DETERMINATION OF SECONDARY METABOLITES AS QUALITY AND TYPICALNESS TRACERS IN AUTOCHTHONOUS VITIS VINIFERA GRAPES AND WINES FROM ISCHIA ISLE

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ABSTRACT

In this study a "metabolomic" approach carried out on secondary metabolites of autochthonous vinegrapes from a terroir with very specific and distinctive characteristics, such as the Ischia isle, permitted us to obtain the characterization on molecular basis of some typical oenological productions. The analysis started from white and red grapes (Arilla grapes from Cuotto and Spadara; Forastera, Don Lunardo, Livella from Panza and Spadara; Guarnaccia from Spadara and Fango), and extended to wines, was effected by means of advanced mass spectrometry techniques (GC/MS, MALDI/TOF-MS; LC/ESI-MS). The characterization of secondary metabolites (volatile compounds and anthocyanins) allowed identification of molecular tracers which can be important for the fingerprinting and protection of qualitative characteristics of these typical productions. Possible relationships between the quantitative composition of these metabolites and some environmental characteristics such as hydric stress conditions were also investigated.

KEYWORDS: wine and grape quality and biodiversity; secondary metabolites; "terroir".

INTRODUCTION

High quality wines acquire a meaningful importance on the market through the diversification of their specific identifiable qualitative characteristics. During the last 30 years, consumer preferences have been oriented towards authochthonous wines with quality trademarks such as the European Appellation of Origin designations (e.g., Italian Controlled and Guaranteed Denomination of Origin, i.e. DOCG) or Controlled Denomination of Origin, i.e. DOC, etc.). The typicalness of these wines is related to the grape variety and specific characteristics of the *terroir* (i.e. soil, location, climate, and specific vineyard-environment interaction). Several studies of viticultural zoning have been carried out by using a multidisciplinary approach to define the best qualitative potential of the oenological productions (Nasi *et al.,* 2010). In grapes and wines the composition of odorous molecules and antocyanin compounds is often used for varietal differentiation being the rationale of these studies that these compounds are constituted by several molecules whose concentration can vary depending on the grape variety (Mamede, Pastore, 2006; Nasi *et al.,* 2008; Oliveira *et al.,* 2004).

Analytical tools can provide new information on molecular basis and when combined with conventional analytical parameters can give a better description of typicalness and quality of food products. 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 carried out through a combined use of techniques based on mass spectrometry (GC/MS, MALDI-TOF-MS), the characterization of secondary metabolites (volatile compounds and antocyanins) in autochthonous grapes and wines from isle of Ischia, allowed identification of molecular tracers which can be important for the fingerprinting and protection of qualitative characteristics of these typical productions. Possible relationships between the quantitative composition of these metabolites and some environmental characteristics such as hydric stress conditions were also investigated.

MATERIALS AND METHODS

Determination of volatile compounds

The SPME (Solid Phase Micro-Extraction) analysis was effected with the holder and fibres (50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS)) purchased from Supelco (Aldrich, Bornem, Belgium) on 130 mL of sample (wine and must). Thermal desorption of the analytes from the fibre inside the GC injection port was carried out in the split mode (1/10) at a desorption temperature of 250 °C for 1 min. All samples were analyzed with an HP 7890A coupled to a 5975C quadrupole HP mass spectrometer (Agilent Technologies, Palo Alto, CA, USA). The gas chromatograph was equipped with an HP-5ms capillary column (30 m x 0.25 mm ID; 0.25 μm Film Thickness) and the carrier gas used was helium. For the analysis of the volatile molecules, 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 method was used (for terpene compounds m/z 59, 69, 93, 121, 136, 154). The NIST library and comparison with spectra and retention times of standards (Sigma-Aldrich; Acros Organics) were used to identify the odorous compounds. Quantitative determinations were obtained by means of calibration curves, in the concentration ranges typical of wines for each compound. In the range of linearity verified seven concentration levels and five replicates per level were used. Multiple replicates (n=3-6) of the samples were analyzed.

Determination of antocyanin compounds

4 g of skins were homogenised in 10 mL of acidified methanol (0,1%, v/v HCl in methanol) and the extraction was effected overnight. The extracted samples were filtered with sodium sulphate anhydrous and analyzed through MALDI/ToF/MS.

MALDI-TOF spectra were recorded using a Voyager DE-Pro spectrometer (PerSeptive BioSystems, Framingham,MA) equipped with a N2 laser (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.

RESULTS

Varietal volatile compounds detected in grape and wine samples are listed in Tab. 1, 2.

The most notable differences in the terpene and norisoprenoid composition were related to the varietal origin.

Among the white grapes and wines considered Don Lunardo grapes and wines had a more complex qualitative and quantitative terpene composition in comparison to the Arilla and Forastera grapes and wines. Identified dominant terpene and norisoprenoid compounds for these varieties were: for Don Lunardo variety, limonene, geraniol, linalool, isobornylacetate, α-pinene, β-myrcene, β-damascenone; for Arilla variety, limonene, isobornylacetate and menthol; for Forastera variety α-pinene, limonene, isobornylacetate, menthol and β-damascenone. Don Lunando grapes are also characterized by the presence of benzaldehyde with potential almond odour. In Arilla wines the dominant terpene was linalool which was not

detected in the grapes but was produced in the wine during the winemaking process through acid and enzymatic hydrolysis from its glycoside precursors.

