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Identification of aroma compounds of *Vitis vinifera* L. flowers by SPME GC-MS analysis

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Summary

Using a gas chromatographic method (GC-MS-analysis), it was possible to determine the volatile constituent of an odorous flower from *Vitis vinifera* varieties growing in Sicily. More than 50 compounds were identified and the technique allowed us to determine that sesquiterpenes, as well as monoterpenes such as limonene and cymene, were the principal components. The odour-profiles allowed us to distinguish between variety groups or even single varieties.

K e y w o r d s : grapevine, flowering, sesquiterpenes, monoterpenes.

Introduction

Wine aroma chemistry has been meticulously studied over the last few years, and several researchers have identified many volatile compounds that are found in grapes and wines of different cultivars. A previous report focused on the olfactory properties and on the identification of character impact odorants attributable to the cultivar (Sánchez-Palomo *et al.* 2010). Although the release of volatiles, including aliphatics, terpenoids, and phenylpropanoids, from anthers and/or pollen has been reported (Dobson *et al.* 2000), the chemical composition of the flower volatiles of *Vitis vinifera*, which are responsible for its odor, has not been substantially investigated until recently (Buchbauer *et al.* 1994a, 1994b, 1995, Lücker *et al.* 2004). Low molecular-weight terpenoids, which include a large array of monoterpenes, sesquiterpenes, and norisoprenoids, are commonly found as volatiles emitted from the flowers, fruits, and leaves of plants (Schrader and Berger 2001). Grapevine flowers produce numerous sesquiterpenoid volatiles [e.g. (E)-β-caryophyllene, α-humulene, (+)-valencene, (E,E)-α-farnesene, and (-)-7-epi-α-selinene], presumably as attractants for pollinators (Buchbauer *et al.* 1994a, 1995, Martin *et al.* 2009). The sesquiterpene valencene is a major volatile emitted from the flowers of white and red varieties of the grapevine *Vitis vinifera* L. (Buchbauer *et al.* 1994a, 1994b, 1995). In this study, we have provided qualitative "odour profiles" in order to obtain more information about the aroma of grape flowers. We report the results of a head space SPME study as a useful tool (short sampling time, small sample amount, and no use of solvents) for an early and preliminary characterisation of volatile compounds.

Material and Methods

Grape inflorescences from eight varieties were collected in 2012 from vineyards located in the Marsala area (West Sicily 100 m a.s.l.). All vines were subjected to the same trellis system, pruning method, and cultivation practices. The eight selected varieties were six white: 'Inzolia', 'Catarratto Comune', 'Grillo', 'Zibibbo' (syn. 'Muscat of Alexandria'), 'Sauvignon Blanc' and 'Fiano'; and two red: 'Frappato' and 'Cabernet Sauvignon'. At full flowering development stage (BBCH 67), three whole inflorescences per replicate (three) and per variety (eight), each with the same light condition and shoot position, were collected from nine different vines. The selected inflorescences were placed in 250 mL vials with silicon septa and stored at 4 °C until analysis.

Preliminary experiments (data not shown) were carried out to select the coating of the fiber and its thickness, as well as to identify the most appropriate conditions, such as the length and temperature of the exposure in the head space over the sample. These experiments were mainly designed to enhance the volatilisation of compounds from the matrix while preventing their decomposition, and to optimise the gas-fiber equilibrium. Before use, the selected SPME fiber, which was coated with divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS, 50 µm, Supelco), was conditioned for 2 h at 250 °C in the inlet of a gas chromatograph. Analyses were performed on the sampled whole inflorescences that were placed in 250 mL glass vials sealed with a silicon septum. After 60 min of equilibration at 25 °C, the SPME fiber was inserted in the silicon septum with the help of a manual holder system. After 5 min at 25 °C, the SPME fiber was recovered and immediately inserted into the injector port of the gas chromatograph, allowing for 1 min desorption at 250 °C. Three replicates of each sample were analysed.

