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Quality attributes of black tea-flavored Chardonnay wine processed by ultrasound

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Abstract

An innovative Chardonnay wine flavored with black tea was prepared under optimal ultrasound conditions at 450.0 W for 22.6 min, and its physicochemical features, antioxidant power, total phenolic content, and volatile composition were systematically examined. A total of 165 phenolic and non-phenolic substances were characterized, including 27 newly formed phenolic substances and 10 non-phenolic substances that were not found in both black tea and Chardonnay wine. Catechin was found to be the phenolic compound with the largest amount detected, which was responsible for the potentiated antioxidant activities. The wine exhibited a profile of 44 volatile compounds, with 13 volatiles as odor-active compounds. Black tea infusion resulted in a decline in fruity, fermented and spicy aromas, but an increase in green and citrus odors, while ultrasound processing further intensified these odors. This study anticipated that ultrasound technology could hold promise for crafting flavored wines with heightened functionalities and appealing flavor profiles.

Keywords Ultrasound, Black tea, Chardonnay wine, Volatile compounds, Phenolic compounds, Antioxidant activity

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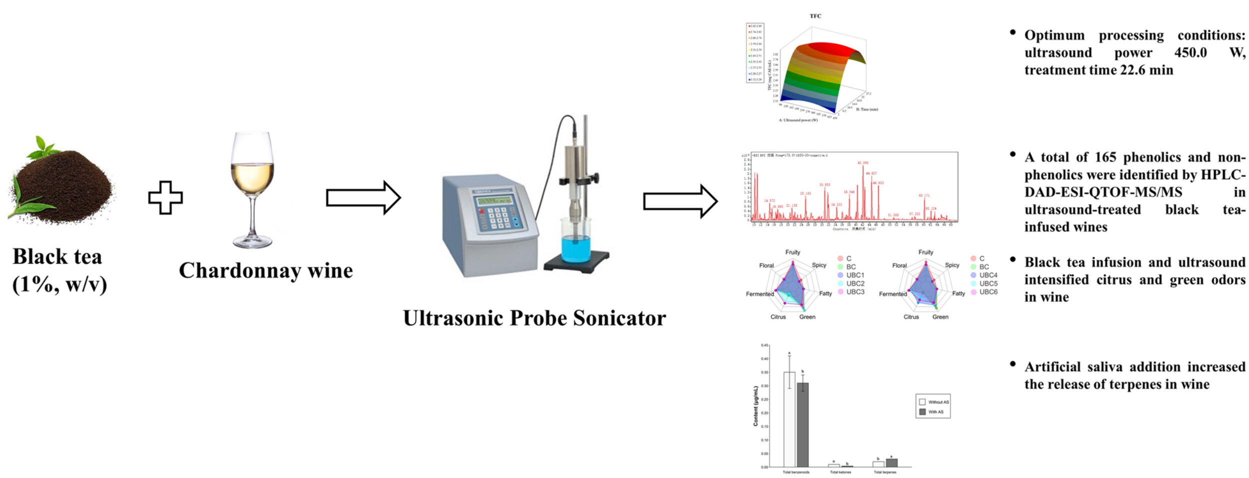
Graphical Abstract

Ultrasound-assisted infusion of black tea potentiated the antioxidant activity and enriched the volatile and phenolic profiles of Chardonnay wine.



Introduction

Wine, originating from the incomplete or complete alcoholic grape fermentation, is a globally popular aromatic beverage. With the advancement of winemaking technology, the traditional wine-producing countries have innovated new categories of wine, such as flavored wine, to meet the growing expectations from consumers and to stand out in the fierce competition. Flavored wine is a style of novel wine intensified with various flavorings, including fruits, spices, herbs and other natural flavor additives. Flavored wines can be produced by infusing non-grape materials into wine before or after alcoholic fermentation, thereby enriching the phenolic and volatile compositions of the wine matrix, which improves the wine mouthfeel, flavor and functionality. As the demand for new flavored wine grew, its global sales increased 40-fold in five years (Liang et al. 2021), indicating a new trend of wine market. However, the use of flavor additives in manufacturing wine products is governed by strict regulations in many countries. In Australia, the wine intensified with flavor additives is either called a “fruit wine” or a “wine product” according to the percentage of wine used. Likewise, the US FDA only permits the infusion of fruits but not herbs into wine (Liang et al. 2021).

Black tea is one of the most preferred non-alcoholic aromatic beverages associated with unique sensory characteristics and nutraceutical potentials, and it contributes to 78% of tea production in the world. When manufacturing black tea, fresh tea leaves are crushed before undergoing fermentation, where over 90% of the catechins are oxidized by polyphenol oxidase and endogenous peroxidase, leading to the generation of dimeric and oligomeric compounds including thearubigins, theasinensins and theaflavins. Such phenolic products account for numerous health-promoting potentials against hypercholesterolemia, cardiovascular disorders, neurological diseases, type 2 diabetes and aging (Liu et al. 2018). Black tea aroma is also a key criterion influencing the intuitive assessment of tea drinkers, which changes from a grassy to a floral, sweet or honey odor via different transformations of volatile compounds in the fermentation process. There are more than 600 volatile substances characterized in black tea, which include ketones, alkanes, aldehydes, alcohols and esters (Chen et al. 2022).Ultrasound has long been perceived as a useful tool in wine production via chemical (formation of free radicals) and physical (micro-mechanical shocks induced by cavitation action) effects, as evidenced by its superior efficiency in improving barrel sanitation and fermentation, extracting bioactive volatile and phenolic compounds, accelerating wine aging process, and controlling microbial and enzyme activity (Raghunath & Mallikarjunan 2020). In 2019, the ultrasound technology was officially permitted by the International Organization of Wine (OIV) for use in treating crushed grapes, which aims at enhancing the extraction of chemical compounds (Natolino & Celotti 2022). In addition, ultrasound was also proved to facilitate the bioactive extraction from black tea with shorter time and higher efficiency (Raghunath & Mallikarjunan

2020). Our previous studies indicated that both green and black teas can be macerated into white wine to develop tea-flavored wine with unique sensory characteristics and health benefits (Liang, Zhang, Zeng et al. 2023; Liang et al. 2024). However, no research has been conducted on the use of ultrasound technology in wine fortification by tea.

In the present work, we used ultrasound technology to prepare an innovative style of Chardonnay wine flavored with black tea. Optimization of processing parameters is essential for achieving efficiency and ensuring product quality at a lower cost, where grey relational analysis, response surface methodology and Taguchi design are the most commonly used techniques. In the current work, response surface methodology was designed for the processing condition optimization. The resultant wines were also investigated to characterize volatile and phenolic profiles. To our knowledge, this was the first research to document the profiles of phenolic and volatile substances, by high performance liquid chromatography diode array detector electrospray-ionization quadrupole time-of-flight mass spectrometry (HPLC–DAD-ESI-QTOF-MS/MS) and headspace-solid phase microextraction gas chromatography mass spectrometry (HS–SPME–GC–MS) respectively, in the black tea-flavored wines treated by ultrasound. This information provides a reference for further investigation on the application of ultrasound as an effective technique in manufacturing novel flavored wine.

Materials and methods

Schematic overview of the experimental program

A comprehensive schematic overview of the experimental program is presented in Fig. 1, displaying the major stages from sample preparation to analytical methods. The process begins with the preparation of Chardonnay wine and black tea powder, followed by ultrasound-assisted fortification, with varying parameters as per the full factorial model. The subsequent steps include the analysis of physicochemical properties, phenolic profiles, and antioxidant activities. The identification and quantification of phenolic compounds were performed using HPLC–DAD-ESI-QTOF-MS/MS and HPLC–DAD, respectively. In addition, volatile compounds were characterized through HS–SPME–GC–MS.