Table 1. Dominant varietal compounds in white varieties. +. Present; (tr). Present only in traces; -. Not detectable

		GRAPE				WINE			
VARIETAL COMPOUND	RT (min)	Arilla Cuotto	Arilla Spadara	Don Lunardo	Forastera	Arilla Cuotto	Arilla Spadara	Don Lunardo	Forastera
α-pinene	9.1	(tr)	(tr)	+	+	(tr)	(tr)	+	+
benzaldehyde	10.9	—	—	+	—	—	—	+	—
β-myrcene	11.6	—	—	+	—	—	—	+	—
limonene	12.8	+	+	+	+	+	+	+	+
linalool	15.4	—	—	+	(tr)	+	+	+	+
menthol	17.6	+	+	(tr)	(tr)	+	+	(tr)	—
geraniol	20.3	—	—	+	—	—	—	+	—
β-damascenone	20.6	—	—	+	+	—	—	+	+
isobornylacetate	20.8	+	+	+	+	+	+	+	+

The quantitative differences observed for the Arilla grapes and wines from Cuotto and Spadara were related to their different geographical origins, and were presumably due to different salinity stress and water deficit conditions (as confirmed by the determination of the quantities in grapes and wines of proline, which is considered a suitable molecular stress indicator; data not shown) and to different ripening rates. In fact the terpene compounds in Arilla grapes and wines that came from Spadara tended to have higher concentrations compared to those from Cuotto (Fig. 1).

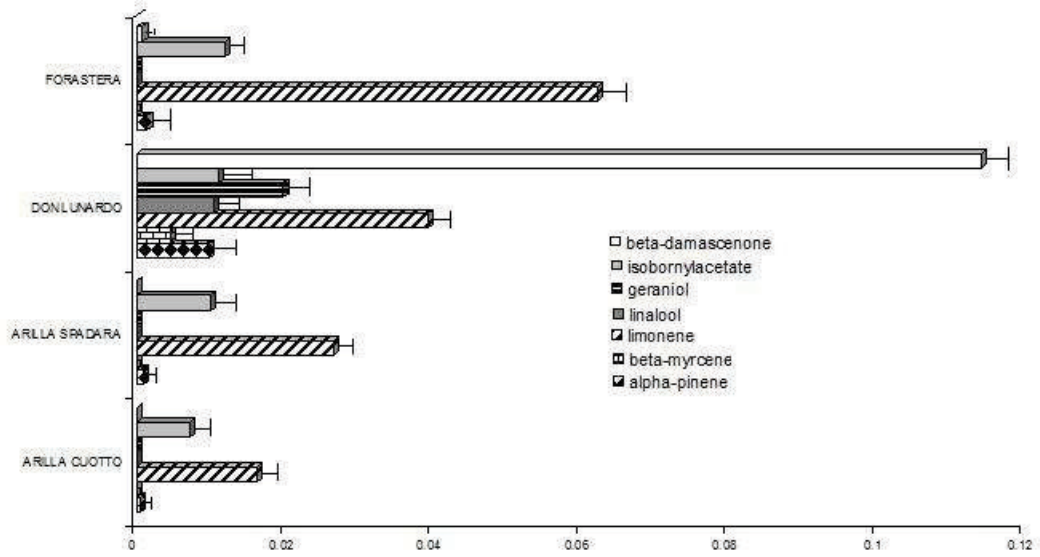

Figure 1. Quantitative data (ppb) for terpene and norisoprenoid compounds in white grapes.

For red varieties, identified dominant terpene and norisoprenoid compounds were: for Livella variety limonene, α-pinene, β-myrcene, isobornylacetate, α-damascenone e di β-

damascenone; for Guarnaccia variety limonene, isobornylacetate, α-damascenone, β-damascenone, α-ionone.

Table 2. Dominant varietal compounds in red varieties. +. Present; (tr). Present only in traces; -. Not detectable.

		GRAPE				WINE		
VARIETAL COMPOUND	RT (min)	Guarnaccia Fango	Guarnaccia Spadara	Livella Panza	Livella Spadara	Livella Panza	Livella Spadara	Guarnaccia Fango
α-pinene	9.1	—	—	+	+	+	+	—
benzaldehyde	10.9	+	+	+	+	+	+	+
β-myrcene	11.5	—	—	+	+	+	+	—
limonene	12.8	+	+	+	+	+	+	+
linalool	15.5	(tr)	(tr)	—	—	—	—	(tr)
α-terpineol	18.1	—	—	(tr)	(tr)	(tr)	(tr)	—
β-damascenone	20.6	+	+	+	+	+	+	+
isobornylacetate	20.8	+	+	+	+	+	+	+
α-damascenone	23.4	+	+	+	+	+	+	+
α-ionone	24.5	+	+	—	—	—	—	+

Anthocyanins extracted from red grapes and wines are listed in Tab. 3 and 4.
M/z 507 and M/z 533 signals, corresponding to delphinidin-3-O-(6-O acetyl)-glucoside and delphinidin-3-glucoside piruvic compound respectively, are present in Livella grapes and wines but absent in Guarnaccia grapes and wines: these anthocyanin compounds can act as varietal molecular markers.Livella and Guarnaccia appeared to be different for composition of diglucosides also: petunidin-3,5-O-diglucoside can act as varietal marker for Livella variety (Tab. 3 and 4).

