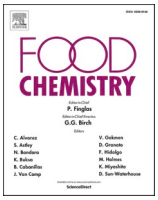
Food Chemistry 406 (2023) 134987

Contents lists available at ScienceDirect

Food Chemistry

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



Metabolomics reveal changes in flavor quality and bioactive components in post-ripening *Torreya grandis* nuts and the underlying mechanism

Jinwei Suo [a,1], Zhenmin Ma [a,1], Bing Zhao [b], Shuang Ma [a], Zuying Zhang [a], Yuanyuan Hu [a], Baoru Yang [c], Weiwu Yu [a], Jiasheng Wu [a,*], Lili Song [a,*]

[a] State Key Laboratory of Subtropical Silviculture, Zhejiang A&F University, Hangzhou, Zhejiang 311300, China
[b] State Key Laboratory of Crop Stress Adaptation and Improvement, Henan Joint International Laboratory for Crop Multi-Omics Research, School of Life Sciences, Henan University, Kaifeng 475001, China
[c] Food Chemistry and Food Development, Department of Life Technologies, University of Turku, FI-20014 Turku, Finland

ARTICLE INFO

Keywords:
Secondary metabolites
Bioactive components
Flavor quality
Torreya grandis nuts
Post-ripening
Metabolomics

ABSTRACT

Secondary metabolites are a group of small molecules with critical roles in plants fitness in addition to their potential bioactivities in humans. Most of these compounds are associated with the flavor and quality formation of fruits or nuts during the development or the postharvest stages. Change in metabolic profiles and shifts underpinning the post-ripening process in *T. grandis* nuts are not yet reported. In this study, a large scale untargeted metabolomics approach was employed in *T. grandis* nuts, revealing for a total of 140 differential accumulated metabolites. Among them, nearly 60% of metabolites belonging to terpenoids, coumarins and phenolic acids, and phytohormones were showed a gradual accumulation pattern, while most of compounds in flavonoids were decreased during post-ripening. An in-depth analysis of changes in these metabolite classes suggest a framework for post-ripening process effect associated with the postharvest quality of *T. grandis* nuts for the first time.

1. Introduction

Secondary metabolites, such as flavonoids, terpenoids, steroids, etc., are a group of natural compounds present in all plant organs, particularly leaf, root, fruit, and seed (Chiocchio et al., 2021). These small molecules also play critical roles in plant physiology i.e., stimulating flowering, attracting pollinators, and fruit-setting and quality formation (Jha and Mohamed, 2022). For example, composition and concentration of flavonoids primarily affect the fruits' taste and organoleptic quality (Kiprovski et al., 2018). Numerous terpenoids serve as likewise as pigments or phytohormones, in addition to their essential part as aroma compounds imparting flavor to fruits in addition to their ecological role to the plants. More importantly, most secondary metabolites are endowed with several biological activities in pharmaceutical applications (Abbas et al., 2017; Chiocchio et al., 2021; Movahedi et al., 2021; Wu et al., 2021). Aiding with the advances in analytics, more tools are available for providing a comprehensive overview of plant metabolome at higher sensitivity (Farag et al., 2022b). Metabolomics as a systems biology approach has been widely employed to provide an in-depth monitoring of changes in plant metabolome (Gonda, 2020). Metabolomics has become a widely applied approach to capture a snapshot of holistic variation in metabolites associated with various plant physiological processes, including plant–microbe interactions, environmental stimulus, reproduction and growth, as well as emerging postharvest biological and technological issues (Gonda, 2020).

Torreya grandis (*T. grandis*) is an ancient economic tree species native to China and has been listed as a national secondary key protected wild plant species, *Torreya grandis* cv. "Merrillii" is a cultivar of wild *T. grandis* and is the commercially important variety for nuts production (Zhang et al., 2020; Suo et al., 2022). Roasted *T. grandis* nuts are one of the most famous world's rarest dry nuts attributed to their unique flavor and high nutritious, and also used as a traditional form of Chinese medicine for their richness in various bioactive compounds, which possessing beneficial effects on curing coughs, excess phlegm, prevent

Abbreviations: ABA, abscisic acid; AGC, automatic gain control; DAM, differential accumulated metabolite; ESI, electrospray ionization; GA, gibberellin; IT, injection time; PCA, principal component analysis; UHPLC-MS/MS, ultra-high performance liquid chromatography and tandem mass spectrometry; VIP, variable importance in projection.

* Corresponding authors.
E-mail addresses: wujs@zafu.edu.cn (J. Wu), lilisong@zafu.edu.cn (L. Song).

[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.foodchem.2022.134987
Received 20 July 2022; Received in revised form 10 October 2022; Accepted 14 November 2022
Available online 17 November 2022

malnutrition, etc. (Lou et al., 2019; Wang et al., 2021; Hu et al., 2022; Suo et al., 2022; Xiao et al., 2022). The harvest period of *T. grandis* nuts typically starts in about mid-September, but the embryo is still underdeveloped at that time (Hu et al., 2018; Zhang et al., 2020). After collection, *T. grandis* nuts undergo a period to complete their physiological maturation, called post-ripening (Zhou et al., 2019; Zhang et al., 2020). This stage is not only the critical period for the accumulation and transformation of oils, proteins, and other nutrients but also a critical stage for reprogramming of its secondary metabolome associated with dramatic changes in nuts organoleptic and sensory characters. Examples of changes include elimination of astringency concurrent with increase in volatile components composition to reach its maximum at 10–15 days post-ripening. (Hu et al., 2022; Suo et al., 2022). Although several bioactive compounds in *T. grandis* nuts have been characterized, the knowledge about non-volatile secondary metabolic profiles and changes underpinning the post-ripening process of *T. grandis* nuts is scarce. Such information is considered pivotal to assess and optimize for the best practices in post-ripening quality of *T. grandis* nuts.

To achieve such goal, ultra-high performance liquid chromatography and tandem mass spectrometry (UHPLC-MS/MS) was employed to profile secondary metabolites at different post-ripening stages in *T. grandis* nuts. Compared to HPLC, UHPLC provides higher peak resolution improving peaks detection level and aided by the higher resolution MS in better peaks identification. The secondary metabolite fingerprinting related to fruit flavor and taste has been identified by the UHPLC-MS/MS method in various plants, such as species in Coffea and Cinnamomum (Farag et al., 2022a; Farag et al., 2022b). In the present study, we aimed to determine the comprehensive secondary metabolic profiles and systematically reveal for the differential changes in secondary metabolites pool during *T. grandis* nut post-ripening. Results from this study provide not only a holistic overview of secondary metabolites associated with the nut flavor and quality formation, but also provided a theoretical basis for the improvement of postharvest quality and post-ripening technologies in *T. grandis* nuts.

