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ORIGINAL ARTICLE

Influence of vinification process over the composition of volatile compounds and sensorial characteristics of greek wines

João Carlos de Souza[1] · Pasquale Crupi[2] · Antonio Colletta[2] · Donato Antonacci[2] · Aline Theodoro Toci[3]

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Abstract Wine is one of the most traditional consumed alcoholic beverages in the world. Millions of wine enthusiasts worldwide duly appreciate a wine of excellent quality in terms of physicochemical and sensorial properties. Different classes of wines have different volatile compositions and sensorial properties, which can be altered, depending on the vinification process and use of additives. Among the widely employed additives in wine production is sulfite (SO_2). The popularity of sulfite lies in its ability to preserve the flavor and freshness of wine; however, depending on the quantity, sulfite can pose serious health risks to consumers and affect the quality of the drink. The present study evaluated and compared the compositions of volatile compounds and sensorial properties of sparkling and traditional wines (with and without SO_2) produced from Greek grapes 'Grechetto', 'Greco bianco' and 'Greco di tufo'. The results obtained of the composition of volatile compounds in these wines pointed to differences between SO_2–containing, SO_2-free and sparkling wines, with different amounts of compounds such as alcohols, esters, fatty acids, phenols and differences in sensorial properties. The 'Grechetto' wine, prepared without SO_2, exhibited greater quality, better volatile composition, and better sensorial properties compared to the wines produced with SO_2.

Keywords Greek grape · Sulfite · Sparkling wine · Volatile composition · Sensory analysis · Flavor

Introduction

Greek grapes (Greco grape family) are varieties of grapes that originated from Greece and imported to Italy by settlers who founded the first cities of Magna Graecia—southern area of the Italian peninsula, which was formerly colonized by the Greeks beginning from the eighth century BC. Currently, these varieties of grapes are widespread in Central Italy, especially in Campania, Abruzzo, Lazio Tuscany, Umbria and Southern Italy (Puglia and Calabria regions). Three varieties of grapes from the Greek grapes have become widely popular and highly appreciated in Italy; these include the 'Grechetto', 'Greco di Tufo', and 'Greco di Bianco' grapes. In general, the wines produced from these grapes are characterized by somewhat intense straw yellow color, pleasant smell, fine taste, fresh, dry and harmonious flavor with fruity notes (Caruso et al. 2012; Cerreti et al. 2017; Esti et al. 2010).

The chemical composition of wine depends on a number of factors; these include the variety of raw materials (grapes) used in the wine-making process, the method of cultivation, and the manufacturing process and technology

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✉ Aline Theodoro Toci
aline.toci@unila.edu.br

[1] Department of Analytical Chemistry, Institute of Chemistry, São Paulo State University (UNESP), Rua Professor Francisco Degni, 55, Araraquara, São Paulo 14800-060, Brazil

[2] Research Unit for Viniculture and Enology in Southern Italy, Agricultural Research Council (CREA), Via Casamassima, 148, 70010 Turi, Italy

[3] Latin American Institute of Life and Nature Sciences (ILACVN), Environmental and Food Interdisciplinary Studies Laboratory (LEIMAA), Federal University of Latin American Integration (UNILA), Av. Tancredo Neves, 6731, Foz do Iguaçu, Paraná 85867-970, Brazil

used in the fermentation and storage of the wine. These factors are found to be primarily important when it comes to the production of a quality drink, with very distinct and peculiar characteristics in terms of aroma and flavor (Boroski et al. 2017; Coletta et al. 2013; Li et al. 2013; Linskens and Jackson 1988; Ribéreau-Gayon et al. 2006; Souza et al. 2019). The fermentation process is regarded an essentially crucial stage; this process can be carried out naturally using different yeasts present in the grapes or through the addition of specific yeasts of interest employed with a view toward providing specific properties to the wine. The most widely employed yeast is the *Saccharomyces cerevisiae*; this yeast is known for being quite resistant to high concentrations of sugars and sulfur dioxide (Linskens and Jackson 1988; Souza et al. 2019). However, in terms of their genetic characteristics, the sensorial properties of the wine may undergo some alterations due to some measures/procedures implemented during the wine-making process and the addition of additives which are meant to help reduce inefficiencies in the production process and enhance the quality and shelf-life of the beverage.

Sulfite (SO_2) is a widely popular additive known to be mainly applied in the production of white wine, and which can be added from the initial stage involving the pressing of the grapes to the final stage of wine bottling. SO_2 is added in the wine-making process in order to protect the wine from the action of microorganisms, diminish the development of yeasts and bacteria, and help avoid the darkening of the wine from the production stage to the storage stage. Furthermore, the addition of SO_2 can help improve the aroma and longevity of the beverage (Boroski et al. 2017).

However, one needs to point out that although the addition of SO_2 comes with some benefits, the use of this food preservative needs to be rationed and regulated properly for the sake of wine consumers; this is because when applied in high doses, SO_2 negatively affects the quality of the beverage in terms of aroma and taste, reduces or undermines the fermentation process, decreases the color intensity in red and rose wines, promotes the production of unpleasant aromas (Li et al. 2005) and may, disturbingly, pose serious health risks to consumers (Boroski et al. 2017; Gao et al. 2002; Sonni et al. 2011; Vally and Thompson 2003). Another major problem associated with the use of SO_2 lies in its ability to trigger allergic reactions; as a result, SO_2 is on the list of allergenic substances regulated by the European Community (Regulation (EU) number 1169/2011) (European Parliament and of The Council 2011). Studies published in the literature have shown that sulfur dioxide can cause a series of adverse effects, including bronchospasm, angioedema, urticaria, nausea, vomiting, headache, migraine, disorientation, increased heart rate, abdominal cramps, and diarrhea (Boroski et al. 2017; Gao et al. 2002; Sonni et al. 2011; Vally and Thompson 2001, 2003).

