

Untargeted Metabolomics Analysis Reveals the Effect of Fixation on the Profile of Volatile Compounds of *Cyclocarya paliurus* Tea

Minge Bai , Liling Wang* 

The Key Laboratory of Biochemical Utilization of Zhejiang Province, Zhejiang Academy of Forestry, Hangzhou 310023, China

Fixation is a necessary tea processing step that affects the quality of tea, including its aroma. However, there are no guidelines to follow whether the *Cyclocarya paliurus* leaves need to be fixated to produce tea. The aim of this study was to determine the volatile metabolites in fixated and unfixated *C. paliurus* tea by gas chromatography-mass spectrometry in order to identify the volatile compounds that differentiate them and establish the key aroma compounds. Compared with the unfixated tea, the fixated tea had higher contents of amines, phenols, nitrogen compounds, ether, alcohol, terpenoids, ester and aromatics. A total of 147 metabolites that differentiated both types of teas were identified using principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA). Among them, 22 and 25 volatile compounds of teas processed without and with fixation, respectively, had relative odor activity values greater than 1. These metabolites had green, fruity, sweet, woody, herbal, floral, fresh, spicy, earthy and waxy odor. This study showed that fixation technology improved the quality of *C. paliurus* tea, which provided the basis for the production of *C. paliurus* tea, and promoted the standardization of the preparation technology of *C. paliurus* tea.

Keywords: fixation technology, HS-SPME-GC-MS, key aroma compounds, OPLS-DA, volatile metabolites

INTRODUCTION

Cyclocarya paliurus is a relict tree species unique to China that survived the Ice Age and is distributed in mountainous areas at an altitude of 420–2500 meters [Zhang *et al.*, 2021]. Due to the presence of various physiological active substances, such as flavonoids, saponins, terpenoids, polysaccharides, *etc.*, beneficial to the human body, its leaves are often used to brew tea. Its golden color and sweet taste make it known as “sweet tea”. *C. paliurus* leaves elicit multiple health benefits, such as preventing and treating diabetes, hypertension, hyperlipidemia, dizziness and swelling and pain, as well as reducing cholesterol level, and modulating the functions of the immune system [Chen *et al.*, 2022b]. The leaves of *C. paliurus* were approved as new food raw material by the National Health and Family Planning Commission of China in 2013. However, the quality of commercially

available *C. paliurus* tea is uneven, and the processing technology for professional production of *C. paliurus* tea has not yet been developed. The traditional processing of *C. paliurus* involves mostly direct drying, but in recent years, some producers have adopted the processing method of green tea including first its fixation followed by drying. It has not been reported in literature whether fixation is suitable for processing of *C. paliurus*, and how the profiles of volatile organic compounds (VOCs) and aroma compounds of *C. paliurus* change after fixation. Such knowledge seems important to improve the technology of processing *C. paliurus* leaves for tea.

Fixation is one of the critical steps in fresh leaves processing for tea. By inactivating enzymes at high temperatures, it prevents tea from turning red [Ouyang *et al.*, 2022]. Simultaneously, a series of chemical reactions occur, such as hydrolysis and oxidation

*Corresponding Author:
e-mail: echo22239@163.com (L. Wang)

Submitted: 7 April 2024
Accepted: 26 July 2024
Published on-line: 23 August 2024



© Copyright by Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences
© 2024 Author(s). This is an open access article licensed under the Creative Commons Attribution-NonCommercial-NoDeriv License (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

of proteins and polysaccharides, polyphenol polymerization, pigment degradation, and generation of simple compounds that are beneficial for tea quality [Wang *et al.*, 2021a]. The main task of fixation is to form an acceptable aroma to satisfy consumers [Xu *et al.*, 2020]. Endeavors have been made to increase the formation of “beneficial” chemicals, such as linalool, phenacetaldehyde and benzoic acid, and to avoid the presence of “unfavorable” chemicals, such as L-isoleucine, L-lysine and L-tyrosine from a tea aroma perspective [Wen *et al.*, 2023; Xue *et al.*, 2022].

Non-targeted metabolomics exhibits a high-sensitivity and wide-coverage, and provides a comprehensive view of metabolite changes, which has been widely utilized in tea research for the unbiased analysis and simultaneous identification of a large number of non-volatile and volatile metabolites [Ouyang *et al.*, 2022; Qin *et al.*, 2020]. Its advantage is that unknown metabolites can be discovered, and differences in metabolites associated with treatment states can be identified through multivariate statistical analysis. Its basic principle is to use high-throughput technology to comprehensively analyze metabolites in samples, including mass spectrometry, nuclear magnetic resonance, and other techniques. These techniques allow detecting thousands of metabolites simultaneously, resulting in comprehensive metabolome information. The analysis of metabolome data provides information on metabolite composition, metabolic pathway, and interaction between metabolites in the sample [Liu *et al.*, 2019]. Untargeted metabolomics is a new approach with a wide range of application prospects, and has been successfully applied in investigating geo-tracing of plants [Cao *et al.*, 2019], food processing [Chen *et al.*, 2020], and plant bioactivity [Hasanpour *et al.*, 2020].

Based on this, the purpose of this study was to compare the volatile compound profiles of *C. paliurus* tea prepared with and without fixation by untargeted metabolomics methods, to explore the impact of fixation on the aroma quality of *C. paliurus* tea, and to provide references for the development of processing standards for *C. paliurus* tea.

MATERIAL AND METHODS

■ Experimental material and chemicals

The *C. paliurus* leaves were randomly picked in Quzhou (Zhejiang, China) in May 2023.

n-Hexane (chromatographic grade) was purchased from Merck (Darmstadt, Germany), and all standards were purchased from BioBioPha Co., Ltd. (KIB, Kunming, China) and Sigma-Aldrich (St. Louis, MO, USA).

■ *C. paliurus* leaves processing

To produce tea using fixation, the fresh *C. paliurus* leaves (50 kg) were spread on bamboo woven round plates (Φ2 m; leaf layer thickness on each plate was 2–5 cm) for 12–24 h at 19–22°C with a relative air humidity of 55% until their water content reached about 15–20 g/100 g. Then, fixation was performed using a roller-hot air coupling fixation machine (6CST-80YJ, Shangyang Machinery Co., LTD, Zhejiang, China), with rolling temperatures of 170–180°C and fixation time of 60–120 s. After

fixing, the *C. paliurus* leaves were dried at 80°C until the moisture content of the finished tea was less than 5 g/100 g.

Tea without the fixation step was produced by spreading out the collected leaves at room temperature as during the fixated treatment and then drying directly at 8°C until the moisture was less than 5 g/100 g.

■ Analysis of volatile metabolites in *C. paliurus* teas

Samples were weighed, immediately frozen in liquid nitrogen, and stored at –80°C until used. Before the analysis, the samples were ground to powder in liquid nitrogen. For the analysis, 500 mg of the powders were transferred immediately to a 20 mL head-space vials (Agilent, Palo Alto, CA, USA) containing NaCl saturated solution, to inhibit any enzyme reaction. The vials were sealed with crimp-top caps of tetrafluoroethylene resin (TFE)-silicone headspace septa (Agilent). The solid-phase micro-extraction (SPME) was carried out by oscillation of each vial at a constant temperature of 60°C for 5 min, then 120 μm VB/CWR/PDMS extraction head (Agilent) was inserted into the sample headspace vial for 15 min of headspace extraction.

The identification and quantification of VOCs in the samples was carried out using an Agilent 7000D triple quadrupole gas chromatography–mass spectrometry (GC/MS) system (Agilent), equipped with a 30 m × 0.25 mm × 0.25 μm (5%-phenyl)-methylpolysiloxane (DB-5MS) capillary column (Agilent). Desorption of VOCs from the fiber coating was performed in the injection port of the chromatograph at 250°C for 5 min in the splitless mode. Injector temperature was maintained at 250°C. The oven temperature was set to 40°C (3.5 min), and then increased to 100°C at 10°C/min, 180°C at 7°C/min, and 280°C at 25°C/min for 5 min. The carrier gas was helium, applied at a flow rate of 1.2 mL/min. The quadrupole mass spectrometer, ion source and transmission line temperatures were set at 150°C, 230°C and 280°C, respectively. The mass spectrum was recorded in an electron shock (EI) ionization mode at 70 eV. The analytes were identified and quantified by mass spectrometry coupled with an ion-monitoring (SIM) mode.

For qualitative analysis, the Agilent MassHunter Unknowns analysis software was used to screen volatile metabolites whose similarity to the NIST11 mass spectrum library standard was >80%. By calculating the linear formula of *n*-alkane (C7–C40), the Kovats retention index of each compound was obtained and compared with the theoretical retention index. For quantitative analysis, the mass concentrations of the volatile metabolites were calculated with reference to the internal standard method using Equations (1) and (2) [Wang *et al.*, 2020]:

$$C_i = \frac{(C_{is} \times A_i)}{A_{is}} \quad (1)$$

$$X_i = \frac{(C_i \times V_i)}{M} \quad (2)$$

where: C_i is the mass concentration of any compound (μg/L), C_{is} is the mass concentration of the internal standard (μg/L) (ethyl caprate, 20 mg/L), A_i is the chromatographic peak area of any compound, A_{is} is the chromatographic peak area of the internal

standard, X_i is the content of compound i in the sample to be measured ($\mu\text{g/g}$), V_i is the volume of the sample i (L), and M is the amount of the sample to be measured (g).