Materials and chemicals

Hydrochloric acid, phenolphthalein, ethanol, iron (III) chloride anhydrous, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$), glacial acetic acid, 2,4,6-tripyridyl-s-triazine (TPTZ), sodium nitrate, sodium hydroxide pellets, sodium carbonate anhydrous, potassium persulphate, Folin & Ciocalteu reagent (FCR), (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), aluminum chloride, and (+)-catechin hydrate were procured from Aladdin (Shanghai, China). Standards of phenolic and volatile compounds, *n*-alkanes (C8–C22), 4-octanol, α-amylase from porcine pancreas, mucin from bovine submaxillary salivary glands were obtained from Sigma-Aldrich (Castle Hill, NSW, Australia). All chemicals were of analytical reagent or HPLC grade.

Samples and preparation

Black tea (Yingde, Yinghong Tea Co., Ltd., Yingde, China) was acquired from a supermarket in Guangzhou, China, while Chardonnay wine (12.5% v/v ethanol, pH

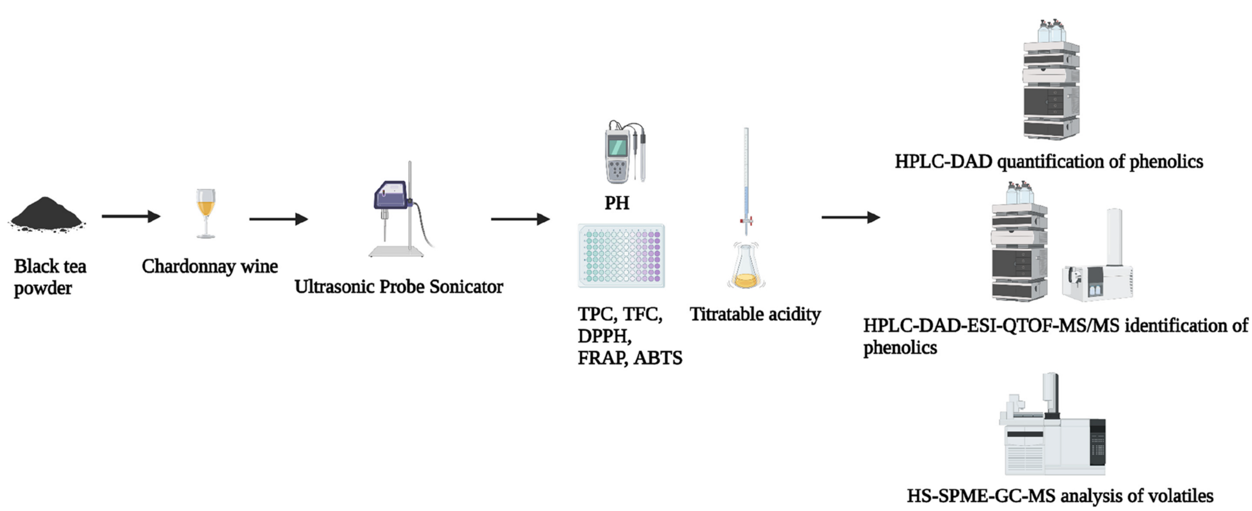


Fig. 1 Flow diagram of studies on quality attributes of black tea-flavored Chardonnay wine processed by ultrasound

3.0) was generously supplied by School of Food Science and Engineering, South China University of Technology (SCUT), Guangzhou, China. For the preparation of model wine, 12.5% (v/v) food grade ethanol was saturated with potassium hydrogen tartrate, followed by the addition of 40% (w/v) tartaric acid to adjust the pH value to 3.0. For the preparation of black tea powder, the black tea leaves were ground, and the resulting powder was sieved through a 500 μm sieve and kept in the dark at 4 ℃ before processing.

Experimental design

The ultrasound-assisted fortification experiment was designed with a full factorial model and performed as previously described (Liang, Zhang, Ma et al. 2023). Briefly, 0.3 g black tea powder was first mixed with 30 mL Chardonnay wine, then the mixture underwent treatment at varying ultrasonic power and treatment time as displayed in Table 1, in an ice bath using an ultrasonic processor (JY92-IIDN, Scientz Biotech Co., Ltd., Ningbo, China) with a Φ 6 mm ultrasound probe at 25 kHz. Afterward, the mixture was centrifuged at 3220 g for 10 min, and the supernatant was filtered and stored at 4 °C before analysis. Control Chardonnay wine (Control) and black tea-infused non-ultrasound-treated Chardonnay wine (BC) were utilized for comparison, with the latter undergone the same conditions as the ultrasound-treated wines but without ultrasonication. The tea-to-wine ratio was set at 1% (w/v) based on a prior study establishing this ratio as optimal for creating a black tea-macerated Chardonnay wine with desired sensory properties and enhanced functionalities (Liang, Zhang, Zeng et al. 2023). To analyze tea phenolics composition and the liberation of phenolics from tea into wine, black tea (1%, w/v) powder was added into model wine and ethanol respectively, then shaken at 150 rpm for 24 h at room temperature. The mixture was subsequently filtered and stored in the dark at 4 °C for analysis.

Table 1 Full factorial design for ultrasound treatment of black tea-infused Chardonnay wine and its runs

Runs	Coded variables		Uncoded variables	
	A	B	A: Ultrasound power (W)	B: Time (min)
C	-	-	-	-
BC	-	-	-	-
UBC1	-1	-1	90	2
UBC2	-1	0	90	10
UBC3	-1	+1	90	30
UBC4	0	-1	270	2
UBC5	0	0	270	10
UBC6	0	+1	270	30
UBC7	+1	-1	450	2
UBC8	+1	0	450	10
UBC9	+1	+1	450	30

C Control Chardonnay wine, *BC* Non-ultrasound treated black tea-infused Chardonnay wine, *UBC* Ultrasound-treated black tea-infused Chardonnay wine

- Not available

Analysis of physicochemical properties, phenolics profile and antioxidant activity

The titratable acidity (TA) and pH values of wine samples were assessed using the analytical techniques proposed by the International Organization of Vine and Wine (OIV) (OIV, 2023). The color parameters of wine samples were evaluated through the CIELAB method (Ayala et al. 1999). The TPC, TFC, DPPH, FRAP and ABTS assays were performed following the procedure outlined by Xiong et al. (2019) and Liang et al. (2024).

HPLC–DAD-ESI-QTOF-MS/MS identification of phenolic compounds

An Agilent 1290 series HPLC system (Agilent Technologies, Singapore) with an Agilent 6546 quadrupole time-of-flight (QTOF) MS system featuring a dual sprayer electrospray ionization (ESI), was employed to identify phenolic compounds in the wine samples. Separation was carried out on a Waters Atlantis T3 C18 column (250 × 4.6 mm i.d., 5 μm particle size) (Waters Corporation, Dublin, Ireland) using a mobile phase of 0.1% acetic acid in water (A) and acetonitrile (B), operated at a temperature of 30 °C. The analysis followed the method of Liang, Zhang, Xiong et al. (2023).

HPLC–DAD quantification of phenolic compounds

The Agilent 1260 Infinity II Prime LC (Agilent Technologies, Santa Clara, CA, USA) coupled with a diode array detector (DAD), was used for the polyphenol quantification in wine samples. The column and gradient program used were consistent with those specified in the preceding HPLC–DAD-ESI-QTOF-MS/MS analysis.