2. Material and methods

2.1. Plant material

In this study, nuts samples were collected from the Merrillii cultivar of *T. grandis* at a commercial orchard at Zhaojia Town, Zhuji City, Zhejiang Province, China, in the middle of September 2021. Nine 14-year-old trees grown with standard fertilization, irrigation, and pest control practices were selected for nut collection. About 30 kg of nuts with aril cracked (mature stage) were picked. The healthy nuts without physical damage, pests, and diseases were selected and left overnight at a cool and ventilated place under room temperature (25℃) for subsequent experiments.

2.2. Treatments

The post-ripening treatment of *T. grandis* nuts adopts a one-time stacking method. About 9 kg of *T. grandis* nut removed from the arils were placed in the greenhouse with a constant temperature of 25℃ ± 1℃ (room temperature). The relative humidity was kept at 90 % ± 2 % and stacked into three 35 cm × 35 cm × 15 cm cubes. The stacked samples were manually stirred daily to ensure enough oxygen and avoid creating anoxic conditions during treatment. Approximately 3 kg of nuts were used in each ripening treatment experiment, and three replications were carried out for each to assess for biological variance.

2.3. Samples collect and photograph

The post-ripening experiment of *T. grandis* nuts was conducted for 30 days, and samples in triplicates were collected every 1 to 2 days and photographed at different time intervals (2 and 5 days) to record the color changes of the middle and internal seed coats of *T. grandis* nuts. The changes of embryo were photographed with a stereoscopic microscope (STEMI 508, Germany) every 5 days of post-ripening. Kernels with removed-seed coats were sectioned and immediately frozen in liquid nitrogen and stored at −80 °C until further analysis.

2.4. Metabolites extraction and metabolomics analysis

The metabolite extraction was performed using the method of Zhao et al. (2021). Briefly, accurately weighed 100 mg kernel sample (Three biological replicates were prepared for each sample), ground into powder, and extracted in 1 mL of 70 % aqueous methanol overnight at 4℃. Extracts were separated and 1 μL was used for injection on an ultra-high performance liquid chromatography (UHPLC) system (Vanquish Horizon UHPLC system, Thermo Scientific™) with a Hypersil Gold Vanquish (2.1 × 100 mm, 1.9 μm; Thermo Scientific™) column. The column temperature was set at 30℃, and the sample volume used for injection. The UHPLC mobile phase included acetonitrile (solvent B) and 0.1 % formic acid in water (solvent A), and the gradient as follows: 90:10 v/v at 0–2 min, 50:50 v/v at 10.0 min, 20:80 v/v at 10.1–13.0 min, 5:95 v/v at 14 min, 90:10 v/v at 14.1–18.0 min, flow rate, 0.3 mL/min. Q Exactive™ Plus Orbitrap mass spectrometer (Thermo Scientific™) simultaneous full scan and tandem MS/MS was used for the metabolite screening. Electrospray ionization was performed with both positive and negative ion modes, the spray voltage was set as 3.5 KV for positive mode and 3.2 KV for negative mode; The capillary temperature was set as 320℃, the auxiliary gas heater temperature was set as 350℃; The sheath gas flow rate was set as 35 units, the auxiliary gas flow was set as 15 units; The S-lens RF level was 50, the AGC target was 1e6, and the maximum inject time used was 100 ms. For DDA acquisition, MS1 spectra were collected in the range of 70–1050 *m/z* at 70,000 resolution, the top 8 ions in each full scan were isolated with a 1.6 *m/z* width at 17,500 resolution of MS2 acquisition. Precursor ions were fragmented with a step-wise collision energy of 20, 40 and 60 units, and a maximum injection time of 50 ms with an AGC target of 2e5 (Zhao et al., 2021).

2.5. Metabolomics data analysis

The acquired raw data from UHPLC-MS/MS were analyzed using Compound Discoverer 3.2 (CD 3.2, Thermo Fisher Scientific). A qualitative analysis of the metabolites was carried out by comparison of the accurate precursor ion, the fragment ion values, the retention time (RT), and the fragmentation pattern based on the self-built standard database (flavonoid and phytohormone related standard database) and the public metabolite databases (including mzCloud, mzVault, Masslists, and Chemspider). For the relative abundance analysis of the metabolites, after the raw data were pre-processing for peaks identification, filtration, alignment, and the ions corresponding to other molecular weight substances were excluded to initially eliminate interference. The parent ions of the compounds were screened, and the peak area of each characteristic peak represents the relative abundance value of a metabolite. To making the quantification more accurate and repeatable, the QC sample was used to create a regression curve of area versus acquisition time for each detected compound and correct the integration of the mass spectral peaks of the same metabolite in different samples. The stability and reliability of the analysis data are guaranteed by checking the relative standard deviation (RSD) of the peak areas data both before and after the correction. Principal component analysis (PCA) and differential accumulation pattern analysis were performed using software on the OmicShare tools platform (https://www.omicshare.com/tools). Variable importance in projection (VIP) was used to detect the discriminatory components. The metabolites with VIP value > 1.0, P less than 0.05, and $|\text{Log2FC}| > 1$ were screened for further differential accumulated metabolites (DAMs) analysis between samples. Heatmaps were drawn using TBtools, and other figures were produced by Microsoft Excel. Statistical analysis was performed by using SPSS statistical software

(version 25.0, IBM SPSS Inc.) by one-way ANOVA. Significant differences were declared at a *p*-value less than 0.05.

