Research Article

Biochemical Markers for Oenological Potentiality in a Grapevine Aromatic Variety under Different Soil Types

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Abstract: Pedo-climatic conditions affect grape and wine quality and in particular the relation soil-grape quality is at the core of the terroir definition. The study focuses on an aromatic and autochthonous grapevine cultivar grown in the north of the Latium region (Centre of Italy), i.e. cv Aleatico, in a heterogeneous environment (PDO ‘Aleatico di Gradoli’), where five sub-areas have been selected as representative of the environmental variability. The ripening grape parameters, volatile and phenolic compounds in wines derived from the grapes of these growing areas, were analysed to assess the relationship among soil traits, biochemical grape and wine parameters. Pedo-climate analysis was carried out following official protocols for soil texture determination and bioclimatic Thermal Index of Winkler computation. The volatile wines composition and phenolic compound were determinate using the SPME technique gas-chromatographic method and standard method, respectively. Data were evaluated through descriptive statistical methods (ANOVA and Pearson’s Coefficient) and multivariate statistical
analysis (PCA and HCA). The results proved that there is a ‘soil effect’ on the grapes and wines’ biochemical composition. The effect of soil on grape ripening parameters was found to be highly significant with regard to total soluble solids and phenols concentration; both exhibited a high correlation to soil’s sand content (%). In particular, soils with a -sandy-loam texture, moderate skeleton content, offer the best wine performance in terms of aroma and phenolic content. The study highlight the importance of the microzonation even in small wine-grape growing areas for better diversified, and therefore more competitive, wine productions. The study enhances the knowledge about the relationship between soil and grapevine aromatic varieties. Data points at identifying biochemical parameters as markers of oenological potential according to geographical origins.

**Key words:** cv Aleatico, berry and wine quality, bioclimatic indexes, HS-GC, polyphenols, SPME

**Introduction**

Current market trends tend to wines with better sensorial features, and high quality standards, like high quality grapes allow to produce. The number of academic papers related to the study of the oenological potential of aromatic grape varieties in the last decade also increased, proving the growing interest of winemakers and grape growers in the aromatic grapevine varieties such as ‘Muscat’ aroma cvs (Crespan and Milani 2001).

The aromatic grapevine varieties are characterized by high monoterpenol’s concentrations; in fact these compounds are identified as the main responsible of this aroma. The more abundant monoterpenols are linalool and geraniol, commonly associated with floral, rose-like flavours (Riberau-Gayon et al. 1998). Numerous studies on the volatile components of
the *Vitis vinifera* have helped the understanding of the complex metabolic pathway for the formation of aromas. Nonetheless, the relationship between individual chemical components and the defining aroma of wines is far from being fully understood, due to the complexity of wine matrices, to the low concentration of some of the volatile compound partially responsible for the wine aroma and flavour, and to complex physical and chemical processes that occur during the grape ripening and wine fermentation.

Wine’s aroma profile derives from a balance of grape’s chemical compounds, in particular volatile fraction and phenolic compounds - precursors of volatile phenols and wine colour (Sagratini et al. 2012). The wine aroma is conditioned by intrinsic factors such as the grape genetic specificity and other extrinsic factors such as the environmental conditions (climate, soil, location) (Perestrelo et al. 2014), vine growing practices (Sala et al. 2004), and winemaking (like fermentation conditions and aging) (Swiegers et al. 2005, Comuzzo et al. 2006, Piñeiro et al. 2006). In this sense, some studies have tried to establish links among viticultural parameters and grape or/wine composition. Several researches found that soil impacts the overall quality of the grape and thus resultant sensory wine quality (Sabon et al. 2002, Van Leeuwen et al. 2004, Wang et al. 2015, Zerihun et al. 2015). This suggests that although macroclimate, mesoclimate, and fruit microclimate all have major impacts on wine quality and varietal peculiarity, soil may also have an independent effect. Micro and macro elements of soil could be used to fingerprint wines from different grape-growing areas. Furthermore, intrinsic geochemical parameters as the strontium isotope analysis would allow to link each wine to its production zone (Bollati et al. 2015). Therefore, if in general the analysis of volatile compounds is used to characterize different cultivars (Arvanitoyannis et al. 1999), these
biochemical compounds could also represent an important biochemical marker for the
differentiation of wines based on geographical origin (Perestrelo et al. 2014), particularly when
testing wine authenticity, pivotal issue after the introduction of European regulations for the
protection of the denomination of origin products. Therefore, fingerprinting techniques, based on
chemical composition can be used to characterize or classify wines according to origin, quality,
variety, type, etc. Furthermore, an interpretation of wine differences based on multivariate data
analysis such as the Principal Component Analysis (PCA) complemented with information
obtained by the Hierarchical Cluster Analysis (HCA) has been efficiently employed in wine
differentiation and classification to geographical origin (Perestrelo et al. 2014).

In the Latium region (Centre of Italy) the red grape variety ‘Aleatico’ is an example of
aromatic autochthonous grapevine germplasm under moderate erosion risk (according to the
Regional Developmental Programme (PSR) Latium 2007/2013 Reg (CE) N. 1698/05). It has
been objected to several viticultural and oenological studies in order to enhance the oenological
potential and to preserve an important biological and economic resource (Bellincontro et al.
2006, Biasi et al. 2007). Probably the Greeks were the first to introduce this variety in ancient
times, as it can be considered a mutation of the ‘Moscato’ grape. The corresponding Greek wine,
from Crete, is ‘non aromatic’. The name may derive from "July" (iouliatico in Greek), the month
in which veraison occurs. In Italy the Protected Denomination of Origin (PDO) ‘Aleatico di
Gradoli’ (D.P.R. 1972 modified with D.M. 2011) is a mono-varietal wine produced in a small
and highly heterogeneous area – mostly in the Gradoli municipality in the northern part of
Latium (Italy). The alcoholic content of this wine ranges between 12 and 17.5%; the wine is
commercially available in different typologies: traditional, liqueur wine, liqueur reserve and passito wine.

The main purpose of this study was to identify and quantify the biochemical traits of grape at ripening time and the volatile fraction and polyphenolic content of an aromatic autochthonous cultivar of Latium region (Italy), i.e. the cv ‘Aleatico’ and to define through Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) the effect of environmental factors, such as the soil, on the wine produced from grapes belonging to different growing areas.

**Materials and Methods**

*Plant material*

The investigation focuses on the aromatic red grapevine variety ‘Aleatico’, an autochthonous genotype of increasing importance due to its suitability to produce a wide range of oenological products, including sweet wines from dehydrated grapes (Bellincontro et al. 2006). This cultivar has been characterized through conventional phenotyping and genotyping procedures (Biasi et al. 2007), however unable to distinguish the high cultivar polymorphism. All the grape samples were from the main typology, i.e. a medium-large wrinkled bunch (170 ± 66 g) with late ripening.

