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Optimization of fermentation conditions, physicochemical profile and sensory quality analysis of seedless wampee wine

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Abstract
The aims of the present stud were to optimize fermentation parameters of seedless wampee wine using response surface methodology (RSM) and evaluate the changes in flavor metabolites during fermentation. Seedless wampee wine of optimal sensory quality was produced using an inoculum concentration of 0.6%, initial sugar levels of 200 g/L, a fermentation temperature of 22 °C, and a fermentation period of 9 days. Then the flavor compound profiles (amino acids, organic acids and volatile aroma compounds) of seedless wampee wine during the fermentation under optimal conditions were analyzed using high performance liquid chromatography (HPLC) and gas chromatography–mass spectrometr (GC-MS). The main fermented phase of fermentation resulted in fluctuations in both total amino acids and organic acids, with stabilization occurring later on. A total of 54 volatile components, including esters, alcohols, terpenes, and acids, were putatively identified. Terpenes were the primary drivers of the flavor characteristics of seedless wampee. The rise of esters and decline of terpenes have the potential to significantly alter the flavor of wine during fermentation. These results would contribute to the further development of seedless wampee wine.

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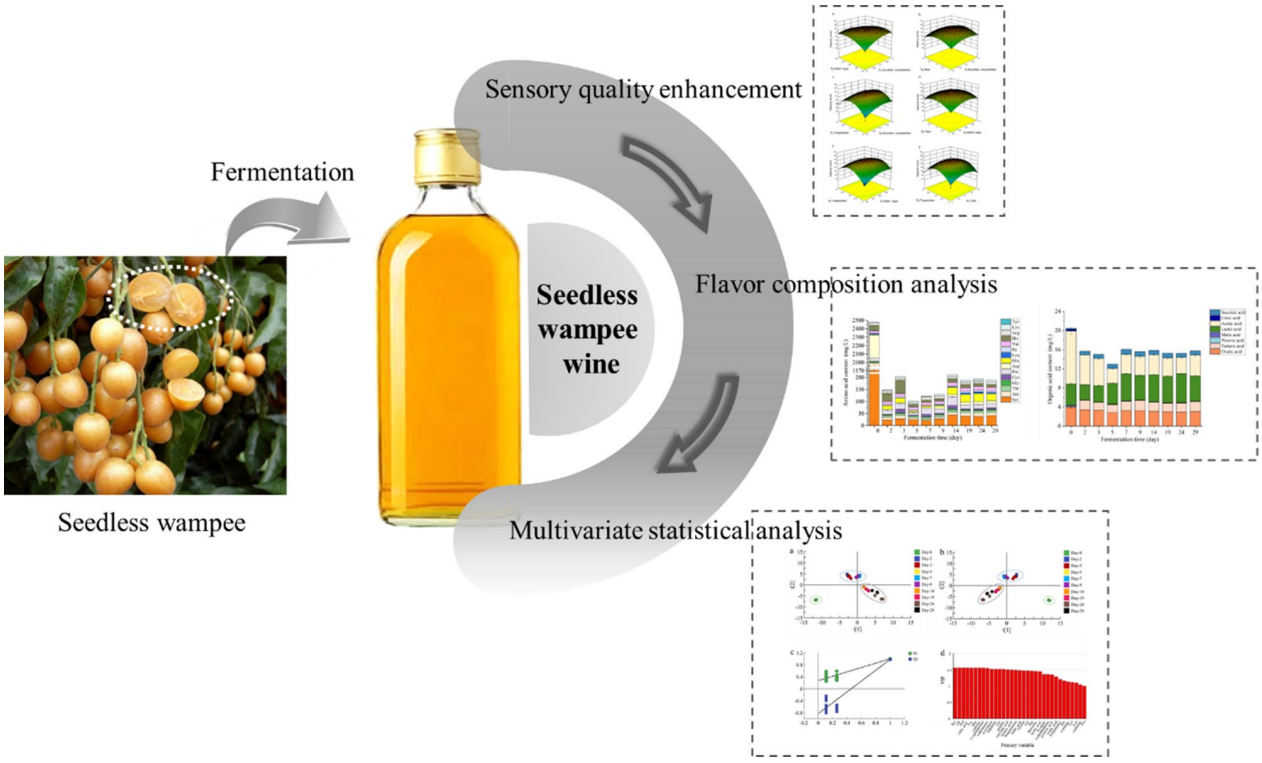
Graphical Abstract



Keywords Seedless wampee wine, Response surface method, Amino acid, Organic acid, Volatile compounds

Introduction

Clausena lansium (Lour.) Skeels, commonly known as wampee, is a fruit crop of the Rutaceae family that is native to southern China, Thailand and Vietnam [1]. Wampee fruit is renowned for its rich taste and flavor, and has been introduced to India, Sri Lanka, Australia, the United States and in Central America [2, 3]. Previous studies have identified multiple key flavor and taste components in different parts of the wampee fruit, which explained the reason that many consumers tend to eat the pulp and peel rather than the seed of wampee [4]. As an edible medicinal and non-medicinal fruit, wampee is nowadays available in various forms such as fruit cups, gelatins, juices, jams, jellies, and pies [2]. Wampee has also been used to treat bronchitis in traditional Chinese and Vietnamese medicine [5]. Recent studies have identified its extensive health benefits, including anti-oxidation, anti-bacterium, anti-inflammation, antihypertension, neuroprotection, and prebiotic effects, which are mainly attributed to the bioactive phenolics, carbazole alkaloids, and polysaccharides [6–10]. Wampee can be classified according to its taste, which can be either sweet or sweet-sour. The sweet variety is typically consumed fresh, while the sweet-sour variety is commonly used as a raw material for the production of processed foods, such as preserved fruit, wine, and vinegar [11]. Seedless wampee (*C. lansium* S. *cv. WuHeHuangPi*), a sweet–sour cultivar, is characterized by its thin skin, thick flesh and distinctive sweet-sour flavor. Due to the expanded cultivation of seedless wampee and the seasonality of the fruit, various wampee-derived products need to be developed urgently.

Nowadays, fruit wine is gaining popularity among consumers because of its special flavor and nutritional benefits. Various types of fruit, such as grapes, apples, durians and red pitayas, can be used for making fruit wine [12–15]. In Vietnam, wampee is fermented with sugar, to produce a beverage that resembles champagne [2]. Fruit wine production not only resolves production, marketing, transportation and preservation issues during the fruit harvest season, but also enhances sensory quality and physical and chemical properties. Flavor metabolites are recognized as crucial indicators of wine quality and play a major role in consumer purchasing decisions [16, 17]. The composition and concentration of primary metabolites, specially carbohydrates, organic acids and amino acids, are closely correlated with the flavor and taste of fruit wine [18, 19]. Additionally, during fermentation, microorganisms can break down

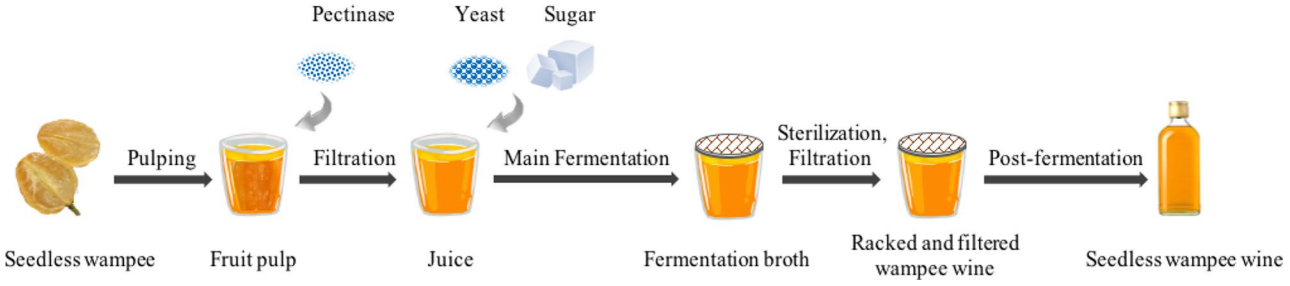


Fig. 1 Preparation of seedless wampee wine

complex macromolecules into smaller molecules with improved bioaccessibility and bioavailability. Enzymes also facilitate the formation and release of flavor compounds to enhance the quality of fruit wine [20]. Over the last decade, researchers have examined the potential health benefits of consuming appropriate fruit wine, especially the non-volatile components, such as phenolic compounds and polysaccharide [21–23]. Nevertheless, limited information is available on the dynamic flavor changes of wampee during fermentation is still unknown, particularly for seedless wampee.

