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Research Article

Variations in Bioactive Compounds and Sensory Properties of Flower-Flavored Chardonnay Wine during Floral Maceration and Bottle Aging

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An innovative flavored wine was developed by macerating six different edible flowers into Chardonnay wine, where the physicochemical characteristics (titratable acidity, pH), antioxidant activity (DPPH, FRAP) and volatile profile were modulated. Bottle aging of the flower-flavored wines were performed for 9 months where a significant ($p < 0.05$) increases of total phenolic content and an opposite trend in antioxidant power (assessed by DPPH and FRAP assays) were observed. A total of 37 volatile substances were characterized in the aged flower-flavored wines. The aging process led to a decline in fruity and floral odors. Among the 12 month-aged wines, 1% (w/v) *O. fragrans*-flavored Chardonnay wine aged for 12 months was perceived as the most-liked product in human sensory analysis. This study manifested a bright future of edible flowers as a novel additive in the development of flavored wine with desirable sensory attributes.

1. Introduction

Flavored wine, a type of specially fortified wine, integrates with additional natural additives (e.g., fruit, herb, spices), thereby improving the aroma, flavor, taste and mouthfeel of wine [1]. It is often prepared by incorporating nongrape materials before or after fermentation, thereby enriching the wine matrix [1]. As the demand for new flavored wine grew, its sales increased by 40-fold in five years [1]. However, many wine-producing countries have implemented stringent regulations regarding the use of flavor additives in wine production. For instance, in Australia, wine fortified with flavoring materials may be classified as either a "wine product" or a "fruit wine" depending on the percentage of wine content [1]. Edible flowers, consisting of different proportions of sugars, acids, polyphenols, volatiles and antioxidants, have serve as aroma and flavor elevators to enhance the flavor, color, appearance, and various health effects of the fortified beverages and food [2]. Our previous research indicated that the polyphenol composition of Chardonnay wine could be successfully modulated via infusion with either black or green tea, potentially amplifying its health-enhancing properties (e.g., antioxidant activity) [3]. However, there is currently limited research on the addition or fortification of the edible flowers in grape winemaking.

Depending on the variety of grapes, wine may age in the bottle from a few months to years from bottling to consumption. During this period of bottle aging, wine can recovery from bottle shock, stabilize and develop new sensory attributes. Darker color, enhanced aroma, softer mouthfeel, and decreased astringency and bitterness are the main perceivable changes in bottle aging of wine. Over time, oxygen ingress triggers a cascade of intricate chemical reactions, leading to creation of new volatiles, polymerization of pigments, condensation of tannins, and breakdown of volatile compounds related to undesirable odors and flavors [4]. The fact that wine is susceptible to oxidation in bottle storage is mainly ascribed to its phenolic profile, and the environmental factors such as temperature, humidity, light

exposure and type of closure [4]. Oaked Chardonnay stands out among white wines as one of the few varieties that benefit from bottle aging, owing to its comparatively subdued aromatic profile, although its relatively lower concentrations of antioxidant compounds especially phenolic compounds compared to red wines may induce oxidative deterioration during prolonged aging [4]. In our previous study, prolonged bottle aging influenced the physicochemical attributes, antioxidant power, phenolic and volatile profiles, as well as the sensory characteristics of Chardonnay wine infused with either green or black tea [5]. Therefore, developing flower-flavored Chardonnay wine should consider the possible effects of bottle aging on the wine quality to reflect the actual bottle aging time from bottling to consumption.

Chrysanthemum indicum, *Jasminum sambac*, *Lavandula angustifolia*, *Sambucus nigra*, *Osmanthus fragrans*, and *Clitoria ternatea* are the representative edible flowers with an intense floral and pleasant aroma, which commonly serve as tea analogue or tea additive [6, 7]. Up to date, there is no research to combine these flowers with red or white wine to develop a new style of flower-flavored wine. Therefore, this study aimed to develop six different novel flower-flavored wines by macerating Chardonnay wine with the above edible flowers, and explore the impact of maceration time, flower concentration and bottle aging time on the physicochemical characteristics (titratable acidity, pH), antioxidant power and volatile composition. Further, sensory evaluations with consumers were established to obtain further insights for the sensory perception of the bottle-aged flower-flavored wines. This research is of potential significance to wine manufacturers and wine market by showing commercial opportunities behind such innovative wine products.

2. Materials and Methods

2.1. Materials and Chemicals. Folin and Ciocalteu reagent (FCR), 2,4,6-tripyridyl-s-triazine (TPTZ), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferrous sulfate heptahydrate ($FeSO_4 \bullet 7H_2O$), iron (III) chloride anhydrous, (+)-catechin hydrate, (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), 4-octanol, *n*-alkanes (C8–C30), SPME fiber assembly polydimethylsiloxane/divinylbenzene (PDMS/DVB; df 65 μm, Fused Silica/SS), and all pure standards of volatile substances were acquired from Sigma-Aldrich (Castle Hill, NSW, Australia). Headspace screw top clear vials (20 mL) and magnetic PTFE/sil hdsp cap were purchased from Agilent Technologies (Santa Clara, CA, USA). All chemicals were of analytical reagent or HPLC grade.

2.2. Samples and Preparation. Commercial Chardonnay wine (Bowler's Run Chardonnay; 12.5% alcohol vol.; pH 3.3; 5.9 g/L of titratable acidity) was purchased from Dan Murphy's (Melbourne, VIC, Australia). *C. indicum* was purchased from Yuan's Market Trading CO. (Coopers Plains, QS, Australia). *J. sambac* was purchased from Guangdong Tea Import and Export Co. Ltd (Toongabblie, NSW, Australia). *O. fragrans* was purchased from Crown Asian Supermarket (Melbourne, VIC, Australia). *L. angustifolia*, *S. nigra* and *C. ternatea* were obtained from commercial seller "The Tea Hut" (Osborne Park, WA, Australia). All flowers were obtained in a dried form.

2.3. Experimental Design

2.3.1. Maceration. The maceration study employed a $6 \times 2 \times 3$ (flower × concentration × maceration time) factorial design (Table 1). A certain amount of flower was weighed and soaked in Chardonnay wine. The concentration of *L. angustifoli* was evaluated by macerating the wine (1 L) with 5 g (0.5% w/v) and 10 g (1% w/v) of this flower, while the concentrations of other flowers were determined by immersing 10 g (1% w/v) and 20 g (2% w/v) of the flowers in the same amount of wine. The maceration process was performed at 25°C for 1 h, 2 h and 3 h (Table 1). Control Chardonnay wine was subjected to the same storage time with no maceration procedure (Figure 1). The concentration and maceration time of the flowers used were selected based on preexperiment, where treatments leading to too weak or overpowered flower-derived aroma in the resultant wines were excluded. After the maceration, all samples were filtered, bottled and stored at 4°C before further analysis.

2.3.2. Aging. Flower-flavored wine was first prepared by infusing different flowers into Chardonnay wine at the flower concentration and maceration time obtained from the maceration study. Control Chardonnay wine was subjected to the same treatment time with no maceration step. Then, the mixture underwent filtration using Whatman No.4 filter paper. Subsequently, the filtered wines were kept in a dark and cool environment prior to analysis. Bottling was performed in December 2021 using 375 mL volume wine bottles with screw cap air-tight closure to ensure rates of consistent oxygen transfer. The wines were subjected to analysis after aging for 1, 3, 6, 9, and 12 months at January 2022, March 2022, June 2022, September 2022 and December 2022 (Table 1) (Figure 1).

2.4. Analysis of Physicochemical Properties, Phenolic Contents, and Antioxidant Activity. The titratable acidity (TA) and pH of wine samples were measured according to the methods of the International Organization of Vine and Wine (OIV) [8]. The total phenolic content (TPC), total flavonoid content (TFC), DPPH radical scavenging activity, and ferric reducing antioxidant power (FRAP) were evaluated based on the procedure described in previous studies [9, 10].

2.5. Analysis of Volatile Compounds by HS-SPME-GC-MS

2.5.1. Sample Preparation and HS-SPME. The wine volatile substances were extracted following a previous method [11] with slight modifications. The wine sample (10 mL) was

Table 1: Composition and codes of flower-macerated wines.

