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Aroma compounds of an Italian wine (*Ruché*) by HS–SPME analysis coupled with GC–ITMS

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Abstract

Headspace solid phase micro extraction (HS–SPME) was used for extraction of aroma compounds characterizing a Piedmont wine (*Ruché*) derived from a non aromatic vine. Extracted compounds were identified by ion trap mass spectrometry (ITMS) after gas-chromatographic analysis. In this way a selection of 59 identified primary aromatic compounds, related to the typical flavour of *Ruché* was made possible. The SPME technique showed peculiar behaviour in that 23 of the 59 compounds identified were not detected by liquid-liquid solvent extraction of the same samples. Subsequent comparison with the aromatic profiles of different wine samples obtained by microvinification from different grape varieties showed similarities between *Ruché* and the wines, *Brachetto* and *Malvasia*, originating from aromatic vines. SPME analysis proved to be useful in understanding aroma compositions of all samples examined, establishing bases for further investigations on the chemical and biochemical mechanisms underlying wine aroma development.

Keywords: SPME; GC–MS; Aroma compounds; Ruché wine

1. Introduction

The flavour of a wine presents an extremely complex chemical pattern in both qualitative and quantitative terms. Over 1000 aroma compounds have been identified, with a wide concentration range varying between hundreds of mg/l to the μg/l or ng/l level (Rapp, 1988). Moreover, wine aroma is represented by several classes of compounds, such as hydrocarbons, alcohols, terpene alcohols, esters, aldehydes, ketones, acids, ethers, lactones, sulphur and nitrogen compounds. Aroma production is influenced by several factors: environment (soil, climate), grape variety, ripeness, fermentation condition, biological factors (yeast strain and other components of the oenological microflora), the wine production process and aging (Rapp, 1988).

All aroma compounds may play a role in the characterization of the specific flavour pattern of each wine type. In some cases has been possible to isolate a few key compounds mostly representing the typical flavour of a wine (Versini, Rapp, & Dalla Serra, 1994; Williams, Strauss, & Wilson, 1980; Wilson, Strauss, & Williams, 1984, 1986), while in the majority of wines several compounds seem to cooperate, with specific ratios between them (Bayonove et al., 1971, Strauss et al., 1988). A better understanding of the key aroma compounds helps to control quality and may have an impact on the viticultural and wine technological processes.

Various extraction methods have been widely used for the analysis of volatile components of wines, such as distillation techniques, solvent extraction or solid phase extraction (SPE) (Dumont & Adda, 1979; Nijssen, 1991; Saxby, 1982).

These techniques allow quantitative data to be obtained, but are often labour-intensive. Besides, chromatographic signals of trace substances may be obscured by high concentrations of low-volatile compounds. Head-space analysis may overcome these disadvantages, allowing analysis of the volatile fraction only. Among head-space sampling techniques, head-space solid phase microextraction (HS-SPME) has specific advantages over conventional static, dynamic and

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purge and trap techniques: it is economic, faster and requires little manipulation of samples (Elmore, Erbahadir, & Mottram, 1997; Jelen, Wlazly, Wasowicz, & Kaminski, 1998; Penton, 1997; Stevenson & Chen, 1996; Xiaogen & Peppard, 1994). For these reasons it has been used for the analysis of a wide range of food products (Bicchi, Panero, Peelegrino, & Vanni, 1997; Chin, Bernhard, & Rosenberg, 1996; Field, Nickerson, James, & Heider, 1996; Jun-Song, Gardner, Holland, & Beaudry, 1997; Matich, Rowan, & Banks, 1996; Miller, Poole, & Pawlowski, 1996; Pelusio, 1995; Ulrich, Krumbein, & Rapp, 1997), including wine and alcoholic beverages (de La Calle-Garcia, Reichenbaecher, & Danzer, 1998a; de La Calle-Garcia, Reichernbaecher, Danzer, Hurlbeck, Bartzsch, & Feller,1998b; Evans, Butze, & Ebeler, 1997; Fischer & Fischer, 1997; Gandini & Riguzzi, 1997; Hayasaka & Bartowsky, 1999; Lay-Keow, Hupe, Harnois, & Moccia, 1996; Ong & Acree, 1999; Steffen & Pawliszyn, 1996; Tateo & Bononi, 1997).

