



Analytical Methods

Analytical characterisation of *Negroamaro* red wines by “Aroma Wheels”

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ABSTRACT

In this work we characterised *Negroamaro* red wines, made by an autochthonous cultivar of Southern Italy, by linking volatile composition to aroma properties. This linking was carried out by picturing “Aroma Wheels”, built by Odour Activity Values (OAVs) of all the identified volatile compounds grouped in “aromatic series” belonging to 13 classes of sensory descriptors. The 18 most active odorants with OAV > 1 were mainly alcohols, fatty acids and their ethyl esters. The “OAVs’ Aroma Wheels” showed that the classes of sensory descriptors are first fruity and floral, next fatty and pungent and minor nutty and caramelised notes. Principal Component Analysis displayed correlations between sensory descriptors and wine samples; the main 7-fruity and 5-floral sensory features of *Negroamaro* wines have negative values of PC1 and they are negatively correlated with the second main sensory feature, i.e. 13-fatty, falling at positive value of PC1; this fit the aroma perception of this varietal.

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1. Introduction

Wine aroma and flavours are some of the main organoleptic characteristics that contribute to typicality concept (Cadot, Caillé, Samson, Barbeau, & Cheynier, 2010; Maitre, Symoneaux, Jourjon, & Mehinagic, 2010); they define the differences among an amazing number of wines and wine styles produced throughout the world. They are formed by hundreds of different volatile compounds, belonging to different chemical categories (alcohols, esters, aldehydes, ketones, fatty acids, terpenes, etc), covering a wide range of concentration (from 1 ng/L to several g/L), polarity, solubility, volatility, and pH (Ortega-Heras, Gonzalez-San Jose, & Beltran, 2002). Wine volatile compounds can be distinguished in: (1) primary (or varietal) compounds, that are aroma compounds found in undamaged cells of the grapes released only by crushing, or produced by enzymatic interactions at crush; (2) pre-fermentative and fermentative secondary compounds, the firsts formed during the processing of the grapes and by chemical, enzymatic and thermal reactions in grape-must, and the latter during the alcoholic fermentation, and (3) tertiary compounds that constitute the maturation “bouquet” caused by chemical reactions during maturation of the wine in the bottle (Pisarnitskii, 2001; Styger, Prior, & Bauer, 2011).

Wine aroma perception is really a intricate matter due to complex matrix effects, and oral human physiological variables; recent research advances in understanding aroma perception are beyond the aim of this work (Muñoz-González, Rodríguez-Bencomo, Moreno-Arribas, & Pozo-Bayón, 2011; Polášková, Herszage,

& Ebeler, 2008). As simplification, the contribution of a specific volatile compound to wine aroma perception can be related to its odour threshold (OTH), which is defined as the lowest concentration that can be detected by smelling. OTHs of wine volatiles are estimated by odour threshold sensory tests (Janusz et al., 2003) in synthetic wine and reported in literature (Rocha, Rodrigues, Coutinho, Delgadillo, & Coimbra, 2004; Gómez García-Carpintero, Sánchez-Palomo, Gómez Gallego, & González-Vinas, 2011).

Another parameter, commonly used to assess the contribution of each compound to wine aroma, is the aroma index (I), also known as “Odour Activity Value” (OAV), defined as concentration/OTH ratio. The concept of “odour activity value” is a useful measure to assess the relative importance of individual chemical components present in a sample. Among all the volatiles present in wine volatile fraction, only a limited number of volatiles can be found in wines at concentrations high enough to be perceived (compounds with OAV ≥ 1) (Gil, Cabellos, Arroyo, & Prodanov, 2006; Gómez-Miguel, Cacho, Ferreira, Vicario, & Heredia, 2007). Obviously, these compounds are the would-be active odorants that will primarily make-up the aroma features of a wine.

The issue of wine aroma characterization is generally dealt with chemical-analytical or sensory descriptive methodologies (Muñoz-González et al., 2011; Saurina, 2010; Sumby, Grbin, & Jiranek, 2010; Swiegers & Pretorius, 2005). The first is normally based on gas chromatographic methods (as GC/MS), that extract, separate, identify and quantify the whole set of volatile compounds released from wine and that may determine the perception of its aroma; hence, it provides a complete chemical profile of wine volatile fraction, that can be represented by a radar plot as a “Volatiles’ Aroma Wheel” (Fig. 1A). The second is based on wine expert panel, who provides a sensory profile of wine, that can be represented by a

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radar plot as a “Sensory Aroma Wheel” (Fig. 1B); the panel use specific descriptive sensory tests and sensory descriptors of perceived wine attributes according to the definition of a common method and a consensual flavours lexicons (Drake & Civille, 2002; Esti, González Airola, Moneta, Paperaio, & Sinesio, 2010; Murray, Delahunty, & Baxter, 2001). Quantitative sensory aroma wheels can be also generated if ratings (panel scores) for each sensory descriptor are evaluated by panel (Barata et al., 2011; Jackson, 2009). “Volatiles’ Aroma Wheel” and “Sensory Aroma Wheel” are schematically reported in Fig. 1A and B, respectively for a generic wine.