Wine code	Flower	Concentration (%, w/v)	Wine	Maceration time (hour)	Aging time (month)
C	N/A	N/A	Chardonnay	N/A	N/A
C_{1m}	N/A	N/A	Chardonnay	N/A	1
C_{3m}	N/A	N/A	Chardonnay	N/A	3
C_{6m}	N/A	N/A	Chardonnay	N/A	6
C_{9m}	N/A	N/A	Chardonnay	N/A	9
C_{12m}	N/A	N/A	Chardonnay	N/A	12
CI_{1p1h}	*Chrysanthemum indicum*	1	Chardonnay	1	N/A
CI_{1p2h}	*Chrysanthemum indicum*	1	Chardonnay	2	N/A
CI_{1p3h}	*Chrysanthemum indicum*	1	Chardonnay	3	N/A
CI_{2p1h}	*Chrysanthemum indicum*	2	Chardonnay	1	N/A
CI_{2p2h}	*Chrysanthemum indicum*	2	Chardonnay	2	N/A
CI_{2p3h}	*Chrysanthemum indicum*	2	Chardonnay	3	N/A
CI_{2p3h1m}	*Chrysanthemum indicum*	2	Chardonnay	3	1
CI_{2p3h3m}	*Chrysanthemum indicum*	2	Chardonnay	3	3
CI_{2p3h6m}	*Chrysanthemum indicum*	2	Chardonnay	3	6
CI_{2p3h9m}	*Chrysanthemum indicum*	2	Chardonnay	3	9
$CI_{2p3h12m}$	*Chrysanthemum indicum*	2	Chardonnay	3	12
JS_{1p1h}	*Jasminum sambac*	1	Chardonnay	1	N/A
JS_{1p2h}	*Jasminum sambac*	1	Chardonnay	2	N/A
JS_{1p3h}	*Jasminum sambac*	1	Chardonnay	3	N/A
JS_{2p1h}	*Jasminum sambac*	2	Chardonnay	1	N/A
JS_{2p2h}	*Jasminum sambac*	2	Chardonnay	2	N/A
JS_{2p3h}	*Jasminum sambac*	2	Chardonnay	3	N/A
JS_{2p3h1m}	*Jasminum sambac*	2	Chardonnay	3	1
JS_{2p3h3m}	*Jasminum sambac*	2	Chardonnay	3	3
JS_{2p3h6m}	*Jasminum sambac*	2	Chardonnay	3	6
JS_{2p3h9m}	*Jasminum sambac*	2	Chardonnay	3	9
$JS_{2p3h12m}$	*Jasminum sambac*	2	Chardonnay	3	12
$LA_{0.5p1h}$	*Lavandula angustifoli*	0.5	Chardonnay	1	N/A
$LA_{0.5p2h}$	*Lavandula angustifoli*	0.5	Chardonnay	2	N/A
$LA_{0.5p3h}$	*Lavandula angustifoli*	0.5	Chardonnay	3	N/A
LA_{1p1h}	*Lavandula angustifoli*	1	Chardonnay	1	N/A
LA_{1p2h}	*Lavandula angustifoli*	1	Chardonnay	2	N/A
LA_{1p3h}	*Lavandula angustifoli*	1	Chardonnay	3	N/A
$LA_{0.5p3h1m}$	*Lavandula angustifoli*	0.5	Chardonnay	3	1
$LA_{0.5p3h3m}$	*Lavandula angustifoli*	0.5	Chardonnay	3	3
$LA_{0.5p3h6m}$	*Lavandula angustifoli*	0.5	Chardonnay	3	6
$LA_{0.5p3h9m}$	*Lavandula angustifoli*	0.5	Chardonnay	3	9
$LA_{0.5p3h12m}$	*Lavandula angustifoli*	0.5	Chardonnay	3	12
CT_{1p1h}	*Clitoria ternatea*	1	Chardonnay	1	N/A
CT_{1p2h}	*Clitoria ternatea*	1	Chardonnay	2	N/A
CT_{1p3h}	*Clitoria ternatea*	1	Chardonnay	3	N/A
CT_{2p1h}	*Clitoria ternatea*	2	Chardonnay	1	N/A
CT_{2p2h}	*Clitoria ternatea*	2	Chardonnay	2	N/A
CT_{2p3h}	*Clitoria ternatea*	2	Chardonnay	3	N/A
CT_{1p2h1m}	*Clitoria ternatea*	1	Chardonnay	2	1
CT_{1p2h3m}	*Clitoria ternatea*	1	Chardonnay	2	3
CT_{1p2h6m}	*Clitoria ternatea*	1	Chardonnay	2	6
CT_{1p2h9m}	*Clitoria ternatea*	1	Chardonnay	2	9
$CT_{1p2h12m}$	*Clitoria ternatea*	1	Chardonnay	2	12
OF_{1p1h}	*Osmanthus fragrans*	1	Chardonnay	1	N/A
OF_{1p2h}	*Osmanthus fragrans*	1	Chardonnay	2	N/A
OF_{1p3h}	*Osmanthus fragrans*	1	Chardonnay	3	N/A
OF_{2p1h}	*Osmanthus fragrans*	2	Chardonnay	1	N/A
OF_{2p2h}	*Osmanthus fragrans*	2	Chardonnay	2	N/A
OF_{2p3h}	*Osmanthus fragrans*	2	Chardonnay	3	N/A
OF_{1p3h1m}	*Osmanthus fragrans*	1	Chardonnay	3	1
OF_{1p3h3m}	*Osmanthus fragrans*	1	Chardonnay	3	3
OF_{1p3h6m}	*Osmanthus fragrans*	1	Chardonnay	3	6

Table 1: Continued.

Wine code	Flower	Concentration (%, w/v)	Wine	Maceration time (hour)	Aging time (month)
OF_{1p3h9m}	*Osmanthus fragrans*	1	Chardonnay	3	9
$OF_{1p3h12m}$	*Osmanthus fragrans*	1	Chardonnay	3	12
SN_{1p1h}	*Sambicus nigra*	1	Chardonnay	1	N/A
SN_{1p2h}	*Sambicus nigra*	1	Chardonnay	2	N/A
SN_{1p3h}	*Sambicus nigra*	1	Chardonnay	3	N/A
SN_{2p1h}	*Sambicus nigra*	2	Chardonnay	1	N/A
SN_{2p2h}	*Sambicus nigra*	2	Chardonnay	2	N/A
SN_{2p3h}	*Sambicus nigra*	2	Chardonnay	3	N/A
SN_{2p2h1m}	*Sambicus nigra*	2	Chardonnay	2	1
SN_{2p2h3m}	*Sambicus nigra*	2	Chardonnay	2	3
SN_{2p2h6m}	*Sambicus nigra*	2	Chardonnay	2	6
SN_{2p2h9m}	*Sambicus nigra*	2	Chardonnay	2	9
$SN_{2p2h12m}$	*Sambicus nigra*	2	Chardonnay	2	12

N/A = not available.

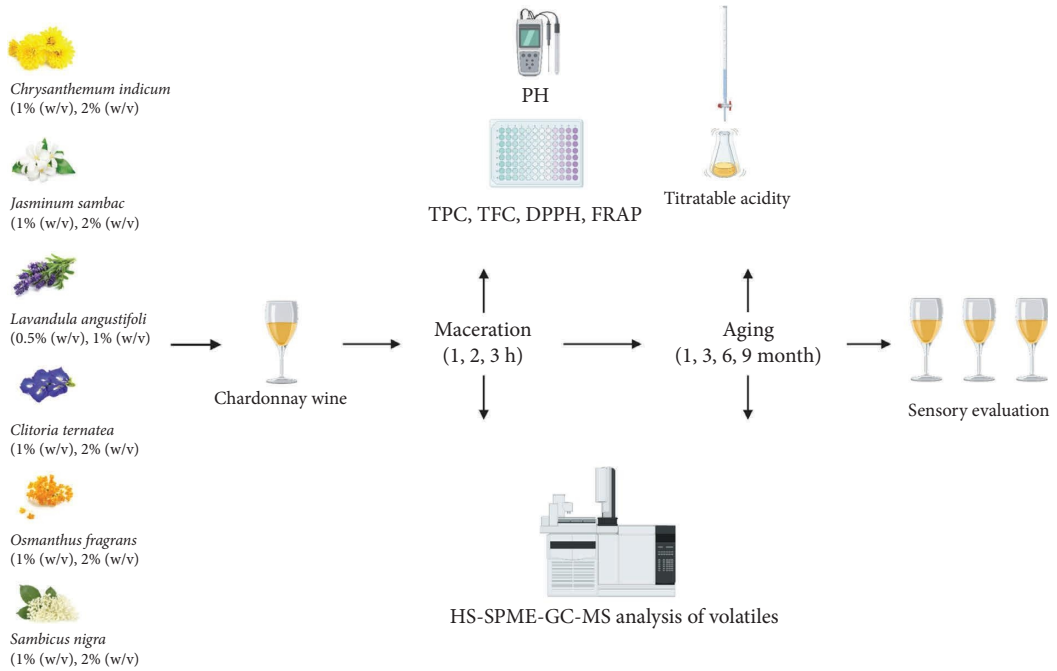

Figure 1: Flow diagram of studies on variations in physicochemical characteristics, antioxidant activity, volatile profiles and sensory attributes of flower-flavored Chardonnay wine during maceration and bottle aging.

mixed with 2 g of sodium chloride and 20 μL of internal standard 4-octanol (100 mg/L in ethanol) in headspace vial. Prior to analysis, the vial was equilibrated at 35°C for 20 min. For adsorption, a 65 μm PDMS/DVB SPME fiber was introduced to the headspace at 35°C for a duration of 30 min. The fully automatic HS-SPME analysis was conducted using a PAL 3 multipurpose automated sampler (Agilent Technologies, Santa Clara, CA, USA). The fiber was preconditioned in the GC injection port at 250°C for 30 min until sample analysis.

