxidant capacity of FPZ post-processing may be related to the increased EA content. This was similar to the report in which the shade-drying method was most favorable for retaining the active ingredients in the roots and rhizomes of *Salvia miltiorrhiza* [32]. However, the hot-air oven drying method was superior to shade drying in improving the antioxidant capacity of navel orange peel [33].

In addition to the antioxidant capacity, different drying methods affected the contents of free amino acids and flavor-producing nucleotides in scallops [34]; the titratable acidity and water activity in apple slices [35]; and the chemical composition of polysaccharides from *Auricularia auricula* [36]. Therefore, it is important to choose the appropriate drying method for each herb or food source.

3.4. Metabolite analysis

In this study, metabolomic analysis was performed on FPZ samples (Original, YD, SD, HD). The superposition analysis of the QC – TIC diagram and sample multi-peak detection diagram shows that the metabolome data measured in this study have good repeatability and reliability (Fig. 5A and B). The composition and proportions of metabolites are sample-specific and in addition, can change during

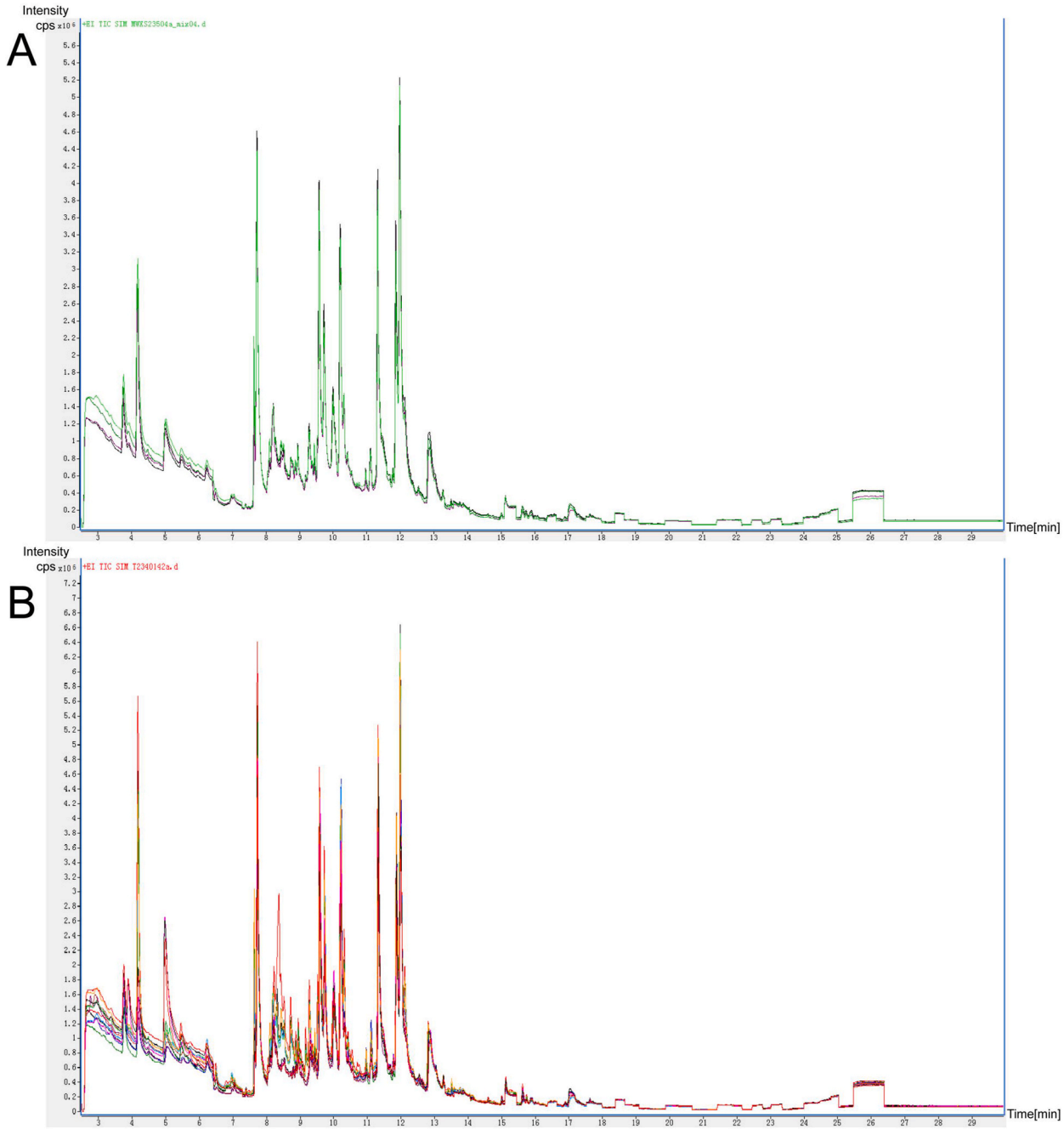

Fig. 5. The QC-TIC diagram (A) and sample multi-peak detection diagram (B).

different physicochemical treatments or biological processes. The analysis of the ratio of the metabolite composition can accurately indicate the distribution of the major metabolites in a sample as a whole. A total of 358 metabolites were detected from FPZ with the three most significant being terpenoids at 20.73 %, heterocyclic compounds at 15.69 %, and esters at 13.45 % (Fig. 6A).