Another analytical methodology for detecting and identifying odour-active compounds in wine is GC-olfactometry, i.e. the coupling of gas chromatography (GC) with the human nose of an individual as a detector. This instrumental-sensory methodology provides a GC-aromagram and may establish which aroma components can be perceived (Bowen & Reynolds, 2012; Craig & Heresztyn, 1984; Francis & Newton, 2005; Lee & Simpson, 1990; Lee & Simpson, 1993).

The role of each volatile compound as odorant in the multiplicity of wine aroma components can be described in terms of one or several sensory descriptors (Franco, Peinado, Medina, & Moreno, 2004). In addition, as reported in several works (Gómez-Míguez et al., 2007; Muñoz, Penaido, Medina, & Moreno, 2007; Penaido, Mauricio, & Moreno, 2006), odorant (or aromatic) series can be defined by grouping all the volatile compounds with similar sensory descriptor, and a generalised Odour Activity Value (generalised OAV) for each aromatic series can be calculated by adding the OAV of each aromatic series component. In such a way an odour profile of wine can be established by plotting in a radar graph the generalised OAV of all the aromatic series; the resulting “OAVs’ Aroma Wheel” allows to link quantitative information get by chemical analysis to sensory perceptions, and provides a valid tool to compare the wine aroma profiles (Fig. 1C).

The aim of the present study is principally to quantify free volatile compounds in monovarietal wines, made from *Negroamaro* grapes grown in Apulia region, relating them to aromatic series and offering a simple method representative of potential aroma profile of this variety by graphing “OAVs’ aroma wheels”. Moreover, this work enters in an oenological wine promotion context, where it is highly felt the need to promote regional productions, giving a contribute to picture the typical aroma profile of *Negroamaro* wines through the chemical analysis of their volatile composition in relationship to odour descriptors. However, the proposed method hasn’t the ambition to become a reference and standardized methodology to describe wine typicality concept, but it should be considered as a tentative “to portrait” the aroma profile of a wine in a first, simple and easy approach without the use of a more advanced and acknowledged sensory analysis, that remains the method commonly used within the framework of the typicality cryptic concept (Cadot et al., 2010; Perrin et al., 2008). According to *Sauvageot*, when the concept of typicality is applied to enology, it represents wine characteristics on the whole, resulting from type of vine, soil, and wine making process (*Sauvageot*, 1994). Thus, the typicality includes sensory, technical, and environmental dimensions and can be defined as a set of properties of belonging and distinction (*Casabianca et al.*, 2005).

2. Materials and methods

2.1. Wine samples

A total of 10 different commercial monovarietal red wines, produced exclusively from *Negroamaro* cultivar, were considered. Moreover, in order to compare the aroma composition of the different wine samples, without considering the aroma evolution due to wine ageing, all the wine samples, considered in our experimental campaign, came from the same vintage, i.e. 2007. All the

