



The effect of in-amphorae aging on oenological parameters, phenolic profile and volatile composition of Minutolo white wine



Antonietta Baiano^{a,*}, Annalisa Mentana^a, Maurizio Quinto^a, Diego Centonze^a, Francesco Longobardi^b, Andrea Ventrella^b, Angela Agostiano^b, Gabriella Varva^a, Antonio De Gianni^a, Carmela Terracone^a, Matteo Alessandro Del Nobile^a

^a Dipartimento di Scienze Agrarie, degli Alimenti e dell'Ambiente, University of Foggia, Via Napoli, 25 - 71122 Foggia, Italy

^b Dipartimento di Chimica, Università degli Studi di Bari "Aldo Moro", Via Orabona, 4, 70126 Bari, Italy

ARTICLE INFO

Article history:

Received 13 January 2015

Received in revised form 13 April 2015

Accepted 18 April 2015

Available online 24 April 2015

Keywords:

Antioxidant

Container

NMR

Phenolic

Volatile

Chemical compounds studied in this article:

Caftaric acid (PubChem CID: 6440397)

Procyanidin B3 (PubChem CID: 146798)

Astilbin (PubChem CID: 119258)

Sulfur dioxide (PubChem CID: 1119)

ABSTRACT

A wine was obtained from cryomacerated *Minutolo* grapes under reductive conditions and aged for 12 months in glass container and in 3 types of amphorae. After aging, wines in glass containers showed the highest alcohol content, volatile acidity, dissolved oxygen, concentrations of aromatics, alcohols, and esters and by the lowest contents of enols and terpenes. They also showed the highest decrease of flavonoids, hydroxycinnamoyl tartaric acids, and procyanidins. Wines in raw amphorae showed the dramatic decrease of flavonoids and flavans reactive with vanillin. The highest antioxidant activity was exhibited by wines in engobe amphorae, while the lowest values were showed by the wines in glass containers and glazed amphorae. Caftaric acid and procyanidin B3 decreased in wine aged under glass while epicatechin mainly reduced in raw amphorae.

According to the Principal Component Analysis, the wines resulted homogeneously grouped as a function of the type of container in which were aged.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Wine contains different chemical substances that influence the sensory characteristics of the final product. Amount and type of these components can be opportunely modified by managing viticultural practices, winemaking process, aging, and type of containers and closures.

Phenolic compounds are important components of wine. They not only contribute to their sensory profiles, such as color, flavor and astringency (Lee & Jaworsky, 1987), but may also act as antioxidants, with mechanisms involving both free-radical scavenging and metal chelation (Benítez, Castro, Sánchez Pazo, & Barroso, 2002). The composition and concentration of phenolic components in wine depends not only on grape variety and wine-making procedures, but also on the chemical reactions that happen during aging (Peña-Neira, Hernández, García-Vallejo, Estrella, & Suarez, 2000).

A fundamental role in wine sensory profile and consumer preferences is also played by volatile compounds. The aromatic profile of wine is the result of important modifications deriving from esterification, hydrolysis, redox reactions, slow and continuous diffusion of oxygen, spontaneous clarification, and CO₂ elimination (Camara, Alves, & Marques, 2006). As a result of these physical and chemical changes, the volatile fraction is extremely complex, accounting for more than 1000 compounds (Poláková, Herszage, & Ebeler, 2008), which belong to different chemical classes, and cover a wide range of polarities, solubility, and volatility values.

Aging can be made in different containers, such as stainless steel tanks, oak barrels, clay vessels, with the aim of enhancing wine flavor. Stainless steel tanks are inert containers while wood and clay interact with wine. Aging in wood changes color, structure, phenolic profile (Tesfaye, Morales, García-Parrilla, & Troncoso, 2002) and aroma (Callejón, Morales, Silva Ferreira, & Troncoso, 2008) since it is a material enable to make a micro-oxygenation of wine and to release phenolic and aromatic substances while adsorbing other wine components. However, in the case of white wines the aging in oak barrels is not always advantageous since both the oxygen could oxidize the wine and

* Corresponding author. Tel.: +39 881 589249.

