Technical Analysis of Detection of Vitis Amurensis Wine Volatiles Based on HS-SPEM-GC-MS

Ming Li (lidaoen@163.com)
Tonghua Normal University
Yanyan Cui
Tonghua Normal University
Jiayi Lei
Tonghua Normal University
Qi Shen
Tonghua Normal University

Research Article

Keywords: Vitis amurensis wine, aroma, volatiles, detection

Posted Date: November 9th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3575116/v1

Additional Declarations: No competing interests reported.

Abstract

Vitis amurensis wine is a Chinese specialty wine product. This study investigated the effects of different sample processing methods on the detection of volatile components of *Vitis amurensis* wines using headspace solid-phase microextraction coupled with gas chromatography-mass spectrometry (HS-SPME-GC-MS). The effects of sample volume, sample concentration, extraction temperature, equilibrium time, adsorption time, stirring speed, sodium chloride, and desorption time on the assay results were explored. The results showed that the sample processing methods significantly impacted the assay results, especially for low-concentration volatiles, which were detected less frequently. However, this effect could be compensated by increasing the adsorption time, adding saturated NaCl and increasing the number of parallel experiments. When the results were statistically analyzed, high matching (probability) reduced the variability of the parameters at different levels, and the detection conditions could not be optimized by regression analysis, but low matching reduced the accuracy of the results. Moreover, the sensitivity of the instrument exerted an effect on the assay results.

1. Introduction

Aroma is one of the most important sensory qualities of wine, determined by the grape variety, origin, and wine-making process. The volatile compounds derived from fermentation constitute the largest percentage of the total aroma composition of wine. Therefore, understanding the composition and formation of aroma is crucial to optimize the wine-making process and improve wine quality. Headspace solid-phase micro-extraction (HS-SPME) coupled with gas chromatography-mass spectrometry (GC-MS) is widely used in the identification of wine aroma compounds. Compared with conventional techniques, HS-SPME offers superior enrichment, is simple, inexpensive, and fast to operate, and requires no solvent (Lancioni, Castells, Candal, & Tascon, 2022).

SPME uses a fiber coated with an extraction phase comprising a pure polymer or adsorptive particles embedded in a polymer. The fiber coating absorbs (extracts) the volatile organic compounds (VOCs) from the sample. The SPME fiber is then inserted directly into the GC injector for thermal desorption and analysis. Selecting an appropriate SPME fiber is essential because the coating determines the absorption selectivity of VOCs. The divinylbenzene/Carboxen/polydimethylsiloxane (DVB/CAR/PDMS) coating is widely used in the identification of wine VOCs owing to its broad range of analyte detection. This mixed-phase sorbent extracts analytes based on van der Waals, π-π, or hydrogen bonding interactions (Lancioni, Castells, Candal, & Tascon, 2022).

In VOC extraction by HS-SPME, multiple factors influence the analysis results, such as the sample volume, SPME fiber coating, extraction time, extraction temperature, stirring, ionic strength, pH of the samples, and fiber-sample distance (Azzi-Achkouty, Estephan, Ouaini, & Rutledge, 2017; Kamgang Nzekoue, Angeloni, Caprioli, Cortese, Maggi, Marconi, et al., 2020). (Azzi-Achkouty, Estephan, Ouaini, & Rutledge, 2017) reported different HS-SPME methods to identify VOCs in wines. Previous studies applied an extraction temperature of 25℃~70℃, an extraction time of 10 ~ 120 min, a salt concentration of 7%~30%, and a sample volume (V_{sample}/V_{vial}) of 5%~75%.

The extraction process consists of two stages, namely the equilibrium and absorption stages. Firstly, the VOCs are released from the sample into headspace until saturation. Since this is an endothermic process, increasing extraction temperature can shorten the equilibrium stage. Secondly, the fiber is inserted into the headspace, and VOCs are absorbed on the fiber. In contrast, this process is exothermic, and heightened temperature decreases absorption. (Marinaki, Sampsonidis, Lioupi, Arapitsas, Thomaidis, Zinoviadou, et al., 2023) demonstrated that the total area of VOCs initially increased with temperature until reaching a maximum and then decreased (30℃~70℃). However, (Rossi, Foschi, Biancolillo, Maggi, & D'Archivio, 2023) reported an increase in total area as the temperature was increased (30℃~50℃). The disparity in their results may arise from the different temperature ranges used. In addition, for some specific applications, SPME is run at lower or moderate temperatures (12℃ or 37℃) to simulate the fermentation temperature of white wines or mouth temperature during tasting, respectively (Comuzzo, Tat, Tonizzo, & Battistutta, 2006). Although some researchers suggest not exceeding an extraction temperature of 45℃ to avoid modifying the original composition of the wine matrix, the low temperature might negatively affect the sensitivity of low-concentration analytes.