■ Calculation of relative odor activity value

Relative odor activity values (rOAVs) of volatile compounds of *C. paliurus* teas were calculated using formula (3) [Xue *et al.*, 2022]:

$$\text{rOAV}_i = \frac{C_i}{T_i} \quad (3)$$

where: rOAV_{*i*} is the relative odor activity value of compound i , C_i is the content of the compound ($\mu\text{g/L}$), and T_i is the threshold of the compound ($\mu\text{g/L}$). Threshold values were taken from the literature [van Gemert, 2011; Yang *et al.*, 2022a; Zhu *et al.*, 2021].

■ Statistical analysis

SIMCA P13 software (Umetrics, Umea, Sweden) and MEV 9.0 software (<https://mev-tm4-org.caas>) were used to analyze the main components and contributions of volatile metabolites in different tea processing, respectively. Three replicates were taken for each sample.

Principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA) were carried out with the Metware Cloud, a free online platform for data analysis (<https://cloud.metware.cn>). Significantly changed metabolites were selected according to variable importance in the project (VIP) ($\text{VIP} \geq 1$ and $p < 0.05$) obtained by the OPLS-DA model. Duncan's multiple test was applied to identify significant differences ($p < 0.05$) among teas. Statistical analysis was performed using SPSS 18 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

■ Types of volatile metabolites in the *C. paliurus* teas

In this study, samples extracted from fixated and unfixated *C. paliurus* tea were analyzed by untargeted metabolomics. A total of 1,222 volatile compounds were detected, including 25 amines (2.05%), 95 alcohols (7.77%), 54 aromatics (4.42%), 29 phenols (2.37%), 10 nitrogen compounds (0.82%), 12 sulfur

compounds (0.98%), 4 halogenated hydrocarbons (0.33%), 7 ethers (0.57%), 92 aldehydes (7.53%), 24 acids (1.96%), 309 terpenoids (25.29%), 85 hydrocarbons (6.96%), 99 ketones (8.1%), 172 heterocyclic compounds (14.08%), 199 esters (16.28%), and 6 others (0.49%).

Using these 1,222 volatile metabolites, PCA was carried out on the *C. paliurus* teas prepared with and without the fixation step, and the resulting plot of PCA is shown in **Figure 1A**. The two processing methods were significantly distinguished from each other, and the percentage change of PC1 and PC2 interpretation was 83.18%; thus, these two principal components represented the main characteristics of the sample. Similarly, OPLS-DA, conducted with 1,222 volatile metabolites as independent variables and with fixated and unfixated teas as dependent variables, showed that there were large differences among sample groups, but small differences within groups (**Figure 1B**). The above results indicate that fixation was the key process in the processing of *C. paliurus* leaves, which had a great influence on the VOCs of the tea.

The numbers of volatile metabolites identified in fixated and unfixated teas and classified into different types are shown in **Figure 2**. Of the 1,222 VOCs, 1,196 metabolites were detected in *C. paliurus* tea prepared without the fixation step, while 1,193 volatile compounds were detected in tea obtained after leaf fixation. The main differences in the numbers of VOCs were found for terpenoids, heterocyclic compounds, amines, and phenols. Overall, there was little difference in numbers of compounds of each type between both tea variants, while the total VOC content in the *C. paliurus* tea obtained after fixation was significantly ($p < 0.05$) higher than in the unfixated tea (**Figure 3**). Considering the different types of VOCs, contents of amines, phenols, nitrogen compounds, ethers, alcohols, terpenoids, esters, aromatics and others were extremely significantly higher ($p < 0.01$) and the content of sulfur compounds was significantly higher ($p < 0.05$) in the fixated tea (**Figure 3A, B**). Previous studies have found that the fixation process, due to the transient high temperature, can promote the hydrolysis, isomerization, substitution and redox reactions, and other physical and chemical

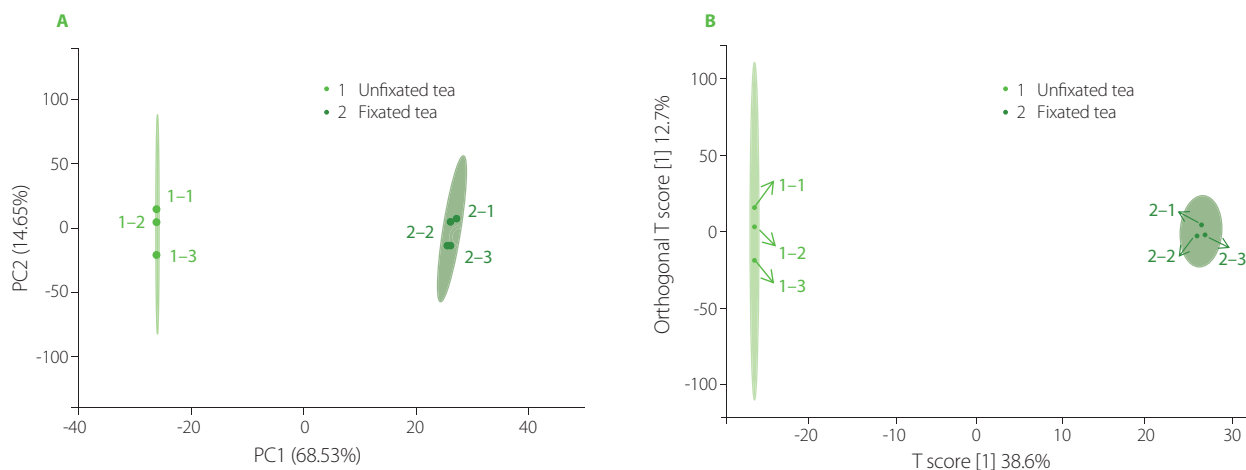


Figure 1. Score plots of principal component analysis (A) and orthogonal partial least squares discriminant analysis (B) based on volatile metabolites of fixated and unfixated *Cyclocarya paliurus* teas.

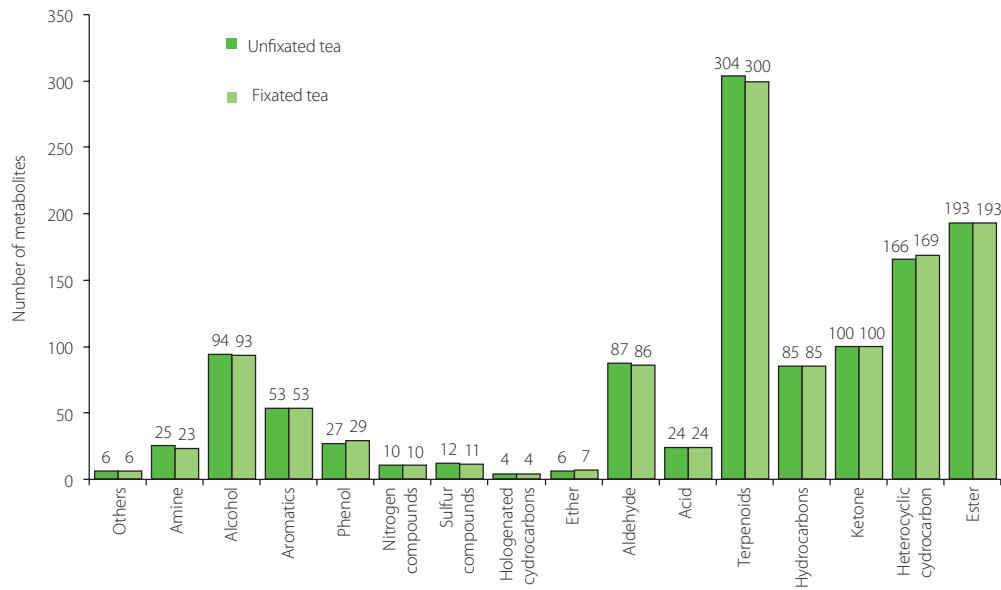


Figure 2. Number of volatile metabolites of different classes identified in *Cyclocarya paliurus* teas produced with and without fixation.