The aim of this work is to identify key aroma compounds of an Italian wine, *Ruché*, focussing on primary aroma compounds. *Ruché* is a red berry vine cultivar, grown near Asti in Piedmont, Northern Italy, whose flavour composition is distinctive and largely unknown. An important feature of this berry vine is that its must is completely non aromatic. So the events leading to the formation of its final very peculiar aromatic pattern must be sought in some of the transformations occurring from the must to the wine. To this end, simplified model wine solutions have been made up to control any fermentation phenomena not directly involved in primary aroma compound production (see Materials and methods section). The gas chromatographic analysis of such solutions shows more simplified patterns of peaks than does whole wine.

Any improvement, in the understanding of composition may allow winemakers to tailor the winemaking process and to control the quality of the product.

This work is part of a project whose aim is to elucidate the complex mechanisms underlying flavour compound production in non-aromatic wines (that is to say without free odorous compounds in the fresh must), focussing attention on the biochemical reactions carried out by selected yeast strains (Delfini, Cocito, & Bonio, 1999; Delfini, Cocito, Bonio, Schellino, Gaia, & Baiocchi, 2001b).

2. Materials and methods

2.1. Samples

Several wine samples, originating from different grape varietes: *Ruché*, *Malvasia*, *Brachetto*, *Cabernet*, from different regions; *Pinot noir*, *Pinot grigio*, *Merlot*, *Croatina*, *Nebbiolo* were *ad hoc* prepared. Their microvinifications were conducted according to Delfini et al. (2001) with *S. cerevisiae* strain BR94 (registered in our microbial collection as CNLBSV-ISEAT 866), previously selected by our institute for vinification of *Ruché*, rigorously and under the same conditions. Microvinification of different grape varieties with the same yeast strain was programmed to substantiate the specificity of the aromatic pattern of the *Ruché* grape variety in contrast to other wines.

Furthermore in order to simulate the production of the key aroma compounds of *Ruché* under the simplest conditions possible, model wine solutions were made up as follows: 500 grammes of grape berries were crushed and separated from pulp and seeds. The obtained skins were stirred 3 h at 30 °C with the following digestion buffer: MES (20 mM), $MgCl_2$ (5 mM), cellulase [1 U/mg (ONOZUKA R-10) (SERVA, Heidelberg, Germany)] pectinase [0.5 U/mg (MACEROZYME R-10) (SERVA, Heidelberg, Germany)], BSA 0.5% (Sigma-Aldrich, Germany)], KOH to pH 5.5, 1:2 w/v. Digestion product was filtered on glass wool, then centrifuged, first at 5000 rpm for 15 min. Supernatant (in the following indicated as FRACTION 1) was lyophilised and resuspended in 50 ml of nutrient synthetic medium (minimal) (NSM/m), (Delfini & Formica, 2001; Delfini, 2001), containing only 5 g/l glucose, and incubated with 40×10^6/ml of actively growing yeast cells for 5 days at 25 °C. The pellet (exausted skins) was then resuspended in 500 ml of digestion buffer, without enzymes, and centrifuged at 20.000 rpm, giving another supernatant (in the following indicated as FRACTION 2) which was treated as FRACTION 1. The pellet was discarded.

Cellulase and pectinase are enzymes that can contribute to the release of aromatic precursors from the vegetable structures increasing their concentration in the must. By comparing the analytical results of FRACTION 1 with those of FRACTION 2 (that actually represent the control (or neutral) sample after the enzymatic treatment) allows optimized experimental conditions to obtain the maximum release of aromatic precursor from the skins into the must. Furthermore, the production of aroma compounds by the yeast in a nutritional synthetic minimal medium (NSM/m) containing only few grammes of glucose will minimise the alcoholic fermentation which can considerably complicate the general pattern of the analysable sustances.

The comparison of their gaschromatographic analysis with those of whole wines, obtained as previously reported, is a fruitful way to elucidate the substances most likely related to the aroma formation process.

2.2. Solid phase micro extraction (SPME)

Three SPME fibre coatings were evaluated and used: 65 μm polydimethylsiloxane (PDMS)/divinylbenzene

(DVB), 65 μm Carbowax/DVB and 100 μm PDMS (Supelco, Bellefonte PA, USA).