PCA is a multivariate method commonly used to summarize the data variance, highlight the differences between groups, and

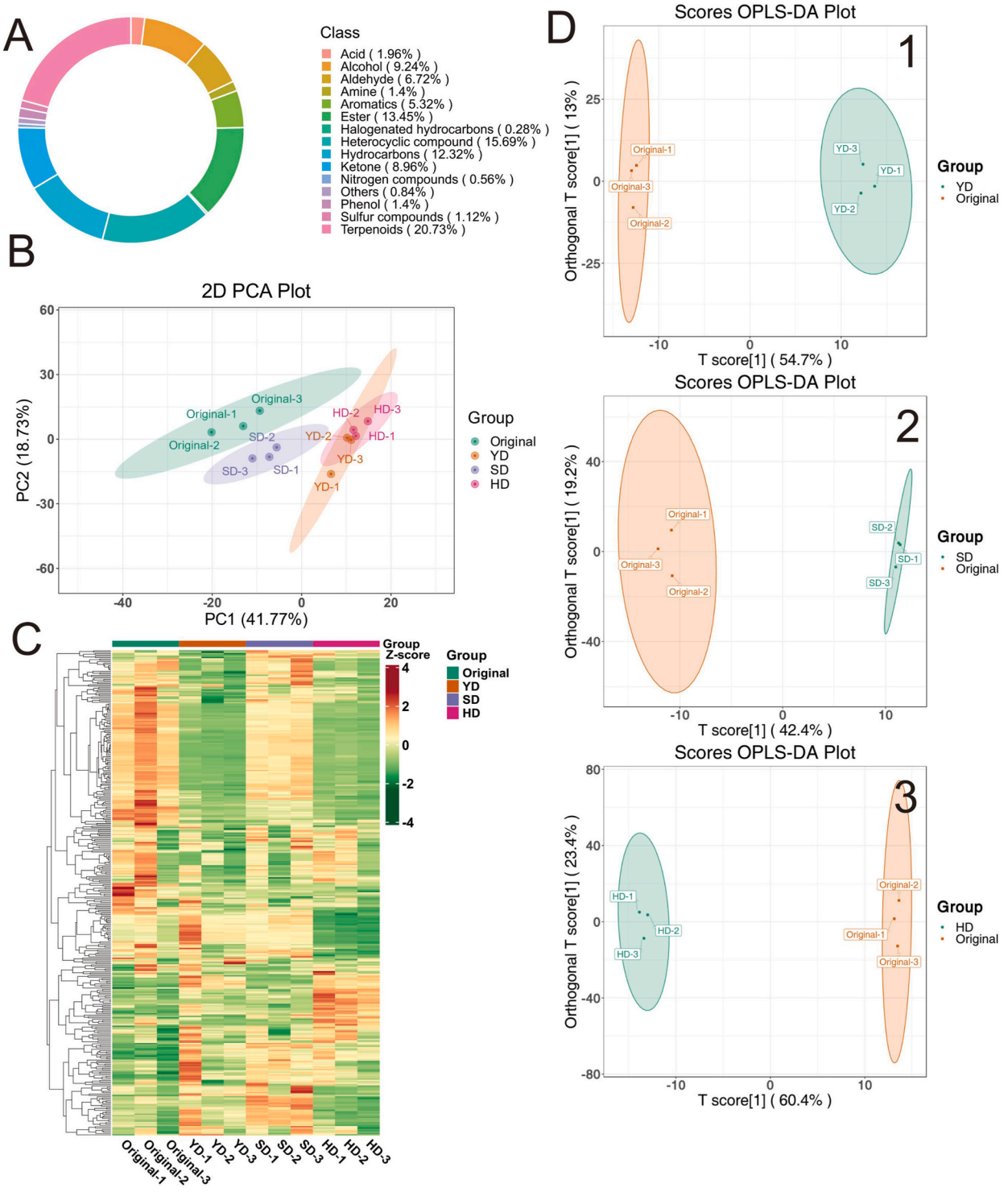

Fig. 6. The composition and proportions of FPZ metabolites (A). Score plots of PCA principal component 1 and principal component 2 (B). HCA of the FPZ metabolites (C). The OPLS-DA between FPZ groups: 1: YD vs Original; 2: SD vs Original; 3: HD vs Original (D).