Owing to these underlying problems related to the use of SO_2, there has been a considerable interest among wine producers regarding the substitution of SO_2 for other less harmful yet equally efficient compounds in the vinification process (Boroski et al. 2017). To this end, different compounds which have the ability to maintain the sensorial properties of wine during storage have been evaluated as potential substitutes of sulfur dioxide. Among these compounds, the use of ascorbic acid, lysozyme and tannins has drawn considerable attention (Boroski et al. 2017; Harbertson et al. 2012; Sonni et al. 2011). Specifically, ascorbic acid has been found to be capable of reducing or preventing the oxidation of *ortho*-quinones and phenolic compounds present in wine (Barril et al. 2012; Li et al. 2008; Ribéreau-Gayon et al. 2006). However, the use of this acid has also been found to have some non-negligible demerits which are particularly related to the oxidation process. Reports in the literature have shown that, during oxidation, ascorbic acid eventually forms hydrogen peroxide (H_2O_2)—a highly oxidizing compound which can oxidize several compounds present in the wine, causing changes in the quality, aroma and flavor of the wine during storage (Barril et al. 2012). Lysozyme has also been widely applied in the fermentation stage of wine as an antimicrobial agent; the compound has been found to inhibit the growth of microorganisms that tend to undermine the quality of the beverage (Gao et al. 2002). Finally, tannins have also been employed in the wine-making process to help clarify the must and the beverage, apart from aiding in preserving and improving the wine color, taste and aroma, especially in red wines (Sonni et al. 2011).

Wine is one of the most widely consumed beverages in the world, and its consumption among the population increases every day (Gmel et al. 2014; Souza et al. 2019). Given its popularity and high consumption rate, consumers are increasingly demanding for good quality wine products. This raises the need for devising and implementing increasingly rigorous, technologically advanced vinification processes aimed at obtaining standardized wine products with proven quality in terms of physicochemical and sensorial properties. In this context, the monitoring and control of the beverage through physical–chemical analysis is found to be of paramount importance (Souza et al. 2016a; b; Souza et al. 2017, 2019).

Owing to the scarcity of information regarding the wines produced from the three aforementioned popular varieties of grapes from the Greco grape family and their organoleptic properties, the objective of this work was to evaluate the sensorial properties of these wines through the conduct of sensorial analysis and determination of volatile compounds present in traditional wines produced with and without the use of SO_2 and sparkling wines.

Materials and methods

Grape production

The research was conducted in Apulia (Southern Italy) in 2011. The three grape varieties from the Greco grape family mentioned above were used for the conduct of the research. ‘Greco bianco’ was cultivated in a commercial vineyard; ‘Greco di tufo’ was cultivated in an experimental vineyard located at the Agricultural Research Council (CREA, Turi—Italy); and ‘Grechetto’ was acquired from a commercial vineyard from Abruzzo (Central Italy). All the vineyards were situated on hilly areas, around 200 m above sea level, lying at the latitude of approximately 41° in the Apulia region and at about 42° in the Abruzzo region. Both the Apulia and Abruzzo regions are characterized by a Mediterranean climate with an annual rainfall of about 650 mm, so water irrigation is necessary for crop cultivation mainly in the months of May to August. The vineyards in these regions require irrigation because rain generally falls during the dormant phase of the growing season and the water storage in the soil is insufficient to meet vineyard evapotranspiration (Etc). In Apulia, according to the average estimate for the last 20 years, a seasonal irrigation volume of 2710 ± 720 m^3 Ha^{-1} was required in order to counterbalance the Etc ratio for the vineyards during the whole productive cycle. In 2011, the Abruzzo region saw a reduction of nearly 18% in Etc (Di Lena and Acutis 2002); as a result, a volume of 900 m^3 Ha^{-1} of water was applied in Apulia in order to counterbalance around 45% of the total amount of water evapotranspired in the region. The same percentage of water integration level (45%) was recorded for Abruzzo, where a volume of 700 m^3 Ha^{-1} of water was applied. Water irrigation was performed by means of a localized irrigation system which consisted of 1.6 L h^{-1} pressure compensated drip emitters placed between the vines. Starting 10 days after the beginning of veraison (with 10% of berry softening) until harvest, the vineyards were irrigated five and six times in Apulia and Abruzzo, respectively. The harvest time for the three grape varieties occurred as follows: September 15th for ‘Greco di tufo’; September 30th for ‘Grechetto’; and October 4th for ‘Greco bianco’.

The three cultivars were evaluated separately. Three randomized replications of variety were applied in the fields; these replications were constituted by two rows of 8 vines each. The vines were trained in a horizontal trellis system (tendone); this consisted of two canes “5 buds”/-plant pruned and 2.50 × 0.50 spaced in Apulia and 2.40 × 2.40 spaced in Abruzzo. The yield did not exceed 3 kg/vine on each production site.

