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Research Article

Impact of Sucrose Addition on the Physiochemical Properties and Volatile Compounds of "Shuangyou" Red Wines

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"Shuangyou," a *Vitis amurensis* Rupr. variety, is widely cultivated in northeastern and western China. Its berries have high acidity and low sugar content. In this study, different proportions of sucrose were added to the must samples during fermentation to investigate the effect of sugar on the physicochemical properties and volatile compounds of "Shuangyou" wines. The addition of sucrose significantly improved yeast growth and alcohol production, altered the color qualities, and slightly decreased titratable acidity during fermentation. The highest tested proportion of added sucrose resulted in the highest maximum yeast counts and final ethanol concentrations. Moreover, 37 volatile compounds (esters, alcohols, fatty acids, ketones, and aldehydes) were identified and quantified by solid-phase microextraction with gas chromatography-mass spectrometry. The concentrations of these compounds were correlated with the addition of sucrose. Furthermore, the addition of 100 g/L sucrose was sufficient for improving the concentrations of the aromatic compounds. The increase in ester, alcohol, and fatty acid concentration led to a positive OAVs impact (odor activity value > 1) at the end of fermentation.

1. Introduction

The "Shuangyou" grape, a *Vitis amurensis* Rupr. grape cultivar, is a member of the Vitaceae family from China [1]. During the mid-1980s, it was successfully selected from the wild *V. amurensis* resources [2]. Nowadays, it is widely cultivated both in western and in northeastern China and is considered the most important cultivar of *V. amurensis*.

V. amurensis grape berries have high acidity (15–30 g/L), a high tannin and polyphenol content, and a low sugar content (80–170 g/L). They are also rich in nutrients (minerals, carotene, vitamins, etc.) [3, 4] that give these grapes a unique fruit fragrance and distinctive taste. Thus, *V. amurensis* grapes have become a good source of grape juice and high-quality red wine. Compared to *V. vinifera* wines, *V. amurensis* wines have a brighter ruby red color, more fragrant aroma, and a mellower and more full-bodied taste. However, this wine is also rich in bioactive substances that often impart high acidity, a very sour taste, and low alcohol content, making it less popular [5]. In order to ferment a high-quality dry red wine, producers try to reduce the acidity and enhance the sugar content in the grapes and wines by a number of methods such as delayed picking time, lactic fermentation, low-temperature treatment, dry treatment, and the addition of sucrose during the fermentation process [6–8]. Although these strategies improve deacidification and alcohol fermentation, they also excessively heighten the pungent taste, dilute the overall flavor of the wine, and lighten the fruit aroma in the final product.

During winemaking, sucrose is added to the must to increase the alcohol content and decrease acidity. In this process, sucrose is hydrolyzed into a reduced sugar that is then assimilated by yeast into ethanol and carbon dioxide [9]; the addition of each 18–20 g/L of sugar into the grape must is predicted to enhance the final concentration of alcohol by 1% (v/v) after fermentation [10]. Some studies have

TABLE 1: Physicochemical characteristics of the initial musts (day 0) and wines added with sucrose and controls (day 18).

	Initial musts (day 0)	Wines (day 18)			
		Controls (no sucrose added)	20 g/L sucrose	60 g/L sucrose	100 g/L sucrose
Yeast count ($\times 10^6$ CFU/mL)	$0.56^a \pm 0.01$	$0.31^a \pm 0.11$	$3.62^b \pm 0.70$	$8.31^c \pm 1.25$	$12.30^d \pm 1.31$
Total sugar content (g/L)	$87.75^b \pm 2.06$	$0.18^a \pm 0.02$	$0.43^a \pm 0.05$	$1.21^a \pm 0.34$	$3.00^a \pm 0.31$
Titratable acidity (g/L)	$14.95^a \pm 0.04$	$13.08^a \pm 0.02$	$13.02^a \pm 0.07$	$13.47^a \pm 0.16$	$13.70^a \pm 0.12$
Ethanol content (% v/v)	Trace	$4.90^a \pm 0.22$	$6.31^b \pm 0.32$	$8.68^c \pm 0.38$	$10.27^d \pm 0.26$
L^* (lightness)	$79.13^b \pm 3.12$	$72.93^a \pm 0.91$	$73.05^a \pm 0.53$	$69.77^a \pm 0.33$	$71.22^a \pm 0.56$
a^* (red/green)	$28.76^a \pm 1.76$	$35.31^b \pm 0.68$	$34.88^b \pm 0.33$	$37.68^b \pm 0.53$	$36.42^b \pm 0.69$
b^* (yellow/blue)	$4.37^a \pm 0.18$	$4.39^a \pm 0.15$	$4.69^a \pm 0.08$	$7.17^b \pm 0.18$	$7.44^b \pm 0.12$

**Values = mean value ± standard deviation (SD). **Different superscript letters in the row indicate that the differences are significant (LSD test, $P < 0.05$).
**Trace indicates that the concentration of the compound is below the detectable level.

suggested that sugar-rich environments play an important role in yeast cell growth during wine fermentation [11, 12]. Thus, high sugar concentrations in grape musts may cause increased yeast counts and alcohol contents [13]. However, the addition of sucrose may also excessively heighten the pungent taste that destroys the other positive qualities of the wine. Furthermore, the addition of sucrose was found to alter the aroma of the final wine product. Most volatile compounds are yeast secondary metabolites whose formation in wine mainly depends on the types of alcohols and acids afforded by sugar assimilation during alcoholic fermentation [14–16].

This paper focuses on the influence of different quantities of sucrose on changes in yeast count, total sugar content, ethanol content, titratable acidity, and color parameters during fermentation. Furthermore, the volatile compounds in the musts and wines at the end of fermentation were identified and quantified by solid-phase microextraction with gas chromatography-mass spectrometry (SPME-GC-MS) and the aromatic profiles of the wines were evaluated. The objective of this study was to evaluate the relevance of adding sucrose and to investigate the resultant characteristics of the "Shuangyou" wine aroma.