*Study area*

The study area is located in central Italy (northern of Rome) (Figure 1A) and is part of the large territory where different PDOs (Protected Denomination of Origin) overlap (i.e. ‘Aleatico di Gradoli’ (Figure 1B), ‘Colli Etruschi Viterbesi’, ‘EST!EST!!EST!!!). In particular, the investigation was carried out within the historical and classic wine-grape growing area for the cv ‘Aleatico’ that includes many localities, namely *S. Antonio* (SA), *La Fratta* (LF), *Macchia del*
Prete (MP), S. Magno (SM), Montemaggiore (MM) (Figure 1B and C). In order to determine the physical characters of the studied DOP area, the following cartographic supports were used: i) the regional geological map 1:50000 scale, ii) the regional topographic map (CTR) 1:10000 scale, iii) the elevation map (DEM) at the resolution of 40 m and iv) the aspect map, resulting from a previous zonation study (Biasi et al. 2007). Aleatico’s vineyards are mostly present between 300 and 450 m a.s.l., on the south-east exposed slope in the 1500 ha wide Landscape System of the Bolsena lake basin. In particular, vineyards for grape sampling were located in the Land Unit ‘Medium and low warmly exposed sides’, following the common zonation methodology for hierarchically organized spaces.

Climate and bioclimatic indexes

The mean precipitations and the average monthly temperatures were calculated from data recorded by a meteorological station (Integrated Service Agrometeorological Lazio Region - SIARL) from 2004 to 2015 based in the study Land Unit (Bolsena, Viterbo, Italy) for a general climatic classification (Fig.2). Furthermore, the Thermal Index of Winkler (IW) (Winkler 1962), also called growing degree-days (GDD) has been calculated to evaluate suitability of grapevine cultivation and classification of grape-wine growing areas (Fregoni and Pezzuto 2000). IW was derived by the formula (1) as the sum of the average daily useful temperatures ($T_{mean} - 10^\circ C$) during the grapevine growing season (from April 1st to October 31).

\[
WI = \sum_{01/04}^{31/10} (T_{mean} - 10)
\]  

(1)
Pedological investigation and vineyard soil characterization

One model vineyard was selected for each locality in the PDO study area (Land Unit ‘Medium and low warmly exposed sides’). The soil pedological profile and typology were classified. The soil texture was determined following the standard methodology for deepness, mean texture, i.e. sand-silt-clay ratio and skeleton percentage measurement (Soil Survey Staff 1998). Soil classification was done according the international soil classification system (USDA-NRCS 1999).

Berry volatile compounds:

The determination of volatile compounds of grapes has been carried out on Grape berry juice (5 g) mixed with 5 mL of saturated CaCl2 (1:1 w/v) or wine alone (10 mL) were homogenized with 200 μL of standard solution (1-penten-3-one). The homogenate was collected in a 25 mL glass miniflask (Supelco, Sigma-Aldrich Co, St Louis, MO, USA) and sealed with a Teflon silicone septum. The sample, grapes or wine, was exposed to a solid phase micro-extraction fiber, respectively, for 30 and 15 min, in a Thermo Haake DL30-V15B water bath (ENCO Spinea, Ve, Italy) maintained at a temperature of 20±2°C. The SPME fiber (PDMS 100 μm) was then inserted into the headspace. During the sampling time (30 min), the sample was stirred at constant speed. After completion of sampling, The fiber, Polydimethylsiloxane (PDMS 100 μm) (Sigma-Aldrich Co), was conditioned in the GC injection port at 250 °C for 2 h prior to use. For as regards the chromatographic conditions they were the same as used for wines.

These method can be useful for monitoring grape maturation and to determine grape composition before wine- making. The aromatic profile of Aleatico’s berry is chiefly characterized by monoterpenes, that represent the 85% of total berry aroma. We focus on five of the most relevant
monoterpenes for Aleatico cultivar: linalool, terpineol, citronellol, nerol and geraniol (Mencarelli and Tonutti 2013).

Samples for microvinifications

Grapes: The experiment was conducted using grapes from the five sub-areas: La Fratta (LF), S. Antonio (SA), Macchia del Prete (MP), S. Magno (SM), Montemaggiore (MM).

Aleatico’s grapes for all tested areas were harvested at technological maturity by monitoring the sugar berry accumulation increment up to it remains constant. Then samples are separately microvinificated. Three vines for sub-area are selected and three replicates of 33 berries each one are randomly collected on three bunches for vines. These replicates have been used to analyze the chemical traits (sugars, pH and total acidity) of berries before the microvinification.

Wine: A total of 85 kg of grapes for each sub-area was destemmed, crushed, divided into five batches one for each sub-area and 10 g·hL⁻¹ of K₂S₂O₅ were added to mash prior the fermentation. All batches were inoculated with yeast (Saccharomyces cerevisiae, 10 g dry wt·hL⁻¹) and punched down three time a day during active fermentation for 5 days, the duration of maceration or contact between solids and liquid was 15 days at 25°C. At the end of maceration the free run of each batches was drained and the pomace was pressed in a tank membrane press in order to obtain the press run. At last the free and press run for each batches have been combined and the wine was stored in 100 L stainless stell tanks at room temperature for five mounths. After 5 months wines were bottled in 750 mL bottles sealed with corks.
Three replicates of wine samples obtained for each sub-area (five batches) has been analyzed to assess chemical traits as titrable acidity, pH, alcohol content, polyphenols, total anthocyanins and aromatic compounds.

Chemical parameters: The titrable acidity of wines were changed between 5.8 and 6.2 g·L⁻¹. The pH values were ranged between 3.5 and 3.6 and alcohol content between 13.2 and 14.4 % vol. The analysis were determined according to the Regulation Official Methods by EU.

Spectrophotometric methods: quantitative estimate of the presence of phenolic compounds was easily carried out involving spectrophotometric detection. A spectrophotometer (Perkin Elmer, mod. Lambda 2) was employed. The methods are described in detail by Di Stefano and Cravero in 1991 and were easily employed.