Wine-making procedure

The ‘Grechetto’ (16.4°Brix), ‘Greco di Tufo’ (19.8°Brix) and ‘Greco bianco’ (18.3°Brix) grapes were harvested under the same ripeness level. For all the varieties, approximately 40 kg of grapes were hand harvested separately. The harvested grapes were stored overnight in a cold room at 4 °C, and were then transferred to the experimental cellar at CREA Research Unit of Turi—Italy. A standard vinification method similar to that employed in experimental cellars was used for the conduct of small-scale vinification. For each grape variety, three wines corresponding to the vineyard replicates were made. After the grapes were destemmed and crushed, 10 g HL^{-1} of potassium metabisulphite (MBK) and 30 g ton^{-1} pectolytic enzyme (Lafazym CL Extract Enzyme, Laffort, Italy) were added to the grape must. The must was left overnight for skin contact (maceration) in a cold room at the temperature of 4 °C; subsequently, the must was pressed with a vertical hydro press at 1 bar, and the pressed juice was placed in 20 L demijohns (carboys). An amount of 4 mL HL^{-1} of the enzyme Rapidase Clear was added to favor the sedimentation of juice overnight (kept in a cold room at 4 °C). Thereafter, the pressed juice was transferred into 20 L stainless-steel fermenters and left idle until the temperature level of the juice reached that of room temperature. At the temperature of 20 °C, the juice was inoculated with a dried yeast strain, QA23 (Lallemand®, Italy), at 30 g HL^{-1}, according to the manufacturer’s recommendations, and placed in a temperature-controlled room of 15 °C. To avoid the assimilation of nitrogen by the yeasts (YAN) and increase the total concentration level of nitrogen in the must, an amount of 250 mg L^{-1} D-Ammonium Phosphate (DAP) was added to the grape juice. The fermentation process was monitored every two days via the quantification of residual sugar through enzymatic analysis (Steroglass, Italy). After that, 15 g HL^{-1} of MBK and 50 mg HL^{-1} of bentonite were added to the racked wine for protein stabilization.

The sparkling wines were bottled at residual sugar levels of approximately 20 g L^{-1} so that a 3 bar of pressure was generated in the bottles. With regard to the traditional wine samples, the alcoholic fermentation was considered terminated when the residual sugar levels were below 5 g L^{-1}. The wines were left idle for their temperature level to reach that of room temperature (18–20 °C), and they were then bottled into 750 mL green glass bottles. Two hours prior to bottling, the green glass bottles were cleaned by water washing and left to drip dry upside down.

The wines were put into the bottles and closed with cork caps.

The traditional wines were placed in a fridge at −4 °C to undergo cold stabilization for two weeks. After two weeks, the wines were racked off the lees and the SO_2 levels were adjusted to 40 mg L^{-1}. Two hours prior to bottling, the green glass bottles used for bottling the wines were cleaned by water washing and left to drip dry upside down. The traditional wines were also left idle for their temperature level to reach that of room temperature (18–20 °C), and they were then bottled into 750 mL green glass bottles and closed with cork caps.

All the bottled wines were stored in a cold room at constant temperature of 15 °C for a period of three to nine months prior to conducting wine sensory and chemical analyses.

For the sulfite-free wines, some amounts of MBK were planned to be added during the wine-making process.

Determination of oenological parameters

Oenological analyses were carried out according to International Organization of Vine and Wine (OIV) methods: free and total sulphurdioxide (AS323-04A); pH (AS313-15); sulphates (AS321-05A).

Analysis of volatile organic compounds

The volatile organic compounds were extracted from the headspace samples by solid phase microextraction (SPME) using divinylbenzene/carboxen/polydimethylsiloxane triple fiber, 50/30 (Sigma Aldrich, St. Louis, Missouri, USA). A volume of 2.0 mL of wine was placed in a 10 mL glass vial and a small magnetic stirring bar was inserted in the vial. The vial was tightly capped with a PTFE septum and placed in a silicone bath with stirrer. The wine sample was kept in the bath for 30 min at 35 °C.

The profile of the volatile organic compounds present in the wine samples was determined by gas chromatography coupled to mass spectrometry (GC–MS). To conduct this analysis, an Agilent 6890 gas chromatograph coupled to an Agilent 5975 mass spectrometry detector (Agilent, Santa Clara, California, USA) was used. The separation of the compounds was performed using DB-WAX (60 m × 0.25 μm) chromatography column – acquired from J & W Scientific (Folsom, California, USA). The analysis was conducted based on the methodology developed by Bosch-Fusté et al (2007) under the following conditions: splitless injection mode for 5 min with injector temperature of 250 °C; temperature ramp of 40 °C (5 min) with an increase of 2 °C min^{-1} up to 200 °C (15 min); and 200 °C with an increase of 1 °C min^{-1} up to 250 °C; mass analyzer quadrupole interface temperature of 280 °C; helium as drag gas applied at flow rate of 1.0 ml min^{-1}; and the ionization of the compounds was performed using electronic impact at 70 eV. Mass spectra were obtained in the range of 1.0 s, in a mass range *m/z* of 28–300 Da. The spectral library of NIST-2014 (National Institute of Standards and Technology, Gaithersburg, Maryland, USA) was used for the identification and confirmation of the compounds. All the analyses were performed in triplicate and the area of each peak was used for the relative quantification of the identified compounds, with the application of the internal standard 2-octanol (Sigma-Aldrich) (Li et al. 2013).

Sensory analysis

The sensorial analyses were conducted by a panel of ten judges—four researchers from CREA (Turi, Italy) and six professional tasters invited by ONAV—National Organization of Wine Tasters (Asti, Italy). The scores were attributed from 0 to 10, with 0 representing the absence of perception and 10 being the maximum perception (Coletta et al. 2013; Dutcosky 2013), taking the following parameters/factors into consideration: color (lightness, intensity and color gradient), aroma (exotic fruits (sweet, bitter, alcoholic, acidic, astringent, mineral structure), citrus, pomegranate, floral, herbaceous, dried fruit, caramelized only for white wines fruity, floral, spicy, caramel, herbaceous, phenolic and sweet, intense, persistent and gustatory), and final considerations (stage of evolution and quality).