2. Materials and Methods

2.1. Sample Collection and Vinification. Some ripe grapes of the "Shuangyou" cultivar were picked by hand in Jian County (Jilin Province, China) during the 2015 harvest season. The vinification process of red wine employed follows methods reported in literature [21, 26]. The stalks were eliminated before the berries were crushed manually and the musts were then transferred into a glass fermenter (5 L, loading volume: ~70–80%). Next, 5% sulfurous acid was added to reach a concentration of 50 mg/L SO_2. The sugar concentration in excellent quality of grape cultivars ranges from 100 to 250 g/L [10]. On the other hand, "Shuangyou" grape berries have a total sugar content of only 87.75 g/L (Table 1). Thus, different proportions of food grade sucrose (20, 60, and 100 g/L; variables) were added to the musts. No sucrose was added in the control. Each must sample was next inoculated with 0.1% of a commercial dry yeast strain (RV171, Angel Yeast Co., Ltd, Yichang, China) for better fermentation [27]. The dry yeast strains were activated and

precultured in synthetic grape must media (110 g/L glucose, 110 g/L fructose, 3 g/L tartaric acid, 0.3 g/L malic acid, 0.5 g/L ammonium chloride, 0.6 g/L yeast extract, 2 g/L tannins, 2 g/L KH_2PO_4, and 0.2 g/L $MgSO_4 \cdot 7H_2O$, pH 3.4) at 1×10^6 colony-forming units (CFU)/mL before inoculation. The must samples were adequately mixed manually every day. After 4 days of maceration, the grape skins were separated from the wine. Finally, alcoholic fermentation was conducted at $18 \pm 2°C$ for 20 days. The physicochemical parameters and yeast counts of the wine samples were analyzed every 2 days until a total sugar content of <4 g/L was reached.

2.2. Yeast Counts and Standard Chemical Parameters Analysis. The enumeration of yeast was carried out using yeast-peptone-dextrose (YPD: 20 g/L glucose, 20 g/L peptone, 10 g/L yeast extract, and 20 g/L agar) plates [28]. Standard chemical parameters (total sugar, ethanol, and titratable acidity) were determined according to the National Standard of the People's Republic of China (GB/T15038-2006, 2006) [22].

2.3. Color Parameter Analysis. The chromatic parameters in the CIELAB color space consisted in recording the absorbance values at four specific wavelengths (450, 520, 570, and 630 nm) and using them to compute the tristimulus measurements [29]. The main CIELAB parameters a^*, b^*, and L^* were calculated; a^* represents the difference between green ($a^* < 0$) and red ($a^* > 0$), b^* indicates the difference between blue ($b^* < 0$) and yellow ($b^* > 0$), and L^* describes the lightness of the color ($L^* = 0$ black and $L^* = 100$ colorless).

2.4. Volatile Compounds Analysis. Volatile compounds were produced by headspace- (HS-) SPME. 4-Methyl-2-pentanol was used as the internal standard substance for semi-quantification. The wine sample (8 mL), 0.8 g NaCl, and 30 μL 4-methyl-2-pentanol (3.2 g/L) were placed into a 15 mL sample vial that was sealed with a polytetrafluorethylene (PTFE)/silicone septum (Supelco, Bellefonte, PA, USA). The samples were agitated with a magnetic stirring bar and preconditioned at 45°C for 10 min in the vial. A 50/30 μm divinylbenzene/carboxen/polymethylsiloxane (DVB/CAR/PDMS) fiber (Supelco, length = 2 cm) was exposed to the

headspace of the vial for 30 min at 45°C; the volatile compounds were absorbed by the fiber. All SPME injections were immediately carried out in splitless mode at 250°C for 10 min.

The volatile compounds were analyzed by GC-MS (Thermo Fisher Science, Trace1300/ ISQ-LT, USA). Thermo TraceFinder easy file sharing (EFS) software was used to operate the system (chromatography column: HP-5 30 m × 0.25 mm × 0.25 μm; carrier gas: helium; and flow rate: 1 mL/min) and was conducted according to the following program: 32°C for 3 min, subsequent raise to 50°C at 2°C/min for 2 min, and further raise to 230°C at 4°C/min for 8 min. The MS analysis conditions were as follows: mass spectra were acquired in electron impact mode (70 eV) and scanned fully at a range of 30–450 m/z at 1 s intervals (ionization source: EI$^+$; transfer line and ion source temperatures: 280 and 230°C, respectively; filament flow: 0.25 μA; detector voltage = 350 V) [30, 31].

The typical samples on day 0 and day 18 were chosen and were studied by GC-MS. After that, the volatile compounds were identified by comparing the retention times and mass spectra of the sample with those found in the mass spectrometer database (NIST 2014) or in literature. Semiquantitative determinations (mg/L of wine) were calculated by the internal standard method (Table 2).

2.5. Odor Activity Values (OAVs). The contribution of volatile compounds to the wine samples was evaluated by determining the OAV of the wine, a measure of importance to typical aromas of wine. Volatile compounds present in wine with OAVs > 1 are generally considered to be likely contributors to the characteristic aroma of the wine [32]. OAVs are calculated as the ratio between the concentration of an individual compound and the perception threshold reported in literature (Table 3) [18–20].

2.6. Statistical Analysis. All analyses were performed in duplicate and the afforded average values for each sample (three samples per treatment) were used to calculate the mean concentration values and their standard deviations. Significant differences were assessed with one-way analysis of variance (ANOVA), while statistical differences between the means were evaluated using least-significant difference (LSD) analysis at the P = 0.05 level. Statistical data processing was performed using SPSS Statistics 19.0 for Windows (IBM, NY, USA) software.

3. Results and Discussion

3.1. Yeast Growth. The growth behavior of the yeast culture is illustrated in Figure 1. The cell counts of all four fermentation processes increased from ~0.5 × 10^5 CFU/mL to ~1 × 10^8 CFU/mL. Cell counts for the controls and samples with 20 and 60 g/L added sucrose reached a maximum (0.464 × 10^8, 1.38 × 10^8, and 1.51 × 10^8 CFU/mL, resp.) on day 6, while the sample with 100 g/L added sucrose reached the maximum (1.59 × 10^8 CFU/mL) on day 8. Notably, during the first 6 days of fermentation, the growth rates were similar for all the samples. A lower growth rate was observed in the controls,
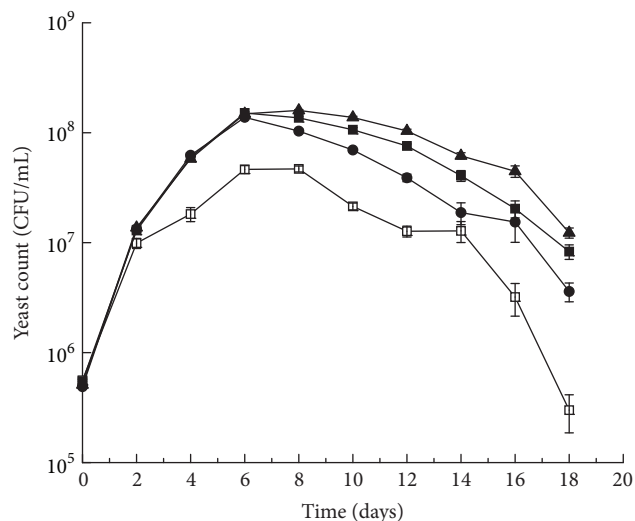

