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ORIGINAL PAPER

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Analysis of volatile components of Petite Arvine wine

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Abstract Petite Arvine is a white grape variety that grows exclusively in the canton of Valais in Switzerland and is used to produce a typical regional wine. In order to elucidate the nature of the flavour compounds that contribute to the characteristic aroma of this wine, the organic extracts were analysed by gas chromatography and olfactometry. The olfactometrically detected zones were further compared with the odour of extracts of vegetal material. 3-Mercaptohexanol, β-ionone and other compounds were identified as main contributors to the characteristic aroma of Petite Arvine wine.

Keywords Petite arvine wine · Wine aroma · Aroma analysis · Olfactometry · 3-Mercaptohexanol · β-Ionone

Introduction

Petite Arvine is an autochthonous grape variety grown in the canton of Valais in Switzerland, which is exclusively used to produce the regional white wine Petite Arvine. This wine is becoming very popular, the acreage of the vineyards and the wine production have more than quintupled in the last few years [1], and it is unlikely that this trend will change in the coming years because of the high demand and consequently the recent increase in the planting of Petite Arvine vines. The characteristic flavour of Petite Arvine wine is described as fruity (grapefruit, wisteria, exotic fruit, rhubarb, quince, etc.). The compounds responsible for the characteristic aroma are still unknown; up to now, their identification has not been the subject of a scientific study. Knowledge of these compounds would allow the study of the genesis of the typical aroma during the wine making and would allow it to be influenced in order to favour the formation of the desired flavour and to prevent the formation of off-flavours. Making Petite Arvine wine is considered to be rather challenging; therefore, the analysis of the characteristic flavour compounds is especially interesting.

Olfactometry is an often-used tool in flavour research of all kinds of foodstuffs. The sniffing of gas chromatography (GC) effluents allows the association of flavour descriptors to chemical constituents as well as the identification of the chemicals that contribute significantly to the overall flavour of a food. The technique has been developed and optimized in parallel to the development of GC [2–8] and has also been applied to alcoholic beverages [9–13]. The olfactometric studies on wine were reviewed by Ferreira et al. [14].

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Materials and methods

Wine samples

The analyses were carried out on 14 samples of Petite Arvine wines. The wines were produced by various winegrowers at different locations in the canton of Valais and were from vintages 2000–2003. The Chasselas and Sylvaner wine samples were also from the canton of Valais, whereas the samples of Chardonnay were partly from the canton of Valais, and partly from other places all over the world.

Extraction of the organic compounds

Wine samples (350 ml) were extracted twice with portions of 100 ml dichloromethane (LiChrosolv, Merck, Darmstadt, Germany) in a 1-l Erlenmeyer flask under magnetic stirring for 5 min. The aqueous and the organic phases were divided in a separating funnel. The combined organic phases were centrifuged at 3,000*g* (Suprafuge 22, Heraeus Sepatech, Osterode, Germany) to break the emulsion. The two phases were again divided in a separating funnel. The resulting organic phase was dried over Na_2SO_4 (Fluka, Buchs, Switzerland) and concentrated to a final volume of 0.350 ml by rotatory evaporation under reduced pressure and a nitrogen stream. For the olfactometric and GC quantification analyses, 2-

octanol (Fluka) was added to the wine samples as an internal standard. For the analysis of the organic compounds, five different wines of Petite Arvine were used, and all the analyses were done in duplicate.

Extraction of thiol compounds

The extraction was carried out according to the method developed and optimized by Tominaga et al. [15, 16]. In this method, the thiols are isolated by a specific complexation with *p*-hydroxymercuric benzoic acid from an organic extract of wine.

Extraction of the organic compounds of quince jelly

Homemade quince jelly (250 g) was extracted with 200 ml dichloromethane (LiChrosolv, Merck) under continuous stirring overnight at room temperature. The organic phase was separated by decanting, filtered through cotton wool and dried over Na_2SO_4. The volume was reduced by rotatory evaporation under reduced pressure and a nitrogen stream to approximately 50 μl.

