



Quantitative analysis of headspace volatile compounds using comprehensive two-dimensional gas chromatography and their contribution to the aroma of Chardonnay wine



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ABSTRACT

The quantitative determination of volatile compounds of Chardonnay wines using HS-SPME-GC × GC/TOFMS along with the determination of odor activity value (OAV) and relative odor contribution (ROC) of volatiles are reported for the first time. The use of GC × GC/TOFMS for the analysis of Chardonnay wine of Serra Gaucha resulted in the tentative identification of 243 compounds, showing the superior performance of this analytical technique for this specific varietal wine, considering that the number of compounds usually separated by 1D-GC for this type of wine is lower. Furthermore, 42 compounds co-eluted in the first dimension and 34 of them were separated in the second dimension, while the others were resolved by spectral deconvolution (8), which indicates that the conventional 1D-GC/MS may result in misleading results. The calculation of OAV and ROC allowed the determination of the volatile compounds that presented the greater contribution to wine aroma. Ethyl octanoate, ethyl hexanoate, ethyl butanoate, and beta-damascenone showed the highest OAV and ROC values, although other 43 compounds showed also potential to contribute to wine aroma. Figures of merit of the developed method were: accuracies from 92.4 to 102.6%, repeatability from 1.2% to 13.4%, LOD from 0.001 $\mu\text{g L}^{-1}$ (ethyl isovalerate and hexanoic acid) to 2.554 $\mu\text{g L}^{-1}$ (ethyl 3-hydroxybutanoate), LOQ from 0.003 $\mu\text{g L}^{-1}$ (ethyl isovalerate and hexanoic acid) to 7.582 $\mu\text{g L}^{-1}$ (ethyl 3-hydroxybutanoate).

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1. Introduction

Wine aroma is one of the most important factors that influence perceived wine quality and consumer acceptance (Bakker & Clarke, 2011; Martínez-Pinilla, Guadalupe, Ayestarán, Pérez-Magariño, & Ortega-Heras, 2013). Volatile compounds play a significant role to wine aroma and the presence, absence or different proportions of volatile compounds can be greatly influenced by both viticultural (climate, soil, cultivar, grape-growing practices) and enological (condition of grapes, fermentation, postfermentation treatments) factors (Jackson, 2008; Malherbe, Menichelli, du Toit, Tredoux, Muller, Næs, et al., 2013).

Hundreds of volatile compounds have been identified in wines (Robinson, Boss, Heymann, Solomon, & Trengove, 2011; Weldegergis, de Villiers, McNeish, Seethapathy, Mostafa, Gorecki, et al., 2011; Welke, Manfroi, Zanus, Lazarotto, & Zini, 2012). However, not all compounds present in wine contribute to aroma (García-Carpintero, Sanchez-Palomo, Gallego, & Gonzalez-Viñas, 2011; Li, Tao, Wang, & Zhang, 2008); Vilanova, Genisheva, Masa, & Oliveira, 2010). The

influence of a volatile compound to the final aroma depends on its concentration in wine and on the perception threshold of this specific compound. The threshold of olfactory perception is defined as the lowest concentration capable of producing an olfactory sensation and that can be detected by human nose for at least 50% of the judges of a panel of sensory evaluation (Guth, 1997).

The determination of odor descriptors has been done by gas chromatography–olfactometry (GC–O), which uses the human nose as detector. The olfactive evaluation of the compounds by GC–O is performed after their separation in a chromatographic column, when their odor descriptors are perceived and registered, e.g., floral, fruity, green, solvent, plastic, toasted and others (Capone, Tufariello, & Siciliano, 2013; Ferreira, Lopez, & Cacho, 2000; Garcia-Carpintero, Sanchez-Palomo, Gallego, & Gonzalez-Viñas, 2011; Jiang & Zhang, 2010; Juan, Cacho, Ferreira, & Escudero, 2012; Li et al., 2008; Peinado, Moreno, Muñoz, Medina, & Moreno, 2004; Vilanova, Genisheva, Masa, & Oliveira, 2010).

The determination of odor activity value (OAV) has also been extensively used to estimate the sensory contribution of aromatic compounds to the overall aroma of wine and it is usually performed in conjunction with 1D-GC/MS (Capone et al., 2013; Ferreira et al., 2000; Guth, 1997; Jiang & Zhang, 2010; Juan et al., 2012). OAV is obtained from the

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ratio between the concentration of an individual compound and its perception threshold. A volatile compound contributes to aroma when its concentration in wine is above the perception threshold, therefore, odorants with OAV > 1 can be perceived (Guth, 1997). In addition to the use of OAV, which represents a quantitative approach of the contribution of volatile compounds to aroma, a qualitative evaluation can be done based on odor descriptors of each component. The relative odor contribution (ROC) represents the percentage of contribution of a particular aroma compound and it is the ratio of OAV percentage of each individual compound and the sum of the OAV of compounds that showed OAV > 1 (Capone et al., 2013; Ferreira et al., 2000; Guth, 1997; Jiang & Zhang, 2010; Juan et al., 2012; Li et al., 2008; Peinado et al., 2004). Capone et al. (2013) have identified 51 volatile compounds in Negroamaro red wines, and among them, only 18 components were perceived as active odorants (OAV > 1). The compounds related to aroma were mainly alcohols, fatty acids and ethyl esters and the cited sensory descriptors were fruity, floral, fatty, pungent, nutty and caramelized notes.

The most suitable gas chromatography technique for untargeted analysis of complex samples, such as wine, has been shown to be comprehensive two-dimensional gas chromatography (GC × GC) (Welke and Zini, 2011). GC × GC has been quite often used in the determination of volatile compounds in different wine varieties, including Cabernet Franc (Ryona, Pan, & Sacks, 2009), Madeira (Perestrelo, Barros, Câmara, & Rocha, 2011), Cabernet Sauvignon (Robinson, Boss, Heymann, Solomon & Trengove, 2011), Pinotage (Weldegergis et al., 2011), Muscat (Bordiga, Rinaldi, Locatelli, Piana, Travaglia, Coisson et al., 2013) and Marsala (Dugo, Franchina, Scandinaro, Bonaccorsi, Cicero, Tranchida et al., 2014) wines. GC × GC offers superior separation capabilities afforded by high peak capacity, selectivity, structural chromatographic peak organization, and sensitivity enhancement in the same analysis time as in 1D-GC (Marriott & Shellie, 2002). Specially valuable are the enrichment in aroma active trace compounds and the possibility of having two retention data, less co-elutions, and an organized distribution of wine volatile compounds in the 2D space, according to their physico-chemical characteristics, which helps the process of identification of unknown compounds. A former work of this research group on Merlot volatiles has been recently published, where the advantages of GC × GC/TOFMS have been highlighted through a detailed characterization of Merlot volatiles. Also, a preliminary approach to the use of multivariate analysis for discrimination of 24 wine samples according to grape variety has been presented (Welke, Manfro, et al., 2012). Other previous works about qualitative determination of volatile compounds using GC × GC include the analysis of wines produced from different grape varieties (Welke, Manfro, Zanús, Lazarotto, & Zini, 2013) and the differentiation of base wines and the respective sparkling wines based on volatile profile (Welke, Zanús, Lazarotto, Pulgati, & Zini, submitted for publication).

To the knowledge of the authors, there is no published work about the quantification of volatile compounds in Chardonnay wines using GC × GC/TOFMS combined with OAV/ROC approach in order to provide a fast overview regarding concentration of volatiles and odorant impact of the most important compounds of wine aroma. The aim of this study was to use the HS-SPME-GC × GC/TOFMS to quantify volatile compounds of wines produced with Chardonnay grapes in Serra Gaúcha region, Brazil.

2. Material and methods

2.1. Samples, analytical reagents, and supplies

Twelve wine samples (~13% ethanol, v/v) produced with Chardonnay grape variety (*Vitis vinifera* L.) of 2009 vintage, produced in Serra Gaúcha region (latitude 29°S, longitude 51°W, altitude 600–800 m) have been investigated. Each one of them was from different production batches. These samples were provided by the Empresa Brasileira de Pesquisa Agropecuária Uva e Vinho (EMBRAPA) in sealed 750 mL bottles and

were chosen as the best wine samples in the National Evaluation of Wines of 2010, an event promoted by the Brazilian Association of Enology.

Standard compounds ethyl acetate, ethyl butanoate, ethyl propanoate, ethyl 2-methylpropanoate (ethyl isobutanoate), ethyl 2-methylbutanoate (ethyl isovalerate), ethyl hexanoate, ethyl 2-hydroxypropanoate (ethyl lactate), ethyl octanoate, ethyl decanoate, diethyl butanedioate (diethyl succinate), ethyl 2-hydroxybutanoate, 3-methylbutyl acetate (isoamyl acetate), ethyl 2-phenyl acetate, 1-propanol, 1-hexanol, 2-phenylethanol, hexanoic acid, octanoic acid, decanoic acid, dodecanoic acid, 4-terpineol, eugenol, and 3-octanol were purchased from Aldrich (Steinheim, Germany). These compounds were chosen considering their importance to flavor characteristics according to scientific reports (Jaffré, Valentin, Meunier, Siliani, Bertuccioli and Le Fur, 2011; Jiang & Zhang, 2010; Li et al., 2008; Vilanova et al., 2010). Individual stock solutions of each compound (10 mg L⁻¹) were prepared in bidistilled ethanol purchased from Nuclear (São Paulo, Brazil). Ethanol 10% in MilliQ deionised water (purification system Millipore, Bedford, MA, USA) had been employed to prepare a solution of 6 g L⁻¹ of (+)-tartaric acid (Synth, São Paulo, Brazil). The pH was adjusted to 3.5 with sodium hydroxide (Nuclear, São Paulo, Brazil). Calibration curves for each one of the analytes had been made through the addition of the 22 standard compounds to the model wine solution.

The SPME fiber (50/30 divinylbenzenecarboxen-polydimethylsiloxane (DVB/CAR/PDMS) StableFlex) was purchased from Supelco (Bellefonte, PA, USA) and was conditioned according to the manufacturer's recommendations prior to its first use. Sodium chloride (NaCl) of analytical grade, purchased from Nuclear was oven dried at 110 °C overnight before use. Twenty microliter headspace vials with magnetic screw caps sealed with silicone septa had been bought from Supelco (Bellefonte, PA, USA).

2.2. Instrumentation

Extraction of volatile compounds from the headspace of the vials containing samples has been performed with a CTC CombiPAL autosampler (CTC Analytics, Zwingen, Switzerland) with an agitator and SPME fiber. The GC × GC system consisted of an Agilent 6890N (Agilent Technologies, Palo Alto, CA, USA) equipped with a Pegasus IV time-of-flight mass spectrometer (Leco Corporation, St. Joseph, MI, USA). A high polarity column (100% polyethylene glycol; 30 m × 0.25 mm × 0.25 μm, J&W Scientific Inc., Folsom, CA, USA) was used as first-dimension (¹D) column, and a DB-17ms (DB17ms (50%-phenyl)-methylpolysiloxane; 1.70 m × 0.18 mm × 0.18 μm, J&W Scientific Inc., Folsom, CA, USA) was employed as a second-dimension (²D) column. The GC system (Agilent 6890N) was equipped with a secondary column oven and non-moving quadjet dual stage thermal modulator. During modulation, cold pulses were generated using dry nitrogen gas cooled by liquid nitrogen (Linde, Canoas, RS, Brazil), whereas heated dry air was used for hot pulses. The injector, transfer line and ion source temperature were at 250 °C. Oven temperature program conditions were as follows: initial temperature of 35 °C for 5 min, programmed at 3 °C min⁻¹ to 250 °C, where it remained for 5 min. The secondary oven was kept 10 °C above the primary oven throughout the chromatographic run. The modulator was offset by +25 °C in relation to primary oven. Helium (99.9999% purity, White Martins, Porto Alegre, RS, Brazil) was used as carrier gas at a constant flow of 1 mL min⁻¹. The MS parameters included electron ionization at 70 eV with ion source temperature at 250 °C, detector voltage of -1750 V, mass range of 45–450 m/z, and acquisition rate of 100 spectra s⁻¹.

2.3. Conditions for the extraction of volatiles

The SPME extraction was performed according to previous work: 1 mL of wine in 20 mL glass headspace vials, 30% of NaCl (m/v), without sample agitation, extraction time of 45 min and extraction temperature

of 45 °C (Welke, Zanus, Lazarotto, Schmitt & Zini, 2012). The wine samples (10 mL measured with a volumetric pipette), were spiked with 10 µL of alcoholic solution of 3-octanol at 1.25 mg L⁻¹, used as internal standard. All samples were kept at 45 °C for 10 min prior to extraction. The headspace was sampled using a 2 cm DVB/CAR/PDMS 50/30 µm fiber. The volatile and semi-volatile compounds were desorbed in the GC inlet at 250 °C for 5 min and the fiber was reconditioned for 5 min at 260 °C prior to each analysis. All samples were analyzed in triplicate.