FIGURE 1: Growth of yeast cells of "Shuangyou" wines added with 20 (●), 60 (■), and 100 (▲) g/L sucrose and controls (□) during fermentation.

probably due to less sugar being available for yeast breeding [12]. These results are in agreement with other studies that have reported that sugar-rich environments are important for yeast cell growth in wine fermentation [11, 12]. After reaching the maximum values, the yeast culture in all the fermentation processes experienced a fast decrease in cell counts. This decline was more marked in the controls where the final cell count was less than that of the initial musts (Table 1). The faster death rate of the yeast cells in the controls indicates that the lack of nutrients in the later stage of fermentation as well as the accumulation of ethanol and other inhibitory substances in the wine could lead to a decrease in yeast cells.

3.2. Sugar Consumption. The changes in total sugar content observed in the different samples during their fermentation processes are displayed in Figure 2(a). The total sugar content in the controls and the sample with 20 g/L added sucrose decreased continuously on the first 6 days of fermentation and remained stable thereafter. Similarly, the total sugar content in the samples with 60 and 100 g/L added sucrose experienced a faster decline in the first 12 days; this was followed by a slight decrease to 1.21 and 3.00 g/L, respectively (Table 1). In fact, the yeast strains yielded the extracellular invertase that hydrolyzed sucrose into a reducing sugar [9]. A large proportion of the reducing sugar was then assimilated by the yeast into ethanol and carbon dioxide [10]. Subsequently, the rate of sugar consumption decreased while the yeast cells died quickly [33]. Thus, the rate of sugar consumption was concluded to be related to the rate of yeast growth [34]. In addition, during fermentation, the highest sugar consumption was observed in the sample with the highest amount (100 g/L) of added sucrose (Figure 2(a) and Table 1). This indicates that the fermentative lifestyle could exhibit a growth advantage in sugar-rich environments due to a higher rate of sugar conversion and energy production [35].

TABLE 2: Volatile compounds of the initial musts (day 0) and wines added with sucrose and controls (day 18).

Number	Volatile compounds	Odor threshold (mg/L)	Descriptor	Initial musts (day 0) (mg/L)	Controls (no sucrose added)	Wines (day 18) (mg/L)		
						20 g/L sucrose	60 g/L sucrose	100 g/L sucrose
	Esters							
1	Ethyl acetate	15.00 [17]	Ethereal-fruity	$8.452^a \pm 0.923$	$32.484^d \pm 2.785$	$32.347^d \pm 1.564$	$18.048^b \pm 2.643$	$23.395^c \pm 1.453$
2	Ethyl butyrate	0.02 [18]	Strawberry, lactic	$0.343^c \pm 0.012$	$0.118^a \pm 0.032$	$0.116^a \pm 0.011$	$0.225^b \pm 0.023$	$0.544^d \pm 0.018$
3	Isoamyl acetate	0.16 [19]	Banana	$8.058^a \pm 0.432$	$9.436^b \pm 0.387$	$12.496^c \pm 1.021$	$13.479^d \pm 0.345$	$21.965^e \pm 1.874$
4	Methyl hexanoate	Unknown	Floral, fruity	$0.159^c \pm 0.012$	$0.061^a \pm 0.002$	$0.057^a \pm 0.003$	$0.099^b \pm 0.004$	$0.090^b \pm 0.003$
5	Ethyl hexanoate	0.08 [20]	Apple peel, fruit	$17.802^d \pm 1.043$	$5.410^b \pm 0.678$	$3.256^a \pm 0.274$	$11.973^c \pm 0.345$	$25.888^e \pm 1.054$
6	Hexyl acetate	0.67 [17]	Fruity, floral	$2.383^e \pm 0.056$	$0.181^a \pm 0.014$	$0.351^c \pm 0.034$	$0.258^b \pm 0.026$	$0.437^d \pm 0.019$
7	Ethyl 2-hexenoate	Unknown	Spicy, candy, earthy	$0.127^a \pm 0.009$	$0.162^a \pm 0.008$	$0.122^a \pm 0.011$	$0.310^b \pm 0.012$	$0.958^c \pm 0.003$
8	Ethyl 2-hydroxyisocaproate	Unknown	Lemon	Trace	$0.122^a \pm 0.006$	$0.177^a \pm 0.004$	$0.538^b \pm 0.008$	$0.980^b \pm 0.009$
9	Ethyl heptanoate	Unknown	Fruity	$0.487^a \pm 0.065$	$1.678^c \pm 0.089$	$0.797^b \pm 0.056$	$1.711^c \pm 0.098$	$3.596^d \pm 0.121$
10	Methyl octanoate	0.20 [21]	Orange	$0.250^b \pm 0.007$	$0.152^a \pm 0.012$	$0.134^a \pm 0.009$	$0.294^b \pm 0.031$	$0.739^c \pm 0.035$
11	Diethyl succinate	1250 [17]	Wine, fruity	Trace	$0.063^a \pm 0.002$	$0.408^b \pm 0.023$	$1.105^c \pm 0.098$	$2.668^d \pm 0.132$
12	Methyl salicylate	Unknown	Cooked fruit	$0.044^a \pm 0.001$	$0.079^c \pm 0.002$	$0.0630^b \pm 0.002$	$0.069^{bc} \pm 0.003$	$0.214^d \pm 0.012$
13	Ethyl octanoate	0.58 [17]	Melon, wood	$15.704^c \pm 1.078$	$10.131^b \pm 1.11$	$7.339^a \pm 0.978$	$28.604^d \pm 1.345$	$73.614^e \pm 3.567$
14	Ethyl 2-phenylacetate	0.65 [22]	Fruity, sweet	Trace	$0.046^a \pm 0.004$	$0.052^a \pm 0.005$	$0.080^b \pm 0.003$	$0.152^c \pm 0.009$
15	3-Methylbutyl hexanoate	Unknown	Cooked meat, mushroom	$0.020^a \pm 0.001$	$0.049^b \pm 0.004$	$0.053^c \pm 0.001$	$0.156^c \pm 0.008$	$0.517^b \pm 0.008$
16	2-Phenylethyl acetate	0.25 [18]	Jammy, plum, floral, fruity	$0.494^a \pm 0.012$	$0.549^a \pm 0.021$	$1.107^b \pm 0.056$	$1.224^b \pm 0.045$	$2.662^c \pm 0.211$
17	Ethyl nonanoate	1.20 [22]	Fruity, rose, waxy, rum, tropical	$0.083^a \pm 0.004$	$0.097^a \pm 0.004$	$0.197^b \pm 0.008$	$0.437^c \pm 0.021$	$0.791^d \pm 0.056$
18	Ethyl decanoate	0.20 [19]	Floral, soap	$2.496^c \pm 0.364$	$0.624^a \pm 0.012$	$0.579^a \pm 0.043$	$1.618^b \pm 0.356$	$4.714^d \pm 0.124$
19	3-Methylbutyl octanoate	0.125 [19]	Sweet, fruity, pineapple, coconut	$0.039^a \pm 0.001$	$0.052^b \pm 0.004$	$0.059^b \pm 0.002$	$0.129^c \pm 0.022$	$0.539^d \pm 0.054$
20	Ethyl dodecanoate	1.50 [19]	Oily, fatty, fruity	$0.219^c \pm 0.089$	$0.108^{ab} \pm 0.012$	$0.090^a \pm 0.001$	$0.131^b \pm 0.005$	$0.305^c \pm 0.013$
	Sum			$57.161^a \pm 4.109$	$61.602^a \pm 5.118$	$59.801^a \pm 4.108$	$80.488^b \pm 5.441$	$164.769^c \pm 8.773$
	Alcohols							
21	Isoamyl alcohol	30.00 [18]	Harsh, nail polish	$14.639^a \pm 2.987$	$77.257^b \pm 8.098$	$88.483^c \pm 9.764$	$227.548^d \pm 25.755$	$400.507^e \pm 31.905$
22	2,3-Butanediol	150.00 [22]	Fruity, sweet, butter	$0.030^a \pm 0.001$	$0.032^a \pm 0.004$	$0.094^b \pm 0.005$	$0.168^c \pm 0.012$	$1.172^d \pm 0.121$
23	1-Hexanol	8.00 [18]	Green, grass	$1.619^a \pm 0.123$	$4.262^c \pm 0.156$	$3.264^b \pm 0.421$	$8.963^d \pm 0.235$	$10.005^e \pm 0.977$
24	1-Octen-3-ol	0.02 [17]	Mushroom	$0.053^a \pm 0.003$	$0.097^b \pm 0.005$	$0.167^c \pm 0.009$	$0.580^e \pm 0.012$	$0.450^d \pm 0.032$
25	Benzyl alcohol	200.00 [20]	Roasted, sweet	$0.069^a \pm 0.002$	$0.234^c \pm 0.009$	$0.199^b \pm 0.008$	$0.228^{bc} \pm 0.003$	$0.308^d \pm 0.003$
26	1-Octanol	0.80 [21]	Chemical, metal, burnt	$0.468^a \pm 0.034$	$1.616^c \pm 0.236$	$1.272^b \pm 0.078$	$2.805^d \pm 0.096$	$3.016^e \pm 0.198$
27	2-Phenylethanol	14.00 [19]	Floral, rose	$1.968^a \pm 0.432$	$18.661^b \pm 1.065$	$19.741^b \pm 3.087$	$35.359^c \pm 4.986$	$54.405^d \pm 4.987.$
28	1-Nonanol	58.00 [22]	Fatty, floral	$0.211^a \pm 0.012$	$1.441^b \pm 0.078$	$1.305^b \pm 0.088$	$2.444^c \pm 0.056$	$3.645^d \pm 0.378$
	Sum			$19.056^a \pm 3.594$	$103.601^b \pm 9.651$	$114.525^b \pm 13.462$	$278.095^c \pm 31.165$	$473.509^d \pm 38.601$
	Acids							
29	Isovaleric acid	3.00 [18]	Acid, rancid	Trace	$0.161^a \pm 0.023$	$0.752^c \pm 0.034$	$0.144^a \pm 0.043$	$0.618^b \pm 0.053$
30	Octanoic acid	0.50 [19]	Butter, almondy	$1.755^a \pm 0.123$	$2.638^b \pm 0.165$	$1.594^a \pm 0.127$	$3.156^c \pm 0.321$	$4.572^a \pm 0.366$