3. Results and discussion

3.1. Morphological and anatomical changes of T. Grandis nuts during post-ripening

The nuts of *T. grandis* are similar to avocado, banana, kiwifruit, and other fruits/seeds, which undergo a special ripening process post-harvest to develop into edible ripe fruit (Zhang et al., 2020). Numerous studies have shown that the post-ripening process usually accompanied by a series of morphological, physiological, and biochemical changes, such as the color transformation of pericarp/seed coat, which is the most direct indicator of fruit/seed ripening (Zhang et al., 2020; Liu et al., 2022). In this study, with the process of post-ripening, seed shell (middle seed coat) of *T. grandis* nuts was gradually changed from bright yellow (0 to 5 d) to dark yellow and unchanged after 10 days (Fig. S1A). While the internal seed coat of *T. grandis* nuts was reddish-brown at harvest, it changed gradually to become dark-brown and remained unchanged after 15 days of post-ripening (Fig. S1A). Seed anatomy revealed that, after collection, embryo development was not mature and appeared conical. With the process of post-ripening, embryo gradually became larger, and the top of the embryo concaves down to form a cotyledon (Fig. S1B) indicating *T. grandis* nuts have undergone the post-ripening process within 20 days consistent with our previous report that the best time for *T. grandis* nuts to finish the post-ripening is 15 to 20 days (Zhang et al., 2020).

3.2. Changes of total secondary metabolites in T. grandis nuts during post-ripening

The post-ripening of *T. grandis* nuts is a critical period for the accumulation and conversion of nutrients in the kernel concurrent with the biosynthesis of many secondary metabolites (Hu et al., 2022; Suo et al., 2022). To explore changes in secondary metabolites during *T. grandis* post-ripening, we conducted metabolomics analysis on *T. grandis* nuts at different stages of post-ripening. PCA modelling of UHPLC-MS derived kernel samples at different stages of post-ripening indicated a clear separation of metabolites at the 0 to 20 days of post-ripening (Fig. 1A). The number of DAMs showed increase gradually with the extension of post-ripening, and the total number of DAMs reached the maximum value of 95 DAMs at 17 days post-ripening, then the total number of DAMs remained stable until the end of post-ripening and fluctuated in the range of 72 to 85 (Fig. 1B; Table S1). Moreover, in all differential metabolites, the number of mainly secondary metabolites, including terpenoids, flavonoids, coumarins and phenolic acids, phytohormones, and steroids accounted for about 60 % (Fig. 1C; Table S1).

Statistical results showed that a total of 140 DAMs were assigned to 8 different profiles, among which the *p*-value of profile 5, 6, and 8 were less than 0.05 that identified as significantly enriched profiles for further analysis (Fig. 2A-B). Profile 5 included 27 DAMs that showed an increase first followed by a decrease pattern, profile 6 included 40 DAMs that showed a gradual accumulation pattern with the progress of *T. grandis* nut post-ripening, with>60 % of secondary metabolites in these two profiles belonging to terpenoids, coumarins and phenolic acids, and phytohormones. Profile 8 included 44 DAMs, mainly flavonoids, that decreased post-ripening (Fig. 2C). The relative abundance changes of metabolites in these metabolic pathways indicate that terpenoid, flavonoid, coumarin and phenolic acid, and phytohormone related

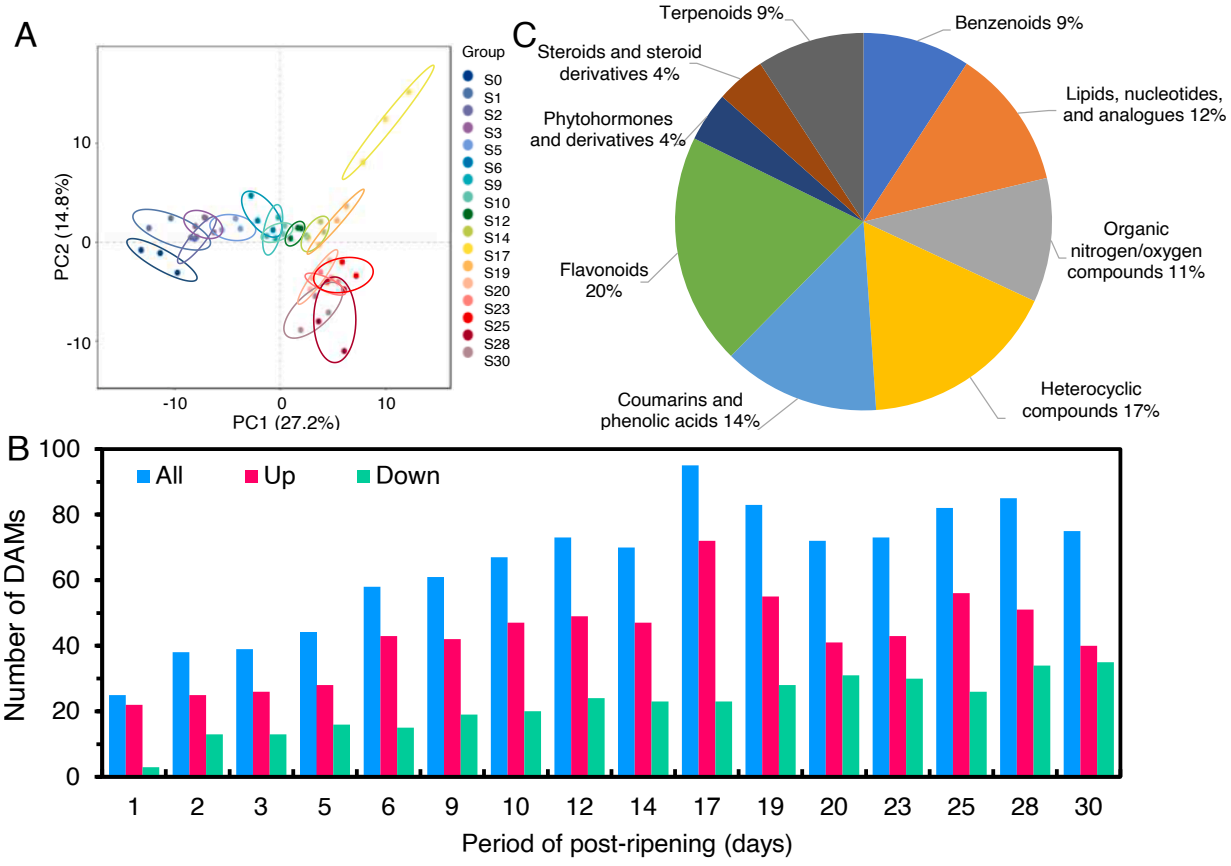


Fig. 1. Metabolomics analysis of *Torreya grandis* nuts during the post-ripening process. (A) Principal component analysis (PCA) of metabolites; (B) Number of differentially accumulated metabolites (DAMs) at different post-ripening stages compared to 0 day; (C) Pie chart depicting the categories of DAMs.