Fibres were exposed to 10 ml of sample in 20 ml gas-tight vials for 30 min at 27 °C, after an equilibration time of 15 min. Before using, fibres were conditioned, following instructions from manufacturers, and cleaned at 250 °C for 5 min. After this, fibres were desorbed in a 1078 split/splitless injector, equipped with deactivated SPME glass inserts (Varian, Walnut creek CA, USA) and analyses were carried out on a 30 m × 0.25 mm ID carbowax column (Supelco). A Varian Star 3400 CX coupled with a Varian Saturn 2000 ion trap mass spectrometer was used. Injection was split/splitless with 0.7 min relay time. GC conditions were: 35 °C for 5 min, 60 °C at 2.5 °C/min, 115 °C at 2 °C/min, 15 min at 115 °C, 180 °C at 2 °C/min, 220 °C at 4 °C/min and 220 °C for 10 min. Injector and transfer line were held at 220 °C, ion trap at 200 °C and the helium flow was 2.0 ml/min.

2.3. Liquid-liquid extraction

Ten to 50 ml of samples were extracted three times with CH_2Cl_2. Emulsion was resolved by centrifuging for 10 min at 3500 rpm and 4 °C. The organic fraction was then concentrated on a Vigreux column. Before extraction, 2-octanol (163 μg/l) was added to the samples as an internal standard. Splitless injection was used. GC conditions were the same as in SPME analysis.

2.4. Gas chromatography–mass spectrometry (GC–MS)

GC–MS analysis and identification were performed on a Varian Star 3400 CX gas cromatograph coupled with a Saturn 2000 ion trap mass spectrometer. Spectra were produced in the electron impact (EI) mode at 70 eV.

For Tandem Mass Spectrometry, a non-resonant excitation method was applied, with collision-induced dissociation (CID) amplitude ranging from 20 to 40 volts. Compounds were identified by comparison with reference spectra (Wiley 5 and NIST92 databases) and with reference standards.

3. Results and discussion

A standard mixture of analytes commonly found in wine flavour volatiles was used for the preliminary study of the extracting properties of different fibres. Three types of coatings were investigated: (1) PDMS/DVB, (2) Carbowax/DVB, (3) PDMS.

The equilibrium time of the system, represented by the polymeric coating, the headspace and the aqueous system, was found to be a fundamental parameter, determined in order to optimise recovery and reproducibility. Different coatings require different equilibration times as a function of the diffusion of the analytes through the system and the number of the molecules extracted (de-La Calle-Gercia, Magnaghi, Reichenbaecher, & Danzer, 1996, de-La Calle-Gercia, Reichenbaecher, Danzer, Hurlbeck, Bartzsch, & Feller, 1997; Jia, Zhang, & Min, 1998).

Table 1 lists the standard constituents examined, corresponding to optimised equilibrium conditions, the relative recovery and the extraction reproducibility typical of the three fibres examined. PDMS/DVB fibre had good selectivity for low-middle molecular weights and semipolar analytes. Carbowax/DVB coating showed better selectivity for more polar compounds, while PDMS fibre had good selectivity for volatile analytes, such as ethyl octanoate and ethyl decanoate. Reproducibility was in the range of 0.10–10.00 for fibre (a), 0.10–4.00 for fibre (b) and 0.10–13.00 for fibre (c), with high values for some fermentation esters which were not considered for the purpose of this work.

PDMS/DVB fibre was then chosen and the entire analytical procedure (sample treatment and GC–MS analysis) so optimised on standard compounds applied to real samples.

Table 2 shows the primary aroma compounds detected in wine samples and in Fraction 1. This fraction was prepared in order to reproduce the aroma pattern of the wine, and most of the compounds listed in Table 2 were selected by their absence in Fraction 2 (not reported), which is sensorially neutral with respect to the characteristic flavour of *Ruche*, and which is mainly constituted of fermentation byproducts. A few compounds associated with the yeast activity are reported too, because they are probably related to the specific composition of the grape berries. Detection only of compounds in Fraction 1 is reported because matrix effects of this wine model solution may influence release of molecules in the headspace (Urruty & Montury, 1996); thus any quantitative data would be of relative significance. On the other hand, model wine solutions were made basically for selection of compounds involved in the aroma pattern of *Ruché*, while more accurate quantitative data and ratios can be obtained only from wine samples.

A total of 59 compounds were selected. Most of them appeared either in wine samples or in Fraction 1 and were selected either because they are primary aroma compounds or potentially aromatic molecules with unknown structure.