Total anthocyanins content of skins of 10 berries were extracted by a 25 ml ethanol/water/HCl (70:30:1 v/v/v) solution for one day in the dark. The solution was diluted in accordance with methods reported by Di Stefano at al. (1991) to register the absorbance spectrum between 230 and 700 nm for estimation of total anthocyanin and flavonoid contents. The Folin–Ciocalteu method proposed by Di Stefano et al. (1989) was used for the determination of the total phenolics in wines. In brief, an aliquot (1 mL) of the appropriate diluted extracts was added to a 10 mL volumetric flask, containing 5 mL of distilled water. Then, 0.5 mL of Folin-Ciocalteu reagent was added and the contents mixed. After 3 min, 1.5 mL Na₂CO₃ solution of concentration 5 g·L⁻¹ was added and made up to a total volume of 10 mL distilled water. After keeping the samples at 50 °C (water bath) for 16 min in sealed flasks and subsequent cooling, their absorbances were read at 765 nm against distilled water as the blank. A calibration curve was constructed using gallic acid standard solutions (0–100 mg·L⁻¹). The concentration of total...
phenolics is expressed as the gallic acid equivalent (GAE) per 1 g of fresh sample. All samples were prepared in triplicate;

The determination of the total anthocyanins in wines was realized by the method proposed by Di Stefano et al. (1989). The samples were diluted with a solution consisting of 70/30/1(v/v/v) ethanol/water/HCl (concentrated) and the absorbance was measured at 540 nm. Due to the lack of a malvidin-3-glucoside standard, the total anthocyanic contents are expressed as malvidin-3-glucoside equivalents and calculated using the following equation:

\[ TA_{540\text{ nm}}(\text{mg·L}^{-1}) = A_{540\text{ nm}} \times 16.7d \]

where \( A_{540\text{ nm}} \) is the absorbance at 540 nm and \( d \) is the dilution.

**Volatile compounds characterization:** the analysis of volatile compounds is normally carried out by gas chromatography (GC). Yet even today, the extraction and concentration of flavour components constitute a problem that has still not been satisfactorily resolved. In recent years, different extraction methods based on solid-phase microextraction (SPME) have been applied to analyse certain types of volatile compounds in wines (Vas et al. 1998, Haggerty et al. 2015) and in particular Vas et al. (1998) reported the use of SPME for fast screening of different wine types, morover SPME is a solvent-free method presenting major advantages, such as small sample volume and higher sensitivity and simplicity.

The analysis of volatile compounds was carried out applying the SPME technique gaschromatographic method and the selected SPME parameters are shown below. Five mL of wine was pipetted and placed into a 25ml glass mini flask (Supelco, Sigma-Aldrich Co, St Louis, MO, USA). Each sample was spiked with 200 μl of a standard solution of 1-penten-3-one (CAS Registry Number: 1629-58-9) (5gL^{-1} in Milli-Q water). A small magnetic stirring bar was also
added. The vial was tightly capped with a PTFE-faced silicone septum and placed in a thermostatted block on a stirrer. The sample was equilibrated for 15 min at sampling temperature in a Thermo Haake DL30-V15B water bath (ENCO Spinea, Ve, Italy) maintained at a temperature of 20 ± 2 °C. The SPME fiber (PDMS 100 μm) was then inserted into the headspace. During the sampling time (30 min), the sample was stirred at constant speed. After completion of sampling, the fiber, Polydimethylsiloxane (PDMS 100 μm) (Sigma-Aldrich Co), was conditioned in the GC injection port at 250 °C for 2 h prior to use.

Chromatography: after the selected extraction time the SPME fiber was insert into the GC injection port and thermally desorbed at 230 °C for 7 min. The splitless injector was mounted on a model 5300 Mega Series gas chromatograph (Carlo Erba Instruments, Milan, Italy). It was equipped with a fused silica capillary column impregnated with a polar phase of Carbowax 20M (Alltech Assoc, Inc, Deerfield, IL, USA), 60m long × 0.25mm id and 0.25 μm film thickness. Helium was used as carrier gas (27 cm·s−1). The temperature was maintained at 40 °C for 7 min, then programmed to reach 230 °C at a rate of 3 °C min−1, with a final isotherm of 30 min. A high sensitivity flame ionization detector (FID) at 260 °C was used. The signal was recorded and integrated by a Mega Series integrator. Compounds identification was achieved by using a Shimadzu 17A GC/MS and a Shimadzu QP 5050A MS and matching against the NIST 107 and NIST 21 libraries. Volatile and semivolatile constituents were identified using mass spectral matching against library standards RI and RI* (values for a Carbowax 20M column after running a carbon alkane standard, also known as Kovats index KI) (Table 1).

Statistical analysis
All determinations were carried out in three replicates and all analytical results were subjected to a statistical study performed at the p<0.05 significance level and carried out by DSAASTAT ver. 1.1 (Onofri 2007), using one-way ANOVA and Fisher’s LSD test.

Correlation analysis (Pearson’s coefficient) were used to identify the aspect of soil (physical) that are associated with berry composition at harvest (sugar, acidity, anthocyanins, polyphenols).

A Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) has been carried out using all the major chemical and aromatic compounds of berries for the five sub-areas at harvest time, in order to detect how the soil type could influence the berry quality.

Finally, in order to reveal the relationship among biochemical parameters of the Gradoli Aleatico’s wines and soil traits of the grapevine growing areas, and to identify the wine components that better concur to characterize wines according to geographical origin, the composition data matrix of five samples (27 variables x 5 wine samples = 135 data) was analysed using the PCA and HCA, based on Euclidian distances. The analyses were performed using R software package

**Results**

*The physical environment of the vineyards*

The climate of the studied grape growing area, i.e. the PDO ‘Aleatico di Gradoli’ was identified as Mediterranean whit mean annual precipitations, over the last 10 years, of 806 ± 230 mm of rain, mainly concentrated in Autumn (November –December). The drought events are concentrated in summer (June – August) where the mean distribution of rainfall was of two-four days per month.
The Winkler index reached in the last 6 years the mean value of 1827.5 ± 130.8 GG (Table 2).

The whole area was characterized by high heterogeneity of steep downslope directions (Figure 1C) although the five areas tested were located on the south-east slopes.

The soil of the study area has evolved from the prominent lithology, i.e. ignimbrite, a volcanic material of different texture (ashes and lapillus) and poorly consistent. In the Land Unit of vineyards (Land Unit ‘Medium and low warmly exposed sides’) were found soils at different evolution stages: from the poor and moderately evolved soils (Entisols and Inceptisols), the most represented, to the highly evolved types (Alfisols) with higher stability (USDA-NRCS 1999).

Based on pedological investigations and soil texture analysis three types of soils proved to characterize the tested vineyards, i.e.: i) a sandy-loam or sandy soil - type ‘lapillo’ (local denomination), ii) a sandy skeletal and shallow soil profile – type ‘pianca’ (local name) and iii) clay loam soil – type ‘volpaio’ (local name).

The characteristics of each soil type and their classification are resumed in Table 3. Three vineyards, i.e. those located in LF, SM and SA sites were characterized by the presence of the ‘lapillo’ soil. The vineyard located in MM had the ‘pianca’ soil and the vineyard located in MP the ‘volpaio’ one.

