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Analytical Methods

Identification of free and bound volatile compounds as typicalness and authenticity markers of non-aromatic grapes and wines through a combined use of mass spectrometric techniques

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ABSTRACT

Molecular approaches by means of a combined use of mass spectrometric techniques can be important in order to open new possibilities in the differentiation and defense of typical products; in this study, a possible approach to the analysis of varietal volatile compounds and some precursors of a non-aromatic grape variety (Falanghina cv., *Vitis vinifera* L.) was traced through a combined use of techniques based on mass spectrometry (GC/MS, LC/ESI-MS, MALDI-TOF-MS). Dominant terpene compounds (limonene, *cis*-furanlinalool oxide, geraniol, 4-carene, myrcene, linalool, α-terpineol), terpene-derivatives (bornyl acetate, menthol), terpene glycosides (glucosides, arabinosylglucosides and rhamnosylglucosides of linalool and geraniol), and norisoprenoids (β-damascenone) were identified in grapes and monovarietal wines, overcoming the analytical difficulties deriving from the low concentration of these compounds strictly related to the variety. The potential release of varietal volatile compounds from the grapes was also explored by enzyme hydrolysis.

1. Introduction

In grapes the composition of odorous molecules is often used for varietal differentiation being the rationale of these studies that the volatile compounds of grapes are constituted by several odorous molecules (alcohols, esters, acids, terpenes, ketones, and aldehydes) whose concentration can vary depending on the variety (Carro, López, Günata, Baumes, & Bayonove, 1996; Câmara, Herbert, Marques, & Alves, 2004; Mamede & Pastore, 2006; Oliveira et al., 2004; Rosillo, Salinas, Garijo, & Alonso, 1999; Sánchez-Palomo, Díaz-Maroto, & Pérez-Coello, 2005). In particular, quali-quantitative composition of terpenes was considered very strictly related to varietal origin (Carro et al., 1996; Câmara et al., 2004; Flamini, 2005; Mateo & Jiménez, 2000). Genes encoding enzymes catalysing the biosynthesis of monoterpenes are responsible of the production of these compounds with large structural diversity. Some studies have been carried out for the identification of genes which encode these enzymes (Lucker et al., 2001). The production of terpenes in plants has been related to an ecological function (Carro et al., 1996; Câmara et al., 2004; Flamini, 2005; Mateo & Jiménez, 2000): terpenes are responsible for attracting pollinating moths and can act as a repellent against aphids and semiochemicals after herbivore attack in some plant species.

In wines the aroma is influenced by many different factors (grape variety, climate, soil, fermentation conditions, yeast strains, and other oenological microflora components, production process). The resulting aromatic profile, in its complexity, with its quali-quantitative descriptors, has a key role in the characterization of a typical wine, and compounds considered strictly related to the variety (terpenes and norisoprenoids) can be important for the expression of varietal characteristics in wine (Christaki & Tzia, 2002; Lopez, Aznar, Cacho, & Ferreira, 2002; Pet̆ka, Ferreira, González-Viñas, & Cacho, 2006; Rapp, 1998).

In grapes and wines, terpenes can be present in free or bound (glycoside) form. After their synthesis they are converted into their more hydrophilic form in order to prevent cell membrane damage (Lucker et al., 2001). Glycosidically conjugated terpenes are not odorous and in most cases they are more abundant than unglycosylated free forms; they give a potential contribution to the aroma of the grape as they are varietal aroma precursors and, during the winemaking process, from these precursors some terpene compounds can be generated through slow enzymatic or chemical hydrolysis (Harborne, 1991; Maicas & Mateo, 2005; Mateo & Di Stefano, 1997; Tamborra, Martino, & Esti, 2004).

In the literature, a few examples of analytical methods for studying the composition of terpene glycosides are present (Carro

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et al., 1996; Fernández-González & Di Stefano, 2004; Mateo, Gentilizi, Huerta, Jiménez, & Di Stefano, 1997; Voirin, Baumes, Günata, Bitteur, & Bayonove, 1992; Voirin, Baumes, Sapis, & Bayonove, 1992) and concern aromatic grapes mainly. Some of these studies were carried out by GC/MS analysis of TMS-derivatives of terpene glycosides (Nasi, Ferranti, & Chianese, 2006; Voirin, Baumes, Günata, et al., 1992) or by analysis of terpenes obtained through hydrolysis of terpene glycosides (Maicas & Mateo, 2005) by means of purified enzymes or commercial enzyme preparations. Fewer studies were carried out on terpenes and terpene glycosides of non-aromatic grapes and up to now LC/ESI-MS or MALDI-TOF-MS techniques which could permit to identify glycosides just like they are, without derivatization, were never applied.

For the varietal characterization of typical non-aromatic grapes and wines several analytical ways can be considered. The development of novel analytical technologies and the progress in scientific knowledge leads to search for new analytical strategies aimed at improving typical food quality and obtaining new information which, combined with conventional analytical parameters, can better describe the typicalness of food products.