E-mail address: antonietta.baiano@unifg.it (A. Baiano).

the wood deriving components completely mask its sensory characteristics (Ortega-Heras, González-Sanjosé, & González-Huerta, 2007).

In the remote past, clay vessels have been used to store, trade, and serve wine. The most common transport container was amphora. “Qvevri” is the vessel used for the Georgian traditional winemaking procedures, which includes fermentation of grape must on all or part of the grape pomace (skins, seeds, stems) and aging of wine. Lanati et al. (Lanati, Marchi, & Mazza, 2001) studied the Georgian white wines produced according to the ancient technology that employs Kakhetian amphorae, during fermentation, maceration and aging. They found that the Kakhetian white wines are characterized by dark, almost orange color, which is very different from those of the other white wines. Today the production of in-amphorae wines is becoming interesting but scientific literature concerning this type of aging system lacks. Baiano et al. (2013), Baiano et al. (2013) studied the effects in-amphorae aging on physico-chemical indices and antioxidant compounds of *Falanghina* and *Fiano passito* wines showed that the characteristics of wines were affected both by aging time and types of containers. The aging wines in three types of amphorae (raw, glazed and engobe) showed similar evolution of physical and chemical characteristics, while those stored in stainless steel tanks had a different trend.

The aim of this research was focused on the study of the effects of in-amphorae aging on quality, phenolic profile and volatile composition of an Italian white wine made from *Minutolo* grapes. The study was performed by conventional analysis, gas chromatography and NMR spectroscopy in combination with multivariate statistical analysis.

2. Materials and methods

2.1. Wine samples

Minutolo grapes produced in vineyards of Apricena (Foggia, Italy) were picked early in the morning in the second week of September 2013 and immediately delivered to a pilot plant (Foggia, Italy) made of a crusher-destemmer, 20 stainless steel vats (100 L-capacity), a temperature management system, and 2 wine presses. At harvesting, grapes had the following characteristics: sugar content 18.5 ± 0.4 °Brix; titratable acidity 6.0 ± 0.3 g tartaric acid/L and pH 3.53 ± 0.07 .

Grapes were submitted to a winemaking procedure, which included a cryomaceration step and a reductive vinification, according to a previous work (Baiano et al. (2013)). After fermentation, the wines were submitted to a first racking and, after four weeks of decantation, they were transferred into the aging containers. Each vinification was repeated two times, using about 120 kg of grapes for each trial, and every time, 3 samples were withdrawn.

According to Baiano et al. (2013), Baiano et al. (2013), wines were stored for 12 months in three types of earthenware amphorae: raw, glazed, and engobe. The wine stored in glass containers was used as a control.

2.2. Conventional analyses of wine

Wines were analyzed before the transfer into the aging containers and each two months during 12-months of aging. Alcoholic strength at 20 °C (expressed as % vol.), titratable acidity (expressed as g of tartaric acid/L), volatile acidity (g acetic acid/L), density (g/L), dry extract (g/L), and free and total sulphur dioxide (mg/L) were determined according to the EEC Regulation 2676, 2676/1990. The residual sugar content was measured through a Digital Wine Refractometer (WM-7, ATAGO, Tokyo, Japan) and expressed as °Brix. Dissolved oxygen (mg/L) was measured by using a LDO-HQ10 portable oxygen meter (Hach, Düsseldorf, Germany). The evaluation of the redox potential (EH) was performed with a CyberScan pH 510 (Eutec Instruments, Nijkerk, Netherlands) equipped with an encapsulated Ag/AgCl electrode (Crison, Lainate, MI, Italy). The EH were expressed in mV. pH values were measured with a CyberScan pH 510 (Eutec Instruments, Nijkerk,

Netherlands) calibrated with buffer solution at pH 4.00 and 7.00 (Crison, Lainate, MI, Italy).

