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Chemical and Sensory Characterization of Vidal Icewines Fermented with Different Yeast Strains

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Abstract: The aim of this study is to comprehensively investigate the aroma composition and sensory attributes of Vidal icewine fermented with four yeast strains (ST, K1, EC1118, and R2). A total of 485 kinds of volatile components were identified by comprehensive two-dimensional gas chromatography-time of flight mass spectrometry, among which 347 kinds of volatile compounds were the same in four kinds of sample. The heat map was conducted with 156 volatile compounds, which have aroma contributions, and the analysis results identified the characteristics of the aroma composition of icewine fermented with different yeasts. Quantitative descriptive analysis was performed with a trained panel to obtain the sensory profiles. The aroma attributes of honey and nut of the icewine fermented by R2 were much higher than others. Partial least squares discriminant analysis further provided 40 compounds that were mainly responsible for the differences of the aroma characteristics of the icewines fermented by four yeasts. This study provides more data on the current status of Vidal icewines by main commercial yeasts.

Keywords: icewine; Vidal; yeast; aroma compounds; sensory analysis

1. Introduction

Icewine is a unique sweet wine, made by delaying grape harvest, freezing, and air-drying the fruits hanging on the branches at −7 °C (EU regulations) and −8 °C (Canada, Vintners Quality Alliance 1999), pressing and low-temperature sugar-preserving fermentation in the freezing state. The ripening condition of icewine grapes at low temperatures is quite different from that of ordinary wine grapes. In this stage, the grapes are dried and shrunken, and the flavor substances such as sugar and aroma are continuously “concentrated”. Therefore, icewine has more unique flavor characteristics than ordinary wine [1]. At present, only a few countries in the world (Canada, Germany, Austria, Switzerland, China, etc.) can produce icewine in a few regions [2]. In addition, the special post-freezing ripening process requires the ice grape berries to have thick skins, easy to preserve, and the ice grape to resist cold. Vidal is a hardy hybrid grape that is grown mainly in Canada and the northeastern United States. It has thick skin, strong resistance to cold, and high natural acidity, thus good for icewine [1,3].

Similar to regular wines, the aroma is also a core factor in determining the quality of icewine [4]. In general, the aroma of icewine is mainly influenced by grape raw material and vinification, and among the vinification, the yeast is one of the important factors to determine the aroma characters of icewine [5]. During fermentation, yeast converts sweet and low-aroma grape juice into high-aroma wine through the glycolytic pathway. In this process, the fructose and glucose are converted to ethanol, carbon dioxide, and volatile metabolites [6–8]. Furthermore, many volatile metabolites are also released from
non-volatile grape-derived precursors by yeast enzymes [9]. Unlike the regular grapes, the grapes for making icewine have to be harvested in their naturally frozen state and pressed while still frozen under a higher-than-normal pressure. Normally, the sugar concentration of grape juice for making icewine reach a minimum 35° Brix, and sometimes it can be as high as 50° Brix [10]. However, the more noteworthy are the challenges of making icewine that is caused by the high reducing sugar and viscosity of ice grape juice. The high sugar fermentation environment means that it takes longer for icewine to reach the expected alcohol content, sometimes even last for several months. Besides, high sugar also can cause high osmotic stress, which leads to the higher volatile acid content of icewine than ordinary wine [11]. For all of this, special measures should be taken to ensure the safety of long-term fermentation, the excellent quality, the harmony of taste, and the volatile acid content of icewine. The main methods are selecting appropriate yeast and controlling fermentation temperature, especially the yeast species that have significant effects on the formation of acetic acid and glycerol, the fermentation speed, and the sensory properties of icewine [1].