Since only the neutral/undissociated compounds are adsorbed/absorbed from the headspace by the SPME fiber, adjusting the pH may improve the sensitivity by converting ions into their neutral forms, thereby decreasing their solubility in hydrophilic matrix and increasing their release (Schmidt & Podmore, 2015; Zhang & Zhang, 2008).

Furthermore, adding salt may enhance the extraction of HS-SPME due to a "salt-out" effect (Schmidt & Podmore, 2015). This effect drives hydrophobic analytes into the headspace by decreasing their solubility in the aqueous phase. However, an opposite effect may be observed at higher salt concentrations. The concentration of analyte compounds in the headspace could be reduced by electrostatic interactions between analyte molecules and ionic salt molecules, and increased viscosity and density of the aqueous phase (Zhang & Zhang, 2008). In general, salt addition increases the extraction of polar compounds, but has no significant effect on non-polar compounds.

The headspace volume also affects the sensitivity of HS-SPME. Under constant sample volume, a lower headspace volume results in higher HS-SPME sensitivity. With a larger headspace volume, a higher proportion of analytes is distributed into the headspace, while a lower proportion is absorbed onto the fiber. Therefore, a small headspace volume could reduce the equilibration time (Yang & Peppard, 1994).

A previous study showed that the distance between the fiber and the sample during extraction significantly affects the quantity of absorbed VOCs. Moreover, the lower the time of extraction, the stronger the dependence on the fiber-sample distance (Kamgang Nzekoue, et al., 2020).

2. Materials and methods

2.1 Materials

The wines were prepared by the traditional red winemaking process using *Vitis amurensis* Rupr Gongniang No.1. The SPME holder for manual sampling, glass vials, and fiber were purchased from Supelco (Aldrich,

Bellefonte, PA, USA). The SPME fibers (Supelco, Bellafonte, PA, USA) were coated with 65 μm DVB/CAR/PDMS.

2.2 HS-SPME extraction

Wine samples (1 ~ 5.0 mL) were accurately measured into 20.0 mL headspace vials, and 0.0 ~ 1.5 g NaCl was added to each vial to promote the volatilization of the flavor components. The sealed headspace vials containing wine samples were equilibrated at 30 ~ 60℃ for 5 ~ 20 mins, and the SPME fiber (65 μm DVB/CAR/PDMS) was exposed to the VOCs for 10 ~ 30 min. Finally, the fiber was withdrawn and introduced to a heated GC injection port at 250℃ to desorb the volatile compounds. The experimental values of the factors in HS-SPME extraction are displayed in Table 1. The effect of pH on HS-SPME extraction was not investigated due to the wine itself being acidic (pH = 3.18).

Table 1
Experimental values of the factors in HS-SPME extraction.

Sample volume (mL)	Sample concentration (%)	NaCl (g)	Agitation (rpm)	Extraction temperature (℃)	Extraction time		Desorption time (min)
					Equilibrium (min)	Absorption (min)	
1	60	0.00	200	30	5	10	2
3	100	0.25	300	40	7	15	5
5		0.50	400	50	10	20	10
		0.75	500	60	15	25	
		1.50	600		20	30	

2.3 GC–MS analysis

The analyses were conducted using a GC-MS (Thermo scientific ISQ series single quadrupole GC-MS) equipped with a split/splitless injector in the splitless mode and set to 250℃ during the chromatographic run. VOCs were separated in a capillary column (DB-WAX, 30 m × 0.25 mm ×0.25 um film thickness) using helium (99.99%) as carrier gas at a 1.5 mL/min flow rate. The oven temperature was set at 40℃ for 1 min, then warmed to 220℃ at 10℃ /min. The ion source and transfer-line temperatures were 250℃ and 230℃, respectively; the range of m/z from 30 to 450 amu was scanned. Compounds were identified by comparing the results with the NIST 15 mass spectrometry database.