2.5.2. GC-MS Analysis. The GC-MS analysis was performed according to a previous method [11] with slight modifications. A combination of an Agilent 6850 GC system (Agilent Technologies, Santa Clara, CA, USA) and a 5973 mass spectrometer was utilized, employing a J&W DB-Wax ultra Inert GC column (30 m × 250 μm × 0.25 μm; Agilent Technologies). The carry gas was helium at a flow rate of 0.8 mL/min, and the SPME fiber desorption was conducted in splitless mode at 200°C for 10 min. The oven temperature program was as follows: an initial temperature at 40°C and kept for 2 min, then raised to 160°C at 3°C/min, before a further increase to 230°C at a rate of 7°C/min and kept for 8 min. The mass acquisition range was 35–350 m/z, and the mass spectrometer was in scan mode at 70 eV. The quadruple temperature, transfer line, and ion source were set at 150, 240, and 230°C, respectively.

2.5.3. Identification and Quantification of Compound. The analysis of the GC-MS data was conducted using the Agilent G1701EA MSD ChemStation software (Version 1.4.20.0). The linear retention index (RI) for each compound was determined employing a range of *n*-alkanes (C8-C30). To generate standard curves, authentic standards were analyzed after serial dilution using model wine. Based on the NIST Chemistry Webbook [12] and NIST reference database (NIST 11.0), the retention indices (RI) and previously reported mass spectra (MS) were used for the identification of each compound. Each compound was also identified via comparison of MS and retention times with external standards (ES). Target ions model was used to integrate the peak areas of volatile compounds. The calibration curve of standard compound was used for the quantification of each corresponding identified compound with the following equation:

$$\text{Conc.}\left(\frac{\mu g}{mL}\right) = \left(\frac{\text{ion peak area of each compound}}{\text{ion peak area of internal standard}} \times \text{Slope}\right) + \text{Intercept.} \quad (1)$$

Standard curves of analogous compounds were utilized to semiquantitatively assess certain identified compounds for which standards were unavailable, as outlined in our prior publication [11].

Further, odor thresholds published in previous studies [10, 13–26] were employed to ascertain the participation of the identified substances in the global aroma of the flower-flavored wines by computing the odor activity values (OAVs) as follows:

$$\text{OAV} = \frac{\text{Concentration of volatile compound}}{\text{Odor detection threshold}}. \quad (2)$$

Odor-active compounds refer to the compounds with OAVs greater than 1 [11], and their sensorial impact is evaluated based on the OAVs and associated odor description.

3. Sensory Analysis

The sensory analysis was performed according to a slightly modified method [27]. Approval for human ethics (No. 2022-23727-28046-4) was obtained from the Human Ethics Committee of the University of Melbourne. A total of 63 consumers (24 males, 39 females) were served with seven 12 month-aged flower-flavored Chardonnay wines (C_{12m}, $CI_{2p3h12m}$, $JS_{2p3h12m}$, $LA_{0.5p3h12m}$, $CT_{1p2h12m}$, $OF_{1p3h12m}$, and $SN_{2p2h12m}$) in University of Melbourne to evaluate sensory attributes using a Just About Right (JAR) scale, with a nonstructured scale. Various quality attributes (mouthfeel/texture, olfactive (taste/flavor), olfactory (aroma), visual and overall perception) of the wines were assessed by the participants based on their respective intensity. The middle of the scale, "Just About Right," was regarded as the ideal of typicality for each descriptor. Each wine sample, approximately 30 mL, was poured into a 3-digit-coded ISO standard wine glass at room temperature (22–24°C). To prevent carry-over effects, the wine samples were served monadically in a balanced presentation order. Participants were instructed to rinse their mouths with plain water between tasting different wine samples. The sensory properties were assessed in the sequence outlined in Table S35.

4. Statistical Analysis

Each treatment was performed in triplicates, and the means ± standard deviations were used to report the results. The significant difference of means between each sample was assessed by one-way ANOVA using Fisher grouping at 95% confidence level with Minitab (Minitab 21.1.0, Sydney, Australia). Principal component analysis (PCA), spider plot, partial least squares-discriminant analysis (PLS-DA) and Pearson's correlation-significance matrix were created using R packages "ggfortify," "fmsb", "mixOmics," and "Hmisc," respectively.

5. Results and Discussions

5.1. Maceration Study

5.1.1. Physicochemical Characteristics, Total Free Phenolic, and Flavonoid Content, Antioxidant Power. A significant increase in pH ($p < 0.05$) was witnessed in all flower-flavored wines compared with the control, except for the wines flavored with *C. indicum*, and there was a corresponding decrease in TA (Table S1). A similar trend was also seen in pH and TA of most flower-flavored wines with the increasing flower concentration and maceration time. Such changes might be ascribed to the esterification of organic acids such as citric and malic acids [28], or their salification with sodium, calcium, potassium and other cations [29]. It is worth mentioning that the pH and TA of *C. ternatea*-treated wines maintained unchanged throughout the maceration, which agreed with a previous study [30] where no significant difference in pH and TA of *C. ternatea* aqueous extracts at different extraction times was reported.

The TPC, TFC, DPPH and FRAP values of all the flower-flavored wines showed a prominent increase ($p < 0.05$) compared to the control (Table S1). As the maceration time and flower concentration increased, these values also showed an increasing trend ($p < 0.05$). The increase in TFC and TPC with maceration time was mainly due to the fact that the plant cell wall was softened and broken down when steeping in wine, and soluble phytochemicals especially phenolics were transferred into wine through osmotic and diffusion process as time increased [31]. The FRAP and DPPH values shared a similar increasing trend with TPC and TFC, suggesting a vital role of the extracted flavonoids and phenolics in enhancing antioxidant activity of the flower-flavored wine.

5.1.2. Alteration of Volatile Profile by Flower Maceration. There were 29, 25, 37, 25, 29 and 38 volatile compounds identified in *C. indicum*-, *J. sambac*-, *L. angustifolia*-, *C. ternatea*-, *O. fragrans*-, and *S. nigra*-flavored wines,