C h r o m a t o g r a p h i c p r o c e d u r e s : A GC instrument (Agilent 6890), equipped with a mass selective detector (Agilent 5975), was used for the chromatographic analyses. A fused silica capillary column Carbowax (30 m length, 0.25 mm internal diameter, and 0.25 µm film thickness; Supelco) served as the stationary phase. The injector was used in splitless mode and had a temperature of 250 °C. Experimental chromatographic conditions were as follows: Helium carrier gas at 1 mL·min^{-1}; and an oven temperature program with a 5 min isotherm at 40 °C followed by a linear temperature increase of 4 °C·min^{-1} up to 200 °C,

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where it was held for 2 min. The MS scan conditions were: source temperature 230 °C, interface temperature 280 °C, EI at 70 eV, and mass scan range 33-350 amu. A commercial library (NIST05) was used interactively with the MS data for compound identification. Standards, required to confirm some assignments, were obtained from Fluka and used without further purification. The relative proportions of the essential oil constituents were expressed as percentages obtained by GC-MS peak area normalisation, with all relative response factors being taken as one.

A hierarchical cluster analysis based on Euclidean distance matrices with the Unweighted Pair Group Method with Arithmetic Averages (UPGMA) was performed using R version 3.0.1 (R Core Team, 2013).

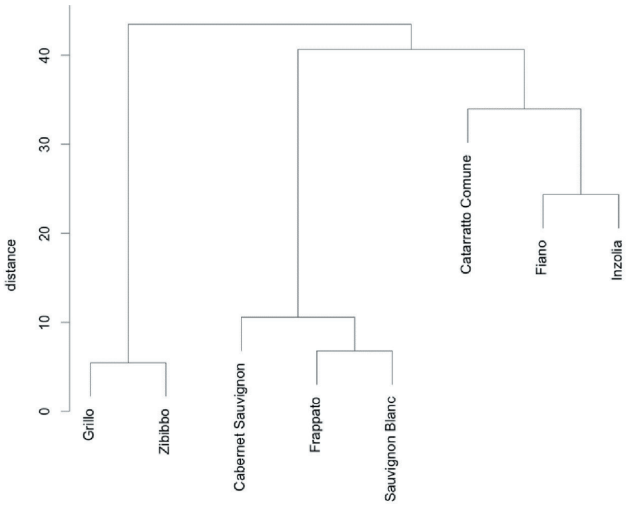

Figure: Hierarchical cluster analysis dendrogram on the Euclidean distance matrix, UPGMA aggregation method.

Results and Discussion

The Table shows the principal constituents found as the average of the three replicates. The percentage of the relative standard deviation was always < 10 %. It is well known that different extraction techniques could result in different quali-quantitative extract compositions and that, because of the equilibrium nature of the SPME method, the GC-MS profiles do not immediately correspond to the composition of the detected substances in the sample or in the head space. Our goal, however, was to take advantage of the ability of the SPME method to extract/concentrate both low and high-eluting components, allowing for comparisons of the chromatographic profiles for different samples under similar experimental conditions.

The Figure shows the clear identification of four groups of varieties according to their different aromatic compounds:

1st Group ('Grillo'-'Zibibbo' (syn. 'Muscat of Alexandria')): 'Grillo' and 'Zibibbo' are characterised by the prevalence of (E)-β-farnesene (exceeding 40 %), low values of (-)-β-caryophyllene (less than 10 %), and modest values of valencene and 7-epi-α-selinene (Table).

2nd Group ('Cabernet Sauvignon'-'Frappato'-'Sauvignon Blanc'-): 'Cabernet Sauvignon' and 'Frappato' show similar profile. They are characterised by the presence of (in order of increasing value): (EE)-α-farnesene, 7-epi-α-selinene, (-)-β-caryophyllene, and (+)-valencene. These account for 78.5 % and 82.3 % of the total compounds in the two varieties, respectively. 'Sauvignon Blanc' has more 7-epi-α-selinene and (+)-valencene than 'Cabernet Sauvignon'. Even if 'Cabernet Sauvignon' is located in the same group, it shows additional compounds, such as 6-methyl-5-hepten-2-one, p-cymene, limonene, and α-trans-bergamotene (Table). However, a prevalence of (+)-valencene has previously been reported (Martin *et al.* 2009).