2.4 Data processing and statistical analysis

The quantity of volatiles and peak area ratio (%) were used to investigate the influence of various factors on the detection results. The volatiles with a probability of ≥ 80% were chosen to calculate the quantity. The “probability” indicated the mass spectral similarity measures (a probability-based matching system), which was used to determine the structure of a compound in GC-MS analysis. Due to the comparatively high probability, the amount of detected volatiles was small, showing no significant difference between the data

(Fig. 1). Therefore, the average values were used to determine the suitable extraction parameters and analyze the effect of factors on the results.

All the measurements were performed at least in triplicate, and the values were plotted using the OriginPro 2021 software. Data were expressed as means ± standard deviations. One-way analysis of variance (ANOVA) was employed to identify significant differences ($p < 0.05$) between data sets with the Tukey test for mean comparison. The ANOVA was performed using the paired comparison plot app in OriginPro 2021 software.

3. Results

3.1 Effect of the extraction temperature

In theory, increasing the temperature would promote the transfer of volatiles from the liquid phase to the gas phase. However, the results showed no significant difference in the amount and peak area % of volatiles with the change in temperature (Fig. 1A).

The extraction temperature test (Table 2) showed that the volatiles obtained were mainly esters. In addition, some compounds showed varying probability at different temperatures, such as ethyl hexanoate and ethyl caprate. In the three parallel experiments, some compounds were not always detected in each test, including ethyl butyrate, isopentyl acetate, and ethyl heptanoate. Despite the poor probability of hexanol (25.50 ~ 44.62 < 80%), its peak was high and it was detected in all test results. In order to reduce operation time and improve experimental efficiency in the subsequent experiments, the extraction was performed at 60℃.

Table 2
The effect of parameters on the detection of volatiles

Compounds		Probability (%)	Peak area (%)	Extraction temperature (°C)
Ethyl butyrate	$C_6H_{12}O_2$	80.98–94.24	0.56–2.70	30, 40, 50, 60(2)
Isopentyl acetate	$C_7H_{14}O_2$	80.93–86.25	3.18-6.00	30, 40, 50(2), 60
Ethyl hexanoate	$C_8H_{16}O_2$	67.37–80.04	16.86–24.30	30, 40, 50, 60
Ethyl heptanoate	$C_9H_{18}O_2$	83.32–88.63	0.36–0.57	30, 50(2), 60
Ethyl caprylate	$C_{10}H_{20}O_2$	86.42–91.16	26.20-36.75	30, 40, 50, 60
Ethyl caprate	$C_{12}H_{24}O_2$	77.79–83.81	3.55–8.46	30, 40, 50, 60
1-Hexanol	$C_6H_{14}O$	25.50-44.62	5.80–9.53	30, 40, 50, 60
Phenylethyl Alcohol	$C_8H_{10}O$	80.43–89.90	1.81–5.77	30, 40, 50, 60
				Equilibrium time (min)
Ethyl butyrate	$C_6H_{12}O_2$	48.92–92.60	0.74–2.91	5, 7, 10, 15, 20(1)
Isopentyl acetate	$C_7H_{14}O_2$	58.04–83.48	2.44–6.54	5, 7, 10, 15, 20
Ethyl hexanoate	$C_8H_{16}O_2$	64.46–82.08	12.91–20.57	5, 7, 10, 15, 20
Ethyl heptanoate	$C_9H_{18}O_2$	82.65–89.78	0.36–0.54	5(1), 7, 10(2), 15, 20
Ethyl caprylate	$C_{10}H_{20}O_2$	80.30-88.89	31.63–41.80	5, 7, 10, 15, 20
Ethyl caprate	$C_{12}H_{24}O_2$	74.11–81.95	6.00-10.70	5, 7, 10, 15, 20
1-Hexanol	$C_6H_{14}O$	33.04–47.75	2.96–7.45	5, 7, 10, 15, 20
Phenylethyl Alcohol	$C_8H_{10}O$	80.44–87.32	1.47–3.67	5, 7, 10(2), 15(2), 20

The range of the values of the probability and peak area % represents all results in a single factor experiment. The value in a parenthesis represents the number (< 3) of times a compound appears in three parallel test.