Due to the ability to ferment under high-sugar conditions, be alcohol-tolerant, and produce relatively lower volatile acidity, V1116 (Saccharomyces cerevisiae), ST (S. cerevisiae), N96 (S. cerevisiae), EC1118 (S. bayanus), R2 (S. bayanus), VL1 (S. cerevisiae), and AWRI 1572 (a hybrid between S. cerevisiae and S. bayanus) are normally utilized to ferment icewine [12–15]. Erasmus et al. (2004) [13] found that N96 and EC1118 could be used to produce high-quality icewine with a strong fruit aroma and low sulfur-like aroma level by studying the differences of seven commercial yeast strains (ST, N96, VIN13, VIN7, EC1118, 71B, V1116) in Riesling icewine production. Crandles et al. (2015) [12] researched the aroma compounds of icewine fermented by different yeasts (V1116, VL1, EC1118, natural fermentation) and found that yeast strain impacted odor-active compounds in Riesling and Vidal icewines, but yeast effects depended upon cultivar and vintage. Synos et al. (2015) [16] used three kinds of yeast (V1116, EC1118, VL1) to ferment and compare with natural fermentation in Cabernet Franc icewine. It was found that the content of aroma active substances in EC1118 and naturally fermented icewine was the highest, but there were great differences between them in the kinds of aroma active substances. Pigeau et al. (2007) [17] studied the effects of ice grape juice with a sugar content over 40 Brix on fermentation, and the results showed that with the increase of ice grape juice sugar content, more acetic acid and glycerol were produced during fermentation, the opposite was that the yeast growth rate and ethanol production were reduced. However, studies assessing yeast effects on icewine aroma compounds are still uncommon, especially in Vidal icewine.

In this work, volatile compounds and sensory were evaluated in Vidal icewine fermented by four different commercial yeasts using comprehensive two-dimensional gas chromatography-time of flight mass spectrometry (GC×GC-TOFMS) and quantitative descriptive analysis (QDA), respectively. Furthermore, relationships between the aroma compounds and sensory attributes were analyzed by multivariate statistical analysis to compare the differences and characteristics of volatile components in icewine fermented by different yeasts. The aim of this study was to comprehensively investigate aroma characteristics in Vidal icewine and to provide more data on the current status of Vidal icewine by main commercial yeasts.

2. Materials and Methods

2.1. Icewine Samples

Experimental Vidal icewines were available from Vidal grapes harvested from ChangYu Winery in Huanren-on-the-Huanlong Lake, Liaoning province, China, in 2018. The total sugar and total acid (in tartaric acid) were 380.5 g/L and 9.1 g/L for the icewine juice, respectively. The icewine samples were fermented by 4 different commercial yeasts (ST, K1, EC1118, R2) at 0.2 g/L—in three replicates for each one (n = 3). The alcoholic fermentation temperature was 13 ± 1 °C in 10 L carboys with 100 mg/L of K2S2O5. Fermentation proceeded until sugar consumption by yeast stopped, after which carboys were moved to a −2 °C chamber for cold stabilization. The icewine samples were then racked off the
gross lees and bottled for analysis. At bottling, 50 mg/L K$_2$S$_2$O$_5$ were added. Details of the icewine samples are provided in Table 1 (the physico-chemical method of wine based on GB/T 15037-2006).

| Treatment | Residual Sugar (g/L) | Ethanol (% (v/v)) | Titratable Acidity (g/L) | Acetic Acid (g/L) | pH |
|-----------|----------------------|-------------------|--------------------------|------------------|----|
| EC1118    | 183.45 ± 1.22        | 10.5 ± 0.1        | 11.2 ± 0.5               | 1.11 ± 0.02      | 3.51|
| R2        | 170.21 ± 1.05        | 11.4 ± 0.2        | 12.8 ± 0.4               | 1.40 ± 0.02      | 3.45|
| K1        | 181.33 ± 2.11        | 10.6 ± 0.1        | 12.1 ± 0.4               | 1.22 ± 0.03      | 3.52|
| ST        | 178.92 ± 1.57        | 10.8 ± 0.1        | 12.2 ± 0.3               | 0.92 ± 0.11      | 3.52|

2.2. Chemicals and Reagents

All chemical standards and internal standards (IS) were of the highest available purity (GC-grade). The analytical standards employed for the positive identification of the aroma compounds were obtained from Sigma-Aldrich (St. Louis, MO, USA) with at least 97% purity. n-hexyl-d13-alcohol (≥98, IS1), 2-methoxy-d3-phenol (≥98, IS2), menthyl acetate (≥98, IS3) were purchased from ANPEL Scientific Instrument Co., Ltd. (Shanghai, China). Ethanol (99.9%, HPLC grade) was purchased from Sigma-Aldrich (Shanghai, China). Sodium chloride (NaCl) was purchased from China National Pharmaceutical Group Corp. Ultrapure water was obtained from a Milli-Q purification system (Millipore, Bedford, MA, USA). Four different S. cerevisiae (ST, K1, EC1118, R2) were purchased from France Lallemand Co., Ltd., Paris, France.