TABLE 2: Continued.

Number	Volatile compounds	Odor threshold (mg/L)	Descriptor	Initial musts (mg/L)	Controls (no sucrose added)	Wines (day 18) (mg/L)		
						20 g/L sucrose	60 g/L sucrose	100 g/L sucrose
31	Hexanoic acid	0.42 [19]	Leafy, wood, varnish	$0.374^b \pm 0.031$	$0.743^d \pm 0.067$	$0.624^c \pm 0.023$	$0.102^a \pm 0.077$	$1.092^e \pm 0.112$
32	Decanoic acid	1.00 [19]	Rancid, fat	$0.149^c \pm 0.021$	$0.044^a \pm 0.008$	$0.041^a \pm 0.002$	$0.040^a \pm 0.007$	$0.053^b \pm 0.002$
	Sum			$2.277^a \pm 0.175$	$3.587^b \pm 0.263$	$3.011^b \pm 0.186$	$3.442^b \pm 0.448$	$6.335^c \pm 0.533$
	Ketones and aldehydes							
33	6-Methyl-5-hepten-2-one	0.05 [23]	Fruity	$0.082^a \pm 0.002$	$0.471^b \pm 0.008$	$0.421^b \pm 0.014$	$0.565^b \pm 0.016$	$1.149^c \pm 0.088$
34	β-Damascenone	0.00014 [24]	Apple, rose, honey	$0.081^a \pm 0.002$	$0.775^b \pm 0.045$	$1.293^c \pm 0.098$	$1.189^c \pm 0.077$	$2.960^d \pm 0.123$
35	Geranyl acetone	0.06 [25]	Floral	$0.047^a \pm 0.005$	$0.094^b \pm 0.005$	$0.085^b \pm 0.003$	$0.129^c \pm 0.012$	$0.167^d \pm 0.011$
36	Phenyl acetaldehyde	Unknown	Rose	$0.037^a \pm 0.002$	$0.114^c \pm 0.003$	$0.054^b \pm 0.004$	$0.134^d \pm 0.008$	$0.212^e \pm 0.009$
37	Decanal	Unknown	Orange	$0.089^a \pm 0.004$	$0.351^b \pm 0.012$	$0.245^b \pm 0.034$	$1.092^d \pm 0.181$	$0.843^c \pm 0.054$
	Sum			$0.335^a \pm 0.015$	$1.806^b \pm 0.073$	$2.098^b \pm 0.153$	$3.095^c \pm 0.294$	$5.331^d \pm 0.285$

**Values = mean value ± standard deviation (SD). **Different superscript letters in the row indicate that the differences are significant (LSD test, $P < 0.05$). **Trace indicates that the concentration of the compound is below the detectable level. **Unknown indicates that the odor threshold of the compound was not found.