Compounds		Probability (%)	Peak area (%)	Extraction temperature (°C)
				Agitation (rpm)
Ethyl butyrate	$C_6H_{12}O_2$	35.56–86.74	0.12–3.27	200, 300(2), 400, 500(2), 600
Isopentyl acetate	$C_7H_{14}O_2$	72.21–83.48	2.44–4.35	200, 300, 400, 500, 600
Ethyl hexanoate	$C_8H_{16}O_2$	64.52–82.08	12.91–21.20	200, 300, 400, 500, 600
Ethyl heptanoate	$C_9H_{18}O_2$	82.25–89.71	0.38–0.55	200, 300(2), 400(2), 500, 600(2)
Ethyl caprylate	$C_{10}H_{20}O_2$	80.30-88.95	36.83–43.37	200, 300, 400, 500, 600
Ethyl caprate	$C_{12}H_{24}O_2$	74.35–81.87	7.67–10.70	200, 300, 400, 500, 600
1-Hexanol	$C_6H_{14}O$	27.00-49.59	4.51–7.45	200, 300, 400, 500, 600
Phenylethyl Alcohol	$C_8H_{10}O$	79.95–88.12	1.42–3.67	200, 300(2), 400(2), 500(2), 600
1,1-diethoxy-3-methyl-Butane	$C_9H_{20}O_2$	85.53	0.04	300(1)
				Absorption time (min)
Ethyl butyrate	$C_6H_{12}O_2$	59.39–92.48	0.99–2.60	10(2), 15, 20, 25, 30
Isobutyl acetate	$C_6H_{12}O_2$	81.94–85.09	0.15–0.17	20(1), 30(1)
Isopentyl acetate	$C_7H_{14}O_2$	68.66–84.31	3.30–5.44	10, 15, 20, 25, 30
Ethyl hexanoate	$C_8H_{16}O_2$	61.33–84.25	15.58–23.75	10, 15, 20, 25, 30
Ethyl heptanoate	$C_9H_{18}O_2$	83.36–88.54	0.44–0.65	10(2), 15, 20, 25, 30
Ethyl caprylate	$C_{10}H_{20}O_2$	86.46–96.71	31.26–39.17	10, 15, 20, 25, 30
Ethyl caprate	$C_{12}H_{24}O_2$	76.38–82.07	7.02–10.36	10, 15, 20, 25, 30

The range of the values of the probability and peak area % represents all results in a single factor experiment. The value in a parenthesis represents the number (< 3) of times a compound appears in three parallel test.

Compounds		Probability (%)	Peak area (%)	Extraction temperature (°C)
1-Hexanol	$C_6H_{14}O$	35.30-55.45	3.37-6.00	10, 15, 20, 25, 30
Phenylethyl Alcohol	$C_8H_{10}O$	79.05–90.40	0.02–3.02	10, 15(2), 20, 25, 30
1,1-diethoxy-3-methyl-Butane	$C_9H_{20}O_2$	84.14–85.49	0.06–0.07	25(1), 30(1)
Homosalate	$C_{16}H_{22}O_3$	86.42	0.08	25(1)
				Sample volumn (mL)
Ethyl butyrate	$C_6H_{12}O_2$	18.62–92.34	1.06–4.79	1(2), 3, 5
Isopentyl acetate	$C_7H_{14}O_2$	68.66–83.85	2.30–4.36	1, 3, 5
Ethyl hexanoate	$C_8H_{16}O_2$	73.80-81.93	0.12–20.51	1, 3(1), 5
Ethyl heptanoate	$C_9H_{18}O_2$	84.41–88.72	0.20–0.63	1(2), 3, 5
Ethyl caprylate	$C_{10}H_{20}O_2$	87.29–89.90	20.36–36.80	1, 3, 5
Ethyl caprate	$C_{12}H_{24}O_2$	76.88–82.39	4.61–8.05	1, 3, 5
1-Hexanol	$C_6H_{14}O$	35.53–55.45	4.00-5.83	1, 3, 5
Phenylethyl Alcohol	$C_8H_{10}O$	81.08–90.13	1.01–3.21	1, 3, 5
1,1-diethoxy-3-methyl-Butane	$C_9H_{20}O_2$	81.62	0.05	3(1)
Homosalate	$C_{16}H_{22}O_3$	87.74	0.15	1(1)
				Concentration of sample (%)
Ethyl butyrate	$C_6H_{12}O_2$	82.66–93.55	0.15–2.03	60, 100
Isopentyl acetate	$C_7H_{14}O_2$	68.66–86.56	2.76–4.79	60, 100
Ethyl hexanoate	$C_8H_{16}O_2$	75.18–82.12	1.07–20.51	60(2), 100

The range of the values of the probability and peak area % represents all results in a single factor experiment. The value in a parenthesis represents the number (< 3) of times a compound appears in three parallel test.

