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Analytical Methods

Aroma impact components of Brazilian Cabernet Sauvignon wines using detection frequency analysis (GC–olfactometry)

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Abstract

The aroma profiles of Cabernet Sauvignon wines from a new grape growing region, Santa Catarina State, Brazil, were established for the first time using a Gas-chromatography–olfactometry (GC–O). Two wines were submmited to detection frequency analysis (DFA) ($n = 8$), one having vegetative characteristics (SJA wine) and one with red fruits and jam aromas (BR wine) in a prior sensory analysis. Fourteen impact aroma descriptors were selected for judging by DFA analysis. Among these, nine compounds were identified using GC–MS, chromatographic retention times and characteristic odours: acetic acid, butyric acid, isovalerianic acid, 2-phenylethanol, methional, 2-methoxy-3-isobutylpyrazine (MIBP), β-damascenone, β-ionone and furaneol. In most, furaneol was associated with jam or caramel aroma by GC–O and its average concentrations in BR wines (252 μg/l) were significantly higher than those in SJA wine (112 μg/l). In contrast, the amount of MIBP, reported as vegetative or bell pepper aroma by GC–O analysis, was much higher in SJA (0.040 μg/l) than BR (0.018 μg/l) wine samples. In the two wines evaluated, β-damascenone was measured at concentrations that are probably responsible for positive fruity notes and by to mask the vegetal aroma of MIBP in BR wine sensory analysis.

Keywords: GC–olfactometry; Detection frequency analysis (DFA); Brazilian Cabernet Sauvignon wines; GC–FID/FPD/MS

1. Introduction

Identification and ranking of odour active components in wines involves both human olfactory perceptions in concert with instrumental measurements. GC–Olfactometry (GC–O or GC-sniffing) quantifies impact odorants in foods using the human nose as a detector. The human nose is often more sensitive than any instrumental detector, and GC–O is a powerful tool for measuring flavours and perfumes, as well as any odoriferous product (Pollien et al., 1997). The GC–O methods that have been developed and applied can be categorized into three general approaches: extract dilution methods, intensity methods, and the detection frequency method. Dilution methods are based on sensory evaluations of stepwise aroma extract dilutions until no odour is perceived (Acree, Cunningham, & Cunningham, 1984; Grosch, 1993). Component ranking is based on the assumption that the higher the dilution at which a compound can be detected by GC–O, the more significant is the odour component. However, the variability of human olfactory sensitivity requires many different evaluators for each GC–O. Psychophysics principles need to be applied to the interpretation of the data (Stevens, 1975). Intensity methods also employ human judges to assess the intensity of eluting odour components in an aroma extract, submitted to GC–O (Pollien et al., 1997). Detection frequency analysis (DFA) (Pollien et al., 1997) is based on the assumption that the relative number of subjects

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detecting an odour at any given retention time, during a GC–O run reflects the relative importance of the odour component. From a preliminary dilution level injection the percentage of panellists who detect an odour is computed over the entire GC run. The unit of olfactogram peak height is the NIF (nasal impact frequency). Because this method is based on the response of a whole panel, the inattention factor, and specific anosmia on the final olfactogram is minimized. However, 6–10 panellists are required for a repeatable result, and this requires the same number of injections (Debonneville, Orsier, Flament, & Chaintreau, 2002). In contrast to the dilution methods, that utilize multiple replicates using the same few individuals, the detection frequency analysis involves only one concentration level.

Impact aroma compounds can be used to support and guide the production of optimum quality wines. Campo, Ferreira, Escudero, and Cacho (2005) considered GC–O to be a useful tool for differentiating Madeira wines (Malvazia, Boal, Verdelho and Sercial grape varieties) and for screening active odorants. The wine aromas were mainly characterized as candy, nutty, woody, toasty, lacquer and dried fruit. Ferreira, Lopez, and Cacho (2000) evaluated Spanish single-variety red wines from Grenache, Tempranillo, Cabernet Sauvignon and Merlot grapes by HRGC–MS to quantify 47 odorants, previously identified as potential aroma contributors, by olfactometric techniques. Within the concentration ranges found in wines, ethyl octanoate, β-damascenone, ethyl hexanoate, isovalerianic acid and isoamyl acetate were dominant. Isoamyl and beta-phenylethyl alcohols, fatty acids, 2,3-butanedione and ethyl butyrate also contributed significantly. In another study done on the flavour of sweet Muscat wines, GC–O was considered a useful tool for evaluating the relationship between the consensual descriptors of flavours and the identifiable volatile substances in these wines (Cutzach, Chatonnet, & Dubourdieu, 1998).