respectively (Tables S2–S7). Compounds including *p*- and *m*-cymene, sulcatone, chrysanthenone, camphor and terpinen-4-ol, were only detected in *C. indicum*-flavored wine but not in the control. Among them, the concentrations of *p*- and *m*-cymene, sulcatone, chrysanthenone, camphor and terpinen-4-ol presented a two-fold increase from CI_{1p1h} to CI_{2p3h}, whereas no significant ($p > 0.05$) treatment effect was witnessed in 2-pheylethanol content. Throughout all the treatments, there were a total of 12 detected volatiles characterized as odor-active compounds in *C. indicum*-flavored wines, thereinto, ethyl octanoate and ethyl hexanoate exhibited stronger impacts on the wine aroma than other volatiles due to their higher OAVs (Table S8). For *J. sambac*-flavored wine and ethyl heptanoate were only observed in the treated wines but absent in the control. The concentration of linalool increased noticeably ($p < 0.05$) in all six treatments compared to the control. In contrast, the concentrations of most alcohols including 2-methyl-1-propanol, 1-octanol, 1-hexanol and 1-butanol were reduced simultaneously. In all the treatments, 13 detected volatiles were characterized as odor-active compounds, in which ethyl hexanoate and ethyl octanoate represented a crucial contribution toward the wine aroma due to their higher OAVs (Table S9). Only 14 compounds absent in the control were found in *L. angustifolia*-flavored wines, notably camphor, (R)-lavendualol, geraniol, *p*-myrcene, nerol acetate, linalool oxide, and nerol oxide. The concentrations of these volatile compounds were positively correlated with the soaking time and flower concentration. A total of 16 volatiles were identified as odor-active compounds throughout the treatments, where ethyl hexanoate and linalool with higher OAVs had a more significant effect on the wine aroma (Table S10). A total of 27 volatile substances were identified, thereinto, 2 compounds were detected only in the *C. ternatea*-flavored wine, which were 3-methylbutyl octanoate and α-terpineol. The concentration of α-terpineol witnessed a two-fold increase across the treatments, while the amount of 3-methylbutyl octanoate showed no significant difference in all six treatments. There were 11 volatiles detected as odor-active compounds over the treatments, and ethyl hexanoate and ethyl octanoate exhibited stronger impacts on the wine aroma due to their higher OAVs (Table S11). Among the 29 volatile compounds detected in *O. fragrans*-flavored wine, only 6 compounds were found in the treated wine, including α-terpineol, geraniol, β-ionone, β-ocimene, D-limonene and theaspirane. Geraniol and β-ionone showed a noticeable increase in concentrations with the increasing flower concentration and maceration time. Compared to the control, the concentrations of three volatile compounds (benzaldehyde, ethyl nonanoate, linalool) were observed with 2 to 10-fold increases in the treated wine, whereas the content of methyl decanoate showed a significantly lower value. A total of 16 volatiles were characterized as odor-active compounds, out of which ethyl hexanoate, ethyl octanoate and theaspirane contributed significantly to the overall wine aroma because of their higher OAVs (Table S12). There were 14 volatiles found only in *S. nigra*-flavored wine by not control, including eucalyptol, terpinolene, ethyl heptanoate, rose oxide, camphor, 3-methylbutyl hexanoate, 3-methylbutyl octanoate, α-terpineol, geranyl acetate, methyl salicylate, citronellol, D-limonene, β-ocimene, and terpinen-4-ol. The concentrations of eucalyptol, camphor and ethyl heptanoate were increased by two-fold as the flower concentration and maceration time increased. Further, the contents of terpinolene, rose oxide, α-terpineol and citronellol were increased by three-fold. There were 18 volatiles identified as odor-active compounds, where ethyl hexanoate, rose oxide and ethyl octanoate with higher OAVs signified a substantial contribution toward the aroma properties of the wine samples (Table S13).

To better understand this complex dataset, Pearson's correlation was employed to explore the association between individual volatile compounds, antioxidant power, physicochemical characteristics, and OAVs in different flower-macerated Chardonnay wines (Figure 2(a)) (Tables S14–S18). FRAP value was mainly contributed by TPC and TFC, while DPPH shared no significant association. The OAV value of "Floral odor" displayed significant ($p < 0.05$) positive association with fatty odor (OAV), waxy odor (OAV), 1-butanol, 2-methyl-1-propanol, ethyl acetate, α-terpinene, β-myrcene, D-limonene, eucalyptol, β-ocimene, *p*-&*m*-cymene, hexyl acetate, terpinolene, rose oxide, 1-hexanol, ethyl octanoate, benzaldehyde, ethyl nonanoate, nerol oxide, 3-methylbutyl hexanoate, linalool, 1-octanol, terpinen-4-ol, methyl decanoate, ethyl decanoate, (R)-lavandulol, nerol acetate, geranyl acetate, methyl salicylate, citronellol, geraniol and octanoic acid. The OAV value of "Citrus odor" was positively associated with sulcatone, chrysanthenone, camphor, 2-phenethyl acetate and 2-phenylethanol, but displayed a negative correlation with linalool oxide, α-terpineol and hexanoic acid. The partial least squares-discriminant analysis (PLS-DA) and sparse principal component analysis (sPCA) biplots of physicochemical characteristics, antioxidant power and characteristic volatile substances in Chardonnay wines flavored with six flowers were also displayed in Figure 2 and Table S19. The PLS-DA analysis revealed clear distinctions among the various flower-flavored wines, with noticeable separation between them. However, there was some partial overlap between the control Chardonnay wine and both *C. indicum*- and *L. angustifolia*-flavored Chardonnay wines (Figure 2(b)). Methyl salicylate, 3-methylbutyl hexanoate, citronellol α-terpineol, rose oxide, eucalyptol, ethyl nonanoate, 3-methylbutyl octanoate, and 2-phenethyl acetate were the main driver of the variation among different flower-flavored wine (Table S19). OAVs sensory profiles of the flower-flavored wines were further illustrated in Figure 3. For *C. indicum*-, *L. angustifolia*- and *O. fragrans*-flavored wines, less pronounced fruity odor was perceived as the flower concentration and maceration time increased, while the floral odor was enhanced simultaneously. A gradual increasing trend was also seen in the citrus aroma of *C. indicum*-flavored wine across the treatments. For *J. sambac*-flavored wine, at 1% (w/v) concentration, the intensity of floral aroma was diminished as the maceration progressed, while the opposite trend was witnessed at 2% (w/v). For *C. ternatea*- and *S. nigra*-flavored wines, both

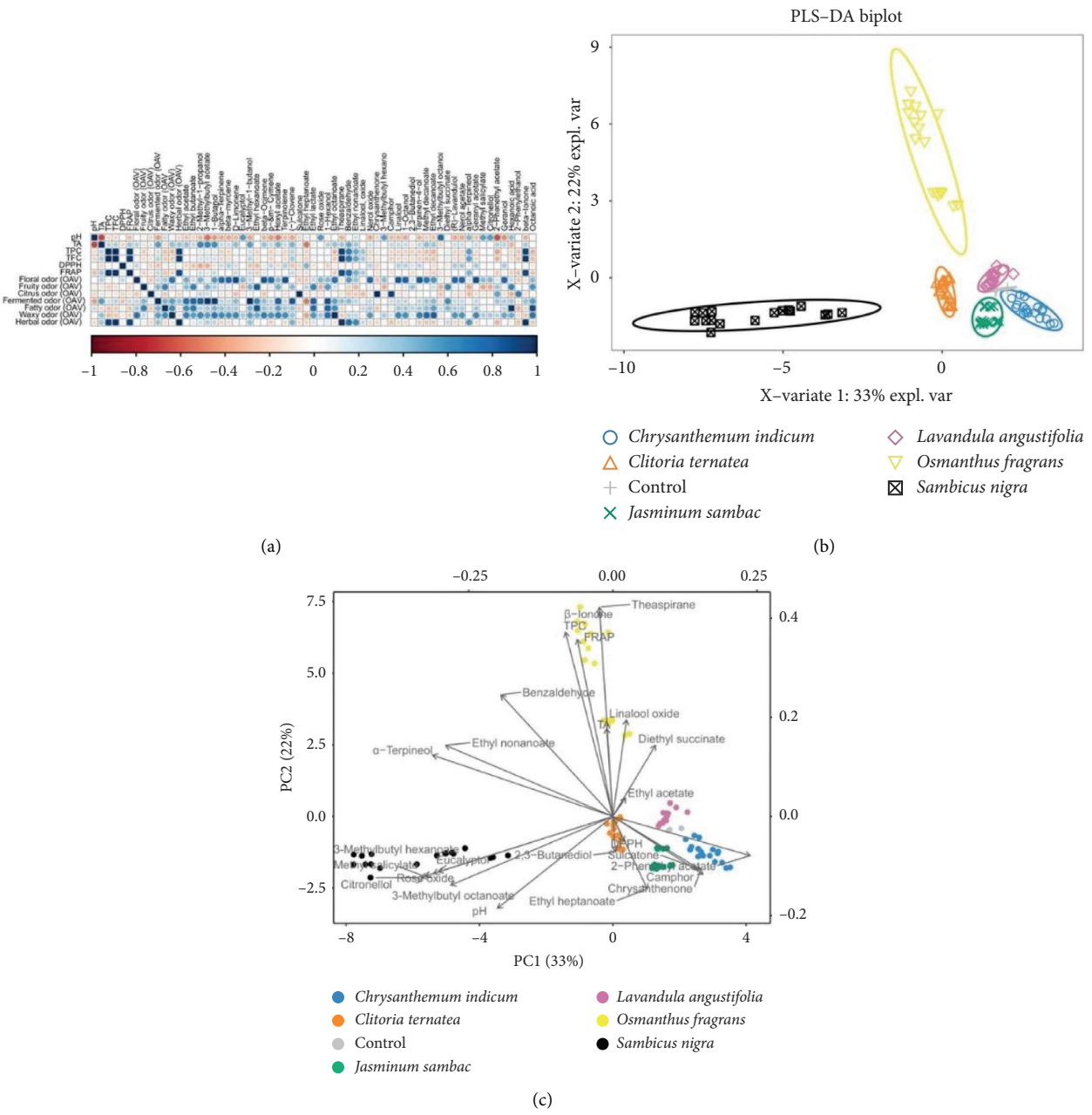

FIGURE 2: Pearson correlation matrix (a), partial least squares-discriminant analysis (b) and sparse principal component analysis (c) of volatile compounds, OAVs, physicochemical properties and tested responses of different flower-macerated Chardonnay wines.

floral and fruity odors were enhanced with the increasing flower concentration.