**Berry biochemical traits**

The berry biochemical characters such as sugar content, acidity, as well as the phenolic fraction concentration (total anthocyanins and polyphenols) are shown in Table 4A. At harvest time the grapes from SM, MP, LF sub-areas exhibited a higher sugar concentrations than those from SA and MM. Significant differences for sugar concentration seems to be related to the soil typology
and to depth (deep versus shallow soil). Berry grown in SA, SM and LF sub-areas, characterized by ‘lapillo’ soil but different for skeleton and sandy content, shown significant difference for total acidity, and didn’t exhibit difference in antocyanins concentration.

The highest total anthocyanins and total poliphenols concentration was associated to berries of MP grape growing sub-areas. In particular total poliphenols concentrations exhibited significant differences according to the soil type. The statistical correlation (pearson’s coefficient) (Figure 3A and 3B) shown as sugars and poliphenols exhibit polynomial trends related to sand soil content. In particular soluble solid and phenols were higher in the clay–loam soil (‘volpaio’ type – MP grape growing sub-area), while the lowest values were obtained in shallow soil with high sand content (‘pianca’ type - MM grape growing sub-area).

*Berry volatile compounds*

The berry aromatic profile is showed in Table 4B, all the volatile compound shown significant difference according to soil type. Berry chemical and aromatic parameters are used to carry out the statistical PCA and HCA, has reported in Figure 4. The I PC (Principal Component) and II PC explain about 83% of the total variation, and the first component are related to linalool, terpineol, citronellol, anthocyanins and polyphenols, while the second to nerol, geraniol, sugar and acidity of berries at harvest time. All the five sub-areas are scattered on Cartesian plane drew by I and II PC, in particular the HCA showed as the berry of this areas could be gather together into two main group. Berries from sub-areas ‘Macchia del Prete’ (MP), the only vineyards characterized by a clay loam soil, are more different from the other ones (sandy-soil).

*Wine volatile compounds*
In order to distinguish a possible classification on the basis of volatile profile, the wines were assigned into three groups, corresponding to different sub-areas with homogeneous soil traits (Figure 5): SA, SM and LF – ‘lapillo’ soil; MM – ‘pianca’ soil and MP – ‘volpaio’ soil.

**Acetaldehyde** (Figure 5A) is an important secondary aroma, it reached concentration of 0.15% and showing significance different among Aleatico di Gradoli wines related to the five growing sub-areas. The composition of aldehyde in the studied Aleatico di Gradoli wine presented uneven values compared to other components of the headspace examined. Despite the fact that total aldehydes amounted to very low values, the total volatile compounds of samples were highest for the decanal and the nonanal in MM, SA and SM, Figure 5A.

The most important flavor and aroma compounds formed from amino acids are higher alcohols (Figure 5C and 5D) and their associated esters (Figure 5B). The esters compound in the Aleatico wine showed the predominance of ethyl caprylate and ethyl decanoate and minor amounts of ethyl acetate (Figure 5B).

Isoamyl alcohol gave the greater contribution to flavor for all Aleatico di Gradoli wines (Figure 5C), in particular, significant differences among the growing sub-areas resulted from the statistic. The ethyl esters for the five wines were the main chemical group followed by isoamyl alcohol. Also for the ethyl esters contribution to total volatile wine profiles it has shown a significance differences related sub-areas origin. The **monoterpenes** compounds (Figure 5E), such as linalool, citronellol, and limonene, were the compounds most important for the Aleatico wines discrimination and all data shown significance differences according to sub-areas wine origins. In particular, citronellol content was from two to four times higher than the respectively geraniol content for each sub-areas. The linalool and limonene contents showed similar evolution
related on soil type, the higher values for wines originate from *Lapillo* soil (sandy and deep soil)

than from *Pianca* and *Volpaio* soils.

**Total phenols and total anthocyanins**

Total phenols and anthocyanins are shown in Figure 5F. All data exhibited significant
differences among them. In particular, it was noted that wines produced with grapes from MM,
SA and SM presented a concentration of total anthocyanins and polyphenols concentrations
significantly higher than in the other samples. On the other hand MP amounted always to the
lower concentration of phenols and anthocyanins.

**Statistical analysis - PCA**

In Table 5 the set of the eigenvalues of PCA is reported along with the amount of inertia
explained by each corresponding axis and the cumulate inertia. We selected two principal axes of
interest, that explain about 76% of the total variation (measured by the inertia), yet 60% is the
minimum cumulate quality of representation of all the parameters and is sufficient for our
exploratory purposes (Camiz et al. 2008). In Table 6 the results of PCA are shown. For each
biochemical parameter and each selected component (axes) (I and II) are reported: (i) its
contribution to the axis and (ii) its correlation with the principal components. The data helps to
interpret the graphical representation, limited here to the principal plane delimited by axes 1 and
2, where all the items are represented.

The contribution of the chosen biochemical markers to the first principal axis (also shown
on the principal plane spanned by axes 1 and 2 in Figure 6) was mostly due most to the
monoterpene and ester fractions, while the contribution to the second principal axis was due to
mainly alcohols and aldehydes. The position of the wines derived from the grapes of the five
tested vineyards, is separated into 3 groups: i) LF, SM and SA ii) MM and iii) MP. In particular 
LF and SM are opposite to MM on the first axis, while MP is opposite to SA.
The samples of the vineyards LF, SM and SA are characterized by the monoterpenes, MP 
samples are characterized mainly by the alcohol and aldehyde components, while the MM 
samples by the phenolic components.

Discussion

Based on the Thermal Index of Winkler (IW) values (Table 2), the PDO area proved to be 
highly vocated for the production of quality wine and in particular for the cultivation of red berry 
variety, following the classification reported in Fregoni and Pezzuto (2000).
Grape volatile compounds are responsible of aroma of wine and depending on the grape 
variety, cultural practices, and climatic or biological factors. The concentration of aromatic 
substances changes according to an optimal sugar/acid ratio (Ribéreau-Gayon et al. 1998). The 
berry quality parameters underlighed the great potential of this cultivar as aromatic grape for 
liqueur and passito wines (De Santis et al. 1999, Bellincontro et al. 2006).
Nowadays it’s known that the wine volatile compound is derived from multiple sources 
and processes but one of the main direct contribution has been given to grape-derived aroma 
compounds, including monoterpenes, norisoprenoids, aliphatics, phenylpropanoids, 
methoxypyrazines, and volatile sulfur compounds (Robinson et al. 2014, Gonzalez-Barreiro et al. 
2015).

In particular, even it is difficult to assess the impact of a single parameter of terroir - such 
as the soil – on grape and wine quality, several researches have highlighted that grape and wine
composition are significantly affected by soil type, which influences the taste of the final product (van Leeuwen et al. 2004, Zerihun et al. 2014, Wang et al. 2015).