TABLE 3: Odour activity values (OAVs) of major volatile compounds in the initial musts (day 0) and wines added with sucrose and controls (day 18).

Volatile compounds	Initial musts (day 0)	Wines (day 18)			
		Controls (no sucrose added)	20 g/L sucrose	60 g/L sucrose	100 g/L sucrose
Esters					
Ethyl acetate	0.563 ± 0.062	2.166 ± 0.186	2.156 ± 0.104	1.203 ± 0.176	1.560 ± 0.097
Ethyl butyrate	17.159 ± 0.600	5.892 ± 1.600	5.822 ± 0.550	11.226 ± 1.150	27.189 ± 0.900
3-Methylbutyl acetate	50.361 ± 2.700	58.976 ± 2.419	78.097 ± 6.381	84.246 ± 2.156	137.281 ± 11.713
Ethyl hexanoate	222.528 ± 13.038	67.631 ± 8.475	40.705 ± 3.425	149.664 ± 4.313	323.604 ± 13.175
Hexyl acetate	3.556 ± 0.084	0.270 ± 0.021	0.524 ± 0.051	0.385 ± 0.039	0.652 ± 0.028
Methyl octanoate	1.249 ± 0.035	0.759 ± 0.060	0.672 ± 0.045	1.469 ± 0.155	3.697 ± 0.175
Ethyl octanoate	27.076 ± 1.859	17.467 ± 1.914	12.654 ± 1.686	49.317 ± 2.319	126.921 ± 6.150
Ethyl 2-phenylacetate	0.000 ± 0.002	0.071 ± 0.006	0.079 ± 0.008	0.123 ± 0.005	0.234 ± 0.014
2-Phenylethyl acetate	1.977 ± 0.048	2.197 ± 0.084	4.427 ± 0.224	4.897 ± 0.180	10.648 ± 0.844
Ethyl nonanoate	0.069 ± 0.003	0.081 ± 0.003	0.164 ± 0.007	0.364 ± 0.018	0.659 ± 0.047
Ethyl decanoate	12.480 ± 1.820	3.118 ± 0.060	2.893 ± 0.215	8.091 ± 1.780	23.569 ± 0.620
3-Methylbutyl octanoate	0.313 ± 0.008	0.412 ± 0.032	0.473 ± 0.016	1.033 ± 0.176	4.310 ± 0.432
Ethyl dodecanoate	0.146 ± 0.059	0.072 ± 0.008	0.060 ± 0.001	0.087 ± 0.003	0.203 ± 0.009
Alcohols					
Isoamyl alcohol	0.488 ± 0.100	2.575 ± 0.270	2.949 ± 0.325	7.585 ± 0.859	13.350 ± 1.064
1-Hexanol	0.202 ± 0.015	0.533 ± 0.020	0.408 ± 0.053	1.120 ± 0.029	1.251 ± 0.122
1-Octen-3-ol	2.637 ± 0.150	4.857 ± 0.250	8.353 ± 0.450	29.000 ± 0.600	22.489 ± 1.600
1-Octanol	0.585 ± 0.043	2.021 ± 0.295	1.590 ± 0.098	3.506 ± 0.120	3.770 ± 0.248
2-Phenylethanol	0.141 ± 0.031	1.333 ± 0.076	1.410 ± 0.221	2.526 ± 0.356	3.886 ± 0.356
1-Nonanol	0.004 ± 0.000	0.025 ± 0.001	0.023 ± 0.002	0.042 ± 0.001	0.063 ± 0.007
Acids					
Isovaleric acid	0.000 ± 0.000	0.054 ± 0.008	0.251 ± 0.011	0.048 ± 0.014	0.206 ± 0.018
Octanoic acid	3.509 ± 0.246	5.277 ± 0.330	3.188 ± 0.254	6.313 ± 0.642	9.144 ± 0.732
Hexanoic acid	0.890 ± 0.074	1.770 ± 0.160	1.487 ± 0.055	0.242 ± 0.183	2.601 ± 0.267
Decanoic acid	0.149 ± 0.0 21	0.044 ± 0.008	0.041 ± 0.002	0.040 ± 0.007	0.053 ± 0.002
Ketones					
6-Methyl-5-hepten-2-one	1.64 ± 0.04	9.42 ± 0.16	8.42 ± 0.28	11.30 ± 0.32	22.98 ± 1.76
β-Damascenone	578.57 ± 14.23	5535.714 ± 330.454	9235.714 ± 123.876	8492.851 ± 165.232	21142.857 ± 465.759
Geranyl acetone	0.783 ± 0.083	1.567 ± 0.067	1.417 ± 0.033	2.150 ± 0.210	2.783 ± 0.183

**Values = mean values ± standard deviation (SD).

3.3. Ethanol Production. The ethanol changes observed in the different samples during their fermentation processes are presented in Figure 2(b). The ethanol in all the fermentation processes increased sharply during the first 8 days of fermentation. Subsequently, the samples with 60 and 100 g/L added sucrose exhibited a marked increase in ethanol. Conversely, the sample with 20 g/L added sucrose and the controls remained stable. The sample with 100 g/L added sucrose exhibited the highest increase in ethanol (10.27% v/v) and the fastest rate of production. This was followed by the samples with 60 g/L added sucrose, 20 g/L added sucrose, and the controls, respectively (Figure 2(b) and Table 1). Compared to the samples with added sucrose, the ethanol contents in the controls were low (4.9% v/v) throughout fermentation, illustrating that the initial musts contained less sugar (87.75 g/L) for the yeasts to assimilate into ethanol (Table 1). Thus, we concluded that if the musts contained

a higher sugar concentration, the yeast would have yielded more ethanol. This is in agreement with the results obtained in previous studies [36, 37].