Overall, based on the results of the total phenolic and flavonoid contents, antioxidant power and volatile contents, 2% (w/v) flower concentration and 3 h maceration time were determined as the optimum treatment conditions for *C. indicum*- and *J. sambac*-flavored wines, 0.5% (w/v) and 3 h were the optimal flower concentration and maceration time for *L. angustifolia*-flavored wine. For *C. ternatea*-flavored wine, 1% (w/v) flower concentration and 2 h maceration time constitute the optimum treatment conditions. For *O. fragrans*-flavored wine, the optimal treatment conditions were established as 1% (w/v) flower concentration and 3 h soaking time. Superior antioxidant power and higher volatile content were found in the *S. nigra*-flavored wine treated at 2% (w/v) flower concentration and 2 h maceration time.

5.2. Aging Study

5.2.1. Physicochemical Characteristics, Total Free Phenolic Content, and Antioxidant Power. In Figures 4(A-I) and 4(A-II), as the aging time increased, the pH values of all flower-flavored Chardonnay wines maintained relatively stable although with fluctuations, which was correlated with no significant difference in TA at different aging times

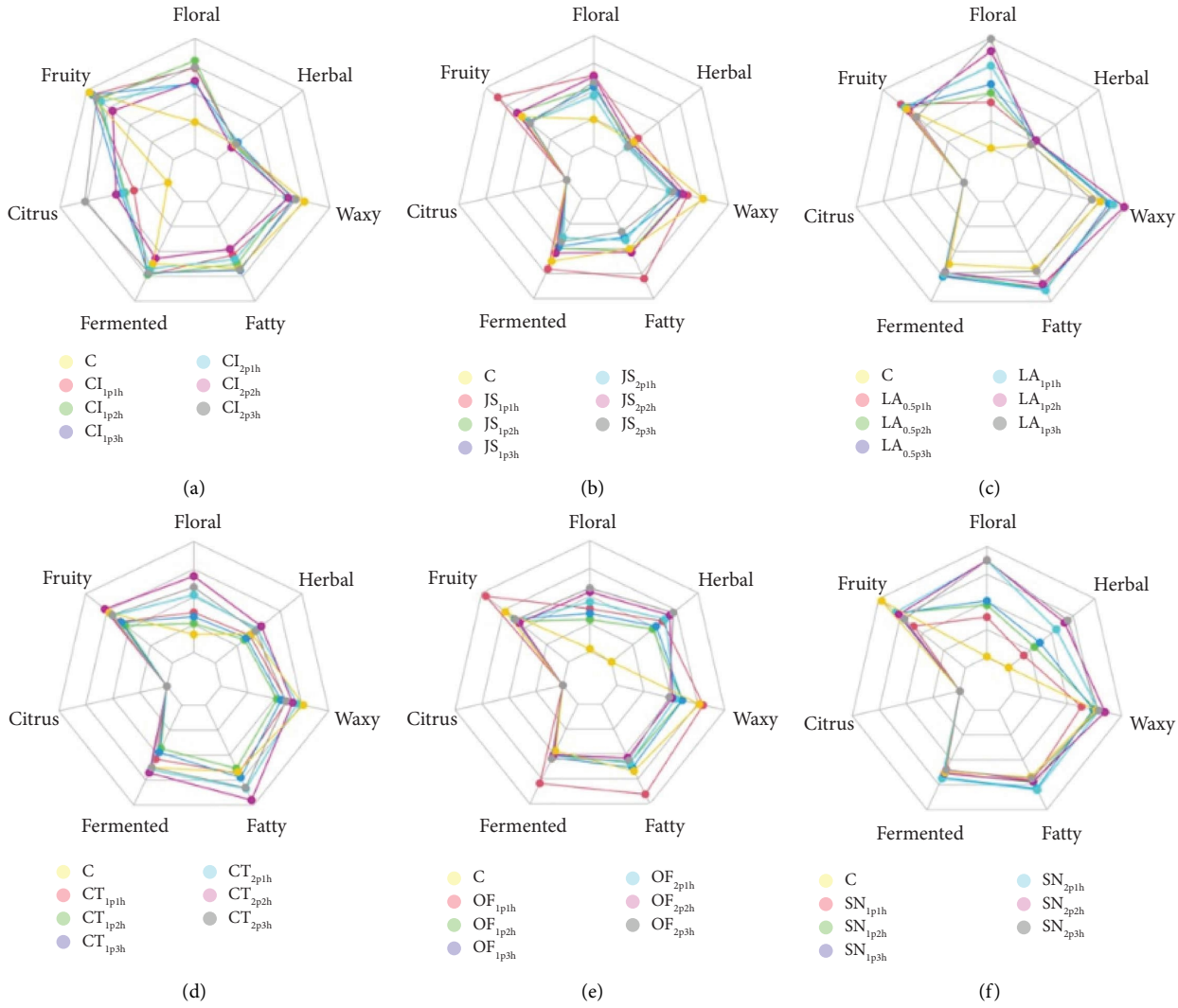

Figure 3: Spider plots of aroma attributes in *Chrysanthemum indicum*-macerated (a), *Jasminum sambac*-macerated (b), *Lavandula angustifolia*-macerated (c), *Clitoria ternatea*-macerated (d), *Osmanthus fragrans*-macerated (e), and *Sambucus nigra*-macerated (f) Chardonnay wines at different concentrations and maceration times. Samples of CI1p1h, JS1p1h, LA0.5p1h etc. refer to Table 1.

(Table S20). The unchanged pH aligned with a previous study [32] reporting no significant change in pH value of a commercial red wine during storage of up to 9 months. Additionally, a previous research also found a similar stable pattern of TA in Meili Rose wine during 160-day bottle aging [33]. Figure 4(A-III) showed a rising trend in the TPC content of the wine samples with the increasing aging time (Table S20), which might be because of the polymerization of polyphenols caused by oxygen ingress over time [34]. In contrast to TPC, a gradual decline was observed in the antioxidant power (DPPH, FRAP) with the increase of aging time (Figures 4(A-IV) and 4(A-V)) (Table S20), implying the wine antioxidant capacity was negatively correlated with the total phenolic content. The unexpected decrease in DPPH and FRAP could also be hypothetically ascribed to the polyphenol polymerization where the phenolic compounds present in the conjugated form have lower reactivity than their free forms. The polymerization process also involves a spatial rearrangement that can elevate the steric hindrance and thus diminish the available active sites for the radical scavenging action [34].

5.2.2. Alteration of Volatile Profile by Aging Time. There were 37 volatile compounds detected in the aged wine samples (Table S21), which can be subdivided into six chemical groups, including 2 acids, 8 alcohols, 16 esters, 1 benzenoid, 1 theaspirane and 9 terpenes. For all flower-flavored wines, the contents of total and individual volatile acids kept decreasing during bottle aging, which agrees with the autoxidation action of volatile acids into carbonyl compounds in white wine after bottle aging for 12 months in a previous study [35]. The levels of total and individual volatile alcohols decreased over the course of bottle aging. This reduction could be attributed to esterification processes occurring between alcohols and acids in the wine during prolonged storage [36]. Both total and individual volatile esters undergone a negative change during bottle storage.