2.4. Data processing and identification of compounds

LECO ChromaTOF version 4.22 software was used for all acquisition control, data processing and Fisher Ratio calculations. Automated peak find and spectral deconvolution with a baseline offset of 0.5 and signal to noise of three have been employed during data treatment.

Twenty-two compounds (listed in Section 2.1) were positively identified through comparison of retention time and mass spectra data of unknown compounds with those of authentic standards. Tentative identification of wine volatile compounds had been achieved by comparing experimental linear temperature programmed retention indices (LTPRI) with retention indices reported in the literature for 1D-GC and a description of this procedure has already been reported elsewhere (von Muhlen, Zini, Caramao, & Marriott, 2008). Retention data of a

series of *n*-alkanes (C9–C24), under the same experimental conditions employed for the chromatographic analysis of wine volatiles had been used for experimental LTPRI calculation. Mass spectrometric information of each chromatographic peak was compared to NIST mass spectra library version 2005, considering a minimum similarity value of 80%. Whenever a LTPRI was not found in the scientific literature in order to match with the experimentally determined LTPRI, only the chemical class of the wine volatile compound was assigned.

2.5. Quantitative analysis

Work solutions of the standard compounds were prepared in model wine and the range of concentrations of each one of the compounds is listed in Table 1. The HS-SPME extraction was performed in five replicates for each one of the sample and standard solutions. 3-Octanol was chosen as internal standard and 10 µL of a 0.625 mg L⁻¹ solution in bidistilled ethanol was added to each standard solution and sample.

The analytical parameters determined were: linearity, limits of detection (LOD) and quantification (LOQ), precision (repeatability and intermediate precision) and accuracy. These procedures were done according to International Conference on Harmonization (ICH) guidelines (ICH – International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use,

Table 1

Figures of merit of the HS-SPME-GC × GC/TOFMS method used for the determination of volatile compounds in Chardonnay wines.

#	Compound	Validation range (µg L ⁻¹)	Regression equation ^a	r ²	LOD (µg L ⁻¹)	LOQ (µg L ⁻¹)	LOQ ^b (µg L ⁻¹)
1	Ethyl acetate	9610–20,880	y = 0.0057x + 149.76	0.992	0.432	1.224	1.52 ^c
2	Ethyl propanoate	1200–20,000	y = 0.0194x + 2.6239	0.992	0.004	0.148	4.1 ^d
3	Ethyl butanoate	2500–25,000	y = 0.0145x + 6.8157	0.994	0.034	0.102	0.128 ^f 0.115 ^d 0.88 ^e 0.82 ^d 1.5 ^e
4	Ethyl 2-methyl propanoate (ethyl isobutanoate)	12–168	y = 0.0259x + 0.7643	0.990	0.005	0.017	0.126 ^f 0.37 ^d 0.098 ^e
5	3-Methyl butyl acetate (isoamyl acetate)	18–490	y = 0.0455x + 1.3978	0.981	0.009	0.029	0.0041 ^c
6	Ethyl 2-methylbutanoate (ethyl isovalerate)	3.5–56	y = 1055x + 0.1067	0.995	0.001	0.003	0.123 ^f
7	Ethyl hexanoate	58–660	y = 0.0524x + 31.081	0.998	0.034	0.102	0.0083 ^d 0.072 ^e
8	Ethyl 2-hydroxy propanoate (ethyl lactate)	59,000–688,800	y = 0.0007x – 0.4042	0.991	0.026	0.079	0.096 ^f 9.542 ^c
9	Ethyl octanoate	52,000–260,000	y = 0.5842x + 14.806	0.990	0.004	0.011	0.281 ^f 0.0031 ^d 0.010 ^e
10	Ethyl decanoate	180–7840	y = 0.3236x – 28.933	0.997	0.007	0.019	0.056 ^f 0.0006 ^d 0.023 ^e
11	Ethyl 3-hydroxy-butanoate	4500–72,700	y = 0.0022x + 0.0547	0.994	2.554	7.582	42 ^f 371 ^e
12	Diethyl succinate	24.88–2300	y = 0.0032x + 1.7595	0.998	0.009	0.028	1.170 ^f 0.58 ^e
13	Ethyl 2-phenyl acetate	14.3–460	y = 0.0931x + 0.3078	0.988	0.002	0.007	0.157 ^f 0.024 ^d 0.057 ^e
14	1-Propanol	15–192	y = 9.10 ⁻⁵ x + 0.4967	0.980	0.048	0.112	–
15	1-Hexanol	51–9340	y = 0.0441x – 2.5073	0.983	0.003	0.010	0.348 ^f 0.28 ^e
16	2-Phenylethanol	1400–360,000	y = 0.0024x + 11.309	0.990	0.094	0.285	0.317 ^f 0.2228 ^c
17	4-Terpineol	5–65.3	y = 0.194 + 0.09	0.977	0.297	0.965	3.1 ^e
18	Eugenol	0.05–5.6	y = 0.100 + 0.108	0.972	0.002	0.007	0.056 ^f
19	Hexanoic acid	8.73–78.6	y = 0.0961x – 0.7158	0.979	0.001	0.003	0.048 ^f
20	Octanoic acid	2744–197,200	y = 0.0011x + 37.349	0.948	0.112	0.340	0.679 ^f
21	Decanoic acid	20.2–1608	y = 0.0667x – 2.2706	0.944	0.006	0.017	0.022 ^f
22	Dodecanoic acid	5.48–49.6	y = 0.2921x + 29.027	0.942	0.017	0.052	–

^a x designates concentration of volatile compounds (µg L⁻¹) and y designates ratio of peak area of a volatile compound and peak area of 3-octanol.

^b LOQ found in literature when HS-SPME-1D-GC/MS was used in the determination of the same volatile compound in the headspace of wine.

^c Howard, Mike, and Riesen (2005).

^d Antalic, Perello, and Revel (2010).

^e Nogueira-Pato, González-Barreiro, Cancho-Grande, and Simal-Gándara (2009).

^f Perestrello, Fernandes, Albuquerque, Marques, and Câmara (2006) used liquid–liquid extraction with dichloromethane and 1D-GC/MS.

Table 2
Tentatively identified compounds of Chardonnay wine headspace with their respective concentration, odor threshold, odor activity value (OAV) and odor descriptor.

Compound ^a	CAS	S ^b	LTPRI (exp) ^c	LTPRI (lit) ^d	Conc. ^e (µg L ⁻¹)	RSD (%) ^f	OTS (µg L ⁻¹) ^h	OAV ^g	Odor descriptor ^h	
<i>Alcohols</i>										
1	2-Propanol (isopropanol) ^{db}	63-63-5	909	926	938 ¹	15.25	2.0	Nf ^k	-	Pungent ⁵⁴
2	1-Propanol ^{db}	71-23-8	934	1038	1036 ²	54.45	3.9	306,000 ⁴⁹	<1	Alcohol, ripe fruit ⁴⁹
3	2-Methyl-1-propanol (isobutyl alcohol) ^{db}	75-65-0	890	1091	1093 ³	110.67	6.7	75,000 ⁴⁹	<1	Alcohol ⁴⁹
4	1-Penten-3-ol ^{db}	616-25-1	934	1102	1112 ⁴	60.09	4.8	400 ^{53,i}	<1	Green ⁵³
5	1-Butanol ^{db}	71-36-3	903	1156	1165 ²	160.88	4.3	150,000 ⁴⁹	<1	Medicinal ⁴⁹
6	3-Methyl-1-butanol (isoamyl alcohol) ^{db}	137-32-6	940	1208	1209 ³	65.72	8.3	60,000 ⁴⁹	<1	Solvent ⁴⁹
7	1-Pentanol (1) ^{db}	71-41-0	913	1245	1233 ⁴	24.45	6.4	64,000 ³²	<1	Almond, synthetic, balsamic ³²
8	3-Methyl-3-buten-1-ol ^{db}	763-32-6	980	1256	1254 ⁵	1200.89	7.5	600 ⁵¹	2.0	Sweet fruity ⁵¹
9	2-Heptanol (18) ^w	543-49-7	934	1315	1316 ⁵	950.21	4.1	200 ⁵¹	4.7	Lemon, orange, copper ⁵¹
10	(E)-2-Penten-1-ol ^w	20273-24-9	850	1326	1337 ⁵⁵	60.87	4.2	89.2 ^{55,i}	<1	Green, plastic ⁵⁵
11	4-Methyl-1-pentanol ^w	626-89-1	980	1333	1330 ²	6740.18	9.5	50,000 ³²	<1	Almond, toasted ³²
12	3-Methyl-1-pentanol ^w	589-35-5	929	1344	1343 ²	9020.90	6.4	50,000 ³²	<1	Vinous, herbaceous, cacao ³²
13	1-Hepten-3-ol (3) ^w	49-3852-7	908	1350	1355 ⁶	95.96	8.5	Nf	-	Nf
14	1-Hexanol ^w	111-27-3	902	1365	1357 ⁵	54.15	6.5	110 ⁴⁹	<1	Herbaceous, grass, woody ⁴⁹
15	3-Methyl-4-penten-1-ol ^w	763-89-3	887	1387	1390 ⁷	690.55	8.2	Nf	-	Nf
16	Cyclohexanol (19) ^l	108-93-0	807	1400	1407 ²⁸	140.12	3.4	300 ⁵²	<1	Camphor like odor, pungent ⁵²
17	3-Ethoxy-1-propanol (4) ^w	111-35-3	935	1405	1409 ²	170.15	9.3	100 ⁵¹	1.7	Fruity ⁵²
18	(Z) 3-Hexen-1-ol (5) ^w	928-96-1	894	1409	1415 ²	880.99	6.9	1000 ⁴⁹	<1	Green, bitter, fatty ⁴⁹
19	Alcohol ^w	-	810	1413	Nf	53.34	4.3	-	-	-
20	(Z)-2-Hexen-1-ol (6) ^w	928-95-0	965	1425	1419 ⁴	70.32	3.1	400 ⁵¹	<1	Green grass, herb ⁵¹
21	1-Octen-3-ol ^w	3391-86-4	971	1439	1444 ⁵⁹	0.97	2.4	160 ⁱ	<1	Chestnut flower, mushroomy ⁵⁹
22	6-Methyl-5-hepten-2-ol (8) ^w	110-93-0	852	1450	1462 ⁶	60.59	1.4	2000 ^{53,i}	<1	Nf
23	2-Ethyl-1-hexanol ^w	104-76-7	899	1478	1474 ⁸	12150.1	7.3	8000 ⁵¹	1.5	Floral ⁴¹ , sweet fruity ⁵¹
24	2-Butyl-1-octanol ^w	3913-02-8	939	1523	1528 ⁹	1.32	6.4	Nf	-	Nf
25	2-Nonanol ^w	628-99-9	955	1526	1530 ⁵	89.23	9.1	58 ^{53,i}	1.5	Fruity, green ⁵³
26	3-Ethyl-4-methylpentanol ^w	100431-87-6	939	1534	1531 ²	9.17	2.9	Nf	-	Nf
27	2-(2-Methoxypropoxy)-1-propanol ^w	83730-60-3	909	1541	1532 ¹⁰	15.07	4.7	Nf	-	Nf
28	2-(2-Methoxy ethoxy)ethanol ^w	111-77-3	872	1568	1576 ¹¹	2.05	5.5	Nf	-	Nf
29	2-Undecen-1-ol ^w	37617-03-1	907	1594	1590 ¹²	8.09	7.6	Nf	-	Nf
30	2,3-Butanediol (9) ^w	513-89-3	932	1600	1598 ²	23.58	8.3	150,000 ³²	<1	Fruity ³²
31	1,2-Propanediol ^w	57-55-6	917	1608	1603 ¹³	10.18	2.3	Nf	-	Nf
32	2,7-Dimethyl-1-octanol (10) ^w	15-250-22-3	878	1618	1628 ¹⁰	44.02	5.3	Nf	-	Nf
33	2-(Ethoxyethoxy)ethanol ^w	111-90-0	876	1637	1636 ¹¹	11.02	1.2	Nf	-	Nf
34	(E)-2-Nonen-1-ol ^w	31502-14-4	905	1690	1697 ¹²	0.95	8.4	Nf	-	Nf
35	4-Butoxy-1-butanol ^w	4161-24-4	897	1706	1701 ¹¹	0.89	3.4	Nf	-	Nf
36	2-Undecanol ^w	1653-30-1	982	1741	1738 ⁵³	98.40	8.4	41 ^{53,i}	2.4	Fruity ⁵³
37	(E)-3-Decenol ^w	10339-60-3	861	1757	1759 ⁶	10.04	6.7	Nf	-	Nf
38	1-Decanol ^w	112-30-1	890	1778	1781 ²	29.89	2.9	400 ⁵²	<1	Sweet, fatty ³
39	(Z)-4-decen-2-ol ^w	57074-37-0	859	1786	1794 ⁵	88.01	7.1	Nf	-	Nf
40	Alcohol (20) ^w	-	858	1805	Nf	10.43	6.7	-	-	-
41	Phenylmethanol (benzylalcohol) ^l	100-51-6	940	1875	1879 ⁵	12376.97	3.4	200,000 ³²	<1	Sweet, fruity ³²
42	3-Methoxy-2-butanol ^w	53778-72-6	941	1889	1903 ¹⁵	81.08	9.0	Nf	-	Nf
43	2-Phenylethanol ^l	60-12-8	930	1947	1946 ³	356,679.48	3.5	200,000 ⁴⁹	1.8	Rose, honey ⁴⁹
44	2,6-Dimethyl-3,7-octadien-2,6-diol ^w	13741-21-4	858	1954	1951 ⁷	20.01	9.6	Nf	-	Nf
45	1-Dodecanol ^w	12-53-8	928	1984	1981 ³	45.98	3.8	1000 ⁵⁷	<1	Unpleasant in higher concentration, flowery in low concentration ⁵⁷
46	1-Tridecanol ^w	112-70-9	853	2066	2063 ¹⁶	10.06	9.3	Nf	-	Nf
47	2-Phenoxyethanol (16) ^l	122-99-6	810	2119	2115 ³⁹	170.49	4.0	Nf	-	Nf
48	Pentadecanol ^w	1653-34-5	986	2200	2197 ¹⁷	9.07	9.2	Nf	-	Nf
49	5-(2-Propynyloxy)-2-pentanol ^w	55702-67-5	876	2272	2273 ¹⁸	6.13	8.8	Nf	-	Nf
50	2-Hexadecanol ^w	14852-31-4	912	2302	2312 ¹⁸	51.40	3.4	Nf	-	Nf
51	1-Phenyl-1,2-propanediol ^l	1855-09-9	943	2465 ^j	2455 ¹⁹	144.15	5.6	Nf	-	Nf
52	1-Heptadecanol ^w	1454-85-9	982	2461 ^j	2475 ²⁰	52.45	3.4	Nf	-	Nf
<i>Acids</i>										
53	4-Methyl-2-oxovaleric acid [§]	816-66-0	876	1434	1421 ²¹	11.48	4.1	Nf	-	Nf
54	Acetic acid [§]	64-19-7	890	1457	1461 ³	5605.98	7.9	20000 ⁵⁰	<1	Pungent, vinegar ³²
55	Propanoic acid [§]	79-09-4	872	1536	1525 ¹⁵	3400.56	6.5	8100 ³²	<1	Pungent, rancid, soy ³²
56	2-Methylpropanoic acid (isobutyric acid) [§]	79-31-2	863	1605	1618 ²	8559.40	3.6	200,000 ⁵⁰	<1	Cheese ²⁶
57	Acid [§]	-	876	1584	Nf	7.94	4.5	-	-	-
58	Butanoic acid (12) [§]	107-92-6	930	1641	1637 ¹⁰	890.89	4.0	10,000 ⁵⁰	<1	Rancid, cheese ³²
59	Acid [§]	-	859	1658	Nf	20.24	3.4	-	-	-
60	3-Methyl butanoic acid (isovaleric acid) (13) [§]	503-74-2	931	1664	1660 ²²	20.87	7.7	3000 ⁵⁰	<1	Rancid, acidic ⁴¹
61	Pentanoic acid [§]	109-52-4	863	1750	1744 ¹⁰	86.76	3.9	3000 ^{60,i}	<1	Cheese ⁵⁸
62	(Z)-2-Methyl-2-butenoic acid (17) [§]	80-59-1	857	1839	1846 ⁵⁹	32.48	6.7	Nf	-	Spicy, pungent ⁵⁹
63	Hexanoic acid [§]	142-62-1	940	1876	1863 ²	49.59	7.3	3000 ⁴⁹	1.5	Cheese, fatty ⁴⁹
64	2-Ethyl-hexanoic acid [§]	149-57-5	979	1956	1950 ³⁵	70.18	1.8	Nf	-	Nf
65	Heptanoic acid [§]	111-14-8	956	1974	1960 ¹⁰	20.34	9.2	Nf	-	Sweaty, cheese ⁵⁸
66	(E)-2-Hexenoic acid [§]	13419-69-7	901	1980	1990 ⁷	12.01	4.7	Nf	-	Nf
67	Octanoic acid [*]	124-07-2	956	2086	2083 ²	160,320.82	6.7	10,000 ⁴⁹	16	Fatty, rancid ⁴⁹
68	Nonanoic acid [*]	112-05-0	904	2090	2091 ²³	1820.65	8.3	3000 ⁴¹	-	Fatty ⁴¹
69	4-Methyl octanoic acid [*]	54947-74-9	963	2167	2173 ²⁴	29.09	6.1	Nf	-	Nf
70	Decanoic acid ^{oo}	334-48-5	942	2287	2296 ²	180.10	5.1	6 ⁴⁹	3	Fatty, rancid ⁴⁹
71	9-Decenoic acid ^{oo}	14436-32-9	917	2350	2348 ²⁵	30.56	9.3	40 ⁵²	<1	Waxy, fatty, soapy ⁵²