In this study, Cabernet Sauvignon wines from five young vineyards in the State of Santa Catarina (southern Brazil) were examined. In this region, *Vitis vinifera* grapes have been raised since 2000. The main objective of this work was to use GC–O analysis to characterize active odorants in Cabernet Sauvignon wines (2004 vintage) from two vineyards from this new grape growing region. Quantitative analysis was also carried out on the highest impact active odorants using GC–FID/FPD/MS. A secondary objective was to evaluate the DFA method for its ability to screen powerful odorants in young Cabernet Sauvignon wines.

2. Material and methods

2.1. Wine samples

Wines from the 2004 vintage of Cabernet Sauvignon variety, taken from five vineyards in Santa Catarina State (SC), Brazil, were analyzed by GC–O: codes SJA (coordinates: 28°16′41″ lat. and 49°55′96″ long.) and SJB (coordinates: 28°19′0″ lat. and 49°34′51″ long.) correspond to São Joaquim vineyards, at 1415 and 1160 m asl, respectively; AD corresponds to Água Doce vineyard (coordinates: 26°43′30″ lat. and 49°55′60″ long.) at 1350 m asl; BR corresponds to Bom Retiro vineyard (coordinates: 27°53′5″lat. and 49°34′51″ long.) at 960 m asl and VID corresponds to Videira vineyard (27°0′14″ lat. and 51°9′0″ long.) at 774 m asl. The wines were produced under the same micro-vinification conditions at EPAGRI (Empresa de Pesquisa e Extensão Agropecuária de Santa Catarina), in Videira, SC, Brazil. The grapes were separated from the stalks, crushed and maintained in a 20 l capacity stainless steel vat. The maceration period was 10 days, with two daily pumpings over at 22 °C. The must was separated from the solid parts and transferred to 13 l capacity stainless steel vats. Prior to initiating alcohol fermentation, a commercial sulfiting agent (20 g/100 kg of must, corresponding to 10 mg/l of free SO_2) (Noxitan, Pascal Biotech, Paris), *Sacharomyces cerevisae* strain (20 g/100 kg) (Fermol Rouge, Pascal Biotech, Paris) and commercial enzymes with pectinolytic activity (2–4 g/hl) (Pectinex SPL/Ultra, Pascal Biotech, Paris) were added to the musts. Malic acid consumption by lactic bacteria occurred spontaneously within 20–25 days. Once alcohol fermentation had finished, the wines were chilled to −4 °C for 10 days, Noxitan (35 mg/l of free SO_2, on average) was added, before bottling. All the samples were 20 months old at the time of analysis. The wine samples were stored at 5 °C prior to analysis and were analyzed at Bordeaux University.

2.2. Reagents

Reagents (and their respective sources) were: dichloromethane (ultra-high-purity, Merck, Darmstadt, Germany), diethyl ether (99.7% min, SDS, France), hexane (99.7%, SDS, France), 2-methoxy-3-methyl pyrazine (Aldrich Chemicals Co., Millwaukee, WI, USA), 2-methoxy-3-isobutylpyrazine (99% pure, Aldrich Chemicals Co., Millwaukee, WI, USA), β-damascenone (77% pure GC, synthesized by Firmenich, Geneva, Switzerland), α-ionone (90% pure) and β-ionone (97% pure) (Aldrich Chemie, Steinheim, Germany), octan-2-one (Sigma, USA), 3-octanol (Aldrich Chemie, Steinheim, Germany), 4-methylsulfanylphenol (Aldrich Chemie, Steinheim, Germany), di-*tert*-butyl-*p*-cresol (BHT) (Aldrich Chemie, Steinheim, Germany) and $(NH_4)_2SO_4$ (Aldrich Chemie, Steinheim, Germany).

2.3. GC–FID–olfactometry (GC–O)

Fifty millilitre samples of wine were extracted with 4, then 2, then 2 ml of dichloromethane by stirring each mixture for 5 min. The organic phases were collected by decantation as emulsions, which were partially clarified with a stirring rod. The stable emulsion was concentrated to 10 times under a nitrogen stream before injection. GC–O

was used to determine odour-active chromatographic zones and to assess olfactory stimulus intensity. The instrument used was an Agilent HP 4890 gas chromatograph, Series II. A flower splitter connected the column exit to a FID detector and an ODO-1 glass-sniffing mask (SGE, Victoria, Australia). The 20 cm of the column was linked to the sniffing port, to permit the judges to sniff all the effluents. The GC effluent was combined with humidified air at the rate of 15 ml/min at the bottom of the mask to avoid nasal dehydration. The column used was a FFAP from SGE (BP 21, 50 m × 0.32 mm × 0.25 μm, Courtaboeuf, France). Two microlitres of the extract were injected in splitless mode. Injector and FID-detector (when the column it was not linked to sniffing port) were both kept at 250 °C. The oven temperature was kept at 40 °C during 1 min and after it was increased at 3 °C/min from 40 °C to 220 °C and held at this temperature for a further 25 min.