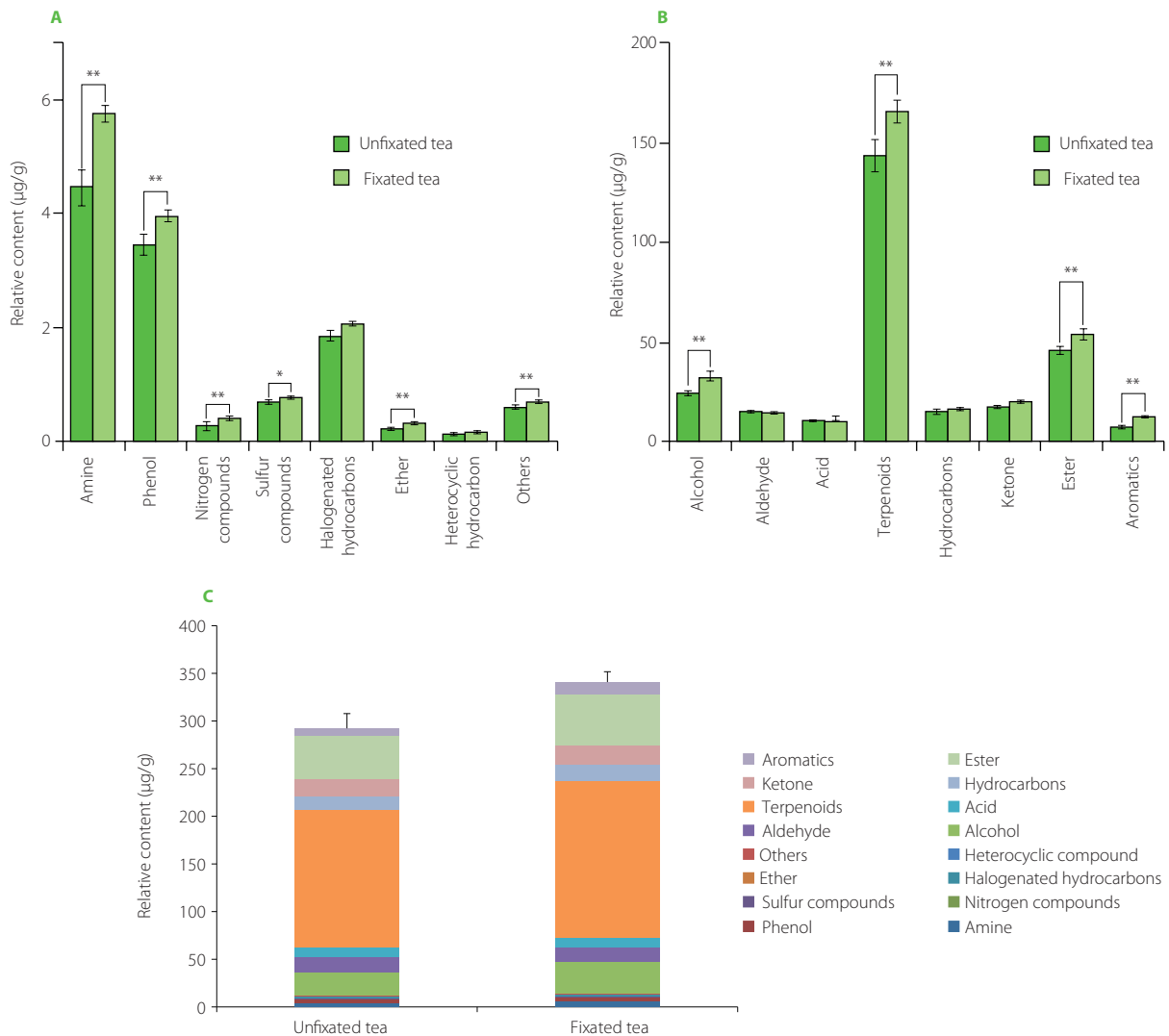


Figure 3. Contents of volatile metabolites of different classes (**A** and **B**) and total content of volatile metabolites (**C**) in *Cyclocarya paliurus* teas produced with and without fixation. Values in pairs (fixated and unfixated teas) marked with * differ significantly at $p < 0.05$ and marked with ** differ significantly at $p < 0.01$.

thermal changes of the non-volatile compounds of green tea leaves, which, in turn, were the key prerequisite compounds for the VOC formation [Wang *et al.*, 2021a]. The increase in the contents of VOCs of the fixated *C. paliurus* tea found in our study can be explained by a similar transformation.

■ Individual volatile metabolites in the *C. paliurus* teas

In order to elucidate the effect of fixation on the volatile metabolites in the processing of *C. paliurus* leaves, the differences of main metabolite contents in the key process of fixation were explored. According to the OPLS-DA screening criteria of metabolites: VIP ≥ 1 and fold change (FC) ≥ 2 or ≤ 0.5 [Geng *et al.*, 2023], a total of 147 tea metabolites were screened, of which 68 were up-regulated and 79 down-regulated after fixation (Figure 4A). Among them, the top ten up-regulated were succinimide, α -ethylidenebenzeneacetaldehyde, (*Z*)-1-methoxy-4-(1-propenyl)benzene, *trans*-anethole, anethole, 1-(4-ethylphenyl)ethanone, 2,2,3-trimethylcyclobutanone, 4-hexen-3-one, diethyl phthalate, benzenepropanoic acid methyl ester, and the top ten down-regulated were 2-hexenal, (*E*)-2-hexenal, *cis-p*-menth-2-en-7-ol, 2-methyl-5-(1-methylethyl)phenol, thymol, isopropenyl ethyl ketone, 1-cyclopropyl-1-propanone, 2,6-dimethyl-2,6-octadiene, acetic acid cyclohexyl ester, and (*E*)-3-octen-2-one (Figure 4B).

As shown in Table S1 in Supplementary Materials, 147 main metabolites included 13 aldehydes, 10 hydrocarbons, 15 heterocyclic compounds, 18 ketones, 18 esters, 9 aromatics, 18 alcohols, 32 terpenoids, 1 nitrogen compound, 1 ether, 2 amines, 2 acids, 3 phenols, 3 sulfurs, and 2 others. In order to observe the differences between metabolite contents of fixated and unfixated teas in a more intuitive way, heat maps of 147 metabolites were obtained and they are show in Figure S1 in Supplementary Materials.

■ Terpenoids

Terpenoids, which were the most abundant among the VOCs of teas (Figure 2 and 3B) mainly included monoterpenoids, sesquiterpenoids and their derivatives. A total of 32 terpenes showed significant differences between tea variants, including 16 up-regulated and 16 down-regulated terpenes after fixation (Table S1, Figure S1). The first group included β -guaiene and β -pinene. Previously, these compounds were detected in black tea flavor profile [Yao *et al.*, 2023]. One of the down-regulated terpenoid was α -terpineol, which was considered to be a non-flavor compound in beverages [Pérez-López *et al.*, 2016], and has been identified as a key component of the stale odor of old green tea [Dai *et al.*, 2020]. In our study, the content of α -terpineol in the *C. paliurus* tea decreased significantly as a result of fixation (Figure S1), indicating that the quality of the *C. paliurus* tea could be improved by this process. In addition, a previous study has shown that terpenoids are important characteristic compounds of *C. paliurus* [Shao *et al.*, 2024], and volatile terpenoids are essential for the aroma quality of tea brews, with typical sweet, floral and woody flavors [Zhu *et al.*, 2017].

■ Alcohols

There was a significant difference in the total alcohol content between the *C. paliurus* tea processed by fixation and the *C. paliurus* tea prepared without the fixation step (Figure 3B), of which 7 compounds were up-regulated and 11 compounds were down-regulated after fixation (Table S1, Figure S1). 2,3-Butanediol was included in the first group. This compound was also observed in the VOC profile of Huaguo tea [Lu *et al.*, 2023], and Fuzhuan brick tea co-fermented with *Moringa oleifera* leaves [Li *et al.*, 2023b]. (*E*)-2-Hexen-1-ol and (*Z*)-2-hexen-1-ol were identified to be more obvious in Huaguo tea [Lu *et al.*, 2023]. After fixation, the down-regulation of some compounds that have adverse

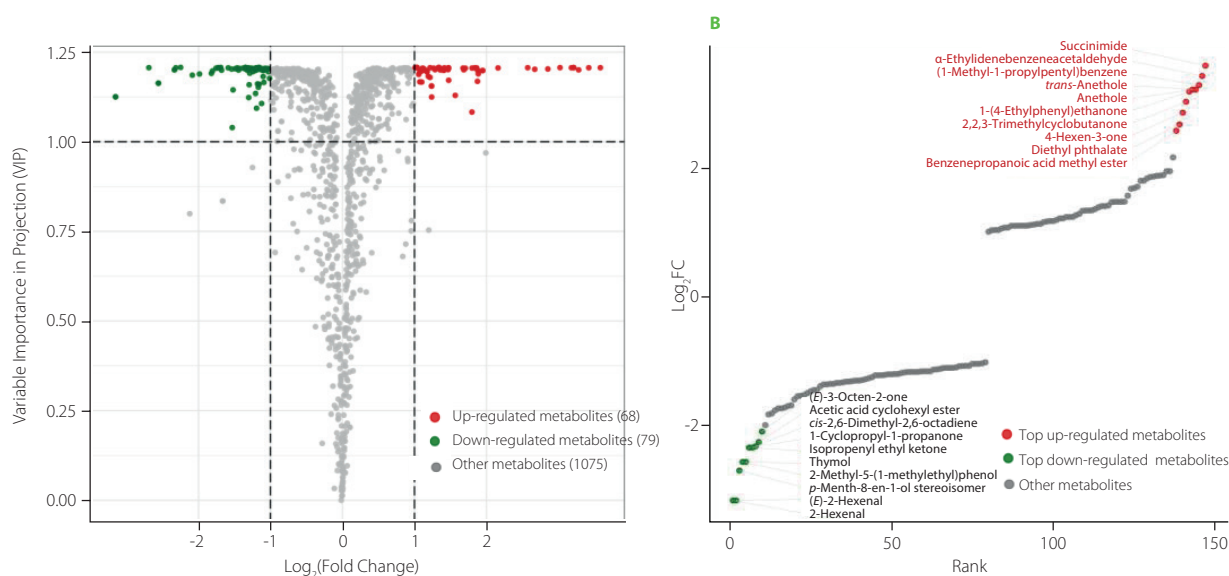


Figure 4. Volcano plot of the volatile metabolites of fixated vs unfixated *Cyclocarya paliurus* teas with indicated compounds significantly up-regulated (red points) and down-regulated (green points) after fixation (A). Dynamic distribution of metabolites significantly differentiating teas with the top 10 metabolites that were up-regulated (red points) and down-regulated (green points) after fixation (B).

reactions to human inhalation was particularly obvious, such as 3-hydroxy-benzenemethanol, *trans*-2-undecen-1-ol, and prenol. Therefore, through the reduction of odor or harmful compounds, fixation offers also an important advantage to improve the quality of *C. paliurus* tea.