Finally, the average values of the parameters assigned to each wine were subjected to a quantitative descriptive analysis (QDA) in order to generate the sensorial profile of each wine. The results obtained were plotted on graphic radar.

Statistical analysis

The data, obtained from the three replicates of each wine, were statistically analyzed using Tukey's honest significance test for a comparison of the average values through the application of the Action Stat, version 3.7 software (Estatcamp—Consultoria Estatística e Qualidade, Campinas, Brazil). Under this test, $p < 0.05$ was considered significant.

Results and discussion

Although the aroma and taste of wine are not determined solely by the action of volatile organic compounds but through a global synergistic effect caused by the simultaneous activity of different and numerous classes of compounds (volatile and non-volatile) present in the beverage at different concentrations (Boroski et al. 2017), the determination of volatile organic compounds is still regarded an important factor when it comes to evaluating the quality of wines. The presence of these compounds in their appropriate blended quantities is intimately related to the flavor, quality and sensory perception of wines. Many volatile compounds found in wines come from grapes (varietal compounds); these compounds have been found to play an interfering role in the fermentation process by forming other (intermediate) compounds. Similar to other types of wines, 'Grechetto', 'Greco bianco' and 'Greco di tufo' wines also have varietal compounds that certainly influence the fermentation, flavor and final aroma of the beverage (Caruso et al. 2012; Ceretti et al. 2017; Esti et al. 2010).

Volatile organic compounds

Thirty eight compounds were identified and quantified in the volatile fraction of 'Grechetto' wines (traditional wines, wines with and without SO_2, and sparkling wines), 'Greco bianco' (traditional) and 'Greco di tufo' (sparkling) wines. The compounds identified were classified into four different categories: alcohols (4 compounds), esters (28 compounds), phenols (3 compounds), and others (3 compounds); this can be found in Table 1. As expected, esters were the most abundant class of volatile compounds found in the wines investigated. This finding is in perfect agreement with the reports published in the literature (Boroski et al. 2017).

The alcoholic fermentation process, carried out through the metabolism of the yeasts used in the vinification process, is one of the main contributors to the formation of volatile compounds such as organic acids, aldehydes, sulfur compounds, ketones, esters, phenols, and alcohols (Boroski et al. 2017; Linskens and Jackson 1988). Along with the esters, the higher alcohols have been shown to be the most important groups of the volatile compounds; the alcohols predominate quantitatively while the esters predominate qualitatively (Linskens and Jackson 1988). In the present study, 4 alcoholic compounds were found in average concentration levels between 53.1 to 100.5 g L^{-1}, and 28 esters recorded concentration levels ranging from 769.2 to 2263.7 mg L^{-1}.

In general, considering the odor activity values (OAV), all the quantified volatile compounds were found in perceptible concentrations, with OAV greater than 1; the only exception being the class of carboxylic acids. This finding points to characteristics that are peculiar to aromatic wines with fruity and floral notes. However, all this aromatic potential may interfere with the harmony of the aromas, as some compounds in high concentrations may inhibit the perception of other compounds, apart from generating unpleasant notes (Boroski et al. 2017; Ruiz et al. 2019; Toci et al. 2012).

The traditional wines presented similar concentration levels of total alcohols which ranged from 75.3 to 79.0 g L^{-1} (Table 1). These concentration levels were found to be superior for the SO_2–free wines (66.9 g L^{-1}) (Table 1); this points to the influence of sulfite on the formation of higher alcohols, as observed in other studies in the literature (Boroski et al. 2017; Ribéreau-Gayon et al. 2006).

The presence of alcohols, such as 2-methyl-1-propanol and 3-methyl-1-butanol, is currently used as a criterion for assessing the quality of wines and other alcoholic beverages (Boroski et al. 2017; Toci et al. 2012), considering that they yield unpleasant notes (including fusel, harsh, bitter and alcoholic notes) (Li et al. 2008). Considering that the presence of higher alcohols at concentrations above 400 mg L^{-1} can impair the organoleptic quality of the drink, causing unpleasant sensations in the aroma of the wine and inhibiting the perception of the aroma of other volatile compounds (Ruiz et al. 2019) and the fact that the analyses performed showed the presence of high amounts of these compounds in the wines, it is likely that all the wines have their peculiar aromas inhibited; this can be clarified in the sensory analysis. One hypothesis for the presence of high concentrations of higher alcohols has to do with the use of YAN. As pointed out in the literature, low concentrations of YAN can increase the formation of higher alcohols, and this often leads to an increase in the final concentration of higher alcohols in the drink (Ruiz et al. 2019). Presumably, other factors, including the synergistic effect of the concentration of YAN, concentration of varietal compounds, and the fermentation process may also play an influential role on the formation and concentrations of higher alcohols present in wines.

Apart from contributing significantly to the final quality of wines, esters are also known to be responsible for providing new wines with fruity aroma (Linskens and Jackson 1988). Ethyl esters, derived from fatty acids, and acetates, derived from higher alcohols, are produced during the fermentation step in the vinification process through the action of the yeasts (Lambrechts and Pretorius 2000; Ruiz et al. 2019). With regard to the total number of esters, the 'Grechetto' grape presented the greatest number of this compound among the grape varieties investigated. When it comes to the total content of esters, sparkling wines were found to present similar contents (with average value of 798.8 mg L^{-1}) and 64% lower content of esters compared to the other wines (with average value of 2170.3 mg L^{-1}).