The commercial value of fruit wine depends on its organoleptic and sensory characteristics which are influenced by a combination of non-volatile and volatile components. The compositions and abundance of these components are closely associated with the fermentation process of fruit wine. Hence, we optimized various variables, such as inoculum concentration, initial sugar, time, and temperature using response surface methodology (RSM) to enhance the quality attributes of seedless wampee wine. Additionally, we evaluated the fluctuation of physicochemical indicators, such as free amino acids, organic acids and volatile aroma compounds. Hopefully, these results would provide a scientific basis for seedless wampee wine production and increase the versatile application of seedless wampee in the food industry.

Materials and methods

Materials and reagents

Seedless wampee was collected in Yunfu, Guangdong Province, China. *Saccharomyces cerevisiae* yeast was purchased from Angel Yeast Co., Ltd. (Hubei, China). Lallzyme EX-V was purchased from Lallemand Inc. (Toulouse, France). The materials used in winemaking were of food grade. Folin-Ciocalteu's phenol reagent (99%), L-ascorbic acid (99%), gallic acid (99%), protocatechuic acid (99%), chlorogenic acid (99%), vanillic acid (99%), syringic acid (99%), coumaric acid (99%), ferulic acid (99%), rutin (99%), quercetin (99%), fluorescein sodium salt (99%), 2,2-azobis(2-methylpropionamidine) dihydrochloride (AAPH) (99%) were procured from Aladdin Ltd. (Shanghai, China). All the chemicals and reagents used in this study were of analytical grade.

Seedless wampee wine preparation

The fermentation process was shown in Fig. 1. Briefly, seedless wampee was washed with water, then water (1:1, v/v) was added to crush and pulp with a homogenizer, after which 100 mg/kg SO_2 was added to inhibit miscellaneous bacteria. Then 0.1% (w/v) Lallzyme EX-V was added and treated at 50 °C for 1 h for clarification, followed by inactivation in a water bath at 80 °C for 10 min. Meanwhile, 1 g of yeast was inoculated into a 100 mL of 3% (v/v) sugar solution and placed in 38 °C for activation. Afterwards, the activated yeast solution (0.5–0.7%, v/v) was inoculated into the wampee pulp, which was adjusted for the initial sugar level (190–210 g/L) and subjected to pre-fermentation at a suitable temperature (21–23 °C) for 8–10 d. In order to analyze changes in physicochemical indicators and sensory quality during fermentation, seedless wampee was fermented under optimal process conditions, and the upper wine layer was collected and subjected to post-fermentation at 20 °C. The samples were collected on day 0, 2, 3, 5, 7, 9, 14, 19, 24 and 29 for further analysis.

Response surface methodology for optimization of fermentation conditions

In this study, Box-Behnken design and response surface methodology (BBD-RSM) was used to investigate the effect of fermentation factors on the quality of seedless wampee wine [24]. The sensory score of seedless wampee wine was taken as the response variable (Y) (Table S1). The experimental design was applied with four independent variable factors including inoculum size, initial sugar, fermentation time, and fermentation temperature at three levels, as indicated in Table 1.

Sensory evaluation

Sensory evaluation of seedless wampee wine was carried out in accordance with national standards of the PRC by ten semi-trained panels, respectively [25]. Plain water was provided to panelists between the evaluations of different samples to avoid lingering aftertaste. Scores were given by evaluators for appearance (0–30), aroma (0–30), taste (0–30) and typicality (0–20), respectively (Table S1). The study was reviewed and approved by the Institutional

Table 1 Treatment combinations with results of the response surface of seedless wampee wine

Run	Independent variable				Response
	X_1 Inoculum size (%)	X_2 Initial sugar (g/L)	X_3 Fermentation time (d)	X_4 Fermentation temperature (°C)	Y Sensory evaluation (scores)
1	0.7	210	9	22	91.5
2	0.6	200	9	22	94.3
3	0.5	200	8	22	90.5
4	0.6	200	9	22	93.5
5	0.7	200	10	22	90.1
6	0.5	210	9	22	93.4
7	0.6	190	9	23	87.4
8	0.7	200	9	21	90.2
9	0.5	190	9	22	88.2
10	0.6	190	10	22	91.8
11	0.6	200	9	22	94.9
12	0.6	200	10	23	89.7
13	0.5	200	10	22	89.3
14	0.6	190	9	21	87.7
15	0.6	200	9	22	94.3
16	0.6	190	9	21	90.8
17	0.6	200	9	22	95.7
18	0.7	200	8	22	91.6
19	0.6	200	8	21	85.6
20	0.6	190	8	22	89.5
21	0.6	200	8	23	88.1
22	0.6	200	10	21	88.3
23	0.6	210	8	22	90.6
24	0.7	190	9	22	91.7
25	0.6	210	9	23	86.1
26	0.6	210	10	22	90.7
27	0.5	200	9	21	85.4
28	0.7	200	9	23	91.1
29	0.5	200	9	23	88.6

Review Board (IRB) of Zhongkai University of Agriculture and Engineering and informed consent was obtained from each subject prior to their participation in the study.

Amino acids analysis

Briefly, concentrations of amino acids in seedless wampee wine were analyzed by the high performance liquid chromatography (HPLC) system (1260, Agilent Technologies, USA), with Advance Bio 3 A amino acid chromatography columns (4.6×100 mm, 2.7 μm, Agilent Technologies, USA) [26]. The sample was centrifuged at a rate of 1000 r/min for 10 min, and then the supernatant was filtered through a 0.45 μm membrane for analysis. Ten millimolars Na_2HPO_4- $Na_2B_4O_7$ (1:1, v/v) which adjust the pH to 8.2 was used as buffer A and acetonitrile-methanol-acetic acid (45:45:10, v/v) was used as buffer B. The flow rate was fixed at 1 mL/min and the column temperature was set at 40 °C. The absorbance was monitored at 338 nm. The program began with 98% A and 2% B. Then the buffer A linearly dropped to 43% from 0 to 13.4 min, dropped to 0% from 13.4 to 15.8 min, and subsequently, rose back to 98% from 15.8 to 20 min. The chromatographic peaks were analyzed qualitatively and quantitatively mainly by comparison with standards.

Organic acids analysis

The method used for the determination of organic acids was based on a previous report [27]. The HPLC system (1260, Agilent Technologies, USA) with ZORBAX SB-Aq columns (250×4.6 mm, 5 μm, Thermo Fisher Scientific, USA) was applied for organic acid detection. Buffer A consisted of 10% methanol and 0.01 mol/L KH_2PO_4 solution was used as buffer B. The isocratic elution program was started with 3% A and 97% B and the absorbance was monitored at 241 nm. The other details were as follows: injection volume was 10 μL, flow rate was 0.8 mL/min, and the column temperature was 30 °C. Identification and quantification were conducted using the external standard method.