2.3. Determination of phenolic compounds, phenolic profile, and antioxidant activity

The total phenolic content was measured at 765 nm through an UV-visible spectrophotometer (Cary 50 SCAN; Varian, Palo Alto, CA) according to the Folin-Ciocalteu method as reported by Singleton and Rossi (1965). Results were expressed as gallic acid equivalents (mg/L of wine). A calibration line was built on the basis of solutions of known and increasing concentrations of gallic acid (Extrasynthèse, Genay, France). The various phenolic classes were analyzed according to the methods of Di Stefano et al. (Di Stefano, Cravero, & Gentilizi, 1989) and Di Stefano and Cravero (1991). When necessary, the extracts were opportunely diluted with aliquots of the extraction solution. The results were expressed as mg per L of wine.

The phenolic profiles of wine were analyzed by HPLC-DAD-ESI-MS/MS. The chromatographic analyses were performed according to the method described by Crupi et al. (2012), with some changes. A Capillary HPLC 1100 Series system, equipped with a degasser, quaternary pump, thermostated column compartment, diode array detector and MSD Trap XCT Plus in a series configuration (Agilent Technologies, Palo Alto, California, U.S.A.) coupled with an ESI interface was used. The reversed stationary phase employed was a Poroshell 120 SB-C18 2.7 μm (150×2.1 mm i.d., Agilent Technologies) thermostated at 40 °C. The following gradient system was used with water containing 1% formic acid (solvent A) and acetonitrile (solvent B): 0 min, 0% B; 2 min, 5% B; 10 min, 13% B; 25 min, 15% B; 30 min, 22% B; 50 min, 22% B; 55 min, 95% B; 65 min, 95% B; 66 min, 5% B; stop time to 66 min followed by washing and re-equilibrating the column. The flow was maintained at 0.2 mL/min; sample injection was 8 μL . Diode array detection was between 250 and 650 nm, and absorbance was recorded at 280, 313, 350 and 520 nm. Both positive and negative electrospray mode were used for ionization of molecules with capillary voltage of 3500 V and a skimmer voltage at 40 V. The nebulizer pressure was 40 psi and the nitrogen flow rate was 8 L/min. Temperature of drying gas was 350 °C. The monitored mass range was from m/z 50 to 1200. Wine samples were filtered through a 0.45 μm syringe Cellulose Acetate filter prior to HPLC injection. Identification was achieved by combining different information: elution pattern, UV-Vis and MS spectra, MS/MS fragmentation patterns and with the help of structural models already hypothesized in the literature. Quantification was made using the external standard method. The calibration curves were obtained by injection of standard solutions under the same conditions used for the samples and over the range of concentrations observed. All phenolic compounds were expressed in (+)-catechin equivalents (CE, mg/L; $R^2 = 0.9945$).

The evaluation of the antioxidant activity was made through the 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Brand-Williams, Cuvelier, & Berset, 1995), and 2,2-azinobis-(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS) (Re et al., 1999) assays and the results were expressed as mmol of Trolox equivalents/L of wine.

2.4. Determination of volatile composition

Volatile composition of wine was analyzed by head-space solid phase microextraction hyphenated with gas chromatography – mass spectrometry (HS-SPME-GC-MS). The chromatographic analysis were performed according to the method described by Canuti et al. (2009) and Tao et al. (Tao, Li, Wang, & Zhang, 2008), opportunely modified. For HS-SPME-GC-MS analyses, wine samples (5.0 mL) were transferred into a 20 mL glass headspace vials containing 1 g of NaCl; 2.5 μL of a octan-3-ol internal standard solution (83 mg/L^{-1} in ethanol) were added to each vial. The mixtures were carefully shaken to dissolve NaCl and then left to equilibrate 1 h in the dark at room temperature before the analysis.