2.3. Descriptive Sensory Analysis (DA)

2.3.1. Panel

Panel candidates were recruited among students and employees of the school of Biotechnology in Jiangnan University. The candidates were selected based on interest, health status, availability, and familiarity with wine using an initial recruitment questionnaire. Thereafter, the candidates were required to complete sensory ability tests for aroma identification, ranking, and response scales (10 cm unstructured scale, ranging from “none” on the left end to “strong” on the right end) [18]. A total of 30 candidates were selected who had achieved at least 70% acuity and were available during the designated time. The general training schedule consisted of 16 h (2 h/week) for introduction to sensory analysis, aroma description and identification, ranking, and triangle tests [19]. Commercial icewines were provided for descriptive tests after 2 to 3 months of general training. The consistency, discernibility, and repeatability of panelists were then evaluated. A total of 12 assessors (5 females and 7 males, aged between 21 and 31 years old) with good sensory performance according to the evaluation were finally selected to participate a further training of descriptive analysis.

2.3.2. Training

The training process was the same as the previous method [20] and took 3 months (once a week). Firstly, 6 descriptors (nut, tropical fruit, apricot, honey, caramel, and rose) of Vidal icewines were obtained by a panel discussing to describe the icewine flavor. Then, assessors were trained for the identification and intensive evaluation of the selected descriptors with reference standards. The reference standards were prepared by adding corresponding aroma standards from Le nez du vin (Jean Lenoir, Provence, France) to 10% v/v aqueous ethanol (pH 3.4) and diluted in series. The sensory reference standards of the 6 descriptors are shown in Table S1. The assessors’ performance was assessed by PanelCheck in terms of their ability in consistency, stability, and repeatability for giving scores before sample evaluation.
2.3.3. Sample Evaluation

Samples were provided to assessors in standard wine glasses covered, coded with random 3-digit numbers, and containing 30 mL icewine (8–12 °C) per glass [21]. Each assessor scored the icewines for each attribute with unipolar 10 cm line scale [22], anchored on the left end with 0 (none) means low intensity, and on the right end with 10 (extreme). Scores were converted to scores from 0 to 10 and exported to an Excel spreadsheet. Between samples, the panelists were asked to take a short break and smell the water to minimize any carry-over effect from the previous sample. All the testing took place in isolated booths illuminated with standard yellow light to eliminate color differences at 20 °C. All sample evaluation was performed in duplicate.

2.4. Analysis of Volatile Aroma Compounds

Headspace solid-phase microextraction coupled with comprehensive 2-dimensional gas chromatography and time-of-flight mass spectrometry (HS-SPME-GC×GC-TOFMS) was employed to determine the volatile profile of the icewine samples, and each sample was analyzed in 3 replicates. Based on previously described methods with slight modifications [23], 5 mL of icewine were placed into 20 mL glass with a silicon septum and saturated with 1.5 g of NaCl. Internal standard mixture (10 μL; IS1: n-hexyl- d13-alcohol, 403.76 μg/L; IS2: 2-methoxy-d3-phenol, 197.6 μg/L; IS3: menthyl acetate, 20.08 μg/L) was added as an internal standard used for the semi-quantification of aroma compounds. A MultiPurpose autosampler (Gerstel GmbH and Co. KG, Mülheim an der Ruhr, Germany) with a 50/30 μm divinylbenzene/Carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber (2 cm; Supelco Inc., Bellefonte, PA, USA) was used to extract the volatile compounds from the headspace of the sample vial based on the previous work with slight modifications. The sample was equilibrated at 50 °C for 5 min and then extracted for 45 min under stirring (250 rpm). After extraction, the fiber was inserted into the gas chromatograph injection port (250 °C) and desorbed for 5 min (splitless) to GC × GC-TOFMS analysis.