Volatile analysis

Volatile compounds were analyzed using the headspace solid-phase microextraction-gas chromatography-mass spectrometry (HS-SPME-GC-MS) system (7890B, Agilent Technologies, USA) [28]. In brief, 7 mL of wine samples were mixed with 0.4 g of NaCl in 15 mL headspace vials. Then, 10 μL of 2-octanol (2.2 mg/L) was added as the internal standard, and the headspace vials were sealed. Subsequently, samples were equilibrated at 45 °C for 50 min, and the volatile compounds were extracted from the headspace to the solid-phase microextraction (SPME) fiber. Separation of volatile components was performed with a DB-WAX UI gas chromatography column (30 m × 250 μm, 0.25 μm, Agilent Technologies, USA). Helium at a flow rate of 1 mL/min was used as the carrier gas. The transfer line was set to 250 °C and the ion-source temperature was set to 230 °C. The ionization energy of the impact was 70 eV, with a scanning range of m/z from 35 to 450. The SPME fiber was placed into a gas chromatograph injection port and desorbed for 5 min at 250 °C. The oven temperature was initially maintained at 40 °C for 5 min, and increased to 120 °C at a rate of 3 °C/min and kept for 3 min, followed by another increase in temperature to 220 °C at a rate of 6 °C/min with a final holding of 5 min. The compounds were identified by comparing their mass spectra against synthetic standards and matches from NIST 2.0 library.

Statistical analysis

Data were expressed as means±standard deviation (SD, $n=3$). Significant differences ($p<0.05$) were analyzed using one-way analysis of variance (ANOVA) and

Duncan's multiple comparison post-test using SPSS statistical software 21.0 (SPSS Inc., Chicago, IL, USA). Design-Expert 8.0.7 was applied to establish the second-order polynomial equation and generate the contour plots based on analysis of variance and the optimization. A dose-effect analysis was performed using Calcusyn software version 2.0 (Biosoft, Cambridge, UK). Multivariate data analysis was performed using SIMCA software 14.0 (Umetrics, Umeaa, Sweden). For GC-MS data, mass spectral resolution and comparison with the NIST 20.0 standard library were used. Results with >80% match were retained for qualitative analysis. The relative content of each component was calculated using the internal standard method.

Results and discussion

Effects of different fermentation conditions on sensory evaluation

Sensory evaluation plays an indispensable role in the quality of fruit wine fermentation. This study indicated that optimization of fermentation conditions can improve the sensory quality of seedless wampee wine. The sensory scores of seedless wampee wine under different fermentation conditions (inoculum size, initial sugar, fermentation time, and fermentation temperature) were shown in Table 1. The second-order polynomial response surface model fitted for sensory quality was displayed in Eq. (1):

$$\begin{aligned} &Y\,(sensoryevaluation, scores)\,= \\ &94.54 + 0.9\,X_1 + 0.57X_2 + 0.33X_3 + 0.25X_4 - \\ &1.35X_1X_2 - 0.075X_1X_3 - 0.58X_1X_4 - 0.55X_2X_3 - \\ &1.1X_2X_4 - 0.28X_3X_4 - 1.57{X_1}^2 - \\ &1.84{X_2}^2 - 2.29{X_3}^2 - 4.39{X_4}^2 \end{aligned} \quad (1)$$

The results of the analysis of variance for the regression model using RSM were presented in Table 2. The regression model was indicated to be significant with a p- value of 0.0016 ($p<0.01$). The quadratic polynomial model for sensory evaluation resulted in a determination coefficient ($R^2=0.8443$), which showed that 84.43% of the change could be explained [29]. The lack of fit, corresponding to p-values of 0.0818, showed non-significance of difference, demonstrating that the experimental data was highly probable. Among the factors explored in the sensory evaluation of seedless wampee wine, inoculum size (X_1) had the greatest effect followed by initial sugar (X_2), fermentation time (X_3), and fermentation temperature (X_4). The combined effects of the tested factors on the sensory scores were visualized in Fig. 2. The quadratic term (X_4^2) displayed highly significance ($p<0.0001$), followed by X_2^2 and X_3^2 ($p<0.01$), and X_4^2 was also significant ($p<0.05$).

According to the response surface and the regression equation, the optimal value for inoculum concentration, initial sugar, fermentation temperature and fermentation time to produce a sensory score value of 94.68 were 0.63%, 200.47 g/L, 22.00 °C and 9.06 d. To ensure the validity of the model equations, three replicate tests were performed under the optimal conditions with slight modification as follows: an inoculum concentration of 0.6%, initial sugar concentration of 200 g/L, fermentation at a temperature of 22 °C, and fermentation for 9 d, taking into account the feasibility of the practical operation. The sensory score of 94.54 was in line with the expected results, suggesting that the established prediction model could effectively predict the sensory score.

Amino acid content of the seedless wampee wine during fermentation

As one of the precursors of volatile compounds, amino acids are recognized for their contribution to the aroma and taste of wine [18, 19]. The sensitivity of amino acids profiles to processing conditions varies depending on the processing methods and materials [30]. Fifteen free amino acids were detected in seedless wampee wine in this study. These fatty acids were categorized according to taste as sweet amino acids (Ser, Ala, Thr, Gly, Cys, Pro), bitter amino acids (Leu, Ile, Val, His, Arg, Lys, Tyr), and umami amino acid (Asp, Glu) [31]. Overall, there was a greater variation in total amino acids during the

Table 2 Variance analysis of response surface model

Source	Sum of square	Degree of freedom	Mean square	F-value	*P*-value
Model	175.32	14	12.52	5.42	0.0016*
X_1	9.72	1	9.72	4.21	0.0595
X_2	3.85	1	3.85	1.67	0.2175
X_3	1.33	1	1.33	0.58	0.4600
X_4	0.75	1	0.75	0.32	0.5779
$X_1 \times_2$	7.29	1	7.29	3.16	0.0974
$X_1 \times_3$	0.022	1	0.022	9.74×10^{-3}	0.9228
$X_1 \times_4$	1.32	1	1.32	0.57	0.4618
$X_2 \times_3$	1.21	1	1.21	0.52	0.4812
$X_2 \times_4$	4.84	1	4.84	2.10	0.1698
$X_3 \times_4$	0.30	1	0.30	0.13	0.7229
X_1^2	15.90	1	15.90	6.88	0.0200*
X_2^2	21.98	1	21.98	9.51	0.0081*
X_3^2	34.04	1	34.04	14.73	0.0018*
X_4^2	125.06	1	125.06	54.13	< 0.0001**
Residual	32.34	14	2.31		
Lack of fit	29.67	10	2.97	4.44	0.0818
Pure Error	2.67	4	0.67		
Cor total	20.74	28			
R^2	0.8443				

*means significant difference ($p<0.05$), **means extremely significant difference ($p<0.01$).