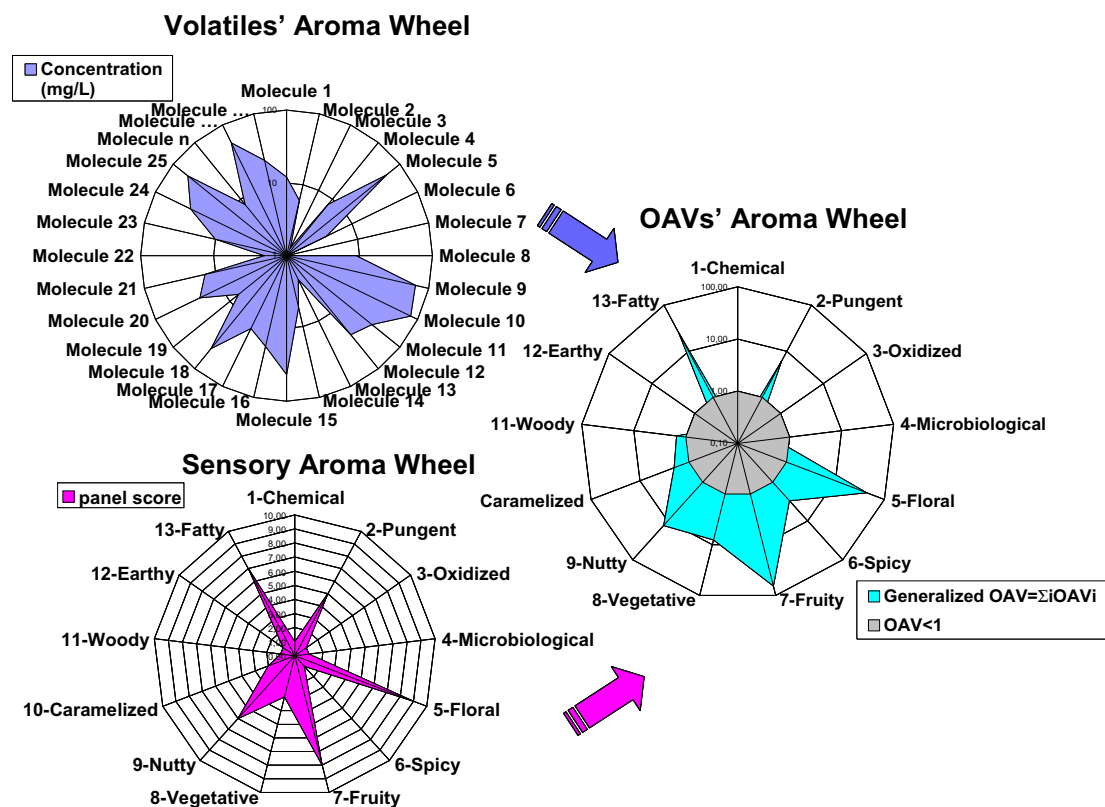


Fig. 1. Building of OAVs' Aroma Wheel starting from Volatiles' and Sensory Aroma Wheels for a generic wine.

selected wine samples had also a Denomination of Controlled Origin (D.O.C.) certification mark, that guarantees the quality and the geographical origin of the *Negroamaro* wines produced in *Salento* (southern area in Apulia region, Italy). In order to protect proprietary interests, winery identities have not been reported. The 10 *Negroamaro* wine samples were numbered with the following identification codes: NA1, NA2, ..., NA10. All samples were stored at 4 °C until analysis.

2.2. Reagents and standards

All reagents were of analytical grade and all solvents were of HPLC grade. Methanol and ethanol 96% were purchased from J.T. Baker (Phillipsburg, NJ, USA); dichloromethane were purchased from Carlo Erba Reactive (Rodano, Italy). Pure water was obtained from a Milli-Q purification system (Millipore, USA). Identification and quantification of the volatile compounds by GC/MS was obtained by using an Internal Standard (I.S.), i.e. a chemical added to the sample at a constant concentration with a distinct retention time to the analyte, and by commercial pure chemical standards. Both the I.S. (octan-2-ol) and commercial pure chemical standards with purity superior of 98% were supplied by Sigma–Aldrich. Hence, a 200 mg/L hydro-alcoholic solution (1:1, v/v) of the I.S. (200 ppm_v I.S. in liquid), was prepared. Moreover, different standard solutions were prepared by dissolving exact volume of the chemical standard compounds in ethanol at concentration three order of magnitude higher than the typically found in wines. Next, for each chemical standard, the standard solution was diluted with water and ethanol adjusting the final alcohol content to 12% (v/v) in order to prepare a set of six standard mixtures containing known concentrations of the chemical standard, covering the typical concentration range found in wine. All the synthetic standard solutions used in the calibration graphs were 6 g/L of tartaric acid and pH 3.2 also adjusted. All these solutions were stored at 4 °C (Tufariello, Capone, & Siciliano, 2012).

2.3. Extraction of volatile compounds

The aroma compounds were separated by adsorption/desorption on preconditioned divinylbenzene cartridges (Strata™-X polymeric SPE sorbents prepacked in 500 mg/6 mL cartridges, Phenomenex).

To 50 mL of each wine sample and to each standard solution were added 400 µL of I.S. solution. The extraction of volatile compounds was carried out by Solid Phase Extraction (SPE), using polymeric sorbents and dichloromethane as elution sorbent; a method developed by Piñeiro et al. (2006), suitably modified in this work, was followed. For the extraction of the wine volatile fraction a Vac Elut 20 station equipment from Varian (Palo Alto, USA) was used. Strata™-X polymeric SPE were first rinsed with 4 mL of dichloromethane, 4 mL of methanol and, finally, 4 mL of a water–ethanol mixture (12%, v/v). Each liquid sample was passed through the SPE cartridge at around 2 mL/min. Afterwards, the sorbent was dried by letting air pass through it. The volatile compounds were recovered by elution with 4 mL of dichloromethane, and concentrated to a final volume of 500 µL under a stream of pure nitrogen (N₂). An aliquot of 2 µL of each extracted wine sample was injected into the GC/MS in splitless mode and analysed for identification and quantification of the volatile compounds contained in the extracted volume. The analyses were performed in triplicate and mean values were used in further data processing.

2.4. GC–MS conditions and quantitative analysis

A 6890N series gas chromatograph (Agilent Technologies) with an Agilent 5973 mass spectrometer selective detector (MSD) and

equipped with a DB-WAX, polyethylene glycol capillary column (60 m × 0.25 mm I.D × 0.25 µm film thickness, Agilent Technologies) was used. The carrier gas was helium at a flow rate of 1.0 mL/min. The injection of the wine extract was made in splitless mode, the injector temperature was 250 °C. The column oven temperature was initially held at 40 °C, then it was programmed to 200 °C at 4 °C/min, with a final holding time of 20 min. Spectra were recorded in the electron impact mode (ionisation energy, 70 eV) in a range of 30–500 amu at 3.2 scans/s. A solvent delay time of 10 min was used to avoid overloading the mass spectrometer with solvent.

The identification of the volatile compounds was achieved by comparing mass spectra with those of the data system library (NIST 98, *P* > 90%), with the retention data of commercially available standards and MS data reported in the literature.