Table 2 (continued)

Compound ^a	CAS	S ^b	LTPRI (exp) ^c	LTPRI (lit) ^d	Conc. ^e (µg L ⁻¹)	RSD (%) ^f	OTS (µg L ⁻¹) ^h	OAV ^g	Odor descriptor ^h	
Acids										
72	Undecanoic acid ^o	112-37-8	901	2369	2365 ²²	42.91	5.3	Nf	–	Oily ³⁰
73	Dodecanoic acid ^o	143-07-7	856	2508 ^l	2517 ²	22.47	6.1	1000 ⁵⁷	<1	Dry, metallic ⁵⁷
74	2-Phenylacetic acid ^o	103-82-2	907	2580 ^l	2585 ²⁶	43.92	8.2	1000 ⁵²	<1	Honey, pollen, roses ²⁶
75	3-Phenylpropanoic acid ^o	501-52-0	865	2613 ^l	2603 ⁸	49.23	4.5	Nf	–	Floral, fruity ⁸
76	Tetradecanoic acid ^o	544-63-8	893	2855 ^l	2847 ²	65.62	5.9	10,000 ^{62,i}	<1	Nf
77	Hexadecanoic acid ^o	57-10-3	845	2870 ^j	2862 ²³	46.61	1.0	10,000 ^{62,i}	<1	Nf
78	–	–	862	2889 ^j	Nf	40.19	2.7	Nf	–	Nf
Aldehydes										
79	Acetaldehyde ^v	75-07-0	871	700 ^j	692 ³	78.65	5.1	500 ⁵⁰	<1	Pungent ³
80	2-Propenal (acrolein) ^v	107-02-8	831	858 ^l	846 ²⁸	79.76	4.3	Nf	–	Nf
81	3-Methyl-1-butanol ^v	590-86-3	901	910	915 ⁸	0.01	8.9	0.35 ^{53,i}	<1	Green, malt ⁴¹
82	2-Butenal ^v	4170-30-3	950	1050	1041 ²⁸	0.11	3.2	0.35 ^{53,i}	<1	Pungent ⁴⁵
83	Hexanal ^v	66-25-1	943	1107	1097 ²⁵	9.71	3.4	4.5 ^{53,i}	2.1	Sweet, orange ³
84	2,3-Dimethylpentanal (2) ^v	32749-94-3	932	1260	1258 ¹⁸	5.12	7.5	Nf	–	Nf
85	Octanal ^v	124-13-0	941	1292	1280 ¹⁰	8.51	5.4	15 ²⁶	<1	Fatty ³
86	Nonanal (19) ^v	124-19-6	960	1399	1408 ³	42.09	5.6	15 ²⁶	2.8	Citrusy, floral ³
87	Decanal ^v	112-31-2	981	1507	1515 ³	0.17	9.7	1 ²⁶	<1	Grassy, orange skin-like ⁴¹
88	4-Ethylbenzaldehyde ^v	4748-78-1	943	1519	1521 ¹⁵	48.86	3.4	Nf	–	Fruity, anised ²⁹
89	Benzaldehyde ^v	100-52-7	967	1516	1529 ²⁸	764.85	5.9	2000 ⁴⁹	<1	Almond ⁴⁹
90	Undecanal ^v	112-44-7	984	1630	1622 ³	3.56	3.8	5 ^{47,i}	<1	Waxy, floral ³
91	2-Phenylacetaldehyde (benzeneacetaldehyde) ^v	122-78-1	896	1638	1631 ²¹	1.67	3.7	1 ²⁶	1.7	Flowery, rose ²⁶
92	4-Methylbenzaldehyde (13) ^v	104-87-0	987	1665	1654 ²⁹	56.12	8.6	Nf	–	Almond ²⁹
93	Dodecanal ^v	112-54-9	864	1733	1729 ³	1.45	5.0	2 ^{47,i}	<1	Floral, waxy ³
94	Tridecanal ^v	10486-19-8	852	1820	1824 ³⁰	30.67	7.3	Nf	–	Sweet, fruity ³⁰
95	Tetradecanal ^v	124-25-4	816	1893	1888 ¹⁴	4.86	5.1	6 ^{29,i}	<1	Floral, waxy ²⁹
96	3-Phenyl-2-propenal (Cinnamaldehyde) ^v	104-55-2	843	1978	1970 ³¹	20.97	7.0	Nf	–	Nf
97	4-Methoxybenzaldehyde (p-anisaldehyde) ^v	123-11-5	874	1984	1986 ³⁹	75.80	3.8	Nf	–	Nf
98	Pentadecanal ^v	2765-11-9	921	2034	2024 ²⁷	64.02	8.2	Nf	–	Nf
99	Hexadecanal ^v	629-80-1	963	2091	2094 ¹⁴	56.98	4.5	75 ^{62,i}	<1	Nf
100	4-Hydroxybenzaldehyde ^v	123-08-0	985	3070	3072 ³²	49.02	8.4	Nf	–	Nf
Esters										
101	Ethyl acetate ⁿ	141-78-6	951	890	885 ²	15,608.09	6.0	12,000 ⁴⁹	1.3	Pineapple, fruity, solvent ⁴⁹
102	Ethyl propanoate ^o	–	930	948	946 ³	10,450.44	7.4	5500 ⁶⁵	1.9	Sweet, fruity ³
103	Ethyl 2-methylpropanoate (ethyl isobutanoate) ^o	97-62-1	896	968	965 ³	30.08	4.8	15 ⁶⁵	2.0	Sweet, fruity ³
104	Ethyl butanoate ^p	105-54-4	982	1028	1022 ²²	15,189.87	5.4	400 ⁴⁹	38.0	Strawberry, apple ⁴⁹
105	Ethyl 2-methylbutanoate (ethyl isovalerate) ^s	7452-79-1	971	1040	1035 ²²	5.31	4.3	1 ⁵⁰	5.3	Apple, sweet ³
106	Ethyl pentanoate ^q	539-82-2	934	1132	1142 ⁵	10.52	3.8	5 ^{62,i}	2.1	Fruity ³ , apple ⁸
107	Butyl ethanoate (Butyl acetate) ^q	123-86-4	925	1063	1074 ³³	14,040.93	8.2	1800 ⁴⁹	7.8	Fruity ⁴⁹
108	3-Methylbutyl acetate (isoamyl acetate) ^r	123-92-2	825	1129	1127 ³⁴	1536.07	5.1	160 ⁴⁹	9.6	Banana, fruity, sweet ⁴⁹
109	Isobutyl 3-methylbutanoate ^r	557-00-6	863	1175	1175 ²⁸	27.55	5.5	Nf	–	Nf
110	Butyl butanoate ^r	109-21-7	841	1199	1212 ¹	34.78	1.4	100 ^{61,i}	<1	Nf
111	Ethyl hexanoate ^t	123-66-0	836	1238	1244 ²	385.96	11.8	8 ⁴⁹	48.2	Fruity, green apple, brandy, wine-like ⁴⁹
112	Ethyl 2-oxopropanoate (1) ^t	617-35-6	973	1247	1242 ¹³	43.53	4.9	Nf	–	Nf
113	Hexyl acetate ^t	142-92-7	919	1281	1270 ⁵	1072.39	4.6	670 ⁴⁹	1.6	Apple, cherry, pear, floral ⁴⁹
114	Ethyl heptanoate (18) ^t	106-30-9	927	1314	1317 ²	5.57	5.2	2.2 ^{61,i}	2.5	Fruity ⁸
115	Propyl hexanoate ^t	626-77-7	934	1322	1321 ³³	124.75	3.2	Nf	–	Fruity ⁸
116	Ethyl 2-hexenoate ^t	27829-72-7	962	1335	1335 ⁵	167.25	4.2	Nf	–	Fruity, pineapple ⁵³
117	Ethyl 2-hydroxypropanoate (ethyl lactate) ^u	97-64-3	921	1364	1363 ²	345127.41	5.7	150,000 ⁴⁹	2.3	Fruity, buttery ⁴⁹
118	Methyl octanoate ^v	111-11-5	804	1371	1378 ²²	280.00	7.2	200 ⁶³	1.4	Fruity, citrus like ⁶³
119	Ethyl 2-hydroxy-3-methylbutanoate (4) ^z	2441-06-7	856	1405	1399 ⁸	2589.78	4.6	1000 ⁵¹	2.6	Pineapple, strawberry, tea, honey ⁵¹
120	Ethyl 2-hydroxybutanoate (5) ^z	52089-54-0	912	1409	1400 ⁸	24875.98	6.6	20,000 ⁶⁵	1.2	Fruity, floral ⁸
121	Propyl heptanoate (6) ^v	7778-87-2	941	1426	1425 ²⁸	60.79	4.5	Nf	–	Nf
122	2-Methylpropyl octanoate (isobutyl octanoate) ^v	5461-06-3	809	1545	1551 ⁵	88.74	7.3	0.8 ⁵⁷	<1	Nf
123	Hexyl 3-methylbutanoate (hexyl isovalerate) (7) ^v	10032-13-0	869	1433	1425 ¹	88.65	7.8	Nf	–	Nf
124	Ethyl 2-(1-ethoxyethoxy)propanoate ^v	37101-80-7	874	1447	1442 ²¹	102.84	9.3	Nf	–	Nf
125	Ethyl octanoate (7) ^v	106-32-1	988	1434	1446 ²	75450.87	6.3	580 ⁴⁹	130	Sweet, fruity, pear ⁴⁹
126	3-Methylbutyl hexanoate (isopentyl hexanoate) ^v	2198-61-0	941	1445	1450 ²	1251.22	2.8	1000 ⁵⁷	1.2	Sweet fruity ³
127	Isobutyl 2-hydroxypropanoate ^z	73523-02-1	988	1455	1455 ²¹	56.49	5.3	Nf	–	Nf
128	3-Methyl octenoate ^v	69668-85-5	976	1475	1483 ³⁶	47.97	7.8	Nf	–	Nf
129	Ethyl 2,2-diethoxyacetate ^x	6065-82-3	852	1480	1487 ⁵	0.65	6.5	Nf	–	Nf
130	Ethyl 6-oxononanoate ^x	4144-59-6	841	1492	1488 ²¹	3.98	4.1	Nf	–	Nf
131	Methyl nonanoate ^x	1731-84-6	863	1494	1493 ²⁸	9.87	9.6	Nf	–	Nf
132	Methyl 3-hydroxy-2-methylpropanoate ^z	72657-23-9	837	1515	1507 ³⁷	87.09	6.7	Nf	–	Floral ⁷¹
133	Butyl 2-hydroxypropanoate (butyl lactate) ^z	138-22-7	935	1522	1520 ²¹	230.78	5.6	1000 ⁴⁹	<1	Creamy, milky, sweet ⁴⁹
134	Ethyl 2-hydroxyhexanoate ^z	6946-90-3	966	1544	1542 ⁵	56.54	9.0	Nf	–	Cashew, cherry ³⁴
135	Ethyl 2-hydroxy-4-methylpentanoate ^z	10348-47-7	924	1554	1547 ³⁴	91.86	3.9	51 ⁷⁰	1.8	Fruity, lemon ³⁴
136	Methyl 2-hydroxyhexanoate ^z	68756-64-9	981	1565	1574 ³⁸	110.32	5.3	Nf	–	Nf
137	Diethyl propanedioate (diethyl malonate) ^Δ	105-53-3	861	1571	1580 ²¹	90.43	6.7	Nf	–	Nf
138	Ethyl nonanoate ^x	123-29-5	841	1578	1581 ²	1603.71	5.2	1300 ⁵⁷	1.2	Floral, fruity ⁸
139	Ethyl 3-hydroxypentanoate ^z	54074-85-0	803	1589	1587 ³⁸	16.98	6.3	Nf	–	Fruity, pineapple-like ³⁸
140	Methyl decanoate ^x	110-42-9	804	1601	1599 ⁵	22.62	4.7	1.2 ⁵⁷	<1	Nf
141	Ethyl 4-oxopentanoate ^t	539-88-8	944	1614	1607 ⁵	78.21	5.6	Nf	–	Nf