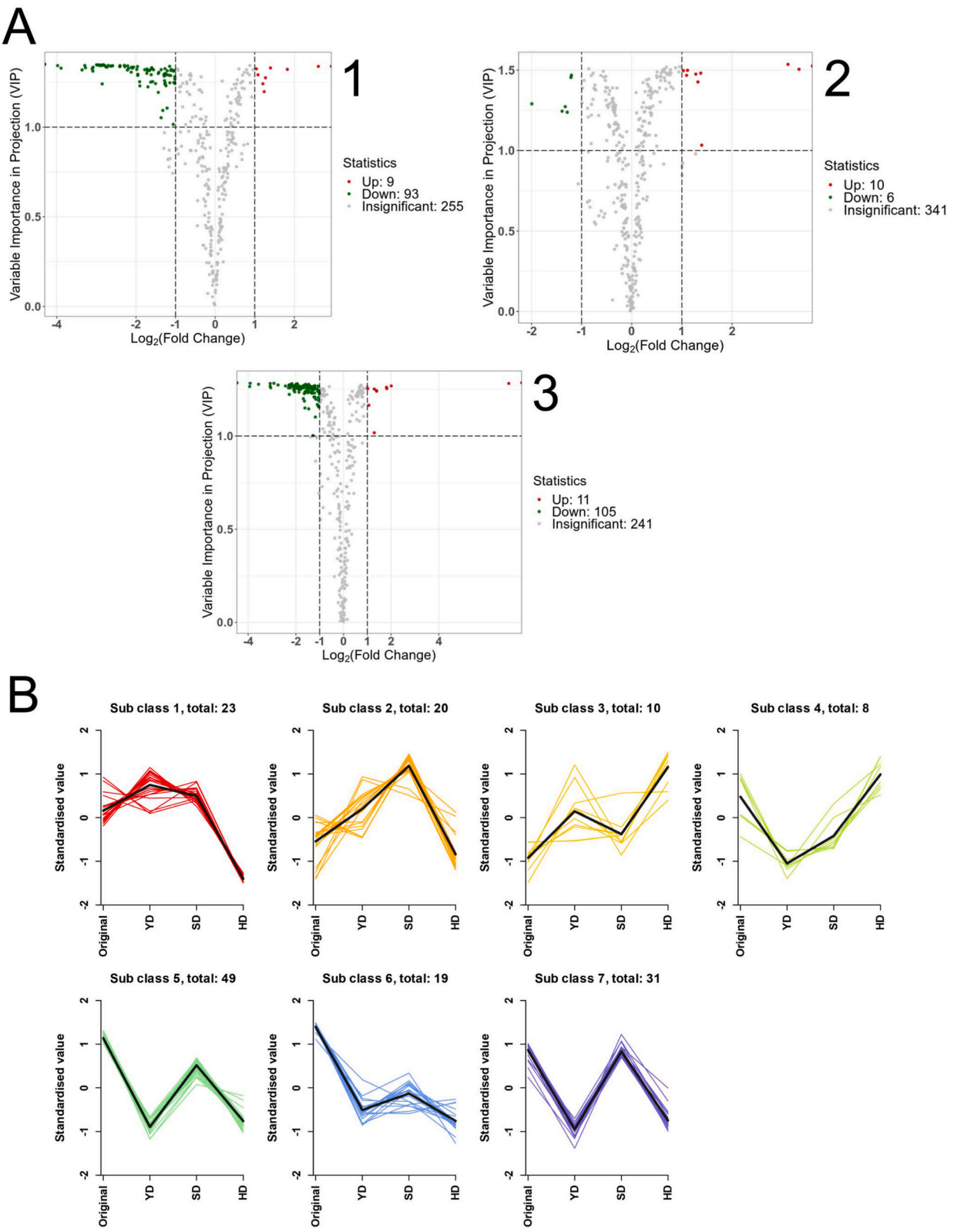

Fig. 7. Volcano plot showing levels of differential FPZ metabolites: 1: YD vs Original; 2; SD vs Original; 3: HD vs Original. Green dots represent down-regulated metabolites, red dots represent up-regulated metabolites, and gray represents metabolites without significant differences (A). K-Means of the relative contents of the FPZ metabolites (B).

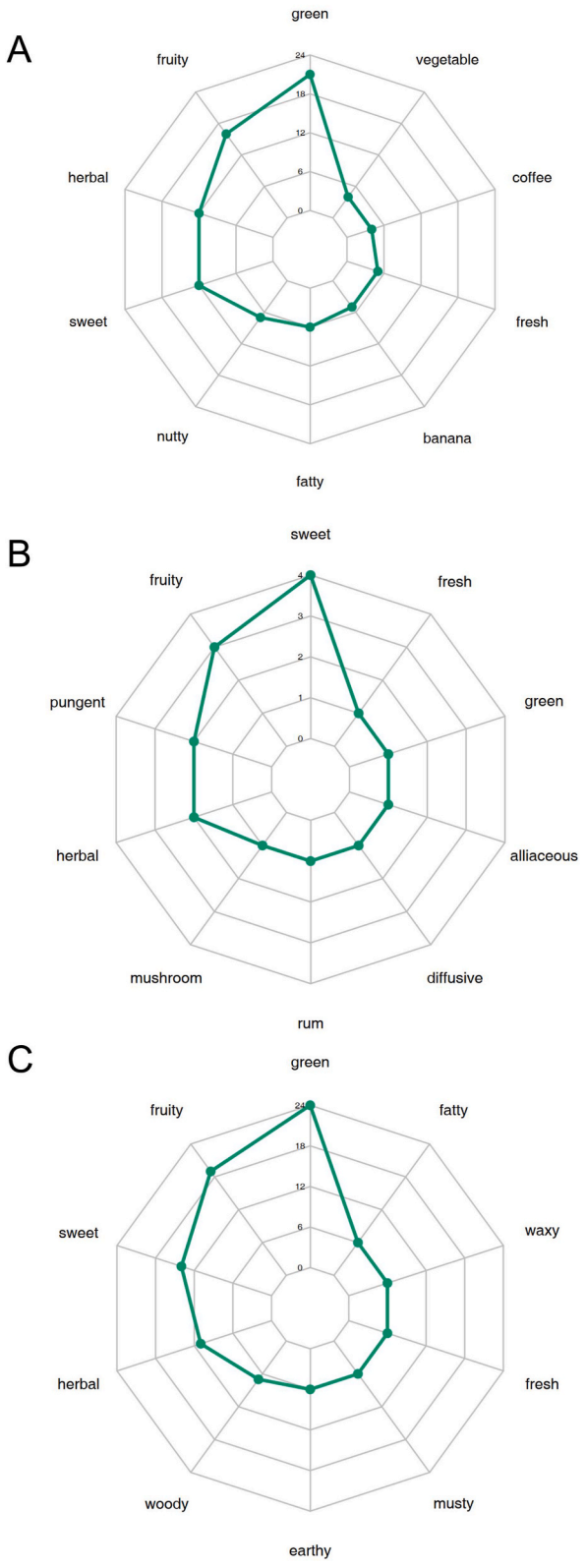

Fig. 8. The radar plotting of the FPZ top ten sensory flavorsin in each comparative group. YD vs Original (A). SD vs Original (B). HD vs Original (C).

measure the sample variability within a group. PC1 and PC2 explained 41.77 % and 18.73 % of the total variance, respectively. Thus, PCA could effectively distinguish between the pre-and post-processed samples, indicating that steps of processing and drying altered the chemical composition of FPZ, with insignificant variations between the replicates, and an acceptable reproducibility. However,

Table 1
The top 10 up VOCs of three drying methods.