Compounds		Probability (%)	Peak area (%)	Extraction temperature (°C)
Ethyl heptanoate	$C_9H_{18}O_2$	84.79–88.93	0.30–0.63	60, 100
Ethyl caprylate	$C_{10}H_{20}O_2$	85.76–90.02	18.85–36.80	60, 100
Ethyl caprate	$C_{12}H_{24}O_2$	76.88–82.48	3.93–8.05	60, 100
1-Hexanol	$C_6H_{14}O$	40.45–55.45	2.76–8.36	60, 100
Phenylethyl Alcohol	$C_8H_{10}O$	81.77–88.50	0.97–2.61	60, 100
Homosalate	$C_{16}H_{22}O_3$	80.48	0.06	60(1)
				NaCl (g)
Ethyl butyrate	$C_6H_{12}O_2$	39.31–95.50	0.15–4.60	0, 0.25, 0.5, 0.75, 1.5(2)
Isobutyl acetate	$C_6H_{12}O_2$	84.27	0.18	0.75(1)
Isopentyl acetate	$C_7H_{14}O_2$	68.66–86.12	4.72–1.48	0, 0.25, 0.5, 0.75, 1.5
Ethyl hexanoate	$C_8H_{16}O_2$	55.87–81.93	14.64–21.66	0, 0.25(2), 0.5, 0.75, 1.5
Ethyl heptanoate	$C_9H_{18}O_2$	81.45–90.23	0.26–0.63	0, 0.75, 1.5
Ethyl caprylate	$C_{10}H_{20}O_2$	87.29–91.27	9.06–36.80	0, 0.25, 0.5, 0.75, 1.5
Ethyl caprate	$C_{12}H_{24}O_2$	55.91–83.98	4.90–8.05	0, 0.25, 0.5, 0.75, 1.5
1-Hexanol	$C_6H_{14}O$	34.82–56.47	2.23–9.66	0, 0.25, 0.5, 0.75, 1.5

The range of the values of the probability and peak area % represents all results in a single factor experiment. The value in a parenthesis represents the number (< 3) of times a compound appears in three parallel test.

Compounds		Probability (%)	Peak area (%)	Extraction temperature (°C)
Phenylethyl Alcohol	$C_8H_{10}O$	80.31–87.94	0.49–5.65	0, 0.25(1), 0.5(2), 0.75, 1.5
Ethyl nonanoate	$C_{11}H_{22}O_2$	84.32	0.10	0.75(1)
1,1-diethoxy-3-methyl-Butane	$C_9H_{20}O_2$	93.10	0.12	0.75(1)
Homosalate	$C_{16}H_{22}O_3$	86.98	0.10	1.5(1)

The range of the values of the probability and peak area % represents all results in a single factor experiment. The value in a parenthesis represents the number (< 3) of times a compound appears in three parallel test.

3.2 Effect of the equilibrium time

The equilibrium time test (Table 2) detected the same volatiles as the extraction temperature experiment. The highest average amount of volatiles and the peak area % was observed when the equilibrium time was set to 7 minutes (Fig. 1B). This may be due to a higher extraction temperature, accelerating the volatilization of the aroma.

3.3 Effect of the agitation

The results showed that agitation had no significant effect on the detection results (Fig. 1C). The largest average amount of volatiles was obtained at 200 and 600 rpm. Compared with the extraction temperature experiment, a new compound (1,1-diethoxy-3-methyl-butane) was detected. However, it only appeared once at 300 rpm. This may be attributed to the low concentration of 1,1-diethoxy-3-methyl-butane (0.04 peak area %), which cannot be readily detected. As shown in Table 2, when the stirring speed was set to 200 rpm, the first 8 volatiles all appeared in the three parallel experiments. Therefore, 200 rpm was selected in subsequent experiments.

3.4 Effect of the absorption time

The average amount of volatiles increased as the absorption time was increased, while the change of peak area % was not significant, as displayed in Fig. 1D. When the absorption time was increased to 20 ~ 30min, two new volatiles (isobutyl acetate and homosalate) were detected (Table 2 absorption time test). This might be due to their low concentration, as they were measured only once in the parallel experiments. An absorption time of 30 min was chosen for subsequent experiments to promote the absorption of volatiles.