(continued on next page)

Table 2 (continued)

Compound ^a	CAS	S ^b	LTPRI (exp) ^c	LTPRI (lit) ^d	Conc. ^e (µg L ⁻¹)	RSD (%) ^f	OTS (µg L ⁻¹) ^h	OAV ^g	Odor descriptor ^h	
<i>Esters</i>										
142	3-Methylbutyl 2-hydroxypropanoate (isopentyl lactate/isoamyl lactate) (10) ^s	19329-89-6	936	1619	1615 ²	294.55	8.9	Nf	-	Nf
143	Butyl octanoate (11) ^x	589-75-3	945	1624	1621 ²⁸	145.63	2.3	700 ^{62,i}	<1	Nf
144	Methyl ethyl butanedioate (methyl ethyl succinate) (11) ^Δ	627-73-6	978	1624	1631 ⁵	124.43	5.6	Nf	-	Nf
145	Ester (12) ^Δ	-	804	1641	1632	15.82	9.7	-	-	-
146	Ester ^Δ	-	807	1646	1635	40.32	9.4	-	-	-
147	Ethyl decanoate ^v	110-38-3	925	1643	1651 ²	1950.32	8.6	500 ⁴⁹	3.9	Fruity, grape ⁴⁹
148	Ethyl benzoate ^T	93-89-0	958	1668	1664 ⁵	29.60	3.9	56 ^{55,i}	<1	Fruity ⁴¹
149	Ester ^T	-	817	1695	Nf	102.53	2.9	-	-	-
150	Ester ^T	-	814	1744	Nf	32.31	5.4	-	-	-
151	3-Methylbutyl octanoate (isoamyl octanoate) ^x	2035-99-6	904	1668	1674 ²	54.96	3.7	152 ⁵¹	<1	Oily ³ , sweet, light fruity, cheese, cream ⁵¹
152	Ethyl (Z)-4-decenoate (14) ^x	7367-84-2	990	1690	1687 ¹⁶	45.65	3.8	Nf	-	Nf
153	Ester ^x	-	824	1702	1693	23.99	8.3	-	-	-
154	Methyl undecanoate ^x	627-90-7	908	1703	1694 ²¹	66.45	8.6	Nf	-	Nf
155	Diethyl butanedioate (diethyl succinate) (14) ^Δ	123-25-1	952	1690	1701 ²	1680.31	6.8	1200 ⁴⁹	1.4	Fruity ⁴⁹
156	Ethyl 9-decenoate ^x	67233-91-4	974	1708	1709 ⁴⁰	191.29	2.8	100 ⁶⁴	1.9	Rose ³
157	Propyl decanoate ^x	30673-60-0	901	1711	1720 ⁵	29.73	5.4	Nf	-	Nf
158	Ethyl undecanoate ^x	627-90-7	967	1739	1732 ²²	102.43	7.2	Nf	-	Nf
159	Methyl phenylacetate ^T	101-41-7	964	1745	1749 ³⁸	43.78	3.9	Nf	-	Nf
160	Methyl 2-hydroxybenzoate ^T	9041-28-5	912	1755	1756 ⁴¹	77.40	6.4	Nf	-	Minty, sweet ⁴¹
161	Diethyl pentanedioate ^x	818-38-2	945	1770	1768 ²²	76.37	9.5	Nf	-	Nf
162	Ester ^x	-	819	1789	Nf	50.64	6.0	-	-	-
163	Methyl dodecanoate ^x	111-82-0	904	1800	1793 ²²	22.19	3.8	Nf	-	Nf
164	Ethyl 2-phenylacetate (20) ^T	103-45-7	985	1796	1805 ²²	3420.04	7.5	1800 ⁴⁹	1.9	Flowery ⁴⁹
165	Isopropyl dodecanoate ^x	10233-13-3	946	1812	1821 ⁴⁰	34.60	4.9	Nf	-	Nf
166	Ethyl 3,3-diethoxypropionate ^x	10601-80-6	917	1833	1829 ⁵	84.54	2.6	Nf	-	Nf
167	Ethyl dodecanoate (ethyl laurate) ^x	106-33-2	928	1839	1835 ²²	5604.11	3.1	3500 ⁵³	1.6	Sweet, floral, fruity cream ⁵⁷
168	Ester ^x	-	814	1845	Nf	10.93	8.6	Nf	-	Nf
169	Isopentyl decanoate ^x	2306-91-4	938	1868	1871 ²	33.65	8.9	Nf	-	Nf
170	Methyl 3-hydroxybutanoate ^z	20257-95-8	937	1882	1880 ¹⁹	5400.88	3.6	Nf	-	Nf
171	Ester ^Δ	-	821	1879	Nf	43.60	5.2	-	-	-
172	Ester ^Δ	-	809	1890	Nf	100.15	6.9	-	-	-
173	Ethyl tridecanoate ^x	28267-29-0	928	1951	1943 ²¹	67.09	1.9	Nf	-	Nf
174	Methyl tetradecanoate ^x	124-10-7	964	2021	2034 ³⁰	49.09	6.5	Nf	-	Waxy ³⁰
175	Diethyl 2-hydroxybutanedioate (diethyl malate) ^Δ	7554-12-3	948	2048	2048 ⁵	1500.92	6.7	760,000 ³²	<1	Over-ripe, peach, cut grass ³²
176	Ethyl tetradecanoate (isopropyl myristate) ^x	124-06-1	987	2059	2054 ⁵	43.09	2.3	800 ⁵⁷	<1	Nf
177	Hexyl benzoate ^T	6789-88-4	925	2057	2056 ⁶	90.31	8.9	Nf	-	Nf
178	2-Phenylethyl hexanoate ^T	6290-37-5	928	2157	2160 ⁸	15.66	4.4	Nf	-	Nf
179	Ethyl pentadecanoate (16) ^x	41114-00-5	967	2149	2151 ⁵	134.33	6.7	Nf	-	Nf
180	Dimethyl decanedioate ^x	106-79-6	899	2209	2220 ⁵	120.18	6.6	Nf	-	Nf
181	Ethyl hexadecanoate ^x	628-97-7	904	2250	2261 ⁵	150.22	3.4	1500 ⁵¹	<1	Fatty, rancid, fruity, sweet ⁵¹
182	Ethyl 9-hexadecenoate ^x	54546-22-4	912	2289	2283 ⁵	9.08	9.0	Nf	-	Nf
183	2-Phenethyl octanoate ^T	5457-70-5	927	2378	2376 ²¹	30.97	5.6	Nf	-	Nf
184	Ethyl octadecanoate ^x	111-61-5	988	2445 ^j	2458 ²¹	192.10	7.8	Nf	-	Nf
185	Ethyl 4-hydroxy-3-methoxybenzoate (ethyl vanillate) ^T	617-05-0	901	2651 ^j	2665 ²⁶	5100.09	6.4	3000 ⁶⁵	1.7	Sweet, honey, vanillin ³²
<i>Ketones</i>										
186	2-Propanone (acetone) ^f	67-64-1	907	815 ^j	816 ¹¹	58.32	9.4	Nf	-	Nf
187	4-Methyl-2-hexanone ^f	110-12-3	889	832 ^j	825 ⁴²	34.01	5.7	Nf	-	Nf
188	2-Butanone ^f	78-93-3	945	908	903 ⁴²	22.56	5.8	80,000 ^{53,i}	<1	Acetone-like ⁵³
189	2,3-Butanedione ^f	431-03-8	908	971	975 ⁴²	187.19	7.3	100 ⁵⁰	8.7	Buttery ³
190	1-Penten-3-one ^f	1629-58-9	975	1000	1008 ¹¹	33.82	5.7	Nf	-	Plastic, pungent ⁴⁷
191	Cyclopentanone ^f	120-92-3	963	1146	1144 ¹	34.80	8.9	Nf	-	Nf
192	5-Methyl-3-heptanone (2) ^s	541-85-5	954	1260	1264 ³³	10.67	7.8	Nf	-	Nf
193	3-Octanone ^s	106-68-3	907	1271	1266 ²	10.34	2.9	21.4 ^{55,i}	<1	Herbal ⁵⁵
194	3-Hydroxy-2-butanone (acetoin) ^u	513-86-0	968	1279	1287 ⁵	180723.42	2.6	150,000 ⁴⁹	1.2	Buttery, cream ⁴⁹
195	1-Hydroxy-2-propanone ^u	116-09-6	966	1315	1323 ⁴³	23.05	7.9	10 ⁶⁰	<1	Nf
196	Cyclohexanone ^s	108-94-1	915	1276	1285 ⁶	9.83	4.6	Nf	-	Nf
197	1-Octen-3-one ^s	4312-99-6	937	1300	1299 ²⁸	4.88	6.6	15 ⁶⁵	<1	Mushroom ⁴¹
198	6-Methyl-5-hepten-2-one ^s	110-93-0	897	1349	1338 ⁴²	30.74	0.9	68 ^{55,i}	<1	Sweet, fruity ⁵⁵
199	4-Hydroxy-4-methyl-2-pentanone (3) ^q	123-42-2	981	1351	1339 ⁴⁰	64.03	0.5	Nf	-	Nf
200	2-Nonanone ^q	821-55-6	884	1391	1394 ²⁸	61.50	7.5	41 ⁵³	1.5	Fruity ⁵³
201	3-Decanone ^q	928-80-3	922	1482	1491 ¹⁴	20.22	4.3	Nf	-	Nf
202	3-Methyl-2-cyclohexenone ^T	1193-18-6	910	1565	1579 ⁶	421.32	3.3	Nf	-	Nf
203	2,3-Dimethyl-2-cyclopenten-1-one ^T	1121-05-7	928	1571	1582 ⁴³	17.44	8.4	Nf	-	Nf
204	2-Hydroxy-2-cyclopenten-1-one ^T	21835-01-8	934	1780	1784 ³⁹	142.32	4.9	Nf	-	Nf
205	2-Dodecanone ^q	6175-49-1	991	1803	1809 ⁴⁴	3.21	0.7	Nf	-	Nf
<i>C13-norisoprenoids</i>										
206	β-Damascenone (17) ⁺	23696-85-7	909	1839	1842 ³³	1.08	3.7	0.05 ⁵⁰	20	Honey, sweet ³
207	Geranylacetone ⁺	3796-70-1	926	1850	1856 ⁵	9.72	5.8	Nf	-	Floral ⁴¹
208	Methyl dihydrojasmonate ⁺	24851-98-7	938	2280	2276 ²³	7.23	1.3	Nf	-	Nf