GC–O–FID retention times were correlated to GC–MS retention times with a standard mixture of potent aroma compounds in the relevant retention time span, analyzed under identical chromatographic conditions. Odorants were identified by comparing their odours, chromatographic retention times and MS spectra with those of pure reference compounds.

The wine sensory profiles, previously assessed by Falcão et al. (2007), showed that the primary difference observed by sensory analysis of SJA, SJB, AD, BR and VID wine samples was the contrast between the vegetative and red fruit aromas. Then, wine samples described as vegetative (SJA) and red fruits or jam aromas (BR) were selected for DFA.

In order to identify zones responsible for fruity and vegetative odours, extracts of the five wine samples were submitted to continuous sniffing for 50 min. One judge was exchanged for another after 25 min. Following this survey, a 25 min chromatographic zone was selected to cover the retention times of the odorants. A panel of eight judges, five women and three men, who were experts in GC–O analysis, sniffed the zones produced from the two wines selected, using DFA. The same wine sample was evaluated twice by each of the eight judges. Each judge carried out one or two 25 min sessions per day. When a judge had two sessions on the same day, they were separated by 5 h. During DFA, the judges recorded the time for onset and end of a perceived odour while sniffing the effluent from the sniffing mask. The judges also noted the eluting odour characters and intensity (1 = weak, hardly recognizable note; 2 = clear but not intense note; 3 = intense note) of each attribute. Sniffing was carried out in a temperature-controlled room (20 °C).

The data from the GC–O evaluations were then compiled into aromagrams having the nasal impact frequency (NIF) as a time function: $NIF = N_t/n \cdot 100$, where N_t is the number of judges recognizing an odour at time t; n is the total number of judges exposed to the GC–O effluent at time t. A NIF score of 100% signifies that all the judges detected an odour at a certain retention time (Pollien et al., 1997). The nasal impact frequency (SNIF) parameter was calculated as the summed minutes that one peak lasted (Nielsen & Poll, 2004).

2.4. Quantitative analysis

2.4.1. GC–MS

For C_{13}-norisoprenoids and 2-methyl-3-isobutylpyrazine (MIBP), the method of Kotseridis, Anocibar Beloqui, Bertrand, and Doazan (1998) was adapted as previously reported (Falcão et al., 2007). The procedure of Guedes de Pinho and Bertrand (1995) was used for furaneol with the following modifications: 10 μl of the internal standard octan-2-one solution (2.014 g/l in 50% ethanol solution) were added to 50 ml of wine sample. The split/splitless injector was held at 250 °C with a division of 30 ml/min and a split time of 0.5 min. The temperature of detector was 250 °C. The carrier gas pressure (Helium 5.6 Alphagaz) was 18 psi with a linear speed of 4.1 ml/min. The oven temperature programme was held for 5 min at 60 °C, then increased at 3 °C/min to 200 °C, then held at this temperature for 15 min. Quantification was carried out in SIM mode, with the following ions: $m/z = 57$, 85 and 128 for the qualifier, using $m/z = 128$ for the quantifier. The mass chromatograms were recorded in the electron impact (E_i) mode ($E_i = 70$ eV). The mass range was 50–600 m/z.

2.4.2. GC–FID

Two hundred microlitres of octan-3-ol solution (400 mg/l in 50% ethanol) and 300 μl of an orthophosphoric acid solution (1/3) were added to the 50 ml wine sample. The sample was extracted consecutively (for 4, then 2, then 2 min) with 5 ml ether/isohexane (1:1, v:v). A Carlo Erba HRGC 5300 gas chromatograph (Thermo Separation Products, Courtabœuf, France), equipped with a FID detector was used. The column was a FFAP capillary column (BP 21, 50 m × 0.32 mm, film thickness 0.25 μm; SGE, Courtabœuf, France). Injection of 2 μL of extract was done in split/splitless mode (division: 30 ml/min and split time: 0.5 min). The carrier gas pressure (hydrogen 5.0) was 8 psi with a linear speed of 1.5 ml/min. The oven was held for 5 min at 40 °C, then raised at 2 °C/min to 220°C, then held at 220 °C for 25 min. The injector and detector temperature were 200 °C and 250 °C, respectively.