3.5 Effect of the sample volume

The sample volume had a significant effect on the peak area % (Fig. 1E), which decreased first and then increased, reaching the highest value when the sample volume was 5 mL. Although 1,1-diethoxy-3-methyl-butane and homosalate were isolated, the detection was not stable. These two compounds only appeared in the 3 mL and 5 mL tests, respectively (Table 2). This discrepancy might be attributed to their low

concentration or the sensitivity of the instrument. Most of the compounds were stably detected with a sample volume of 5 mL, indicating that this sample volume was suitable for most analyses.

3.6 Effect of the sample concentration

In this experiment, the samples were diluted to 60% (3 mL sample was mixed with 2 mL water) and compared with the undiluted sample. Figure 1F showed that the diluted sample produced a lower peak area %. Homosalate was only determined in a diluted sample (Table 2).

3.7 Effect of the NaCl

More compounds were detected when NaCl was added (Table 2). As shown in Fig. 1G, low NaCl concentration (0.25 g, unsaturated) had a significant impact on the amount and peak area % of compounds. When NaCl reached saturation (0.5 ~ 1.5 g), the number of compounds and peak area % showed an increasing trend as NaCl was added. Moreover, some volatiles with low peak area%, such as isobutyl acetate, ethyl nonanoate, 1,1-diethoxy-3-methyl-butane, and homosalate, were detected as NaCl reached saturation (0.75 ~ 1.5 g). Therefore, the “salt-out” effect of NaCl might promote the volatilization of low-concentration compounds.

3.8 Effect of the desorption time

The desorption time was set to 2 ~ 10 min during the GC-MS analysis. No significant difference was observed in the results. However, the lowest amount of volatiles was observed with an adsorption time of 2 minutes. Therefore, the desorption time was set to 5 min, as displayed in Fig. 1H.

4. Discussion

In the experiments, a probability of 80% was defined as the criterion to ensure the accuracy of compound structure identification. Different factors or even different levels of the same factor had a significant effect on the matching of volatiles. This could be due to a number of complex reasons, such as instrument sensitivity, changes in conditions, or unavoidable human error. Increasing the extraction temperature could promote the volatilization of aroma and thus shorten the equilibration time. Agitation increased the amount of volatiles detected, but increasing the agitation speed did not have a significant effect on the detection results. Furthermore, extending the absorption time could improve the detection and facilitate the absorption of some low-concentration compounds. Increasing the sample volume improved the stability of the assay results, but impaired the detection of low-concentration substances. This may be due to the fact that high concentrations of some substances hindered the absorption of other low-concentration compounds. This resistance decreased as the sample volume was decreased or the sample was diluted, thus favoring the absorption of volatiles at low concentrations. Saturated NaCl increased the number of detected compounds, while low concentrations of NaCl had the opposite effect, which could be attributed to the low concentration of NaCl increasing the ionic strength of the sample and hindering the transfer of compounds from the liquid phase to the gas phase. Under saturated NaCl conditions, the salting effect reduced the solubility of volatiles and promoted the volatilization of these substances, thus improving the sensitivity of the detection.

Since the differences between the levels in the one-way experiments were not significant, the assay parameters could not be optimized using regression analysis like response surface experiments, so the best assay conditions could only be determined by analyzing the results of the one-way experiments.

As shown in Table 2, an extraction temperature of 60℃ and an equilibrium time of 20 min were chosen for promoting volatilization and enrichment of compounds in the headspace. The agitation was set to 300 rpm to detect 1,1-diethoxy-3-methyl-Butane. In addition, an absorption time of 30 min was selected to ensure adequate absorption of the compound by the fiber. To ensure sufficient volatiles in the headspace, the sample volume, concentration of the sample, and NaCl were set to 5 mL, 100%, and 1.5g, respectively. The results are displayed in Table 3.

Table 3 Experimental results after optimization.