Table 2 (continued)

Compound ^a	CAS	S ^b	LTPRI (exp) ^c	LTPRI (lit) ^d	Conc. ^e (µg L ⁻¹)	RSD (%) ^f	OTS (µg L ⁻¹) ^h	OAV ^g	Odor descriptor ^h	
<i>Terpenes</i>										
209	3-Carene [♦]	13466-78-9	901	1134	1146 ¹	70.40	2.3	44 ^{69,i}	1.6	Mango leaf-like, sweet, green ⁶⁹
210	ρ-Cymene [♦]	99-87-6	893	1250	1262 ³⁸	27.36	5.4	11.40 ^{62,i}	2.4	Fruity, sweet ³⁰
211	3,3,5-Trimethyl-2-cyclohexen-1-one (isoforone) [♦]	78-59-1	981	1393	1381 ²¹	8.65	7.8	Nf	–	Nf
212	(Z)-Linaloloxide [♦]	5989-33-3	888	1429	1438 ³⁸	19.29	8.3	500 ⁵¹	<1	Rose, wood ⁵¹
213	Hotrienol [♦]	29957-43-5	934	1460	1449 ¹³	8.67	2.3	Nf	–	Nf
214	Linalool [♦]	78-70-6	907	1539	1540 ²²	28.50	3.7	15 ⁴⁹	1.9	Citrus, floral, sweet, grape-like ⁴⁹
215	β-Elementol (9) [♦]	515-13-9	952	1600	1595 ³⁰	5.44	5.3	Nf	–	Nf
216	4-Terpineol [♦]	562-74-3	887	1609	1602 ⁵	40.82	6.3	250 ⁶⁸	<1	Sweet, herbaceous ⁴¹
217	α-Terpineol [♦]	98-55-5	899	1713	1706 ³³	1100.67	5.4	1000 ⁴⁹	1.1	Floral, sweet ⁴⁹
218	Dihydrocarveol [♦]	38049-26-2	936	1715	1720 ⁴⁵	4.21	3.3	100 ⁵⁰	<1	Nf
219	α-Citronellol [♦]	106-22-9	907	1774	1771 ³⁰	123.98	6.3	100 ⁵⁰	1.2	Sweet, citrus-like ³⁰
220	Nerol [♦]	106-25-2	922	1785	1797 ⁵	34.21	4.2	400 ⁶⁷	<1	Rose, lime ⁶⁷
221	β-Ionone [♦]	106-28-5	883	1920	1924 ⁴¹	12.93	5.3	5 ⁴⁹	<1	Balsamic, rose, violet ⁴⁹
222	(E)-Nerolidol [♦]	40716-66-3	884	2062	2058 ²	1630.22	8.7	1000 ⁴⁹	1.6	Rose, apple, green, citrus ⁴⁹
223	(E)-Farnesol [♦]	4602-84-0	931	2335	2345 ²⁶	2881.10	2.6	2400 ⁴⁹	1.2	Floral ⁴⁹
224	(Z)-α-Santalol [♦]	115-71-9	928	2348	2350 ⁴⁷	33.90	7.4	Nf	–	Incense, woody ⁴⁷
<i>Phenols</i>										
225	2-Methoxy phenol (guaiacol) [†]	90-05-1	907	1861	1860 ¹⁰	3.87	5.2	10 ⁵⁰	<1	Smoke, sweet, medicine ⁶⁷
226	4-Methylguaiacol [†]	108-95-2	894	2002	2007 ⁸	8.90	4.5	Nf	–	Nf
227	4-Ethylguaiacol [†]	2785-89-9	881	2039	2033 ⁵	3.67	2.1	33 ⁵¹	<1	Medicine, wood, clove, smoky ⁵¹
228	Eugenol [†]	97-53-0	963	2155	2157 ⁵	1.29	1.8	5 ³⁰	<1	Spices, clove, honey ³²
229	4-Ethylphenol [†]	123-07-9	914	2154	2167 ³⁸	2.04	0.9	610 ⁵²	<1	Phenolic ⁴¹
230	4-Vinylguaiacol [†]	7786-61-0	915	2195	2190 ¹⁹	5.80	4.5	40 ⁵⁰	<1	Spices, curry ³²
231	5-Isopropyl-3-methylphenol [†]	3228-03-3	847	2282	2287 ⁴⁵	2.89	0.9	Nf	–	Nf
<i>Pyrrroles</i>										
232	Ethyl-pyrrole [♦]	617-92-5	991	1198	1194 ⁴³	8.93	9.3	Nf	–	Nf
233	Pyrrole-2-carboxaldehyde [♦]	1003-29-8	974	1996	1990 ³⁹	16.78	4.1	Nf	–	Nf
234	2-Pyrrolidinone [♦]	616-45-5	892	2037	2041 ⁴⁶	19.22	4.7	Nf	–	Nf
<i>Sulfur compounds</i>										
235	Ethyl (methylthio)acetate (8) ^q	4455-13-4	814	1450	1438 ³⁸	20.76	7.2	Nf	–	Cucumber-like ⁶⁶
236	Ethyl 3-(methylthio)propanoate ^q	13532-18-8	917	1566	1558 ³⁸	18.91	5.6	7 ⁶³	2.7	Pineapple-like ⁶³
237	3-(Methylthio)-1-propanol (15) ^q	505-10-2	965	1729	1738 ²	28.35	4.4	500 ⁵⁰	<1	Cooked vegetable ³²
238	2-Thiophenecarboxaldehyde (15) ^q	98-03-3	985	1729	1734 ⁴³	30.32	2.1	Nf	–	Nf
<i>Furans</i>										
239	2-Methyl-furan [♦]	534-22-5	892	861	858 ⁴³	4.32	1.9	Nf	–	Nf
240	2-Pentyl-furan (1) [♦]	3777-69-3	935	1246	1240 ¹⁰	5.87	0.9	6 ^{60,i}	<1	Sweet ¹²
241	2-(Diethoxymethyl)furan [♦]	13529-27-6	987	1438	1442 ⁸	75.01	4.3	Nf	–	Roasted ⁸
242	Ethyl 2-furoate [†]	614-99-3	965	1617	1618 ⁵	235.43	7.4	16,000 ⁶⁵	<1	Balsamic ⁸
243	2-Furanmethanol [♦]	98-00-0	981	1640	1647 ⁸	32.43	2.8	Nf	–	Burnt sugar ⁸

CAS: Chemical Abstract Service.

Whenever one of the following symbols is placed by the side of the name of a tentatively identified compound, it designates that its quantification was accomplished taking into consideration the calibration curve of this standard: ^qethyl acetate, ^rethyl propanoate, ^sethyl butanoate, ^tethyl isobutanoate, ^uisoamyl acetate, ^vethyl isovalerate, ^wethyl hexanoate, ^xethyl lactate, ^yethyl octanoate, ^zethyl decanoate, ^{aa}ethyl 2-hydroxybutanoate, ^{ab}diethyl succinate, ^{ac}ethyl 2-phenyl acetate, ^{ad}1-propanol, ^{ae}1-hexanol, ^{af}2-phenylethanol, ^{ag}4-terpineol, ^{ah}eugenol, ^{ai}hexanoic acid, ^{aj}octanoic acid, ^{ak}decanoic acid, ^{al}dodecanoic acid.

^a Co-elutes were numbered from 1 to 20 and these numbers are written between parentheses after the compound's name. Whenever compounds are followed by the same number, they co-elute.

^b Similarity.

^c LTPRI exp: Experimental linear temperature programmed retention index (LTPRI) calculated using *n*-alkanes (C9–C24) in polar × (DB-Wax, 100% polyethyleneglycol) × medium polar (DB-17 ms, (50%-phenyl)-methylpolysiloxane) column set.

^d LTPRI: literature LTPRI on a DB-WAX column or equivalent stationary phase: ¹Frohlich, Duque, & Schreier, 1989; ²Tao, Li, Wang, & Zhang, 2008; ³Gurbuz, Rouseff, & Rouseff, 2006; ⁴Weckerle, Bastl-Borrmann, Richling, Hor, Ruff and Schreier, 2001; ⁵Zhao, Xu, Li, Fan, & Jiang, 2009; ⁶Werkhoff, Guntert, Krammer, Sommer, & Kaulen, 1998; ⁷Garcia, Quek, Stevenson, & Winz, 2011; ⁸Fan & Qian, 2006; ⁹Guo, Wu, Han, Cao, Rahman and Qin, 2008; ¹⁰Pozo-Bayon, Ruiz-Rodriguez, Pernin, & Cayot, 2007; ¹¹Vekari, Oreopoulou, Kourkoutas, Kamoun, Msallem, Psimouli, et al., 2010; ¹²Annan, Poll, Plahar, & Jakobsen, 2003; ¹³Selli, Canbas, Cabaroglu, Erten, & Gunata, 2006; ¹⁴Brunton, Cronin, & Monahan, 2002; ¹⁵Fu, Yoon, & Bazemore, 2002; ¹⁶Verzera, Ziino, Scacco, Lanza, Mazzaglia, Romeo, et al., 2008; ¹⁷Jang, Ka, & Lee, 2008; ¹⁸Lee & Shibamoto, 2000; ¹⁹Mayorga, Knapp, Winterhalter, & Duque, 2001; ²⁰Peng, Yang, & Ding, 1991; ²¹Ledauphin, Saint-Clair, Lablanquie, Guichard, Fournier, Guichard, et al., 2004; ²²Ferrari, Lablanquie, Cantagrel, Ledauphin, Payot, Fournier, et al., 2004; ²³Selli, Bagatar, Sen, & Kelebek, 2001; ²⁴Whetstone, Karagul-Yuceer, Avsar, & Drake, 2003; ²⁵Mahajan, Goddik, & Qian, 2004; ²⁶Cullere, Escudero, Cacho, & Ferreira, 2004; ²⁷Takahashi, Mizuib, & Miyazawa, 2010; ²⁸Bianchi, Careni, Mangia, & Musci, 2007; ²⁹Varlet, Prost, & Serot, 2007; ³⁰Choi, 2003; ³¹Meret, Brat, Mertz, Lebrun, & Guenata, 2011; ³²Garcia-Carpintero et al., 2011; ³³Kumazawa, Ito, Nishimura, & Hamaguchi, 2008; ³⁴Sampaio, Garruti, Franco, Janzantti, & Da Silva, 2011; ³⁵Saidana, Mahjoub, Boussaada, Chriaa, Mahjoub, Cheraif, et al., 2008; ³⁶Bendahou, Muselli, Grignon-Dubois, Benyoucef, Desiobert, Bernardini, et al., 2008; ³⁷Chyau, Chen, & Wu, 1992; ³⁸Umano, Hagi, Nakahara, Shoji, & Shibamoto, 1992; ³⁹Simon, Esteruelas, Munoz, Cadahia, & Sanz, 2009; ⁴⁰Wu, Krings, Zorn, & Berger, 2005; ⁴¹Fan, Xu, Jiang, & Li, 2010; ⁴²Conduro, Verzera, Romeo, Ziino, & Conte, 2008; ⁴³Sanz, Ansoarena, Bello, & Cid, 2001; ⁴⁴Selli & Cayhan, 2009; ⁴⁵Lee, Umano, Shibamoto, & Lee, 2005; ⁴⁶Liu, Jin, Liu, Hunag, Wang, Mao, et al., 2011; ⁴⁷Wacharananun, Cadwallader, Huangrak, Kim, & Lorjaroenphon, 2009; ⁴⁸Iwaoka, Hagi, Umano, & Shibamoto, 1994.