The SPME fiber coating used in this study was made of polydimethylsiloxane (PDMS), 100 μm thickness and 23 gauge (Supelco, Bellefonte, PA, USA). The wine samples were warmed to 40 °C for 10 min before exposure of the SPME fiber to the headspace. Extraction times of 30 min with continuous stirring (250 rpm) were applied. GC-MS was performed using an Agilent 6890 N gas chromatograph (Little Falls, DE, USA) equipped with a Gerstel MPS autosampler (Gerstel, Baltimore, MD, USA) coupled with an Agilent 5975 mass selective detector. The software used was MSD ChemStation (Agilent). SPME injections were made in splitless mode using a SPME injection sleeve (0.75 mm I.D.) at 250 °C for 350 s. During this time, the thermal desorption of analytes from the fiber occurred in a DB-Wax column (60 m \times 0.25 mm I.D., 0.25 μm film thickness) (J & W Scientific, Folsom, CA, USA). Helium carrier gas was used with a total flow of 1.0 ml/min. The oven parameters were the following: initial temperature, 40 °C for 1.0 min, increase to 200 °C at a rate of 4 °C/min⁻¹, maintenance at 200 °C for 20 min before returning to the initial temperature. The total cycle time was 61 min. The MS detector operated in scan mode (mass range 30–500) and the transfer line to the MS system was maintained at 250 °C. The identity of peaks was assigned using the NIST 05 Library.

The relative areas of the compounds were normalized with respect to the internal standard area. The samples were analyzed in duplicate and blank runs were made with empty glass vial before each analysis.

2.5. NMR spectroscopic analysis

The application of nuclear magnetic resonance (NMR) spectroscopy to the wines makes possible the fast and simultaneous determination of a wide number of compounds, providing qualitative and quantitative information, in a non-invasive way (Gemma, Clark, Barnett, Niere, & Adams, 2008). This analysis was performed according to a previous work (Baiano et al., 2012). The wine samples were analyzed by using ¹H NMR spectroscopy with the purpose to obtain indications about samples aged in the different types of containers. From a spectroscopic point of view, the useful multi-suppression of the intense signals due to water and ethanol by the NOESYGPPS sequence allowed to enlarge the dynamic range of the analysis by increasing the receiver gain value. Moreover, the pulse sequence made it possible to gain clear NMR information without time-consuming sample pre-treatments, such as freeze-drying processes (and consequent re-dissolution in deuterated solvents), not only avoiding to evaporate the solvent, but also minimizing the risk to lose volatile species during the evaporation process. A bucket data set was generated from the NMR spectra, considering the spectral region 0.1–10 ppm, with the exclusions of the spectral portions where the residues of water (about 4.8 ppm) and ethanol signals (about 1.17 and 3.65 ppm) were observable; the bucket width was 0.04 ppm and the spectra were all scaled to the total intensity before proceeding with the bucketing.

2.6. Statistical analysis

Each analysis was replicated at least five times, where not differently specified. The averages and the standard deviations were calculated using Excel software V. 11.5.1 (Microsoft, Redmond, WA). The statistical treatment of the results of the conventional analysis was performed using the package Statistica for Windows V. 8.0. (Statsoft Inc., Tulsa, OK) and SCAN software from Minitab Inc. (State College, PA, USA).

Concerning NMR data, the generation of input variables for statistical analysis was done via bucketing using AMIX 3.9.7 (Bruker BioSpin GmbH) and the obtained bucket tables were subjected to PCA by using Statistica for Windows, V. 8.0.

3. Results and discussion

3.1. Conventional analysis of wine

The wine samples were analyzed over a 12-months storage time for parameters with oenological meaning (Table 1).