A few aroma compounds detected in *Ruché* samples remain unidentified or only partially characterized and their spectral data are reported in Table 3.

Compounds 39 and 49 are likely cyclic norisoprenoids related to β-damascone (39) and β-damascenone (49); compound 17 is an unidentified terpenoid; compound 21 is a terpene acetate; compound 22 and its isomer, 23, are partially characterized terpineyl acetates, while compound 42 is of unknown structure and origin.

Table 1
Specificity and reproducibility of three SPME coatings used for head-space analysis of selected volatile compounds

Compound	PDMS/DVB		CARBOWAX/DVB		PDMS	
	Relative peak area (%)[a]	RSD[b]	Relative peak area (%)	RSD	Relative peak area (%)	RSD
Isobutyl acetate	39.00	0.57	15.50	0.57	55.15	0.49
Ethyl propanoate	44.45	0.35	14.20	0.85	55.95	2.33
Isoamyl acetate	113.30	1.84	31.85	2.05	148.15	4.17
3 + 2-Methylbutanol	13.15	0.14	22.35	0.35	12.75	0.35
Ethyl hexanoate	226.20	2.40	65.75	0.49	195.00	2.47
Hexyl acetate	234.70	9.94	68.75	0.49	188.00	7.50
Ethyl lactate	1.40	0.57	0.85	0.04	1.65	0.07
1-Hexanol	13.00	0.28	20.45	0.21	12.05	0.07
Cis Hexen-1-ol	5.35	0.07	10.30	0.14	4.60	0.14
Ethyl octanoate	287.30	10.32	152.40	1.98	384.60	2.83
1-Heptanol	51.20	0.57	84.05	3.75	48.10	0.14
Benzaldehyde	71.25	0.35	76.90	0.71	36.70	0.71
Linalool	100.45	5.44	109.05	1.77	104.50	2.83
1-Octanol	126.30	2.69	161.45	0.64	113.10	7.07
2,3 Butandiol	1.95	0.07	2.35	0.49	0.25	0.07
Ethyl decanoate	223.25	7.42	167.65	5.30	281.30	6.22
Furfuryl alcohol	46.25	1.48	36.25	2.19	25.70	1.27
Diethyl succinate	13.00	0.42	17.20	0.57	13.00	1.84
α-Terpineol	70.20	5.37	95.90	3.82	88.50	9.76
Citronellol	67.80	7.92	86.70	0.99	57.95	8.98
Nerol	38.60	1.70	53.90	1.27	33.35	4.03
Phenethyl acetate	84.90	0.99	116.20	2.26	71.70	13.15
Hexanoic acid	1.95	0.07	4.10	0.14	1.00	0.07
Geraniol	22.0	2,6	34.00	1.41	18.90	2.26
Phenylmethanol	3.60	0.85	6.50	0.28	1.55	0.07
2-Phenylethanol	6.40	1.27	7.65	0.78	5.15	0.21
Octanoic acid	4.20	0.42	3.20	0.28	0.35	0.07

[a] Relative peak area is normalized to the peak of internal standard taken as 100 (2-octanol, 1 mg/l).
[b] Relative standard deviation (RSD, $n = 3$).

SPE and liquid–liquid extraction gave comparable results. However, some compounds were detected only by SPME analysis (or may be present in liquid–liquid extracts in undetectable traces, Table 4). Fig. 1 shows these peaks in a GC–SPME chromatogram of a wine sample.

For comparison with the primary aroma profile of *Ruché*, selected as described earlier, several micro-vinifications with different grape varieties were conducted and then analysed by HS–SPME. Results are shown in Table 5.

Ruché shows similarities to *Malvasia* and, to a lesser extent, *Brachetto*, wines considered to be aromatic. On the other hand, it is clearly distinct from the other wines analysed, except for some commonly found terpenes and fermentation byproducts. We may therefore consider *Ruché* to be a non-aromatic wine (because its berries do not release free odorous markers into the must), having an aromatic pattern related to *Malvasia*, a grape variety with a very different ampelographic description. Some distinct chemical features may be observed too, probably associated with the typical aroma of *Ruché*.