^e Concentration.

^f Relative standard deviation.

^g Odor activity value.

^h OTS = odor threshold determined in 10–12% (v/v) ethanol obtained from literature: ⁴⁹Peinado et al., 2004; ⁵⁰Guth; ⁵¹Tao and Li, 2009; ⁵²Samapitto & Butkhop, 2010; ⁵³Qian & Wang, 2005; ⁵⁴Chastrette, Cretin, & El Aidi, 1996; ⁵⁵Giri, Osako, & Ohshima, 2010; ⁵⁶Etiévant, 1991; ⁵⁷Li et al., 2008; ⁵⁸Niu, Zhang, Xiao, Song, Eric, Jia, et al., 2011; ⁵⁹Lee, Shin, & Oh, 2008; ⁶⁰Buttery, Orts, Takeoka, & Nam, 1999; ⁶¹Takeoka, Flath, Mon, Teranishi, & Guentert, 1990; ⁶²Pino & Quijano, 2012; ⁶³Zheng, Sun, Liu, Lv, Yang, Zhao, et al., 2012; ⁶⁴Tao & Zhang, 2010; ⁶⁵Juan et al., 2012; ⁶⁶Kourkoutas et al., 2006; ⁶⁷Vilanova et al., 2010; ⁶⁸Ferreira et al., 2000; ⁶⁹Boonbumrung et al., 2001; ⁷⁰Lytra, Tempere, Revel, & Barbe, 2012; ⁷¹Morales & Duque, 2002.

ⁱ Odor threshold obtained in water.

^j Extrapolated LTPRI for compounds with LTPRI <900 and >2400.

^k Not found: LTPRI, odor threshold or odor descriptor are not available in the literature.

1996). Calibration graphs with at least six concentration levels for each standard compound were constructed by least square linear regression. The relative area of each compound (area compound/area internal standard) was plotted against the respective compound concentration. For each standard compound a calibration curve equation, determination coefficient (r^2), and linear range were calculated.

Determination of the LOD and LOQ were based on the standard deviation the intersection of the analytical curve (s) and on the slope of the curve (S). The LOD was expressed as $3.3 (s/S)$ and LOQ as 10 times (s/S).

The precision of the method was evaluated in terms of repeatability and intermediate precision expressed as relative standard deviation (RSD). Precision was evaluated as repeatability by carrying out six independent assays performed under the same analytical conditions in a short period of time on the same day, whereas intermediate precision was determined on two different days. Precision and accuracy assays were determined for two levels of concentration, including the lower and the higher concentrations of the calibration curves. Accuracy has been evaluated by addition of standard volatile compounds in a model wine solution and was determined after five extractions and analysis procedures for each level of concentration, for each one of the 22 standard compounds. It was calculated as the percentage deviation between the calculated value and the nominal value obtained in the calibration curves.

Quantitative data for the 22 positively identified compounds (Table 1) were obtained by interpolation of the calibration graphs built with pure reference compounds. The concentration of the tentatively identified volatile compounds, for which there was no pure reference, was obtained using calibration graphs of the available standard compounds. In this case, calibration graphs were chosen according to the chemical structure of the tentatively identified volatile compound to be quantified, trying to choose the standard compound that would be most similar to the tentatively identified one. The linear regression equations and other parameters concerning the calibration curves of the standard compounds that were used in the quantification of each volatile compound are indicated in Table 1.

2.6. Odor activity value (OAV) and relative odor contribution (ROC)

OAV was calculated by dividing the mean concentration ($n = 5$) of a compound by its odor threshold value, published in the scientific literature. Odor thresholds obtained in hydroalcoholic solution (matrix similar to wine with 10–12% ethanol) were used whenever available in the scientific literature.

3. Results and discussion

3.1. Identification of volatile compounds

In the headspace of Chardonnay wines, 243 compounds were tentatively identified using GC \times GC/TOFMS, and they are presented in Table 2, according to their chemical classes and in order of increasing LTPRI. Among them, 22 compounds were positively identified using standard compounds. Similarity values between the mass spectra of sample compounds and NIST library, as well as calculated LTPRI and literature LTPRI for each compound are also shown in Table 2. All these data have contributed to the tentative identification of volatile compounds. LTPRI from scientific literature have been found in 1D-GC papers and this approach has been already discussed in a previous work of this research group (Welke, Manfroi, et al., 2012). Differences between calculated LTPRI and literature LTPRI were accepted for the process of tentative identification when they were less than 14 units. Only 13% of the tentatively identified compounds showed LTPRI values greater than 10 units. Some of them were acids, including 4-methyl-2-oxovaleric acid, isobutyric acid, hexanoic acid and heptanoic acid, which have presented the highest differences between experimental and literature LTPRI. It is well known that polar column LTPRI are more prone to variations (von Muhlen et al., 2008), and in the case of this work, some variability could be expected, as two polar columns were coupled.

Some studies about volatile composition of Chardonnay wines using 1D-GC have been published and the number of compounds tentatively identified was approximately 50 (Jaffré et al., 2011; Jiang & Zhang, 2010; Li et al., 2008). This number suggests that former GC/MS methods were able to identify only part of the volatile compounds that may be identified when GC \times GC/TOFMS is employed in Chardonnay wine headspace analysis.

Among all the chemical groups found in the volatile content of Chardonnay wines of Serra Gaúcha, esters were present in higher number (85), followed by alcohols (52), acids (26), aldehydes (22), ketones (20), terpenes (16), phenols (7), furans (5), sulfur compounds (4), pyrroles (3) and C13-norisoprenoids (3). Predominant presence of alcohols, esters and acids in Chardonnay wine has already been observed in previous studies, although in this case, the number of alcohols was higher than that of esters (Jiang & Zhang, 2010; Li et al., 2008).

A color plot obtained of a Chardonnay wine analyzed by HS-SPME-GC \times GC/TOFMS is shown in Fig. 1A. Some co-elutions that might have happened with the use of one-dimensional GC can be viewed in this color plot. Forty-two compounds co-eluted in 1D and 8 of them also co-eluted in 2D . In Table 2, compounds that co-elute with other components are numbered and this number is informed after the compound name, between parentheses. Co-elutions are numbered from (1) until (20).

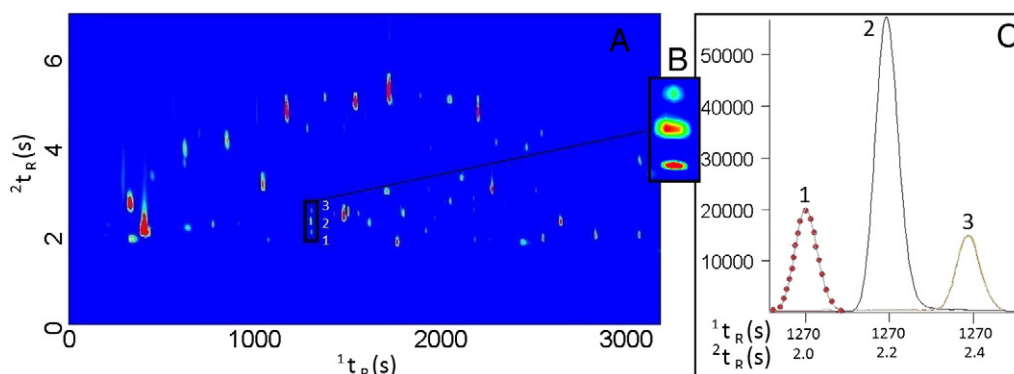


Fig. 1. Separation of volatile compounds of Chardonnay wine headspace. (A) GC \times GC/TOFMS total ion current chromatogram (TIC) color plot. The color gradient reflects the intensity of the TOFMS signal (Z-axis) from low (blue) to high (red). Some trace volatile compounds are not visible in this chromatogram due to the higher abundance of esters and alcohols (Table 2). Chromatographic conditions are described in Section 2.2. (B) Expanded region of color plot shows in detail the three co-eluted peaks in the first dimension. (C) Separation of (1, red line) ethyl 2-oxopropanoate, (2, black line) 2-pentyl furan and (3, yellow line) 1-pentanol that co-eluted in 1D and were separated in 2D . These compounds (co-elution #1) correspond to numbers 112, 240 and 7 in Table 2.

An interesting example of co-elution of compounds of the headspace of Chardonnay wines in the first dimension ($^1t_R = 1270$ s) and also of their separation in the second dimension is given by co-elution (1), which includes 1-pentanol (# 7 in Table 2, $^2t_R = 2.4$ s, LTPRI experimental 1245, LTPRI of literature 1233 (Weckerle et al., 2001), 2-pentyl-furan (# 240 of Table 2, $^2t_R = 2.2$ s, LTPRI experimental 1246, LTPRI of literature 1240 (Pozo-Bayon et al., 2007) and ethyl 2-oxopropanoate (# 112 in Table 2, $^2t_R = 2.0$ s, LTPRI experimental 1247, LTPRI of literature 1242 (Selli et al., 2006)). These three co-eluting compounds are highlighted in the color plot of Fig. 1A and their separation in the second dimension is shown in detail in Fig. 1B. Regarding their contribution to wine aroma, 1-pentanol can contribute negatively to wine aroma because its odor perception is described as synthetic and balsamic (Garcia-Carpintero et al., 2011). Another co-eluting compound, 2-pentylfuran, may have a positive contribution to aroma, as its odor description is known as sweet (Annan et al., 2003). No information was found in the scientific literature about the contribution of ethyl 2-oxopropanoate to wine aroma. This example highlights the importance of resolution enhancement provided by GC \times GC, whenever tentative identification of aroma active compounds that may co-elute in the first dimension is necessary.

3.2. Method validation

The performance of the method in terms of linearity, limits of detection (LOD) and quantification (LOQ), precision (repeatability and intermediate precision) and accuracy are shown in Tables 1 and 3.

The choice of the concentration ranges for each volatile compound was based on previously published works (Guth, 1997; Juan et al., 2012; Li et al., 2008; Vilanova et al., 2010). Preliminary analyses were performed to verify if the concentration of volatile compounds found in wines from Serra Gaucha was within the range of concentrations used in the calibration curves. In some cases, such as ethyl acetate, ethyl hexanoate and ethyl octanoate, the range of concentration had to be adjusted. In the case of ethyl acetate, the concentration found in the Serra Gaucha samples was lower than that previously reported and for ethyl hexanoate and ethyl octanoate, the levels were higher than in other works (Guth, 1997; Juan et al., 2012; Li et al., 2008; Vilanova et al., 2010).

The calibration curves were found to have good linearity in the range of studied concentrations. The determination coefficient (R^2) values were in the range of 0.998 to 0.972 for the evaluated standard compounds (Table 1), with the exception of organic acids that presented

Table 3

Average values (n = 6) for recoveries (%), repeatability and intermediate precision of standard compounds determined by HS-SPME-GC \times GC/TOFMS, using solutions of synthetic wine.

