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Analytical Methods

Volatile components of *Negroamaro* red wines produced in Apulian *Salento* area

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ABSTRACT

Volatile composition of monovarietal young red wines made from *Negroamaro* cultivar, an autochthonous grape variety of *Vitis vinifera* grown exclusively in *Salento* area (southeast of Italy in Apulia region), was investigated. Volatile compounds were extracted following a solid phase extraction (SPE) method, and then analysed by gas chromatography–mass spectrometry (GC/MS). Results showed a complex aroma profile rich in alcohols, esters and fatty acids, with a minor contribute from aldehydes, lactones, volatile phenols and sulphur compounds. For the first time, aromatic patterns that characterise wines produced from *Negroamaro* autochthonous grape variety were established, starting a fundamental register of typicity and geographical identity of Apulians wines. Statistical data analysis techniques (Principal component analysis (PCA) and ANOVA) showed the structure of the experimental data and the significant differences for each compound in the different wines.

1. Introduction

The wine industry, worldwide, is fastly getting better, thanks to an increased knowledge on wine analytical chemistry from scientific research in the field and to advanced technological innovations in the production process. At present, the challenge is to improve wine quality and safety, satisfying both consumer preferences and market requirements, spreading commercial end-products at superior quality/price ratios. In addition to these issues, the certification of the geographical origin of wine has recently been arousing increasing interest, due to the introduction of European regulations for the protection in the common market of the denomination of origin for agricultural products. The development of precise methods for the classification of wines on a scientific basis, according to the linked parameters of geographical origin and vine variety, is becoming very important for the assignment of a "Denomination of Controlled Origin" trade mark and in general for the characterisation of typical wine varietals (Brescia et al., 2002; Gil, Cabellos, Arroyo, & Prodanov, 2006; Lukić, Plavša, Sladonja, Radeka, & Peršurić, 2008).

In Italy DOC wines are produced in specific well-defined regions, according to specific rules designed to preserve the traditional wine-making practices of the individual regions. The question of geographical identification of wines becomes more interesting when it relates to small production areas. In fact, often, there are several DOC areas in one region and they are not far from each other. In Italy, the regional wine producers devote their efforts to bring out the local wine productions made by autochthonous grape varieties. Many of these varieties are so climate-specific that they cannot be transplanted to another region. That is why wines produced from different Italian regions have their own distinctive aromas. Hence, there is a great interest to characterise and typicality the volatile fraction of the Italian regional wines.

In particular, wine production in Apulia is – in terms of quantity – among the first ones in Italy, a characteristic which made the region become the "wine tank", not only of Italy, but also of Europe. For many years the wine from Apulia was shipped to the north and to the other Italian regions, as well as to some areas of Europe, in order to be used for the blending with local wines, therefore giving colour, body and alcohol. In recent years the oenological situation of Apulia is changing, finally aware of the great wine potentials of the region, Apulian producers are now focusing on quality production. After all, Apulia is rich of autochthonous grapes having great potentials, such as *Negroamaro*, *Primitivo* and *Uva di Troia* (*Vitis Vinifera* L.), which in the last years have become more and more popular, therefore contributing to the rebirth of Apulian wines.

Wine production of Apulia is influenced by an extremely particular climate and by the quantity of sun rays the soil receives in the course of the year; Apulia is in fact among the most sunny regions of Italy and Europe. Ideally located in the "heel of the boot" of Italian peninsula, Apulian Mediterranean climate is influenced by the effects of the sea. Identification of Apulian wine aroma components and the relationships between their relative content may be a useful tool in differentiating the Apulian wines from different Italian or European varieties and establishing criteria of genuineness in order to improve the quality of the wines, prevent fraud and guarantee their geographical origin and typicity.

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Such work aims to start a systematic characterisation of Apulian monocultivar wines by building a registry the complete wine odour profile by GC/MS analysis to check whether or not there are differences between the wines according to the production area. This activity is part of a Strategic Project of the research founding program of Apulia region government: "Innovative biotechnological approaches to improve quality and safety of typical Apulian wines" – acronym INNOWINE.

In our knowledge, at the moment, this is the first systematic study of the volatile composition of red wines made by *Negroamaro*, an Italian indigenous grape varietal grown mainly in *Salento* area in Apulia region. Therefore, given the lack of information about the aroma profile of these *Negroamaro* wines, this work aims to identify and quantify the free aroma compounds that could have relevance on the organoleptic profile of these typical Apulian wines, whose quality is well appreciated from market and are more and more meeting the consumer preferences. We proposed a procedure to characterise the volatile fraction of *Negroamaro* wines that uses a solid phase extraction (SPE) technique, followed by capillary GC/MS analysis.