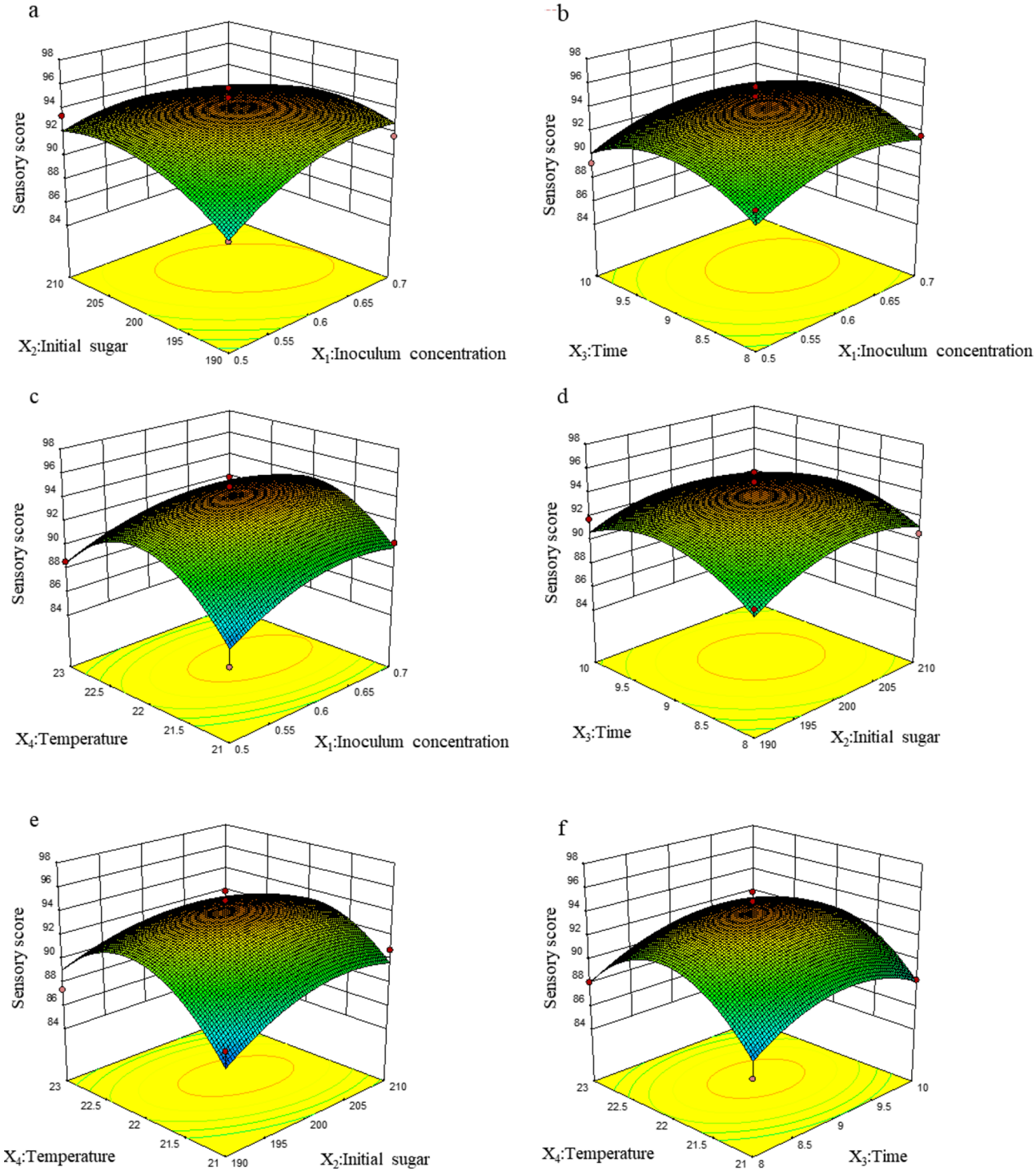


Fig. 2 3D surface plots for the effect of independent variables (a. inoculum concentration and initial sugar; b. inoculum concentration and fermentation time; c. inoculum concentration and fermentation temperature; d. initial sugar and fermentation time; e. initial sugar and fermentation temperature; f. fermentation time and fermentation temperature.) on sensory score of seedless wampee wine

main fermentation, ranging from 100.98 mg/L on day 5 to 2492.36 mg/L on day 0, but the levels remained relatively stable during the post-fermentation period, ranging from 187.38 to 210.74 mg/L (Fig. 3a). Regarding amino acids responsible for taste, the percentage of sweet amino acids decreased from 82% on day 0 to 48% on day 29, which could be partly attributed to the significant reductions of Ser and Ala (Fig. 3b). On the 9th day, the volatile

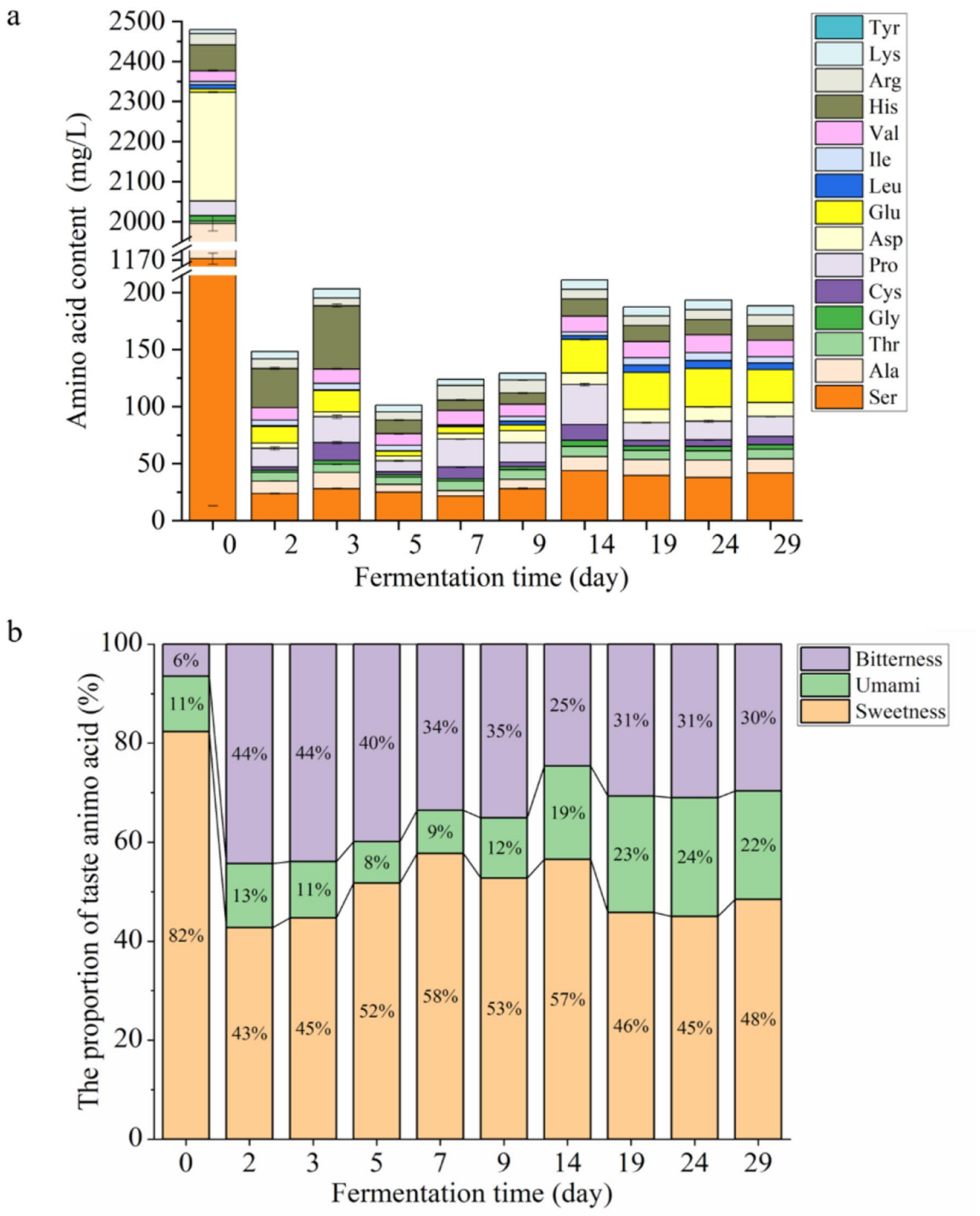


Fig. 3 Changes in amino acid composition (**a**) and the proportion of taste amino acid (**b**) during seedless wampee wine fermentation