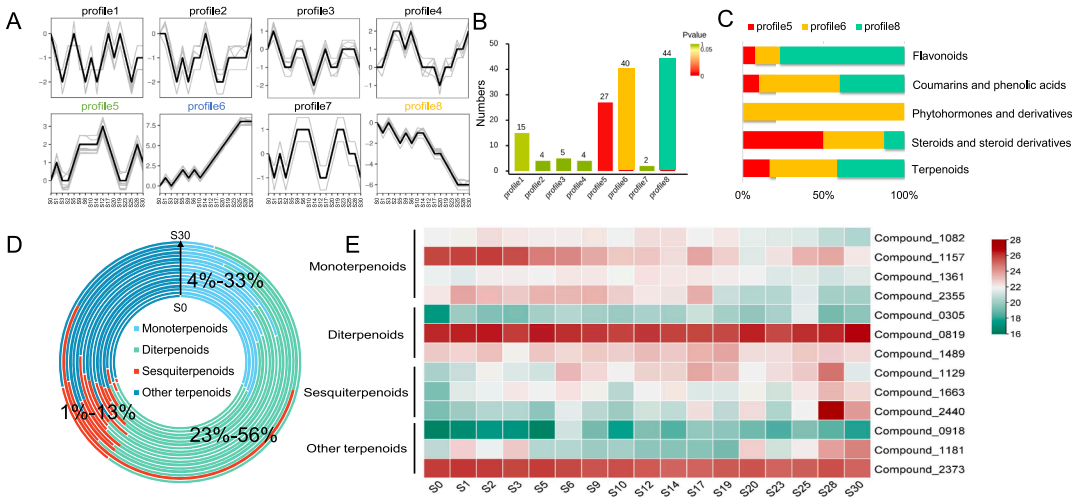


Fig. 2. Statistical analysis of differential accumulated metabolites (DAMs) with different changing profiles and heat map analysis of terpenoids. (A-B) Identify different profiles and DAMs numbers in each profile, profiles 5, 6, and 8 showed significant p-values (p less than 0.05); (C) The proportion of relative abundance of different secondary metabolite categories in profiles 5, 6, and 8. (D) The relative abundance ratio of different terpenoid categories; (E) Heat map analysis of monoterpenoid, diterpenoid, and sesquiterpenoid compounds.

pathways may play an essential role in *T. grandis* nut post-ripening process. Next sections shall discuss in details changes in each metabolic pathway highlighting for any potential role of involved chemicals in nuts post-ripening process.

3.3. Terpenoids metabolism in T. Grandis nuts during post-ripening

Our previous study revealed that terpenoids were the predominant aroma compounds of *T. grandis* nuts by the gas chromatography-mass spectrometer (GC–MS) analysis (Hu et al., 2022). In this study, 13 terpenoid compounds, including four monoterpenoids, three diterpenoids, three sesquiterpenoids, and three other terpenoids were identified differential accumulated during *T. grandis* nuts post-ripening (Table 1). The relative abundance of monoterpenoids, diterpenoids, and sesquiterpenoids accounted for 4 ~ 33 %, 23 ~ 56 %, and 1 ~ 13 %, of the total terpenoids, respectively (Fig. 2D). Further analysis identified several monoterpenes and diterpenoids volatiles to show a relative high accumulation with the post-ripening. Among them, the relative abundance of, dehydrocostus lactone (Compound_1181), geranic acid (Compound_1361), and abietic acid (Compound_0819) was increased gradually during the process of post-ripening, the relative abundance of citral (Compound_1082) was reached its maximum level from 6 to 19 days of post-ripening, while relative abundance of camphor (Compound_1157) and thymol (Compound_2355) was decreased gradually after 6 days of post-ripening (Fig. 2E).

Most of these volatile compounds have been identified as major aroma components in some nuts, fruits, and vegetables, and likely to contribute to *T. grandis* nut aroma (Kesen et al., 2018; Li et al., 2021). The relatively high accumulation of these compounds in *T. grandis* nut suggested their important contribution to the aroma formation, and the variation of their accumulation patterns also indicated that the major aroma components of *T. grandis* nuts maybe different with the processing of post-ripening. In addition, the relatively high level of these compounds might be also help to eliminate harmful microorganisms in the post-ripening process. Indeed, several of the aroma compounds, such as camphor, abietic acid, citral, and thymol exert potential antimicrobial actions (Kumar and Kudachikar, 2020; Li et al., 2021). Combining with the accumulation patterns of these aroma compounds during the post-ripening process, indicating that these compounds may play different roles in *T. grandis* nut aroma formation and post-ripening quality maintaining, which needs further research.

3.4. Flavonoids metabolism in T. grandis nuts during post-ripening

Flavonoids are versatile natural polyphenolic secondary metabolites with extensive pharmacological activities in addition to their ecological functions (Wang et al., 2020). More importantly, studies have shown that some specialized flavonoids are important flavor components that affect fruits' taste and quality (Majumder et al., 2022). In this study, several flavonoids were identified during *T. grandis* nut post-ripening, including catechin (Compound_1047), epicatechin (Compound_1274), epigallocatechin (Compound_1278), naringenin (Compound_1751), naringenin chalcone (Compound_1752), hesperetin (Compound_1399), nobiletin (Compound_1932). Decrease in relative levels of these flavonoids mostly belonging to flavanone and flavonol subclasses was observed during post-ripening process (Fig. 3; Table 1), and to account for the minimal astringent taste in *T. grandis* nuts that completing post-ripening. In contrast, other flavonoids such as chrysin (Compound_1076) and tangeritin isomer (Compound_2323) showed a relative high accumulation during the post-ripening process (Fig. 3; Table 1).

Further analysis found that most of those metabolites belong to flavonoid biosynthesis pathway, in which cinnamoyl-CoA, naringenin chalcone, and naringenin are common intermediates for the biosynthesis of bitterness and astringent substances (catechin, epicatechin, epigallocatechin, etc.) and bioactive compounds of chrysin and taxifolin (Fig. 3). Therefore, decreasing bitterness and astringent compounds might also contribute to accumulating of other bioactive substances during the process of *T. grandis* nut post-ripening. Conclusively, post-ripening process not only improves the flavor quality of *T. grandis* nuts but also contributes to differential accumulation of bioactive flavonoids. These metabolites are important bioactive compounds and were reported with a wide range of pharmacological activities i.e., chrysin exerts cytotoxic and anti-inflammatory effects (Naz et al., 2019), whereas taxifolin have gained increasing attention for its antioxidant, antibacterial, antifungal, antitumor, and anticancer activities (Das et al., 2021). However, whether a differential response occur in flavonoid biosynthesis with post-ripening leading to accumulation of certain flavonoids or their classes i.e.,increase in flavones versus decline in flavanols has yet to be confirmed using gene expression analysis of enzymes involved in flavonoids biosynthesis.