Extraction of the organic compounds of wisteria flowers

Wisteria flowers were picked from a home garden, the blossoms separated from the stalk and leaves and analysed the same day. The blossoms (10 g) were soaked in 250 ml diethyl ether (Siegfried, Zofingen, Switzerland) and extracted at room temperature under gentle stirring for 10 min. The organic phase was separated by decanting, filtered through cotton wool and dried over Na_2SO_4. The volume was reduced by rotatory evaporation under reduced pressure and a nitrogen stream to approximately 50 μl.

Analysis of the volatile compounds by GC–flame ionization detection and olfactometry

The organic extracts were injected into a GC system (HRGC 5300 Mega Series, Carlo Erba Instruments, Milan, Italy), equipped with a BP 20 column (50 m×0.22 mm×0.25 μm; SGE, Melbourne, Australia) and operated under the following conditions: initial temperature 40 °C for 1 min, temperature program at a rate of 3 °C/min to 230 °C and 230 °C for 10 min. The injector port was heated to 240 °C. Helium was used as a carrier gas at a pressure of 100 kPa. The injection volume was 2 μl. A flame ionization detector at 300 °C and supplemented with air (100 kPa) and hydrogen (80 kPa) was used. The data were acquired using ChromCard, version 1.07 (Thermo Quest, Milan, Italy). For olfactometric analyses the GC system was extended with a sniffer port (Sniffer 9000, Brechbühler, Schlieren, Switzerland). The intensities of the olfactometrically detected zones were registered on an integrator (HP 3394 A Integrator, Hewlett-Packard, Palo Alto, USA). The surfaces of the peaks were corrected by the surface of the internal standard (2-octanol) detected by the flame ionization detector to prevent biases due to extraction and injection differences. Nitrogen was used as a make-up gas at 50 kPa and the sniffer port was supplemented with humidified air at 100 kPa.

Analysis of thiol compounds by GC-flame photometric detection

The thiol compounds were analysed using the same GC system as described earlier. An aliquot of 3 μl was injected in the splitless mode. A flame photometer detector was used supplemented with air (70 kPa) and hydrogen (150 kPa), at a top temperature of 160 °C; the bottom temperature being 300 °C.

Identification and quantification of the volatile compounds by GC-mass spectrometry

The volatile compounds were analysed by GC–mass spectrometry (MS) using the following conditions: GC HP Series II 5890 (Hewlett-Packard), supplemented with a mass-selective detector, HP 5971A (Hewlett-Packard). The gas chromatograph was equipped with a HP-WAX column (25 m×0.22 mm×0.4 μm; Hewlett-Packard). This column corresponds in its properties to the BP 20 column used for GC–flame ionization detection (FID) and olfactometry (see the section "Analysis of the volatile compounds by GC–flame ionization detection and olfactometry"). Helium was used as the carrier gas at 85 kPa. The injector and the detector had temperatures of 250 and 300 °C, respectively. The same temperature profile was used as for the GC–FID/flame photometric detection and GC–olfactometry analyses (see the section "Analysis of the volatile compounds by GC–flame ionization detection and olfactometry").

The sample volume injected into the GC was 0.2 μl. For a semi-quantitative estimation of the amounts of the compounds identified the area of the peaks was compared with the area of the internal standard, and the concentrations were calculated using a response factor of 1. The samples were analysed in duplicate, and the mean value was calculated.