■ Esters

A total of 18 esters were detected in the fixated and unfixated teas, with 9 up-regulated and 9 down-regulated after fixation (Table S1, Figure S1). Previous studies have shown that high temperature and long-term treatment are conducive to the formation of ester volatiles [Zhou *et al.*, 2023b], and fixation process results in a large amount of dissipation of low boiling point, grassy volatile esters [Ouyang *et al.*, 2022]. These esters, together with ketones, aldehydes, and other metabolites, were responsible for chestnut-like, flowery, and fruity aromas of tea [Wang *et al.*, 2021a]. Among up-regulated esters of *C. paliurus* tea, butyrolactone was determined (Figure S1). This compound was also found in the volatile compound profile of Congou black tea [Zhou *et al.*, 2023a], green tea [He *et al.*, 2023] and Dianhong Congou tea [Chen *et al.*, 2022a].

■ Ketones

Ketones, which mainly contribute to woody and floral aromas [Liu *et al.*, 2023], represent a common class of volatile compounds. In the study, it was found that 15 ketones were significantly down-regulated and only 3 were significantly up-regulated after fixation (Table S1, Figure S1). Among them, 2-heptanone down-regulated in the fixated tea, is commonly found in the VOCs of fruit and vegetable food products [Fella *et al.*, 2022; Wang *et al.*, 2021b] and is a flavor compound associated with fresh fruit aroma, with special banana aroma. 2-Heptanone can be formed by thermal β -oxidation of fatty acids, then decarboxylation [Valero *et al.*, 2001], and can bind to proteins [Wang & Arntfield, 2015]. This may explain its decreasing content after high temperature treatment in our study. The three up-regulated ketones were 4-hexen-3-one, 2,2,3-trimethylcyclobutanone, and 1-(4-ethylphenyl)ethanone (Figure S1). 4-Hexen-3-one is an unsaturated aldehyde that was reported to occur in peels of *Citrus aurantiifolia* fruit [Sandoval-Montemayor *et al.*, 2012], 1-(4-Ethylphenyl)ethanone was found in *Rhus potaninii* Maxim [Zhu *et al.*, 2020], while 2,2,3-trimethylcyclobutanone has not been reported in literature concerning VOCs of plants.

■ Heterocyclic compounds

Heterocyclic compounds are also important aroma compounds, and occupy an important place in the VOCs of various varieties of tea [Guo *et al.*, 2021]. A total of 15 heterocyclic compounds with significant differences were detected in *C. paliurus* tea variants, of which 8 were up-regulated and 7 were down-regulated after fixation (Table S1, Figure S1). The up-regulated compound, methylpyrazine, has nutty, roast and sweet aromas [Hu *et al.*, 2021]; hence, its up-regulation endows the tea a unique nutty aroma. It was discovered in chestnut-like aroma green tea [Yang *et al.*, 2022b]. 1*H*-Pyrrole-2-carboxaldehyde is a heat-induced

compound (a previous study has shown that its content was higher in tomatoes obtained by heat pump drying [Jeyaprakash *et al.*, 2020]). This may explain up-regulation of this compound after fixation in this study.

5-Ethylhydantoin, succinimide, 2-acetyl-3-ethylpyrazine, 1*H*-pyrrole-2,5-dione, and (3*R*,6*S*)-2,2,6-trimethyl-6-vinyltetrahydro-2*H*-pyran-3-ol were the five significantly up-regulated heterocyclic compounds (Figure S1) that have not been reported in the volatility studies of other species, and can be used as characteristic compounds of *C. paliurus* tea obtained through fixation. Among the down-regulated heterocyclic compounds, betahistine, which is used in western medicine for the treatment of cardiovascular disease, was found in *C. paliurus* leaves for the first time. 2-*n*-Heptylfuran is only produced in a thermal reaction at 14°C and is associated with the flavor of cocoa and roasted nuts [Li *et al.*, 2023a]. However, the temperature of the fixation reached 170–180°C; hence, the content of this compound in tea decreased after processing. 1-Methyl-2-pyrrolidinone, 4-(*N*-nitroso-*N*-methylamino)-1-(3-pyridyl)-1-butanone, 2-furanpropanoic acid ethyl ester, dihydro-5-pentyl-2(3*H*)-furanone, and 1,5,6-trimethyl-azacyclohexan-3-one were the five down-regulated heterocyclic compounds (Figure S1) that have not been reported in the volatile compound profile of other species.

■ Aldehydes

Aldehydes with low odor threshold [Culleré *et al.*, 2011] can provide special flavor for *C. paliurus* tea. In addition, they perform a variety of biological functions, *e.g.*, cinnamaldehyde and *trans*-cinnamaldehyde showed antifungal and antibacterial activities [Gu *et al.*, 2024; Mu *et al.*, 2023]. The first of the mentioned aldehydes was up-regulated after fixation in our study (Table S1, Figure S1). Generally, in the *C. paliurus* teas, 6 aldehydes were significantly up-regulated and 7 others were significantly down-regulated after fixation. Up-regulated 2,4-dimethylbenzaldehyde was also found in a previous study as an odor active compound of the sour bamboo shoot fermentation broth [Long *et al.*, 2023]. 4-Isopropylcyclohexa-1,3-dienecarbaldehyde and 8-isopropyl-1-methyltricyclo[4.4.0.0^{2,7}]dec-3-ene-3-carbaldehyde were two significantly up-regulated aldehydes that have not been reported in studies of VOSs of other species, and can be used as the characteristic compounds derived from *C. paliurus* tea.

2-Hexenal and (*E*)-2-hexenal are common volatile aldehydes, which have been recognized as signature characteristic compounds in tea processing [Rong *et al.*, 2023; Yang *et al.*, 2023]. Similarly, 2-undecenal and (*E*)-2-undecenal (the *cis-trans* isomers) are also typical volatile aldehydes sensitive to heating [Zhou *et al.*, 2021], and this is the first time when they had been detected in the VOCs of *C. paliurus* leaves. Studies have shown that (*E,E*)-2,4-nonadienal appeared to be mainly responsible for the stale odor in green tea [Liu *et al.*, 2023], which indicated that the significant down-regulation of (*E,E*)-2,4-nonadienal could increase the flavor quality of *C. paliurus* fixated tea. (*E*)-2-Decenal was the main aroma compound in *Angelica keiskei* tea [Rong *et al.*, 2021], presenting typical flower, fruity, and sweet flavor.

The significant down-regulation of this compound after fixation in our study, may be related to its properties. After heating, it can continue to oxidize to form volatile substances with short carbon chains [Wang *et al.*, 2023]. 5-(Propan-2-yl)bicyclo[3.1.0]hex-2-ene-2-carbaldehyde has not yet been reported in the VOCs of *C. paliurus* leaves.

■ Hydrocarbons

Weakly polar hydrocarbons are also characteristic flavor compounds in teas [Guo *et al.*, 2021]. The 10 individual hydrocarbons (Table S1, Figure S1) included down-regulated after fixation olefins (4 compounds) and alkanes (2 compounds), as well up-regulated alkenes (3 compounds) and alkane (1 compound). Among them, 2,6-dimethyl-2,6-octadiene is produced during the thermal decomposition of glycosides [Hattori *et al.*, 2004]. Heating at 130°C/10 min + 110°C/30 min was conducive to its formation in black tea [Yang *et al.*, 2020]. In this study, the fixation temperature was higher (170°C–180°C); hence, its content decreased significantly. Albene has not been found in the VOCs of other species, especially tea, except in the roots of yarrow [Kindlovits *et al.*, 2018]. In addition, other up-regulated olefins and alkanes are rare in the study of volatile compound profile, and can be used as the characteristic compounds of *C. paliurus* tea for further study.

■ Aromatics

Contents of nine aromatics in *C. paliurus* tea variants showed significant differences, of which six were up-regulated after fixation (Table S1, Figure S1). Surprisingly, anethole and *trans*-anethole, which exists in fennel seeds [Odeh & Allaf, 2017], were found in *C. paliurus* tea, and they were significantly up-regulated after fixation. Due to their sweet, herbaceous, and smooth odor profile, anethole and *trans*-anethole exhibited a comparatively high rOAV, indicating their crucial role in creating distinctive flavors and making them the primary contributor to the overall flavor of Yunan and Fujian tea [Kfoury *et al.*, 2018]. Estragole, a volatile phenylpropanoid contained in a variety of edible herbs [Gross *et al.*, 2009; Yamani *et al.*, 2014], was suspected to be carcinogenic and genotoxic, according to the European Union Committee on Herbal Medicinal Products (<https://www.ema.europa.eu/en/committees/committee-herbal-medicinal-products-hmpc>). Therefore, further studies are needed on a safe daily intake of *C. paliurus* herbal tea. The 1,2-dihydro-1,1,6-trimethyl-naphthalene was found as a key aroma compound of Pu-erh tea (ripe tea) [Wang *et al.*, 2022]. It was also significantly up-regulated in *C. paliurus* tea after fixation. The other two up-regulated aromatics, *i.e.*, 1,2,3,4-tetrahydronaphthalene and 2-phenylpropenal, have not been reported in any tea volatile compound profile. The aromatics down-regulated after fixation were 2-methoxy-4-vinylphenol, (1-methyl-1-propylpentyl) benzene, and 1,1'-(1,3-propanediyl)bis-benzene. Among them, 2-methoxy-4-vinylphenol has the clove-like flavor, which has been reported to be a landmark compound of canned black tea after heating [Kumazawa & Masuda, 2001]. The fixation temperature was significantly higher than that of black tea sterilization;

thus, the changes of 2-methoxy-4-vinylphenol caused by the two treatments were inconsistent.