Findings underlined, according to van Leeuwen et al. 2004, that different soil types resulted in significant differences in total sugars and phenols concentration of berries. Correlation analysis among berry ripening parameters (sugar and polyphenols content) and sand soil content shown as this soil physical trait impact the overall quality of the grape and thus the resultant sensory quality of the wines, according to Gomez-Miguez et al. 2007.

Aleatico grapes and wines, produced from vines growing on several soil types but in the similar climatic condition, as the five sub-areas, could be discriminated from each other. Multivariate data analysis is confirmed to be a discriminating method of the origin of grape and wines. PCA and HCA carried out on berry at harvest time help to analyse the soil effect on berry quality.

In particular, clay loam soils with low skeleton content - MP sub-areas - are beneficial for improving grape quality, inasmuch it seems to optimize (best values for many berry quality parameters – Table 4) in this sub-areas as resulted also from cluster analysis (Figure 4). It might be influenced by the soil texture: deep and shallow sandy soil, characterized by a moderate-high amount of skeleton (LF, SA, SM and MM) and with lower clay seem to promote higher vigour (Fraga et al. 2014), that in these grape-growing area could be undermine the Aleatico berry quality.

All ripening parameters are pivotal factors for wine composition, but total soluble solids (sugars) and polyphenols being the most important and discriminating substances.
Since it is difficult to ‘unhook’ a soil effect on grape composition from possible contributions of many other factors (climate and cultivars), or established if it has an indirect effect of vine attribute (Zerihun et al. 2014), this does not preclude an effect of soil factors on technological and phenolic components of berries and wines. Moreover the microbial communities on grapes may be affected by a large number of factors such as the geographic location and its traits (soil and climate) (Barata et al. 2012), in addition to genetic traits of the berry itself. The study of wine flavors is a critical issue because it is the result of a complex mixture of volatile compounds. Among the 800 volatile molecules identified in wine, a relatively limited number of them called varietal (or primary) aromas, play a crucial role in wine flavor and typicality.

Findings underlined a consistent presence of esters and a low participation of aldehydes, debt levels of higher alcohols and monoterpenes. These flavor profiles are consistent with the average composition of red wines (Bonino et al. 2003). The values of the individual constituents, expressed in percentage terms, highlights the complex composition of the volatile fraction for the sub-areas samples observed. In particular for the monoterpenic alcohols (Figure 4 E), that represents the most common varietal aromas in the ‘Muscat’ varieties (Bonino et al. 2003).

Acetaldehyde (Figure 4A) amounted to good concentration, contributing to diversify the aroma complexity of wines and producing stable pigments in red wine, enhancing the wines quality (Bakker and Clarke 2011). Acetaldehyde is normally present in large quantities in wine as a metabolite of fermentation, because many of them are generated by fermentation, maturation and aging in wood. As the fermentation process ends, acetaldehyde produced is introduced into
the yeast cell and reduced to ethanol, so that the content falls to lower levels at the end of vinification.

The esters profile (Figure 4B) in the Aleatico wines show high level of esters able to develop fruity notes to wines, such as ethyl octanoate, erthyl decanoate and dodecanoate gave a positive contribution to wines quality. Most of them, in fact, have mature fruit flavor nuances that are responsible for the ‘fruity’ and ‘floral’ sensory properties of wine (Sagratini et al. 2012). While acetate esters - isoamylacetate and hexylacetate- are responsible for tropical fruit and banana-like aromas.

Esters are the product of the condensation between carboxyl groups of organic acids and hydroxyl groups of alcohols or phenols. These, in small quantities and some with a rather high threshold of perception, make a slight contribution to the aromatic composition of wine.

The production of esters during fermentation is influenced by many factors: the grape ripeness, the esterase activity of strains of yeast and fermentation temperatures are the elements of greatest influence. Low temperatures may encourage the formation of fruity "ester" as isoamyl, isobutyl and ehyl acetate, while high temperatures favor the formation of esters with higher molecular weight as ethyl octanoate, ethyl decanoate and phenylacetate. High temperatures can also depress the formation of esters favoring hydrolysis. Low levels of SO₂ and clarification of the wort can both promote the synthesis and accumulation of foreign as well as intracellular fermentation (carbonic maceration) and the lack of oxygen during fermentation.

Although esters in wine are mainly produced by yeast metabolism their production can be influenced also by the grape variety (Swiegers et al. 2005).
The higher alcohols (Figure 4C and D) are generally found to be responsible for aroma due to the fact that they are found in quantities above perception threshold, rather it is the minimal concentration in which the component can be detected. At low levels (< 300 mg·L⁻¹), straight chain higher alcohols generally add complexity to the bouquet of a wine (Bakker and Clarke 2011), such as for the Aleatico di Gradoli wines, where the greater contribution is given by Isoamyl alcohol (Figure 4C), that generally accounts for more than 50% of all alcohol fractions - and it is found nascent in the grape, but the majority is formed by yeast during alcoholic fermentation and help to determine identity and complexity of wine (Bakker and Clarke 2011).

The formation of higher alcohols during fermentation is markedly influenced by the wine-making practices: the presence of oxygen, high temperature fermentation and materials suspended in the fermenting juice promoting the proliferation of alcohols through the reduction of aldehydes grape, reductive de-nitrification synthesis of amino acids or sugars.

Aleatico wines with a similar higher alcohol content produced in the five sub-areas, have a distribution that differs only slightly from higher values in heptanol in the samples from the SM and MP areas and an increase in hexanol in the LF area (Figure 4C and 4D).

The mono-terpenes compounds were the most important compounds for the differentiation according to sub-areas classification. The aromatic grape varieties, such as Muscat, Riesling and Gewürztraminer, contain large amounts of the monoterpenes geraniol and nerol.

Geraniol has aromas described as rose-like and linalool aromas described as rose, whereas linalool oxides are described as camphorous and nerol oxides as vegetative. In general,
more bound glycosides are found than the free terpenoids, and the ratios of bound to free
terpenoids can also vary amongst different grape cultivars. Muscat of Alexandria grapes, for
example, have a ratio of 5:1, whereas some non-Muscat varieties have a ratio of 1:1. Varietal
wine aroma from muscat-related grapes, for example, is mainly due to the presence of various
isoprenoid monoterpenes in the grapes, with the most important being linalool, geraniol, nerol,
and citronellol.

The de novo biosynthesis of flavor and aroma compounds is probably the most important,
because, in general, fermentation-derived volatiles make up the largest percentage of total aroma
composition of wine in terms of numbers. All of these compounds have been proved to be odor-
active compounds in musts and wines (García-Muños et al. 2011).

Among the tested sub-areas, SM, SA and LF optimized the grape and wine whole. An
explanation might be the fact that these sub-areas, which are managed with the same agronomic
techniques, have the same soil type – ‘lapillo’. As confirmed by other authors (Morlat and Bodin
2006), the total content of anthocyanins is strongly related to environmental factors, as also
influenced by vineyard management. This might be due to the synthesis of the enzymes involved
in the various stages of anthocyanin’s synthesis closely linked to environmental and
physiological factors. This concept implies that there is a strong relationship between the
composition of the grape, the characteristics of the wine and the territory of production.