At racking, the wines had high dry extract, good alcohol strength and titratable acidity, and low pH and volatile acidity values (Table 1). According to these data, the potential life expectancy of wines was long although it is well known that it also depends on storage conditions. Based on the residual sugar content at racking, vinification did not proceed until complete dryness but this behaviour was in agreement with Baiano et al. (2012), Baiano et al. (2013), who found that the low temperature of the cryogen promoted the later liberation of cellular components such as phenols, mannoproteins, sugars, and aromatic compounds in the liquid phase. Concerning the redox status of the wines, the management of the contact with oxygen during production and aging is a crucial factor for their final characteristics due to their phenolic concentration. Browning is the immediate consequence of the enzymatic oxidation of phenols and flavanols and is due to the formation of quinones and their addition products between oxidized and non-oxidized phenols (Rigaud, Cheynier, Souquet, & Moutounet, 1991). In the present study, the application of cryomaceration was chosen in order to enhance the varietal aroma of *Minutolo* wine since it come from a semi-aromatic grape variety. Nevertheless, it also determined a greater extraction of phenolics that, in a white wine, can be detrimental during storage since they can react through radical chain reactions thus producing hydrogen peroxide. The latter reacts with polyphenols and other wine components such as ethanol, oxidizing them (Singleton, 1987). Cryomaceration was therefore combined with a reductive vinification but, since the use of ascorbic acid only is not recommended (it initially decreases the redox potential but successively acts as an oxidant agent), sulphur dioxide, which is able to neutralize the reactive forms of oxygen, was also added (Peng, Duncan, Pocock, & Sefton, 1998). As a consequence of the preservation activity of ascorbic acid against oxidation of the free SO₂ and in accordance with Baiano et al. (2012; 1013a), wines at racking had a higher free/total ratio (38.7%), a very low redox potential, and low dissolved oxygen (the oxygen concentration in wine saturated with air is about 8.4 mg/L (Cheynier, Atanasova, Fulcrand, Mazauric, & Moutounet, 2002)).

During aging, a significant decrease of the alcohol content was observed, especially in wines stored in amphorae, due to evaporation and diffusion through the container walls. In addition, the reduction of alcohol strength in glass containers would suggest that it could be partially due to its oxidation to acetaldehyde by direct chemical reaction with air. This reaction occurs at an appreciable rate only by a coupled autoxidation of certain phenolic substances present in wine. First, oxygen interacts with phenolic compounds producing hydrogen peroxide as a by-product. Then, hydrogen peroxide reacts with ethanol and produces acetaldehyde and water (Wildenradt & Singleton, 1974). Obviously, density showed an inverse trend with respect to the alcohol content and decreased during aging. The volatile acidity significantly increased between 6 (data not shown) and 12 months of storage though it remained far below the sensory perception threshold (1 g/L). After 12 months, the highest volatile acidity (0.47 \pm 0.06 g/L) was observed in wines stored in glass containers. Titratable acidity decreased in wines aged in amphorae but not in glass containers. As highlighted in previous studies (Carrol & Starkey, 1971), clays react with acids and a cation exchange occurs, with the removal of small amounts of SiO₂, Al₂O₃, Fe₂O₃, and the formation of H-clays. The free SO₂ decreased during aging independently on the containers, whereas the total SO₂ increased, resulting in a decrease of the ratio between the two forms. The reason for the increase of the total and thus of the bound SO₂ forms could be the presence of acetaldehyde produced in the completely fermented wines by the already discussed chemical oxidation of ethanol. The lowest free/total SO₂ ratios were found in engobe (6%) and

Table 1Quality parameters of *Minutolo* wines aged in three types of amphorae and in glass containers at 0 and 12 months.