Table 1
A list of differential accumulation of secondary metabolites during the post-ripening of *Torreya grandis* nuts.

Compound ID	Compound Name	Class	Molecular Formula	Molecular Weight (Da)	Retention Time	Ionization mode	Identification method
Compound_1047	Catechin	Flavonoids	$C_{15}H_{14}O_6$	290.079	2.99	pos	Standard
Compound_1274	Epicatechin	Flavonoids	$C_{15}H_{14}O_6$	290.079	4.53	neg	Standard
Compound_1278	Epigallocatechin	Flavonoids	$C_{15}H_{14}O_7$	306.074	2.21	neg	Manual identification
Compound_1751	Naringenin	Flavonoids	$C_{15}H_{12}O_5$	272.069	9.19	neg	Standard
Compound_1752	Naringenin chalcone	Flavonoids	$C_{15}H_{12}O_5$	272.068	9.22	pos	Manual identification
Compound_1399	Hesperetin	Flavonoids	$C_{16}H_{14}O_6$	302.079	9.33	pos	Standard
Compound_1932	Nobiletin	Flavonoids	$C_{21}H_{22}O_8$	402.131	10.95	pos	Manual identification
Compound_2323	Taxifolin isomer	Flavonoids	$C_{15}H_{12}O_7$	304.058	1.85	pos	Manual identification
Compound_1076	Chrysin	Flavonoids	$C_{15}H_{10}O_4$	254.058	11.24	pos	Manual identification
Compound_1365	Gibberellin A7	Phytohormones	$C_{19}H_{22}O_5$	330.147	8.83	neg	Manual identification
Compound_0821	Abscisic acid	Phytohormones	$C_{15}H_{20}O_4$	264.136	8.10	pos	Standard
Compound_1485	L-Tryptophan	IAA precusors	$C_{11}H_{12}N_2O_2$	204.090	2.44	pos	Standard
Compound_1114	Coumarin isomer	Coumarins and phenolic acids	$C_9H_6O_2$	146.037	1.65	pos	Manual identification
Compound_1452	Isofraxidin	Coumarins and phenolic acids	$C_{11}H_{10}O_5$	222.052	6.24	neg	Manual identification
Compound_0649	4-Methylcoumarin	Coumarins and phenolic acids	$C_{10}H_8O_2$	160.052	8.45	pos	Manual identification
Compound_0766	7-Hydroxy-4-methylcoumarin	Coumarins and phenolic acids	$C_{10}H_8O_3$	176.047	9.18	pos	Manual identification
Compound_2365	P-Coumaric acid isomer	Coumarins and phenolic acids	$C_9H_8O_3$	164.047	1.35	pos	Manual identification
Compound_0770	7-Methoxy-4-methylcoumarin	Coumarins and phenolic acids	$C_{11}H_{10}O_3$	190.063	9.57	pos	Manual identification
Compound_1629	Methylcinnamate	Coumarins and phenolic acids	$C_{10}H_{10}O_2$	162.068	8.11	pos	Manual identification
Compound_0819	Abietic acid	Terpenoids	$C_{20}H_{30}O_2$	302.225	13.52	neg	Manual identification
Compound_1181	Dehydrocostus lactone	Terpenoids	$C_{15}H_{18}O_2$	230.131	10.17	pos	Manual identification
Compound_1157	Camphor	Terpenoids	$C_{10}H_{16}O$	152.120	5.73	pos	Manual identification
Compound_1361	Geranic acid	Terpenoids	$C_{10}H_{16}O_2$	168.115	10.18	pos	Manual identification
Compound_1082	Citral	Terpenoids	$C_{10}H_{16}O$	152.120	11.02	pos	Manual identification
Compound_2355	Thymol	Terpenoids	$C_{10}H_{14}O$	150.105	6.45	pos	Manual identification

Note: 'Standard' indicates the metabolite was confirmed by self-built chemical standard database. 'Manual identification' indicates the metabolite was confirmed by comparison of the accurate precursor ion, fragment ion values, the retention time (RT), and fragmentation pattern based on the metabolite database. The MS/MS spectra for each metabolite in this table have been provided in Fig. S2.

3.5. Coumarins and phenolic acids metabolism in T. grandis nuts during post-ripening

Coumarins and phenolic acids are natural phenylpropanoid secondary metabolites with a wide range of biological/pharmacological activities (Krieger et al., 2013; Togna et al., 2014). In this study, several hydroxylated and methylated coumarins, including coumarin isomer, isofraxidin, 4-methylcoumarin, 7-methoxy-4-methylcoumarin, and 7-Hydroxy-4-methylcoumarin, along with their precursor phenolic acids, like *p*-coumaric acid and methylcinnamate were detected during the process of *T. grandis* nut post-ripening (Fig. 4; Table 1). The level of coumarin isomer (Compound_1114) and most of its derivatives (Compound_0770 and Compound_0766) were increased gradually during post-ripening process, but the relative abundance of these compounds was not very high. In contrast, the phenolic acids were showed significant enrichment, especially of p-coumaric acid isomer (Compound_2365) (Fig. 4).

Numerous studies have shown that phenolic acids such as p-coumaric acids are found to maintain the organoleptic quality of post-harvest sweet cherry fruit (Liu et al., 2020) and whether similar effect occurs in *T. grandis* nut should be examined. Most of phenolic acids are potential antioxidants in addition to their antimicrobial action to aid avoid against microbial infestations during post-harvest ripening storage (Zielińska et al., 2021; Zhang et al., 2022). Since the biosynthesis of coumarins and phenolic acids share the same substrate, it seems that more phenolic acids were accumulated during *T. grandis* nut post-ripening process. Thus, the relatively high abundance of these metabolites in the post-ripening *T. grandis* nut appears of value to maintaining post-ripening nut quality.