Quantification analysis is based on the principle that the area under a peak is proportional to the amount of the analyte present in the sample. The quantification was carried out following the internal standard quantification method. The different sets of standard mixtures, previously prepared and containing known concentrations of the chemical standard (C_{analyte}) and the I.S. concentration (C_{I.S.}), were analysed and their peak areas (A_{analyte}) recorded. For each chemical standard, a six points calibration graph of relative peak area (A_{analyte}/A_{I.S.}) versus analyte concentration (C_{analyte}) was drawn to confirm a linear detector response and from which the amount of the analyte can be determined. The unknown concentration of an analyte (C_{analyte, x}) is calculated according to the interpolation of the calibration plots:

$$C_{\text{analyte},x} = \left(\frac{A_{\text{analyte},x}}{A_{\text{I.S.}}} \right) \cdot \frac{C_{\text{I.S.}}}{\text{DRF}_{\text{analyte}}} \quad (1)$$

where DRF_{analyte} is the Detector Response Factor for an individual analyte. The concentration of volatile compounds for which there was no pure reference chemical standard available, was obtained by using the same calibration plot as one of the compounds with the most similar chemical structure belonging to the same molecular family. The obtained coefficients of regression (*R*²) were >0.999 and the relative standard deviations (RSD) were <5%.

2.5. Statistical analysis

To reveal the relationship among the *Negroamaro* wine samples based on compositions of volatile fraction, and to identify the constituents that are the chemotypical factors, the composition data matrix of ten samples was analysed using Principal Component Analysis (PCA) with STATISTICA 7.0 software package. Two-dimensional PCA biplots, including both wine samples and chemical compounds, were produced as results.

3. Results and discussion

3.1. Active odorants (impact aromatic molecules)

The results reporting the concentrations of wine volatiles have been published in a previous work (Tufariello et al., 2012). In this work, we extended the characterization of *Negroamaro* wines by combining the chemical-analytical analysis to the study of the would-be active odorants. In order to identify the most important wine odorants of *Negroamaro* wines, the aroma index (I), i.e. the Odour Activity Value (OAV), defined as the ratio between the concentration of the volatile compound (C_{odour}) and its odour perception threshold, was calculated for all the identified chemical species (Table 1). On the basis of their odour description and OAV, the most active odorants in the analysed *Negroamaro* wines were determined. As can be seen in Table 1, only 18 (pointed by

arrows in the table) of the 51 identified have an OAV ≥ 1 . Volatiles with OAV ≥ 1 are commonly considered the most important compounds contribute to wine aroma (Ferreira, L pez, & Cacho, 2000; Gil et al., 2006; G mez-Miguez et al., 2007; Romano, Suzzi, Domizio, & Fatichenti, 1997; Styger et al., 2011), although interactions between the different aroma components may occur. Compounds with high OAVs may be suppressed due to masking by other components (antagonistic effects), whereas compounds with lower OAVs can be revealed due to additive or synergic effects of the volatile components in the wine matrix. Therefore, the identification of the most active odorants only on the basis of their OAVs should be considered as a tentative study (Francis & Newton, 2005; G mez Garc a-Carpintero et al., 2011; G mez-Miguez et al., 2007).

In *Negroamaro* wine samples, the would-be impact odorants (i.e. those with OAV ≥ 1), are some compounds generated during alcoholic fermentation, already known to be the most important contributors to the aroma of young red wines with limited varietal aroma potential (G mez-Miguez, Vicario & Heredia, 2007; Ferreira, Fernandez, Pena, Escudero, & Cacho, 1995; G mez-Miguez, Cacho, Ferreira, Vicario & Heredia, 2007; Falqu  & Fern ndez, 1995). As exception we registered the contribution of 1-hexanol, which proceeds from the grapes (Baumes, 2000); its concentration in the wines depends on the conditions of fermentation, the composition of the must and the yeast biota.

Among compounds with high odour activities, we identified some alcohols (our code: A4, A5, A11), several esters (code: B2, B3, B4, B5, B12) and acids (code: C2, C3, C5, C6, C7, C8), a few aldehydes (code: E1, E2, E3), and one sulphur compound (code: G1).