compounds had 52.74% of sweetness amino acids, 12.12% of umami amino acids and 35.07% of bitterness amino acids. Although the proportion of bitter amino acids increased during the initial stage of fermentation, their concentration decreased significantly during fermentation, dropping from 161.18 mg/L on day 0 to 54.93 mg/L on day 29, which represents a 65.9% reduction. It has been suggested that the bitterness threshold is adjusted through the acidity threshold [32], and that the increase of bitter amino acids may balance the acidity of seedless wampee, resulting in a more harmonious flavor of wampee wine. Furthermore, the percentage of umami amino acids significantly increased during fermentation, from 11% on day 0 to 22% on day 29. Previous studies

have demonstrated that fermented beverages with prolonged yeast exposure contain high levels of free Glu, which may enhance umami more than beverages with limited or no yeast exposure. This is consistent with the results of our study, where the freshness amino acids increased significantly during the late fermentation of wampee wine [33]. Amino acids not only contribute to aroma formation, but are also precursors of a variety of flavor compounds, mainly due to their role in microbial growth and metabolism as nitrogen sources [17]. The profile of individual amino acids in fruit wine was influenced by various factors, including yeast, fermentation conditions, and carbon source [34, 35]. In our study, decreases in bitter amino acid content during fermentation might contribute to the taste of seedless wampee wine.

Organic acid content of the seedless wampee wine during fermentation

The presence of an adequate amount of organic acids has been shown to hinder the growth of contaminating bacteria and improve the mellowness and flavor of wine [36]. In order to investigate the variations in organic acid levels in the fermentation process of seedless wampee wine, eight organic acids, namely oxalic acid, tartaric acid, pyruvic acid, malic acid, lactic acid, acetic acid, citric acid, and succinic acid, were identified through HPLC analysis of the seedless wampee wine. Overall, the concentration of organic acids exhibited a modest decline during the fermentation process and then maintained a relatively stable, fluctuating between 15.24 and 16.08 mg/L after the seventh day, suggesting that the microbial community involved in the fermentation of wampee wine had achieved a state of equilibrium (Fig. 4a).

As for the concentrations of individual organic acids, tartaric acid, lactic acid, and succinic acid, were observed to be significantly increased ($p<0.05$). Tartaric acid exhibited the highest increase, rising from 0.14 mg/L on day 0 to 2.05 mg/L on day 29, indicating a 13.64-fold increase. The peel of seedless wampee is a possible source of the higher tartaric acid concertation [37]. On the other hand, the levels of oxalic acid, pyruvic acid, malic acid, acetic acid, and citric acid were significantly reduced ($p<0.05$), and malic acid exhibited the most significant decline, decreasing from 0.22 mg/L on day 0 to 0.04 mg/L on day 29.

After the fermentation process, the predominant organic acid found in seedless wampee wine was changed from initially acetic acid to lactic acid, with the percentage of acetic acid decreasing from 54% on day 0 to 28% on day 29 and the percentage of lactic acid increasing from 23% on day 0 to 34% on day 29 (Fig. 4b). The increase in alcohol content during fermentation could lead to the solubility of lactic acid in wampee. As reported, most organic acids in beverages were not directly correlated with sensory characteristics, however, the ratio of acetic acid to total organic acid content exhibited a strong correlation with sensory characteristics [38]. The ratio of acetic acid to total organic acid content remained steady (22-28%) from day 5 to day 29, suggesting that organic acids have a minimal impact on the sensory features of seedless wampee wine during this stage. Furthermore, it should be noted that acetic acid contributes to the synthesis of ethyl acetate, and the reduction of acetic acid during fermentation is accompanied by an increase in ethyl acetate concentration, which may ultimately result in improved fruit aroma of wampee wine [39].

Dynamic changes of aroma compounds in seedless wampee wine during fermentation

There was a strong relationship between the sensorial properties and aroma compounds of fruit wine [40]. The main volatile aroma compounds of seedless wampee wine during fermentation were determined by HS-SPME-GC–MS system, including 14 esters, 10 alcohols, 27 terpenes, and 3 acids (Table 3). In general, the composition of volatile aroma components varied during the fermentation process. The flavor components of seedless wampee fruit presented a fruity and floral aroma that is characteristic of terpenes, with lower levels of alcohols, esters and acids [41]. During the fermentation process, more than10 esters, 3 alcohols, 2 acids and 4 terpenes being produced in seedless wampee wine, whereas 9 terpenes found in wampee juice were not detected in the resulting wine. The fermentation process resulted in the gradual development of a delicate and mellow flavor of seedless wampee wine, which was achieved by day 29.

Esters play a major role in providing fresh and fruity fragrances to wine. They are primarily produced during yeast metabolism through the fatty acid acyl- and acetyl-coenzyme A (CoA) pathways [42, 43]. The seedless wampee wine contained twelve esters, eight of which were ethyl esters of fatty acid. Ethyl decanoate and ethyl octanoate were promoted most significantly after fermentation compared with seedless wampee juice, followed by ethyl 9-decenoate, ethyl palmitate and ethyl tetradecanoate (>1%). The similar trend of change for ethyl decanoate and ethyl octanoate was observed during wine fermentation, which brought out grape and fat odors to the seedless wampee wine [23].

The alcohols present in fruit wine that are derived from yeast`s amino acid metabolism are associated with the variety of fruit [44]. Phenylethyl alcohol and n-pentanol were the prominent higher alcohols found in seedless wampee wine as they were the byproducts of alcoholic fermentation. A moderate amount of these compounds contributes to the mellow and sweet taste of fruit wine. For instance, phenylethyl alcohol is known