The PCA distribution of the biochemical characters of the wine coming from different
physical environments for soil typology (Figure 5) shows a ‘soil effect’ from sandy-loam soil
type (‘lapillo’) (LF, SM and SA), to sandy skeletal and shallows soil (MM) (‘pianca’), to a clay-
loam soil (MP) type ('volpaio'). The soil texture seems to be therefore strictly correlated to the biochemical characters of the oenological products.

The phenolic components related to the grater skeletal and sandy content as in the vineyards of MM and SA, SM, probably owing to the positive effect of a light water deficit (stress) on berry phenolic composition, as found in previous work (Biasi et al. 2010). Soluble phenolic compounds are crucial to determine some aspects of berry quality and influence the volatile metabolites that are responsible for wine varietal aroma (Koundouras et al. 2006).

In particular when the Aleatico grapevine grows and undergoes water stress, like in sandy soil, the highest values for phenolics and mono-terpenes components are reached and they concur to improve the quality and variety of traits of the grape and wine production.

On a final note, the aromatic complexity of Aleatico’s wine is a result of the presence in the headspace of different volatile chemicals.

Not all substances are perceived in the same way. The perception, in fact, depends on the complexity of our olfactory system and is the result of the presence of a volatile substance in space, the "smell" of a food, and our ability to appreciate.

Several studies proved that the terpenoid compounds express the link between the variety and the aroma of wine (Bakker and Clarck 2011). This class is complex and largely glycosidic bound in an odorless form. The aroma of a wine varietal is the result of the combination of various quantitative and qualitative free monoterpenes present in the must. However, the terpene fraction undergoes slight transformations by enzymatic hydrolysis and acid, as well as isomerization and cyclization. These changes are slow and influenced by several factors.
including light, temperature and microbial flora. Nonetheless this does not change the impression that this class of components gives to the various types of vines.

The wine’s aroma is the result of labile and complex processes that build on several elements such as grape variety, the winemaking process, and the grape maturation and aging stages.

The monoterpenes, the minor constituents of the plants, follow the common biosynthetic way and generally free and bound fraction increases during the ripening of berries. During the winemaking process the monoterpenes may undergo various transformations: acid or enzymatic hydrolysis, isomerization and cyclization.

Aging and storage provide time for their slow transformation. It is, however, well known that the hydrolysis of glycosides in aromatic monoterpenes is faster than the isomerization and rearrangement. After a long time, related terpenes have slowly converted into aromatic terpenes, which rearrange much more slowly into new compounds. Some of them may also be less odorous molecules contrarily to those from which they originate. Other factors that could influence this important fraction are the fermentation process, the storage temperature, the pH value and composition of the wine. All these elements of variability are still considered by many authors, influential on the sparsely-quantitative composition terpenes. The description of this class of aromatic components is extremely interesting because it reveals the real differences between aromatic grape wines, indisputably linked to the variety. Wanting to decipher this component in the Aleatico aromatic wine, one recognizes a strong fruity note, tempered by a combination of smells: roses and a hint of citrus and balsamic. This verbal description-olfactory attempts to translate the terpene composition and could be used to describe the aroma variety of
other important wines such as Muscat or Gewurztraminer for which the qualifying note is rose,
geraniol and nerol or Malvasia, where linalool predominates with its pronounced floral notes,
tempered by a citrus smell. Without wishing to invest this kind of description as highly scientific,
we can still conclude that the varietal mark set by terpenes is a strong element of distinction and
categorization. This parameter, also for its stability in wine, is potentially capable of being
effectively used to discriminate varietal wines made in analytical purity.

Conclusions

This study allowed us to determine the effect of soil on Aleatico’s berry ripening
parameters and wines. Among the berry biochemical traits at harvest time sugar and phenols
concentration were discriminant variables able to have a direct influence on wine quality. Grape
quality potential (high grape sugar and phenols) is high on the soil that induce water deficit,
especially on clay-loam (MP) and sandy-loam (SM) soil where water deficit occur early in the
season but are moderate.

Aleatico wines, produced in the PDO sub-district maintain unique and comparable
biochemical traits, therefore preserving the identity of a territorial production. The exception of
the wine produced from grapes from the sub-area, La Fratta (LF), which deviates from many
values observed, in respect to the total sample values recorded, may be attributed to a slight
"immaturity" compared to the others. Nonetheless, the terpene compounds, i.e. linalool,
citronellol, and limonene, were the most important compounds for the Aleatico wines
discrimination and all data shown significant differences according to sub-area grape origins.

Taken together our results proved that berry biochemical traits, volatile and phenolic
compounds may be considered as biochemical markers for high quality wine from highly
vocated environments. Based on the strong relationship among berry ripening traits, volatile and phenolic compounds and structural soil characters, they provide not only an indication for oenological potential but also they could represent pivotal indexes suitable to understand the ‘soil effect’ on grape and wine quality, and to identify the best terroir conditions (following microzonation) also within small although highly heterogenic grape-wine producing areas, like the ones we investigated. The knowledge of the environment physical characters, mainly the soil in our study, which better affect the quality of productions could address farmer’s efforts to exploit the variability of the viticultural environment and to assure a sustainable use of the grapevine genetic resources.

**Literature Cited**


Fraga, H., Malheiro, A. C., Moutinho-Pereira, J., Cardoso, R. M., Soares, P. M., Cancela, J. J. and Santos, J. A. 2014. Integrated analysis of climate, soil, topography and vegetative growth in Iberian viticultural regions. PloS one 9:


Winkler, A. J. 1962 General Viticulture. University of California, USA.