Quantification of phenylethanol and phenylethyl acetate

Phenylethanol and phenylethyl acetate were quantified after solid-phase microextraction and GC analysis as described by Rodriguez-Benecomo et al. [17] but with slight modifications. The wine sample (10 ml) and NaCl (2.9 g, Fluka) were placed in a 20-ml vial (BGB-Analytik, Adliswil, Switzerland). The volatiles present in the headspace of the vial were adsorbed by a polydimethylsiloxane 100 fibre (Supelco, Bellefonte, USA) at a sample temperature of 30 °C for 40 min. The volatiles were analysed by GC–FID after desorption of the fibre in the splitless injector of the gas chromatograph at 300 °C for 2 min. The GC (6890 N, Agilent Technologies, Palo Alto, USA) was equipped with a DB-Wax column (30 m×0.25 mm×0.25 μm, J&W Scientific, Folsom, USA) and operated at the following conditions. The initial temperature was 40 °C for 1 min. Then, the temperature was raised to 230 °C at a rate of 5 °C/min. Helium was used as the carrier gas at a flow rate of 1 ml/min. The flame ionization detector (300 °C) was supplemented with 40 ml/min hydrogen and 400 ml/min air. Quantification was done by using 2-phenylethyl acetate and 2-phenylethanol (both Fluka) as external standards and a calibration curve. All analyses were carried out in duplicate and the mean values were used for calculation.

Results and discussion

Compounds present in the organic extract of Petite Arvine wine

The gas chromatogram of an organic extract of Petite Arvine wine is shown in Fig. 1. The corresponding substances are listed in Table 1. The retention times correspond to the conditions used for the GC–MS analyses.

A total of 44 compounds were detected by GC–MS. All the compounds identified are well-known constituents of the organic fraction of wine and have been described in numerous publications and reviews [18–21]. These compounds are mainly formed during alcoholic fermentation. The concentrations found in Petite Arvine extracts are on the same order of magnitude as the values found for other white wines [20–24].

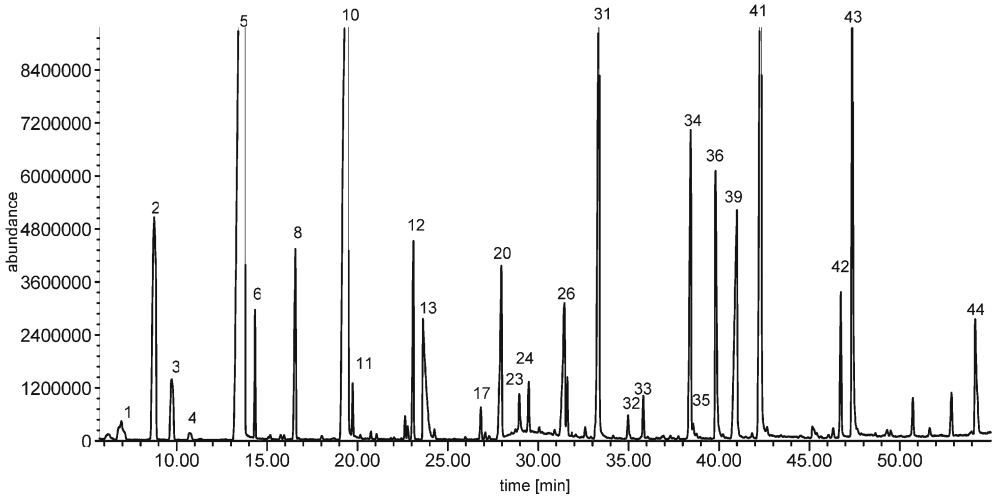

Fig. 1 Gas chromatogram of an organic extract of Petite Arvine wine. The *numbers* correspond to the peak numbers in Table 1. For reasons of legibility, not all peaks are numbered (for experimental details see the text).

Table 1 Identification and amounts of compounds present in Petite Arvine wines. A total of five wines were analysed (n=2).