Most esters are pleasant-smelling molecules. Here, our attention will be focused on the compounds that contain significant amounts of esters above 10 mg L^{-1}. All the wines produced and analyzed exhibited very high

Table 1 Amount of volatile organic compounds (mg L^{-1}) found in the varieties 'Grechetto', 'Greco bianco' and 'Greco di tufo' in different wines making

	Rt (min)	Compounds	Odour	Grechetto						Greco bianco		Greco di tufo	
			Threshold*	SO_2-free	OAV	Traditional	OAV	Sparkling	OAV	Traditional	OAV	Sparkling	OAV
Alcohols	7.80	2-methyl-1-propanol	4.9	1587 ± 86.2^{b}	324	1604 ± 102^{b}	327	1223 ± 72.3^{c}	250	1451 ± 93.4^{b}	296	2247 ± 73.2^{a}	459
	13.70	3-methyl-butanol	150	$63{,}004 \pm 250^{c}$	420	$71{,}190 \pm 1405^{b}$	475	$49{,}494 \pm 1600^{d}$	330	$73{,}579 \pm 1657^{b}$	491	$93{,}017 \pm 2005^{a}$	620
	25.20	1-hexanol	1.2	2334 ± 57.4^{c}	1945	2468 ± 151^{c}	2057	2358 ± 180^{c}	1965	3294 ± 124^{b}	2745	4663 ± 243^{a}	3885
	28.06	3-hexen-1-ol	0.2**	n.d		n.d		n.d		707.6 ± 48.2^{a}	3538	553.7 ± 35.1^{b}	2769
		Total contents		$66{,}924 \pm 204^{d}$		$75{,}263 \pm 1380^{c}$		$53{,}076 \pm 1569^{e}$		$79{,}031 \pm 1531^{b}$		$100{,}480 \pm 1237^{a}$	
Esters	8.30	3-methyl-1-butanol acetate (isoamyl acetate)	0.06	50.8 ± 9.4^{b}	847	118.6 ± 7.8^{a}	1977	22.7 ± 5.2^{c}	378	112.5 ± 6.2^{a}	1875	33.0 ± 8.6^{c}	550
	14.70	Ethyl hexanoate	0.65	328.3 ± 23.7^{a}	505	276.4 ± 12.6^{b}	425	195.0 ± 21.8^{c}	300	254.2 ± 9.3^{b}	391	110.8 ± 13.5^{d}	170
	17.50	Hexyl acetate	0.15	7.1 ± 1.0^{d}	47	19.5 ± 1.2^{b}	130	5.5 ± 1.2^{d}	37	42.2 ± 3.9^{a}	281	11.3 ± 1.7^{c}	75
	21.45	3-hexen-1-ol acetate		n.d		n.d		n.d		3.2 ± 0.4		n.d	
	22.70	Ethyl heptanoate		1.1 ± 0.2^{a}		0.7 ± 0.1^{b}		0.6 ± 0.1^{b}		0.5 ± 0.1^{b}		0.9 ± 0.1^{ab}	
	23.76	Ethyl 2-hexanoate		n.d		n.d		0.6 ± 0.1^{a}		0.3 ± 0.1^{b}		0.3 ± 0.1^{b}	
	24.50	Ethyl 2-hydroxy-propanoate		8.8 ± 0.3^{a}		4.7 ± 0.2^{b}		0.6 ± 0.1^{d}		4.3 ± 0.2^{b}		0.9 ± 0.1^{c}	
	33.50	Ethyl octanoate	1.2	1377 ± 143^{a}	1148	1368 ± 110^{a}	1140	474.5 ± 37.0^{c}	395	1167 ± 50.1^{b}	972	342.4 ± 60.3^{d}	285
	34.80	Isopentyl hexanoate	0.002	2.6 ± 0.3^{b}	1300	2.6 ± 0.4^{b}	1300	0.7 ± 0.1^{d}	350	1.6 ± 0.2^{c}	800	6.1 ± 0.5^{a}	3050
	38.10	Ethyl 2,4-hexadienoate		0.7 ± 0.1		n.d		n.d		n.d		n.d	
	39.10	Propryl octanoate	0.001	0.7 ± 0.1^{a}	700	0.7 ± 0.1^{a}	700	0.3 ± 0.0^{a}	300	n.d		n.d	
	40.20	Ethyl nonanoate	0.002	2.2 ± 0.4^{a}	1100	0.8 ± 0.2^{b}	400	0.3 ± 0.0^{c}	150	n.d		n.d	
	41.10	Isobutyl octanoate	0.02	0.4 ± 0.0^{b}	20	0.7 ± 0.1^{a}	35	0.1 ± 0.0^{c}	5	n.d		n.d	
	41.20	3,7-dimethyl-1,6-octadien-3-ol acetate	0.1**	0.7 ± 0.1^{b}	7	1.0 ± 0.1^{a}	10	0.2 ± 0.0^{c}	2	1.1 ± 0.2^{a}	11	0.6 ± 0.1^{b}	6
	43.30	Methyl decanoate	0.002	0.6 ± 0.1^{b}	300	1.1 ± 0.1^{a}	550	n.d		1.0 ± 0.1^{a}	500	n.d	
	44.72	Ethyl 2-furancarboxyrate		0.4 ± 0.0^{a}		0.5 ± 0.0^{a}		0.3 ± 0.0^{b}		0.5 ± 0.0^{a}		n.d	
	45.70	Ethyl decanoate	0.3	325.1 ± 25.1^{c}	1084	423.2 ± 15.8^{b}	1411	54.1 ± 12.3^{e}	180	474.8 ± 24.6^{a}	1583	145.2 ± 14.0^{d}	484
	46.40	Isopentyl octanoate	0.07	2.7 ± 0.3^{b}	39	3.7 ± 0.4^{a}	53	0.6 ± 0.2^{d}	9	3.0 ± 0.3^{b}	43	1.4 ± 0.2^{c}	20
	47.40	Diethyl butanoate	1.7	23.7 ± 6.7^{b}	14	8.1 ± 0.5^{c}	5	5.7 ± 0.3^{d}	3	2.9 ± 0.3^{e}	2	135.4 ± 31.2^{a}	80