HS–SPME–GC–MS analysis of volatile compounds

Artificial saliva was prepared and the volatile compounds in wine were extracted as previously described (Liang, Zhang, Ma et al. 2023). The GC–MS analysis was carried out according to the protocol outlined by Liang et al. (2020). An Agilent 6850 GC system (Agilent Technologies) coupled with a 5973 mass spectrometer and a J&W DB-Wax Ultra Inert GC column (30 m × 250 μm × 0.25 μm) was utilized. The analysis of GC–MS data was conducted using the Agilent G1701EA MSD ChemStation software (Version 1.4.20.0).

Also, published odor thresholds (Crandles et al. 2015; Dragone et al. 2009; Guth 1997; Hu et al. 2022; Javed et al. 2018; Jiang & Zhang 2010; Li et al. 2008; Liang et al. 2020; Wang et al. 2015; Zea et al. 2001) were used to determine the contribution of the identified compounds to the overall aroma of wine samples via the calculation of the odor activity values (OAVs) as previously specified (Liang, Zhang, Ma et al. 2023).

Statistical analysis

Each treatment was performed in triplicates, and the outcomes were presented as the means ± standard deviations. To evaluate the significant differences between each sample, one-way ANOVA with Fisher grouping at a 95% confidence level was employed, utilizing Minitab (Minitab 21.1.0, Sydney, Australia). Pearson's correlation-significance matrix, principal component analysis (PCA), heatmap and spider plot were created using R packages, specifically 'Hmisc', 'factoextra', 'pheatmap' and 'fmsb', respectively.

Results and discussions

Physicochemical properties, total free phenolic and flavonoid content, antioxidant activity

A significant increase ($p = 0.01$) in pH was detected in the wine samples after mixing with black tea and ultrasound treatment, whereas the titratable acidity (TA) maintained relatively stable (Fig. 2a, b). The mixing with black tea and ultrasound treatment also led to a considerable decline ($p = 0.01$) in the L* values of the wine samples, while the a* and b* values as the increasing treatment time increased (Fig. 2c-e). It is worth noting that the lowest L* value was observed at 270 W and 30 min, which was paralleled with the highest a* and b* values under the same condition. Accordingly, the ultrasound-treated wines were featured by higher intensity of orange and amber hue and darker color, which might be ascribed to the presence of brown-colored substances (e.g., theaflavins, thearubigins) from the polyphenols in black tea (Liu et al. 2018), which will also be discussed later.

Optimization data outputs

The optimization data outputs are presented in Fig. 3. Ultrasound power and treatment time were only found to be significantly interacted with each other in DPPH and FRAP assays (Fig. 3c-I & d-I). The 3D surface plots and contour charts revealed that the combination of high ultrasound power (450.0 W) and long treatment time (22.6 min) could maximize the volatile content, antioxidant power and phenolic content in the treated wine samples. Ultrasound power can lead to the breakdown of cell wall structures, and thus boosting the efficiency of endogenous bioactive compounds like phenolics diffusing into the cell membranes (Raghunath & Mallikarjunan 2020). Rising trend was observed in TPC, TFC, DPPH and FRAP assays as the ultrasound treatment time increased from 2 to 22.6 min, but decreased afterward (Table S1). This might be due to the fact that phytochemicals had been completely extracted into the wines after 22.6 min of sonication. In the study of Raghunath and Mallikarjunan (2020), the optimum sonication time for maximum ABTS and DPPH radical scavenging activities were found to be 30 min when applying ultrasound in the cold-brewed black tea, while ultrasound was also claimed to optimally facilitate the release of most volatile components from black tea dry leaves at the extraction time of 21 min (Sereshti et al. 2013). These findings are in alignment with the present study. Time was the major factor influencing total volatile esters, alcohols, benzenoids and terpenes, which had increasing trend with increased treatment time. The interplay between ultrasound power and treatment time was mainly observed in total ketones, which reached the maximum level at around 16 min and decreased afterward. Ultrasound power was the major factor influencing total volatile acids, which showed an increasing trend with ultrasound power. The rising trend observed with processing time might be due to the fact that the prolonged ultrasonication is responsible for the enhanced permeability of the cell walls, resulting in the liberation of more volatile compounds (Natrella et al. 2023). The increase of total acids with ultrasound power might be that ultrasound irradiation leads to the oxidation of unsaturated aldehydes and alcohols. The mechanical and cavitation actions of ultrasound can result in an extreme microenvironment of high pressure and temperature, which can activate reactive molecules (Zheng et al. 2014).

Influence of ultrasound on the concentration of phenolics and volatiles

HPLC–DAD-ESI-QTOF-MS/MS had identified a total of 165 substances, encompassing 32 phenolic acids, 81 flavonoids and caffeine, in Chardonnay wine treated with ultrasound and infused with black tea (Table S2) (Fig. S1, S2 and S3). There were a total of 44 volatiles identified from the ultrasound-treated black tea-infused Chardonnay wines, with 26 compounds identified through authentic standards, NIST library 11.0 and NIST Webbook (NIST 2022). Additionally, 18 compounds were tentatively assigned based on MS and RI of the library (Table S4) (Fig. S4). These identified compounds were categorized into six chemical groups: 2 terpenes, 2 ketones, 13 esters, 7 benzenoids, 16 alcohols and 4 acids.

A total of 28 phenolic compounds, 35 volatiles and caffeine were either quantified or semi-quantified in Chardonnay wine treated with ultrasound and infused with