■ Other compounds

In addition to the volatile compounds mentioned above, the other up-regulated volatile compounds of teas after fixation included carbonochloridodithioic acid methyl ester, ethanediamide, benzoic acid, 2-(1-methylpropyl)phenol, *cis-p*-menth-2-en-7-ol, dipropyl disulfide, (*Z*)-1-methoxy-4-(1-propenyl)benzene, benzyl nitrile, and ethyl 1-methylethyl disulfide (Table S1, Figure S1). Among them, benzoic acid has a floral and fruity flavor, and its content was also significantly up-regulated after green tea and yellow tea fixation [Wen *et al.*, 2023], which is consistent with our findings. Benzyl nitrile has a pungent odor [Li & Wang, 2020], which has been identified as a key marker for the floral odor of Fenghuang Dancong tea [Qin *et al.*, 2023]. The significantly up-regulated *cis-p*-menth-2-en-7-ol, has been previously found in *Ephedra sinica* Stapf. [Miyazawa *et al.*, 1997].

Four volatiles of *C. paliurus* teas were down-regulated after fixation included butanoic anhydride, *N*-phenylacetamide, (*E*)-2-hexenoic acid, and 2-(1,1-dimethylethyl)phenol. (*E*)-2-Hexenoic acid, with a fruity, herbal odor, has also been found in ferns [Fons *et al.*, 2010].

■ Aroma compounds of *C. paliurus* teas

Although a variety of volatile components were screened in different processed products of *C. paliurus* tea, not all of the volatile compounds were the material basis for the odor difference between fixated and unfixated *C. paliurus* teas. Therefore, the rOAV method was used to determine the contribution of volatile components to the overall odor of teas, and then to identify the key aroma compounds. In general, rOAV ≥ 1 indicates that the compound has a direct contribution to the sample flavor, and 0.1 ≤ rOAV < 1 can be considered to have an important modification effect on the aroma of the sample [Huang *et al.*, 2022; Xue *et al.*, 2022]. As shown in Table 1, there were 46 volatile compounds with rOAV value greater than 0.1 in the unfixated *C. paliurus* tea, among which 22 volatile compounds had rOAV greater than 1. In the fixated *C. paliurus* tea, there were 44 volatile compounds with rOAV greater than 0.1, and 25 volatile compounds with rOAV greater than 1.

Many identified ketones, aldehydes, terpenes and other compounds in the tea produced by the two processing technologies had a high content and a relatively low threshold; hence, they contributed a lot to the overall flavor of the tea. The compound that contributed the most was 1-hexen-3-one (ketone), which mainly presents a cooked, vegetable, and metallic aroma. Followed by 3-octen-2-one, also a ketone, mainly presents earthy, spicy, herbal, sweet, mushroom, hay, and blueberry aroma. In addition, 5 terpenoids (2-methylisoborneol, 6,6-dimethyl-bicyclo[3.1.1]hept-2-ene-2-methanol, β -pinene, α -terpineol and ι - α -terpineol), 6 aldehydes (2-hexenal, (*E*)-2-undecenal, (*E,E*)-2,4-nonadienal, (*E*)-2-hexenal, 2-undecenal, (*E*)-2-decenal), 4 aromatics (anethole, 2-methoxy-4-vinylphenol, *trans*-anethole, and 1,2-dihydro-1,1-6-trimethyl-naphthalene), 2 esters (acetic acid cyclohexyl ester

Table 1. Threshold, odor description and relative odor activity value (rOAV) of volatile compounds identified in *Cyclocarya paliurus* tea produced with and without fixation.

Compound	Class	Odor	Threshold ($\mu\text{g/L}$)	rOAV*	
				Unfixated tea	Fixated tea
1-Hexen-3-one	Ketones	Cooked, vegetable, metallic	2×10^{-5}	25,371 \pm 1826	13,291 \pm 74
3-Octen-2-one	Ketones	Earthy, spicy, herbal, sweet, mushroom, hay, blueberry	3×10^{-5}	5,419 \pm 607	1,810 \pm 119
2-Methylisoborneol	Terpenoids	Earthy, musty	0.0005	551 \pm 27	273.8 \pm 8.3
Anethole	Aromatics	Sweet, exotic, flowery, stewed	0.015	21.3 \pm 1.4	212.8 \pm 7.8
(<i>E,E</i>)-2,4-Nonadienal	Aldehydes	Fatty, melon, waxy, green, violet, leafy, cucumber, tropical, fruity, chicken	0.0002	272 \pm 12	143.4 \pm 4.3
(<i>E</i>)-2-Undecenal	Aldehydes	Fresh, fruity, citrus, orange peel	0.0008	296 \pm 16	143.1 \pm 4.2
Heptanoic acid methyl ester	Esters	Sweet, fruity, green, orris, waxy, floral, berry	0.004	39.0 \pm 2.7	88.6 \pm 5.7
(<i>E</i>)-6,10-Dimethyl-5,9-undecadien-2-one	Ketones	Fresh, green, fruity, waxy, rose, woody, magnolia, tropical	0.01	162.5 \pm 8.1	68.3 \pm 2.4
<i>trans</i> -Anethole	Aromatics	Sweet, anisic, licorice, mimosa	0.057	5.62 \pm 0.37	56.0 \pm 2.1
(<i>E</i>)-2-Hexenal	Aldehydes	Green, grassy	0.0031	388 \pm 193	47.8 \pm 2.1
Acetic acid cyclohexyl ester	Esters	Fruity, sweet, musty, ethereal	0.0016	117.6 \pm 9.0	26.6 \pm 1.1
β -Pinene	Terpenoids	Dry, woody, resinous, pine, hay, green	0.14	7.32 \pm 0.46	16.15 \pm 0.50
1,2-Dihydro-1,1,6-trimethyl-naphthalene	Aromatics	Licorice	0.0025	5.19 \pm 0.23	12.16 \pm 0.40
2-Undecenal	Aldehydes	Fresh, fruity, orange, peel	0.01	23.1 \pm 1.3	11.17 \pm 0.33
2-Hexenal	Aldehydes	Sweet, almond, fruity, green, leafy, apple, plum, vegetable	0.017	71 \pm 35	8.72 \pm 0.38
2-Methoxy-4-vinylphenol	Aromatics	Spicy, raisin	0.003	15.62 \pm 0.60	8.02 \pm 0.34
(<i>E</i>)-2-Decenal	Aldehydes	Waxy, fatty, earthy, green, cilantro, mushroom, aldehydic, fried, chicken, fatty, tallow	0.005	14.21 \pm 0.68	7.28 \pm 0.37
6,6-Dimethyl-bicyclo[3.1.1]hept-2-ene-2-methanol	Terpenoids	Woody, minty	0.007	8.20 \pm 0.45	3.56 \pm 0.23
Acetic acid 4-methylphenyl ester	Esters	Narcissus, phenol, animalic	0.025	0.766 \pm 0.069	2.941 \pm 0.090
Diethyl phthalate	Esters	Bitter	0.33	0.384 \pm 0.033	2.65 \pm 0.25
3-Phenyl-2-propenal	Aldehydes	Sweet, spicy, aldehydic, aromatic, balsamic, cinnamyl, resinous, honey, powdery	0.024	0.58 \pm 0.11	2.392 \pm 0.099
Dipropyl disulfide	Sulfur compounds	Sulfury, earthy, burnt, green, onion	0.0195	0.495 \pm 0.038	1.348 \pm 0.051
α -Ethylidenebenzeneacetaldehyde	Aldehydes	Sweet, narcissus, cortex, beany, honey, cocoa, nutty, radish	0.5	0.110 \pm 0.007	1.271 \pm 0.052
Estragole	Aromatics	Sweet, saffrass, anisic, spice, green, herbal, fennel	0.035	0.414 \pm 0.025	1.188 \pm 0.032
Eucalyptol	Terpenoids	Eucalyptus, herbal, camphor, medicinal	0.015	0.365 \pm 0.032	1.040 \pm 0.058
α -Terpineol	Terpenoids	Pine, iris, teil	0.3	2.28 \pm 0.18	0.964 \pm 0.032
(<i>E</i>)-2-Hexen-1-ol	Alcohols	Fresh, green, leafy, fruity, unripe banana	0.1	0.330 \pm 0.015	0.897 \pm 0.005
(<i>Z</i>)-2-Hexen-1-ol	Alcohols	Green, cortex, leafy, beany, nasturtium, herbal, soapy, aldehydic, narcissus, phenol	0.1	0.330 \pm 0.015	0.897 \pm 0.005
Dihydro-5-pentyl-2(3 <i>H</i>)-furanone	Heterocyclic compounds	Coconut, woody	0.0079	2.42 \pm 0.27	0.892 \pm 0.067
L- α -Terpineol	Terpenoids	Lilac, floral, terpenic	0.33	2.07 \pm 0.16	0.876 \pm 0.030
Methylpyrazine	Heterocyclic compounds	Nutty, cocoa, roasted, chocolate, peanut, green	0.06	0.306 \pm 0.037	0.689 \pm 0.020

Table 1 cont. Threshold, odor description and relative odor activity value (rOAV) of volatile compounds identified in *Cyclocarya paliurus* tea produced with and without fixation.