In this study we applied an analytical approach, based on a combined use of mass spectrometric techniques (GC/MS, LC/ESI-MS, and MALDI-TOF-MS), suitable for a characterization of varietal volatile compounds and their precursors in non-aromatic varieties, aimed at characterizing Falanghina grapes (autochthonous grape variety of the Campania region in Italy) and monovarietal wines derived from these grapes (with trademarks of quality such as Appellation of Origin denominations) from different production areas and from different wine trading firms, in order to obtain useful analytical information and possible molecular markers which can describe the typicalness and the authenticity of these products.

2. Materials and methods

2.1. Chemicals and reagents

The SPME holder and fibers (100 μm polydimethylsiloxane (PDMS), 65 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB), 75 μm carboxen/polydimethylsiloxane (CAR/PDMS), 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS)) were purchased from Supelco (Sigma-Aldrich, Bornem, Belgium). The trimethylsilyl-derivatives (TMS-derivatives) of terpene glycosides were obtained with *N*,*O*-bis (trimethyl-silyl) trifluoroacetamide 1% chlorotrimethylsilane (Sigma).

Grapes and monovarietal wines were supplied by different producers in Campania region (Italy).

2.2. Aromatic characterization of grapes and wines

Different strategies of extraction and a combined use of different techniques were used in order to obtain more information (Bonino et al., 2003; Ferreira, Fernández, & Cacho, 1996; Ferreira, Ortega, Escudero, & Cacho, 2000; Lopez et al., 2002).

Liquid/liquid extraction was carried out with 2.5 ml of dichloromethane and 50 ml of wine on a vortex for 1 h.

SPME extraction was carried out in the following conditions: the fiber was immersed in the headspace (HS) of the samples using 250 ml of wine until equilibrium was reached. Thermal desorption of the analytes from the fiber inside the GC injection port was carried out in the split mode (1/10) at a desorption temperature of 250 °C for 1 min.

Falanghina grapes in good sanitary conditions from Benevento area (BN, Benevento, Italy) and Campi Flegrei area (CF, Napoli, Italy) were collected and stored at −20 °C until analysed. For the analysis with static headspace 10 ml of grape juice were mixed with 2 g of NaCl and introduced in vial for headspace autosampler Agilent 7694E (Agilent Technologies, Santa Clara, CA, USA). The SPME analysis was carried out from 50 ml of grape juice adding 15 g of NaCl; furthermore the SPME analysis was carried out on 3 g of skins homogenised in 10 ml of water and 4 g of NaCl. All samples were analysed with an HP 6890 coupled to a 5973N quadrupole HP mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). The gas chromatograph was equipped with an HP-5ms capillary column (30 m × 0.25 mm ID) and the carrier gas used was helium.

For the analysis of free compounds the GC oven temperature was programmed from 40 °C (held for 7 min) to 180 °C at 5 °C/min. The masses were scanned on *m*/*z* range of 45–350 amu. In other cases a SIM (selected ion method) method was used (for terpene compounds *m*/*z* 93, 121, 136). For the identification of odorous components the NIST library and/or comparison with spectra and retention times of standards (Acros Organics, Geel, Belgium) were used. Quantitative determinations of terpenes were obtained by means of calibration curves constructed in matrix, in the concentration ranges typical of wines for each compound, using seven concentration levels and five replicates per level, in the verified range of linearity; multiple replicates (n = 3–6) of the samples were analysed.

Discriminant analysis was effected by means of SPSS statistical software (version 12.0) (SPSS Inc., Chicago, IL).

2.3. Analysis of terpene precursors

Terpene glycosides were extracted by fractionated extraction with a C18 cartridge (Baker, Deventer, Netherlands; 500 mg/3 ml) starting from 15 ml of grape juice. The washing step was effected with 15 ml of water and with 25 ml of dichloromethane in order to elute free odorous compounds. The elution of glycosides was carried out in four steps with 10 ml of methanol respectively at 20%, 30% and 40% in water for the first three steps, and 100% methanol in the final step (Fernández-González & Di Stefano, 2004; Ferreira et al., 1996; Mateo et al., 1997).

The eluted fractions were dried; TMS-derivatives were obtained with *N*,*O*-bis (trimethyl-silyl) trifluoroacetamide 1% chlorotrimethylsilane (Sigma) at 60 °C for 45 min (Nasi et al., 2006).

For the analysis of glycoside compounds the GC oven temperature was programmed from 120 °C (held for 4 min) to 300 °C at 3 °C/min. The mass spectrometer was operated in electron ionization mode (EI, 70 eV) and the masses were scanned on *m*/*z* range of 45–600 amu.

The enzymatic reaction of the glycoside fraction was obtained using a pectinolytic enzyme preparation (Rohapect, Seitz Perdomini,Verona, Italy): the eluted fractions were dried and dissolved in 2 ml of 0.2 M citrate-phosphate buffer with 1 mg of enzymatic preparation; terpenols formed by enzymatic hydrolysis were analysed by means of SPME–GC/MS analysis.