These results should still be considered cautiously when drawing conclusions about the typical aroma composition of this wine. In previous works (Vas, Koteleky, Farkas, Dobo, & Vekey, 1998; Calle-Garcia, Reichenbaecher, & Danzer, 1998; De-la Calle-Garcia, Reichenbaecher, Danzer et al., 1998a,b), HS–SPME analysis has been successfully used for geographycal and varietal characterization of wines. Nevertheless, further investigations are needed to better isolate key aroma compounds, whose olfactory characteristics really affect the typical sensorial profile of the wine. Aroma composition is affected, in these grape cultivars, by several factors, modifying the aromatic pattern and ratios between aroma compounds. Geographical origin, agronomical practices, winemaking processes, specific yeast strains used for fermentation and aging, after fermentation, are all critical factors able to after the final aroma pattern. Therefore results here presented are to be considered only in the experimental and analytical context adopted in this work. Furthermore, artifact formation (Verhoeven, Beuerle, & Schwab, 1997), influence of ethanol on aroma extraction (Urruty & Montury, 1996) and chemical selectivity of polymers coating SPME fibres, are all factors which may lead to misinterpretations about molecular composition of wine aroma bouquet.

Table 2
Primary aroma compounds detected in headspace by HS–SPME

No.	Compound[a]	Microvinification (relative amount)[b]	FRACTION 1(detection)[c]
1	2,6,6-Trimethyl-2-vinyl-4H-pyran	5.2	D
2	β-Myrcene	3.3	D
3	β-Pinene	4.6	D
4	d/i-Limonene	3.6	D
5	*cis*-Ocimene	3.3	D
6	*trans*-Ocimene	5.9	D
7	α-Terpinolene	7.3	D
8	2-Octanone	33	D
9	5-Hepten-2-one, 6-methyl	9.9	D
10	Linalyl ethyl ether	20.3	D
11	Ethyl 2-hexenoate	7.9	D
12	*cis*-Rose oxide	7.2	D
13	*trans*-Rose oxide	3.3	D
14	*cis*-3-Hexenol	6.6	D
15	*trans*-3-Hexenol	8.9	D
16	Terpenoid (unidentified)	2.0	ND
19	Nerol oxide	13.5	D
20	6-Methyl-5-hepten-2-ol	12.5	D
21	Terpene acetate (unidentified)	4.1	ND
22	Terpineyl acetate 1	6.6	D
23	Terpineyl acetate 2	21.4	D
24	Vitispiran 1	33.4	D
25	Vitispiran 2	9.8	D
26	2-Methyl-4H-thiophen-3-one	4.3	D
27	Linalool	89.1	D
28	Ethyl 3-methyltiopropanoate	1.8	D
29	Hotrienol	3.3	D
30	Myrcenol	20.5	D
31	Riesling acetale	3.7	ND
32	Safranale	1.8	ND
33	Citronellyl acetate	4.6	D
34	*cis*-Ocimenol	3.3	D
35	Menthenol 1 (unidentified)	8.6	D
36	*trans*-Ocimenol	9.9	D
37	Menthenol 2 (unidentified)	9.9	D
38	α-Terpineol	56.1	D
39	Norisoprenoid (unidentified)	3.7	ND
40	Propanol, 3-methylthio	4.3	D
41	Neryl acetate	3.3	D
42	Unidentified	2.7	ND
43	Ethyl geranate	21.5	ND
44	Linalool oxide c	3.3	D
45	Phenyl methyl acetate	9.9	D
46	Methyl, 2-hydroxy-benzoate	10.6	D
47	Geranyl acetate	1.2	D
48	Citronellol	95.7	D
49	Norisoprenoid (unidentified)	4.0	ND
50	Nerol	90.7	D
51	β-Damascenone	13.5	D
52	Geraniol	110.5	D
53	4-Ethyl-2-methoxyphenol	5.6	ND
54	4-Ethylphenol	17.4	ND
55	Methyl-(α-OH-3-phenyl) ketone	7.3	D
56	Ethyl, 3-phenyl-2-OH-propionate	9.9	D
57	Geranic acid	21.5	D
58	Methylvanillate	1.7	D
59	Ethylvanillate	1.7	D

[a] Compounds are reported in order of retention time.
[b] Relative amount, expressed as percentage with respect to the area of internal standard (2-octanol, 163 mg/l)
[c] D = detected, ND = not detected.