Dry method	Formula	Compounds	Class I	CAS	NIST_RI	Odor
YD	C_5H_8O	2-Pentyn-1-ol	Alcohol	6261-22-9	778	–
	$C_{10}H_{20}O_2$	2-Nonanone, 3-(hydroxymethyl)-	Ketone	67801-33-6	1093	fresh, sweet, green, weedy, earthy, herbal
	$C_{10}H_{18}O$	Cyclobutaneethanol, 1-methyl-2-(1-methylethenyl)-, *cis*-	Alcohol	30820-22-5	1183	–
	$C_{13}H_{28}$	Tridecane	Hydrocarbons	629-50-5	1300	alkane
	$C_8H_{12}O$	3,5-Octadien-2-one	Ketone	38284-27-4	1091	fruity, fatty, mushroom
	C_5H_8O	2-Butenal, 3-methyl-	Aldehyde	107-86-8	782	sweet, fruity, pungent, brown, nutty, almond, cherry
	$C_7H_8O_2$	2-methoxy-Phenol	Phenol	90-05-1	1089	nutty
	C_6H_6OS	Ethanone, 1-(2-thienyl)-	Heterocyclic compound	88-15-3	1092	sulfurous, nutty, hazelnut, walnut
	C_8H_{10}	Benzene, 1,3-dimethyl	Aromatics	108-38-3	866	plastic
	$C_{10}H_8$	Naphthalene	Aromatics	91-20-3	1182	pungent, dry, tarry
SD	C_5H_8O	2-Pentyn-1-ol	Alcohol	6261-22-9	778	–
	C_5H_8O	2-Butenal, 3-methyl-	Aldehyde	107-86-8	782	sweet, fruity, pungent, brown, nutty, almond, cherry
	$C_{10}H_{20}O_2$	2-Nonanone, 3-(hydroxymethyl)-	Ketone	67801-33-6	1093	fresh, sweet, green, weedy, earthy, herbal
	$C_{11}H_{14}O_2$	p-Tolyl isobutyrate	Ester	103-93-5	1290	aromatic, fruity, phenolicnote
	$C_8H_{12}O$	3,5-Octadien-2-one	Ketone	38284-27-4	1091	fruity, fatty, mushroom
	$C_{10}H_{18}O$	(1.alpha.,2.beta.,5.alpha.)-2-methyl-5-(1-methylethenyl)-Cyclohexanol	Terpenoids	38049-26-2	1192	–
	$C_{13}H_{28}$	Tridecane	Hydrocarbons	629-50-5	1300	alkane
	$C_{10}H_{18}O$	3-Cyclohexene-1-ethanol, .beta.,4-dimethyl	Alcohol	18479-68-0	1295	fruity, herbal
	$C_{14}H_{30}$	Dodecane, 4,6-dimethyl-	Hydrocarbons	61141-72-8	1325	–
	$C_6H_6N_2O2$	2-Picoline, 6-nitro	Heterocyclic compound	18368-61-1	1183	–
HD	C_5H_8O	2-Pentyn-1-ol	Alcohol	6261-22-9	778	–
	C_5H_8O	2-Butenal, 3-methyl-	Aldehyde	107-86-8	782	sweet, fruity, pungent, brown, nutty, almond, cherry
	C_8H_8	Styrene	Aromatics	100-42-5	893	penetrating, balsamic, gasoline
	$C_{10}H_{20}O_2$	2-Nonanone, 3-(hydroxymethyl)-	Ketone	67801-33-6	1093	fresh, sweet, green, weedy, earthy, herbal
	$C_7H_8O_2$	2-methoxy-Phenol	Phenol	90-05-1	1089	nutty
	$C_8H_{12}O$	3,5-Octadien-2-one	Ketone	38284-27-4	1091	fruity, fatty, mushroom
	$C_{10}H_{18}O$	Cyclobutaneethanol, 1-methyl-2-(1-methylethenyl)-, *cis*-	Alcohol	30820-22-5	1183	–
	$C_{11}H_{24}O$	6-Undecanol	Alcohol	23708-56-7	1277	–
	$C_5H_4O_2$	3-FurAldehyde	Heterocyclic compound	498-60-2	831	–
	$C_{15}H_{24}$	1H-3a,7-Methanoazulene, octahydro-3,8,8-trimethyl-6-methylene-, [3R-(3.alpha.,3a.beta.,7.beta.,8a.alpha.)]-	Terpenoids	546-28-1	1421	–

- No-identified.
Odor description were obtained from literature data (http://www.flavornet.org./flavornet.html and http://www.odour.org.uk/).

overlap existed between the YD and HD samples, indicating that both the drying methods similarly affected the composition of FPZ (Fig. 6B).

HCA of the metabolites also clearly indicated the consistency between the replicates and the variations detected pre-and post-processing of FPZ (Fig. 6C). The OPLS-DA analysis of the maximization of variation between the groups of FPZ facilitated the screening of the DDMs after the processing of FPZ using three drying methods (Fig. 6D1,2,3). The OPLS-DA model was used to analyze the levels of all 358 metabolites detected in the processing and drying of FPZ. The comparative Q2 values of all the groups exceeded 0.9, proving that the model was stable and reliable for further screening of the DDMs. The YD, SD, and HD samples on the left could be clearly distinguished from the Original samples on the right, indicating that the raw FPZ was significantly different from the processed and dried FPZ.

3.5. Differential metabolite analysis using volcano plots and K-means

A volcano plot was used to indicate the differences in the relative contents of metabolites in the two groups and determine their statistical significance. The metabolites with a VIP value > 1 and a fold change ≥ 2.0 or fold change ≤ 0.5 were selected. The More metabolites were down-regulated in YD and HD than those up-regulated, while it was the opposite in SD indicating significant variations in the DDMs caused by the three drying methods (Fig. 7A1,2,3).

To further investigate these trends, the relative contents of the metabolites were subjected to unit-variance scaling (UVS) followed by k-means clustering algorithm (K-Means) clustering. Post-processing, the highest relative content of YD was in subclass 1; SD was in subclasses 2 and 7; and HD was in subclasses 3 and 4 (Fig. 7B).