3.6. Phytohormones metabolism in T. grandis nuts during post-ripening

Furthermore, similar to ginkgo seeds, the post-ripening process of *T. grandis* nut is a physiological maturation process that prepares for seed germination as exemplified by several changes in plant hormones occur during this period (Miransari and Smith, 2014). In this study, metabolites profiling identified increase in GA (Compound_1365) and auxin amino acid precursor (tryptophan) (Compound_1485) with *T. grandis* nut post-ripening, concurrent with a decrease in ABA (Compound_0821) level gradually especially in extended post-ripening time (Table 1; Table S1). In general, exogenous abscisic acid (ABA) application can inhibit seed germination, high gibberellin (GA) levels or GA

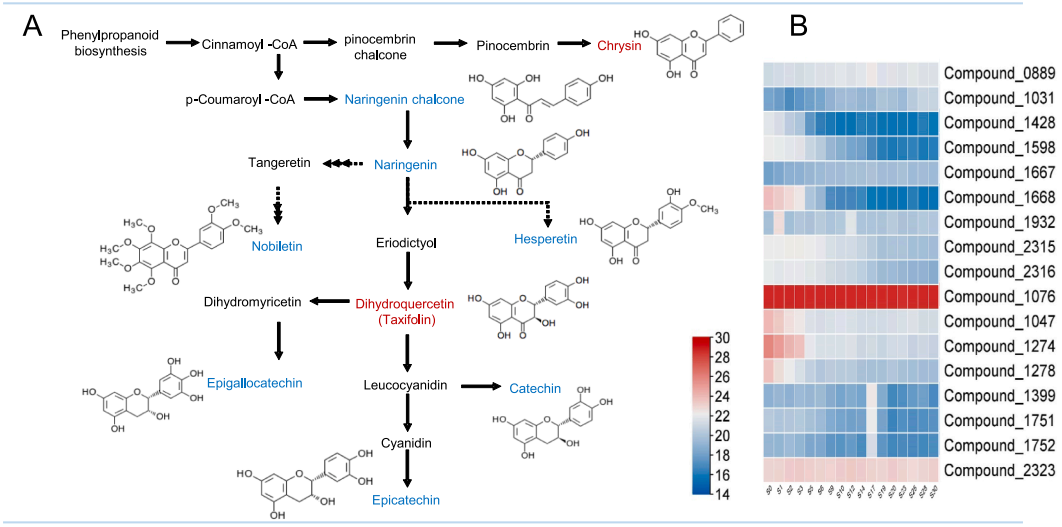


Fig. 3. Schematic diagram of flavonoid biosynthesis pathway during *Torreya grandis* nut post-ripening process. (A) Flavonoid biosynthesis pathway; (B) Heat map analysis of metabolites in the biosynthetic pathways, red indicates a relative high level, and blue indicates a relative low level. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

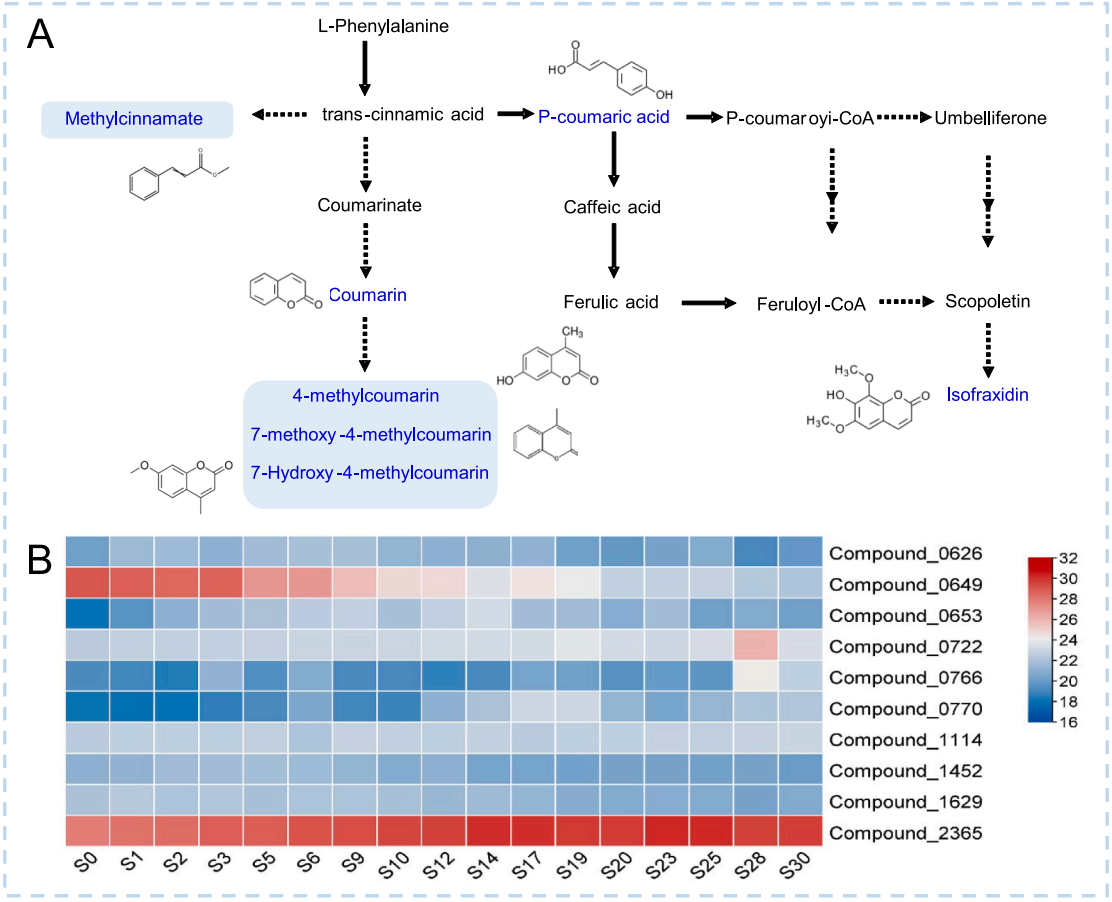


Fig. 4. Coumarin and phenolic acid biosynthesis pathway analysis during *Torreya grandis* nut post-ripening process. (A) Coumarin and phenolic acid biosynthesis pathway, the identified metabolites in this study are marked in blue; (B) Heat map analysis of metabolites in the biosynthetic pathways, red indicates a high level, and blue indicates a low level. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

signaling promote seed germination, and it showed a negative correlation between auxin content/signaling and seed germination (Shu et al., 2016). These results indicate that changes in these plant hormones during *T. grandis* nut post-ripening are closely related to the physiological ripening process, while the complex interaction among ABA, GA, and auxin, as well as the specific regulation mechanism on seed physiological ripening, remain to be further studied.