Peak number	Retention time	Compound	Concentration (mg/l)
1	6.73	Ethylbutyrate[a,b,c]	0.4–1.2
2	8.45	2-Methyl-1-propanol[a,b,c]	2.3–10.2
3	9.69	Isoamylacetate[a,b,c]	0.5–3.0
4	10.42	Butanol[b]	0.09–0.3
5	13.46	3-Metylbutanol[a,b,c]	55–75
6	14.32	Ethylhexanoate[b,c]	0.079–1.8
7	15.90	Hexylacetate[b]	1.0–1.78
8	16.04	3-Hydroxy-2-butanone[b]	0.5–4.5
9	16.39	2-Octanone[b]	0.83–0.9
10	18.88	Ethylactate[b]	11.4–40
11	19.44	1-Hexanol[b]	0.8–2.5
12	22.94	Ethyloctanoate[b]	1.3–7.2
13	23.29	Acetic acid[b,c]	2.5–4.3
14	23.81	Furfural[b]	0–2.9
15	23.89	Heptan-1-ol[b]	3.1–3.7
16	24.10	ni	0.14–0.51
17	26.39	3-Hydroxybutanoic acid, ethyl ester[b]	0.17–0.58
18	27.08	2*H*-Thiopyran-3-(4*H*)-one dihydro[b]	ni–0.13
19	27.72	2-(Methylthio)ethanol[b]	0.05–0.31
20	28.39	1,3-Butandiol[b]	1.3–4.7
21	28.51	1-Octanol[b]	0.06–0.14
22	28.71	ni	0.09–0.17
23	28.95	Dimethyl propandioic acid[b]	0.34–0.98
24	29.84	2,3-Butandiol[b]	0.13–1.1
25	30.00	1,2-Propandiol[b]	0.07–0.1
26	30.74	Butyrolactone[b]	1.0–4.6
27	31.42	Butanoic acid[b]	ni–0.27
28	31.68	Decanoic acid, ethyl ester[b]	0.64–1.44
29	32.56	ni	0.84–0.24
30	33.08	3-Methylbutanoate[b]	0.08–0.52
31	33.23	Diethylsuccinate[b]	0.57–15
32	34.43	3-Methylthiopropanol[b,c]	0.23–0.65
33	35.25	ni	0.44–1.6
34	37.85	Methyl-2-tetrahydrothiophene[b]	0.67–7.1
35	38.25	2-Phenylethyl acetate[a,b,c]	0.24–1.6
36	39.30	Hexanoic acid[a,b,c]	0–6.3
37	39.67	Ethyldodecanoate[b]	0–0.24
38	40.22	ni	0.19–0.86
39	40.33	Phenylmethanol[b]	0.27–0.53
40	40.50	4-Hydroxy-3-methyl-2-butanone[b]	0.15–0.23
41	41.79	Phenylethanol[a,b,c]	17–58
42	46.33	Diethylmalate[b]	3.9–7.2
43	47.0	Octanoic acid[a,b]	0.07–1.92
44	54.23	Decanoic acid[b]	0.16–3.2

ni not identified
[a] Identification by comparing reference substances
[b] Identification by gas chromatography (*GC*)–mass spectrometry (*MS*) by comparing the spectrum with the spectra in the nbs library
[c] Identification by olfactometry

Table 2 Olfactometrically perceived odours in an organic extract of a Petite Arvine wine

Peak number	Retention time	Odour perceived	Compound identified	Intensity	Olfactometric zone
1	14.5	Sweet caramel	Ethyl butyrate[a,b,c]	m	
2	15.32	Sweet	ni	w	W
3	16.00	Alcoholic, fruity	2-Methyl-1-propnaol[a,b,c]	m	
4	16.75	Alcoholic	ni	w	
5	18.2	Banana, ester	Isoamyl acetate[a,b,c]	s	
6	20.2	Flowery, wisteria, burnt	ni	w	W
7	22.5	Rancid almonds, pungent	3-Methylbutanol[a,b,c]	s	
8	23.90	Pear, ester	Ethyl hexanoate[b,c]	m	
9	28.9	Flowery, wisteria	ni	w	W
10	32.8	Vinegar	Acetic acid[b,c]	s	
11	33.2	Exotic fruit, passion fruit	Ethyl octanoate[b]	m	
12	43.17	Sourish, stinky	ni	s	
13	45.19	Sweaty, rubbery, stinky	ni	s	
14	48.68	Jelly, quince	ni	s	Q
15	50.25	Flowery	ni	w	
16	50.57	Slightly pungent, rancid	ni	w	
17	51.26	Rose, flowery, honey	Phenylethyl acetate[a,b,c]	s	W
18	52.86	Rancid	Hexanoic acid[a,b,c]	m	
19	53.22	Thiolic, burnt	ni	w	
20	53.3	Rhubarb, fruity	3-Mercaptohexanol[a,c]	s	
21	56.15	Rose	Phenylethanol[a,b,c]	s	W
22	57.39	Violet	β-Ionone[a,c]	s	W