Table 3
Mass spectral data of non identified or partially characterized compounds

	Mass spectra
Compound 16 terpenoid	69 (100), 87 (50), 136 (30), 121 (23), 93 (23), 39 (16), 67 (14), 79 (12), 107 (4)
Compound 21 terpene acetate	93 (100), 121 (82), 41 (73), 67 (50), 79 (40), 136 (40), 107 (23), 53 (17)
Compound 22 terpineyl acetate 1	121 (100), 93 (85), 136 (64), 79 (30), 43 (25), 65 (7), 51 (7)
Compound 23 terpineyl acetate 2	121 (100), 93 (85), 136 (64), 79 (30), 43 (25), 65 (7), 51 (7)
Compound 35 menthenol 1	93 (100), 79 (82), 67 (68), 121 (58), 136 (35), 39 (30), 107 (21), 55 (15)
Compound 37 menthenol 2	81 (100), 123 (65), 93 (52), 39 (48), 67 (32), 107 (20), 55 (17), 136 (17)
Compound 39 norisoprenoid	192 (100), 177 (71), 149 (57), 131 (40), 91 (32), 107 (30), 43 (27), 119 (23), 57 (19), 77 (19), 159 (16)
Compound 42 unidentified	145 (100), 160 (44), 105 (43), 119 (36), 185 (34), 218 (34), 39 (32), 91 (31), 77 (30), 131 (17), 69 (17), 200 (13), 175 (8)
Compound 49 norisoprenoid	190 (100), 91 (72), 107 (70), 175 (56), 77 (33), 120 (26), 55 (24), 133 (20), 147 (20), 161 (20), 39 (18), 65 (18)

Identifications were made by using reference spectra libraries (Nist92 and Wiley5), together with personal interpretation. Many standards were first used to check the reliability of mass spectra obtained with the ion trap mass analyser as against those obtained with a quadrupole detector (typical of the libraries).

The mass results were comparable providing that low concentrations were injected. However some differences were present due to the greater life times of the ions collected in the ion trap: lower intensities, in some cases, of molecular ions and some differencies in the relative intensities of the fragments present. These discrepancies arose almost exclusively with terpenes and terpenoids so their identification was in all cases confirmed by means of pure standards.

In future, the systematic use of tandem mass spectrometry will help to resolve interpretative ambiguity of spectra of certain compounds.

Table 4
Primary aroma compounds detected only by HS–SPME

No.	Compound	Rel. amt.
1	Linalyl ethyl ether	20.3
2	2-Ethyl hexenoate	7.9
3	*cis*-Rose oxide	7.2
4	*trans*-Rose oxide	3.3
5	Compound 16 (terpenoid)	20.0
6	Nerol oxide	13.5
7	Compound 21 (terpene acetate)	4.1
8	Compound 22 (terpineyl acetate 1)	6.6
9	Compound 23 (terpineyl acetate 2)	21.4
10	Vitispiran 1	33.4
11	Vitispiran 2	9.8
12	Ethyl 3-methylthiopropanoate	1.8
13	Riesling acetate	3.7
14	Safranale	1.8
15	Citronellyl acetate	4.6
16	Compound 39 (norisoprenoid)	3.7
17	Neryl acetate	3.3
18	Compound 42 (unidentified)	2.7
19	Ethyl geranate	21.5
20	Phenyl methyl acetate	9.9
21	Geranyl acetate	1.2
22	Compound 49 (norisoprenoid)	4.0
23	β-Damascenone	13.5

4. Conclusions

The results obtained, though preliminary, confirm the correctness of the analytical procedure adopted, both in terms of efficiency of extraction and in terms of chromatographic analysis.

HS–SPME allowed detection of trace aroma compounds, not detectable by conventional liquid–liquid extraction, while the use of wine model solutions helped to select molecules involved in the characterization of the aroma profile of the wine under study.

On the basis of experimental data obtained, it was possible to assign a role of primary importance to the skin of the grape vine berries as a source of varietal aromatic precursors, easily releasable in the maceration medium in the presence of the enzymes cellulase and pectinase.

Hence these data will allow further investigations of the complex mechanisms underlying the release, into the must, of aromatic precursors and the production of substances related to the aromatic pattern of aromatic or non aromatic wines, focussing on the biochemical reactions carried out by specific yeast strains.