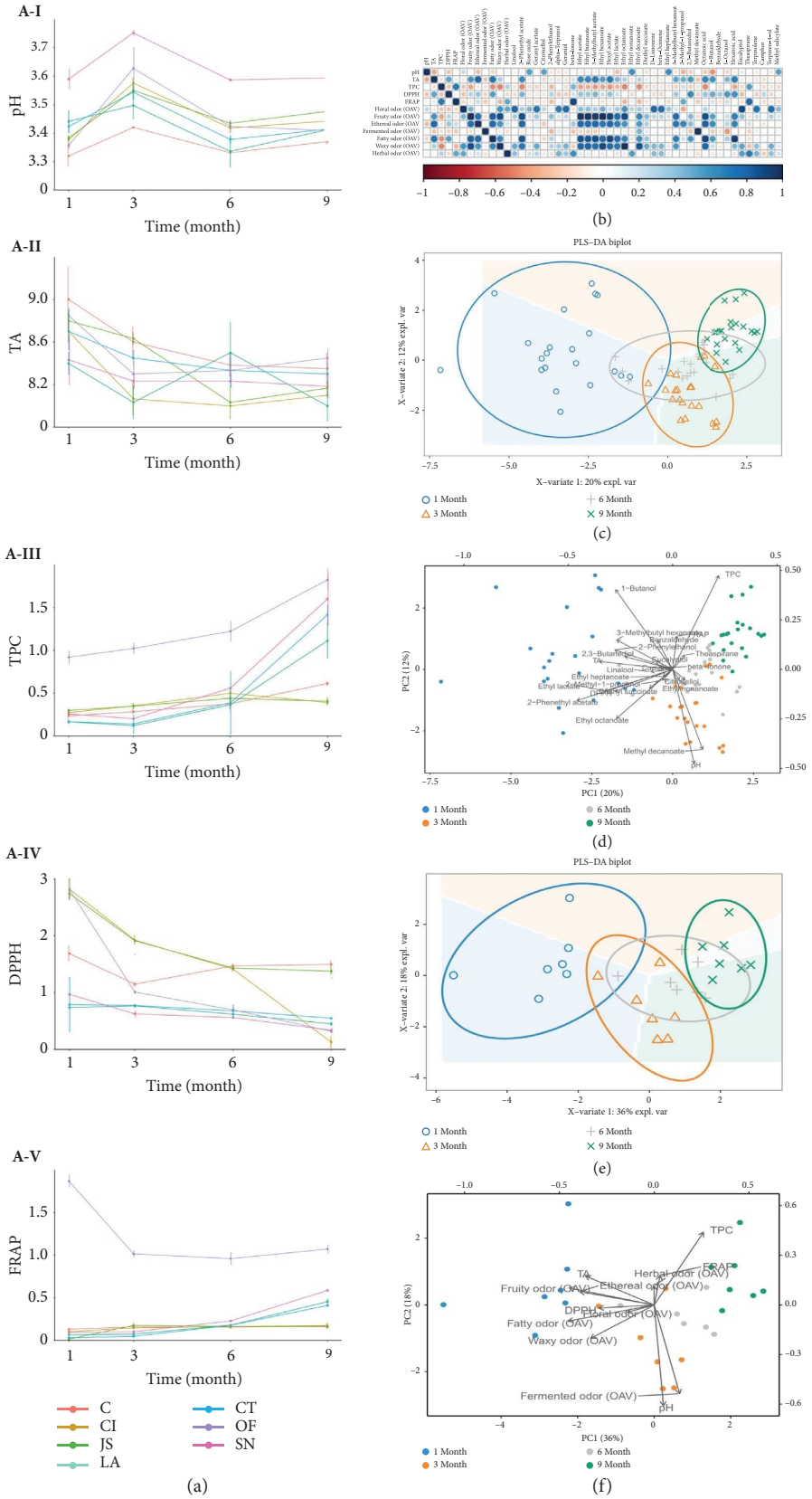

Figure 4: The pH (A-I), titratable acidity (TA) (A-II), TPC (A-III), DPPH (A-IV) and FRAP (A-V) of different flower-macerated Chardonnay wines at different aging times (a). Pearson correlation matrix (b), partial least squares-discriminant analysis (c) and sparse principal component analysis (d) of volatile compounds, OAVs, physicochemical properties and tested responses of different flower-macerated Chardonnay wines at different aging times. Partial least squares-discriminant analysis (e) and sparse principal component analysis (f) of OAVs, physicochemical properties and tested responses of different flower-macerated Chardonnay wines at different aging times.

Indeed, the autoxidation of volatile esters was proved to diminish their amount in bottle storage of white wine [35]. The total terpenes of the flower-favored wines also witnessed a gradual decline, except for *J. sambac*- and *C. ternatea*-flavored wines. The reduction might be ascribed to hydrolysis or conversion of terpenes into other aroma-active compounds. For instance, a monoterpene conversion is readily induced by the acid-catalyzed reactions during prolonged wine storage [37]. On the contrary, the increase in terpenes might be due to the hydrolysis of terpene glycosides or the conversion of other terpenes [38]. It is worth mentioning that rose oxide experienced a considerable increase during the last three months' aging in *J. sambac*-flavored wine. Based on a previous study [39], the aroma of rose oxide in wine can be masked by linalool and α-terpineol. During aging for 6 to 9 months, the linalool concentration was decreased, along with the unchanged α-terpineol content and enhanced rose oxide content. The altered concentration ratio of linalool, α-terpineol and rose oxide may explain the surge in rose oxide concentration.

During the aging period, there were 15, 20, 22, 16, 17, and 18 detected volatiles identified as odor-active substances in *C. indicum*-, *J. sambac*-, *L. angustifolia*-, *C. ternatea*-, *O. fragrans*- and *S. nigra*-flavored wines, respectively (Tables S22–S28). Fruity aromas represent the major aroma group because of greater diversity and higher OAV values, while fatty, fermented, floral, waxy, herbal, terpenic, creamy, ethereal, camphoreous, minty and spicy odors were also responsible for the overall aroma but with inferior sensorial impact. For *C. indicum*-, *J. sambac*- and *C. ternatea*-flavored wines, ethyl hexanoate and ethyl octanoate with higher OAVs exhibited more significant impacts on the wine aroma than other volatiles. The OAVs of both ethyl hexanoate and ethyl octanoate kept rising in bottle storage, albeit at a fluctuating pace. Linalool and geraniol were witnessed to constitute a significant contribution toward the aroma properties of *L. angustifolia*-flavored wines, both of which experienced a decreasing trend in the OAVs as the wine was aged. As the characteristic aroma compounds in lavender flower, linalool and geraniol give a sweet, floral and fruity scent [6]. In *O. fragrans*-flavored wine, ethyl hexanoate, ethyl octanoate and theaspirane had a prominent sensorial impact due to their high OAVs. The OAV of ethyl hexanoate was gradually decreased during bottle storage, while the OAV of ethyl octanoate and theaspirane experienced a sudden surge in the first three months before a significant decline. Theaspirane as one of the key odorants in *Osmanthus* absolute gives a fresh and green odor [40]. For *S. nigra*-flavored wine, ethyl hexanoate, rose oxide and ethyl octanoate acted a primary role in releasing the wine aroma, all of which witnessed a gradual decline throughout the bottle storage. The occurrence of rose oxide is consistent with a previous research stating it as one of the main volatiles for *S. nigra* flower [41].

The Pearson's correlation was carried out to understand the correlation between physicochemical characteristics, antioxidant power, individual volatile compounds and their OAVs in the aged wine samples (Figure 4(b)) (Tables S29–S32). There was a significant ($p < 0.05$) association between the TPC and FRAP, whereas DPPH demonstrated no significant association. The OAV value of "Floral odor" demonstrated significant ($p < 0.05$) positive association with fruity odor (OAV), waxy odor (OAV), herbal odor (OAV), linalool, rose oxide, geranyl acetate, α-terpineol, geraniol, ethyl octanoate, ethyl hexanoate, ethyl butanoate, ethyl acetate, D-limonene, β-ocimene, ethyl heptanoate, eucalyptol, terpinolene, terpinen-4-ol, and methyl salicylate, while establishing a negative correlation with 2-methyl-1-propanol. The OAV value of "Herbal odor" was also found to be positively correlated to floral odor (OAV), linalool, α-terpineol, β-ionone, ethyl nonanoate, D-limonene, β-ocimene, eucalyptol, theaspirane, terpinolene and terpinen-4-ol. Figures 4(c) and 4(d) were the PLS-DA and sPCA biplots of physicochemical characteristics, antioxidant power and representative volatile substances in the bottle-aged wines at four aging times. The PLS-DA indicated a well separation of 1 month-aged wine samples from 9 month-aged ones, while both of them showed partial overlapping with 3 month- and 6 month-aged wines (Figure 4(c)). This variation was mainly driven by 2-phenethyl acetate, TA, ethyl lactate, 2-phenylethanol, DPPH, 1-butanol, ethyl octanoate, 3-methylbutyl hexanoate, 2,3-butanediol, and TPC (Table S33). Figures 4(e) and 4(f) and Table S34 also revealed the main odor drivers of this variation were fatty odor, fruity odor, ethereal odor and waxy odor. This implies the various effects of these aging times on physicochemical characteristics, antioxidant power and volatile composition of different types of flower-flavor wine.