Compounds		Probability (%)	Peak area (%)	Number of occurrences in 5 parallel experiments
Ethyl butyrate	$C_6H_{12}O_2$	86.17	0.5	1
Isobutyl acetate	$C_6H_{12}O_2$	49.32-72.15	0.19±0.04	4
Isopentyl acetate	$C_7H_{14}O_2$	80.58-86.63	3.44±0.26	4
Ethyl hexanoate	$C_8H_{16}O_2$	77.34-84.10	16.75±1.61	5
Ethyl heptanoate	$C_9H_{18}O_2$	65.20-77.85	0.33±0.05	5
Ethyl caprylate	$C_{10}H_{20}O_2$	87.22-92.72	28.98±4.06	5
Ethyl caprate	$C_{12}H_{24}O_2$	53.01-63.48	4.88±0.71	5
1-Hexanol	$C_6H_{14}O$	23.98-46.03	6.68±0.85	5
Phenylethyl Alcohol	$C_8H_{10}O$	77.98-84.74	3.97±0.92	5
1,1-diethoxy-3-methyl-Butane	$C_9H_{20}O_2$	83.42-88.16	0.15±0.36	3
Homosalate	$C_{16}H_{22}O_3$	89.18-90.36	0.08±0.007	2
Diethyl succinate	$C_8H_{14}O_4$	83.21	3.55	1
4-Hepten-2-one	$C_7H_{12}O$	80.97	0.03	1

Except for ethyl nonanoate, all compounds in Table 2 were detected, with two additional substances, namely diethyl succinate and 4-Hepten-2-one. Among them, isobutyl acetate, ethyl heptanoate, ethyl caprate and 1-Hexanol showed a decreased match. Furthermore, compounds with small peak area % appeared less frequently in the parallel experiments. These phenomena may be related to low compound concentration and the sensitivity of the instrument.

5. Conclusion

HS-SPME-GC-MS is widely used for the detection of wine flavor components. The results of the study showed that the sample treatment had a significant effect on the amount of detected flavor components, especially for low-concentration compounds. The results are influenced by the sample treatment methods and the sensitivity of the instrument. In addition, the choice of match (probability) has an impact on the results. For very low levels of volatile component repeatability of parallel experiments is low, but they can be detected by increasing the adsorption time, adding saturated NaCl and increasing the number of parallel experiments.

Declarations

Acknowledgments

This research was supported by the project of Scientific Research of Education Department of Jilin Province (2023) “Comparative Study on the Effect of Selective Addition of Sulfur Dioxide on the Quality of Mountain Wine” (JJKH20230594KJ).

References

1. Azzi-Achkouty, S., Estephan, N., Ouaini, N., & Rutledge, D. N. (2017). Headspace solid-phase microextraction for wine volatile analysis. *Critical Reviews in Food Science and Nutrition, 57*(10), 2009-2020.
2. Comuzzo, P., Tat, L., Tonizzo, A., & Battistutta, F. (2006). Yeast derivatives (extracts and autolysates) in winemaking: Release of volatile compounds and effects on wine aroma volatility. *Food Chemistry, 99*(2), 217-230.
3. Kamgang Nzekoue, F., Angeloni, S., Caprioli, G., Cortese, M., Maggi, F., Marconi, U. M. B., Perali, A., Ricciutelli, M., Sagratini, G., & Vittori, S. (2020). Fiber–Sample Distance, An Important Parameter To Be Considered in Headspace Solid-Phase Microextraction Applications. *Analytical Chemistry, 92*(11), 7478-7484.
4. Lancioni, C., Castells, C., Candal, R., & Tascon, M. (2022). Headspace solid-phase microextraction: Fundamentals and recent advances. *Advances in Sample Preparation, 3*, 100035.
5. Marinaki, M., Sampsonidis, I., Lioupi, A., Arapitsas, P., Thomaidis, N., Zinoviadou, K., & Theodoridis, G. (2023). Development of two-level Design of Experiments for the optimization of a HS-SPME-GC-MS method to study Greek monovarietal PDO and PGI wines. *Talanta, 253*, 123987.

6. Rossi, L., Foschi, M., Biancolillo, A., Maggi, M. A., & D’Archivio, A. A. (2023). Optimization of HS-SPME-GC/MS Analysis of Wine Volatiles Supported by Chemometrics for the Aroma Profiling of Trebbiano d’Abruzzo and Pecorino White Wines Produced in Abruzzo (Italy). In *Molecules*, vol. 28).
7. Schmidt, K., & Podmore, I. (2015). Solid Phase Microextraction (SPME) Method Development in Analysis of Volatile Organic Compounds (VOCS) as Potential Biomarkers of Cancer. *Journal of Molecular Biomarkers & Diagnosis, 06*.
8. Yang, X., & Peppard, T. (1994). Solid-Phase Microextraction for Flavor Analysis. *Journal of Agricultural and Food Chemistry, 42*(9), 1925-1930.
9. Zhang, Y., & Zhang, J. (2008). Optimization of headspace solid-phase microextraction for analysis of ethyl carbamate in alcoholic beverages using a face-centered cube central composite design. *Analytica Chimica Acta, 627*(2), 212-218.