3.4. Acid Consumption. The changes in titratable acidity for the different fermentation process of the different samples are illustrated in Figure 2(c). The titratable acidity in all the samples decreased from ~15 g/L to ~13 g/L (Table 1). This decrease was attributed to the reduction in tartaric acid and is related to the complexation of this acid with other compounds (such as potassium to form potassium bitartrate). In addition, this result was justified by the reduction in the volatile acid content in the musts during fermentation [38]. The titratable acidity for the samples with 60 and 100 g/L added sucrose decreased slightly during the first 10 days of fermentation (Figure 2(c)). No significant changes were observed in the sugar-rich environments, probably due to the
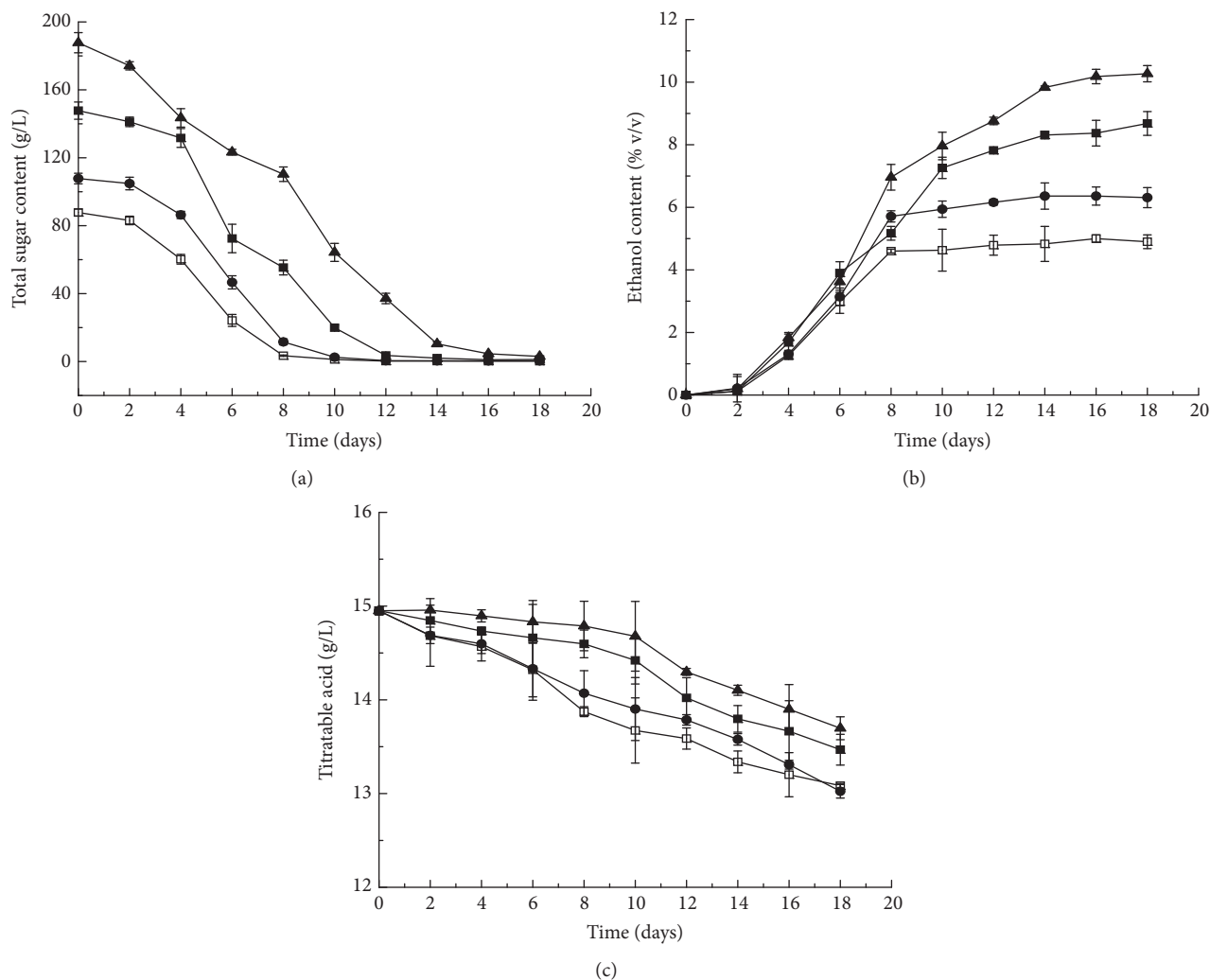
(a)

(b)

(c)

Figure 2: Changes of chemical parameters including total sugar content (a), ethanol content (b), and titrable acidity (c) of "Shuangyou" wines added with 20 (●), 60 (■), and 100 (▲) g/L sucrose and controls (□) during fermentation.

excretion of the intermediate (α-ketoglutaric acid, succinic acid, acetoin, diacetyl, etc.) from the tricarboxylic acid (TCA) [39].

3.5. Color Evolution. The changes in color parameters [a^* (a), b^* (b), L^* (c)] in the different fermentation samples are displayed in Figure 3. For all the samples, an increase in a^* (red/green) and a decrease in b^* (yellow/blue) and L^* (lightness) were observed on the first four days of the fermentation process. These changes were attributed to the mass of the pigments that leach from the grape skin during maceration (4 days) [40]. After this process, a slight decrease in a^* was observed. This was due to the formation of a more complex mixture of pigments that are involved in the burgundy color of red wine [41]. Conversely, the value for b^* increased sharply. This increase was more marked in the samples with 60 and 100 g/L added sucrose and is likely due to the formation of yellow-orange pigments during the later stages of fermentation that are relevant to sugar assimilation [42]. Moreover, an increase in L^*, attributed

to the precipitation of insoluble substances in the wine samples, was also observed. Notably, the wine from the musts with 60 and 100 g/L added sucrose exhibited the highest a^* and b^* values (Table 1). This indicated that the main CIELAB parameters of the wine were related to the sugar concentration added in the must samples.

3.6. Volatile Compounds. The concentration of volatile compounds and the OAVs of the major volatile compounds in the different fermentation samples are listed in Tables 2 and 3, respectively. A total of 37 volatile compounds, including esters, alcohols, acids, ketones, and aldehydes, were identified and quantified. Among these compounds, alcohols were the most abundant in the wine samples (samples with added sucrose and the controls), while esters were the most abundant compounds in the musts (Table 2).