2.4.3. GC–FPD

The method used was one described by Beloqui and Bertrand (1995). The 50 μl wine sample was supplemented with the following before extraction: 50 μl of 4-(methylsulfanyl) phenol at 702 mg/l (hydroalcoholic solution, 50% of ethanol) as internal standard, 200 μl of di-tercbutyl-para-cresol (BHT) at 1.1 mg/l and 300 μl of orthophosphoric acid (1/3) (v/v with water). Dichloromethane, 5 ml, was added and the mixture was shaken for 5 min and the aqueous layer was reshaken with fresh dichloromethane. The combined extract was dried with 5.0 g anhydrous sodium sulphate and concentrated under nitrogen to

Table 1
GC–O observations in Brazilian Cabernet Sauvignon wines

Stimuli nos.	GC–O RT (min)	Odour description	Wine samples[a]				
			SJA	SJB	BR	VID	AD
1	3.4–3.6	Fruity	1	1	3	–	1
2	4.9	Menthol	1	–	2	–	–
3	5.4–5.7	Banana, cooked banana	**1**	**2**	**3**	**2**	**1**
4	8.1	Garlic, toasted	–	1	1	–	–
5	6.3	?	1	–	1	–	1
6	8.4	Jam, cooked red fruits	1	–	2	–	–
7	8.4–8.7	Heated milk, roasted	1	1		1	–
8	11.8–12.15	Fruity, strawred fruits	**1**	**1**	**1**	**1**	**1**
9	13.1–13.2	Vinegar	**1**	**2**	**3**	**3**	**1**
10	14.1–15.8	Vegetable, green pepper	**3**	**3**	**1**	**1**	**2**
11	16.2–16.6	Flowery	–	1	–	1	–
12	16.7–17.5	Grilled, coffee torrefied	1	–	1	2	–
13	17.4–17.5	Strawred fruits, fruity	**1**	**1**	**2**	**1**	**2**
14	17.4–17.9	Unpleasant, sulphur	1	–	1	–	–
15	17.5–18.7	coffee torrefied	1	–	3	1	–
16	18.8	Green, vegetable	2	–	–	1	–
17	19.1–19.6	Cheese, sulphur	**1**	**1**	**1**	**1**	**1**
18	19.7–21.1	Dirty socks, old cheese	**1**	**3**	**3**	**3**	**3**
19	22.3	Pear, soaped	–	–	1	–	–
20	22.8	Vegetable, green wood	1	–	1	3	–
21	23.1–23.2	Flowery	**3**	**1**	–	**1**	**1**
22	23.3–23.4	Spices	–	–	1	–	1
23	23.5–23.8	Pleasant, soap, rose	–	–	1	1	–
24	24.7	Vegetative	–	–	–	1	–
25	24.8–26.1	Peach, canned apple	**3**	**2**	**3**	**2**	**1**
26	25.75	Strawred fruits, cherry	–	–	–	1	–
27	25.85	Violet	–	–	–	1	1
28	26.4–27.1	Spices, flowery	–	1	–	1	–
29	27.2–27.3	Vegetable, onions	**3**	**1**	**1**	**1**	**3**
30	27.6	Cooked	1	–	–	–	–
31	27.9–28.0	Animal, sweat	**2**	**1**	–	**1**	**1**
32	28.2	Spices, black pepper	1	–	2	–	1
33	28.4	landly, dust	–	–	–	–	1
34	30.4	Honey	–	1	–	–	–
35	30.7	Spices	–	–	–	1	–
36	31.0	Rose	**2**	**3**	**3**	**3**	**3**
37	32.9–33.29	Flowery, violet	–	1	–	1	–
38	33.3–33.6	Pear, peach	1	–	1	1	–
39	34.4–36.6	Caramel, cooked strawred fruits	**1**	**3**	**3**	**3**	**2**
40	35.1	Spices	–	–	–	1	–
41	35.5	Leather	–	–	–	1	–
42	35.9–36.3	Plastic, rubber	1	–	–	2	–
43	36.3	Toasted almond	–	–	–	1	–
44	36.8	Sulphur	–	–	–	–	1
45	36.9	Flowery	1	–	–	–	–
46	38.1	Animal, sweat	–	–	1	–	2
47	39.1	Jam	–	–	1	–	–
48	38.1–41.4	Curry, spicy	**1**	**3**	**2**	**3**	**3**
49	40.8	Smoky	–	–	1	–	–
59	41.4–41.8	Spicy, black pepper	1	–	1	–	–
51	41.0-41.7	Leather, animal	–	1	–	1	–
52	42.8	Smoky	–	–	1	1	–
53	43.3	Vegetable, green beans		1	–	1	–
54	44.6	Sulphur	–	1	–	–	
55	43.8	Grilled	–	–	1	–	–
56	46.5–47.0	Spicy	–	–	1	–	1
57	47.3	Sulphur				1	
58	50.2–51.9	Caramel, chocolate, faded rose	**1**	**1**	**1**	**3**	**3**
59	51.6–51.8	Groove	–	–	–	1	–
60	52.7	Cooked	–	–	1	–	–
61	53.7	Flowery	–	–	1	–	–
62	54.0	Sulphur	–	–	–	–	1

? = Compound not described. 1 = weak, hardly recognizable note; 2 = clear but not intense note; 3 = intense note. In bold, odorant zones common for every wine.