A LECO Pegasus 4D GC × GC-TOFMS instrument (LECO Corporation, St. Joseph, MI, USA) equipped with an Agilent 7890B gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) was used in GC × GC-TOFMS analysis. A polar/moderately polar column set was optimized for the GC × GC separation. The first dimension (1D) column was DB-FFAP column (60 m × 0.25 mm i.d. and 0.25 μm film thickness, Agilent Technologies Inc., Santa Clara, CA, USA), and the second dimension (2D) column was Rxi-17Sil MS Cap. column (1.5 m × 0.25 mm i.d. and 0.25 μm film thickness, Restek Technologies Inc, Bellefonte, PA, USA). The oven temperature of the first column was held at 45 °C for 2 min, ramped at 4 °C/min to 230 °C, held for 15 min. The temperature of the second column was held at 40 °C for 2 min, ramped at 5 °C/min to 250 °C, held for 5 min. A quad-jet, dual-stage thermal modulator was equipped between the 1D and 2D columns. The modulation period as set for 4 s with a 1 s hot pulse time. Helium (99.9995% purity) was used as the carrier gas at a constant flow of 1.0 mL/min. For the TOFMS system, the temperatures of the transfer line and ionization sources were 280 and 230 °C, respectively. Electron impact mass spectra were recorded at 70 eV, and the acquisitions were performed over an m/z scan range of 35–400 amu.

LECO ChromaTOFTM Workstation (version 4.44) was used for all acquisition control and data processing. Automated peak detection and spectral deconvolution were employed. The minimum value for the signal to noise (S/N) ratio necessary to record a chromatographic peak was set as 50 in GC × GC. The positive identification of the compounds was achieved by comparing the retention data and mass spectra of the standard compounds. For unavailable standards, tentative identification of the compounds was achieved by comparing their experimental retention indices (I) with the retention indices reported in the scientific literature (IT). A compound was considered to be tentatively identified if the similarity between mass spectrometric information of each chromatographic peak and the National Institute of Standards and Technology (NIST) mass spectra library was at least 75%, and the difference between I and IT did not exceed 30 units. Semi-quantitative
analysis was performed in triplicate, and average values of concentration were used in further data elaboration.

2.5. Statistical Analysis

Panelcheck (version 1.4.2, Nofima Mat and DTU-Informatics and Mathematical Modelling, Norway) was used to assess the consistency, discernibility, and repeatability of the results from panelists. Partial least squares (PLS) regression analysis was conducted using XLSTAT software (version 2014; Addinsoft, Paris, France), employed to investigate the relationships between the compositional variables (x data) and the sensory attributes (y data) in icewine samples fermented by 4 different yeast strains. Heatmap were visualized with Gephi (Version 0.9.1) using classic FruchtermanReingold algorithm.

3. Results and Discussion

3.1. Samples of Icewines

The four commercial yeast strains produced icewines with small differences in residual sugar values (170.21–183.45 g/L), pH (3.45–3.52), ethanol values (10.5–11.4%), and TA (11.2–12.8 g/L) (Table 1). These results are consistent with Fuleki (1994) [14], who also found that little differences in Riesling icewines fermented by five commercial yeasts. Furthermore, R2 icewines contained the lowest residual sugar, had the lowest pH, and highest ethanol.

Acetic acid is a key indicator during the wine-making progress, and its content determines the quality of wine directly. Equally, acetic acid is important to the production of icewine. The yeast will be exposed to a high osmotic pressure due to the high sugar concentration of ice grape juice, which results in yeast metabolism abnormality. On the one hand, achieving the desired alcohol content needs to take more time. On the other hand, high osmotic pressure will lead to the formation of a large number of volatile acids that are dominated by acetic acid [13,24]. Appropriate acetic acid will not have a bad effect on icewine. Under the catalysis of ethanol acetyl transferase, acetic acid can react with ethanol to produce ethyl acetate, which will bring a fruit flavor for icewine. In Canada, VQA (1999) limits that the volatile acid content in icewine must be below 2.1 g/L. However, Cliff and Pickering (2006) [25] found that the threshold of acetic acid in icewine was 3.185 g/L, meaning the acetic acid content in most icewine would not be above this value. A previous study of Canadian icewine reported that there was a wide range of acetic acid in Canadian icewine, from 0.49 to 2.29 g/L. The average level was around 1.3 g/L, which was still far below the sensory threshold and will not have a great change on the flavor of icewine [3]. Table 1 shows that the concentration of acetic acid was between 0.92 and 1.40 g/L in the four yeast-fermented icewine samples, also far below the 3.185 g/L. The content of acetic acid in icewine fermented by ST yeast was the lowest, which was only 0.92 g/L, and EC1118 yeast also had a good effect on the control of acetic acid, which was 1.11 g/L. The results were the same as Erasmus et al. (2004) [13], who compared the differences of acetic acid in icewines that fermented by seven different commercial yeasts and found that ST yeast produced the least acetic acid, followed by N96 and EC1118.