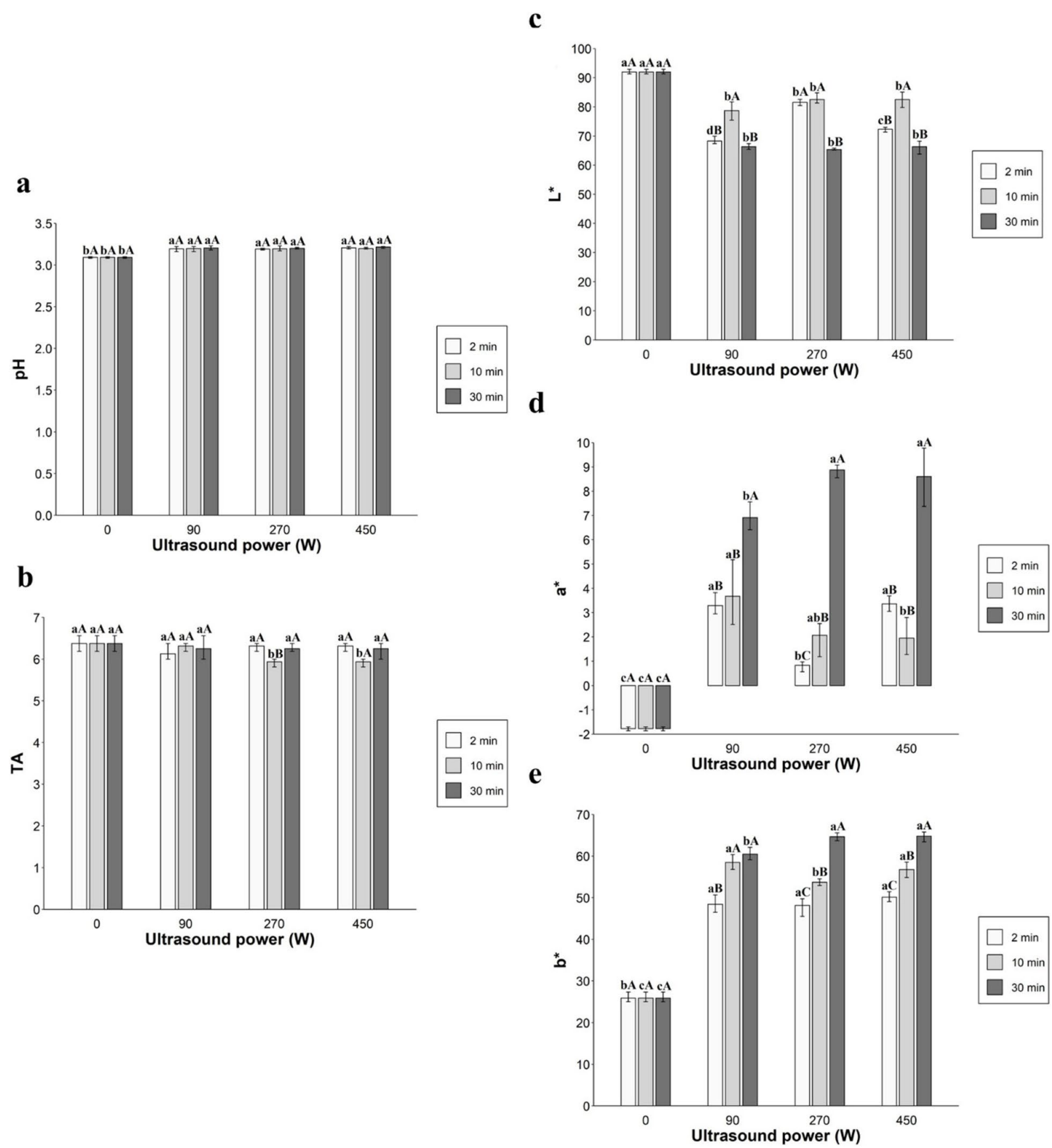


Fig. 2 The pH (**a**), titratable acidity (TA) (**b**), lightness L* (**c**), redness a* (**d**) and yellowness b* (**e**) of different ultrasound-treated black tea-flavored Chardonnay wines

black tea (Fig. 4a) (Tables S3 and S4). Overall, UBC1, UBC9, UBC3, UBC4, UBC7, UBC6 and UBC8 treatments displayed similar patterns, distinctly different from UBC2 and UBC5 (Fig. 4a). Several specific compounds including 1-propanol, catechin, quinic acid, 3-methyl-1-butanol, caffeine, rutin, quercetin 3-*O*-glucoside, unknown quercetin conjugate, procyanidin B2, epicatechin, gallocatechin gallate, *p*-hydroxybenzoic acid, kaempferol 3-*O*-glucoside, epicatechin gallate, gallic acid, phenylethyl alcohol, 3,4-dihydroxybenzoic acid, caffeic acid, diethyl succinate, hexanoic acid, octanoic acid, gallocatechin, procyanidin B3, procyanidin B1 and

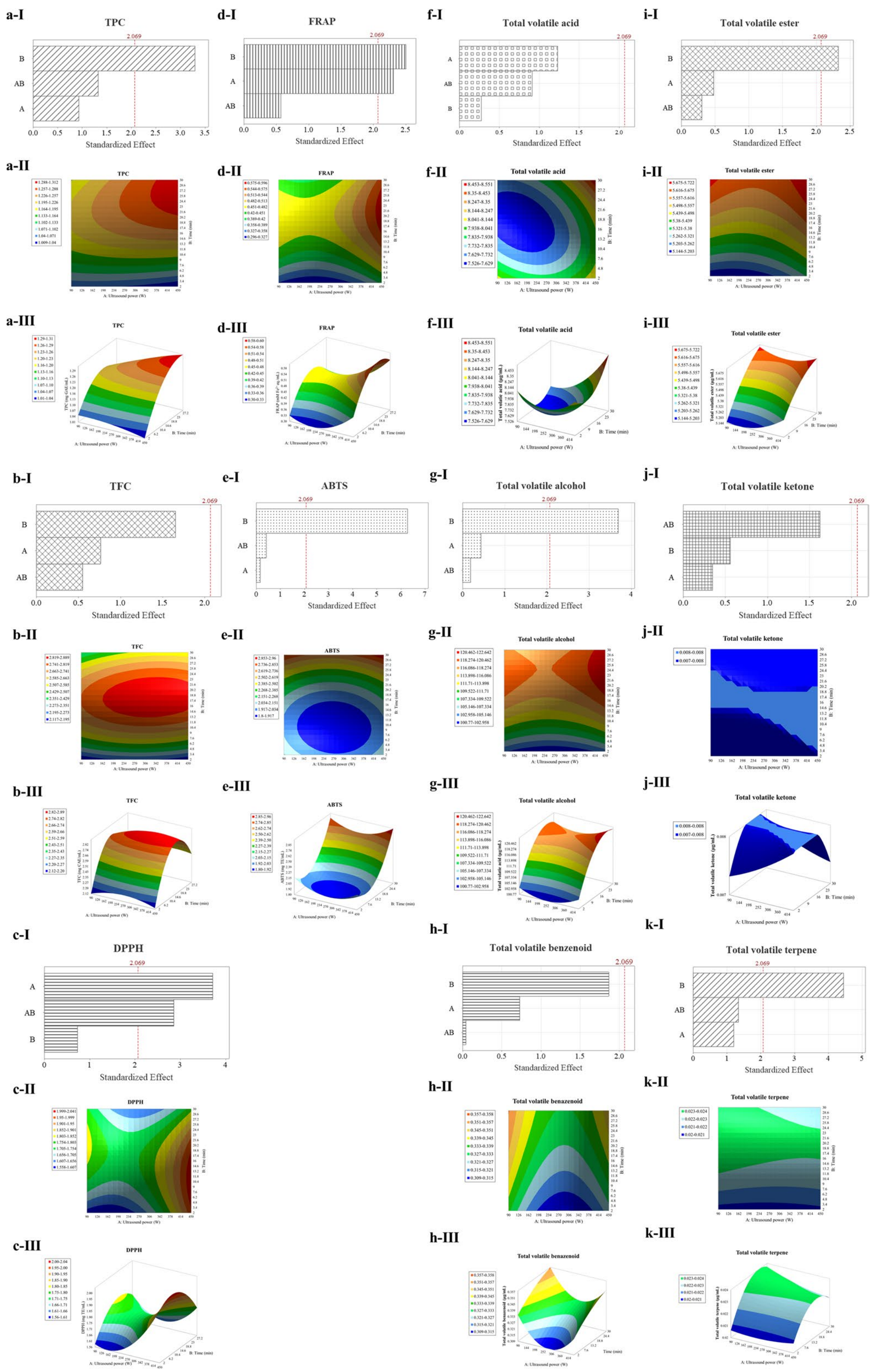


Fig. 3 Pareto chart of standardized effects (**a-I**, **b-I**, **c-I**, **d-I**, **e-I**, **f-I**, **g-I**, **h-I**, **i-I**, **j-I**, **k-I**) suggest the contribution of selected parameters to the ultrasound-treated black tea-infused Chardonnay wines (A: Ultrasound power, B: Treatment time, AB: Ultrasound power and time interaction effect). Contour plots (**a-II**, **b-II**, **c-II**, **d-II**, **e-II**, **f-II**, **g-II**, **h-II**, **i-II**, **j-II**, **k-II**) and 3D response surface plots (**a-III**, **b-III**, **c-III**, **d-III**, **e-III**, **f-III**, **g-III**, **h-III**, **i-III**, **j-III**, **k-III**) indicate the role of ultrasound power and treatment time on the value of responses