Aging time (months)	Alcohol (%vol)	Density (g/mL)	Redox pot.(mV)	Volatile ac. (g acetic ac./L)	Titrateable ac. (g tartaric ac./L)	Dry extract (g/L)	Free SO ₂ (mg/L)	Total SO ₂ (mg/L)	Residual sugar (°Brix)	pH	Dissolved O ₂ (mg/L)
In raw amphorae											
0	12.4 ± 0.4 b ¹	0.987 a	152.4 ± 0.1 a	0.26 ± 0.04 a	5.4 ± 0.4 b	20.2 ± 0.4 a	35.0 ± 1.3 b	90.5 ± 6.0 a	7.0 b	3.37 ± 0.02 a	5.62 ± 0.83 a
12	11.4 ± 0.6 a	0.993 b	157.2 ± 1.8 b	0.37 ± 0.06 b	4.9 ± 0.1 a	21.0 ± 0.8 b	31.4 ± 3.8 a	158.1 ± 3.8 b	6.3 ± 0.1 a	3.38 ± 0.06 a	6.77 ± 0.05 b
In glazed amphorae											
0	12.4 ± 0.4 b	0.987 a	152.4 ± 0.1 a	0.26 ± 0.04 a	5.4 ± 0.4 b	20.2 ± 0.4 a	35.0 ± 1.3 b	90.5 ± 6.0 a	7.0 b	3.37 ± 0.02 a	5.62 ± 0.83 b
12	11.1 ± 0.1 a	0.994 b	152.2 ± 1.2 a	0.34 ± 0.03 b	4.8 ± 0.6 a	24.4 ± 1.6 b	9.0 ± 1.5 a	129.9 ± 5.7 b	6.5 a	3.47 ± 0.22 a	4.75 ± 0.62 a
In engobe amphorae											
0	12.4 ± 0.4 b	0.987 a	152.4 ± 0.1 a	0.26 ± 0.04 a	5.4 ± 0.4 b	20.2 ± 0.4 a	35.0 ± 1.3 b	90.5 ± 6.0 a	7.0 b	3.37 ± 0.02 a	5.62 ± 0.83 b
12	11.0 ± 0.7 a	0.995 b	157.0 ± 0.9 b	0.34 ± 0.02 b	4.8 ± 0.3 a	24.6 ± 2.3 b	10.9 ± 1.3 a	190.1 ± 6.4 b	6.3 a	3.38 ± 0.01 a	4.79 ± 0.58 a
In glass containers											
0	12.4 ± 0.4 b	0.987 a	152.4 ± 0.1 a	0.26 ± 0.04 a	5.4 ± 0.4 a	20.2 ± 0.4 a	35.0 ± 1.3 b	90.5 ± 6.0 a	7.0 b	3.37 ± 0.02 b	5.62 ± 0.83 a
12	11.8 ± 0.4 a	0.994 b	171.1 ± 0.3 b	0.47 ± 0.06 b	5.1 ± 0.2 a	22.8 ± 0.3 b	19.2 ± 1.5 a	166.6 ± 5.5 b	6.4 a	3.21 ± 0.02 a	7.22 ± 0.02 b

¹ In column, for each type of container, different letters indicate significant differences at $p < 0.05$ by LSD multiple range test.

glazed amphorae (7%). It means that those wines had the highest amounts of bound SO₂, a result that was well correlated with their lowest levels of dissolved oxygen whose consumption was due to the occurred oxidation reactions. The free/total SO₂ values for wines in glass containers and raw amphorae were 11 and 20%, respectively. These samples also showed the highest values of dissolved oxygen and, in the case of wines aged in glass containers, the highest redox potential.

3.2. Phenolic content and antioxidant activity of wines

Redox potential, amount of dissolved oxygen, and concentrations of native antioxidants determine the resistance of wine to oxidation (Oliveira, Silva Ferreir, Guedes de Pinho, & Hogg, 2002). In particular, phenolics exhibit a great capacity to consume oxygen due to the presence of hydroxyl groups (Vivas & Glories, 1996). The phenolic composition greatly changes in grapes and wines as a consequence of ripening, drying process, and aging. For example, the study of the raisining process on Garnacha Tintorera grapes highlighted increases of anthocyanins, flavonols, esters of hydroxycinnamic acids, flavan-3-olmonomers and proanthocyanidins. The lower increase was observed for esters of hydroxycinnamic acids, which undergone strong enzymatic degradations (Figueiredo-González, Cancho-Grande, & Simal-Gándara, 2013). Table 2 shows the evolution of several parameters related to the wines antioxidants (concentration of several phenolic classes, total phenolic content, antioxidant activity) during aging in amphorae and in glass containers. All the phenolic classes exhibited significant changes of the concentrations during aging although the more severe reduction occurred in the concentration of flavans reactive with vanillin and proanthocyanidins, while the hydroxycinnamoyl tartaric acids exhibited only limited decreases. In particular, after 12 months, flavonoids