[a] Average results of two experienced sniffers (in duplicate).

one-fourth of its initial volume. Two microlitres of the sample extract were injected into an Agilent Tech., HP 6890 gas chromatograph Series II, fitted with a flame photometric detector (FPD). The column was the same as that used above. The oven temperature was held for 1 min from 40 °C, then increased at 3 °C /min at 40–220 °C, then held at 220 °C for 20 min. The carrier gas was helium 5.6 (1.5 ml/min). The injector was a splitless system: the splitless time was 20 s and the split vent 30 ml/min. The injector and detector temperatures were 250 °C and 220 °C, respectively.

Quantitative data for the identified compounds were obtained by square root of the relative area interpolation versus the internal standard area. The identification was confirmed by retention times compared with those of standards and compared also with their mass spectra (NBS75K library) in SCAN mode.

3. Results and discussion

3.1. Olfactometric data selection of Cabernet Sauvignon wines

This analysis had two complementary objectives. The first was to characterize the odorant zones of the five Cabernet Sauvignon wines that were recently produced in Santa Catarina State, Brazil. SJA and BR wine samples were previously reported to be distinguished, respectively by vegetative and red fruits/jam notes in sensorial analysis (Falcão et al., 2007). The second was to determine what 25 min chromatographic zone revealed the common vegetative, red fruits and jam odorants of these wines. In this step, two judges, experienced in GC–O analysis, carried out olfactory evaluation using a three points scale on the five wines. The data included chromatographic retention times of odour detections, odour descriptions and intensities (Table 1). Sixty-two different olfactometry-signals were detected (each one associated with one or more descriptors). The number of odours detected in GC–O was large because each chemical compound was perceived independently. This analysis is different from a classical sensory analysis, since odorants can have a synergistic effect in sensory analysis but not in GC–O analysis. In addition, the combination of two or more odorants can have an effect on the final aroma (more or less intense) and with a different nuance of the odorants separately. Among the 62 olfactometry signals, 14 active odours were common for every wine (stimuli nos. 3, 7, 9, 10, 13, 17 18, 21, 25, 27, 36, 39, 48, 58 shown in Table 1) and, from these, 12 were perceived between the 10th and 35th minute retention time. This 25 min zone was therefore selected for DFA.

3.2. Ranking of odorants by DFA

Concentrated dichloromethane extracts of the BR and SJA wines were evaluated by DFA, using eight judges

Table 2
The main odorant active zones in two Cabernet Sauvignon wine previously distinguished by sensory analysis

Peak	BR wine					Peak	SJ(A) wine				
	Odour description	Compound	Int.*	NIF	SNIF		Odour description	Compound	Int.*	NIF	SNIF
Total minutes					54.4						56.4
1	Green apple, artificial fruit, coconut	ni	0.9	67.0	1.5	1	Artificial fruit	ni	0.9	73.2	2.3
2	Vinegar	Acetic acid[a,b,c]	1.6	85.7	2.0	2	Vinegar, solvent	Acetic acid [a,b,c]	1.5	93.8	2.8
3	Bell pepper, vegetative, gas	MIBP[b,c]	0.9	67.0	2.2	3	Bell pepper, vegetative	MIBP[b,c]	1.7	67.9	4.0
							Animal, mouse, unpleasant	ni	0.7	26.8	1.4
4	Coffee torrefied, smoky	ni	1.3	66.1	1.4	4	Toasted broad	ni	0.2	33.0	0.8
5	Cheese, rancid	Butyric acid[a,b,c]	1.5	73.2	5.2	5	Cheese, rancid	Butyric acid[a,b,c]	1.2	67.0	5.7
6	Dirty socks, old cheese	Isovalerianic acid[a,b,c]	2.9	100.0	10.7	6	Dirty socks, old cheese	Isovalerianic acid[a,b,c]	2.9	100.0	9.5
	Flowery	ni	0.6	46.4	1.3						
7	Cooked potato, soup	Methional[a,b,c]	1.1	51.8	2.2	7	Sulphur, cooked potato	Methional[a,b,c]	1.1	45.5	2.1
8	Peach, canned apple	β-Damascenone[a,b,c]	2.3	80.4	4.1	8	Peach, canned apple	β-Damascenone[a,b,c]	2.2	100.0	7.8
	Vegetable, green	ni	1.2	66.1	2.3		Peach, flower	ni	0.4	19.6	0.9
9	Animal, sweat, spicy	ni	1.3	80.4	2.8	9	Animal, spicy, 'Knorr'	ni	0.8	66.1	3.9
10	Rose, flowery	2-Phenylethanol[a,b,c]	2.4	87.5	8.3	10	Rose, flowery	2-Phenylethanol[a,b,c]	2.4	100.0	10.0
11	Violet, flowery	β-Ionone[b,c]	0.8	46.4	1.3	11	Violet, flowery	β-Ionone[b,c]	0.6	47.3	1.7
12	Jam, cooked red fruits, caramel	Furaneol[a,b,c]	1.9	81.3	8.3	12	Jam, red fruits, caramel	Furaneol[a,b,c]	1.1	52.7	2.6