The LC/MS analysis was carried out by means of a LC/MS instrumentation (HP1100-MSD, Agilent Technologies, Santa Clara, CA, USA) with single quadrupole by using C18 column (Vydac, Hesperia CA, USA; 2.1 × 250 mm) and a gradient from 5% Acetonitrile (0.2% formic acid, buffer A) and 95% water (0.2% formic acid, buffer B) to 70% buffer B in 45 min, with a flow of 0.2 ml/min.

MALDI-TOF spectra were recorded in positive-ion mode, using a Voyager DE-Pro spectrometer (PerSeptive BioSystems, Framingham, MA) equipped with a N_2 laser (ψ = 337 nm); α-ciano-4-hydroxy-cinnamic acid (Fluka, Buchs SG, Switzerland) was used as matrix prepared by dissolving 5 mg in 1 ml of aqueous 50%, v/v, acetonitrile/0.1%, v/v, TFA). The instrument operated with an accelerating voltage of 20 kV. Mass spectrum acquisition was performed in both positive linear and reflectron mode. External mass calibration was performed with mass peptide standards (Sigma). The

post-source decay measurements were performed with the same probes on the template as used for peptide mass determination. PSD fragment ion spectra were obtained after isolation of the appropriate precursor using timed ion selection. Fragment ions were refocused onto the final detector by stepping the voltage applied to the reflector in the following ratios: 1.0000 (precursor ion segment); 0.9126, 0.6049, 0.4125, 0.2738, 0.1975 (fragment ion segments), recording data at the digitization rate of 20 MHz. All precursor ion segments were acquired at low laser power to avoid saturating the detector; the laser power was increased for all of the remaining segments of the PSD acquisitions. Typically, 200 laser shots were gathered for each fragment-ion segment. The individual segments were finally stitched together using the software developed by PerSeptive BioSystems furnished with the instrument.

3. Results and discussion

3.1. Grape volatile compounds

In order to obtain possible varietal volatile markers which can describe the typicalness and the authenticity of a non-aromatic wine, the analysis of volatile compounds in grapes (carried out on both juice and skins separately) represented a preliminary step for obtaining structural data.