w weak, *m* medium, *s* strong, *W* olfactometric zone also detected in an organic wisteria flower extract, *Q* olfactometric zone also detected in an organic quince jelly extract
[a] Identification by injecting reference substances
[b] Identification by GC-MS by comparing the spectrum with spectra in the nbs library
[c] Identification by olfactometry

Compounds present in the thiol extract

Thiols have been identified to be important for the characteristic aroma of Sauvignon blanc and other wines [15, 23, 25]. They have a low odour threshold and occur only in traces in wine. In the thiol extract of Petite Arvine, four thiol compounds could be detected, three of which were identified by injecting reference substances: mercaptoethyl acetate (retention time 36.3 min), 3-mercaptopropanyl acetate (41.9 min), 3-mercaptohexanol (53.3 min) and one unidentified compound (54.5 min).

Mercaptoethyl acetate and 3-mercaptopropanyl acetate have been previously identified in Sauvignon blanc, where they are responsible for the "grilled" and "roasted meat" aroma [26]. In the present study these two substances were not detected by olfactometry (see later), and can therefore be considered as not important for the characteristic aroma of Petite Arvine wine. The olfactometric zone of 3-mercaptohexanol, however, was very strong, reminiscent of rhubarb. 3-Mercaptohexanol was identified first in passion fruit [27], and later in Sauvignon blanc [15] and other wines [23, 25]. Comparing the chromatograms of the thiol extract of Petite Arvine with that of Sauvignon blanc, we could detect fewer substances, and the amounts of the different thiols are considerably lower in Petite Arvine than in Sauvignon blanc (results not shown).

Olfactometry

Olfactometry is an excellent tool to associate the substances detected in the form of peaks in gas chromatograms with their odour. In this way, the aroma-relevant substances of a foodstuff can be distinguished from the irrelevant ones. The most important peaks detected by GC–olfactometry of organic extracts of Petite Arvine wine are shown in Fig. 2 and Table 2. Only zones that could be detected in several samples are reported. The retention times correspond to the conditions used in GC–FID coupled to olfactometry.

About 50% of the detected olfactometric zones could not be attributed to an identified flavour compound. Furthermore, some of the olfactometric zones do not seem to make an important contribution to the typical flavour of Petite Arvine wine. Either the odour is unpleasant (peaks 4, 12, 13, 16) or the zone was only perceived weakly (peaks 2, 6, 9, 15, 19). Peak 14, however, was very strong and had an aroma (quince jelly note) that had been described in sensory evaluation of Petite Arvine wines and was correlated positively with the typicity (results not shown). In addition, peaks 20 and 22 seem to contribute to the typical aroma of Petite Arvine wine as well. These zones could not be detected in organic extracts of other wine varieties, such as Chasselas and Chardonnay (data not shown).