Alcohols are reported to contribute the wine odour intensity (Etievant, 1991; Styger et al., 2011); isoamyl-alcohols (code A4), phenylethanol (code A11) and 1-hexanol (A5) were the only ones found contributing to the analysed *Negroamaro* wines. The first two are fusel alcohols, which are usually present in wines as a result of yeast metabolism during alcoholic fermentation. Concentrations above 300 mg/L in these alcohols have an adverse impact on wine aroma and flavour (specifically, a pungent smell and taste) (Rapp & Versini, 1991); on the other hand, concentrations below that level can have a positive impact by imparting the wine with fruity and floral notes. 1-hexanol (code A5) exhibits an small herbaceous and greasy odour (Kotseridis and Baumes (2000)) (OAV slightly higher to 1). Esters are responsible for the fruity aroma in wines, particularly acetate esters are correlated with the freshness and fruitiness of young wines (Rapp, 1990) ($28 < \text{OAV} < 114$) and their origin being mainly fermentative. Esters formation during fermentation is influenced by several factors, such as the physicochemical composition of musts and fermentation conditions (Ugliano & Henschke, 2009). In *Negroamaro* wines this class of odorous compounds is well represented by different active species, as for example isoamyl acetate (code B4) with its characteristic “banana” odour and ethyl hexanoate (code B5) with its characteristic green apple odour. Within the family of fatty acids, 2-methylpropanoic, 2+3 methylbutyric, hexanoic and octanoic acids were notable for their high concentrations in all wines. Fatty acids production is governed by the initial composition of the must and by fermentation conditions (Schneider, 1979) and have been described with fruity, cheese, fatty, and rancid notes (Rocha et al., 2004). Among acids ($28 < \text{OAV} < 114$), we mention as relevant active odorants 2+3-methylbutyric (code C5), butyric (code C3) and octanoic (code C7) acids. In recent studies on Bordeaux red wines carried out by Kotseridis and Baumes (2000), the importance of fatty acids as impact odorants were reported. In particular, they found that 2+3-methylbutyric acid was very intense at the sniffing port, and its odour descriptors were fatty, rancid and cheesy, whereas butyric and isobutyric acids were associated with, cheesy odour descriptors (Table 1). Finally, we point put the relevant content of the aldehyde vanillin (code E3) in the *Negroamaro* samples

NA1 and NA4, in which they impress a sensorial contribution described as vanilla, sweet, chocolate.

The presence of these 18 potential aroma-contributing substances in *Negroamaro* sample corroborates the typical fruity and floral main nuances associated to this wine, perceived together to fatty and pungent notes as well as a distinctive but pleasant bitterish note. However, it should be highlighted that the balance and relative amount of aroma compounds present in a wine, rather than the presence of specific impact odorants is important.

3.2. OAV's Aroma Wheel

In order to link volatile composition to aroma descriptors, we designed an approach based on picturing a tentative odour profile representative of wine aroma characteristics. In particular, likewise to the approach used by other authors (Mu oz et al., 2007; Penaido et al., 2006), we propose a method that groups odorous compounds with similar sensory descriptors into classes (aromatic series). In Table 1 for each compound we affiliated one or more membership sensorial classes. As sensory classes we considered the standardized classes of sensory descriptors used in the well known “Aroma Wheel”, developed at the University of California at Davis by Ann C. Noble in the early Eighties, as a standard used to describe wine in uniform, non-judgmental terms (<http://winearomawheel.com/>). The “Aroma Wheel” provides a visual graphic of the different categories and aroma components that one can encounter in wine. It consists of three concentric circles divided into segments for different classes of both bouquet and aroma. The terminology used is standardized for use by both professionals and amateur wine tasters. The wheel breaks down wine aromas into 12 basic categories and then further sub-divided into different aromas that can fall into those main categories. In our opinion an additional thirteenth category (13-fatty) has to be integrate in the aroma wheel to complete the organoleptic description of wine volatile fraction.

Afterward, in reference to the 13 defined standardized classes of sensory descriptors, an Odour Activity Value for each class of sensory descriptors (i.e. generalised OAV) was calculated by adding up the OAVs of all the compounds that form that class. In this computation, also the compounds with OAV < 1 were considered in agreed with the acknowledged theory that sub-thresholds compounds may also contribute to wine aroma through additive effects of compounds with similar structure or odour (Francis & Newton, 2005). Finally, we graphed them in a radar plot called “OAVs' Aroma Wheel”, since it is based neither on chemical analytical data (i.e. compound concentrations get by chromatographic quantification) nor on sensory data based on intensity marks given by panel judgments, but on hybrid chemical/sensory parameter as generalised OAV is. A radar chart is a useful graphical method of displaying multivariate data in the form of a n -dimensional chart of n -quantitative variables represented on axes (rays) starting from the same point. This gives the plot a star-like appearance. In this case the variables are the thirteen classes of sensory descriptors and the quantitative input values are the generalised OAVs of each sensorial class. It's evident how such type of plots are useful to answer the following questions: a) what sensory classes are dominant for a given wine sample?, b) which wine samples are most similar, c) are there outliers?

Fig.2 shows both the individual Analytical Aroma Wheel for each *Negroamaro* wine sample and a global Analytical Aroma Wheel overlapping the radar plots of all the *Negroamaro* wine samples. In particular, we chose a logarithmic scale in order to enhance minor variables flattened by predominant variables. The organoleptic profile of *Negroamaro* wine obtained by this method is compatible with the popular *Negroamaro* characteristics, focused on the name itself literally meaning “black” and “bitter”, to emphasise both the deep colour of the grape and the wine, as well as its