Table 5
Profiling of several red wines, based on HS–SPME analysis of primary aroma compounds

Compound	*Ruché*	*Malvasia*	*Brachetto*	*Pinot grigio*	*Pinot noir*	*Nebbiolo*	*Merlot*	*Croatina*	*CabernetAosta*	*Cabernet*Piemonte
2,6,6-trimethyl-2-vinyl-4H-pyran	5.2[a]	4.5	0	0	0	0	0	0	0	0
β-myrcene	3.3	3.3	4.8	0	0	0	0	0	0	0
β-pinene	4.6	4.5	2.4	0	0	3.2	0	<0.1[b]	0	0
α-terpinene	3.3	5.3	3.6	0	0	0	0	0	0	0
d/i-Limonene	3.6	10.5	12.8	2.8	2.8	52.8	2.9	1.6	2.2	1.6
Ocimene 1	3.3	Nd	4	0	0	0	0	0	0	0
Ocimene 2	5.9	7.3	2.8	0	0	0	0	0	0	0
α-Terpinolene	7.3	16.4	6	0	0	0	0	0	0	0
2-Ottanone	33	32.2	0	1.7	<0.1	0	<0.1	2.6	3.1	2.8
Linalyl ethyl ether	20.3	88.8	0	0	0	0	0	0	0	0
5-Hepten-2-one, 6-methyl	9.9	16.3	6	1.7	2	2	1.8	<0.1	1.7	0.8
Ethyl 2-hexenoate	7.9	22.3	7.2	15.4	6	6	6.5	1.6	2.2	2
cis-Rose oxide	7.3	14.4	18	3.5	2.4	<0.1	3.6	<0.1	2.2	0
trans-rose oxide	3.3	3.3	8.8	0	0	0	0	0	0	0
cis-3-Hexen-1-ol	6.6	<0.1	4	3.5	1.2	2	1.8	1.6	2.2	2
2-Nonanone	4.9	3.3	4	0	0	0	9.3	2.6	1.7	2
trans-3-Hexen-1-ol	8.9	0	6	3.5	<0.1	0	<0.1	0	0	0.8
Compound 16 (terpenoid)	20.0	19.8	0	0	0	0	0	0	0	0
nerol oxide	13.5	17.5	16.8	0	0	0	0	0	0	0
trans-Linalool oxide	3.3	0	0	0	0	0	0	0	0	0
5-Hepten-2-ol, 6-methyl-	9.9	11.1	4	2.4	0	0	0	1.6	0	0
Compound 21 (terpene acetate)	4.1	8.3	0	0	0	0	0	0	0	0
Compound 22 (terpineyl acetate 1)	6.6	18	0	0	0	0	0	0	0	0
Compound 23 (terpineyl acetate 2)	21.4	79.4	0	0	0	0	0	0	0	0
Vitispiran1	33.4	0	0	0	0	0	0	<0.1	0	0
Vitispiran2	9.8	0	0	0	0	0	0	0	0	0
2-Methyl-4H-thiophen-3-one	4.3	8.9	8	3.5	0	7.2	3.6	1.6	5.2	0
linalool	89.1	631	40	0	16	6	7.2	6.4	6.5	0
Ethyl 3-methyltiopropanoate	1.8	0	0	0	0	0	0	0	0	0
Hotrienol	3.3	8.9	4	0	0	0	0	0	0	0
Riesling acetale	3.7	1.7	0	0	0	0	0	0	0	0
Safranale	1.8	0	0	0	0	0	0	0	0	0
Citronellyl acetate	4.6	22.2	200	1.7	1.2	<0.1	<0.1	<0.1	0	0
cis-**Ocimenol**	3.3	0	0	0	0	0	0	0	0	0
trans-Ocimenol	9.9	0	0	0	0	0	0	0	4.3	0
Compound 35 (menthenol 1)	8.6	2.7	4.8	0	0	0	0	0	0	0
Compound 37 (menthenol 2)	9.9	5.2	8	0	0	0	0	0	0	0
α-Terpineol	16.4	60.6	49	41	15	30	16	6.9	12	8
Compound 39 (norisoprenoid)	3.7	2.0	0	0	0	0	0	0	0	0
Propanol, 3-methylthio-	4.3	5.1	2.4	2.1	8.8	10	8.3	1.6	8.7	8
Neryl acetate	3.3	5.1	64	0	0	0	0	0	0	0
Compound 42 (unidentified)	2.7	<0.1	0	0	0	0	0	0	0	0
Ethyl geranate	21.5	6.1	0	0	0	0	0	0	0	0
Linalool oxide C	3.3	0	0	0	0	0	0	0	0	0
Phenyl methyl acetate	9.9	0	2	<0.1	0	<0.1	0	0	3.5	0
Methyl 2-hydroxy-benzoate	10.6	3.9	2	<0.1	2	1.2	4	<0.1	0	0
Geranyl acetate	3.3	5.1	30	0	0	0	0	0	0	0
Citronellol	95.7	202	84	10.5	6	2.4	5.4	5.4	0	4
Ethyl 2-OH-benzoate	3.3	0	0	0	0	<0.1	1.1	1	0	0.8
Compound 49 (norisoprenoid)	4.0	1.5	0	0	<0.1	0	0	0	0.9	0
Nerol	90.7	66.3	64	0	2	<0.1	<0.1	<0.1	0	0
β-Damascone	0	44.8	0	0	0	0	0	0	0	0
β-Damascenone	13.5	34.1	8	3.5	8	8	7.2	2.6	8.7	1.2
Geraniol	110.5	84.3	32	<0.1	4	0	0	1.6	6.5	2
4-Ethyl-2-methoxyphenol	5.6	0	0	0	0	0	0	0	0	0
4-Ethylphenol	17.4	0	0	0	0	0	0	0	0	0
Ethyl 3-phenylpropionate	1.6	0	0	<0.1	<0.1	1.2	<0.1	<0.1	0	0.8
Ethyl 3-OH-octanoate	1.7	0	2	<0.1	<0.1	<0.1	<0.1	<0.1	0	0
Methyl-(α-OH-3-phenyl) Ketone	7.3	0	0	<0.1	<0.1	<0.1	<0.1	<0.1	2.2	0.8
Ethyl, 3-phenyl-2-OH-propionate	9.9	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	1.7	2.4
Geranic acid	21.5	22.2	9.6	0	0	0	0	0	0	0
Methylvanillate	1.7	0	0	0	0	0	0	0	0	0
Ethylvanillate	1.7	0	<0.1	0	0	0	0	0	0	0