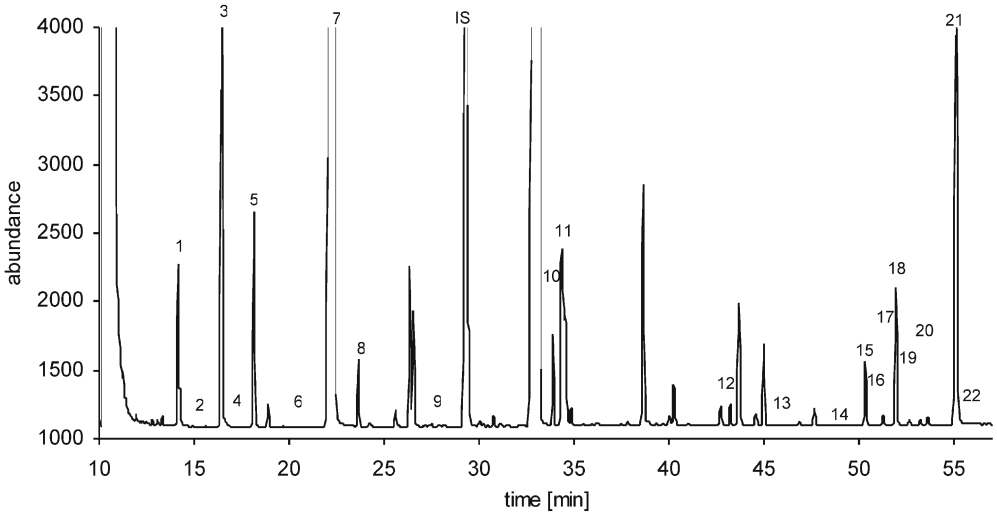

Fig. 2 Chromatogram of an organic extract of Petite Arvine wine, with the olfactometric zones. The *numbers* correspond to those in Table 2. For the conditions of the gas chromatography–flame ionization detection analysis see the "Materials and methods".

Identification and quantification of some aroma compounds which contribute to the characteristic flavour of Petite Arvine

Olfactometric analyses of the wine extracts revealed an intense zone at a retention time of 48 min (peak 14, Fig. 2, Table 2). The zone exhibited a typical quince (*Cydonia oblonga*) jelly aroma. To identify the chemical nature of this compound, quince jelly was extracted and analysed under the same conditions. In the organic extract of quince jelly a peak exhibiting the same olfactometric properties was detected at the same retention time as in the wine extract. However, the substance could not be identified up to now. Tsuneya et al. [28, 29] identified the so-called marmelo lactones [(+)- and (−)-2,7-dimethyl-4-hydroxy-5,7-octadienoic acid lactone] as important aroma compounds in quince extracts. However, these substances were identified at another retention time in our organic quince extract. Schreyen et al.[30] found ethyl-2-methyl-2-butenoate to be an important contributor to the quince aroma. Since the concentration determined in the quince extract was high [30], and the odour threshold of 65 μl/l is relatively high [31], it is improbable that this ester is responsible for the olfactometric zone in which peak 14 was included. Furthermore, it can be excluded that peak 14 corresponds to a thiol, since no peak was detected at a retention time of 48 min in the thiol extract (see the section "Compounds present in the thiol extract"). Additional work has therefore to be invested in the identification of the substance exhibiting a strong quince jelly aroma.

In tastings of Petite Arvine wine the aroma of wisteria flower (*Wisteria sinensis*) is often mentioned. To check the nature of the substances responsible for this association, an organic extract of wisteria flower was prepared and analysed by GC–olfactometry and compared with the wine extract. Several olfactometric zones were identical to zones perceived in the organic extract of Petite Arvine wine. Some of the substances responsible for these odours were identified. The intensities of the unidentified olfactometric zones are weak and therefore not of interest.

Phenylethanol and phenylethyl acetate are well-known products of alcoholic fermentation. Both substances are degradation products of the amino acid phenylalanine [32] and have a strong flowery characteristic reminiscent of rose. Phenylethanol has been isolated from rose oil, the essential oil of other flowers and from alcoholic beverages [33]. Both compounds have been described to be present in wisteria flower as well [18].

Phenylethyl acetate and phenylethanol were shown to be present in considerable quantities in Petite Arvine wine and in two other wine varieties (Chardonnay and Sylvaner) as well (Table 3).

The concentrations of both compounds are not significantly different between the three wine varieties. The relative standards are high, especially for the concentrations of phenylethyl acetate.