5.2.3. Alteration of OAVs Sensory Profile by Aging Time. OAVs sensory profiles of flower-flavor wine at different aging time were displayed in Figure 5(a). For *C. indicum*-, *C. ternatea*- and *O. fragrans*-flavored wines, less pronounced floral and fruity odors were detected as the aging time increased, while the herbal odor remained nearly unchanged. Less pronounced fruity odor was also found in *J. sambac*-flavored wine, while the floral and herbal odors kept diminished and unchanged in the first 6 months, respectively, followed by a surge in the last three months. Less pronounced floral, fruity and herbal odors were detected in *L. angustifoli*- and *S. nigra*-flavored wines.

5.2.4. Sensory Analysis. Figure 5(b) and Table S35 showed the JAR scale percentages of responses classified in five levels of different flower-flavored Chardonnay wines aged at 12 months. Within the C_{12m} group, the majority of parameters received predominant scores in the JAR category, ranging from 29% (floral smell, vegetal taste and flavor) to 60% (astringency). In all the flower-flavored wine groups, lower proportions of JAR responses were found as compared to C_{12m} for most of the properties assessed. Most of the consumers considered the color intensity of flower-flavored wines as "Just about right", except for $SN_{2p2h12m}$ where "A little dark" was perceived by 35% of the consumers. For $CI_{2p3h12m}$, most consumers perceived the "Strong" fruit flavor and "Weak" bitterness, whereas the most responses to

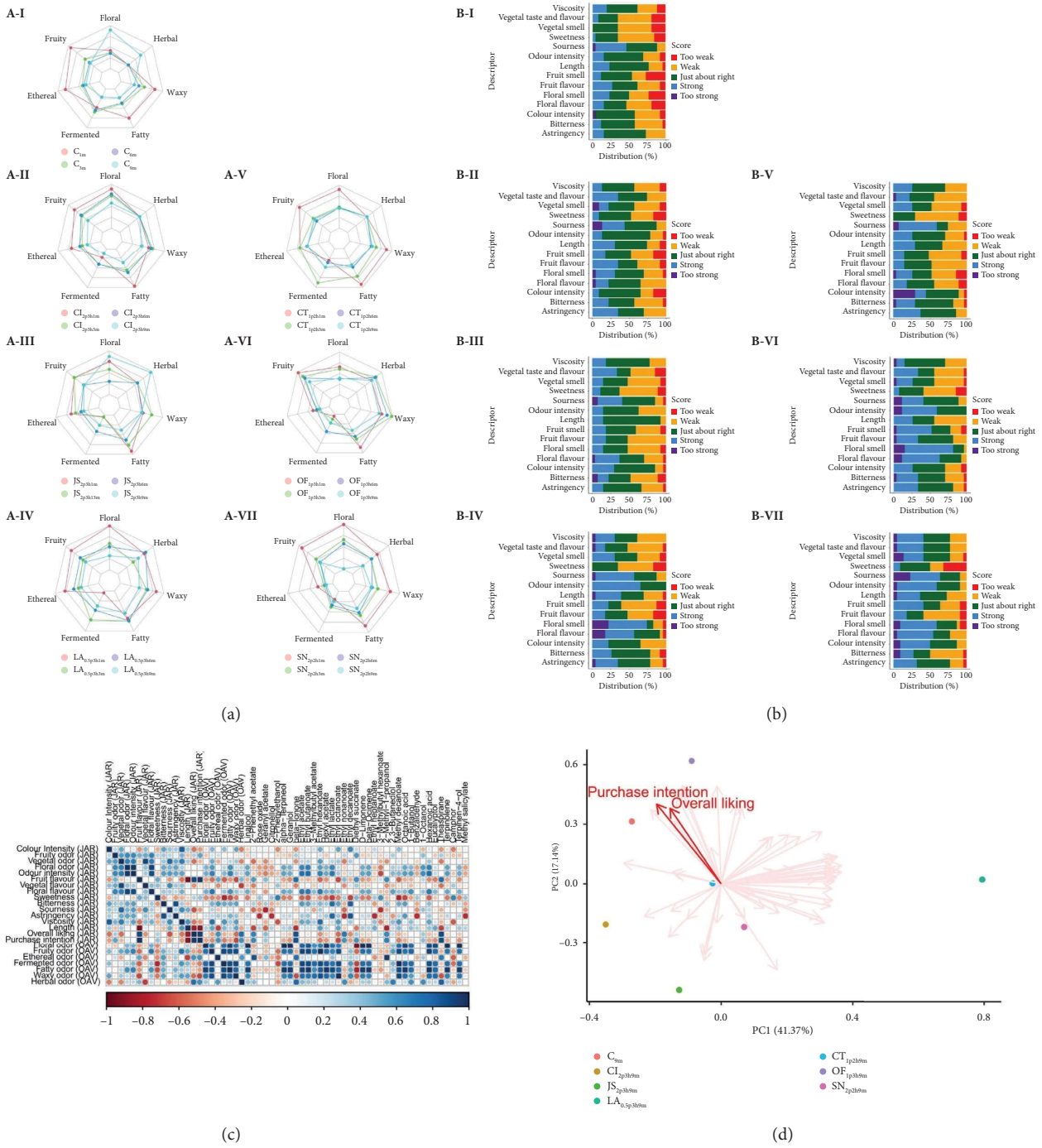

Figure 5: Spider plots of aroma attributes in flower-macerated Chardonnay wines at different aging times (a). (b) Just-about-right (JAR) scale percentages of responses grouped in five levels of C9m (B-I), CI2p3h9m (B-II), JS2p3h9m (B-III), LA0.5p3h9m (B-IV), CT1p2h9m (B-V), OF1p3h9m (B-VI) and SN2p2h9m (B-VII). Pearson correlation matrix (c) and principal component analysis (d) of JAR scores, OAVs and individual volatile compounds of different 9 month-aged flower-macerated Chardonnay wines. Samples of C9m, CI2p3h9m, JS2p3h9m etc. refer to Table 1.

the other attributes were "Just about right." The $JS_{2p3h12m}$ scored the higher proportion of response "Weak" regarding vegetal flavor and smell, floral smell, odor intensity, fruit flavor, sweetness and bitterness, while astringency, sourness, floral flavor and smell, viscosity and length were mainly perceived as "Just about right." The "Strong" vegetable taste and flavor was also documented for $JS_{2p3h12m}$ in most cases. Most consumers thought that $LA_{0.5p3h12m}$ had "Weak" fruit smell and flavor, vegetable taste and flavor, sweetness and viscosity, "Just about right" vegetable smell, bitterness, astringency and length, and "Strong" floral smell, odor intensity, floral flavor, strong sourness and length. The mostly reported intensity of fruit, vegetable smell and flavor, floral smell and sweetness in $CT_{1p2h12m}$ were "Weak," while the

odor intensity, floral flavor, bitterness, astringency, viscosity and length were mainly distributed in "Just about right." The sourness of $CT_{1p2h12m}$ was mainly determined as "Strong." Most consumers considered $OF_{1p3h12m}$ to have "Weak" vegetable smell and flavor, sweetness and length, "Just about right" fruit flavor, bitterness, sourness, astringency and viscosity, "Strong" fruit and floral smell, odor intensity and floral flavor. For $SN_{2p2h12m}$, the "Weak" fruit flavor and bitterness, "Just about right" vegetable smell and flavor, sweetness, astringency, viscosity and length, and "Strong" floral and fruit smell, odor intensity, vegetable and floral flavor, sourness and viscosity were documented for the majority of consumers. As displayed in Table S35, the overall liking of $CI_{2p3h12m}$ and $JS_{2p3h12m}$ was mainly distributed in "Neither like nor dislike," whereas most responses to $LA_{0.5p3h12m}$ and $CT_{1p2h12m}$ were only scored in the "Dislike slightly" category. On the contrary, 34 of 62 consumers displayed their likings for $OF_{1p3h12m}$, where 17 consumers "Like very much." For $SN_{2p2h12m}$, 17 of 62 consumers liked the wine slightly. Accordingly, 30 of 63 consumers thought that they were "likely" to buy $OF_{1p3h12m}$, whereas most consumers only had neutral purchase intentions toward other flower-flavored wines.