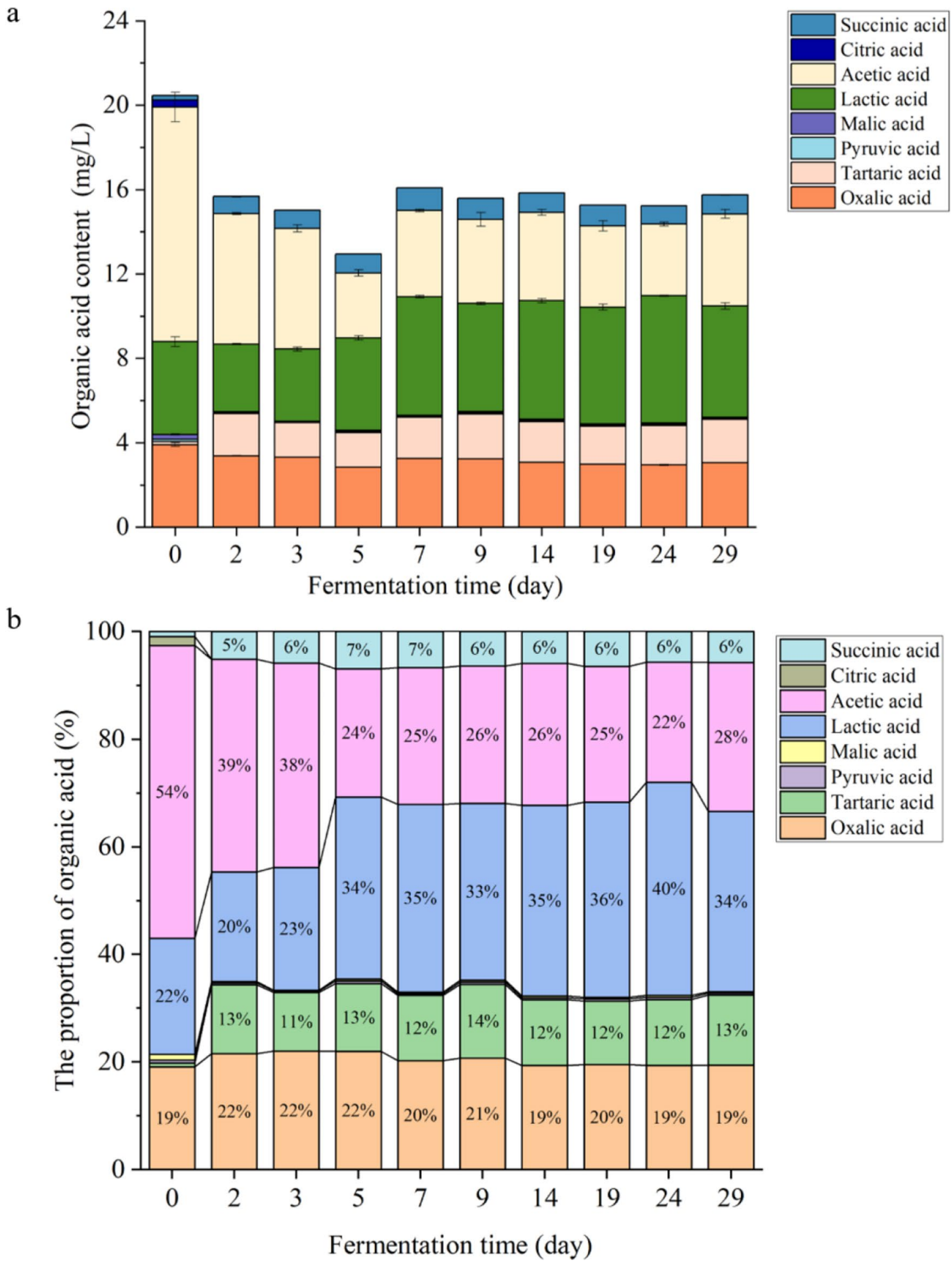


Fig. 4 Changes in organic acid composition (**a**) and the proportion of organic acid (**b**) during seedless wampee wine fermentation

for its rose-like aroma and jasmine aroma [45], while n-pentanol plays a role in providing bitter almond and fat flavor [46]. Additionally, various alcohols including 4-terpinenol, linalool, (-)-α-cadinol, spathulenol, and α-bisabolol were derived from wampee juice, although there were some losses during the fermentation process.

Terpenes have a unique aroma with a low flavor threshold and are reported as the characteristic flavor for ripened fruit and wine [47]. In seedless wampee

Table 3 Relative contents (%) of aroma components in seedless wampee wine during fermentation process

Category	Aroma component	RI values	Published RI values	Fermentation time (day)									
				0	2	3	5	7	9	14	19	24	29
Esters	L-bornyl acetate	1296	1283	0.42 ± 0.03	1.53 ± 0.06	1.06 ± 0.27	nd	nd	nd	nd	0.67 ± 0.15	0.53 ± 0.03	0.60 ± 0.06
	ethyl palmitate	2191	2137	0.06 ± 0.01	nd	0.10 ± 0.04	0.25 ± 0.13	0.19 ± 0.01	1.49 ± 0.32	0.23 ± 0.03	0.39 ± 0.03	1.13 ± 0.28	1.36 ± 0.84
	isoamyl acetate	1369	1356	nd	1.18 ± 0.76	0.66 ± 0.15	1.26 ± 0.51	0.65 ± 0.11	1.03 ± 0.05	0.45 ± 0.08	0.35 ± 0.16	0.62 ± 0.02	0.61 ± 0.02
	ethyl acetate	1209	1237	nd	0.12 ± 0.02	nd	0.03 ± 0.02	0.17 ± 0.04	0.23 ± 0.03	0.06 ± 0.02	0.07 ± 0.01	0.06 ± 0.04	0.03 ± 0.02
	ethyl decanoate	1668	1657	nd	nd	6.74 ± 0.46	12.74 ± 2.44	14.97 ± 0.4	0.20 ± 0.01	15.86 ± 1.33	15.83 ± 0.62	15.64 ± 1.46	15.05 ± 0.15
	ethyl octanoate	1392	1384	nd	nd	3.10 ± 0.85	10.48 ± 0.81	7.25 ± 0.35	0.13 ± 0.01	10.21 ± 0.21	6.15 ± 2.89	8.51 ± 1	8.2 ± 0.04
	ethyl laurate	1313	1349	nd	nd	1.35 ± 0.56	4.36 ± 0.57	4.28 ± 0.33	0.28 ± 0.05	4.58 ± 0.01	6.74 ± 0.01	7.72 ± 1.11	0.16 ± 0.01
	isoamyl decanoate	1628	1579	nd	nd	nd	0.14 ± 0.01	nd	0.67 ± 0.17	0.14 ± 0.01	nd	nd	0.2 ± 0.02
	ethyl tetradecanoate	1571	1649	nd	nd	nd	nd	0.5 ± 0.04	0.48 ± 0.14	0.57 ± 0.03	1.31 ± 0.02	2.59 ± 0.69	1.04 ± 0.17
	ethyl 9-decenoate	1747	1764	nd	nd	nd	nd	nd	5.62 ± 0.28	2.39 ± 1.33	5.09 ± 0.30	3.84 ± 0.38	3.45 ± 0.21
	isoamyl octanoate	2108	2186	nd	nd	nd	0.96 ± 0.40	1.39 ± 0.17	0.96 ± 0.03	1.03 ± 0.29	1.17 ± 0.03	8.51 ± 0.07	0.51 ± 0.10
	ethyl nonanoate	1288	1367	nd	nd	nd	nd	nd	nd	nd	nd	0.06 ± 0.01	0.08 ± 0.01
	ethyl caproate	1357	1425	nd	nd	0.92 ± 0.07	1.62 ± 0.48	0.73 ± 0.04	0.25 ± 0.06	0.96 ± 0.18	nd	nd	nd
	propyl caprylate	1349	1394	nd	nd	nd	nd	nd	0.24 ± 0.04	0.06 ± 0.01	nd	0.17 ± 0.02	nd
Alcohols	4-terpinenol	1820	1856	9.92 ± 0.26	4.92 ± 0.36	nd	2.71 ± 0.95	3.21 ± 0.37	0.61 ± 0.22	nd	nd	0.03 ± 0.19	5.06 ± 0.28
	linalool	1537	1438	3.10 ± 0.34	1.56 ± 0.40	1.35 ± 0.32	0.83 ± 0.17	0.6 ± 0.21	5.87 ± 0.48	nd	nd	0.67 ± 0.15	1.01 ± 0.15
	(-)-α-cadinol	1336	1346	1.42 ± 0.05	0.98 ± 0.38	1.88 ± 0.04	0.61 ± 0.06	0.43 ± 0.26	0.28 ± 0.03	0.36 ± 0.21	0.31 ± 0.05	0.26 ± 0.14	0.96 ± 0.08
	spathulenol	1676	1598	0.39 ± 0.02	nd	0.54 ± 0.47	nd	nd	nd	0.09 ± 0.03	nd	nd	0.31 ± 0.01
	α-bisabolol	1412	1436	0.22 ± 0.06	0.18 ± 0.03	0.19 ± 0.1	0.13 ± 0.02	0.07 ± 0.02	0.36 ± 0.33	0.08 ± 0.01	0.3 ± 0.11	nd	0.15 ± 0.04
	palustrol	1295	1357	nd	0.41 ± 0.15	0.15 ± 0.03	0.45 ± 0.14	0.33 ± 0.02	2.48 ± 0.24	0.33 ± 0.04	0.5 ± 0.01	0.59 ± 0.08	1.19 ± 0.05
	phenethyl alcohol	2152	2048	nd	nd	0.42 ± 0.12	0.62 ± 0.10	0.99 ± 0.3	0.55 ± 0.02	0.36 ± 0.05	0.44 ± 0.05	0.39 ± 0.07	0.66 ± 0.11
	santalol	1612	1698	nd	nd	nd	0.11 ± 0.03	0.09 ± 0.04	0.44 ± 0.10	nd	nd	0.14 ± 0.04	0.10 ± 0.01
	n-pentanol	1581	1548	nd	nd	0.35 ± 0.14	nd	nd	nd	1.07 ± 0.48	nd	nd	1.51 ± 0.16
	α-terpineol	1430	1364	nd	nd	nd	nd	0.04 ± 0.02	2.19 ± 0.14	nd	nd	nd	nd
Terpenes	α-ocimene	1921	1953	12.93 ± 0.38	nd	nd	nd	1.95 ± 0.27	0.27 ± 0.18	nd	nd	nd	1.98 ± 0.56
	α-phellandrene	1744	1648	9.94 ± 0.20	nd	nd	nd	nd	nd	3.23 ± 1.09	3.23 ± 0.32	3.54 ± 1.73	2.33 ± 0.04
	4-carene	1418	1358	4.99 ± 0.24	nd	nd	nd	nd	1.69 ± 0.17	1.62 ± 0.43	1.40 ± 0.06	0.84 ± 0.38	1.80 ± 0.12
	calamenene	1433	1488	3.14 ± 0.16	nd	nd	nd	nd	nd	0.25 ± 0.01	0.35 ± 0.07	0.28 ± 0.10	0.44 ± 0.01
	α-pinene	1211	1269	2.72 ± 0.37	0.14 ± 0.02	1.78 ± 0.57	0.7 ± 0.06	0.65 ± 0.34	1.29 ± 0.25	0.73 ± 0.28	0.19 ± 0.03	1.41 ± 0.06	1.10 ± 0.24
	cadinene	1406	1385	1.40 ± 0.08	nd	nd	nd	nd	nd	0.28 ± 0.18	nd	nd	1.34 ± 0.78
	β-sesquiphellandrene	1586	1648	0.66 ± 0.10	4.17 ± 0.12	2.84 ± 0.2	3.33 ± 0.18	3.28 ± 0.27	nd	nd	3.37 ± 0.15	3.41 ± 0.53	2.55 ± 0.25
	α-caryophyllene	1725	1665	0.54 ± 0.22	nd	nd	nd	1.73 ± 0.3	nd	2.43 ± 0.59	2.00 ± 0.75	0.42 ± 0.07	0.95 ± 0.25
	terpinolene	1337	1354	0.28 ± 0.10	nd	1.49 ± 0.07	0.15 ± 0.08	0.25 ± 0.08	0.30 ± 0.14	0.34 ± 0.28	0.15 ± 0.02	0.14 ± 0.05	0.14 ± 0.03
	α-cedrene	2050	1954	0.10 ± 0.03	nd	0.11 ± 0.05	0.13 ± 0.04	0.11 ± 0.01	0.29 ± 0.09	nd	nd	0.13 ± 0.09	0.21 ± 0.04
	α-farnesene	2005	2045	0.06 ± 0.04	1.28 ± 0.04	4.08 ± 1.54	0.21 ± 0.13	1.01 ± 0.03	1.37 ± 0.16	0.62 ± 0.52	0.66 ± 0.6	1.39 ± 0.19	0.21 ± 0.12
	camphene	2106	2164	0.04 ± 0.02	nd	nd	nd	nd	nd	nd	nd	nd	0.22 ± 0.15
	α- piperidine	1241	1268	nd	nd	0.97 ± 0.49	1.22 ± 0.54	0.65 ± 0.04	0.31 ± 0.10	0.58 ± 0.12	0.8 ± 0.23	0.22 ± 0.02	1.77 ± 0.15
	α-sabinene	1737	1795	nd	0.17 ± 0.06	nd	nd	nd	nd	nd	nd	nd	1.21 ± 0.56