2424	D6	45	vinylphenol 2,6-Dimethoxyphenol (E) Aldehydes	Phenolic	0.57		0.12	0.63	0.33	0.70	0.04	0.37	0.05	0.02	0.14	0.12	11
1458	E1	16	Furfural	Caramel	3		0.167	0.09	1.07	0.8	1.3	0.8	2.233	0.627	1.53	0.97	10
1503	E2	18	Benzaldehyde	Bitter almond	0.35	ⁱ	1.71	1.11	1.43	1.71	1.66	1.43	1.71	1.14	1.14	1.11	9
2511	E3	48	Vanillin (F) Terpenes	Vanilla	0.2	^c	12.50	<0.1	<0.1	14.80	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	10
2007	F1	41	3,7-Dimethyl,1,7-octanediol	Floral	N/A		<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	5
1715	F2	28	α -Terpineol (G) Sulphur compounds	Floral	0.25	^e	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	5
1725	G1	29	3-Methylthio-1-propanol (H) Lactones	Potato, baked cabbage	0,5	^c	0.42	0.96	0.60	0.24	1	0.12	1	0.40	<0.1	0.14	8
1635	H1	23	Butyrolactone	Sweet, buttery	20	^c	0.18	0.17	0.23	0.20	0.18	<0.1	0.16	0.46	0.19	0.24	11
1974	H2	37	cis-Methyl-octanolide (I) Ketons	woody	0.067		<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	11
	I1	10	Acetoin	Buttery	155	^c	0.09	0.12	0.05	0.01	<0.1	0.02	<0.1	0.04	0.10	0.13	10
2685	I2	51	Acetovanillon	Caramel, vanilla	1	^g	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	10

Notes: LRI: linear retention index on a DB wax column.

Peak: numbering peak according to elution time.

Code: identification code assigned to each compound following the numbering inside the classes (A-Alcohols; B-Esters; C-Fatty acids; D-Phenols; E-Aldehydes; F-Tepernes; G-Sulphur compounds; H-Lactones; I-Ketons).

Odour Description (ODE) and Odour Threshold (OTH) as reported in the literature references (Ref.):

Odour Activity Value (OAV): defined as ratio between odour concentration (C_{odour}) and OTH.

Each compound was attributed to 1 or more classes of the following 13 classes of sensory descriptors: 1, chemical; 2, pungent; 3, oxidised; 4, microbiological; 5, floral; 6, spicy; 7, fruity; 8, vegetative; 9, nutty; 10, caramelized; 11, woody; 12, earthy; 13, fatty.

^a Rocha et al. (2004).

^b Penaido et al. (2006).

^c Guth (1997).

^d Moyano, Zea, Moreno, and Medina (2002).

^e Günata, Bayonove, Baumes, and Cordonnier (1985).

^f Vilanova and Sieiro (2006).

^g González Álvarez, González-Barreiro, Cancho-Grande, and Simal-Gándara (2011).

^h Gómez-Míguez et al. (2007).

ⁱ García-Carpintero et al. (2011).