3.2. Sensory Analysis

In order to describe the sensory differences of Vidal icewine fermented by different yeasts, QDA was used to analyze the flavor of icewine samples. Based on the average strength of six aroma descriptors (nut, tropical fruit, apricot, honey, caramel, and rose) in icewine [4,20] to plot a spider web (Figure 1). It shows that the aroma intensity of honey and nut was the highest in icewine fermented by R2 yeast, especially the honey aroma, was much higher than others, and the aroma intensity of rose and tropical fruit was ranked only second to EC1118 yeast-fermented wine. For the icewine fermented with EC1118 yeast, it has a stronger aroma intensity on tropical fruits and caramel than others. However, the aroma intensity in icewine fermented by K1 was much lower than others except for the apricot aroma.
3.3. Chemical Analysis

3.3.1. Volatile Profiling of Icewines Fermented by Different Yeasts

Due to the complexity of wine aroma composition and matrix, there were some problems in the separation and detection of wine by one-dimensional gas chromatography, such as insufficient peak capacity, co-outflow, low sensitivity, and so on. Therefore, this method cannot satisfy our needs for the study of wine flavor chemistry. However, the emergence of GC×GC-TOFMS provides a useful tool for better separating and identifying these complex components [26]. In this study, the volatile compounds of four different Vidal icewines fermented by four yeasts (ST, K1, EC1118, R2) were analyzed by GC×GC-TOFMS. A total of 3784 chromatographic peaks were detected. Figure S1A shows the chromatogram of the volatile components in R2 fermented icewine. It can be seen from Figure S1B that the three compounds, 4-methyl-1-pentanol, 1-butanol, and ethyl valerate, co-flowed in one dimension and were difficult to identify and quantify, but they were well separated in the two-dimensional chromatography. Consequently, it can be proved that GC×GC-TOFMS can effectively solve the co-outflow phenomenon and improve the accuracy of qualitative analysis.

Finally, 485 volatile compounds were identified in four icewine samples, including 123 esters, 26 terpenes, 105 alcohols, 115 carbonyl compounds, 23 furans, 21 nitrogen-containing compounds, 19 volatile phenols, 7 sulfides, 35 aromatic compounds, and 11 lactones. A total of 391, 399, 364, and 457 volatile compounds were detected in four icewines, which fermented by K1, EC1118, ST, and R2 yeast, respectively (Table 2). It can be seen that the number of volatile compounds in icewine fermented by R2 was much higher than the other. In addition, there were 347 compounds in common, accounting for 72% of the total volatile compounds. It can also be seen from Table 2 that the icewines fermented by different yeasts differed greatly in yeast fermentation products such as esters, alcohols, carbonyls (aldehydes and ketones), and nitrogen-containing compounds. In previous studies, the aroma compounds were analyzed using GC-MS in Riesling, Vidal, and Cabernet Franc icewines, which was fermented by different yeasts, the differences of aroma compounds mainly in alcohol and ester compounds, and the sum of these two classes of compounds accounted for more than 50% of the total [12,16]. The results of this study were basically consistent with the above research.

Figure 1. Sensory profiles of Vidal icewines fermented by different yeasts.
Table 2. Volatile compounds in Vidal icewine fermented by different yeasts.

|                  | K1 | EC1118 | ST | R2 |
|------------------|----|--------|----|----|
| Esters           | 97 | 91     | 93 | 117|
| Alcohols         | 79 | 81     | 73 | 105|
| Ketones          | 50 | 49     | 46 | 55 |
| Aromatic compounds | 34 | 35     | 32 | 31 |
| Terpenes         | 25 | 24     | 24 | 26 |
| Aldehydes        | 18 | 23     | 14 | 26 |
| Purins           | 18 | 19     | 19 | 23 |
| Acids            | 19 | 21     | 19 | 22 |
| Nitrogen-containing compounds | 17 | 21 | 10 | 18 |
| Phenols          | 17 | 18     | 17 | 17 |
| Lactones         | 11 | 11     | 11 | 11 |
| Sulfides         | 6  | 6      | 6  | 6  |
| Total            | 391| 399    | 364| 457|

3.3.2. Chemical Characteristic of Icewines Fermented by Different Yeasts

Among the 485 volatile compounds identified in all icewine samples, not all of them have aroma contributions, and the study of flavor pays more attention to the compounds with aroma contributions [27]. Therefore, based on the qualitative results and matched it with the aroma substance database (Flavornet, http://www.flavornet.org/flavornet.html, accessed on 11 December 2018), 156 volatile compounds were selected to further analyze, details can be seen in Table S2.