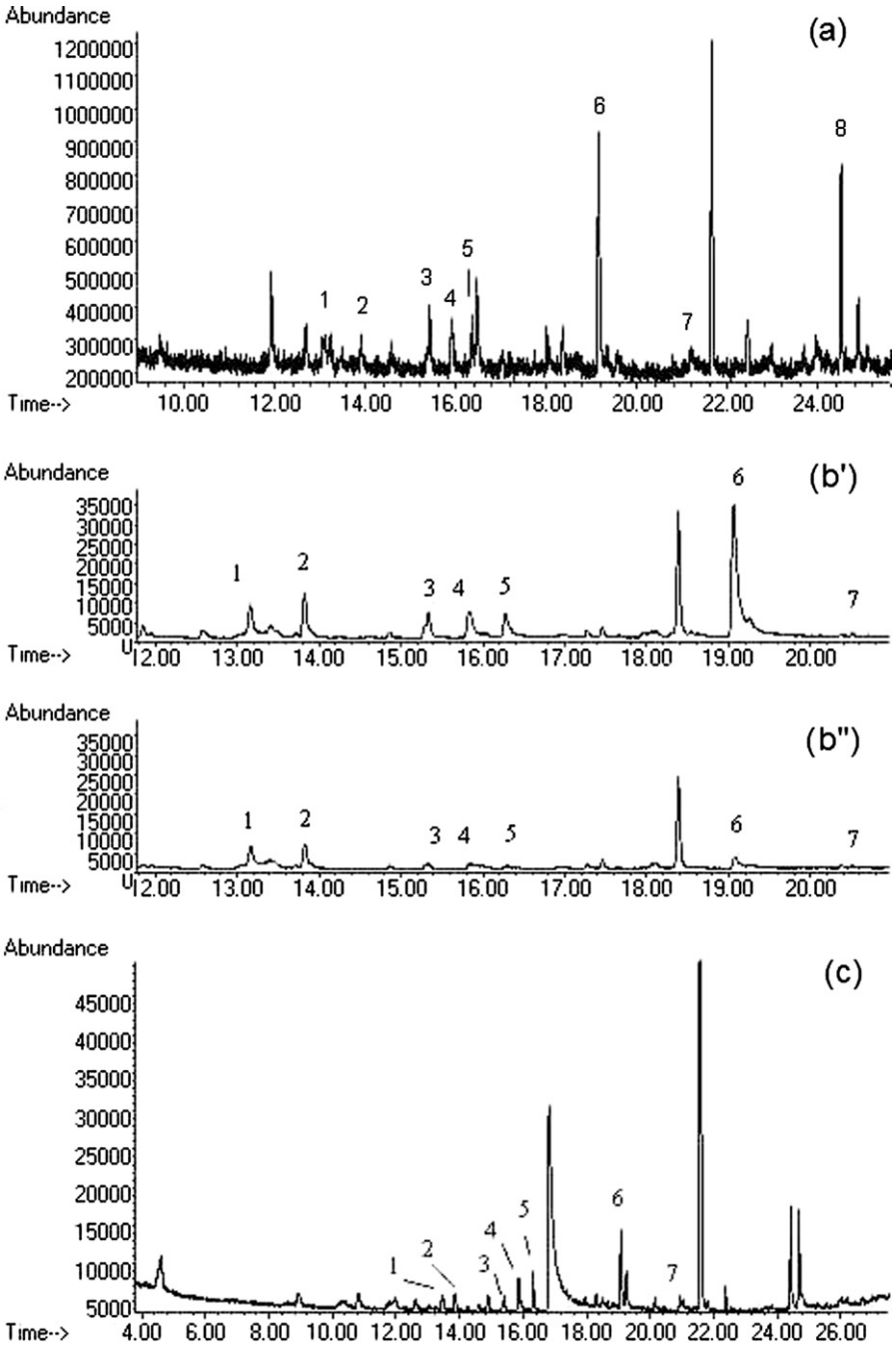


Fig. 1. TIC chromatogram obtained with SCAN method (m/z 45–350) by means of static headspace-GC/MS analysis on a BN Falanghina grape sample (a), with SIM method (m/z 93, 121, 136) on Falanghina grapes of Benevento area (b′) and Campi Flegrei area (b″); TIC chromatogram obtained by means of HS-SPME–GC/MS analysis with SIM method (m/z 93, 121, 136) on a BN Falanghina wine sample (c). 1, β-Myrcene; 2, limonene; 3, linalyl oxide; 4, 4-carene; 5, linalool; 6, α-terpineol; 7, geraniol; 8, β-damascenone.

However, the low quantities of varietal molecules could not provide immediate evidence of the presence of these compounds among many other odorous molecules at high concentrations in the headspace of the grape extracts; in fact sometimes the background spectra and the presence of coeluting chromatographic peaks needed a very accurate analysis of spectrometric data.

Terpenes and norisoprenoids detected in grapes are indicated in Fig. 1a where the TIC chromatogram obtained for a Falanghina grape sample through static headspace-GC/MS analysis (SCAN method) is reported (the presence of β-damascenone, norisoprenoid compound related to floral and fruit odorous nuances, with odor threshold 0.05 ppb (Lopez et al., 2002) was also observed).

In Table 1 varietal odorous molecules for Falanghina grape samples from two geographical areas (Benevento and Campi Flegrei (Napoli)) are reported. Volatile compounds detected for other grape varieties (Coda di Volpe, Greco) through the same analytical method are also reported in the Table 1 for comparison. The terpene composition of Falanghina grape appeared more complex in comparison with the other non-aromatic autochthonous grape considered in this study (Coda di Volpe, Greco); some terpenes, myrcene, furanlinalool oxide, geraniol, 4-carene, resulted detectable in Falanghina grapes and not in Coda di Volpe and Greco grapes. Among Falanghina grape samples from different geographical areas (Benevento (BN) and Campi Flegrei (CF)) only quantitative differences were observed for these compounds. The presence of all terpenes identified (limonene, geraniol, linalool, myrcene, 4-carene, *cis*-linalool oxide, and alpha-terpineol) was higher in samples from Benevento area in comparison with samples from Campi Flegrei. In Fig. 1b′ and b″ the SIM chromatograms obtained through headspace (HS)-SPME–GC/MS analysis carried out on a Falanghina grape sample from Benevento area (b′) and from Campi Flegrei (b″) are shown.

In the TIC chromatograms obtained for the three non-aromatic grapes considered, through headspace-SPME–GC/MS analysis, peaks with major area correspond to C6 compounds (hexanal, 1-hexanol, 2-hexenal) with odorous descriptor "herbaceus", the con-

Table 1
Composition (in ppb) of varietal aroma compounds of Falanghina (BN, Benevento; CF, Campi Flegrei), Coda di Volpe and Greco grapes

	BN Falanghina variety	CF Falanghina variety	Coda di Volpe variety	Greco variety
Limonene	50 ± 9	33 ± 7	11 ± 9	33 ± 8
cis-Linalool oxide	3 ± 1	1 ± 1	–	–
Geraniol	14 ± 3	8 ± 2	–	–
4-Carene	4 ± 1	2 ± 1	–	2 ± 1
Myrcene	5 ± 1	2 ± 1	–	–
Linalool	180 ± 9	60 ± 5	36 ± 5	18 ± 5
α-Terpineol	14 ± 7	3 ± 2	In traces	–
Menthol	In traces	In traces	In traces	–
Bornyl acetate	In traces	In traces	In traces	In traces
β-Damascenone	2 ± 1	2 ± 1	–	–