Compound	Class	Odor	Threshold (µg/L)	rOAV*	
				Unfixed tea	Fixed tea
Prenol	Alcohols	Fruity, green, lavender	0.25	1.37±0.10	0.664±0.020
α-Methyl-benzenemethanol	Alcohols	Fresh, sweet, gardenia, hyacinth	0.479	0.238±0.031	0.621±0.023
(1S)-6,6-Dimethyl-2-methylene-bicyclo[3.1.1]heptane	Terpenoids	Dry, woody, fresh, pine, hay, green, resinous	4.16	0.246±0.016	0.544±0.017
4-Methyl-1-(1-methylethyl)-bicyclo[3.1.0]hex-3-en-2-one	Terpenoids	Minty, pungent	0.75	0.158±0.009	0.469±0.034
3,7-Dimethyl-1,5,7-octatrien-3-ol	Alcohols	Mouldy	0.11	0.875±0.016	0.421±0.066
Hotrienol	Alcohols	Sweet, tropical, ocimene, fennel, ginger, myrcene	0.11	0.875±0.016	0.421±0.066
4-Methylene-1-(1-methylethyl)-bicyclo[3.1.0]hexane	Terpenoids	Woody, terpene, citrus, pine, spice	0.98	0.144±0.013	0.360±0.010
4-Methyl-1-(1-methylethyl)-bicyclo[3.1.0]hex-2-ene	Hydrocarbons	-	0.98	0.118±0.008	0.260±0.010
Isopentyl hexanoate	Esters	Fruity, banana, apple, pineapple, green	0.32	0.53±0.10	0.251±0.005
(E)-3,7,11-Trimethyl-1,6,10-dodecatrien-3-ol	Terpenoids	Floral, green, citrus, woody, waxy	0.25	0.478±0.0145	0.241±0.037
2-(1,1-Dimethylethyl)phenol	Phenols	-	0.05	0.421±0.040	0.202±0.030
(Z)-3-Hexen-1-ol benzoate	Esters	Fresh, green, leafy, floral, orchid, balsamic, fatty	0.5	0.046±0.011	0.183±0.009
(E)-2-Hexenoic acid	Acids	Powerful, fruity, sweet, warm, herbal	1.9	0.409±0.019	0.175±0.011
3-Hexanone	Ketones	Sweet, fruity, waxy, rummy, grape	0.041	0.396±0.030	0.148±0.041
1,2,3,4-Tetrahydronaphthalene	Aromatics	-	0.05	0.054±0.006	0.135±0.009
Benzyl nitrile	Nitrogen compounds	-	1.2	0.031±0.001	0.105±0.004
Acetic acid 2-ethylhexyl ester	Esters	Earthy, herbal, humus, undergrowth	0.047	0.162±0.017	0.058±0.003
Thymol	Terpenoids	Herbal, thyme, phenol, medicinal, camphor	0.188	0.220±0.070	0.040±0.003

*Values are shown as mean ± standard deviation.

and heptanoic acid methyl ester), 1 heterocyclic compound (dihydro-5-pentyl-2(3H)-furanone), 1 alcohol (prenol), and 1 ketone ((E)-6,10-dimethyl-5,9-undecadien-2-one) in the unfixed *C. paliurus* tea are its characteristic aroma substances. Green and fruity were the two main aromas, which were presented by a variety of substances. In the fixed *C. paliurus* tea, 8 aldehydes ((E,E)-2,4-nonadienal, (E)-2-undecenal, (E)-2-hexenal, 2-undecenal, 2-hexenal, (E)-2-decenal, 3-phenyl-2-propenal, and α-Et hydridenebenzeneacetaldehyde), 5 aromatics (anethole, *trans*-anethole, 1,2-dihydro-1,1,6-trimethyl-naphthalene, 2-methoxy-4-vinylphenol, and estragole), 4 terpenoids (2-methylisoborneol, β-pinene, 6,6-dimethyl-bicyclo[3.1.1]hept-2-ene-2-methanol, and eucalyptol), 4 esters (heptanoic acid methyl ester, acetic acid cyclohexyl ester, acetic acid 4-methylphenyl ester, and diethyl phthalate), 1 sulfur compound (dipropyl disulfide), and 1 ketone ((E)-6,10-dimethyl-5,9-undecadien-2-one) were its characteristic aroma compounds. Green and sweet were the two main aromas, which were presented by a variety of compounds.

CONCLUSIONS

Herein, the effect of fixation on the volatile compounds of *C. paliurus* tea was investigated and analyzed comprehensively for the first time. A total of 1,222 volatile metabolites were detected and 147 individual volatile metabolites were screened. Fixation had a strong effect on the content of volatile compounds in *C. paliurus* tea. After fixation, the content of amines, phenol, nitrogen, ether, alcohols, terpenoids, ester, aromatics increased significantly. The difference of metabolites before and after fixation indicates that fixation was a key step in *C. paliurus* tea processing. In addition, the study also found a batch of unique volatile compounds of *C. paliurus* tea and key aroma compounds, which present mainly green and sweet odor. The results of this study provided a new theoretical basis for the development of processing technology of *C. paliurus* leaves into tea, which could be used for quality control during the processing of *C. paliurus* leaves. In the follow-up study, we will investigate the potential effects of amino acids, lipids *etc.* added during the fixation step on the flavor characteristics of *C. paliurus* tea.

SUPPLEMENTARY MATERIALS

The following are available online at <https://journal.pan.olsztyn.pl/Untargeted-Metabolomics-Analysis-Reveals-the-Effect-of-Fixation-on-the-Profile-of,191754,0,2.html>.

Table S1. Profile of volatile metabolites of *Cyclocarya paliurus* teas produced with fixation and without fixation. **Figure S1.** Heatmaps of the significantly up-regulated (red cells) and down-regulated (green cells) volatile metabolites after fixation of *Cyclocarya paliurus* teas; aldehydes (A), hydrocarbons (B), heterocyclic compounds (C), ketones (D), aromatics (E), esters (F), alcohols (G), terpenoids (H) and others including one nitrogen compound, one ether, two amines, two acids, three phenols, and three sulfur compounds (I).

RESEARCH FUNDING

This work was supported by the Zhejiang Provincial Science and Technology Project (No. 2024F1065-3).

ADDITIONAL INFORMATION

Data supporting the findings of this study are available upon request from the corresponding author.

CONFLICT OF INTERESTS

The authors declare no competing interests.