The semi-quantitative results were obtained by the internal standard method and then drawn on a heat map (Figure 2). In the figure, the rows represent the compounds, and the columns represent the yeasts used for fermentation. The distance measure used in clustering rows (compounds) was the Euclidean method, and the data were standardized before cluster analysis. According to the results of heat map analysis, the 156 compounds can be divided into three classes, the compounds of class A were the highest content in R2 yeast fermented icewine, class B substances were highest in ST yeast fermented icewine, and class C aroma components had the highest content in K1 and EC1118 yeast fermented icewine.

In class A, most of the compounds were esters, alcohols, terpenes, and carbonyl compounds. In this group, ethyl 2-methylbutyrate, ethyl isobutyrate, ethyl octanoate, and ethyl caproate were all the major aroma contributors in Vidal icewine, which smells similar to the apple, pineapples, and so on. In addition, the 1-octen-3-ol and 1-octen-3-one showed a mushroom characteristic, 1-hexanol showed a resin characteristic, β-damascenone, linalool, and geraniol with the aroma characteristics of honey, lavender, and rose, respectively. α-Terpinene, γ-terpinene, and 1,4-cineole were the aroma characteristics of pine. Benzaldehyde showed the aroma characteristics of almonds. These compounds were all key aroma compounds reported in Vidal icewine [4,20], especially β-damascenone, which has been reported in many studies that is the strongest aroma in Vidal icewine [4,20,28]. In red wines, β-damascenone has been found to enhance fruit aroma while can also inhibit the plant aroma produced by methoxypyrazine [29]. Similar phenomena have also been found in the study of icewine, β-damascenone not only affects the perception of honey but also affects the perception of other aromas such as apricot peach [4].

Class B is mainly composed of aromatic compounds and ester compounds, including seven aromatic compounds, six ester, four alcohol, six aldehydes and ketones, two nitrogen compounds, and one volatile phenol, of which phenethyl acetate (flowers), homofuran (sweet), ethyl valerate (fruit) were reported as the important aroma compounds in icewine [4]. Class C compounds gather all lactones, which usually have aroma characteristics such as apricot [4]. From the above analysis, we can see that some key aromas, which has reported on Vidal icewine were significantly higher in R2 than other samples, such as 1-octen-3-ol, 1-hexanol, β-damascenone, linalool, geraniol, 1-octen-3-one, and so on, and the alcohol, ester, and carbonyl compounds in the wine also had obvious advantages.
homofuraneol (sweet), ethyl valerate (fruit) were reported as the important aroma compounds in icewine [4]. Class C compounds gather all lactones, which usually have aroma characteristics such as apricot [4]. From the above analysis, we can see that some key aromas, which have been reported on Vidal icewine, were significantly higher in R2 than other samples, such as 1-octen-3-ol, 1-hexanol, β-damascenone, linalool, geraniol, 1-octen-3-one, and so on, and the alcohol, ester, and carbonyl compounds in the wine also had obvious advantages.

Figure 2. Heat map of 156 compounds with different aroma characteristics of four different yeast fermented icewines. In the figure, the rows represent the compounds, and the columns represent the yeasts used for fermentation. Distance measure used in clustering rows (compounds) was Euclidean method, and the data were standardized before cluster analysis. (A), the compounds were higher in R2 yeast fermented icewine; (B), the compounds were higher in ST yeast fermented icewine; (C), the compounds were higher in K1 and EC1118 yeast fermented icewine.
3.4. Relationships between the Sensory Attributes and Aroma Compounds

To further understand the chemical origin of the sensory descriptors, PLS regression analysis was employed to determine the associations between the aroma attributes (y variables, n = 6) and the significantly different aroma compounds (x variables, n = 65) in four different icewine samples. The result showed that differences existing in Vidal icewines fermented by different yeasts could be revealed by the chemometric methods. The aroma compounds plotted in the vicinity of the sensory descriptors were positively associated with those attributes (Figure 3).