Table 3 (continued)

Category	Aroma component	RI values	Published RI values	Fermentation time (day)									
				0	2	3	5	7	9	14	19	24	29
	ledenee	1588	1649	nd	nd	0.54 ± 0.09	nd	nd	nd	nd	0.64 ± 0.04	0.65 ± 0.09	1.52 ± 0.61
	(+)-aromadendrene	1433	1498	nd	0.23 ± 0.11	0.25 ± 0.1	0.15 ± 0.02	nd	nd	nd	nd	0.06 ± 0.03	0.26 ± 0.04
	(-)-germacrene	1378	1397	1.22 ± 0.10	nd	nd	nd	nd	nd	nd	nd	nd	nd
	calarene	1639	1578	0.82 ± 0.27	nd	0.81 ± 0.31	0.59	nd	nd	nd	nd	0.11 ± 0.01	nd
	guaiene	1168	1248	0.59 ± 0.16	nd	nd	nd	nd	nd	nd	nd	nd	nd
	terpinene	2016	1974	0.56 ± 0.15	nd	nd	nd	nd	nd	nd	nd	nd	nd
	caryophyllene	1350	1387	0.52 ± 0.03	nd	nd	nd	nd	2.28 ± 0.23	2.43 ± 0.59	nd	nd	nd
	cedrene	1409	1354	0.45 ± 0.01	nd	nd	nd	nd	nd	nd	nd	nd	nd
	bergamotene	1730	1784	0.39 ± 0.14	nd	nd	nd	nd	nd	nd	nd	nd	nd
	limonene	1724	1648	0.18 ± 0.08	nd	nd	nd	nd	nd	nd	nd	nd	nd
	styrene	1921	2048	0.15 ± 0.06	nd	nd	nd	nd	nd	nd	nd	nd	nd
	cadinene	2475	2454	nd	8.47 ± 0.59	0.75 ± 0.34	0.51 ± 0.08	0.51 ± 0.08	nd	nd	nd	nd	nd
	caryophylene oxide	2150	2104	nd	0.08 ± 0.02	0.06 ± 0.01	0.08 ± 0.03	0.10 ± 0.03	nd	0.06 ± 0.01	0.05 ± 0.01	nd	nd
Acids	decanoic acid	1988	1975	nd	1.57 ± 0.25	1.52 ± 0.60	0.63 ± 0.03	0.6 ± 0.05	0.60 ± 0.07	0.25 ± 0.03	0.23 ± 0.04	0.22 ± 0.04	0.45 ± 0.12
	octanoic acid	1433	1348	nd	2.74 ± 0.66	2.28 ± 0.89	1.60 ± 0.37	1.03 ± 0.29	0.40 ± 0.07	0.38 ± 0.04	nd	nd	0.4 ± 0.01
	lauric acid	1643	1687	nd	0.46 ± 0.04	0.38 ± 0.15	0.19 ± 0.03	0.23 ± 0.05	0.40 ± 0.14	0.08 ± 0.01	0.06 ± 0.01	nd	nd

nd means not detected

wine, there was an overall downward trend in terpenes (from 41.68 to 18.03%) compared to day 0. This decrease could be attributed to the sharp decline in α-ocimene, α-phellandrene, 4-carene, calamenene and α-pinene. A decline in terpenes during wine fermentation was attributed to either volatility or transformation into different metabolites [48]. Additionally, the release of glycocide-bound terpenes in fruit by enzymatic hydrolysis during fermentation may partially explain the accumulation of terpenes in wampee wine [49].