4. Conclusions

In this study, metabolomics approach using UHPLC-MS/MS analysis unraveled changes in metabolite profiles in *T. grandis* nuts during the post-ripening process for the first time. The differential accumulated secondary metabolites belonged to metabolites from several classed i.e., terpenoids, flavonoids, coumarin, phytohormones, etc. These secondary metabolites were potentially associated with the flavor and quality formation during *T. grandis* nut post-ripening, mainly including the components of taste, aroma, and other potentially bioactive compounds. Identification of the regulation mechanism as other cellular levels i.e., genes and proteins shall aid confirm the hypothesis generated using such chemical profiling approach. Post-harvest ripening is a complex developmental process controlled by a plethora of genetic and epigenetic factors, and aided by omics technologies and functional genomics tool better understanding of such intricate physiological process can be achieved. An in-depth understanding of these small molecules' changes and their biological functions underlying *T. grandis* nut post-ripening will further improve its postharvest quality and promote future utilization of these functional nuts.

CRediT authorship contribution statement

Jinwei Suo: Methodology, Investigation, Writing – review & editing. **Zhenmin Ma:** Investigation, Formal analysis, Writing – original draft. **Bing Zhao:** Investigation, Visualization, Validation. **Shuang Ma:** Investigation, Formal analysis. **Zuying Zhang:** Visualization, Validation. **Yuanyuan Hu:** Visualization, Data curation. **Baoru Yang:** Investigation, Validation. **Weiwu Yu:** Data curation, Validation. **Jiasheng Wu:** Conceptualization, Funding acquisition, Supervision, Resources. **Lili Song:** Conceptualization, Funding acquisition, Methodology, Supervision, Writing – review & editing.

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.

Data availability

Data will be made available on request.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (31971699, U20A2049, 32001349); the Zhejiang Provincial Key R&D Projects (2020C2019, 2022C02061); the Zhejiang Provincial Public Welfare Projects (LGN20C160002); the Zhejiang Provincial Academy Cooperative Forestry Science and Technology Project (2021SY01, 2021SY11); the Selective Breeding of New Cultivars in Torreya grandis (2021C02066-11); the Zhejiang provincial ten thousand people plan (2019R52025). The project also supported by the National Industry Innovation Alliance for Torreya grandis and the SFGA research center for *Torreya grandis*.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2022.134987.

References

Abbas, F., Ke, Y., Yu, R., Yue, Y., Amanullah, S., Jahangir, M. M., & Fan, Y. (2017). Volatile terpenoids: Multiple functions, biosynthesis, modulation and manipulation by genetic engineering. *Planta, 246*, 803–816. https://doi.org/10.1007/s00425-017-2749-x

Chiocchio, I., Mandrone, M., Tomasi, P., Marincich, L., & Poli, F. (2021). Plant secondary metabolites: An opportunity for circular economy. *Molecules, 26*(2), 495. https://doi.org/10.3390/molecules26020495

Das, A., Baidya, R., Chakraborty, T., Samanta, A. K., & Roy, S. (2021). Pharmacological basis and new insights of taxifolin: A comprehensive review. *Biomedicine and Pharmacotherapy, 142*, Article 112004. https://doi.org/10.1016/j.biopha.2021.112004

Farag, M. A., Kabbash, E. M., Mediani, A., Döll, S., Esatbeyoglu, T., & Afifi, S. M. (2022a). Comparative metabolite fingerprinting of four different cinnamon species analyzed via UPLC–MS and GC–MS and chemometric tools. *Molecules, 27*(9), 2935. https://doi.org/10.3390/molecules27092935

Farag M. A., Zayed A., Sallam I. E., Abdelwareth A., Wessjohann L. A. (2022b). Metabolomics-based approach for coffee beverage improvement in the context of processing, brewing methods, and quality attributes. *Foods*, 11(6), 864. Doi: 10.3390/foods11060864.

Gonda, S. (2020). Special issue: plant metabolomics. *Metabolites, 10*(11), 467. https://doi.org/10.3390/metabo10110467

Hu, Y., Zhang, Y., Yu, W., Hnninen, H., Song, L., … Wu, J. (2018). Novel Insights into the Influence of Seed Sarcotesta Photosynthesis on Accumulation of Seed Dry Matter and Oil Content in *Torreya grandis* cv. "Merrillii". *Frontiers. Plant Science, 8*, 2179. https://doi.org/10.3389/fpls.2017.02179

Hu, Y., Zhang, Z., Hua, B., Tao, L., Chen, W., Gao, Y., … Song, L. (2022). The interaction of temperature and relative humidity affects the main aromatic components in postharvest *Torreya grandis* nuts. *Food Chemistry, 368*, Article 130836. https://doi.org/10.1016/j.foodchem.2021.130836

Jha, Y., & Mohamed, H. I. (2022). Plant Secondary Metabolites as a Tool to Investigate Biotic Stress Tolerance in Plants: A Review. *Gesunde Pflanzen*. https://doi.org/10.1007/s10343-022-00669-4

Kesen, S., Amanpour, A., & Selli, S. (2018). Comparative evaluation of the fatty acids and aroma compounds in selected Iranian nut oils. *European Journal of Lipid Science & Technology, 120*(10), 1–21. https://doi.org/10.1002/ejlt.201800152

Kiprovski, B., Borkovi, B., Maleni, O., Veberi, R., Tampar, F., & Mikuli-Petkovek, M. (2018). Postharvest changes in primary and secondary metabolites of sweet cherry cultivars induced by *Monilinia laxa*. *Postharvest Biology and Technology, 144*, 46–54. https://doi.org/10.1016/j.postharvbio.2018.05.012

Krieger, S., Hayen, H., & Schmitz, O. J. (2013). Quantification of coumarin in cinnamon and woodruff beverages using DIP-APCI-MS and LC-MS. *Analytical and Bioanalytical Chemistry, 405*(25), 8337–8345. https://doi.org/10.1007/s00216-013-7238-x

Kumar, A., & Kudachikar, V. (2020). Efficacy of aroma compounds for postharvest management of mango anthracnose. *Journal of Plant Diseases and Protection, 127*, 245–256. https://doi.org/10.1007/s41348-019-00286-w