3.6. Flavoromics

The aroma produced post-processing is closely associated with the quality. The composition of the aroma can objectively reflect the flavor characteristics of the different samples and is an important index for evaluating flavor quality [37]. The sensory analysis of the DDMs can be used to ascertain the sensory flavor characteristics in the samples.

Based on the MWGC and NIST data system library, the determined retention time (RT) and qualitative and quantitative ions were selected for precise scanning in the selective ion detection mode. One quantitative ion and two to three qualitative ions were selected for each compound respectively. The top ten sensory flavors with the highest number of annotations were selected for the radar plotting based on the sensory flavor characteristics of the DDMs and the annotated sensory flavor characteristics in each comparative group (Fig. 8A,B,C). Overall, processing had the greatest impact on the green and fruity odors. HD and YD concentrated a significantly higher number of compounds than SD. The significant odors specific to YD were nutty and banana-, coffee-, and vegetable-like while those specific to HD were woody, earthy, musty, and waxy.

The top 10 up VOCs of three drying methods were selected according to DDMs screening criteria (Table 1). The metabolite upregulated after processing and drying were 2-nonanone,3-(hydroxymethyl)- in YD which could be used as a major flavoring agent for producing the green, herbal, sweet, and fresh odors; 2-butenel, 3-methyl in SD that could be used as a major flavoring agent for producing the green, fruity, and pungent odors; and ionone and 3,5-octadiene-2-one in HD that could be used as major flavoring compounds, affecting the sweet, woody, and fatty odors.

Most of the green odor in foods is produced by the metabolism of the polyunsaturated fatty acids through the lipoxygenase pathway [38]. It has therapeutic and preventive effects on depression in rats and demonstrates certain anti-stress effects in humans [39]. However, it can be rejected by consumers of Western countries in soy products due to its green color and fishy taste, and hence the green odor needs to be controlled. The fruity odor was widely prevalent in yellow tamarillo fruit [40] and wine and can be enhanced by the lipase treatment of essential oils in the citrus, which has a certain antibacterial activity. Therefore, green and fruity odors in FPZ post-processing may relate to these biological activities.

The nutty odor was detected in peeled walnut kernels, black ripe olives [41], fresh tea leaves picked in the summer, and during the processing of large-leaf yellow tea using old fire roasting [42]. The banana-like odor was detected in mature coconut water reprocessed into coconut water vinegar [43]. The coffee odor can improve abnormal olfactory behaviors in heterozygous oxytocin receptor knock-in micewhile [44] while the vegetable-like was detected in bell peppers, green tea and *Eriocheir sinensis*. The woody odor was observed in grapefruit, orange, apple, and mango. Similarly, deep fermentation was effective to enhance woody odor of Wuyi rock tea. A waxy odor was observed in wheat and beeswax while the earthy and musty odors were more sharply associated with microorganisms.

Similar techniques and compound results were reported in a study of the VOCs detected in *Rubus*. GC–MS and gas-chromatography–olfactometry were used todetermine and identify the *Rubus idaeus* honey odor-active compounds. *Rubus idaeus* honeys had more "green'' notes. The changes in the levels of the free and bound VOCs during the ripening of the fruits of *Rubus corchorifolius* were determined by HS-SPME-GC-MS. Hexanal, 2-heptanone, ethyl hexanoate, 4-terpineol, geranial and methyleugenol were identified as the characteristic free aroma compounds of ripened red fruit. The final aroma of the fruits at all stages of ripening was mainly attributable to free aroma compounds, including β-damascenes, hexenal, 2-hexenal, and linalool.

There are some new techniques and analyses that can be further applied in future studies. The major aroma-associated active compounds in the clear red raspberry juice were identified through a combined strategy consisting of the quantitative descriptive analysis and an e-nose, with floral and herbaceous flavors dominating the overall aroma [45]. The proton transfer reaction time-of-flight mass spectrometry (PTR-ToF-MS) technique has also been applied for the assessment of the VOCs of raspberry. Combined with SPME-GC-MS and PTR-ToF-MS evaluations was used to study the complexity and obtain the composition of the aromas of

raspberries at the fruit ripening stages of pink, ripe, and overripe [46]. Statistical analysis has been used to evaluate the dispersion of quantitative data in HS-SPME-GC-MS. It is verified in *Rubus ulmifolius* Schott analysis [47].

4. Conclusions

In this study, the three processing and drying methods of YD, SD, and HD were selected considering their advantages of easy productivity and high-volume operability. The color, standard ingredient, antioxidant capacity, and flavor of FPZ pro- and post-processing and drying were compared and characterized; they were observed to be different. The contents of KR slightly decreased, those of EA significantly increased and antioxidant capacity significantly increased after the processing of FPZ. Among them, YD had the best effect and had a certain impact on color. The methods imparted varied flavors to the FPZ, and unique flavor-producing compounds were selected through detection using HS-SPME-GC-MS combined with flavoromics. The processing and drying steps greatly impacted the green and fruity odors; of which HD and YD led to a more significant enhancement in the number of compounds than SD. The significant odors specific to YD were nutty and banana-, coffee-, and vegetable-like; while those specific to HD were woody, earthy, musty, and waxy. The compounds 2-nonanone,3-(hydroxymethyl)-; 2-butenel,3-methyl-; ionone; and 3,5-octadiene-2-one can be significant flavor-producing substances. The results of this study helped in selecting the suitable processing methods for FPZ to obtain the natural antioxidants and supplementation with extracted compounds for a high commercial value and a special flavor. However, more detailed biochemical and biophysical studies are needed to further elucidate the specific mechanisms that induce changes in FPZ.

Data availability statement

Data will be made available on request. Mendeley Data, V1, https://doi.org/10.17632/wcxcjnw927.1. Mendeley Data, V1, https://doi.org/10.17632/jrvyxhjyzx.1.