Int. = Intensity. *Scale of 1–3 (average values, $n = 8$, two repetitions). ni = compound not identified; NIF = nasal impact frequency; SNIF = summed minutes that one peak lasted.
[a] Identified by the coincidence of GC–MS spectra and retention times of pure compounds.
[b] Identified by the peak coincidence between retention times data and characteristic aroma.
[c] Literature.

experienced in GC–O analysis (two repetitions). Quantitative analysis employing coincidence of GC–MS spectra, retention times of pure compounds and aroma characteristics resulted in the positive identification of 9 wine compounds (Table 2). All these compounds could be directly associated with odours detected by the judges in GC–O. The NIF and SNIF scores are presented in Table 2. Five of the 14 odours detected by the judges, were not identified by GC–MS, probably because their concentrations were below the method detection limit. The olfactometry profile varied between SJA and BR wines. In general, fermentative compounds, which generally occur in young wines were the most powerful odorants detected. Fourteen odours for the two wines selected by DFA were detected by at least two of the eight judges for calculated NIF scores of 45% or higher (Fig. 1, Table 2).

SNIF was more efficacy than NIF parameter in the differentiation of the wines, mainly for odours decrypted as 'vegetal' and 'jam/red fruits/caramel' (Table 2). According to the SNIF values, the most important odorants (considered to have a mean value $\geqslant 4.0$ min) in the BR wines were: isovalerianic acid < furaneol = 2-phenylethanol < butyric acid < β-damascenone, which were associated with cheese, rancid, dirty socks or old cheese, peach or canned apple, rose or flowery, jam or caramel notes, respectively. In the SJA wines, the most important zones according to SNIF values were: 2-phenylethyl alcohol < isovalerianic acid < β-damascenone < butyric acid < 2-methoxy-3-isobutylpyrazine (MIBP), associated with rose or flowery, dirty socks or old cheese, peach or canned apple, cheese or rancid and bell pepper or vegetative, respectively. These aroma profiles revealed that BR wine had a high SNIF value for