Table 1 continued

	Rt (min)	Compounds	Odour	Grechetto						Greco bianco		Greco di tufo	
			Threshold*	SO_2-free	OAV	Traditional	OAV	Sparkling	OAV	Traditional	OAV	Sparkling	OAV
	47.90	Ethyl 9-decenoate	0.02	4.5 ± 0.2^{b}	225	2.0 ± 0.5^{c}	100	2.4 ± 0.6^{c}	120	1.8 ± 0.3^{c}	90	5.5 ± 0.6^{a}	275
	48.08	Ethyl 2-hydroxy-4-methyl-pentanoate		n.d		0.3 ± 0.0^{a}		0.2 ± 0.0^{a}		0.4 ± 0.1^{a}		n.d	
	50.01	Ethenyl decanoate (Vinyl decanoate)		0.8 ± 0.3^{a}		1.1 ± 0.2^{a}		0.1 ± 0.0^{b}		0.1 ± 0.0^{b}		n.d	
	51.70	Ethyl benzeneacetate		0.7 ± 0.2^{b}		0.9 ± 0.1^{ab}		0.6 ± 0.2^{b}		1.1 ± 0.1^{a}		0.8 ± 0.1^{b}	
	52.80	2-phenylethyl acetate	0.65	3.4 ± 0.4^{d}	5	13.9 ± 1.2^{a}	21	1.4 ± 0.5^{e}	2	7.5 ± 0.7^{b}	12	5.0 ± 0.8^{c}	8
	54.00	Ethyl dodecanoate	0.09	3.9 ± 0.7^{c}	43	8.6 ± 0.8^{b}	96	0.9 ± 0.4^{d}	10	15.1 ± 1.8^{a}	168	8.1 ± 1.0^{b}	90
	54.70	3-methylbutyl pentadecanoate		0.4 ± 0.0^{c}		0.7 ± 0.1^{a}		0.1 ± 0.0^{d}		0.8 ± 0.1^{a}		0.6 ± 0.0^{b}	
	55.10	1-[2-(Isobutyryloxy)-1-methylethyl]-2,2-dimethylpropyl 2-methylpropanoate		3.5 ± 0.6^{a}		2.4 ± 0.5^{b}		1.7 ± 0.2^{c}		1.5 ± 0.3^{c}		1.3 ± 0.2^{c}	
	65.43	Bis(2-ethylhexyl) hexanedioate		n.d		3.6 ± 0.5		n.d		n.d		n.d	
	66.10	Ethyl hexadecanoate	0.002	n.d		n.d		n.d		n.d		0.72 ± 0.3	360
		Total		2150 ± 134^{a}		2264 ± 104^{a}		769.2 ± 27.8^{b}		2097 ± 53.4^{a}		810.3 ± 57.5^{b}	
Phenols	56.30	Phenylethyl alcohol	34	3678 ± 189^{d}	108	$12,828 \pm 257^{a}$	377	2648 ± 178^{e}	78	5031 ± 130^{c}	148	8807 ± 123^{b}	259
	60.50	4-ethyl-2-methoxyphenol	0.04	n.d		n.d		n.d		n.d		123.1 ± 31.5	3078
	67.10	2,4-bis(1,1-dimethylethyl)-phenol	0.06	812.4 ± 43.4^{b}	13,540	1137 ± 204^{a}	18,948	n.d		1325 ± 167^{a}	22,083	n.d	
		Total		4490 ± 173^{d}		$13,965 \pm 251^{a}$		2648 ± 147^{e}		6356 ± 201^{c}		8930 ± 110^{b}	
Others	38.60	Benzaldehyde	0.35	0.2 ± 0.0^{a}	0,6	0.1 ± 0.0^{a}	0,3	n.d		n.d		n.d	
	64.10	Octanoic acid	26	30.0 ± 8.0^{b}	1,2	58.7 ± 9.9^{a}	2,3	14.4 ± 1.5^{c}	0,6	33.8 ± 7.0^{b}	1,3	6.7 ± 1.0^{d}	0,3
	68.00	Decanoic acid	2.6	8.4 ± 1.3^{c}	3,2	18.7 ± 2.3^{a}	7,2	5.1 ± 1.0^{d}	2,0	14.6 ± 2.8^{b}	5,6	5.3 ± 0.5^{d}	2,0
		Total		38.6 ± 5.1^{c}		77.5 ± 8.3^{a}		19.5 ± 1.4^{d}		48.4 ± 6.4^{b}		12.0 ± 0.9^{e}	

*Threshold values, comprising odour, wine and artificial wine solution threshold, found in the searched literature (Li et al. 2008; Zhang et al. 2020);

** Threshold in water;

The values represent the mean of three repetitions. Values with the same superscript letters within lines do not differ significantly at $p < 0.05$;

Rt – Retention time; n.d. – not detected concentrations of isoamyl acetate (concentration values between 22.7 and 118.6 mg L^{-1}, with banana and apple notes), diethyl butanoate (between 2.0 and 135.4 mg L^{-1}, with fruity notes), 2-phenylethylacetate (between 1.4 and 13.9, with rose notes), ethyl hexanoate (between 110.8 and 328.3 mg L^{-1}, with green apple notes), ethyl octanoate (between 342.4 and 1377.1 mg L^{-1}, with pineapple, sweet and fruity notes), and ethyl decanoate (between 54.1 and 474.8 mg L^{-1}, with floral notes) (Lambrechts and Pretorius 2000; Ruiz et al. 2019). As aforementioned, sparkling wines presented relatively lower concentrations of these esters (average concentration values ranging from 199.4 to 241.2 mg L^{-1}); the only exception was in the case of diethyl butanoate, where the sparkling 'Greco di Tufo' wine presented the lowest concentration of this compound: 135.4 mg L^{-1} (Table 1).