Negroamaro is a non-aromatic red wine grape variety native to southeast Italy (Gomez Garcia-Carpintero, Sanchez-Palomo, & Gonzalez-Vinas, 2011; Tamborra & Esti, 2010). It is grown almost exclusively in Apulia and particularly in "*Salento*" (southern area of Apulia). This grape variety can produce wines very deep in colour, that tend to be very rustic in character, combining perfume with an earthy bitterness, with a pleasant organoleptic complexity appreciated by the consumers. *Salento* producers have increasingly put the accent on premium quality, and by now *Negroamaro* wines are well acknowledged by the international experts as some of the best Italian red wines. The interest in *Negroamaro* wine is also due its beneficial effects on health, since it has a high content of resveratrol, a substance known for its anti-inflammatory and anti-cancerogenous characteristics.

The volatile fraction is one of the most important quality attributes that defines wine organoleptic character and typicity and have a great influence on its appreciation by consumers. The composition of aroma wine is very complex since different classes of volatile compounds (aldehydes, ketones, alcohols, esters, terpens, acids, etc.) are present in wine chemical pattern (Ferreira, Lòpez, & Cacho, 2000; Romano, Suzzi, Domizio, & Fatichenti, 1997). Over 1000 volatile compounds have been found in wines, with a wide concentration range varying from hundreds of mg/L to the μg/l or ng/l level (Bayonove, Baumes, Crouzet, & Günata, 1998). Hence, the identification of wine volatile compounds is a delicate matter since extraction and concentration of the volatile compounds is needed before analysis, and the completeness and representativeness of the experimental aromatic profile depends on the effectiveness of the used sample extraction method (Falqué & Fernández, 1999; Ferreira, Fernandez, Pena, Escudero, & Cacho, 1995; Genovese, Dimaggio, Lisanti, Piombino, & Moio, 2005; Gómez-Míguez, Vicario, & Heredia, 2007). Wine volatile profile is so complex and intricate because it's the result of many processes and factors: environment (soil and climate), grape variety, ripeness, fermentation conditions, biological factors (yeast and other components of the oenological microflora (Lambrechts & Pretorius, 2000; Swiegers & Pretorius, 2005), the wine production process and ageing. Primary (or varietal) aromas are odours, intrinsic to the grape, which are transmitted directly to wine; primary aromas are the source of so-called varietal character of certain wines. Secondary aromas are odours produced during winewaking procedures, distinguished in pre-fermentative and fermentative odours, the first formed during the processing of grapes and by chemical, enzymatic-chemical and thermal reaction in grape must, the latter formed during alcoholic fermentation by the action of yeasts. Tertiary aromas are post-fermentation odours formed during maturation of wine in barrel and bottle. The formation of these particular aromas is an integral part of the evolution of fine wine and they are generally most intense in mature wines. They are the result of the chemical action of enzymes on the natural components of the wine, and in the case of wood barrel-aged wines, they are the compounds released from the wood.

In this study free volatile compounds of different monovarietal *Negroamaro* wines (2007 vintage) were extracted by solid phase extraction method (SPE) and analysed by gas chromatography–mass spectrometry (GC–MS), allowing a chemical characterisation of these local wines. The complete volatile profile of all *Negroamaro* wine samples were obtained together with a quantitative analysis by internal standardisation method.

2. Materials and methods

2.1. Wine samples

Since we intend to focus the attention on the "bouquet" typicity of *Negroamaro* wines get by a SPE–GC/MS analysis, a total of 10 different commercial monovarietal red wines, i.e., 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 selected wine samples had also a Denomination of Controlled Origin (DOC) certification mark, that guarantees the quality and the geographical origin of the *Negroamaro* wines produced in *Salento* (southern area in Apulia region, Italy). To protect proprietary interests, winery identities have not been reported. All samples were stored at 4 °C until analysis. The list of identification codes of the wine samples together with their specific geographical area production and the geographical *Salento* area where *Negroamaro* wines are produced is shown in Fig. 1.

2.2. Reagents and standards

All reagents used were of analytical quality. Methanol and ethanol 96% were purchased from J.T. Baker (Phillipsburg, NJ, USA); dichloromethane was HPLC grade quality was 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 hydroalcoholic 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, listed in Table 2, 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.

2.3. Extraction of volatile compounds

To 50 ml of each wine sample and to each standard solution were added 400 μl of I.S. solution. The extraction of volatile