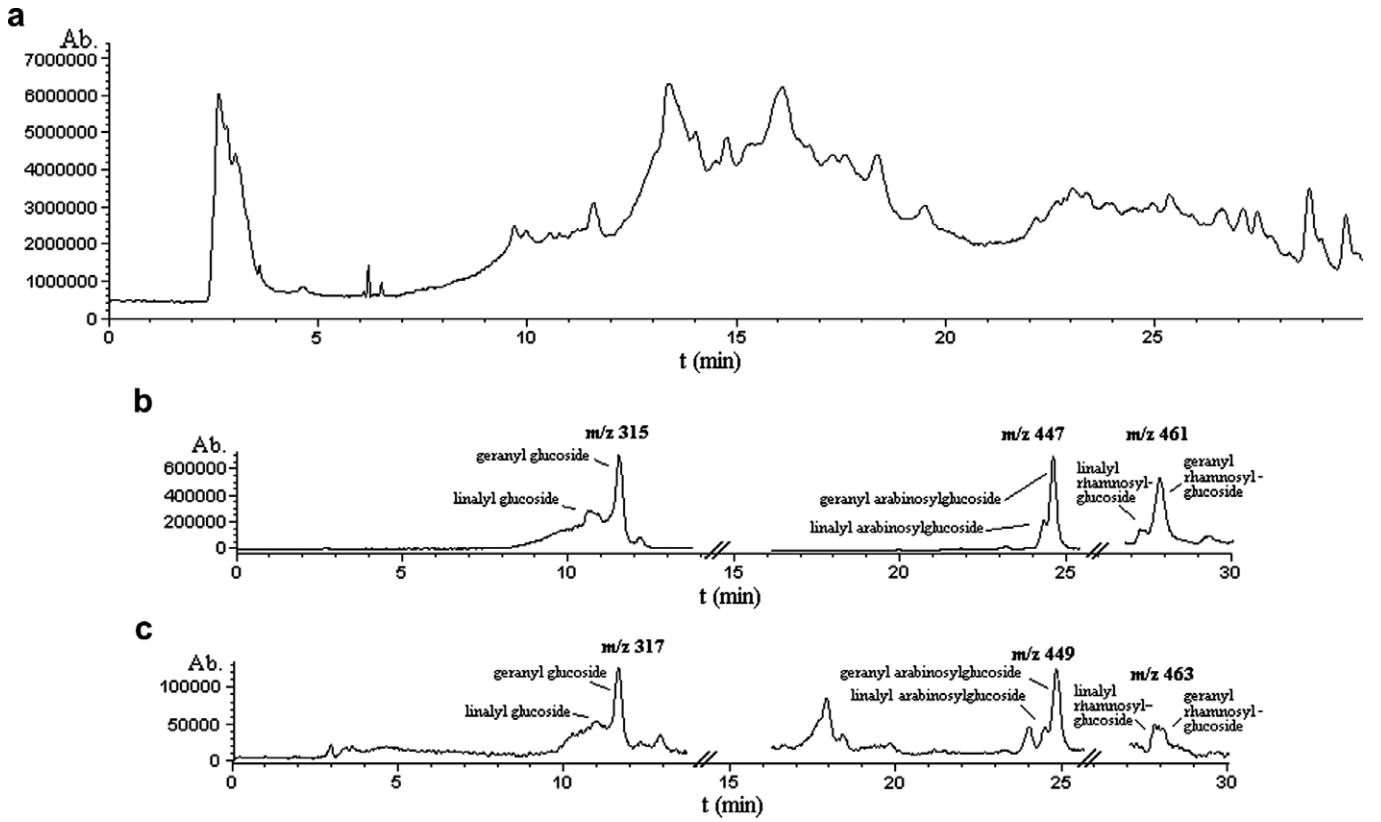


Fig. 2. TIC chromatogram obtained by LC/ESI-MS analysis in negative ion mode for an extract of Falanghina grape (a). Signals obtained for some terpene glycosides by LC/ESI-MS analysis in negative ion mode (b) and in positive ion mode (c).

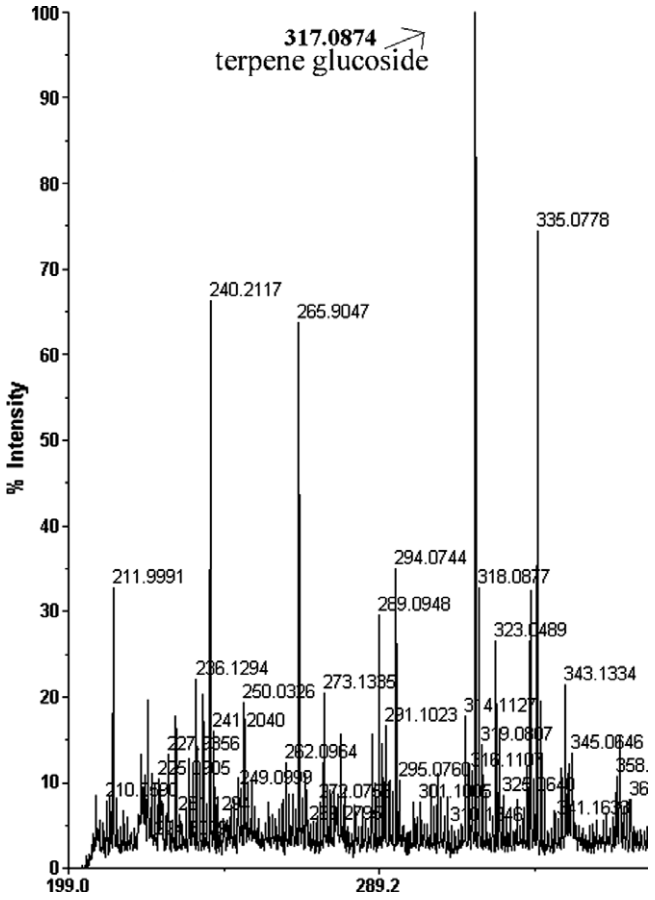


Fig. 3. MALDI-TOF-MS signals observed for the eluate obtained with C18 SPE (methanol 100% step). The signal at *m*/*z* value of 317 Da, whose PSD fragmentation produced a loss of 162 Da, was related to the presence of glucosides of terpenols with Mw 154 Da, for example linalool.