Lou, H., Ding, M., Wu, J., Zhang, F., Chen, W., Yang, Y., … Song, L. (2019). Full-length transcriptome analysis of the genes involved in tocopherol biosynthesis in *Torreya grandis*. *Journal of Agricultural and Food Chemistry, 67*(7), 1877–1888. https://doi.org/10.1021/acs.jafc.8b06138

Li, X. Q., Chen, Y., Dai, G. C., Zhou, B. B., & Tan, R. X. (2021). Abietic acid ameliorates psoriasis-like inflammation and modulates gut microbiota in mice. *Journal of Ethnopharmacology, 272*, Article 113934. https://doi.org/10.1016/j.jep.2021.113934

Liu, G. S., Li, H. L., Grierson, D., & Fu, D. Q. (2022). NAC Transcription Factor Family Regulation of Fruit Ripening and Quality: A Review. *Cells, 11*(3), 525. https://doi.org/10.3390/cells11030525

Liu, X., Ji, D., Cui, X., Zhang, Z., Li, B.o., Xu, Y., … Tian, S. (2020). p-Coumaric acid induces antioxidant capacity and defense responses of sweet cherry fruit to fungal pathogens. *Postharvest Biology and Technology, 169*, Article 111297. https://doi.org/10.1016/j.postharvbio.2020.111297

Majumder, A., Mondal, S. K., Mukhoty, S., Bag, S., Mondal, A., Begum, Y., … Banik, A. (2022). Virtual screening and docking analysis of novel ligands for selective enhancement of tea (*Camellia sinensis*) flavonoids. *Food Chemistry, 13*, Article 100212. https://doi.org/10.1016/j.fochx.2022.100212

Miransari, M., & Smith, D. L. (2014). Plant hormones and seed germination. *Environmental and Experimental Botany, 99*(3), 110–121. https://doi.org/10.1016/j.envexpbot.2013.11.005

Movahedi, A., Yaghuti, A., Hui, W., Rutland, P., & Qiang, Z. (2021). Plant secondary metabolites with an overview of populus. *International Journal of Molecular Sciences, 22*(13), 6890. https://doi.org/10.3390/ijms22136890

Naz, S., Imran, M., Rauf, A., Orhan, I. E., Shariati, M. A., Haq, I. U., … Heydari, M. (2019). Chrysin: Pharmacological and therapeutic properties. *Life Sciences, 235*, Article 116797. https://doi.org/10.1016/j.lfs.2019.116797

Shu, K., Liu, X. D., Xie, Q., & He, Z. H. (2016). Two faces of one seed: Hormonal regulation of dormancy and germination. *Molecular Plant, 9*(1), 34–45. https://doi.org/10.1016/j.molp.2015.08.010

Suo, J., Gao, Y., Zhang, H., Wang, G., Cheng, H., Hu, Y., … Wu, J. (2022). New insights into the accumulation of vitamin B_3 in *Torreya grandis* nuts via ethylene induced key gene expression. *Food Chemistry, 371*, Article 131050. https://doi.org/10.1016/j.foodchem.2021.131050

Togna, A. R., Firuzi, O., Latina, V., Parmar, V. S., Prasad, A. K., Salemme, A., … Saso, L. (2014). 4-methylcoumarin derivatives with anti-inflammatory effects in activated microglial cells. *Biological and Pharmaceutical Bulletin, 37*(1), 60–66. https://doi.org/10.1248/bpb.b13-00568

Wang, J. F., Liu, S. S., Song, Z. Q., Xu, T. C., & Wu, S. H. (2020). Naturally occurring flavonoids and isoflavonoids and their microbial transformation: A review. *Molecules, 25*(21), 5112. https://doi.org/10.3390/molecules25215112

Wang, Y., Yao, X., Yang, L., Fei, X., Cao, Y., Wang, K., & Guo, S. (2021). Effects of harvest time on the yield, quality and active substance of *Torreya grandis* nut and its oil. *Journal of Oleo Science, 70*(2), 175–184. https://doi.org/10.5650/jos.ess20155

Wu, Y., Zhang, C., Huang, Z., Lyu, L., & Wu, W. (2021). The color difference of *rubus* fruits is closely related to the composition of flavonoids including anthocyanins. *LWT- Food Science and Technology, 149*, Article 111825. https://doi.org/10.1016/j.lwt.2021.111825

Xiao, M., Huang, M., Huan, W., Dong, J., Xiao, J., Wu, J., Wang, D., & Song, L. (2022). Effects of *Torreya grandis* kernel oil on lipid metabolism and intestinal flora in C57BL/6J Mice. *Oxidative Medicine and Cellular Longevity, 2022*, 4472751. https://doi.org/10.1155/2022/4472751

Zhang, M., Liu, H., Yue, Z., Wang, X., & Zhou, H. (2022). The effects of caffeic acid and epicatechin treatment on gray mold resistance and antioxidant metabolism in apples. *Journal of Plant Pathology, 104*(2), 661–670. https://doi.org/10.1007/s42161-022-01054-w

Zhang, Z., Jin, H., Suo, J., Yu, W., Zhou, M., Dai, W., … Wu, J. (2020). Effect of temperature and humidity on oil quality of harvested *Torreya grandis* cv. "Merrillii" nuts during the after-ripening stage. *Frontiers in plant science, 11*, Article 573681. https://doi.org/10.3389/fpls.2020.573681

Zhao, B., Zhang, S., Yang, W., Li, B., Lan, C., Zhang, J., … Zhang, X. (2021). Multi-omic dissection of the drought resistance traits of soybean landrace LX. *Plant, Cell & Environment, 44*(5), 1379–1398. https://doi.org/10.1111/pce.14025

Zhou, M., Song, L., Ye, S., Zeng, W., Hännien, H., Yu, W., … Wu, J. (2019). New sights into lipid metabolism regulation by low temperature in harvested Torreya grandis nuts. *Journal of the Science of Food and Agriculture, 99*(9), 4226–4234. https://doi.org/10.1002/jsfa.9653

Zielińska, D., Zieliński, H., Laparra-Llopis, J. M., Szawara-Nowak, D., Honke, J., & Giménez-Bastida, J. A. (2021). Caffeic acid modulates processes associated with intestinal inflammation. *Nutrients, 13*(2), 554. https://doi.org/10.3390/nu13020554