Because they are the primary source of fruity and floral aromas, esters are an important group of volatile compounds that greatly influence the wine aroma [43–45]. The results revealed that the wines were rich in esters; 20 esters were
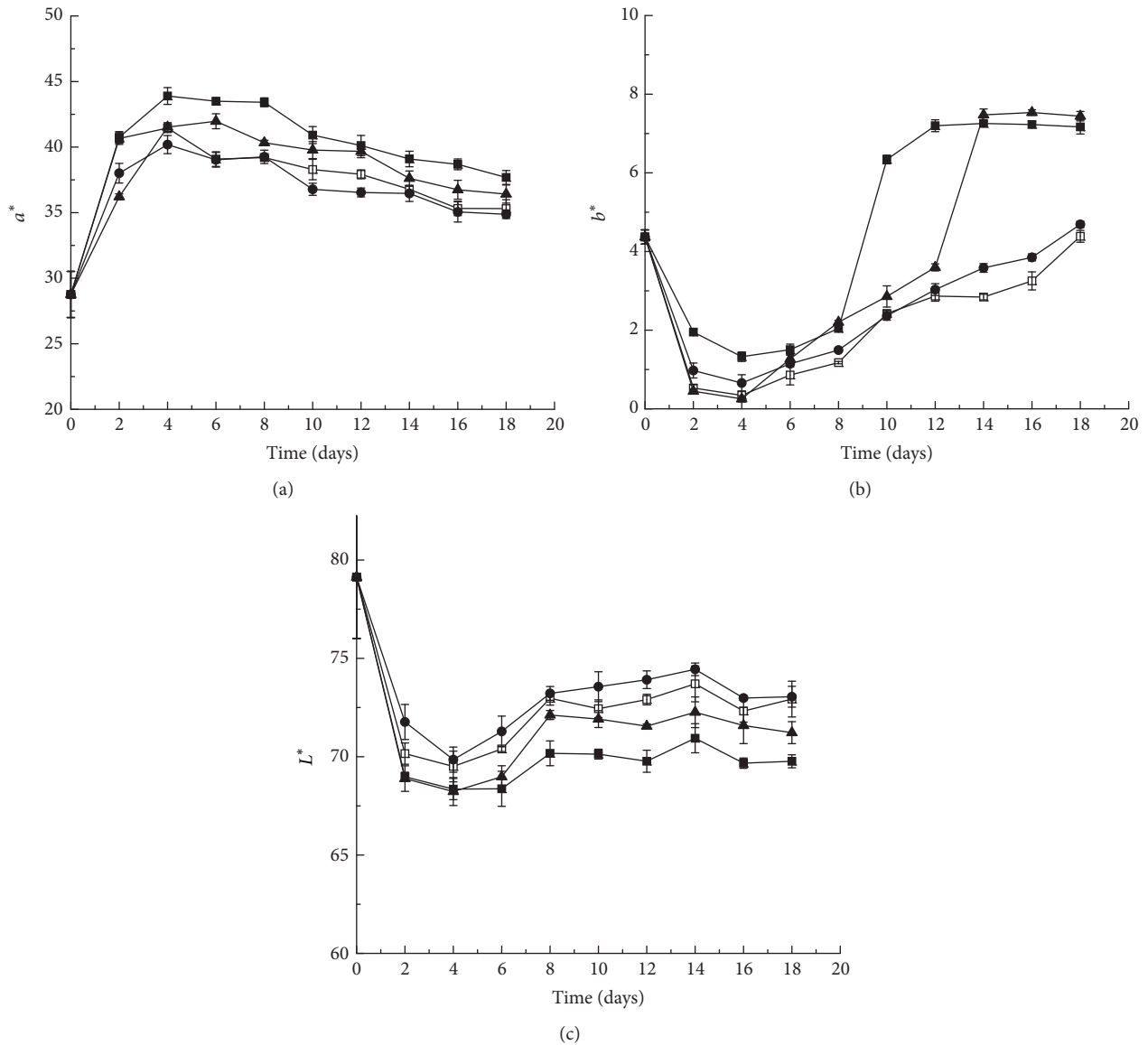
(a)

(b)

(c)

FIGURE 3: Changes of the color parameters including: a^* (red/green, (a)), b^* (yellow/blue, (b)), and L^* (lightness, (c)) of "Shuangyou" wines added with 20 (●), 60 (■), and 100 (▲) g/L sucrose and controls (□) during fermentation.

identified (Table 3), 9 of which contribute to the wine aroma directly or synergistically due to their relatively high OAVs (>1). Ethyl acetate, ethyl octanoate, ethyl hexanoate, and isoamyl acetate were the dominant compounds. Compared to the controls, the concentrations of the total esters and the odor activity values of esters (OAVs > 1) in the wines added sucrose were higher. Furthermore, the wines with 100 g/L added sucrose during fermentation exhibited the highest concentration of total esters (164.77 mg/L). The odor activity values of esters in the wines with 100 g/L added sucrose were 2.33–10.46 times higher than that of the controls. Previous studies have reported that most esters are secondary metabolites whose formation in wine mainly depends on the number of alcohols and acids that are generated by yeast assimilated sugar during alcoholic fermentation [14–16]. Thus, during esterification, a larger number of esters

formed in the wine samples that contained a high sugar content; these results were in agreement with the results in this study.

In terms of alcohols, significant differences were observed between the wines with added sucrose and the controls. The wines from the samples with 100 g/L added sucrose comprised the highest concentration of total alcohols (474.51 mg/L). This demonstrates that the addition of sucrose improves the alcohol concentration in wines. C_4, C_5, and C_6 alcohols typically exhibit a number of characteristics (harsh, fruity, green, and grass aromas) that negatively affect the wine aroma [14]; C_4, C_5, and C_6 alcohols in this study were identified as 2,3-butanediol, isoamyl alcohol, and 1-hexanol, respectively. The highest alcohol concentrations reached at the end of the fermentation process were observed in the wine samples with 100 g/L added sucrose (2,3-butanediol

= 1.17 mg/L, isoamyl alcohol = 400.51 mg/L, and 1-hexanol = 10.01 mg/L). These were followed, in descending order, by the samples with 60 g/L added sucrose, 20 g/L added sucrose, controls, and musts (Table 2). The increase of these compounds in sugar-rich environments may be due to extra oxygen dissolved in the must samples. Thus, the more oxygen is dissolved in the musts, the more C_6 aldehydes would be generated through the degeneration of lipid acids. In addition, the results in our study demonstrated that the concentrations of isoamyl alcohol and 1-hexanol in wines (Table 3) exceeded their threshold (OAVs > 1). The odor activity values of isoamyl alcohol and 1-hexanol in the wines with 100 g/L added sucrose were 5.18 and 2.35 times higher, respectively, than that of the controls. Higher alcohols (>C_6, fusel alcohols) usually have a strong and pungent odor character. These compounds are secondary products of yeast alcoholic fermentation and can have a positive or negative effect on wine aroma depending on their concentration. At low concentrations (<300 mg/L), they can contribute to the desirable complexity of the wine aroma. On the other hand, at higher concentrations (>400 mg/L), they become negative aroma quality factors [19]. Five higher alcohols were identified in this study, namely, 1-octen-3-ol, benzyl alcohol, 1-octanol, 2-phenylethanol, and 1-nonanol (Table 2); these alcohols usually have rose, floral, mushroom, and burnt aromas. The higher concentrations of the different alcohols in all treatments were <400 mg/L (at 0.45–55.44 mg/L, Table 2). At the end of fermentation, the wines with 100 g/L added sucrose exhibited the highest total concentration. Furthermore, the results revealed that the concentrations of 1-octen-3-ol, 1-octanol, and 2-phenylethanol (Table 3) in the wine samples all exceeded their threshold values (OAVs > 1). The odor activity values of 1-octen-3-ol, 1-octanol, and 2-phenylethanol in the wines with 100 g/L added sucrose were 4.63, 1.87, and 2.92 times higher, respectively, than that of the controls. This effect indicates that the addition of sucrose strengthens the desired wine aroma.