Figures

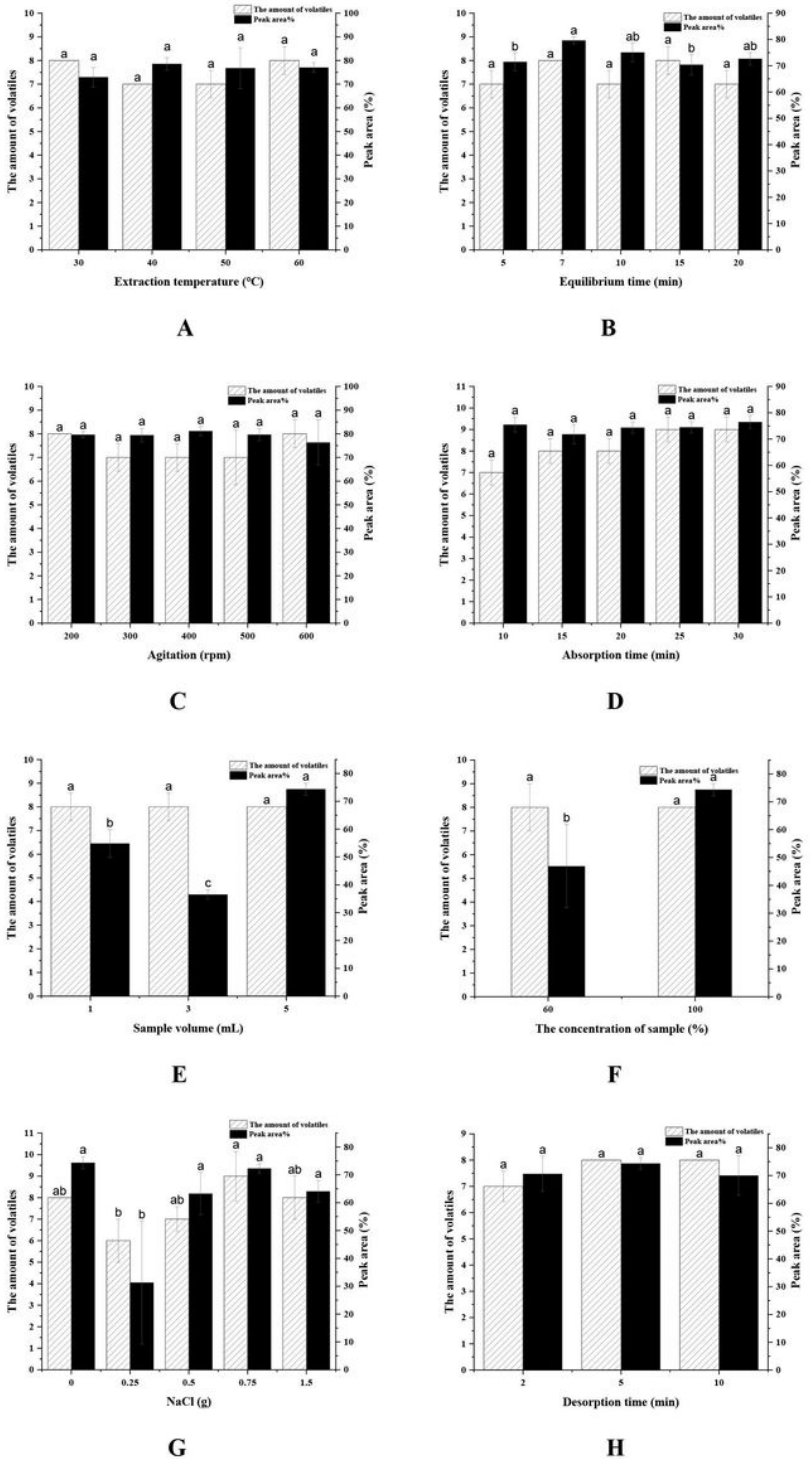

Figure 1

The effect of different factors on the detection of volatiles: (A) extraction time, (B) equilibrium time, (C) agitation, (D) absorption tiem, (E) sample volumn, (F) the concentration of sample, (G) the addition of NaCl.