tribution of which to the aromatic profile is considered dominant in non-aromatic grapes in comparison with aromatic varieties. Furthermore their formation seems to increase in the presence of oxygen and of lipoxygenase-like enzymatic systems (Carro et al., 1996). Among dominant aroma compounds detected for Falanghina samples, ethyl hexanoate, benzeneacetaldehyde, nonanal, phenyl ethyl alcohol, ethyl octanoate, decanal, ethyl decanoate were also detected.

3.2. Grape terpene glycosides

The analysis of glycosidically conjugated terpenes, varietal aroma precursors, in grapes presents more analytical difficulties related to the yield of extraction and to the effectiveness of purification. For the analysis of terpene glycosides of Falanghina grapes, the fractionated extraction (obtained through stepwise elution at 20%, 30%, 40%, 100% methanol) with C18 SPE was needed in order to allow a reduction of interferences which create difficulties for non- aromatic grapes where the terpene compounds are present in low concentration in comparison with aromatic varieties. The eluate obtained with methanol 100% presented decisively less interferences in comparison with the other steps of elution with different percentage of methanol. Identity of terpene glycosides was obtained by means of a combined use of mass spectrometric techniques. At first the identification of glycosides was effected through GC/MS analysis of TMS-derivatives considering fragmentation spectra for TMS-derivatives of these compounds reported in the literature (Voirin et al., 1992a,b); further indications were obtained through LC/ESI-MS analysis in both positive and negative ion mode, and MALDI-TOF-MS.

The analysis by GC/MS on the TMS-derivatives of terpene glycosides showed the presence of glucosides, arabinosylglucosides, rhamnosylglucosides of linalool and geraniol.

The eluate corresponding to the step of elution with methanol 100%, containing the native terpene glycosides, was also analysed by LC/ESI-MS (Fig. 2). The best results were obtained in negative ion mode. In Fig. 2a the TIC chromatogram obtained in negative ion mode is shown. In Fig. 2b and c signals, obtained in negative ion mode ($[M\text{-}H]^-$) and in positive ion mode respectively, are indicated for some terpene glycosides. The MALDI-TOF-MS spectrum (Fig. 3) obtained with the eluate with methanol 100%, showed a signal at *m*/*z* value of 317 Da, whose PSD fragmentation produced a loss of 162 Da, corresponding to the presence of glucosides of terpenols with Mw 154 Da, for example linalool.

The presence of rhamnosylglucosides and arabinosylglucosides of terpenes has been already reported also in some other non-aromatic varieties (Carro et al., 1996), and our findings are supported also by studies effected on Falanghina wine with α-L-rhamnopiranosidase, β-D-glucopiranosidase and α-L-arabinofuranosidase from *Aspergillus niger,* indicating that some quantities of terpenols in Falanghina grapes are present in glycoside form (glucosides, rhutinosides and arabinofuranosylglucosides) and can be released after enzymatic hydrolysis (Cabaroglu, Selli, Canbas, Lepoutre, & Gunata, 2003; Martino et al., 2000).

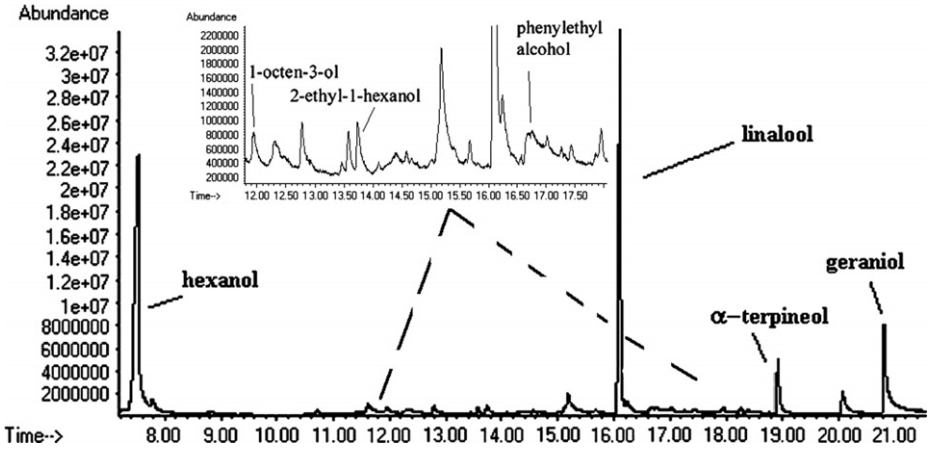


Fig. 4. Dominant terpenols obtained through enzymatic hydrolysis of an extract of glycosides from Falanghina grape and detected by HS-SPME–GC/MS analysis.