![Figure 3. Projection on the PLS regression between sensory attributes and aroma compounds (p < 0.05) of four different yeast fermented icewines (A); the VIP values were calculated from the PLS regression model (B).](image-url)
As we can see from Figure 3A, the aroma attributes of honey and nut can be used to distinguish the aroma of the icewine fermented by R2 and other icewines. At the same time, R2 was also well related to β-damascenone, 2-hydroxybenzoic acid methyl ester, 2-methylpyrazine, Butanediolic acid diethyl ester, 5-hexylidihydro-2(3H)-furanone, 3-hydroxy-butyric acid ethyl ester, and 3-octanol. The icewines fermented by EC1118 had high correlation with some sweet aromas such as caramel and tropical fruit. The caramel was well related to dihydro-2(3H)-furanone and hexanoic acid, which showed the aroma of caramel and sweet. The esters, such as ethyl vanillate, hexanoic acid hexyl ester, octanoic acid ethyl ester, hexyl octanoate together with butylcaprylate were associated with the “tropical fruit” aroma. The esters generally contributed to the fruity aroma of the wine, which conforms to the PLS regression results. In previous studies, Huang et al. (2018) [20] find the sensory profiles of icewines from China were characterized as nut and honey aromas, while icewines from Canada expressed caramel and rose aromas. Bowen and Reynolds (2015) [30] stress the importance of time of harvest, whereby early-harvested fruit (e.g., following a “cold snap” in late November) typically produces wines that were fruit-forward (citrus, peach, etc.), whereas fruit harvested after several freeze-thaw cycles normally produce wines that were more caramel, nutty, and sherry-like. According to the results of this study, the possible reason for the different aroma characteristics of Chinese and Canadian Vidal icewine was not only the harvest time but also the selection of different yeast during the fermentation.

In addition, the variable importance for projection (VIP) values was obtained from the PLS regression model (Figure 3B). The VIP scores for the 40 aroma compounds were >1, including 11 esters (butylcaprylate, butanoic acid ethyl ester, hexyl octanoate, octanoic acid methyl ester, butanoic acid 2-methyl- ethyl ester, ethyl vanillate, benzeneacetic acid ethyl ester, hexanoic acid hexyl ester, octanoic acid ethyl ester, butanoic acid 3-hydroxy-ethyl ester, butanediolic acid diethyl ester), five alcohols (3-octanol, 1-undecanol, 1-octen-3-ol, 1-decanol, 3-ethoxy-1-propanol), five terpenes (α-terpinene, pipertone, geranyl acetone, α-terpinolene, 1,8-cineole), five aldehydes and ketones (pentanal, 3-methyl-butanal, 4-methyl-3-penten-2-one, 6-heptyltetrahydro-2H-pyran-2-one, 2-hexanone), four furans (5-ethyl-2(5H)-furanone, 5-hexylidihydro-2(3H)-furanone, dihydro-2(3H)-furanone, (Z)-dihydro-5-(2-octenyl)-2(3H)-furanone), four phenols (3-ethyl-phenol, 2-methoxy-4-vinylphenol, phenol, 2-methyl-phenol), three acids (hexanoic acid, octanoic acid, henzoic acid), and three sulfides (trisulfide dimethyl, 1-propanol 3-(methylthio)-, benzothiazole). These aroma compounds can be considered as being mainly responsible for the differences in the aroma characteristics between the icewines fermented by four yeasts.

4. Conclusions

In this study, four different yeast fermented icewines were further analyzed by evaluating the chemical and sensory profiles and then the potential associations between them. A total of 485 volatile components were identified by HS-SPME-GC×GC-TOFMS, among which 347 kinds of volatile compounds were the same in four kinds of the sample. Matching it with aroma substance database [http://www.flavornet.org/flavornet.html, accessed on 22 September 2021], finally, 156 aromas were selected to draw the heat map, and the analysis results identified the characteristics of aroma composition of icewines fermented by different yeasts. The sensory differences of different Vidal icewine samples were distinguished by QDA. PLS-DA also provided some candidate compounds that were correlated with particular sensory characteristics in different yeast fermented icewine samples. This study not only comprehensively investigates aroma characteristics in icewine fermented by different yeasts but also provides a theoretical reference for the production of icewine.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/fermentation7040211/s1. Figure S1: Analytical ion chromatogram contour plot analysis of volatile components in icewine fermented R2 yeast (A) and modulated peaks of 3 compounds found in icewine (B) and deconvoluted mass spectra of 3 compounds(C). Table S1: Aroma descriptor reference. Table S2: Information on 156 compounds with aroma characteristics (µg·L⁻¹).
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