Additional information

No additional information is available for this paper.

CRediT authorship contribution statement

Can Qian: Writing – original draft, Visualization, Validation, Software, Conceptualization. **Hongfa Li:** Methodology, Investigation. **Zhuoni Hou:** Writing – review & editing, Resources. **Zongsuo Liang:** Writing – review & editing, Project administration, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Zongsuo Liang reports financial support was provided by Shaanxi Province Key Research and Development Projects. Zongsuo Liang reports financial support was provided by The Major Science and Technology Projects of Breeding New Varieties of Agriculture in Zhejiang Province. Zhuoni Hou reports financial support was provided by Zhejiang Provincial Department of Agriculture and Rural Affairs - Major technology collaborative promotion plan project - General project. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by Shaanxi Province Key R&D Project (2019TSLSF02-02, 2020ZDLSF05-02), The Major Science and Technology Projects of Breeding New Varieties of Agriculture in Zhejiang Province (2021C02074), Fundamental Research Funds of Zhejiang Sci-Tech University (2022Q024, 23042097-Y) and Zhejiang Provincial Department of Agriculture and Rural Affairs - Major technology collaborative promotion plan project - General project (2022XTTGZYC01-2). The funder had no role in data collection and analysis, decision to publish or preparation of the manuscript. We thank Bullet Edits Limited for the linguistic editing and proofreading of the manuscript.

References

[1] L. Wang, T. Lei, G. Han, J. Yue, X. Zhang, Q. Yang, H. Ruan, C. Gu, Q. Zhang, T. Qian, N. Zhang, W. Qian, Q. Wang, X. Pang, Y. Shu, L. Gao, Y. Wang, The chromosome-scale reference genome of *Rubus chingii* Hu provides insight into the biosynthetic pathway of hydrolyzable tannins, Plant J. 107 (2021) 1466–1477, https://doi.org/10.1111/tpj.15394.

[2] B. He, L. Dai, L. Jin, Y. Liu, X. Li, M. Luo, Z. Wang, G. Kai, Bioactive components, pharmacological effects, and drug development of traditional herbal medicine *Rubus chingii* Hu (Fu-Pen-Zi), Front Nutr 9 (2023 Jan 9) 1052504, https://doi.org/10.3389/fnut.2022.1052504.

[3] Z. Liu, Z. Mi, N. Han, J. Zhai, S. Li, J. Yin, Diterpenoid glucosides with anti-inflammatory activity from Rubi Fructus, Fitoterapia 164 (2023) 105325, https://doi.org/10.1016/j.fitote.2022.105325.

[4] T. Popović, B. Šarić, J.D. Martačić, A. Arsić, P. Jovanov, E. Stokić, A. Mišan, A. Mandić, Potential health benefits of blueberry and raspberry pomace as functional food ingredients: dietetic intervention study on healthy women volunteers, Front. Nutr. 9 (2022) 969996, https://doi.org/10.3389/fnut.2022.969996.

[5] J.-Y. Sheng, S.-Q. Wang, K.-H. Liu, B. Zhu, Q.-Y. Zhang, L.-P. Qin, J.-J. Wu, *Rubus chingii* Hu: an overview of botany, traditional uses, phytochemistry, and pharmacology, Chin. J. Nat. Med. 18 (2020) 401–416, https://doi.org/10.1016/S1875-5364(20)30048-0.

[6] G. Yu, Z. Luo, W. Wang, Y. Li, Y. Zhou, Y. Shi, *Rubus chingii* Hu: a review of the phytochemistry and pharmacology, Front. Pharmacol. 10 (2019) 799, https://doi.org/10.3389/fphar.2019.00799.

[7] Y. Kong, Y. Hu, J. Li, J. Cai, Y. Qiu, C. Dong, Anti-inflammatory effect of a novel pectin polysaccharide from *Rubus chingii* Hu on colitis mice, Front. Nutr. 9 (2022) 868657, https://doi.org/10.3389/fnut.2022.868657.

[8] H. Zeng, Z. Liu, Y. Wang, D. Yang, R. Yang, L. Qu, Studies on the anti-aging activity of a glycoprotein isolated from Fupenzi (*Rubus chingii* Hu.) and its regulation on klotho gene expression in mice kidney, Int. J. Biol. Macromol. 119 (2018) 470–476, https://doi.org/10.1016/j.ijbiomac.2018.07.157.

[9] J. Ma, Y. Deng, Y. Wang, Q. Liu, J. An, M. Li, N. Song, J. Zhang, L. Cheng, K. Ma, A comparative study on ingredient and efficiency difference between fresh and steamed *Gastrodia elata* Blume: an herbal material to a novel functional food, J. Funct.Foods 82 (5) (2021) 104512, https://doi.org/10.1016/j.jff.2021.104512.

[10] J. Qiao, G. Lu, G. Wu, H. Liu, W. Wang, T. Zhang, G. Xie, M. Qin, Influence of different pretreatments and drying methods on the chemical compositions and bioactivities of Smilacis Glabrae Rhizoma, Chin. Med. 17 (2022) 54, https://doi.org/10.1186/s13020-022-00614-7.

[11] L. Zhu, M. Li, W. Yang, J. Zhang, X. Yang, Q. Zhang, H. Wang, Effects of different drying methods on drying characteristics and quality of *Glycyrrhiza uralensis* (Licorice), Foods 12 (2023) 1652, https://doi.org/10.3390/foods12081652.

[12] M. Zhao, L. Li, W. Cao, Z. Wang, Q. Chu, B. Bhandari, G. Ren, X. Duan, Effects of different drying methods on the properties, stability, and controlled release of *Cornus officinalis* flavonoids microparticles, J. Food Sci. 88 (2023) 2313–2324, https://doi.org/10.1111/1750-3841.16611.