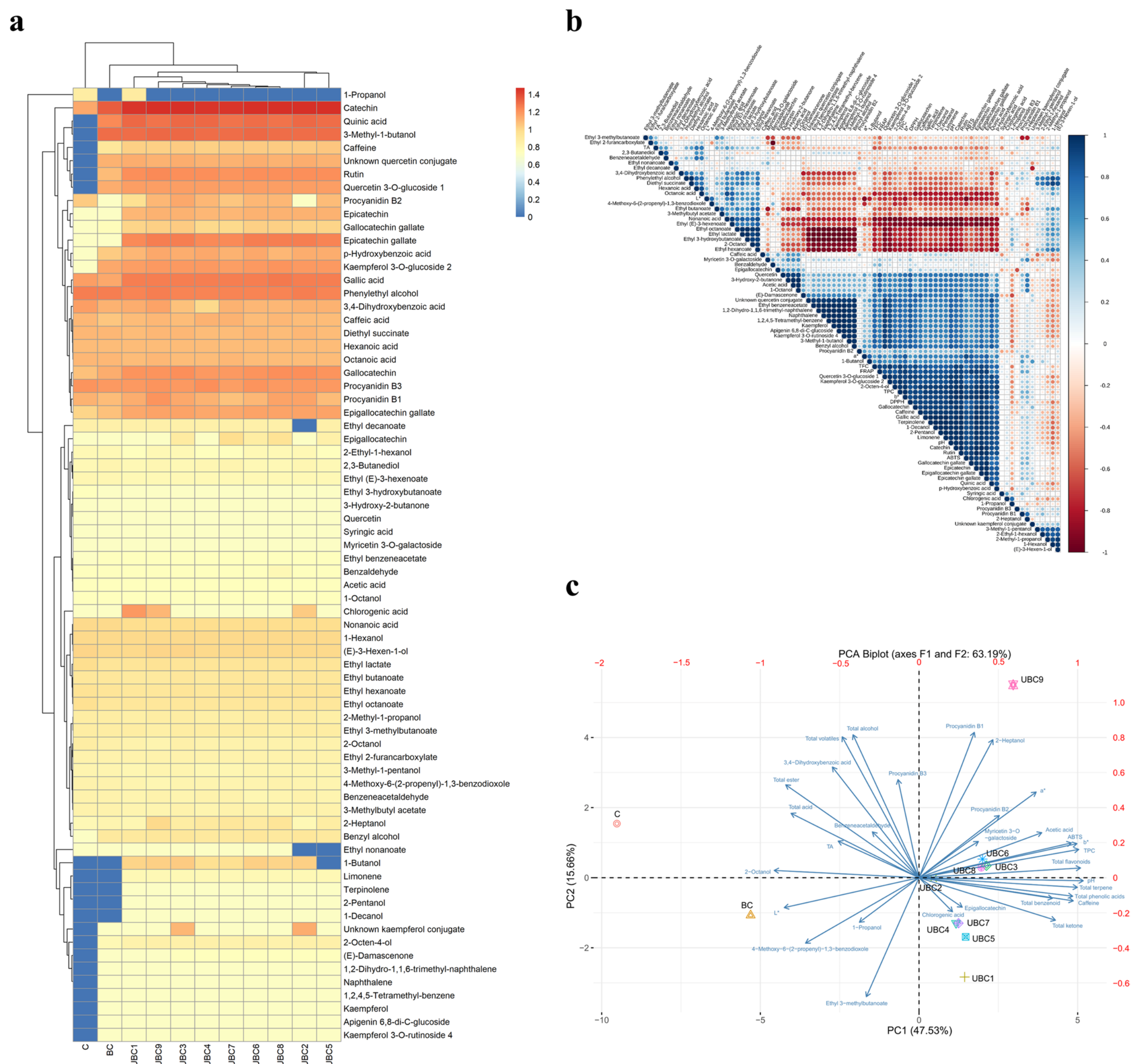


Fig. 4 Heatmap (**a**) showing the concentrations of all quantified phenolic and volatile compounds from control, non-ultrasound and ultrasound-treated black tea-infused Chardonnay wines. Pearson correlation matrix (**b**) and principal component analysis (**c**) of quantified phenolic and volatile compounds, physicochemical properties and tested responses of control, non-ultrasound and ultrasound-treated black tea-infused Chardonnay wines

epigallocatechin gallate showed similar distribution patterns among the treatment groups (Fig. 4a).

The predominant phenolics are catechin and quinic acid. As the primary phenolic acid, quinic acid was detected with the concentration range between 56.86 ± 1.44 and 86.02 ± 47.61 μg/mL observed in ultrasound-treated wine samples, which was significantly lower than the levels detected in 2% (w/v) black tea water infusion (81.25 ± 0.88—283.75 ± 4.42 μg/mL) as reported by Jeszka-Skowron et al. (2015). Catechins, the main constituents of tea polyphenols, were led by catechin, reaching concentrations between 400.03 ± 31.05 and 492.62 ± 10.61 μg/mL. This surpasses the values reported by Del Rio et al. (2004) for a 5.5% (w/v) black tea water infusion (12 ± 0.1 mg/L). The ultrasound-treated wines also recorded higher contents of gallocatechin (15.74 ± 3.73—22.24 ± 1.63 μg/mL) and epicatechin gallate (26.07 ± 2.17—42.36 ± 1.55 μg/mL) compared to the values reported by Del Rio et al. (2004), while the other catechins were quantified at lower concentrations.

Caffeine was also detected in ultrasound-treated wines with the highest value of 2.07 ± 0.61 μg/mL, which was significantly lower than the values (0.361–0.617 mg/mL) for the 2.5% (w/v) black tea water infusion (Carloni et al. 2013). This discrepancy is speculated to derive from the various tea cultivars and tea concentration. In black tea fermentation, various processing methods such as orthodox and crush-tear-curl, may also contribute to the loss of extractable caffeine to different extents (Carloni et al. 2013).

Diethyl succinate (34), octanoic acid (43), hexanoic acid (40), phenylethyl alcohol (42) and 3-methyl-1-butanol (9) are the major volatiles identified in ultrasound-treated Chardonnay wine infused with black tea (Fig. 4a) (Table S4). The decreasing trend was observed for phenylethyl alcohol and 3-methyl-1-butanol after ultrasound treatment, followed by a continuous decrease with the increasing ultrasound power and sonication time, whereas a considerable increase was witnessed at the treatment time of 30 min. The finding aligned with a previous study documenting the degradation effect of ultrasound irradiation on the higher alcohols in red wine (Zhang et al. 2020). Indeed, higher power ultrasound could potentiate the cavitation energy, leading to greater sonochemical impact on the stability of alcohols. Similarly, there was also a significant decline ($p = 0.01$) in the contents of hexanoic acid and octanoic acid after ultrasound processing, which contradicted the study of Zheng et al. (2014) where the total acid content in steeped greengage wine was increased after ultrasound processing. However, as the ultrasound power increased, the level of total acids climbed up at the treatment time of 2 and 30 min. When the ultrasound was applied, the content of diethyl succinate undergone a negative change induced by a possible degassing effect (García Martín & Sun 2013), although with an upswing as the sonication time increased. It was observed that the prolonged ultrasound processing can intensify the esterification between alcohols and acids in wine (Zheng et al. 2014), which agrees with the current study.