Table

Percentage of compounds divided in sesquiterpenes, monoterpenes, ketones and esters

Compounds	Grillo %	Zibibbo %	Cabernet S. %	Frappato %	Sauvignon B. %	Catarratto C. %	Fiano %	Inzolia %
Sesquiterpenes								
(-)-β-caryophyllene	9.0	7.7	21.0	21.1	18.5	36.1	14.2	8.4
(E.E)-α-farnesene	10.4	7.7	14.6	9.0	12.1	15.7	27.2	45.2
(E)-β-farnesene	41.0	45.2	2.5	7.4	6.7	13.9	12.1	17.8
α-humulene	3.1	4.5	3.7	3.3	4.2	5.0	5.1	2.9
α-selinene	4.5	4.4	0.0	4.3	3.6	8.7	10.5	3.9
7-epi-α-selinene	11.4	11.3	17.6	20.2	19.2	3.1	0.0	4.4
(+)-valencene	15.6	16.0	25.3	32.0	27.9	4.3	0.7	6.1
Monoterpenes								
α-trans-bergamotene	1.2	1.2	3.0	0.0	2.0	2.2	5.2	5.0
p-cymene	0.8	0.6	4.8	0.2	2.4	4.4	7.2	2.3
limonene	1.7	1.1	3.5	0.7	1.7	3.5	5.8	1.0
Ketones								
6-methyl-5-hepten-2-one	0.7	0.2	3.2	1.4	1.0	2.5	8.8	1.3
Esters								
ethyl benzoate	0.6	0.0	0.6	0.3	0.5	0.6	3.3	1.5

3rd Group ('Catarratto C.'): 'Catarratto Comune' is represented by the prevalence of (-)-β-caryophyllene (36.1 %), (EE)-α-farnesene (15.7 %), (E)-β-farnesene (13.9 %), α-selinene, and α-humulene. Its profile was qualitatively close to the 4th group, but it differed quantitatively (Table).

4th Group ('Fiano'-'Inzolia'): 'Fiano' and 'Inzolia' are both characterised by the dominance of (EE)-α-farnesene, though at different percentages. Particularly, 'Inzolia' is characterised by the prevalence of (EE)-α-farnesene (45.2 %) and (E)-β-farnesene (17.8 %), followed by (-)-β-caryophyllene, (+)-valencene and α-trans-bergamotene. 'Fiano' is the variety with the greater diversity of compounds, having (EE)-α-farnesene (27.2 %), (-)-β-caryophyllene (14.2 %), (E)-β-farnesene (12.1 %), α-selinene (10.5 %), p-cymene, limonene, α-trans-bergamotene, α-humulene and the highest presence of 6-methyl-5-hepten-2-one (8.8 %) than the other varieties (Table).

Conclusions

The technique we used (SPME GC-MS analysis) was useful in distinguishing between different odorous profiles and, therefore, enabled the discrimination of the grape varieties we examined. The results showed that the predominant flower aroma compounds can be used to distinguish between variety groups, or even single varieties, growing under identical conditions. It is interesting to note the similar aroma profiles between 'Cabernet Sauvignon' and 'Sauvignon Blanc' (Bowers and Meredith 1997), and between 'Grillo' and 'Zibibbo' (syn. 'Muscat of Alexandria') (Di Vecchi Staraz *et al.* 2007). Further studies are needed to expand both the varietal data set and the list of known aroma compounds to additionally contribute to our knowledge of variety behaviours and how external factors (such as climate, vineyard management, etc.) can impact on the composition of flower aroma profiles.

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