[13] J.-W. Bai, Y.-C. Wang, J.-R. Cai, L. Zhang, Y. Dai, X.-Y. Tian, H.-W. Xiao, Three-dimensional appearance and physicochemical properties of *Pleurotus eryngii* under different drying methods, Foods 12 (2023) 1999, https://doi.org/10.3390/foods12101999.

[14] Z. Geng, L. Zhu, J. Wang, X. Yu, M. Li, W. Yang, B. Hu, Q. Zhang, X. Yang, Drying sea buckthorn berries (*Hippophae rhamnoides* L.): effects of different drying methods on drying kinetics, physicochemical properties, and microstructure, Front. Nutr. 10 (2023) 1106009, https://doi.org/10.3389/fnut.2023.1106009.

[15] Q. Shen, Z. He, Y. Ding, L. Sun, Effect of different drying methods on the quality and nonvolatile flavor components of *Oudemansiella raphanipes*, Foods 12 (2023) 676, https://doi.org/10.3390/foods12030676.

[16] P. Wang, Y. Li, R. Yu, D. Huang, S. Chen, S. Zhu, Effects of different drying methods on the selenium bioaccessibility and antioxidant activity of *Cardamine violifolia*, Foods 12 (2023) 758, https://doi.org/10.3390/foods12040758.

[17] M. Larrosa, M.T. García-Conesa, J.C. Espín, F.A. Tomás-Barberán, Ellagitannins, ellagic acid and vascular health, Mol. Aspect. Med. 31 (2010) 513–539, https://doi.org/10.1016/j.mam.2010.09.005.

[18] N.H. Juhari, H.J. Martens, M.A. Petersen, Changes in physicochemical properties and volatile compounds of roselle (*Hibiscus sabdariffa* L.) calyx during different drying methods, Molecules 26 (2021) 6260, https://doi.org/10.3390/molecules26206260.

[19] H. Qin, T. Yang, S. Yang, M. Yang, Y. Wang, J. Zhang, Effects of different pre-drying and drying methods on volatile compounds in the pericarp and kernel of *Amomum tsao-ko*, Front. Plant Sci. 13 (2022) 803776, https://doi.org/10.3389/fpls.2022.803776.

[20] H. Li, W. Geng, S.A. Haruna, C. Zhou, Y. Wang, Q. Ouyang, Q. Chen, Identification of characteristic volatiles and metabolomic pathway during pork storage using HS-SPME-GC/MS coupled with multivariate analysis, Food Chem. 373 (2022) 131431, https://doi.org/10.1016/j.foodchem.2021.131431.

[21] P. Xia, Q. Li, Z. Liang, X. Zhang, K. Yan, Spaceflight breeding could improve the volatile constituents of *Andrographis paniculata*, Ind. Crops Prod. 171 (2021) 113967, https://doi.org/10.1016/j.indcrop.2021.113967.

[22] S.-H. Chang, H.-Y. Ho, C.-C. Chang, C.-Z. Zang, Y.-H. Hsu, M.-C. Lin, S.-H. Tseng, D.-Y. Wang, Evaluation and optimization of a HS-SPME-assisted GC-MS/MS method for monitoring nitrosamine impurities in diverse pharmaceuticals, J. Pharm. Biomed. Anal. 221 (2022) 115003, https://doi.org/10.1016/j.jpba.2022.115003.

[23] S. Liao, J. Han, C. Jiang, B. Zhou, Z. Jiang, J. Tang, W. Ding, Z. Che, H. Lin, HS-SPME-GC × GC/MS combined with multivariate statistics analysis to investigate the flavor formation mechanism of tank-fermented broad bean paste, Food Chem. X 17 (2023) 100556, https://doi.org/10.1016/j.fochx.2022.100556.

[24] M. Sharma, L. Li, J. Celver, C. Killian, A. Kovoor, N.P. Seeram, Effects of fruit ellagitannin extracts, ellagic acid, and their colonic metabolite, urolithin A, on wnt signaling, J. Agric. Food Chem. 58 (2010) 3965–3969, https://doi.org/10.1021/jf902857v.

[25] K.S. Feldman, Recent progress in ellagitannin chemistry, Phytochemistry 66 (2005) 1984–2000, https://doi.org/10.1016/j.phytochem.2004.11.015.

[26] J.-L. Ríos, R. Giner, M. Marín, M. Recio, A pharmacological update of ellagic acid, Planta Med. 84 (2018) 1068–1093, https://doi.org/10.1055/a-0633-9492.

[27] N. Yang, R. Qiu, S. Yang, K. Zhou, C. Wang, S. Ou, J. Zheng, Influences of stir-frying and baking on flavonoid profile, antioxidant property, and hydroxymethylfurfural formation during preparation of blueberry-filled pastries, Food Chem. 287 (2019) 167–175, https://doi.org/10.1016/j.foodchem.2019.02.053.

[28] W. Zhou, X. Cao, MdN. Islam, H. Zheng, J. Li, F. Liu, Y. Cao, Y. Dai, Comparison of hydrability, antioxidants, microstructure, and sensory quality of barley grass powder using ultra-microcrushing combined with hot air and freeze drying, Food Sci. Nutr. 9 (2021) 1870–1880, https://doi.org/10.1002/fsn3.2138.

[29] R. Kaur, K. Kaur, Effect of processing on color, rheology and bioactive compounds of different sweet pepper purees, Plant Foods Hum. Nutr. 75 (2020) 369–375, https://doi.org/10.1007/s11130-020-00824-0.

[30] O.T. Balaban, A. Kamiloğlu, H.H. Kara, Changes of some bioactive and physicochemical properties during the black tea processing, J. Food Sci. 87 (2022) 2474–2483, https://doi.org/10.1111/1750-3841.16151.