The relationships among physicochemical characteristics, individual volatile and phenolic compounds, and antioxidant capacities were investigated with Pearson's correlation (Fig. 4b). The color parameters including L*, a* and b*, exhibited significant ($p = 0.01$) correlation with pH, TPC, gallic acid, *p*-hydroxybenzoic acid, caffeine, epicatechin gallate, gallocatechin gallate, epigallocatechin gallate, quercetin 3-*O*-glucoside 1, epicatechin, catechin, and rutin, implying the essential role of these substances in varying the chromatic characteristics of ultrasound-treated wines. The potentiated antioxidant activities of ultrasound-treated wines were driven by catechin, which scored the highest coefficients ($r = 0.902$–0.936) in terms of antioxidant assays. Two major volatile substances, ethyl hexanoate and octanoic acid displayed significantly ($p = 0.01$) negative association with gallic acid, quinic acid, *p*-hydroxybenzoic acid, epigallocatechin gallate, gallocatechin, epicatechin, catechin, gallocatechin gallate, caffeine, unknown quercetin conjugate, quercetin 3-*O*-glucoside 1, kaempferol 3-*O*-glucoside 2, epicatechin gallate and rutin.

The antioxidant capacities, physicochemical properties and representative volatile and phenolic compounds was displayed using principal component analysis (PCA) biplots in Fig. 4c. F1 and F2 accounted for 63.19% of the total variance, out of which F1 (47.53%) was contributed by L*, pH, 4-methoxy-6-(2-propenyl)-1,3-benzodioxole, 1-propanol, ethyl 3-methylbutanoate, chlorogenic acid, epigallocatechin, total ketone, total benzenoid, caffeine, total terpene and total phenolic acids, and F2 (15.66%) was mostly related to 2-octanol, TA, total acid, benzeneacetaldehyde, total ester, 3,4-dihydroxybenzoic acid, total volatiles, total alcohol, procyanidin B3, procyanidin B1, 2-heptanol, procyanidin B2, a*, b*, myricetin 3-*O*-galactoside, acetic acid, ABTS, TPC and total flavonoids. In the PCA, non-ultrasound-treated black tea-infused Chardonnay wine and control Chardonnay wine were well-distinguished from ultrasound-treated wines along the F1 axis. There was some partial overlapping between UBC3, UBC6 and UBC8, suggesting the similar effect of these treatments on physicochemical properties, antioxidant capacities, volatile and phenolic profiles of wine. Additionally, UBC4, UBC5, and UBC7 exhibited a similar effect, as evident from their clear clustering and overlapping. Meanwhile, the distances between other treatment groups were investigated. The distance between UBC1 and UBC9 was the farthest, signifying a notable difference in the impact of these two treatments.

Alteration of phenolic and volatile profiles by ultrasound

Alteration of phenolic profile by ultrasound

Out of the 131 phenolic compounds characterized in Chardonnay wine subjected to ultrasound with black tea infusion, 51 phenolics were exclusively identified in ultrasound-treated wine but absent in untreated Chardonnay wines infused with and without black tea (Table S2). These newly observed phenolic substances can be subdivided into 8 phenolic acids, 37 flavonoids and 6 others, where 24 were present in black tea-macerated model wines and 21 can be found in black tea ethanolic extracts (Table S2). This suggested that the 24 compounds identified in black tea-macerated model wines were possibly originated from tea, and the other 27 compounds (51–24 = 27) might be generated through interaction of wine and tea phenolics when exposing to ultrasound. Apart from these phenolics, 10 new non-phenolic substances were exclusively found in ultrasound-treated black tea-infused Chardonnay wine, but absent in black tea-infused model wine, non-ultrasound-treated black tea-infused Chardonnay, control Chardonnay nor black tea ethanolic extract (Table S2).

Among these 27 compounds, coniferyl alcohol (38), umbelliferone (66), procyanidin C1 (74), myricetin (110), *N*-ethyl-2-pyrrolidinone-epicatechin 3-*O*-gallate (165) and tiliroside (179) stand out as particularly intriguing. Coniferyl alcohol (38) was speculated to derive from caffeic acid (76) that were found in Chardonnay and black tea. A dual methylation pathway might be responsible for the synthesis. One pathway involves the conversion of caffeic acid (76) into caffeoyl CoA via 4-coumarate-CoA ligase, followed by methylation through hydroxycinnamic acids/hydroxycinnamoyl CoA esters *O*-methyltransferase (AEOMT) to feruloyl CoA. This is then transformed into coniferyl alcohol through the actions of cinnamyl-alcohol dehydrogenase (CAD) and caffeoyl-CoA *O*-methyltransferase (CCOMT) (Fig. 5a) (Liu et al. 2021). In the alternate pathway, caffeic acid (76) is methylated by AEOMT to ferulic acid, then converted to feruloyl CoA by 4-coumarate-CoA ligase, ultimately leading to coniferyl alcohol as described above (Fig. 5b) (Liu et al. 2021). For the possible synthesis of umbelliferone (66), coumaric acid (52) was converted to 2,4-dihydroxycinnamic acid via ortho-hydroxylation, then transformed into umbelliferone by non-enzymatic lactonization (Fig. 5c) (Vialart et al. 2012). Procyanidin C1 (74) was believed to be the condensation product of three epicatechin (78) units linked by C4-C8 interflavan bonds (Fig. 5d), and myricetin (110) was degraded from myricetin 3-*O*-galactoside (109) and myricetin 3-*O*-glucoside (107) identified in black tea. *N*-ethyl-2-pyrrolidinone-epicatechin 3-*O*-gallate (165) might be synthesized via the nucleophilic reaction of epicatechin gallate (136) with theanine, the predominant amino acid in unfermented teas and fresh tea leaves. Theanine is first transformed into an aldehyde via Strecker degradation, then converted to *N*-ethyl-2-pyrrolidinone by spontaneous cyclization. The *N*-ethyl-2-pyrrolidinone is then captured by epicatechin gallate in nucleophilic reaction where the C6 or C8 position on the A-ring is substituted to form *N*-ethyl-2-pyrrolidinone-epicatechin 3-*O*-gallate (Fig. 5e) (Meng et al. 2019). These sequential reactions are common during tea fermentation, resulting in theanine remarkedly decreased or hardly detectable in fermented tea products such as dark tea and black tea, which justify the absence of theanine in black tea-infused wines with or without ultrasound treatment. Considering no *N*-ethyl-2-pyrrolidinone-epicatechin 3-*O*-gallate was found in black tea ethanolic extract and black tea-infused model wine, a conceivable scenario is that the content of ultrasound extracted *N*-ethyl-2-pyrrolidinone-epicatechin 3-*O*-gallate was sufficient to reach the detection threshold. For the synthesis of tiliroside (179), astragalin was first derived from the attachment of glucose from uridine diphosphate (UDP)-glucose to the 3-hydroxyl group of kaempferol (188) by the action of flavonol 3-*O*-glucosyltransferase derived from tea, followed by the transformation into tiliroside via acylation of the 6″ group of astragalin's glucose with a *p*-coumaroyl moiety (Fig. 5f) (Grochowski et al. 2018). The presence of coniferyl alcohol, umbelliferone and myricetin aligned with our previous study in which the synthesis of these compounds was confirmed in Chardonnay wine macerated with black tea (Liang et al. 2024). Chlorogenic acid 1 (10), caffeic acid 1 (29), theaflavic acid 1 (45), *p*-coumaroylquinic acid 4 (50), (epi)gallocatechin-(epi)catechin gallate 2 (72), *p*-hydroxybenzoic acid 2 (79), apigenin 7-*O*-apiosyl-glucoside 1 (93), procyanidin B1 3-*O*-gallate 2 (98), quercetin 1 (113), kaempferol 3-*O*-rutinoside 3 (144) and theaflavin 3-gallate 1 (176) were the isomers of the corresponding substances detected in the control and non-ultrasound-treated Chardonnay samples, black tea ethanolic extract and black tea-infused model wine. The mechanism behind the production of other newly formed substances is still unknown. The use of ultrasound is claimed to induce violent acoustic cavitation with a bubble implosion effect, which could generate damaging oxidative free radicals (e.g., H_2O_2) and thus increase the oxidative stress. These stress markers could lead to significant activities of phenolic triggering enzymes participated in the phenylpropanoid pathway which is the predominant biosynthetic process of proanthocyanidins, flavonoids and phenolic acids (Ampofo & Ngadi 2020). Therefore, ultrasound can facilitate the formation of these phenolics within a short time. Overall, ultrasound exhibited a pronounced effect on the enrichment of phenolic profile in black tea-infused Chardonnay wine, by aiding the interaction between wine and tea phenolics and the transfer of tea phenolics.