Previous studies have shown that the production and presence of fatty acids depends on the composition of the grape musts and the fermentation conditions [46]. Volatile fatty acids have a negative effect on the wine aroma when above their thresholds [19]. Nevertheless, they still have a harsh, fruity, green, grass aroma character that causes a complex wine bouquet even when they exceed the threshold values (OAVs > 1). Four fatty acids, including octanoic acid, hexanoic acid, decanoic acid, and isovaleric acid, were identified in the wines in this study (Table 2). Among these, octanoic acid was the dominant acid, followed by hexanoic acid, isovaleric acid, and decanoic acid, respectively. Except for decanoic acid, the amount of fatty acids in the wine samples was higher than that observed in the musts. Thus, these compounds were thought to be generated from the yeast assimilated sugar during the fermentation process. Conversely, decanoic acid formed esters, resulting in a decrease in its content at the end of fermentation. Furthermore, the wine sample with 100 g/L of added sucrose had the highest amounts of each fatty acid except decanoic acid. Moreover, the concentration of octanoic acid and hexanoic acid (Table 3) in the wine samples significantly exceeded their threshold values (OAVs > 1). The odor activity values of octanoic acid and hexanoic acid in the wines with 100 g/L added sucrose were 1.73 and 1.47 times higher, respectively, than that of the controls. Usually, the presence of fatty acids in wine is related to unpleasant odors, even though these compounds do not have a direct negative effect on the flavor of the wines since they are present in low concentrations. The wine samples with added sucrose produced higher relative amounts of fatty acids that have the potential to destroy the wine aroma.

Other volatile compounds found in the wine samples included three ketones and two aldehydes (6-methyl-5-hepten-2-one, β-damascenone, geranylacetone, phenyl acetaldehyde, and decanal, resp.). The amounts of these compounds were higher in the wines than in the musts. This indicates that these compounds are generated by yeast assimilated sugar during fermentation. Compared to the controls, the ketones and aldehydes in the wine samples with 100 g/L added sucrose comprised the largest amounts of these compounds (Table 2). Furthermore, the concentration of 6-methyl-5-hepten-2-one, β-damascenone, and geranylacetone (Table 3) in the wine samples significantly exceeded their threshold values (OAVs > 1). The odor activity values of 6-methyl-5-hepten-2-one, β-damascenone, and geranylacetone in the wines with 100 g/L added sucrose were 2.44, 3.82, and 1.78 times higher, respectively, than that of the controls.

4. Conclusions

The results of this work reveal that the addition of sucrose during the fermentation process has a significant influence on the physicochemical properties and volatile compounds of "Shuangyou" wine. The study indicated that yeast could convert sucrose into a reducing sugar that was then assimilated into alcohol by yeast. Therefore, we concluded that the addition of sucrose contributes to yeast growth and alcohol production. The addition of 100 g/L sucrose afforded the highest concentrations of total sugar and ethanol at the end of fermentation that theoretically could be metabolized by the yeast. The titratable acidity of all the fermentation processes decreased slightly. Moreover, the higher amounts of added sucrose (60 or 100 g/L) altered the color of the wine samples, making them darker and deeper than that those with lower or no sucrose. A total of 37 individual aroma compounds rich in different volatile compounds (esters, alcohol, fatty acids, ketones, and aldehydes) were detected in "Shuangyou" wines by HS-GC-MS analysis. The addition of sucrose influenced the aroma concentration of these wines. Furthermore, the addition of 100 g/L sucrose was sufficient to improve the concentrations of the aroma compounds. This was evidenced by volatile quantification and OAVs evaluation. Most of the aroma compounds in the wine samples with the highest amount of added sucrose made a positive impact on the wine quality, especially reinforcing the global aromatic complexity of the wine. In addition, sucrose addition seems to produce a stronger pungent taste impact during fermentation, based on the results of the present study. Hence, it should elucidate in greater detail the relationship between volatile aroma and poor sensory quality of wines. Technologies like sensory

panel may be of a great advantage to researchers when dealing with samples with unpleasant taste. Further study is needed to confirm our hypothesis.

Additional Points

Practical Applications. The "Shuangyou" grape cultivar is widely cultivated both in western and in northeastern China and its grapes are a source of grape juice and high-quality red wine. Compared to *V. vinifera* wines, *V. amurensis* wines have a bright ruby red color, fine fragrant aroma, and a mellow and full-bodied taste. However, this wine is also rich in bioactive substances that often impart high acidity, a very sour taste, and low alcohol content, making it less popular. The results reveal that when sucrose is added during the winemaking process, so that the sugar concentration reaches concentrations observed in more commonly grown wine, the quality of "Shuangyou" wines is improved. This increases the potential popularity of these wines.

Conflicts of Interest

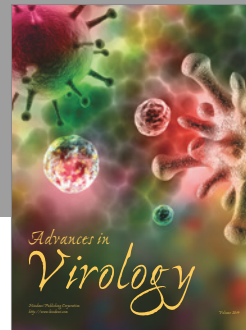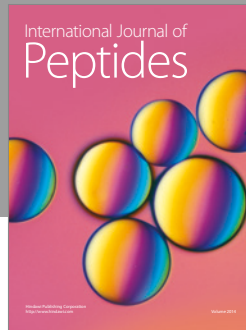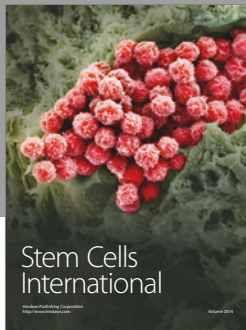The authors declare that they have no conflicts of interest.

Acknowledgments

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