The Pearson's correlation was performed to understand the association between individual volatile compounds, OAVs and JAR scores (Figure 5(c)). The JAR score of "Floral odor" was significantly ($p < 0.05$) positively correlated with odor intensity (JAR) and floral flavor (JAR), while the JAR score of "Vegetable odor" was positively associated with vegetable flavor (JAR) and sourness (JAR). Sweetness (JAR) established a negative correlation with fermented odor (OAV) and methyl decanoate, whereas bitterness (JAR) was positively related to astringency (JAR) and ethyl decanoate. The purchase intention and overall liking were determined by fruit flavor (JAR), β-ionone and theaspirane, suggesting the indispensable role of these volatiles and properties in the consumer preference when assessing the wines. A negative correlation was also found between them and length (JAR). The relationship of the purchase intention and overall liking with different flower-flavored wines was shown in PCA biplot (Figure 5(d)). The results indicated a positive correlation between purchase intention and overall liking with the cluster of $OF_{1p3h12m}$ to the greatest extent. Therefore, $OF_{1p3h12m}$ which was the 1% (w/v) *O. fragrans*-infused Chardonnay wine and bottle-aged for 12 months (Table 1) was preferred by most consumers, which manifests the successful entry of this flavored wine product into the wine market in the future.

6. Conclusion

Edible flower has long been applied as an aroma enhancer in a wide variety of food or beverages, unveiling its vast potential as a novel wine additive. Bottle aging is a sensible and complicated process that prominently influences the properties of grape wine. In this study, maceration with six different edible flowers successfully modulated the physicochemical characteristics, antioxidant activity and volatile profile of Chardonnay wine. However, prolonged bottle storage induced no variations in the physicochemical characteristics of all the flower-flavored wines, but exhibited the contrasting impacts on the antioxidant power and total free phenolic content. A total of 37 volatiles were identified in the aged flower-flavored wines, including 15, 20, 22, 16, 17 and 18 odor-active compounds detected in *C. indicum*-, *J. sambac*-, *L. angustifolia*-, *C. ternatea*-, *O. fragrans*-, and *S. nigra*-flavored wines. In all the flower-flavored wines, the aging process led to a decline in fruity and floral aromas. In the 12 month-aged wines, $OF_{1p3h12m}$ was regarded as the well perceived product because of its positive relation with purchase intention and overall liking in consumer sensory study. This research offers an essential prerequisite for the future utilization of dried edible flowers to develop novel flower-based grape wine, and explores the time-dependent effect of bottle storage on different attributes of such wine, revealing its bright prospect as a high valued commercial wine product.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethical Approval

Approval for human ethics (Grant no. 2022-23727-28046-4) was obtained from the Human Ethics Committee of the University of Melbourne.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Zijian Liang, Shuge Yang, Ying Chia, and Jingning Xiao proposed the methodology, validated the study, investigated the study, curated the data, performed formal analysis, visualized the study, wrote the original draft, and wrote, reviewed, and edited this study. Yipeng Chen proposed the methodology, validated the study, investigated the study, and curated the data curation. Zhongxiang Fang proposed the methodology, supervised the study, wrote, reviewed, and edited the study, and administered the project. Pangzhen Zhang: conceptualized the study, proposed the methodology, investigated the study, curated the data, performed formal analysis, supervised the study, wrote, reviewed, and edited the study, and administered the project.

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Supplementary Materials

Table S1: Effect of different processing treatments on pH, titratable acidity (TA), total phenolic and flavonoid contents (TPC, TFC) and antioxidant activities (DPPH, FRAP) of different flower-macerated wines. Table S2: Effect of different treatments on volatile profile of *Chrysanthemum indicum*-flavored wine (μg/L). Table S3: Effect of different treatments on volatile profile of *Jasminum sambac*-flavored wine (μg/L). Table S4: Effect of different treatments on volatile profile of *Lavandula angustifolia* (LA)-flavoured wine (μg/L). Table S5: Effect of different treatments on volatile profile of *Clitoria ternatea* (CT)-flavored wine (μg/L). Table S6: Effect of different treatments on volatile profile of *Osmanthus fragrans* (OF)-flavored wine (μg/L). Table S7: Effect of different treatments on volatile profile of *Sambicus nigra* (SN)-flavored wine (μg/L). Table S8: Odor active values (OAVs) of volatile compounds in *Chrysanthemum indicum* (CI)-flavored wine. Table S9: Odor active values (OAVs) of volatile compounds in *Jasminum sambac* (JS)-flavoured wine. Table S10 Odor active values (OAVs) of volatile compounds in *Lavandula angustifolia* (LA)-flavoured wine. Table S11: Odor active values (OAVs) of volatile compounds in *Clitoria ternatea* (CT)-flavored wine. Table S12: Odor active values (OAVs) of volatile compounds in *Osmanthus fragrans* (OF)-flavored wine. Table S13: Odor active values (OAVs) of volatile compounds in *Sambicus nigra* (SN)-flavored wine. Table S14: Correlation matrix of quantified volatile compounds, OAVs, physicochemical properties and tested responses of flower-macerated Chardonnay wines (continued 1/5). Table S15: Correlation matrix of quantified volatile compounds, OAVs, physicochemical properties and tested responses of flower-macerated Chardonnay wines (continued 2/5). Table S16: Correlation matrix of quantified volatile compounds, OAVs, physicochemical properties and tested responses of flower-macerated Chardonnay wines (continued 3/5). Table S17: Correlation matrix of quantified volatile compounds, OAVs, physicochemical properties and tested responses of flower-macerated Chardonnay wines (continued 4/5). Table S18: Correlation matrix of quantified volatile compounds, OAVs, physicochemical properties and tested responses of flower-macerated Chardonnay wines (continued 5/5). Table S19: PLS-DA results for flower-macerated Chardonnay wines: variables importance in projection (VIP > 1) and standardized coefficient (β). Table S20: Effect of different aging times on pH, titratable acidity (TA), total phenolic content (TPC) and antioxidant activities (DPPH, FRAP) of different flower-macerated wines. Table S21: Effect of aging time on volatile compounds of flower-flavored wine (μg/L). Table S22: Odor active values (OAVs) of volatile compounds in control Chardonnay wine at different aging times. Table S23: Odor active values (OAVs) of volatile compounds in *Chrysanthemum indicum*-flavored Chardonnay wine at different aging times. Table S24: Odor active values (OAVs) of volatile compounds in *Jasminum sambac*-flavored Chardonnay wine at different aging times. Table S25: Odor active values (OAVs) of volatile compounds in *Lavandula angustifolia*-flavored Chardonnay wine at different aging times. Table S26: Odor active values (OAVs) of volatile compounds in *Clitoria ternatea*-flavored Chardonnay wine at different aging times. Table S27: Odor active values (OAVs) of volatile compounds in *Osmanthus fragrans*-flavored Chardonnay wine at different aging times. Table S28: Odor active values (OAVs) of volatile compounds in *Sambicus nigra*-flavored Chardonnay wine at different aging times. Table S29: Correlation matrix of quantified volatile compounds, OAVs, physicochemical properties and tested responses of bottle-aged flower-macerated Chardonnay wines (continued 1/4). Table S30: Correlation matrix of quantified volatile compounds, OAVs, physicochemical properties and tested responses of bottle-aged flower-macerated Chardonnay wines (continued 2/4). Table S31: Correlation matrix of quantified volatile compounds, OAVs, physicochemical properties and tested responses of bottle-aged flower-macerated Chardonnay wines (continued 3/4). Table S32: Correlation matrix of quantified volatile compounds, OAVs, physicochemical properties and tested responses of bottle-aged flower-macerated Chardonnay wines (continued 4/4). Table S33: PLS-DA results for volatile compounds, OAVs, physicochemical properties and tested responses of bottle-aged flower-macerated Chardonnay wines: variables importance in projection (VIP > 1) and standardized coefficient (β). Table S34: PLS-DA results for OAVs, physicochemical properties and tested responses of bottle-aged flower-macerated Chardonnay wines: variables importance in projection (VIP > 1) and standardized coefficient (β). Table S35: The distribution of 63 consumers by different categories in JAR analysis. (*Supplementary Materials*)

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