Alteration of volatile profile by ultrasound

Volatile profile of wine samples A total of 13 volatiles were detected and identified as odor-active substances, with fruity aromas being the major odor group because of both greater diversity and higher values. Spicy, fatty, green, citrus, fermented and floral aromas are also responsible for the overall olfactory profile but with inferior sensorial impact (Table S5). Overall, ethyl hexanoate (10), ethyl octanoate (22) and (*E*)-damascenone (39) with higher OAVs exerted stronger impacts on the wine aroma as compared to other volatiles. The OAV of ethyl hexanoate was decreased after ultrasound treatment, before a gradual increase as the treatment time increased.

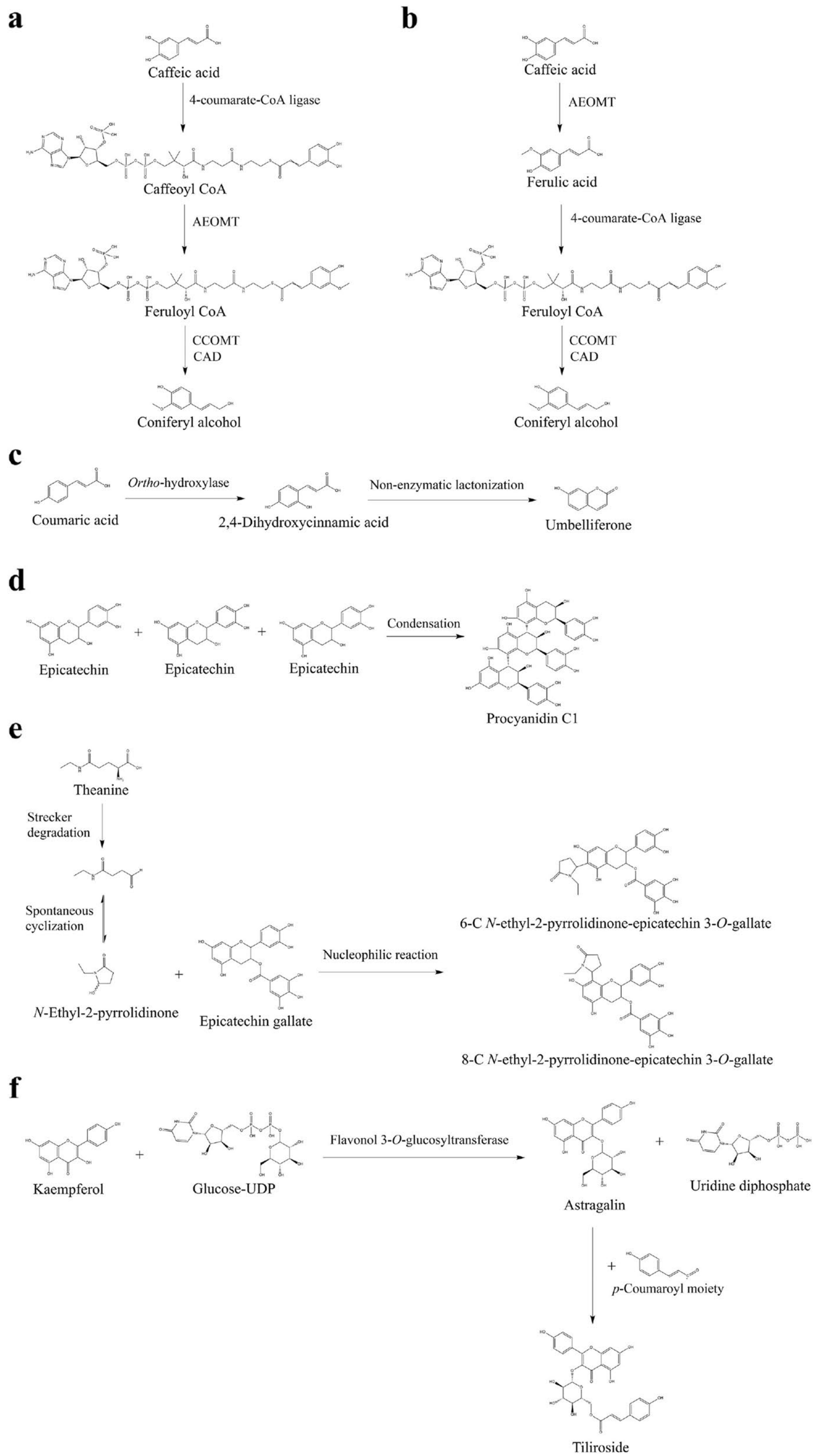


Fig. 5 Schematic diagram of the biosynthesis of coniferyl alcohol (**a**, **b**), umbelliferone (**c**), procyanidin C1 (**d**), *N*-ethyl-2-pyrrolidinone-epicatechin 3-*O*-gallate (**e**) and tiliroside (**f**) in the ultrasound-treated black tea-infused Chardonnay wines (AEOMT, hydroxycinnamic acids/hydroxycinnamoyl CoA esters *O*-methyltransferase; CCOMT, caffeoyl-CoA *O*-methyltransferase; CAD, cinnamyl-alcohol dehydrogenase)

Additionally, a decline of its OAV was also observed with the increasing ultrasound power. On the contrary, ethyl octanoate and (*E*)-damascenone maintained nearly unchanged throughout the ultrasound treatment. Interestingly, 2-heptanol (17) showed a trend of OAV fluctuation where its value was below 1 under mild ultrasound conditions (90 W, 2 and 10 min) and then rebounded to over 1 with the increasing power and time. This finding further confirmed the efficiency of high intensity ultrasound to transfer volatiles from tea to wine during infusion.

Figure 6a further displays the OAVs sensory profile of the sample wine. Less pronounced spicy, fermented, floral and fruity odors were detected after infusing black tea into Chardonnay wine, while the green, citrus and fatty aromas were enhanced simultaneously. The application of ultrasound seemed to intensify the citrus and green aromas in contrast to the non-ultrasound-treated