**Table 1** Volatile compounds identified in the analysis of red wine made from Aleatico grapes.

<table>
<thead>
<tr>
<th></th>
<th>LF</th>
<th>MM</th>
<th>MP</th>
<th>SA</th>
<th>SM</th>
<th>RI</th>
<th>RI*</th>
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<tbody>
<tr>
<td>1</td>
<td>Ethyl acetate</td>
<td>3.15E+07</td>
<td>2.76E+07</td>
<td>2.41E+07</td>
<td>2.51E+07</td>
<td>3.39E+07</td>
<td>900</td>
</tr>
<tr>
<td>2</td>
<td>Isoamyl acetate</td>
<td>4.20E+06</td>
<td>8.32E+06</td>
<td>1.08E+07</td>
<td>2.85E+06</td>
<td>6.07E+06</td>
<td>1136</td>
</tr>
<tr>
<td>3</td>
<td>Ethyl caproate</td>
<td>1.81E+07</td>
<td>1.50E+07</td>
<td>1.61E+07</td>
<td>1.55E+07</td>
<td>1.61E+07</td>
<td>1243</td>
</tr>
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<td>4</td>
<td>Hexyl acetate</td>
<td>2.55E+06</td>
<td>1.35E+06</td>
<td>1.99E+06</td>
<td>2.63E+06</td>
<td>1.93E+06</td>
<td>1327</td>
</tr>
<tr>
<td>5</td>
<td>Ethyl lactate</td>
<td>4.98E+06</td>
<td>4.06E+06</td>
<td>4.13E+06</td>
<td>5.20E+06</td>
<td>5.91E+06</td>
<td>1343</td>
</tr>
<tr>
<td>6</td>
<td>Ethyl caprylate</td>
<td>1.43E+08</td>
<td>1.13E+08</td>
<td>1.25E+08</td>
<td>1.09E+08</td>
<td>1.26E+08</td>
<td>1454</td>
</tr>
<tr>
<td>7</td>
<td>Ethyl decanoate</td>
<td>7.06E+07</td>
<td>8.35E+07</td>
<td>8.32E+07</td>
<td>5.04E+07</td>
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<td>8</td>
<td>Ethyl laurate</td>
<td>4.79E+06</td>
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<td>2.05E+06</td>
<td>4.08E+06</td>
<td>1858</td>
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<tr>
<td>9</td>
<td>Acetaldehyde</td>
<td>2.07E+06</td>
<td>2.01E+06</td>
<td>2.25E+06</td>
<td>1.54E+06</td>
<td>1.72E+06</td>
<td>714</td>
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<tr>
<td>10</td>
<td>Heptanal</td>
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<td>3.41E+05</td>
<td>2.56E+05</td>
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<tr>
<td>11</td>
<td>Nonanal</td>
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<td>4.00E+06</td>
<td>8.23E+05</td>
<td>1.97E+06</td>
<td>1.30E+06</td>
<td>1416</td>
</tr>
<tr>
<td>12</td>
<td>Decanal</td>
<td>9.27E+05</td>
<td>4.68E+06</td>
<td>2.70E+06</td>
<td>4.29E+06</td>
<td>2.98E+06</td>
<td>1524</td>
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<tr>
<td>13</td>
<td>α-terpinene</td>
<td>1.39E+06</td>
<td>8.31E+05</td>
<td>9.07E+05</td>
<td>1.66E+06</td>
<td>1.11E+06</td>
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<tr>
<td>14</td>
<td>Linalool</td>
<td>3.18E+06</td>
<td>1.94E+06</td>
<td>2.06E+06</td>
<td>3.05E+06</td>
<td>2.59E+06</td>
<td>1553</td>
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<td>15</td>
<td>Terpineol</td>
<td>1.84E+06</td>
<td>1.09E+06</td>
<td>1.11E+06</td>
<td>1.70E+06</td>
<td>1.19E+06</td>
<td>1646</td>
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<tr>
<td>16</td>
<td>Citronellol</td>
<td>6.21E+06</td>
<td>6.54E+06</td>
<td>3.94E+06</td>
<td>6.39E+06</td>
<td>6.26E+06</td>
<td>1750</td>
</tr>
<tr>
<td>17</td>
<td>Geraniol</td>
<td>3.87E+05</td>
<td>2.73E+05</td>
<td>2.11E+05</td>
<td>9.48E+05</td>
<td>6.26E+05</td>
<td>1847</td>
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<tr>
<td>18</td>
<td>Limonene</td>
<td>2.98E+06</td>
<td>1.85E+06</td>
<td>2.01E+06</td>
<td>3.42E+06</td>
<td>2.44E+06</td>
<td>1201</td>
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<tr>
<td>19</td>
<td>Ethanol</td>
<td>1.92E+09</td>
<td>2.20E+09</td>
<td>1.85E+09</td>
<td>1.80E+09</td>
<td>1.77E+09</td>
<td>954</td>
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<tr>
<td>20</td>
<td>Isoamyl alcohol</td>
<td>4.71E+07</td>
<td>5.73E+07</td>
<td>7.09E+07</td>
<td>5.94E+07</td>
<td>7.17E+07</td>
<td>1216</td>
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<tr>
<td>21</td>
<td>1-Hexanal</td>
<td>3.67E+06</td>
<td>1.42E+06</td>
<td>1.66E+06</td>
<td>1.93E+06</td>
<td>2.18E+06</td>
<td>1354</td>
</tr>
<tr>
<td>22</td>
<td>Heptanol</td>
<td>2.12E+05</td>
<td>2.99E+05</td>
<td>5.07E+06</td>
<td>2.18E+05</td>
<td>5.22E+06</td>
<td>1313</td>
</tr>
<tr>
<td>23</td>
<td>Octanol</td>
<td>1.03E+06</td>
<td>2.13E+05</td>
<td>4.66E+05</td>
<td>6.01E+05</td>
<td>5.92E+05</td>
<td>1570</td>
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<tr>
<td>24</td>
<td>Nonanol</td>
<td>3.65E+05</td>
<td>1.88E+05</td>
<td>7.51E+05</td>
<td>3.71E+05</td>
<td>2.92E+05</td>
<td>1527</td>
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<tr>
<td>25</td>
<td>Phenylethanol</td>
<td>1.64E+07</td>
<td>2.69E+07</td>
<td>3.75E+07</td>
<td>1.05E+07</td>
<td>2.03E+07</td>
<td>1956</td>
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<tr>
<td>26</td>
<td>Total</td>
<td>2.29E+09</td>
<td>2.57E+09</td>
<td>2.25E+09</td>
<td>2.11E+09</td>
<td>2.15E+09</td>
<td>900</td>
</tr>
</tbody>
</table>

RI (mass spectral matching against library standards) and RI* (values for a Carbowax 20M column after running a carbon alkane standard. also known as Kovats index KI).
Table 2  Winkler index values for the cv Aleatico native growing area.

<table>
<thead>
<tr>
<th>Year</th>
<th>Mean</th>
<th>SD</th>
</tr>
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<tbody>
<tr>
<td>2007</td>
<td>1718.9</td>
<td></td>
</tr>
<tr>
<td>2008</td>
<td>1696.1</td>
<td></td>
</tr>
<tr>
<td>2009</td>
<td>1991.3</td>
<td></td>
</tr>
<tr>
<td>2010</td>
<td>1746.9</td>
<td></td>
</tr>
<tr>
<td>2011</td>
<td>1979.7</td>
<td></td>
</tr>
<tr>
<td>2012</td>
<td>1832.0</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>1827.5</td>
<td>130.8</td>
</tr>
</tbody>
</table>

Table 3  Main soil physical characteristics for the three typology identified in the study area and in each sample vineyard of the five sub-areas. SA, Sant’Antonio; LF, La Fratta; MP, Macchia del Prete; SM, San Magno; MM, Montemaggiore.