The compound 3,7-dimethyl-1,6-octadien-3-ol acetate (linalool acetate) is a typical varietal compound derived from the linalool; this compound has sweet, floral-fruity odor which resembles that of bergamot and pear (Zhang et al. 2020). This compound was found in all the wines investigated, with concentrations ranging from 0.2 to 1.1 mg L^{-1}; the highest concentrations of linalool acetate were recorded in the traditional wines (Table 1). Some studies have shown that the varietal aromas of grapes, such as those of the Greek grapes, can also have a suppressive effect on other esters and volatile compounds in wine (Lambrechts and Pretorius 2000; Ruiz et al. 2019), which, in this case, may have a positive effect on the wine composition and quality.

Phenols are regarded compounds of great relevance when it comes to the composition of wines. The presence of these compounds has a direct influence on the color, astringency, bitterness and oxidation level of the beverage. Furthermore, the quantity of phenols is strongly influenced by the conditions involving the fruit cultivation and the wine-making process (Li et al. 2011). Compared to the other classes of compounds analyzed previously, phenols exert much less influence over the aroma and odor of wines, though they contribute strongly when it comes to the pungency of the drink. One will note, however, that wines with high amounts of total phenols present low organoleptic quality because they are often rough, bitter and astringent; this makes them less popular and less attractive among wine consumers (Singleton and Noble 1976).

All the wines analyzed exhibited a high concentration of phenylethyl alcohol—with special reference to the traditional 'Grechetto' (12.8 g L^{-1}) and the sparkling 'Greco di tufo" (8.8 g L^{-1}); this provided them with good positive floral and rose notes (Li et al. 2008) (Table 1). None of the sparkling wines contained the compound 2,4-bis(1,1-dimethylethyl)-phenol—which indicates the presence of phenols and confers the corresponding positive grade related to phenolic content in the wine composition. The compound 4-ethyl-2-methoxyphenol was found only in 'Greco di tufo' sparkling wine (123 mg L^{-1}) (Table 1). Greek grapes are among the grape species with the highest amounts of phenolic compounds. The quantity and concentration of these phenols vary according to the grape species, based on the genetic makeup of that particular grape variety.

Fatty acids are also produced in relatively high amounts during the fermentation process, at a stage prior to the formation of their respective esters, derived from acetyl-coA, followed by a hydrolysis reaction. Following the breakdown of acetyl-coA by hydrolysis, the cleavage of coA-SH from the alcohols finally generates the esters (Boroski et al. 2017; Linskens and Jackson 1988); this directly leads to an increase in the production of ethyl esters, derived from fatty acids and acetates, with the increase of the amount of ethanol during the fermentation process (Linskens and Jackson 1988).

With regard to fatty acids, the traditional 'Grechetto' and 'Greco bianco' wines exhibited relatively higher concentrations of octanoic (58.7 and 33.8 mg L^{-1}, respectively) and decanoic fatty acids (18.7 and 14.6 mg L^{-1}, respectively) compared to the following wines: 'Grechetto' sparkling wine (14.4 and 5.1 mg L^{-1}, respectively), 'Greco di tufo' sparkling wine (6.7 and 5.3 mg L^{-1}, respectively) and SO_2-free 'Grechetto' wine (30.0 and 8.4 mg L^{-1}, respectively) (Table 1). Interestingly, compared to the other wines investigated, sparkling wines exhibited the lowest concentration levels of fatty acids, with the 'Grechetto' variety exhibiting fatty acids levels 75% lower than the other classes of wines. This result points to a clear influence of the wine-making process in the formation of these acids. Studies have shown that the amount of fatty acids present in wines largely depends on factors such as pH, dissolved oxygen tension, temperature, type of yeast used, and yeast nutrient concentration (Lambrechts and Pretorius 2000; Ruiz et al. 2019). Here, the pH difference does not justify this variation, as 'Grechetto' and 'Greco di tufo' sparkling wines have the lowest pH (3.20 and 3.12, respectively), but the SO_2-free 'Grechetto' wine has the highest pH when compared (3.40) (Table S1). When present in concentrations above 100 mg L^{-1}, the fatty acids can significantly undermine the aroma of the beverage, leading to a decline in the drink quality (Ruiz et al. 2019); in addition, octanoic and decanoic acids have been found to present unpleasant aromas, such as rancid, stale and mould (Ruiz et al. 2019).

The analysis of volatile organic compounds has proven to be an important tool for distinguishing one type of wine from another; these compounds have been found to influence the vinification process relative to the composition of