Multivariate statistical analysis of seedless wampee wine during fermentation

Multivariate data analysis was carried out to analyze the flavor composition, including volatile aroma components and non-volatile aroma components (amino acids and organic acids), to map the samples from seedless wampee wine fermentation and gain understanding of the basic principles underlying the differences observed (Fig. 5).

According to the results of the principal component analysis (PCA), the samples from different fermentation periods were distributed across four quadrants (Fig. 5a). The wampee juice samples were situated in the third quadrant, while the samples that underwent fermentation for 2–9 days could be found in the first and second quadrants, and the samples that fermented for 14–29 days were situated in the fourth quadrant. Three distinct regions were observed in the seedless wampee wine: unfermented, main fermented and post-fermented phase, indicating significant variations in the flavor compounds, including organic acids, amino acids and volatile flavor compounds among the different fermentation stages. To further characterize these samples, a partial least squares - discriminant analysis (PLS-DA) model contrasted with an R^2 of 98.4% and a Q^2 of 93.9% (Fig. 5b). Clearly, the unfermented samples, as well as those fermented for 2–9 days and 14–29 days, exhibited distinct characteristics, and the flavor variations observed in the different stages of wampee fruit wine fermentation were clearly separated. These findings were consistent with the results obtained from the PCA model. After conducting the alignment test and 200 alignment experiments, it was found that the intersection of the Q^2 regression line with the vertical axis was less than 0, and the y-values of the left simulation points of R^2 and Q^2 were lower than the rightmost origin (Fig. 5c). These results indicated that the

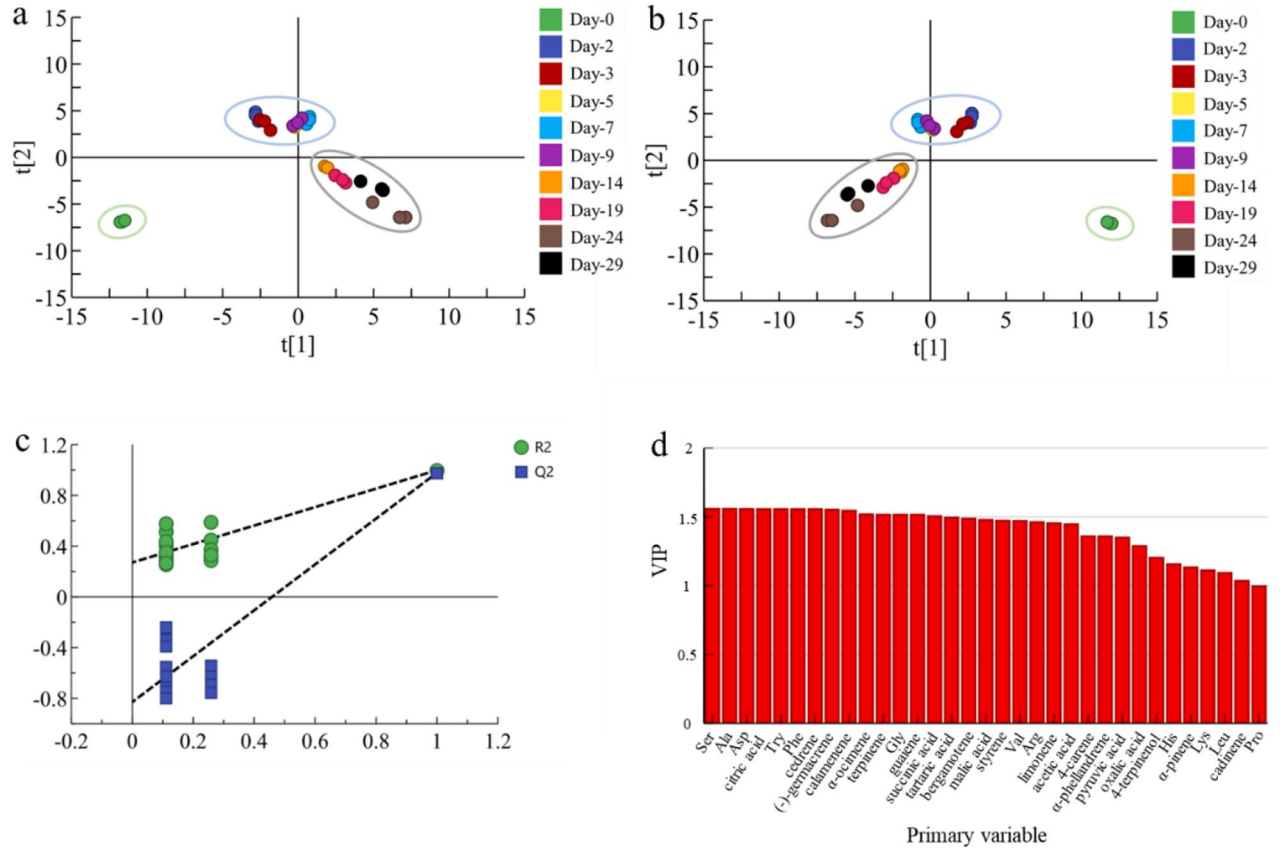


Fig. 5 Multivariate statistical analysis of seedless wampee wine during seedless wampee fermentation. (a) Principle component analysis (PCA) score plot, (b) Partial least squares - discriminant analysis (PLS-DA) score plot, (c) Model validation diagram and (d) Variable importance plot(VIP)through PLS-DA analysis

PLS-DA model had strong predictive ability with no signs of overfitting. Therefore, it can used for flavor analysis of seedless wampee wine. The VIP values indicate the varying contributions of different flavor compounds, with a VIP value greater than 1 indicating a more significant discriminatory contribution. A total of 33 major flavor compounds, comprising 6 organic acids, 12 amino acids, and 15 volatile flavor compounds, processed a VIP value above 1 (Fig. 5d). Organic acids, including citric acid, succinic acid and tartaric acid, along with amino acids such as Ser, Ala and Asp, and volatile flavor compounds such as cedrene, (-)-germacrene and calamenene, comprised the vital flavor components of seedless wampee wine.

The present study investigated the application of seedless wampee in fruit wine fermentation. The optimal fermentation conditions for seedless wampee wine were established, including an inoculum concentration of 0.6%, an initial sugar level of 200 g/L, a fermentation temperature of 22 °C, and a fermentation period of 9 days. Under these conditions, the sensory score can reach 94.68. Then the changes of physicochemical profile and sensory properties of seedless wampee wine were evaluated under optimal fermentation conditions. Notably, the non-volatile components, including amino acids and organic acids exhibited significant changes during the main-fermented process. Regarding volatile aroma components, the number and concentration of esters showed a significant increase after fermentation, whereas the number and content of terpenes relatively decreased in seedless wampee wine. These results enhance our understanding of the flavor formation of seedless wampee wine. Further studies could focus on the bioactive components and potential health benefits of seedless wampee wine.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13765-024-00938-y.

Supplementary Material 1

Author contributions

Hong Wang: Methodology; Writing — original draft; Writing — review & editing, Visualization. Xiang Liao: Writing — original draft; Writing — review & editing; Visualization. Chunyao Lin: Methodology; Software; Writing — original draft; Writing — review & editing. Weidong Bai: Writing — review & editing; Supervision. Gengsheng Xiao: Funding acquisition; Supervision. Xingyuan Huang: Investigation. Gongliang Liu: Funding acquisition. All authors reviewed the manuscript.

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Declarations

Conflict of interest

The authors declare no conflict of interest.

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