ORCID IDs

M. Bai
L. Wang

<https://orcid.org/0009-0007-6478-2602>
<https://orcid.org/0000-0003-2166-2284>

REFERENCES

- Cao, T., Liu, J., Zhang, X., Wei, X., Qi, Y., Zhang, B., Liu, H., Xiao, P. (2019). Metabolomics characterization of different geographical origins of *Flos Carthami* using UPLC-QTOF/MS and their correlation with climate factors. *Analytical Methods*, 11(20), 2700-2711. <https://doi.org/10.1039/C9AY00368A>
- Chen, J., Yang, Y., Deng, Y., Liu, Z., Shen, S., Zhu, J., Yuan, H., Jiang, Y. (2022a). Characterization of the key differential volatile components in different grades of Dianhong Congou tea infusions by the combination of sensory evaluation, comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry, and odor activity value. *LWT – Food Science and Technology*, 165, art. no. 113755. <https://doi.org/10.1016/j.lwt.2022.113755>
- Chen, Q., Shi, J., Mu, B., Chen, Z., Dai, W., Lin, Z. (2020). Metabolomics combined with proteomics provides a novel interpretation of the changes in nonvolatile compounds during white tea processing. *Food Chemistry*, 332, art. no. 127412. <https://doi.org/10.1016/j.foodchem.2020.127412>
- Chen, Z., Jian, Y., Wu, Q., Wu, J., Sheng, W., Jiang, S., Shehla, N., Aman, S., Wang, W. (2022b). *Cyclocarya paliurus* (Batalin) Iljinskaja: Botany, ethnopharmacology, phytochemistry and pharmacology. *Journal of Ethnopharmacology*, 285, art. no. 114912. <https://doi.org/10.1016/j.jep.2021.114912>
- Culleré, L., Ferreira, V., Cacho, J. (2011). Analysis, occurrence and potential sensory significance of aliphatic aldehydes in white wines. *Food Chemistry*, 127(3), 1397-1403. <https://doi.org/10.1016/j.foodchem.2011.01.133>
- Dai, Q., Jin, H., Gao, J., Ning, J., Yang, X., Xia, T. (2020). Investigating volatile compounds' contributions to the stale odour of green tea. *International Journal of Food Science and Technology*, 55(4), 1606-1616. <https://doi.org/10.1111/ijfs.14387>
- Fella, P., Kaikiti, K., Stylianou, M., Agapiou, A. (2022). HS-SPME-GC/MS analysis for revealing carob's ripening. *Metabolites*, 12(7), art. no. 656. <https://doi.org/10.3390/metabo12070656>
- Fons, F., Froissard, D., Bessiere, J., Buatois, B., Rapior, S. (2010). Biodiversity of volatile organic compounds from five French ferns. *Natural Product Communications*, 5(10), 1655-1658. <https://doi.org/10.1177/1934578X1000501028>
- Geng, Z., Wang, J., Zhu, L., Yu, X., Zhang, Q., Li, M., Hu, B., Yang, X. (2023). Metabolomics provide a novel interpretation of the changes in flavonoids during sea buckthorn (*Hippophae rhamnoides* L.) drying. *Food Chemistry*, 413, art. no. 135598. <https://doi.org/10.1016/j.foodchem.2023.135598>
- Gross, M., Lewinsohn, E., Tadmor, Y., Bar, E., Dudai, N., Cohen, Y., Friedman, J. (2009). The inheritance of volatile phenylpropenes in bitter fennel (*Foeniculum vulgare* Mill. var. *vulgare*, Apiaceae) chemotypes and their distribution within the plant. *Biochemical Systematics and Ecology*, 37(4), 308-316. <https://doi.org/10.1016/j.bse.2009.05.007>
- Gu, K., Feng, S., Zhang, X., Peng, Y., Sun, P., Liu, W., Wu, Y., Yu, Y., Liu, X., Liu, X., Deng, G., Zheng, J., Li, B., Zhao, L. (2024). Deciphering the antifungal mechanism and functional components of *Cinnamomum cassia* essential oil against *Candida albicans* through integration of network-based metabolomics and pharmacology, the greedy algorithm, and molecular docking. *Journal of Ethnopharmacology*, 319(Part 2), art. no. 117156. <https://doi.org/10.1016/j.jep.2023.117156>
- Guo, X., Ho, C., Schwab, W., Wan, X. (2021). Aroma profiles of green tea made with fresh tea leaves plucked in summer. *Food Chemistry*, 363, art. no. 130328. <https://doi.org/10.1016/j.foodchem.2021.130328>
- Hasanpour, M., Iranshahy, M., Iranshahi, M. (2020). The application of metabolomics in investigating anti-diabetic activity of medicinal plants. *Biomedicine & Pharmacotherapy*, 128, art. no. 110263. <https://doi.org/10.1016/j.biopha.2020.110263>
- Hattori, S., Kawaharada, C., Tazaki, H., Fujimori, T., Kimura, K., Ohnishi, M., Nabeta, K. (2004). Formation mechanism of 2,6-dimethyl-2,6-octadienes from thermal decomposition of linallyl β -D-glucopyranoside. *Bioscience, Biotechnology & Biochemistry*, 68(12), 2656-2659. <https://doi.org/10.1271/bbb.68.2656>
- He, Y., Li, J., Mei, H., Zhuang, J., Zhao, Z., Jeyaraj, A., Wang, Y., Chen, X., Li, X., Liu, Z. (2023). Effects of leaf-spreading on the volatile aroma components of green tea under red light of different intensities. *Food Research International*, 168, art. no. 112759. <https://doi.org/10.1016/j.foodres.2023.112759>
- Hu, H., Shi, A., Liu, H., Liu, L., Fauconnier, M.L., Wang, Q. (2021) Study on key aroma compounds and its precursors of peanut oil prepared with normal- and high-oleic peanuts. *Foods*, 10(12), art. no. 3036. <https://doi.org/10.3390/foods10123036>
- Huang, W.J., Fang, S.M., Wang, J., Zhuo, C., Luo, Y., Yu, Y., Li, L., Wang, Y., Deng, W., Ning, J. (2022). Sensomics analysis of the effect of the withering method on the aroma components of Keemun black tea. *Food Chemistry*, 395, art. no. 133549. <https://doi.org/10.1016/j.foodchem.2022.133549>
- Jeyaparakash, S., Heffernan, J.E., Driscoll, R.H., Frank, D.C. (2020). Impact of drying technologies on tomato flavor composition and sensory quality. *LWT – Food Science and Technology*, 120, art. no. 108888. <https://doi.org/10.1016/j.lwt.2019.108888>
- Kfoury, N., Baydakov, E., Gankin, Y., Robbat, A.Jr. (2018). Differentiation of key biomarkers in tea infusions using a target/nontarget gas chromatography/mass spectrometry workflow. *Food Research International*, 113, 414-423. <https://doi.org/10.1016/j.foodres.2018.07.028>
- Kindlovits, S., Sárosi, S., Inotai, K., Petrović, G., Stojanović, G., Németh, E. (2018). Phytochemical characteristics of root volatiles and extracts of *Achillea collina* Becker genotypes. *Journal of Essential Oil Research*, 30(5), 330-340. <https://doi.org/10.1080/10412905.2018.1470581>
- Kumazawa, K., Masuda, H. (2001). Change in the flavor of black tea drink during heat processing. *Journal of Agricultural and Food Chemistry*, 49(7), 3304-3309. <https://doi.org/10.1021/jf001323h>
- Li, J., Ma, Y., Xiao, F., Fu, B., Xu, X. (2023a). Formation and conversion of flavour related compounds of thermal cooked *Ruditia pesphilippinarum* soup. *International Journal of Food Science, Technology*, 58(12), 6421-6431. <https://doi.org/10.1111/ijfs.16753>
- Li, X., Shi, C., Wang, S., Wang, S., Wang, X., Lü, X. (2023b). Uncovering the effect of *Moringa oleifera* Lam. leaf addition to Fuzhuan Brick Tea on sensory properties, volatile profiles and anti-obesity activity. *Food & Function*, 14(13), art. no. 6274. <https://doi.org/10.1039/D3FO90057F>
- Li, Z., Wang, J. (2020). Identification and similarity analysis of aroma substances in main types of Fenghuang Dancong tea. *PLoS One*, 15(12), art. no. 244224. <https://doi.org/10.1371/journal.pone.0244224>
- Liu, P., Feng, L., Xu, Y., Zheng, L., Yin, P., Ye, F., Gui, A., Wang, S., Wang, X., Teng, J., Xue, J., Gao, S., Zheng, P. (2023). Characterization of stale odor in green tea formed during storage: Unraveling improvements arising from reprocessing by baking. *LWT – Food Science and Technology*, 174, art. no. 114458. <https://doi.org/10.1016/j.lwt.2023.114458>
- Liu, X., Zhou, L., Shi, X., Xu, G. (2019). New advances in analytical methods for mass spectrometry-based large-scale metabolomics study. *TrAC Trends in Analytical Chemistry*, 121, art. no. 115665. <https://doi.org/10.1016/j.trac.2019.115665>