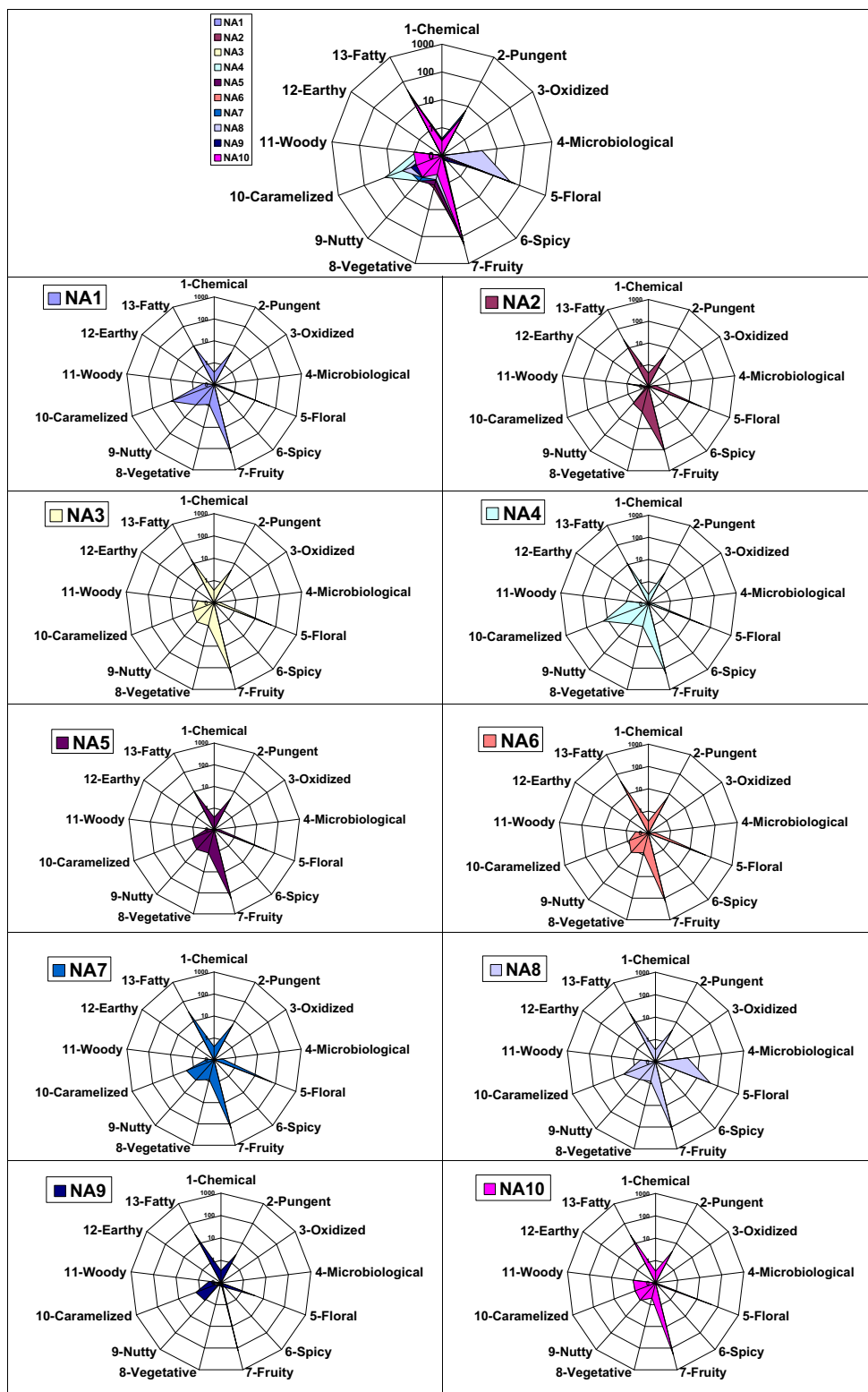


Fig. 2. Analytical "Aroma Wheel" of the *Negroamaro* wines based on the OAVs values of each class of sensory descriptors.

basically bitter flavour. Indeed, as can be observed all the analytical aroma wheels of *Negroamaro* wines show high 7-fruity and 5-floral characteristics, as well as typical 13-fatty and 2-pungent sensory classes with minor harmonious 10-caramelised and 9-nutty features. Of course some differentiations among the analysed *Negroamaro* wines are visible.

The proposed method based on "OAVs' aroma wheel" doesn't aim to become a reference and standardized methodology to describe wine aroma characteristics, but it could be used by chemical analysts to draw the aroma fingerprint of any wine by linking the chemical information to sensory parameters (i.e. Odour Thresholds-OTHs reported in literature). This fast and simplified

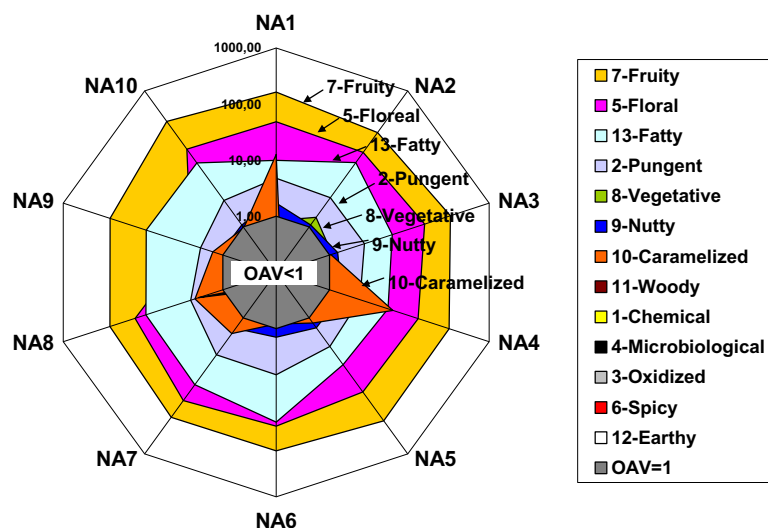


Fig. 3. Radar plot of the OAVs of the 13 standardized classes of sensory descriptors for the different *Negroamaro* wines analysed in this work.