Finally in order to obtain further information about bound terpenols and other indications about varietal "aromatic potentialities" of Falanghina grapes, enzymatic hydrolysis of terpene glycosides was carried out by using a pectinolytic enzyme preparation. Pectinolytic preparations (obtained generally from GRAS microorganisms *Aspergillus* spp.) are largely used in winemaking process in order to improve must clarification, must yield, and color extraction (Cabaroglu et al., 2003). In addition to their main activities, these preparations possess other enzymatic "side activities" including glycosidase activity, which are remarkably stable at the wine pH in contrast with those from plant and yeast. It is important to notice that different pectinolytic enzyme preparations differ largerly in glycosidase activities involved in aroma release since they are formulated as a function of their pectinase activities. In this work a pectinase enzyme preparation from *A. niger* was used to study the liberation of glycosidally bound aroma compounds in winemaking process.

In Fig. 4 the TIC chromatogram obtained through HS-SPME–GC/MS analysis of terpenols formed by means of enzymatic hydrolysis of an extract of glycosides from Falanghina grapes is shown. Dominant terpenes formed were linalool, α-terpineol and geraniol (which were detected as glycoside forms also, see above) and α-terpineol as well (indicating the presence of α-terpineol in glycoside form as well).

Some other odorous compounds formed by means of enzymatic hydrolysis were detected: 1-hexanol, phenyl ethyl alcohol, and some other alcohols in traces (1-octen-3-ol and 2-ethyl-1-hexanol).

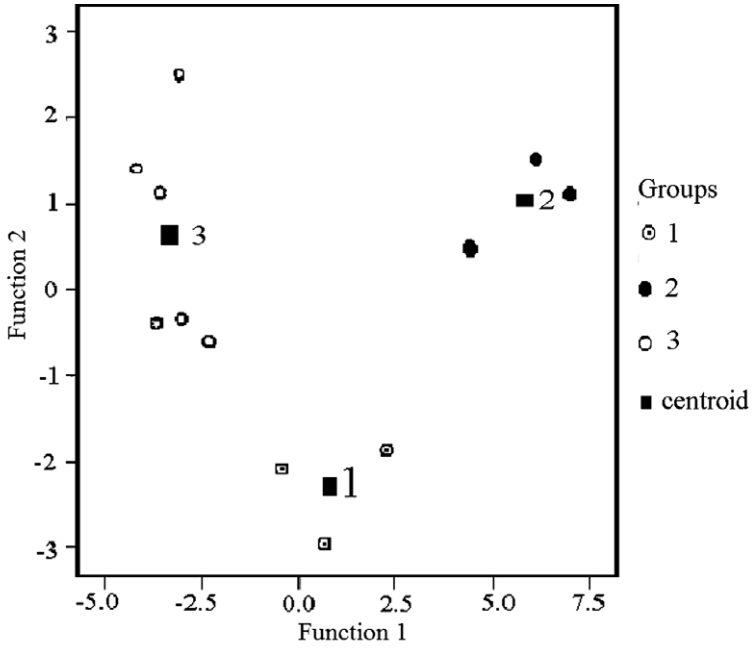
The comparison of results obtained through the analysis of terpene glycosides and enzymatic hydrolysis, with data obtained with other non-aromatic grapes, indicated that the composition of terpene glycosides can depend on the grape variety and a varietal differentiation can be carried out by means of these analytical tools.

3.3. Falanghina wines

The characterization of a wine requires the determination of a large number of different components (varietal and non-varietal); for the identification of volatile markers of Falanghina wine different methods of extraction and concentration were used.

The HS-SPME analysis carried out by means of PDMS and PDMS/DVB fibers (non-polar and semipolar fiber) gave no significant qualitative differences for the analysis of odorous molecules in the analysed wine samples.

By extraction with dichloromethane not all compounds were detected, nevertheless with the obtained analytical data some possible wine descriptors for analysed Falanghina wine samples were identified; in fact the application of the discriminant analysis, effected on some aroma compounds in aromatic bouquet, ethyl butanoate, iso-