decreased by about 32–34% in wines aged in raw amphorae and in glass containers and by about 26% in wines aged in glazed and engobe amphorae; the concentrations of flavans reactive with vanillin dropped by 76% in raw and engobe amphorae, 70% in glazed amphorae, and 60% in glass containers; hydroxycinnamoyl tartaric acids were reduced by 9% in glass containers, 8% in engobe amphorae, and 6% in raw amphorae, while remained unchanged in the glazed ones; proanthocyanidins decreased by 53% in glass containers, 46–47% in raw and glazed amphorae, 37% in engobe amphorae; the total phenolic content showed decreases of 14% in glazed amphorae and glass containers, 11% in raw amphorae, and 9% in engobe amphorae. The high loss of flavans reactive with vanillin and proanthocyanidins during aging was due to their strong antiperoxidative activity (Saija et al., 1995). Riedl et al. (Riedl, Carando, Alessio, McCarthy, & Hagerman, 2002) demonstrated that both hydrolysable and condensed tannins scavenge free radicals in a kinetically involving both a fast and a slow scavenging step. Four moles of radical were scavenged per ortho-substituted diphenol group of monomeric and polymeric phenolic compounds.

The antioxidant activity of wines was measured according to the DPPH and ABTS assays (Table 2). Both assays use a stable free radical and give information on the radical scavenging or antiradical activity. DPPH is a stable radical whose absorption at 515 nm decreases when it is reduced by an antioxidant and is widely used to determine the antiradical or antioxidant activity of purified phenolics or of natural extracts while the ABTS assay measures the ability of antioxidants to scavenge the ABTS generated in aqueous phase. The data reported in Table 2 highlight the decrease of antiradical/antioxidant activity during aging but also the absence of correlations between the antiradical/antioxidant activity measured by the two techniques thus suggesting a reducing effect on only one of the two color reactants, according with the finding of

Table 2Phenolic classes and antioxidant activity of *Minutolo* wines aged in three types of amphorae and in glass containers at 0 and 12 months.

Aging time (months)	Flavonoids (mg (+) -catechin/L)	Flavans reactive with vanillin (mg (+) -catechin/L)	Hydroxycinnamoyl-tartaric acids (mg caffeic ac./L)	Total phenolic component (mg gallic ac./L)	Proanthocyanidins (mg cyanidin chloride/L)	DPPH antiox. activity (mmol trolox/L)	ABTS antiox. activity (mmol trolox/L)
In raw amphorae							
0	167.4 ± 10.0 b ¹	181.8 ± 16.0 b	76.5 ± 11.7 b	557.8 ± 12.7 b	317.9 ± 49.7 b	0.05 b	2.32 ± 0.21 b
12	110.8 ± 8.5 a	43.6 ± 0.7 a	71.5 ± 8.0 a	497.5 ± 5.9 a	167.0 ± 11.6 a	0.04 ± 0.01 a	1.80 ± 0.18 a
In glazed amphorae							
0	167.4 ± 10.0 b	181.8 ± 16.0 b	76.5 ± 11.7 a	557.8 ± 12.7 b	317.9 ± 49.7 b	0.05 b	2.32 ± 0.21 b
12	123.6 ± 4.9 a	53.6 ± 1.7 a	77.0 ± 5.1 a	479.5 ± 19.3 a	170.4 ± 38.4 a	0.04 ± 0.01 a	1.65 ± 0.13 a
In engobe amphorae							
0	167.4 ± 10.0 b	181.8 ± 16.0 b	76.5 ± 11.7 b	557.8 ± 12.7 b	317.9 ± 49.7 b	0.05 b	2.32 ± 0.21 b
12	123.6 ± 4.9 a	43.7 ± 10.5 a	70.3 ± 3.8 a	507.6 ± 17.2 a	200.4 ± 32.7 a	0.04 ± 0.01 a	1.93 ± 0.18 a
In glass containers							
0	167.4 ± 10.0 b	181.8 ± 16.0 b	76.5 ± 11.7 b	557.8 ± 12.7 b	317.9 ± 49.7 b	0.05 b	2.32 ± 0.21 b
12	112.9 ± 12.8 a	73.4 ± 7.4 a	69.5 ± 6.2 a	478.7 ± 8.2 a	150.3 ± 20.0 a	0.04 ± 0.01 a	1.69 ± 0.26 a