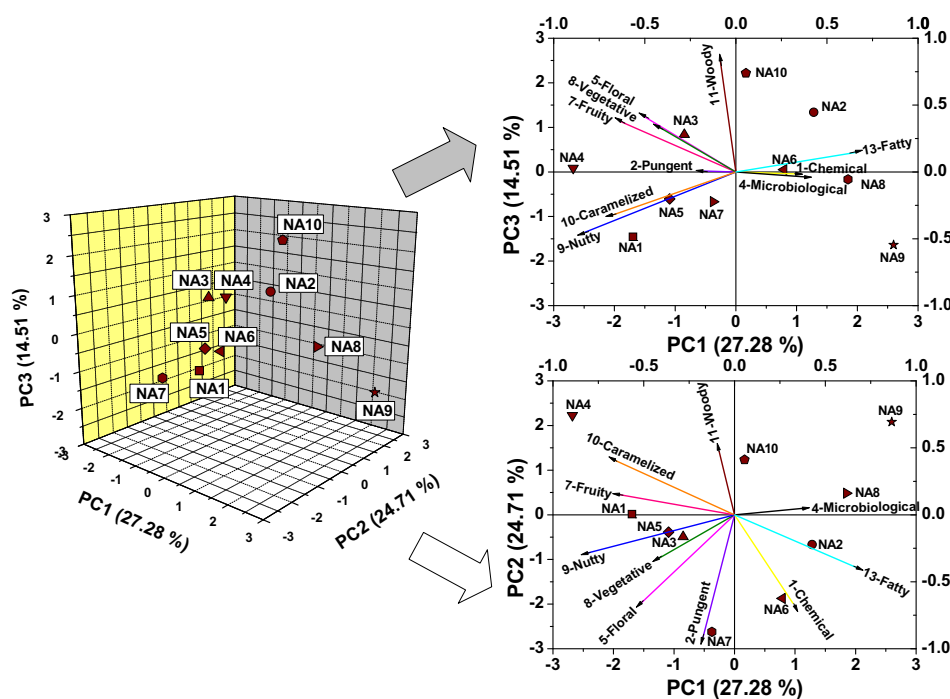


Fig. 4. PCA results based on the generalized OAVs of the 13 sensory descriptors. Left: 3-D score plot of the different *Negroamaro* wines; right: 2-D biplots on PC1-PC2 and PC1-PC3 plane overlapping scores and loadings.

comparison between wines is not substitutive of pure sensory analysis, but it is preliminary to it. It could become useful in many contexts where a sensory analysis isn't available or affordable and a first raw fingerprint of wine aroma peculiarity has to be sketched.

Moreover, in Fig. 3 we graphed an inverse radar plot by using the 10 analysed *Negroamaro* samples as variables and the 13 classes of sensory descriptors as samples. Here, it can more easily be seen which are the prevalent classes of sensory descriptors and the classes with insignificant sensory impact falling inside the $OAV < 1$ area.

3.3. Principal Component Analysis

Principal Component Analysis (PCA), was the multivariate data analysis technique used for dimensionality reduction and display

relationships/correlations between the 13 standardized classes of sensory descriptors and the considered *Negroamaro* wine samples. PCA was applied to the data matrix containing the generalised OAVs of each aromatic series.

Fig. 4 (left) shows the PCA score plot in the space of the first three Principal Components (PCs). An only class of data was explored by PCA; no clear clustering of *Negroamaro* wine samples according to local areas of *Salento* was observed. This is consistent with the aroma profile typicality of a generic *Negroamaro* wine, that seems not depend on the microarea of production inside *Salento* geographical macroarea. Data related to the different *Negroamaro* wines resulted rather spread since seven Principal Components were extracted explaining 100% of the total variance of the initial data set. The first three PC1, PC2 and PC3 accounted for 66.50% of total variance and they were considered sufficient for further discussion.

The little dissimilarities/similarities (mainly due to the diversification of the wine-making process operated by the different producers) among the different *Negroamaro* wine samples can be better resolved by reporting score plots combined with loadings plots (biplots), since in such a way it's more easy to display correlations between the 13 standardized sensory descriptors and the wine samples, and between the sensory descriptors and the PCs. Fig. 4 right shows the biplot on the PC1–PC2 and PC1–PC3 planes. As can be seen, the main 7-fruity and 5-floral sensory features of *Negroamaro* wines have negative values of PC1 and they are negatively correlated with the second main sensory feature, i.e. 13-fatty, falling at positive value of PC1; this fits the traditional and known aroma perception of this varietal. Wine samples NA8, NA9, NA10 are the only wines with minor correlation with the main *Negroamaro* aroma notes, being scattered in the I and IV quadrant in both PC1–PC2 and PC1–PC3 plane. NA10 seems also characterised by 11-woody notes, NA2 is more correlated to 13-fatty notes compared to the other wines whereas NA6 to 1-chemical notes. Among the wines falling in the II and III quadrant, we highlight samples NA1 and NA4 that show characteristic correlations with 10-caramelised notes. Moreover sample NA5 has typical correlated with 9-Nutty nuances, whereas sample NA7 with 2-pungent notes.

4. Conclusions

In conclusions, the results obtained by SPE-GC/MS analysis on *Negroamaro* red wines (an Italian indigenous grape varietal grown mainly in Salento area in Apulia Region) showed that the aroma composition of these monovarietal wines is dominated by different categories of compounds originating from different fermentation processes, mainly alcohols, ethyl esters and fatty acids. Other classes of compounds found in minor percentage were: ketones, aldehydes and lactones. Based on the sensory OTH values of each compound, the Odour Activity Values (OAVs) of all the quantified volatile compounds were calculated and eighteen would-be impact odorants (with OAV > 1) were determined. The identified key odorants of *Negroamaro* wines are associated with fruity (ranging from dark plum to prune and blackberries with a distinctive peppery note), floral, fatty and pungent notes with a pleasantly bitterish vein, that represents the known main characteristic aromatic feature of *Negroamaro* wines.

In this work the chemical analysis by GC–MS on *Negroamaro* wines was functional to the development of a method to built up “volatile/sensory aroma wheels” as pictures of odour wine typicality. We get *Negroamaro* organoleptic profile in a more objective way by relating the quantitative information get by chemical analysis to OTHs. The comparison of the volatile/sensory aroma wheels of different wines may be a easy method to discriminate wines that differ for: cultivar, area growing, wine making, *terroir*, ageing, etc.

Principal Component Analysis (PCA), applied to the data matrix containing the generalised OAVs of each aromatic series, was used for dimensionality reduction and allowed us to display and discuss relationships/correlations between the 13 standardized classes of sensory descriptors and the considered *Negroamaro* wine samples.

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