Compound	Concentration ($\mu\text{g L}^{-1}$)	Recovery (%)	Repeatability RSD (%) ^a	Intermediate precision RSD (%) ^b
Ethyl acetate	9610	99.3	2.6	4.5
	20,880	98.3	9.3	9.9
Ethyl propanoate	1200	99.8	1.6	3.4
	20,000	99.8	9.6	9.8
Ethyl butanoate	2500	98.6	5.5	6.4
	25,000	95.7	3.4	4.5
Ethyl 2-methyl propanoate (ethyl isobutanoate)	12.0	97.7	5.4	3.3
	168	98.1	8.7	6.7
3-Methyl butyl acetate (isoamyl acetate)	18.0	96.4	2.9	4.5
	490.0	95.5	4.4	5.1
Ethyl 2-methylbutanoate (ethyl isovalerate)	3.5	95.5	8.5	5.7
	56	96.2	2.5	3.2
Ethyl hexanoate	58	97.3	8.9	9.1
	660	98.7	5.7	6.4
Ethyl 2-hydroxy propanoate (ethyl lactate)	59,000	99.2	5.7	5.5
	688,800	96.8	4.8	5.3
Ethyl octanoate	52,000	96.1	7.8	3.2
	260,000	102.6	4.0	6.5
Ethyl decanoate	180	95.7	1.2	2.8
	7840	97.5	7.7	7.8
Ethyl 2-hydroxy-butanoate	4500	96.3	5.7	6.5
	72,700	95.7	3.3	3.7
Diethyl succinate	24.88	98.6	6.7	7.1
	2300	93.7	2.4	3.4
Ethyl 2-phenyl acetate	14.3	96.3	1.6	2.3
	460	95.4	7.9	7.8
1-Propanol	15	99.7	1.3	3.2
	192	97.7	3.8	3.9
1-Hexanol	51	98.3	7.9	7.6
	9340	95.6	9.0	8.9
2-Phenylethanol	1400	96.9	8.9	8.6
	360,000	98.9	8.8	8.3
4-Terpineol	5	99.7	7.8	7.4
	65.3	97.4	7.8	8.3
Eugenol	0.05	98.6	6.7	7.4
	5.6	96.7	6.4	9.8
Hexanoic acid	8.73	94.9	5.6	6.7
	78.6	93.4	6.6	7.6
Octanoic acid	2744	93.1	10.9	11.3
	197,200	92.5	11.1	11.4
Decanoic acid	20.2	93.9	9.9	10.3
	1608	93.2	8.7	9.4
Dodecanoic acid	5.48	90.8	13.4	13.8
	49.6	92.4	9.7	10.2

^a Relative standard deviation of assay done on the same day.

^b Relative standard deviation of assay done on two different days.

values from 0.948 to 0.942. Noguero-Pato et al. (2009) found r^2 higher than 0.99 for terpenes, C-13 norisoprenoids, alcohols, esters and phenols. Antalick et al. (2010) investigated only esters and obtained r^2 that was also higher than 0.99, while in this work r^2 ranged from 0.998 to 0.981. However, Pozo-Bayon, Pueyo, Martín-Álvarez, and Polo (2001) showed lower r^2 values, such as 0.96 for aldehydes and esters, while the r^2 of acids was in the range of 0.966 to 0.936. Howard et al. (2005) reported that acids presented poor extraction and chromatographic behavior, besides being present in low concentrations in wines, and these were the reasons why they had not included fatty acids in their analytical method. On the other hand, Olivero and Trujillo (2011) developed a method for the determination of nine short-chain fatty acids (acetic, propionic, isobutyric, butyric, isovaleric, 2-methylbutyric, hexanoic, octanoic and decanoic acids) in wines using the HS/SPME-GC/ion trap MS and found $r^2 > 0.997$. In this case, the method was focused only on fatty acids and all conditions involved in extraction and analyses were optimized having acids as a goal, instead of having all the volatile compound classes as target analytes, as is the case in the present study.

The lower r^2 values were found for acids (octanoic, decanoic, dodecanoic acid) and this has probably happened because of chromatographic tailing due to their stronger interaction with the stationary phase. The calculated LOD and LOQ for each one of the 22 compounds are shown in Table 1. The values of LOD ranged from 0.001 $\mu\text{g L}^{-1}$ for ethyl isovalerate and hexanoic acid to 2.554 $\mu\text{g L}^{-1}$ for ethyl 3-hydroxybutanoate. LOQ ranged from 0.003 $\mu\text{g L}^{-1}$ for ethyl isovalerate and hexanoic acid to 7.582 $\mu\text{g L}^{-1}$ for ethyl 3-hydroxybutanoate. The LOQ and LOD values determined for all 22 compounds (Table 1) were found to be lower than the ones reported in other scientific works that have also employed HS-SPME-1D-GC/MS (Antalick et al., 2010; Howard et al., 2005; Noguero-Pato et al., 2009; Perestrelo et al., 2006).

The precision test (repeatability and intermediate precision) has been performed in two different concentration levels (low and high) for each standard compound and results are shown in Table 3. These levels were used because they correspond to the lowest and the highest concentrations of each volatile compound that was used for the calibration curve. Repeatability ranged from 1.2% for ethyl decanoate to 13.4% for dodecanoic acid and intermediate precision ranged from 2.3% for ethyl 2-phenyl acetate to 13.8% for dodecanoic acid (Table 3). The only RSD values higher than 10% were found for octanoic acid and dodecanoic acid and the reason for that is probably the same mentioned for the lower r^2 found for carboxylic acids: its stronger interaction with the stationary phase that causes chromatographic tailing. Lower accuracies were also observed for acids.

Table 3 shows the accuracies for each one of the 22 standard compounds in two levels of concentration (low and high). The accuracy percent ranged from 92.4% for dodecanoic acid to 102.6% for ethyl octanoate. These accuracy results are in accord with data reported by other researchers that also used HS-SPME (DVB-CAR-PDMS) and GC/MS to analyze wines (Antalick et al., 2010; Howard et al., 2005; Noguero-Pato et al., 2009).

3.3. Volatile compound quantification and odor activity value evaluation

The concentration of the volatile compounds of the headspace of a Chardonnay wine is shown in Table 2 and is expressed in $\mu\text{g L}^{-1}$ as the average of five analytical replicates. OAV can be used to establish which compounds contribute to aroma because OAV calculation depends both on measured concentration and on the odor threshold of a specific compound in a certain matrix. Odor threshold and odor descriptor for the calculation of OAV of each tentatively identified compound were obtained from the scientific literature. Calculated OAV and literature references are given in Table 2. A volatile compound will contribute to the final wine aroma if its concentration is above its threshold perception (Guth, 1997; Li et al., 2008; Vilanova & Martinez, 2007). A compound should present an odor activity value > 1 in order

to be perceived by human nose. Forty-seven compounds of Chardonnay wine showed OAV > 1 and this number corresponds to 19% of total identified compounds (Table 2). Among them, esters are the class that includes the larger number of odorant components (51%), especially acetates and ethyl esters. The higher OAV values for ethyl esters are the ones of ethyl octanoate (# 125 in Table 2, OAV = 130.0, sweet, fruity or pear aroma (Peinado et al., 2004), ethyl hexanoate (# 111 in Table 2, OAV = 48.2, fruity, green apple, brandy or wine-like aroma (Peinado et al., 2004) and ethyl butanoate (# 104 in Table 2, OAV = 38.0, strawberry or apple aroma (Peinado et al., 2004) that are enzymatically produced during yeast fermentation and also during ethanolic acyl-CoA, that is formed during fatty acids synthesis or degradation. Their concentration is dependent on several factors, mainly: yeast strain, fermentation temperature, aeration degree, and sugar content (Etievant, 1991; Bakker & Clarke, 2011).

Acetates (isoamyl acetate, OAV = 9.6 and butyl acetate, OAV = 7.8) also are among the compounds with higher OAV (Table 2) and are the result of the reaction of acetyl-CoA with alcohols and are formed from degradation of amino acids and carbohydrates (Etievant, 1991). In general, acetate and ethyl esters contribute to the fruity aroma of wine (Gurbuz et al., 2006; Peinado et al., 2004; Tao & Zhang, 2010).

The acids that showed OAV > 1 were octanoic, decanoic and hexanoic acid (OAV = 16.0, 3.0 and 1.5, # 67, 70, 63 in Table 2, respectively). Volatile acids are produced during alcoholic fermentation and the contribution for the aroma depends on their concentration range in wine (Etievant, 1991). Shinohara (1985) showed that at concentrations of 4 to 10 mg L^{-1} , C₆ to C₁₀ acids provide mild and pleasant aroma to wine. However, the impact of the presence of volatile acids may be negative when the concentration of these compounds is greater than 20 mg L^{-1} (Shinohara, 1985). In this study, only octanoic acid was found in a concentration that may negatively affect the wine aroma (160 mg L^{-1}).

Aldehydes and ketones may be formed by decarboxylation of acids carried by yeast, such as α -keto acids including α -ketolactic, α -ketobutyric, α -ketoisovaleric, α -ketoisocaproic acids and others (Bakker & Clarke, 2004). Nonanal (# 86 in Table 2, OAV = 2.8), hexanal (# 83 in Table 2, OAV = 2.1) and 2-phenylacetaldehyde (# 91 in Table 2, OAV = 1.7) are among the most important aldehydes for wine aroma, whose odor descriptors are positive.

The ketone that showed higher OAV is 2,3-butanedione (# 189 in Table 2, OAV = 8.7). This compound, also called diacetyl ketone, is important to the wine aroma and its contribution depends on its concentration in wine. Undesirable aroma can be perceived when the concentration of 2,3 butanedione is above 7.5 mg L^{-1} . However, when present at concentrations below 4 mg L^{-1} , it contributes positively with buttery or caramel aroma (Davis, Wibowo, Eschenbruch, Lee, & Fleet, 1985). In the case of Chardonnay wines evaluated in this work, 2,3-butanedione was found in 0.18 mg L^{-1} , which can result in pleasant odor perception.

The terpenes found in Chardonnay wines, in concentrations above odor threshold were: *p*-cymene (# 210 in Table 2, OAV = 2.4), linalool (# 214 in Table 2, OAV = 1.9), (*E*)-nerolidol (# 222 in Table 2, OAV = 1.6), 3-carene (# 209 in Table 2, OAV = 1.6), α -citronellol (# 219 in Table 2, OAV = 1.2), (*E*)-farnesol (# 223 in Table 2, OAV = 1.2), and α -terpineol (# 217 in Table 2, OAV = 1.1). These compounds tend to contribute positive floral aromas to wine aroma and their odor description is reported in Table 2. Terpene and C13-norisoprenoids are part of the grape varietal aroma and may undergo fermentation without substantial changes. They may be found in grape skin and maceration is an essential step for the transference of these compounds to the grape must (Bakker & Clarke, 2004).

β -Damascenone (# 206 in Table 2, OAV = 20) is the only C13-norisoprenoid with OAV able to positively contribute to the aroma of the wine. However, this compound can synergistically change the aroma of other compounds. In hydroalcoholic solution, β -damascenone has enhanced fruity notes of ethyl cinnamate and caproate and masked