[X]/Ref	Lacryma Christi1	Lacryma Christi2	Lacryma Christi3	greco1	greco2	greco3	Falanghina 1	Falanghina 2	Falanghina 3	Falanghina 4	Falanghina 5	Falanghina 6	Group 1 Mean	Group 2 Mean	Group 3 Mean	Global Mean
[A]	0,00000	0,00000	0,00000	0,00000	0,00000	0,00000	0,00770	0,00250	0,01400	0,00770	0,00810	0,00640	0,00000	0,00000	0,00773	0,00386667
[B]	0,00000	0,00000	0,00000	0,00000	0,00000	0,03000	0,05600	0,00610	0,17000	0,03600	0,06800	0,03400	0,00000	0,03000	0,07168	0,04334167
[C]	0,01100	0,00000	0,06400	0,00000	0,01100	0,02500	0,05500	0,01600	0,11000	0,03300	0,03400	0,05400	0,02500	0,01200	0,05033	0,03441667
[D]	0,61000	0,38000	0,63000	0,29000	0,45000	0,02300	0,26000	0,12000	0,20000	0,19000	0,01400	0,04028	0,54000	0,25633	0,13738	0,26777315
[E]	0,06300	0,01900	0,03900	0,12000	0,05800	0,07400	0,53000	0,03500	0,19000	0,09200	0,05000	0,00903	0,04033	0,08400	0,15100	0,10658565
[G]	0,00660	0,00000	0,00000	0,00000	0,02300	0,03300	0,01400	0,00530	0,03800	0,01900	0,03500	0,01600	0,00220	0,01867	0,02122	0,01582500
[H]	0,01200	0,00000	0,00000	0,18000	0,01200	0,01500	0,04800	0,01100	0,06100	0,02000	0,01700	0,02700	0,00400	0,06900	0,03067	0,03358333
Effective Group	1	1	1	2	2	2	3	3	3	3	3	3				
Forecasted Group	1	1	1	2	2	2	3	3	3	3	3	3				
Probability	0,33500	0,44100	0,80200	0,84900	0,31700	0,54200	0,84900	0,61000	0,50600	0,56300	0,16500	0,28400				

Fig. 5. Application of discriminant analysis effected on some samples of Falanghina wines (group 3) and other two typical wines (Lacryma Christi wine produced with Coda di Volpe grapes, and Greco di Tufo wine produced with Greco grapes) (group 1, group 2). [X]Ref = [X]/[Ref]. [X = A–E, G, and H] Ref: ethyl butanoate, isoamyl acetate, ethyl hexanoate, ethyl succinate, ethyl octanoate, phenyl ethyl acetate, ethyl decanoate, phenyl ethyl alcohol, respectively. Group mean and global mean: averages observed on variables [A], [B],. . ., [H], respectively, for wine groups and for all wines. Effective group: a-priori classification of wines. Forecasted group: classification of wines based on discriminant analysis. Probability: probability that a wine belongs to the forecasted group.

amyl acetate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, ethyl succinate, phenyl ethyl acetate, phenyl ethyl alcohol, with significant impact in the aroma profile of Falanghina wines (Martino et al., 2000), permitted us to provide a further possible tool for differentiation of the analysed typical Falanghina wines from some others. The application of discriminant analysis (Rencher, 2002) effected on some samples of Falanghina wines (group 3) and other two typical wines (Lacryma Christi wine produced with Coda di Volpe grapes (see above), and Greco di Tufo wine produced with Greco grapes (see above) (group 1, group 2) is indicated in Fig. 5. The identified discriminant descriptors with mainly fermentative origin for the analysed samples (through extraction with dichloromethane) were the following: ethyl butanoate, isoamyl acetate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, ethyl succinate, phenyl ethyl acetate, and phenyl ethyl alcohol.

In the TIC chromatograms, obtained with the used strategies of extraction and concentration and through SCAN method, several times terpenes were co-eluting with other compounds; in this case, for the detection of terpenes, co-eluting peaks were resolved spectrometrically at the expected retention times of terpenes, and the intensity ratios of diagnostic fragmentation ions were verified to be in accordance with the expected intensity ratios.

Dominant terpenes and norisoprenoids (varietal volatile markers) observed in Falanghina grapes were detected also in all samples of Falanghina wines analysed (Fig. 1c). Some of the detected varietal odorous molecules were not observed in Falanghina wines previously (limonene, myrcene, 4-carene, and some terpene derivatives such as isobornyl acetate and menthol) (Moio, Ugliano, Gambuti, Genovese, & Piombino, 2004a; Moio et al., 2004b).

In wine samples also, the concentration of all terpenes identified (limonene, geraniol, linalool, myrcene, 4-carene, *cis*-linalool oxide, alpha-terpineol) was higher in samples from Benevento area (in order to give some quantitative indications, 0.5 mg/l for linalool) than samples from Campi Flegrei (0.3 mg/l for linalool).

In conclusion, these studies showed that molecular approaches based on advanced mass spectrometric techniques could open new possibilities in the differentiation, valorization and defense of the typical products.

The combined use of advanced mass spectrometric techniques (GC/MS, LC/ESI-MS, and MALDI-TOF-MS) in conjunction with specific methodologies of extraction and purification permitted us to overcome the analytical difficulties deriving from the extremely low concentration of metabolites strictly related to the variety, in complex matrices, such as grapes and wines.

This is of outmost importance, considering that the defense of commercial typical food products is accomplished through a differentiation process and the identification of distinctive qualitative characters. In this respect, the metabolomic approach presented here permitted us to identify varietal molecular markers in grapes and wines, providing possible tools for checking the authenticity and origin of typical oenological products with quality trademarks (such as the European Appellation of Origin designations, for example the Italian Controlled and Guaranteed Denomination of Origin, i.e. DOCG, or Controlled Denomination of Origin, i.e. DOC, etc.).

Furthermore, the methodology used, being based on the determination of odorous varietal metabolites and their precursors, can permit to explore intimately the potential and specific expression of the aroma in a wine, which is particularly important under the technological and qualitative aspects.

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