Table 3. Anthocyanin compounds identified in the metanolic extract of red grape skins.

ANTOCYANIN COMPOUNDS	M+	LIVELLA SPADARA	LIVELLA PANZA	GUARNACCIA SPADARA	GUARNACCIA FANGO
malvidin	331	+	+	+	+
cyanidin-3-O-glucoside	449	+	(tr)	+	+
peonidin-3-glucoside	463	(-)	(-)	+	+
petunidin-3-O-glucoside	479	(tr)	+	+	+
malvidin-3-O-glucoside	493	+	+	+	+
peonidin-3-O-(6-O acetyl) glucoside	505	+	+	+	+
delphinidin-3-O-(6-O acetyl)-glucoside	507	+	+	(-)	(-)
peonidin-3-glucoside piruvic compound	531	+	+	+	+
delphinidin-3-glucoside piruvic compound	533	+	+	(-)	(-)
malvidin-3-acetylglucoside	535	+	+	+	+
cyanidin-3,5-O-diglucoside or delphinidin-3-O-(6-O p-coumaroyl)-glucoside or cyanidin-3-O-(6-O p-caffeoyl)-glucoside	611	+	+	+	+
malvidin-3glucoside-4-vinylguaiacyl	639	+	+	+	+
petunidin-3,5-O-diglucoside	641	(-)	(-)	+	+
peonidin-3-(p-coumaroyl)-glucoside piruvic compound	677	+	+	+	+
+ present; (-) not detectable; (tr) only in traces.					

Table 4. Anthocyanin compounds identified in the metanolic extract of red wines.

ANTOCYANIN COMPOUNDS in WINES	M+	LIVELLA SPADARA	LIVELLA PANZA	GUARNACCIA FANGO
malvidin	331	+	+	+
malvidin-4-vinylphenyl	447	+	+	+
cyanidin-3-O-glucoside	449	+	+	(-)
peonidin-3-O-glucoside	463	+	+	+
petunidin-3-O-glucoside	479	+	+	+
cyanidin-3-O-glucoside	491	+	+	+
malvidin-3-O-glucoside	493	+	+	+
peonidin-3-O-(6-O acetyl) glucoside	505	+	+	+
delphinidin-3-O-(6-O acetyl)-glucoside	507	+	+	(-)
malvidin-3-glucoside-4-vinyl	517	+	+	+
peonidin-3-glucoside piruvic compound	531	+	+	+
delphinidin-3-glucoside piruvic compound	533	+	+	(-)
malvidin-3-acetylglucoside	535	+	+	+
malvidin-3-glucoside piruvic compound	561	+	+	+
malvidin-3-glucoside-4-vinylphenyl or peonidin-3-glucoside-4-vinylguaiacyl	609	+	+	+
cyanidin-3,5-O-diglucoside or delphinidin-3-O-(6-O p-coumaroyl)-glucoside or cyanidin-3-O-(6-O p-caffeoyl)-glucoside	611	+	+	+
petunidin-3-(p-coumaroyl)-glucoside	625	+	+	+
delphinidin-3,5-O-diglucoside or delphinidin-3-O-(6-O p-caffeoyl)-glucoside	627	+	+	+
malvidin-3-glucoside-4- vinylguaiacyl	639	+	+	+
petunidin-3,5-O-diglucoside	641	+	(tr)	(-)
malvidin-4 vinyl-catechin or epicatechin	643	+	+	+
malvidin-3-(p-coumaroyl)glucoside-4-vinylphenyl	755	+	+	(-)
malvidin-3-glucoside4-vinylcatechin or epicatechin	805	+	+	+
malvidin-3-glucoside-4-ethyl-catechin or epicatechin or malvidin-3-glucoside-8-ethyl-catechin or epicatechin	809	+	+	(-)
malvidin-3-(acetyl)glucoside-4-vinylcatechin or epicatechin	847	(-)	(-)	+
unknown	889	+	+	+
malvidin-4-vinyl-procyanidin dimer	931	+	+	+
malvidin-3-(p-coumaroyl)-glucoside-4-vinylcatechin or epicatechin	951	(-)	(-)	+
malvidin-3-glucoside-4-vinyl- procyanidin dimer	1093	+	+	+
+ present; (-) not detectable; (tr) only in traces.				

CONCLUSIONS

Suitable quality and authenticity molecular markers of typical food products can furnish useful analytical tools which can provide quality control and commercial protection. The metabolomic approach carried out in this study on autochthonous grapes and wines from Isle of Ischia indicated that the varietal metabolites identified, which express the distinctive vine genetic characteristics and grapevine biodiversity, could act as quality and origin tracers for these typical oenological products.

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