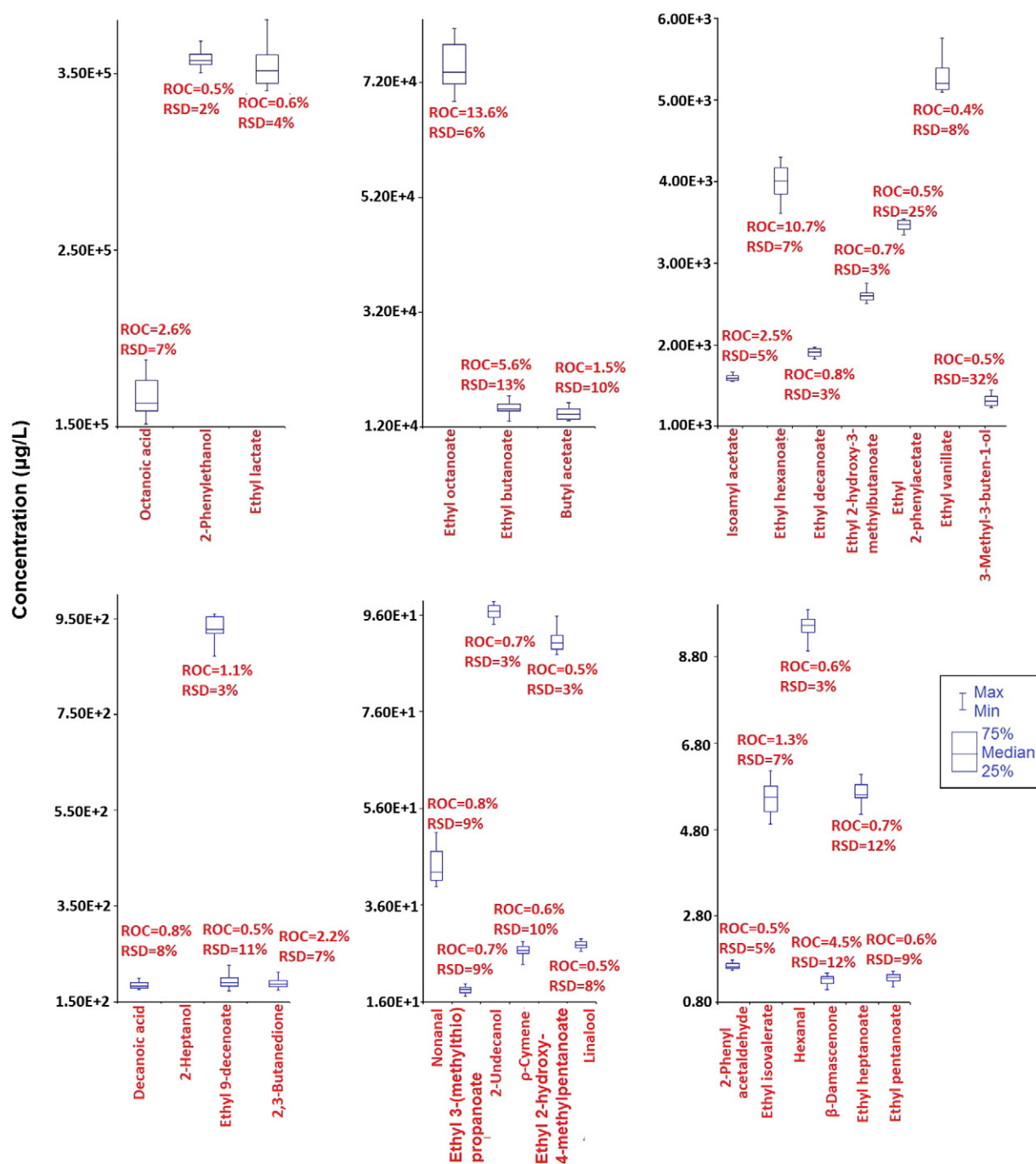


Fig. 2. Box-plots that present the distribution of concentrations of volatile compounds with highest relative odor contribution (ROC) in 12 Chardonnay wines. ROC and relative standard deviation (RSD) are shown for each compound.

the herbaceous aroma of 3-isobutyl-2-methoxypyrazine (IBMP) (Pineau, Barbe, Leeuwen, & Dubourdieu, 2007).

The contribution of each volatile compound with OAV > 1 to wine aroma can be evaluated qualitatively by means of its associate descriptor, and quantitatively by means of its OAV or ROC (Table 2). In Fig. 2, the quantified compounds were grouped according to their concentration range (in the following order: 10⁵, 10⁴, 10³, 10², 10¹ and <10 µg L⁻¹). This approach was necessary due to the great variability of concentration among different tentatively identified compounds, ranging from 0.89 to more than 37,000 µg L⁻¹. The box plot of Fig. 2 shows 29 compounds with the highest OAV (>1.7) that were quantified in twelve Chardonnay wine samples of the Serra Gaúcha region, including those found in the order of 10⁵ µg L⁻¹: octanoic acid (OAV = 16, # 67 in

Table 2), 2-phenylethanol (OAV = 1.8, # 43), ethyl lactate (OAV = 2.3, # 117); 10⁴ µg L⁻¹: ethyl octanoate (OAV = 130, # 125), ethyl butanoate (OAV = 38, # 104), butyl acetate (OAV = 7.8, # 107); 10³ µg L⁻¹: isoamyl acetate (OAV = 9.6, # 108), ethyl hexanoate (OAV = 48.2, # 111), ethyl decanoate (OAV = 3.9, # 147), ethyl 2-hydroxy-3-methylbutanoate (OAV = 2.6, # 119), ethyl 2-phenylacetate (OAV = 1.9, # 164), ethyl vanillate, 3-methyl-3-buten-1-ol; 10² µg L⁻¹: decanoic acid (OAV = 3, # 70), heptanol (OAV = 4.7, # 9), ethyl 9-decenoate (OAV = 1.9, # 156), 2,3-butanedione (OAV = 8.7, # 189); 10 µg L⁻¹: nonanal (OAV = 2.8, # 86), ethyl 3-(methylthio)propanoate (OAV = 2.7, # 236), 2-undecanol (OAV = 2.4, # 36), p-cymene (OAV = 2.4, # 210), ethyl 2-hydroxy-4-methyl-pentanoate (OAV = 2.1, # 106), linalool (OAV = 1.9, # 214);

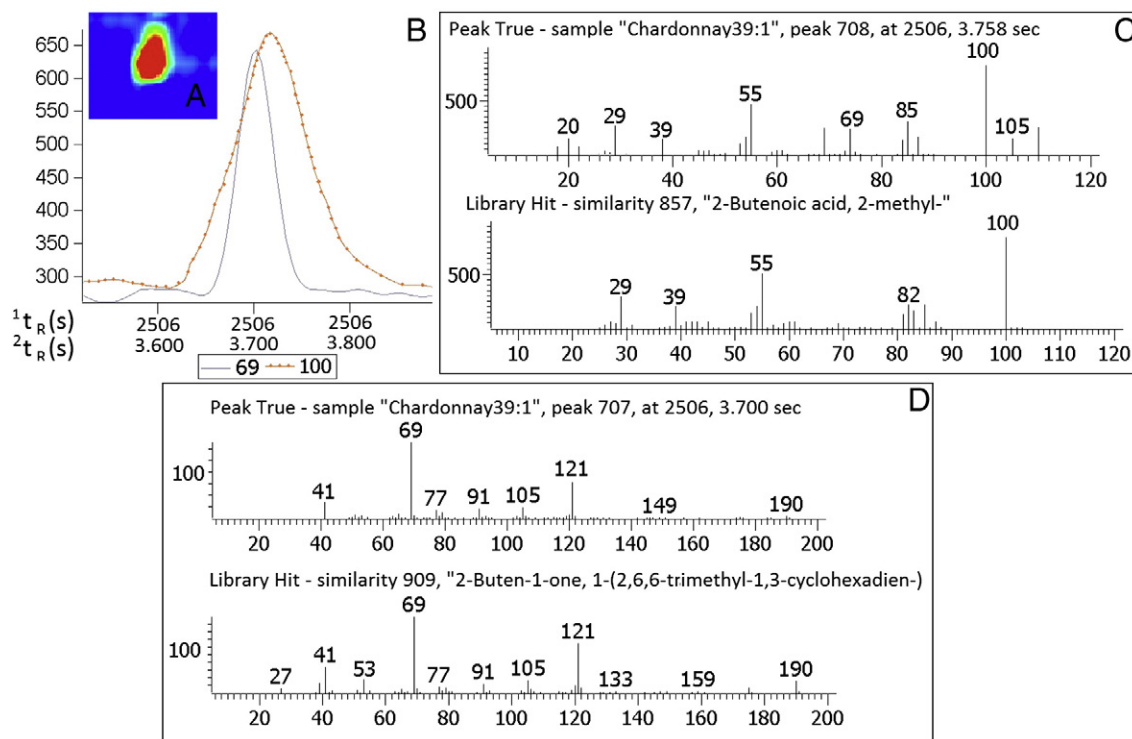


Fig. 3. Co-elution # 17 (Table 2) in the first and second dimensions of two volatile compounds of Chardonnay wines. (A) Part of GC \times GC/TOFMS total ion current chromatogram (TIC) showing the two co-eluted compounds in both dimensions. (B) Modulated peaks of (*Z*)-2-methyl-2-butenic acid (orange line, compound # 62 in Table 2) and β -damascenone [(*E*)-1-(2,6,6-trimethyl-1-cyclohexa-1,3-dien)-but-2-en-1-one, blue line, compound # 206 in Table 2] found in the headspace of Chardonnay wine. Deconvoluted mass spectra of (C) (*Z*)-2-methyl-2-butenic acid and (D) β -damascenone.

$<10 \mu\text{g L}^{-1}$: 2-phenylacetaldehyde (OAV = 1.7, # 91), ethyl isovalerate (OAV = 5.3, # 105), hexanal (OAV = 2.1, # 83), β -damascenone (OAV = 20, # 206), ethyl heptanoate (OAV = 2.5, # 114) and ethyl pentanoate (OAV = 2.1, # 106). The box-plot provides information on dispersion and asymmetry of the quantitative data. The top and bottom rows of the box indicate that 75% and 25%, respectively, of the samples have lower concentration than the value expressed by these bars.

The ROC, that represents the contribution percentage of the each volatile compound to aroma, is also shown in Fig. 2. Ethyl octanoate is the compound that showed the highest contribution to aroma (ROC = 13.6%), followed by ethyl hexanoate (ROC = 10.7%) and ethyl butanoate (ROC = 5.6%). Ethyl octanoate and ethyl hexanoate are also the compounds with more expressive contribution to the aroma of Cabernet Sauvignon, Cabernet Gernischt and Chardonnay wines from China (Jiang & Zhang, 2010). However, the contributions of these compounds to the aroma of Chinese wines were 92.9 and 93.3%, respectively. Several aspects may explain differences between the aroma of Brazilian and Chinese Chardonnay, as for example climate, enological practices, etc. Furthermore, extraction and analysis of the Chinese wines were performed with HS-SPME-GC/MS and only twelve among the 42 tentatively identified volatile compounds showed OAV >1 . In the present work a higher number of compounds (243) were tentatively identified using HS-SPME-GC \times GC/TOFMS and 47 of them had OAV >1 . Then, as a higher number of compounds contribute to the flavor of the Serra Gaucha Chardonnay, the percentage contribution of each compound to the overall aroma, expressed by the ROC, is reduced.

ROC relative standard deviation (RSD) for each compound in twelve Chardonnay wines is shown in Fig. 2. The variation in the concentration of the majority of the volatile compounds responsible for the aroma of the twelve Chardonnay wines was less than 10% for 83% of the compounds. These data show that these twelve Chardonnays produced in the Serra Gaucha region presented a reasonable similarity among them regarding aroma. Ethyl 2-phenylacetate (RSD = 25%) and

3-methyl-3-buten-1-ol were the compounds with the highest RSD for ROC values. However their contribution to aroma is not among the most expressive, as their ROC values are low (0.5% for each compound).

β -Damascenone was the fourth compound with highest ROC (ROC = 4.5%) (Fig. 2). This C13-norisoprenoid (# 206 in Table 2) co-eluted with (*Z*)-2-methyl-2-butenic acid (# 62 in Table 2) in both first and second dimension (Fig. 3). Fig. 3A and B shows the superimposed chromatographic peaks and Fig. 3C and D, the mass spectra of the two compounds compared with their mass spectra in the NIST library. Spectral deconvolution based on mass spectra differences was quite useful in this case, as chromatographic selectivity was not enough to achieve separation, even though two different stationary phases have been employed for analysis. The odor of (*Z*)-2-methyl-2-butenic acid is described as spicy, pungent (Lee et al., 2008), while β -damascenone can contribute to sweet or honey odor (Gurbuz et al., 2006). Correct separation and identification of these compounds are crucial for a fair characterization of important aroma characteristics and this illustrates the importance of spectral deconvolution.

4. Conclusions

The well known higher capacity of GC \times GC/TOFMS for the analysis of complex samples proved to be appropriate for this first quantitative analysis of volatile compounds of Chardonnay wines, as separation of first dimension co-elutions were achieved through the selectivity of the second dimension column and also through spectral deconvolution, rendering an adequate method validation for a detailed analysis. Co-elutions in ^1D point to difficulties that might arise whenever only 1D-GC/MS is employed, possibly resulting in insufficient chromatographic separation and consequently incorrect identification and quantitative results. A higher number of compounds with OAV >1 than are usually found by 1D-GC/MS were verified and this also might be attributed to the higher efficiency of GC \times GC/TOFMS.

The combination of GC × GC/TOFMS for qualitative and quantitative analyses of Chardonnay wines along with the OAV and ROC approaches has been performed for the first time and has been shown to be advantageous, as it allowed a fast overview of the linking between concentration, odor descriptors, and odorant impact for each volatile compound, resulting in the discovery of the most important compounds for the aroma of Chardonnay wines and their concentrations. A detailed quantitative analysis combined with OAV and ROC determination for a varietal wine, followed by the same procedure focused only on the most important odorant compounds for a higher number of wine samples of the same varietal wine paves the way to facilitate the implementation of meaningful quality control for the wine industry. Future work for a better characterization of the aromatic profile of wines, using olfactometric detector with 1D-GC and/or GC × GC will be important for a more detailed description of the contribution of each compound to the aroma. Furthermore, the effect of synergistic interactions and sensory evaluation by trained panelists should be considered to complement these studies of the aroma of Brazilian wines.

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