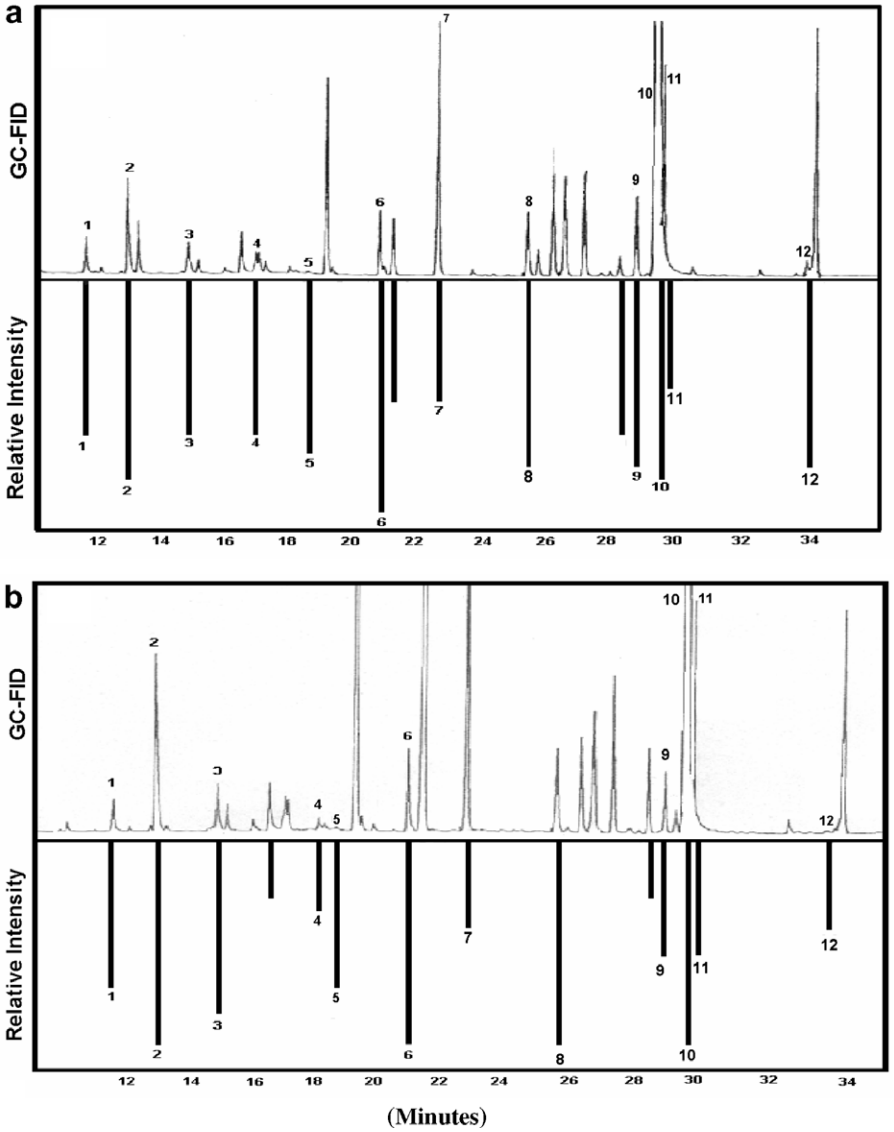

Fig. 1. GC–FID and average time-intensities of two repetitions by eight panellists of Cabernet Sauvignon wines on FFAP (BP 21) column. (a) BR wines and (b) SJ(A) wines. The numbers refer to compounds in Table 2.

furaneol, associated with jam or caramel notes. In SJA wine, this compound has a SNIF value significantly lower and the bell pepper or vegetative notes are dominant in the active odorant ranking.

3.3. Chemical quantification of active odorants

Except for isovalerianic acid and 2-phenylethanol (the two main compounds in the BR and SJA wines by GC–O analysis), all the compounds detected by GC–O gave values above the sensory olfactometry thresholds described in the literature. Table 3 shows the concentrations and corresponding threshold values of eight odorants identified in the SJA and BR wines. These data confirm that most of the data obtained in the FDA study were appropriate for GC–O analysis. Most of the compounds with high NIF and SNIF scores were also detected by GC–MS.

Escudero, Hernández-Orte, Cacho, and Ferreira (2000), demonstrated that the key odorant in oxidized wine was methional, which causes an off-flavour reminiscent of cooked vegetables. In this study, methional, in both BR and SJA wines, was described by GC–O as cooked potato. Methional could not be detected by GC-FPD in the SJA wine. MIBP was measured at 0.040 μg/l by GC–MS, well above the olfaction threshold (0.015 μg/l) in SJA wine. In BR wine, this value was 0.018 mg/l only slightly above to the olfaction threshold. Therefore, the SNIF value for MIBP was 46% higher in SJA wines than in BR wines. MIBP levels in the wines were higher than those obtained by Kotseridis, Baumes, Bertrand, and Skouroumounis (1999) in Cabernet Sauvignon and Merlot wines (0.002–0.014 μg/l), but within the range found for Cabernet Sauvignon wines by Allen, Lacey, and Boyd (1994) (0.003–0.036 μg/l) and for Japanese red wines evaluated by Hashizume and Umeda (1996) (0.0036–0.0563 μg/l).

C_{13}-norisoprenoid levels probably benefited the Cabernet Sauvignon wines evaluated in this study. From Table 3, β-damascenone levels are well above their odour threshold values (4–7 μg/l) (Ferreira, Ardanuy, Lopez, & Cacho, 1998; Pineau, Barbe, Van Leeuwen, & Dubordieu, 2007). The β-damascenone levels were higher than those found by Sabon, De Revel, Kotseridis, and Bertrand (2002) for Grenache wines (1.35–4.17 μg/l) and by Kotseridis et al., 1998 (0.2–1.3 μg/l for Merlot wines) and very much lower than those found by Perestelo, Fernandes, Albuquerque, Marques, and Câmara (2006) (500–112,100 μg/l) for Tinta Negra Mole wines. β-Damascenone may manifest itself differently in different cultivars. Odour nuance for this compound is likely to depend on its concentration and on the general composition of the wine. Our GC–O data clearly associate β-damascenone with peach or canned apple notes, similar to the results of Ferreira et al. (2000). Research carried out by Pineau et al. (2007) indicated that, in model media, β-damascenone can act as an aroma enhancer for fruity notes and can to mask the vegetal aroma of MIBP. As showed in the Table 3, in our study, MIBP was detected in concentration slightly above their olfactory threshold in the BR wine and well above in SJA wine (Table 3). But, in a previous classic sensory analysis (Falcão et al., 2007), the BR wine ("960 wine") was considered as "fruity/jam aromas". It signifies that the judges of the sensory panel were not capable of detect the vegetal aroma of MIBP in the BR wine. Probably, the presence of β-damascenone at concentration well above their olfactory threshold can mask the vegetal aroma of MIBP in this wine.