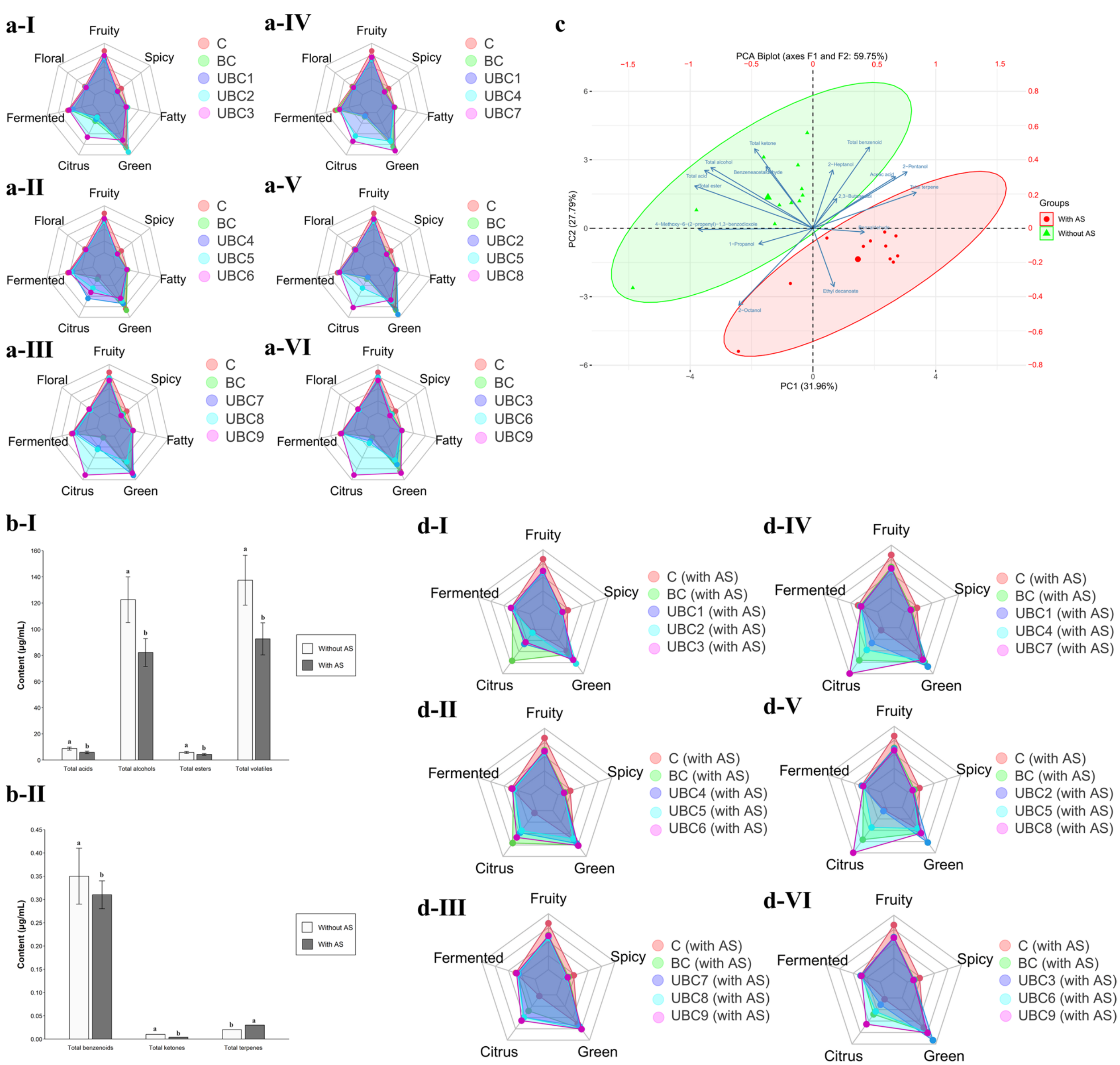


Fig. 6 Spider plots (**a**) of aroma attributes in control, non-ultrasound and ultrasound-treated black tea-infused Chardonnay wines. Contents of volatile compounds (**b**) in ultrasound-treated black tea-infused Chardonnay wines with and without artificial saliva addition at 450 W and 30 min. Principal component analysis (**c**) of selected quantified volatile compounds and spider plots (**d**) of aroma attributes in control, non-ultrasound and ultrasound-treated black tea-infused Chardonnay wines with artificial saliva addition

black tea-infused Chardonnay wine. In contrast, the diminished floral, fermented, fatty and spicy odors were witnessed after ultrasound treatment, although with an upswing in the intensity as the ultrasound power and sonication time increased. Therefore, the ultrasound processing could transfer more intense citrus and green aromas from black tea to Chardonnay wine.

Aroma release in simulated oral environment The release of volatiles in oral environment was simulated by spiking wine samples with artificial saliva (Table S6). The addition of saliva reduced the liberation of volatile esters, alcohols, acids, benzenoids and ketones to different extents, while it exerted an increasing effect on the total amount of terpenes (Fig. 6b). This difference can be clearly observed in PCA biplots (Fig. 6c). The decreased volatile concentrations in spiked samples were mainly because of mucin and α-amylase retaining the aroma via hydrophobic effect. Mucin, consisting of a lengthy glycosylated polypeptidic chain in the central region, creates hydrophobic domains for aroma molecule binding. α-Amylase is a globular protein with a three-dimensional structure providing one or several hydrophobic domain(s) to bind aroma substances (Liang et al. 2021; Pagès-Hélary et al. 2014).

After spiking, only 11 odor-active substances were remained in the wine samples, with fruity aromas continuing to dominate the aroma profile. Fermented, spicy, green, citrus aromas also contribute to the overall wine aroma but with inferior sensorial effect (Table S7). Notably, ethyl octanoate (22), ethyl hexanoate (10) and (*E*)-damascenone (39) scored the highest OAVs in the odor-active substances. The OAVs of ethyl hexanoate and ethyl octanoate maintained nearly unchanged throughout the ultrasound treatment. In contrast, the OAV of (*E*)-damascenone was elevated after ultrasound treatment, before a continuous increase as the ultrasound power and sonication time increased. The fermented, spicy and fruity aromas of the spiked wine samples undergone a negative change after black tea infusion, whereas a considerable rise was observed in the green and citrus odors after black tea infusion and ultrasound treatment (Fig. 6d). These results reveal that the ultrasound processing could exert similar impacts on the aroma attributes of wine samples, regardless of the presence or absence of artificial saliva.

Conclusion

Ultrasound as an efficient extraction technique holds a vast potential in developing novel flavored wine. This study explored the impact of ultrasound on the physicochemical properties, antioxidant power, phenolic, and volatile profiles of Chardonnay wine infused with black tea under optimal treatment conditions (450.0 W ultrasound power, 22.6 min treatment time). A comprehensive analysis identified 165 non-volatile compounds in ultrasound-treated wine samples. The application of ultrasound also resulted in the formation of 27 phenolic substances and 10 non-phenolic substances that were not present in either Chardonnay wine or black tea. In addition, catechin emerged as the most abundant phenolic substance that showed positive ($p < 0.05$) association with the boosted antioxidant activity of ultrasound-treated wines ($r = 0.902–0.936$). The ultrasound treatment identified 44 volatiles, including 13 odor-active substances in the wines. Black tea infusion, with or without saliva spiking, led to a reduction in fruity, fermented, and spicy odors, accompanied by a prominent elevation in green and citrus scents. Ultrasound processing facilitated the transfer of more pronounced green and citrus odors from tea into wine. This study reveals the potential utilization of ultrasound in commercially manufacturing tea-flavored wine with enhanced aromas and functionalities. However, the detection of unknown phenolic substances by HPLC–DAD-ESI-QTOF-MS/MS requires further exploration of these substances and their biological effects in future research. Sensory evaluations with consumers and professional panel could also be performed to observe further perspectives for the perception of ultrasound-treated black tea-infused Chardonnay wine in terms of aroma attributes.

Supplementary Information

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Supplementary materials 1.

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Authors' contributions
ZL: investigation, validation, methodology, data curation, formal analysis, visualization, writing-original draft, writing-reviewing & editing; PZ: supervision, methodology, investigation, writing-reviewing & editing; WM: resources, investigation; XZ: project administration, supervision, resources, writing-reviewing & editing; ZF: project administration, supervision, methodology, conceptualization, writing-reviewing & editing. All authors have thoroughly reviewed and approved the manuscript.

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Declarations

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Competing interests
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