<table>
<thead>
<tr>
<th>Soil local denomination</th>
<th>Soil depth</th>
<th>Skeleton content</th>
<th>Texture</th>
<th>Soil classification (†)</th>
<th>Vineyards’ locality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lapillo</td>
<td>Deep</td>
<td>High</td>
<td>Sandy</td>
<td>Vitrandic Xerorthent sandy skeletal, mesic</td>
<td>SA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Moderate</td>
<td>Sandy</td>
<td>Typic Xeropsamment, mixed, mesic</td>
<td>SM, LF</td>
</tr>
<tr>
<td>Volpaio</td>
<td>Deep</td>
<td>Low</td>
<td>Clay loam</td>
<td>Typic Hapludalf fine loamy, mesic</td>
<td>MP</td>
</tr>
<tr>
<td>Pianca</td>
<td>Shallow</td>
<td>High</td>
<td>Sandy</td>
<td>Vitrandic Xerorthent sandy skeletal, mesic</td>
<td>MM</td>
</tr>
</tbody>
</table>

Table 4 (A-B)  Main soil physical characteristics for the three typology identified in the five sub-areas related to berry biochemical traits [A] and volatile compounds [B] at harvest time (Mean, Standard deviation and significance among sub-areas (p<0.05)). SA, Sant’Antonio; LF, La Fratta; MP, Macchia del Prete; SM, San Magno; MM, Montemaggiore.

[A]

<table>
<thead>
<tr>
<th>Vineyards’ locality</th>
<th>Texture</th>
<th>Sugar (°Brix)</th>
<th>Acidity (g·L⁻¹ tartaric acid)</th>
<th>Antocyanins (mg·L⁻¹)</th>
<th>Poliphenols (mg·L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SA</td>
<td>Sandy</td>
<td>20.9 ± 2.7 b</td>
<td>8.8 ± 2.1 a</td>
<td>605.4 ± 212.6 a</td>
<td>1564.0 ± 198.0 b</td>
</tr>
<tr>
<td>SM</td>
<td>Sandy-loam</td>
<td>25.4 ± 3.8 a</td>
<td>4.2 ± 1.1 b</td>
<td>739.4 ± 174.2 a</td>
<td>1514.3 ± 145.8 b</td>
</tr>
<tr>
<td>LF</td>
<td>Sandy-loam</td>
<td>22.0 ± 2.8 ab</td>
<td>4.8 ± 1.1 b</td>
<td>692.7 ± 207.9 a</td>
<td>1566.0 ± 171.1 b</td>
</tr>
<tr>
<td>MP</td>
<td>Clay-loam</td>
<td>25.4 ± 1.8 a</td>
<td>4.1 ± 0.9 b</td>
<td>820.0 ± 278.9 a</td>
<td>1824.1 ± 221.8 a</td>
</tr>
<tr>
<td>MM</td>
<td>Sandy</td>
<td>21.5 ± 3.4 b</td>
<td>7.1 ± 1.7 ab</td>
<td>742.4 ± 354.9 a</td>
<td>1374.5 ± 106.8 c</td>
</tr>
</tbody>
</table>

[B]

<table>
<thead>
<tr>
<th></th>
<th>Linalool</th>
<th>Terpineol</th>
<th>Citronellol</th>
<th>Nerol</th>
<th>Geraniol</th>
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</thead>
<tbody>
<tr>
<td>SA</td>
<td>17.9% e</td>
<td>3.2% b</td>
<td>67.7% a</td>
<td>10.2% bc</td>
<td>1.0% b</td>
</tr>
<tr>
<td>SM</td>
<td>30.9% b</td>
<td>3.9% ab</td>
<td>52.0% c</td>
<td>11.1% b</td>
<td>2.2% b</td>
</tr>
<tr>
<td>LF</td>
<td>44.9% a</td>
<td>5.7% a</td>
<td>5.5% d</td>
<td>39.5% a</td>
<td>4.4% a</td>
</tr>
<tr>
<td>MP</td>
<td>24.8% d</td>
<td>5.3% a</td>
<td>57.3% b</td>
<td>8.6% c</td>
<td>4.0% a</td>
</tr>
<tr>
<td>MM</td>
<td>28.5% c</td>
<td>5.2% a</td>
<td>53.7% c</td>
<td>11.5% b</td>
<td>1.1% b</td>
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</table>

Table 5 – PCA: eigenvalues. explained and cumulate inertia.
Table 6 – PCA: contribution and correlation of the indicators in respect to the two principal axes selected.

<table>
<thead>
<tr>
<th></th>
<th>I Axis</th>
<th>II Axis</th>
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</thead>
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<td>Correlation</td>
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<td>2</td>
<td>Isoamyl acetate</td>
<td>704</td>
</tr>
<tr>
<td>3</td>
<td>Ethyl caproate</td>
<td>351</td>
</tr>
<tr>
<td>4</td>
<td>Hexyl acetate</td>
<td>545</td>
</tr>
<tr>
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Figure 1  Map of the study area (A). localization of the sub-areas within the PDO ‘Aleatico di Gradoli’ (B) and spatial distribution of tested vineyards in respect to steepest downslope direction (C). SA. Sant’Antonio; LF. La Fratta; MP. Macchia del Prete; SM. San Magno; MM. Montemaggiore.
Figure 2  Ombrothermic diagram based on meteorological data from a weather station close to Gradoli. The rainfall (dotted grey line) and temperature (continue black line) represent the mean value of the last 11 years (2004-2015).

Figure 3  The correlation analysis (Pearson’s coefficient) between biochemical berry traits (sugar [A] and poliphenols [B]) and soil’s sand content in the tested vineyards. SA. Sant’Antonio; LF. La Fratta; MP. Macchia del Prete; SM. San Magno; MM. Montemaggiore.
Figure 4 PCA: representation of all berry quality parameters (chemical and volatile compounds) at harvest time. In the box the cluster dendrogram.
Figure 5 Volatile and phenolic compounds determined in Aleatico di Gradoli (PDO) wines produced in the five sub-areas (SA. Sant’Antonio; LF. La Fratta; MP. Macchia del Prete; SM. San Magno; MM. Montemaggiore) and three soils type: Lapillo (fill color: white); Volpaio (fill color: black); Pianca (fill color: dark gray). Different letters indicate significantly differences at $p < 0.05$. [A] Aldehydes. [B] Esters. [C] and [D] Higher alcohols. [E] Monoterpenes. [F] Anthocyanins and Polyphenols.
Figure 6 - PCA: representation of all indicators on the plane spanned by the principal axes 1 and 2. HCA (dotted line): representation of cluster analysis.