[a] Data expressed as percent respect to the area of IS (2-ottanol, 163 mg/l) and calculated as media on three replicates.
[b] Signals at the level of background.

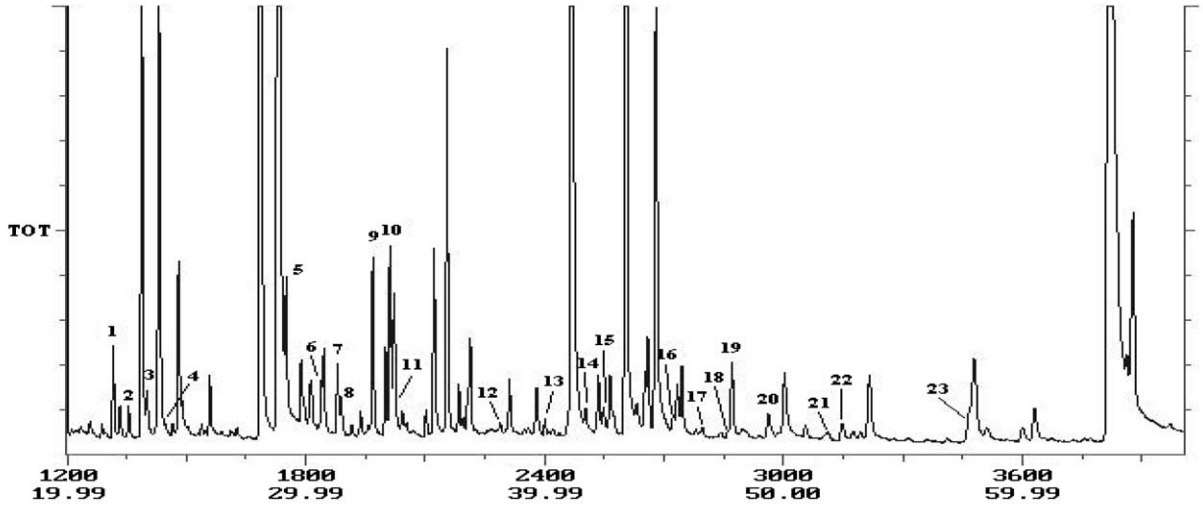

Fig. 1. GC-ITMS chromatogram of Ruché headspace extracted with SPME. Numbered compounds correspond to those of Table 4.

Further studies in this direction are actively in progress in our laboratories.

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