This result indicates neither phenylethyl acetate nor phenylethanol plays a role in the typical aroma of Petite Arvine wine. The concentration of phenylethyl acetate in Petite Arvine is in all samples below the threshold value of 250 μg/l [22], whereas the concentration of phenylethanol is consistently above the threshold value of 10 mg/l [22]. Phenylethanol probably plays a role in the overall flowery aroma of wines. According to Rapp and Güntert [35] the concentrations of both compounds are considerably higher in very young wines. This may explain the differences in the concentrations of phenylethanol found in the wines shown in Table 1, where one wine sample was very young. In microvinification experiments samples with very high concentrations up to 940 and 21 mg/l of phenylethyl acetate and phenylethanol, respectively, were measured (data not shown). According to the literature the concentrations of esters and fusel alcohols are high immediately after alcoholic fermentation and decrease over time [36].

β-Ionone was perceived olfactometrically in the extract of Petite Arvine wine as well as in the organic extract of wisteria flowers. The presence of β-ionone in

Table 3 Concentration of phenylethyl acetate and phenylethanol in different samples of Petite Arvine, Chardonnay, and Sylvaner wines (n=2)

Wine sample, origin and vintage	Phenylethyl acetate (μg/l)	Phenylethanol (mg/l)
Chardonnay		
Chardonnay, Chamoson, 2001	72	9.85
Chardonnay, Fully, 2000	54	9.85
Chardonnay, Saillon, 2001	147	12.0
Chardonnay, Sierre, 2001	139	16.7
Chardonnay, Saillon, 2001	71	9.81
Chardonnay, Leytron, 2001	97	11.9
Mean value	96.7 (rsd 39.8)	11.7 (rsd 22.8)
Petite Arvine		
Petite Arvine, Corin/Sierre, 2002	131	12.6
Petite Arvine, Saillon, 2002	169	10.5
Petite Arvine, Fully, 2000	47	11.1
Petite Arvine, Muraz/Sierre, 2002	181	18.0
Petite Arvine, Sierre, 2000	179	19.9
Petite Arvine, Ollon, 2001	87	13.2
Petite Arvine, Chamoson, 2000	139	14.5
Petite Arvine Leytron, 2000	53	11.8
Petite Arvine, Vétroz, 2000	46	14.5
Petite Arvine, Sion, 2000	109	11.2
Petite Arvine, Loèche, 2000	116	13.3
Mean value	114 (rsd 44.8)	13.7 (rsd 21.5)
Sylvaner		
Sylvaner, Chamoson, 2001	287	21.0
Sylvaner, Chamoson, 2002	143	11.9
Sylvaner, Sierre, 2001	142	17.2
Sylvaner, Chamoson, 2002	185	14.7
Mean value	189 (rsd 36.0)	16.2 (rsd 23.8)

rsd relative standard deviation

wisteria flowers has been described previously [34]. This compound has a very low threshold value of 30 ng/l in water [37] and of 90 ng/l in wine [38, 39]. The compound has also been identified in other food items, such as tomatoes [40], rhubarb [41] and grapefruit oil [42] as well as in red [38, 39, 43–45] and white wines [46]. The concentrations found in red wine reached 337 ng/l [39].

In the present study β-ionone was identified by olfactometry (strong intensity) and by injection of a reference substance; however, the concentration of β-ionone in the organic extract of Petite Arvine was too low to be detected by GC-MS.

Conclusions

The organic extract of Petite Arvine wine was analysed by GC–olfactometry and GC–MS. Most of the olfactometrically detected zones could be attributed to an identified flavour compound. 3-Mercaptohexanol and β-ionone seem to contribute to the typical aroma of this wine variety together with some hitherto unidentified compounds. The contribution of phenylethanol and phenylethyl acetate to the typical Petite Arvine aroma was disproved. These two aroma compounds have been shown to be present in two other wine varieties in similar concentrations.

The identification of the as yet unidentified compounds should be pursued further in order to elucidate the overall typicity of the Petite Arvine wine aroma.

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