2-Phenylethanol was detected in every wine in relatively higher concentration (42,730–90,160 μg/l) than their odour threshold values in hydro-alcoholic solution (10,000 μg/l) (Guth, 1997). This indicates that it plays an important role in wine bouquets. Furaneol was found at particularly high

Table 3
Quantification of the 8 principals compounds responsible for the odorant active zones in Cabernet Sauvignon wines and odour thresholds

Compounds (μg/l)	BR wine	SJA wine	Odour threshold value (μg/l)
Acetic acid	n.a.	n.a.	200,000 (Guth, 1997)[a]
3-Methoxy 2-isobutyl pyrazine***	0.018 ± 0.00	0.040 ± 0.00	0.015 (Roujou de Boubée et al., 2000)[b]
Butyric acid*	8160 ± 220	11430 ± 600	173.0 (Ferreira et al., 2000)[c]
Isovalerianic acid*	8830 ± 290	9330 ± 990	2.0 (Devos et al., 1990)[d]
Methional**	153.00 ± 0.00	n.d.	0.5 (Escudero et al., 2000)[e]
β-Damascenone***	13.33 ± 0.47	17.20 ± 1.91	4.0 (Ferreira et al., 1998)[f]; 4–7 (Pineau et al., 2007)[h]
2-Phenylethanol*	90,160 ± 63810	42,730 ± 8140	10,000 (Guth, 1997)[a]
β-Ionone***	0.08 ± 0.01	0.14 ± 0.00	0.090 (Ferreira et al., 2000)[c]
Furaneol***	252.21 ± 3.90	111.47 ± 2.10	37.0 (Kotseridis & Baumes, 2000)[g]

n.a. = not analyzed. n.d. = not detected. Compounds analyzed by *GC–FID; **GC–FPD; ***GC–MS.

[a] The matrix was hydroalcoholic solution (10% ethanol).

[b] Matrix was ethanol at 12%, with 5 g of tartaric acid, pH 3.5.

[c] The matrix was a 11% water/ethanol solution containing 7 g/l glycerol, 5 g/l tartaric acid, pH adjusted to 3.4 with 1 M NaOH.

[d] Matrix was water.

[e] In synthetic wine.

[f] Matrix was ethanol at 12%, with 5 g of tartaric acid, pH 3.5 adjusted with 1 M NaOH.

[g] Matrix was a water/ethanol mixture 89:11, containing 4 g of tartaric acid and pH adjusted to 3.5 with K_2CO_3.

[h] Different matrix were utilized: hydroalcoholic solution was a water/ethanol mixture (88:12, v:v), with 4 g/l tartaric acid, pH adjusted to 3.5 (0.5 N KOH); three model wines (two red and one white) and a merlot red wine.

concentration compared to its threshold in wine (37 μg/l) (Kotseridis & Baumes, 2000) and it was higher in BR than SJA wines (Table 3). Furaneol can be found in quantities above 1 mg/l in wines from with hybrid grapes. At this concentration it produces a disagreeable strawred fruits scent (Rapp, Kripser, Engel, Ullemeyer, & Heimann, 1980). This compound was strongly linked to the caramel or red fruits jam aroma by GC–O and was responsible for this odour characteristic in sensory analysis.

Butyric acid has an unpleasant odour, described as cheese or rancid, and it is present in higher amounts in BR than in SJA wines. Measured levels are well above their threshold values, according to Ferreira et al. (2000).

In conclusion, this work evaluated the differences among wines from various sites by GC–O analysis. These differences were compared to quantitative data from GC–FPD/FID/MS. The location of the vineyard has a significant influence on the quality and amount of active odours in the wines. The DFA method resulted in the detection of 25 odours with a NIF score at or above 45% (in BR and SJA wines) where butyric acid, isovalerianic acid, 2-phenylethanol, methional, 2-methoxy-3-isobutylpyrazine, β-damascenone, β-ionone and furaneol were identified. It was clear that MIBP and furaneol were the compounds responsible, respectively, for the vegetative and red fruits/caramel notes in SJA and BR wines. β-Damascenone was present in the two wines evaluated in concentrations that could add beneficial fruity notes to the wine. In BR wine, where MIBP was detected at concentration slightly above their detection threshold, β-damascenone can be masking their vegetal odour. The aromatic profile obtained by DFA confirmed the previous difference observed by classical sensory analysis between BR and SJA as being the contrast of "fruity/jam or caramel" and "vegetative" notes, respectively. These findings help to assess the aroma profile of Cabernet Sauvignon, which has been recently produced in this new grape growing region. DFA (GC–O–FID) is a useful complementary detection technique that will help to explain wine aroma diversity.

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