[31] J. Zhong, Y. Wang, C. Li, Q. Yu, J. Xie, R. Dong, Y. Xie, B. Li, J. Tian, Y. Chen, Natural variation on free, esterified, glycosylated and insoluble-bound phenolics of *Rubus chingii* Hu: correlation between phenolic constituents and antioxidant activities, Food Res. Int. 162 (2022) 112043, https://doi.org/10.1016/j.foodres.2022.112043.

[32] L. Zhang, X. Zhang, N. Begum, P. Xia, J. Liu, Z. Liang, Effects of different processing methods based on different drying conditions on the active ingredients of *Salvia miltiorrhiza* bunge, Molecules 27 (2022) 4860, https://doi.org/10.3390/molecules27154860.

[33] C. Lai, Y. Liang, L. Zhang, J. Huang, K. Kaliaperumal, Y. Jiang, J. Zhang, Variations of bioactive phytochemicals and antioxidant capacity of navel orange peel in response to different drying methods, Antioxidants 11 (2022) 1543, https://doi.org/10.3390/antiox11081543.

[34] Z. Chen, Y. Zhu, W. Cao, L. Zhou, C. Zhang, X. Qin, H. Zheng, H. Lin, J. Gao, Novel insight into the role of processing stages in nutritional components changes and characteristic flavors formation of noble scallop *Chlamys nobilis* adductors, Food Chem. 378 (2022) 132049, https://doi.org/10.1016/j.foodchem.2022.132049.

[35] O. Kahraman, A. Malvandi, L. Vargas, H. Feng, Drying characteristics and quality attributes of apple slices dried by a non-thermal ultrasonic contact drying method, Ultrason. Sonochem. 73 (2021) 105510, https://doi.org/10.1016/j.ultsonch.2021.105510.

[36] Y. Zhang, Q. Shi, W. Jiang, J. Yao, J. Zeng, W. Wang, Y. Zhang, Comparison of the chemical composition and antioxidant stress ability of polysaccharides from *Auricularia auricula* under different drying methods, Food Funct. 13 (2022) 2938–2951, https://doi.org/10.1039/D1FO03956C.

[37] M. Flaig, S. Qi, G. Wei, X. Yang, P. Schieberle, Characterization of the Key odorants in a high-grade Chinese green tea beverage (*Camellia sinensis; Jingshan cha*) by means of the sensomics approach and elucidation of odorant changes in tea leaves caused by the tea manufacturing process, J. Agric. Food Chem. 68 (18) (2020) 5168–5179, https://doi.org/10.1021/acs.jafc.0c01300.

[38] N.J. Salas, C. Sa, D.L. Garci, N. Aparicio, Impact of the suppression of lipoxygenase and hydroperoxide lyase on the quality of the green odor in green leaves, J. Agric. Food Chem. 53 (5) (2005 Mar 9) 1648–1655, https://doi.org/10.1021/jf040331l.

[39] T. Watanabe, M. Fujihara, E. Murakami, M. Miyoshi, Y. Tanaka, S. Koba, H. Tachibana, Green odor and depressive-like state in rats: toward an evidence-based alternative medicine? Behav. Brain Res. 224 (2) (2011 Oct 31) 290–306, https://doi.org/10.1016/j.bbr.2011.06.001.

[40] JM. García, LJ. Prieto, A. Guevara, D. Malagon, C. Osorio, Chemical studies of yellow Tamarillo (Solanum betaceum Cav.) fruit flavor by using a molecular sensory approach, Molecules 21 (12) (2016 Dec 16) 1729, https://doi.org/10.3390/molecules21121729.

[41] A.H. Sánchez, Aroma profile and volatile composition of black ripe olives (Manzanilla and Hojiblanca cultivars), Food Res. Int. 127 (2020 Jan) 108733, https://doi.org/10.1016/j.foodres.2019.108733.
[42] X. Guo, CT. Ho, W. Schwab, X. Wan, Effect of the roasting degree on flavor quality of large-leaf yellow tea, Food Chem. 347 (2021 Jun 15) 129016, https://doi.org/10.1016/j.foodchem.
[43] K. Osada, R. Kujirai, A. Hosono, M. Tsuda, M. Ohata, T. Ohta, K. Nishimori, Repeated exposure to kairomone-containing coffee odor improves abnormal olfactory behaviors in heterozygous oxytocin receptor knock-in mice, Front. Behav. Neurosci. 16 (2023) 983421, https://doi.org/10.3389/fnbeh.2022.983421.
[44] K. Osada, R. Kujirai, A. Hosono, M. Tsuda, M. Ohata, T. Ohta, K. Nishimori, Repeated exposure to kairomone-containing coffee odor improves abnormal olfactory behaviors in heterozygous oxytocin receptor knock-in mice, Front Behav. Neurosci. 16 (2023 Feb 1) 983421, https://doi.org/10.3389/fnbeh.2022.983421.
[45] B. Farneti, I. Khomenko, M. Ajelli, K.E. Wells, E. Betta, E. Aprea, L. Giongo, F. Biasioli, Volatilomics of raspberry fruit germplasm by combining chromatographic and direct-injection mass spectrometric techniques, Front. Mol. Biosci. 10 (2023) 1155564, https://doi.org/10.3389/fmolb.2023.1155564.
[46] B. Farneti, I. Khomenko, M. Ajelli, KE. Wells, E. Betta, E. Aprea, L. Giongo, F. Biasioli, Volatilomics of raspberry fruit germplasm by combining chromatographic and direct-injection mass spectrometric techniques, Front. Mol. Biosci. 10 (2023 Apr 13) 1155564, https://doi.org/10.3389/fmolb.2023.1155564.
[47] M.F. D'Agostino, J. Sanz, I. Martínez-Castro, A.M. Giuffrè, V. Sicari, A.C. Soria, Statistical analysis for improving data precision in the SPME GC-MS analysis of blackberry (Rubus ulmifolius Schott) volatiles, Talanta 125 (2014 Jul) 248–256, https://doi.org/10.1016/j.talanta.2014.02.058.