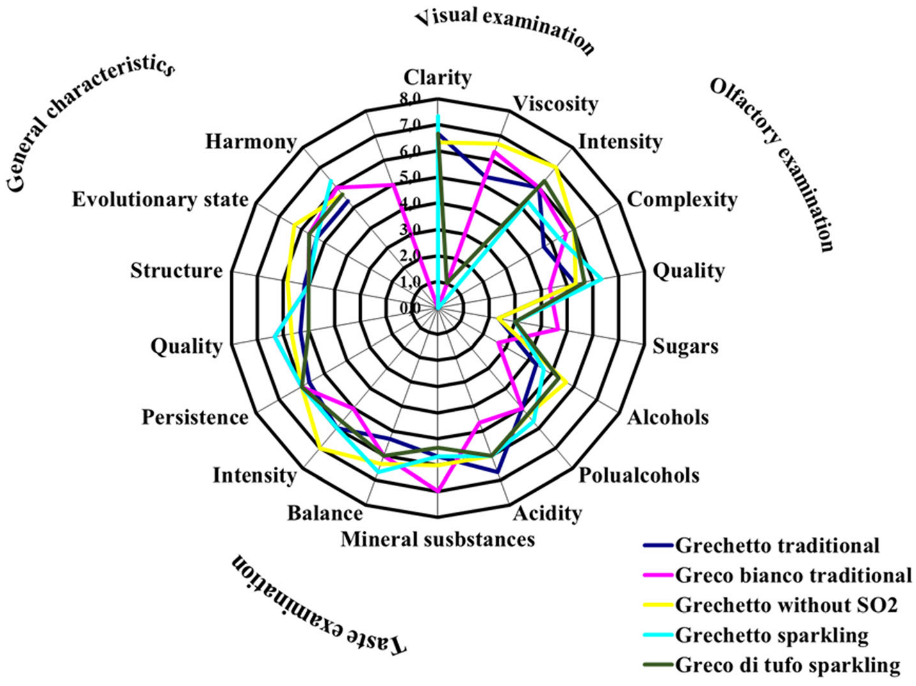

Fig. 1 Radar graph showing the sensory profile of the following wine varieties: 'Grechetto', 'Greco bianco', and 'Greco di tufo' in different wine-making processes

the volatile fraction of the beverage as a whole. This is evidenced by the presence of different concentrations and the absence of several volatile compounds in each class of wine investigated.

However, one needs to point out the difficulty involved in establishing a direct relationship between the volatile fraction present in each wine class and the wine-making process to which it has been subjected; this is because the aroma and taste of the wine are not solely determined by the compounds that make up the volatile fraction of the beverage, but also by the overall synergistic effect of the compound composition and how these compounds interact with each other before the wine production stages (Boroski et al. 2017; Linskens and Jackson 1988).

Sensory analysis

The organoleptic characteristics of wines, including flavor and aroma, depend on the quantity and concentration of the compounds present in their composition—for example, the volatile organic compounds of different classes: alcohols, esters and phenols. As stated above, volatile compounds are responsible for providing the beverage with various characteristics such as color, astringency, bitterness and acidity (Boroski et al. 2017; Linskens and Jackson 1988; Singleton and Noble 1976; Toci et al. 2012).

The conduct of sensory analysis enables one to evaluate and interpret the taste and aroma quality of wines, taking into account the reactions displayed by the human senses, mainly in terms of vision, smell, and taste (Linskens and Jackson 1988). In this sense, sensory analysis is regarded a tool of great importance for the evaluation of the organoleptic characteristics of wines.

Figure 1 shows the radar chart for the sensory analysis of different attributes associated with the 'Grechetto' wines (without SO_2, traditional and sparkling wines), the 'Greco bianco' traditional wines, and the 'Greco di tufo' sparkling wines.

In the radar graph, Fig. 1, one can clearly observe that there are differences in the attributes of the wines investigated. In general, one will notice that relatively lower notes were assigned to the final harmony; this may be attributed to the high concentrations of volatile impact compounds, with OAV much higher than 1.0.

A further interesting point worth mentioning has to do with the SO_2–free "Grechetto" wine, which presented some positive characteristics, including high aromatic and taste intensity, complexity, good structure and evolutionary state. This result demonstrates the potential of wine making in the absence of SO_2 (Table S1), as demonstrated in other studies published in the literature (Boroski et al. 2017); the characteristics exhibited by SO_2-free Grechetto wine can be attributed to the substitution of SO_2 by the tannins and ascorbic acid in the wine-making process (Boroski et al. 2017; Harbertson et al. 2012).

Some characteristics identified in the analysis of volatile organic compounds were revealed in the sensory analysis. For illustrative purposes, the results obtained from the analyses showed that 'Grechetto' traditional wine presented the highest acidity in terms of taste and highest concentrations of carboxylic acids among all the wines investigated. The 'Grechetto' sparkling wine exhibited the best harmony, gustative and olfactory quality, and balance; coincidentally, this was the wine with the lowest total concentration levels of all classes of volatile compounds. This shows that high concentrations of volatile compounds affect the overall positive characteristics of wines.

The ‘Greco bianco’ traditional wine exhibited the lowest clarity, acidity, olfactory quality, and intensity among all the wines investigated; however, this class of wine presented a high content of sugars, polyalcohols, and minerals.

Conclusion

The present study showed that the ‘Greco bianco’, ‘Grechetto’ and ‘Greco di tufo’ grape varieties presented significant quantities of volatile organic compounds, mainly from the alcohol and esters classes, in all the vinification processes investigated; the study also led to the identification of the presence of linalool acetate (which characterizes the varietal aroma) in the grape varieties. The results obtained from the analysis of volatile organic compounds and sensorial experiments enabled us to identify the characteristics and distinction of each wine; this helped critically evaluate the quality of the wines investigated.

With regard to the type of wine-making process, the SO_2–free ‘Grechetto’ wine presented relatively higher quality, better volatile composition, with higher levels of volatile esters and better sensorial aspects compared to wines produced with SO_2, which contained higher levels of alcohols. Thus, the replacement of SO_2 with ascorbic acid, lysozyme and tannins significantly improved the quality of the wines.

Although the use of sulfite in the wine-making process has become a routine and widespread practice, the results obtained show that one can produce sulfite-free wines with superior flavor and aroma compared to sulfite-containing wines.

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Declarations

Conflicts of interest The authors declare that they have no conflicts of interests.

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