27. Long, Z., Zhao, S., Xu, X., Du, W., Chen, Q., Hu, S. (2023). Dynamic changes in flavor and microbiota in traditionally fermented bamboo shoots (*Chimonobambusa szechuanensis* (Rendle) Keng f.). *Foods*, 12(16), art. no. 3035. <https://doi.org/10.3390/foods12163035>
28. Lu, W., Chen, J., Li, X., Qi, Y., Jiang, R. (2023). Flavor components detection and discrimination of isomers in Huaguo tea using headspace-gas chromatography-ion mobility spectrometry and multivariate statistical analysis. *Analytica Chimica Acta*, 1243, art. no. 340842. <https://doi.org/10.1016/j.aca.2023.340842>
29. Miyazawa, M., Minamino, Y., Kameoka, H. (1997). Volatile components of *Ephedra sinica* Stapf. *Flavour and Fragrance Journal*, 12(1), 15–17. [https://doi.org/10.1002/\(SICI\)1099-1026\(199701\)12:1<15::AID-FFJ604>3.0.CO;2-5](https://doi.org/10.1002/(SICI)1099-1026(199701)12:1<15::AID-FFJ604>3.0.CO;2-5)
30. Mu, R., Zhang, H., Zhang, Z., Li, X., Ji, J., Wang, X., Gu, Y., Qin, X. (2023). Trans-cinnamaldehyde loaded chitosan based nanocapsules display antibacterial and antibiofilm effects against cavity-causing *Streptococcus mutans*. *Journal of Oral Microbiology*, 15(1), art. no. 2243067. <https://doi.org/10.1080/20002297.2023.2243067>
31. Odeh, A., Allaf, A.W. (2017). Determination of polyphenol component fractions and integral antioxidant capacity of Syrian aniseed and fennel seed extracts using GC-MS, HPLC analysis, and photochemiluminescence assay. *Chemical Papers*, 71(9), 1731–1737. <https://doi.org/10.1007/s11696-017-0169-9>
32. Ouyang, W., Yu, Y., Wang, H., Jiang, Y., Hua, J., Ning, J., Yuan, H. (2022). Analysis of volatile metabolite variations in strip green tea during processing and effect of rubbing degree using untargeted and targeted metabolomics. *Food Research International*, 162(Part B), art. no. 112099. <https://doi.org/10.1016/j.foodres.2022.112099>
33. Pérez-López, A.J., Saura, D., Lorente, J. Carbonell-Barrachina, Á.A. (2006). Limonene, linalool, α -terpineol, and terpinen-4-ol as quality control parameters in mandarin juice processing. *European Food Research and Technology*, 222, 281–285. <https://doi.org/10.1007/s00217-005-0055-5>
34. Qin, D., Wang, Q., Jiang, X., Ni, E., Fang, K., Li, H., Wang, Q., Pan, C., Li, B., Wu, H. (2023). Identification of key volatile and odor-active compounds in 10 main fragrance types of Fenghuang Dancong tea using HS-SPME/GC-MS combined with multivariate analysis. *Food Research International*, 173(Part 1), art. no. 113356. <https://doi.org/10.1016/j.foodres.2023.113356>
35. Qin, D., Wang, Q., Li, H., Jiang, X., Fang, K., Wang, Q., Li, B., Pan, C., Wu, H. (2020). Identification of key metabolites based on non-targeted metabolomics and chemometrics analyses provides insights into bitterness in Kucha [*Camellia kucha* (Chang et Wang) Chang]. *Food Research International*, 138(Part B), art. no. 109789. <https://doi.org/10.1016/j.foodres.2020.109789>
36. Rong, Y., Gu, X., Li, D., Chen, L., Zhang, Y., Wang, Z. (2021). Characterization of aroma, sensory and taste properties of *Angelica keiskei* tea. *European Food Research and Technology*, 247(7), 1665–1677. <https://doi.org/10.1007/s00217-021-03737-7>
37. Rong, Y., Xie, J., Yuan, H., Wang, L., Liu, F., Deng, Y., Jiang, Y., Yang, Y. (2023). Characterization of volatile metabolites in Pu-erh teas with different storage years by combining GC-E-Nose, GC-MS, and GC-IMS. *Food Chemistry: X*, 18, art. no. 100693. <https://doi.org/10.1016/j.fochx.2023.100693>
38. Sandoval-Montemayor, N.E., García, A., Elizondo-Treviño, E., Garza-González, E., Alvarez, L., Del Rayo Camacho-Corona, M. (2012). Chemical composition of hexane extract of *Citrus aurantifolia* and anti-*Mycobacterium tuberculosis* activity of some of its constituents. *Molecules*, 17(9), 11173–11184. <https://doi.org/10.3390/molecules170911173>
39. Shao, Y., Li, T., Wu, C., Fang, S., Fan, G., Shang, X., Yang, W. (2024). A review on extraction and biological activities of triterpenoid from *Cyclocarya paliurus*. *Food Reviews International*, 40, art. no. 2213307. <https://doi.org/10.1080/87559129.2023.2213307>
40. Valero, E., Villamiel, M., Miralles, B., Sanz, J., Martínez-Castro, I. (2001). Changes in flavour and volatile components during storage of whole and skimmed UHT milk. *Food Chemistry*, 72(1), 51–58. [https://doi.org/10.1016/S0308-8146\(00\)00203-X](https://doi.org/10.1016/S0308-8146(00)00203-X)
41. van Gemert, L.J. (Ed.), (2011). *Odour Thresholds: Compilations of Odour Threshold Values in Air, Water and Other Media*. 2nd edition, Oliemans Punter, Partners BV, Zeist, The Netherlands.
42. Wang, B., Wang, S., Wang, Y., Zhang, S., Lin, X., Xu, X., Ji, C., Liang, H., Dong, L. (2023). Deep exploration of lipid oxidation into flavor compounds: A density functional theory study on (E)-2-decenal thermal oxidative reaction. *Food Chemistry*, 428, art. no. 136725. <https://doi.org/10.1016/j.foodchem.2023.136725>
43. Wang, H., Hua, J., Jiang, Y., Yang, Y., Wang, J., Yuan, H. (2020). Influence of fixation methods on the chestnut-like aroma of green tea and dynamics of key aroma substances. *Food Research International*, 136, art. no. 109479. <https://doi.org/10.1016/j.foodres.2020.109479>
44. Wang, H., Hua, J., Yu, Q., Li, J., Wang, J., Deng, Y., Yuan, H., Jiang, Y. (2021a). Widely targeted metabolomic analysis reveals dynamic changes in non-volatile and volatile metabolites during green tea processing. *Food Chemistry*, 363, art. no. 130131. <https://doi.org/10.1016/j.foodchem.2021.130131>
45. Wang, K., Arntfield, S.D. (2015). Effect of salts and pH on selected ketone flavours binding to salt-extracted pea proteins: The role of non-covalent forces. *Food Research International*, 77(Part 1), 1–9. <https://doi.org/10.1016/j.foodres.2015.03.017>
46. Wang, Z., Dou, R., Yang, R., Cai, K., Li, C., Li, W. (2021b). Changes in phenols, polysaccharides and volatile profiles of noni (*Morinda citrifolia* L.) juice during fermentation. *Molecules*, 26(9), art. no. 2604. <https://doi.org/10.3390/molecules26092604>
47. Wang, Z., Su, D., Ren, H., Lv Q., Ren L., Li, Y., Zhou, H. (2022). Effect of different drying methods after fermentation on the aroma of Pu-erh tea (ripe tea). *LWT – Food Science and Technology*, 171, art. no. 114129. <https://doi.org/10.1016/j.lwt.2022.114129>
48. Wen, S., Sun, L., Zhang, S., Chen, Z., Chen, R., Li, Z., Lai, X., Zhang, Z., Cao, J., Li, Q., Sun, S., Lai, Z., Li, Q. (2023). The formation mechanism of aroma quality of green and yellow teas based on GC-MS/MS metabolomics. *Food Research International*, 172, art. no. 113137. <https://doi.org/10.1016/j.foodres.2023.113137>
49. Xu, Q., Zhou, Y.J., Wang, J.H., (2020). The impact of fixation method on the biochemical characteristics of hawk tea (*Litsea coreana*). *International Journal of Food Engineering*, 16(8), art. no. 20190290. <https://doi.org/10.1515/ijfe-2019-0290>
50. Xue, J., Liu, P., Yin, J., Wang, W., Zhang, J., Wang, W., Le, T., Ni, D., Jiang, H. (2022). Dynamic changes in volatile compounds of shaken black tea during its manufacture by GC \times GC–TOFMS and multivariate data analysis. *Foods*, 11(9), art. no. 1228. <https://doi.org/10.3390/foods11091228>
51. Yamani, H., Mantri, N., Morrison, P.D., Pang, E. (2014). Analysis of the volatile organic compounds from leaves, flower spikes, and nectar of Australian grown *Agastache rugosa*. *BMC Complementary and Alternative Medicine*, 14, art. no. 495. <https://doi.org/10.1186/1472-6882-14-495>
52. Yang, G., Zhou, M., Shi, J., Peng, Q., Lin, Z., Lv, H., Simal-Gandara, J. (2023). How anaerobic treatment is controlling the volatile components and key odorants of purple-colored leaf tea. *Journal of Food Composition and Analysis*, 122, art. no. 105451. <https://doi.org/10.1016/j.jfca.2023.105451>
53. Yang, Y., Hua, J., Deng, Y., Jiang, Y., Qian, M.C., Wang, J., Li, J., Zhang, M., Dong, C., Yuan, H. (2020). Aroma dynamic characteristics during the process of variable-temperature final firing of Congou black tea by electronic nose and comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry. *Food Research International*, 137, art. no. 109656. <https://doi.org/10.1016/j.foodres.2020.109656>
54. Yang, Y., Qian, M.C., Deng, Y., Yuan, H., Jiang, Y. (2022b). Insight into aroma dynamic changes during the whole manufacturing process of chestnut-like aroma green tea by combining GC-E-Nose, GC-IMS, and GC \times GC-TOFMS. *Food Chemistry*, 387, art. no. 132813. <https://doi.org/10.1016/j.foodchem.2022.132813>
55. Yang, Y.J., Ai, L.Z., Mu, Z.Y., Liu, H.D., Yan, X., Ni, L., Zhang, H., Xia, Y.J. (2022a). Flavor compounds with high odor activity values (OAV > 1) dominate the aroma of aged Chinese rice wine (Huangjiu) by molecular association. *Food Chemistry*, 383, art. no. 132370. <https://doi.org/10.1016/j.foodchem.2022.132370>
56. Yao, H., Su, H., Ma, J., Zheng, J., He, W., Wu, C., Hou, Z., Zhao, R., Zhou, Q. (2023). Widely targeted volatileomics analysis reveals the typical aroma formation of Xinyang black tea during fermentation. *Food Research International*, 164, art. no. 112387. <https://doi.org/10.1016/j.foodres.2022.112387>
57. Yu, J., Chu, M., Park, H., Park, J., Lee, K. (2021). Analysis of volatile compounds in coffee prepared by various brewing and roasting methods. *Foods*, 10(6), art. no. 1347. <https://doi.org/10.3390/foods10061347>
58. Zhang, L., Zhang, Z., Fang, S., Liu, Y., Shang, X. (2021). Integrative analysis of metabolome and transcriptome reveals molecular regulatory mechanism of flavonoid biosynthesis in *Cyclocarya paliurus* under salt stress. *Industrial Crops and Products*, 170, art. no. 113823. <https://doi.org/10.1016/j.indcrop.2021.113823>
59. Zhou, J., He, C., Qin, M., Luo, Q., Jiang, X., Zhu, J., Qiu, L., Yu, Z., Zhang, D., Chen, Y., Ni, D. (2023a). Characterizing and decoding the effects of different fermentation levels on key aroma substances of Congou black tea by sensomics. *Journal of Agricultural and Food Chemistry*, 71(40), 14706–14719. <https://doi.org/10.1021/acs.jafc.3c02813>
60. Zhou, J., Zhang, X., Liu, W., Zhang, Q., Wu, L., (2023b). Effect of different drying methods on color difference, microorganisms and volatile metabolites profiles of Ganpu Tea. *Food Bioscience*, 56, art. no. 103314. <https://doi.org/10.1016/j.fbio.2023.103314>

61. Zhou, X., Zhang, Q., Chen, X., Li, X., Han, C. (2021). *In-situ* assessment of olive oil adulteration with soybean oil based on thermogravimetric-gas chromatography/mass spectrometry combined with chemometrics. *Food Control*, 130, art. no. 108251. <https://doi.org/10.1016/j.foodcont.2021.108251>
62. Zhu, J.C., Niu, Y.W., Xiao, Z.B. (2021). Characterization of the key aroma compounds in Laoshan green teas by application of odour activity value (OAV), gas chromatography-mass spectrometry-olfactometry (GC-MS-O) and comprehensive two-dimensional gas chromatography mass spectrometry (GC×GC-qMS). *Food Chemistry*, 339, art. no. 128136. <https://doi.org/10.1016/j.foodchem.2020.128136>
63. Zhu, X., Li, L., Hsiang, T., Zha, Y.P., Zhou, Z.X., Chen, R., Wang, X., Wu, Q.L., Li, J.K. (2020). Chemical composition and attractant activity of volatiles from *Rhus potaninii* to the spring aphid *Kaburagia rhusicola*. *Molecules*, 25(15), art. no. 3412. <https://doi.org/10.3390/molecules25153412>
64. Zhu, Y., Shao, C.Y., Lv, H.P., Zhang, Y., Dai, W.D., Guo, L., Tan, J.F., Peng, Q.H., Lin, Z. (2017). Enantiomeric and quantitative analysis of volatile terpenoids in different teas (*Camellia sinensis*). *Journal of Chromatography A*, 1490, 177-190. <https://doi.org/10.1016/j.chroma.2017.02.013>