¹ In column, for each type of container, different letters indicate significant differences at $p < 0.05$ by LSD multiple range test.

Martysiak-Zurowska and Wenta (2012). In particular, the DPPH assay gave lower antiradical/antioxidant values than the ABTS assay and identical results for aging in all the types of containers, probably due to its lower sensitivity and slower reaction with antioxidants. According to the ABTS assay, the highest antioxidant activity was observed in wines aged in engobe amphorae, followed by those aged in raw amphorae. The lowest values were showed by the wines aged in glass containers and glazed amphorae. These stronger decrease of antioxidant activity observed in the types of container that would be better protect wine against oxygen injuries, could be related to the hypothesis that part of the oxygen that permeated through the amphorae walls was consumed for the oxidation of ethanol to acetaldehyde catalyzed by transition metals and thus it did not involve the phenolic oxidation and other detrimental changes.

In the analysis of individual phenolic contents, 12 compounds were identified and quantified as follows: caftaric acid, procyanidins B2, B3 and B4, catechin, epicatechin, fertaric + ferulic, *cis*-coutaric, *trans*-coutaric, and coumaric acids, and astilbin. Table 3 includes the mean phenolic contents and standard deviations thus showing that, during aging, the phenolic profiles significantly changed in all the types of containers due to hydrolysis, oxidation, and complexation reactions (Zafrilla et al., 2003). Compounds such as caftaric acid, procyanidin B4 (a dimeric procyanidin), and astilbin decreased in all the wines. Caftaric acid was quickly oxidized, and its concentrations decreased by 18, 12, 11, and 9% in glass containers, engobe, raw, and glazed amphorae, respectively. Caftaric acid could hydrolyze to caffeic acid and its methyl ester but those compounds were under the detection limits in the wine samples also after 12 months. The concentrations of procyanidin B4, and astilbin fell below the detection limits. The content of procyanidin B3 was reduced by 13 and 10% in glass containers and raw amphorae, respectively, while it remained unchanged in glazed and engobe amphorae. The results concerning procyanidins B3 and B4 can be understood on the base of the activation energies of their degradative reactions (Dallas, Ricardo Da Silva, & Laureano, 1995). First of all, the degradation of oligomeric procyanidins is always a first-order kinetic reaction, but procyanidin B3 has a higher activation energy value than B4. This means that B3 is a more stable procyanidin than B4. Also the degradation process of astilbin follows a first-order kinetic model and its reduction under the detection limit could be related to the presence of metal ions (Zhang, Fu, Huang, Shangguang, & Guo, 2013). Epicatechin showed a reduction of 45, 39, and 17% in raw amphorae, engobe, and glazed amphorae. Instead, the concentration of procyanidin B2 considerably increased during aging in all the containers. The reason could be related to its high stability, as indicated by the activation energy of its degradative reactions and to the hydrolysis of galloylated dimeric and trimeric procyanidins that occur at wine pH (Dallas et al., 1995). Also, the content of coumaric acid increased with aging in all the wines due to the possible hydrolysis of the corresponding hydroxycinnamic ester

(coutaric), as reported by Budic-Leto and Lovric (2002). In fact, although the concentrations of the *trans*-coutaric form remained substantially unchanged during aging in the different types of containers, the concentrations of the *cis*- form decreased with time in all the containers with the exception of the glazed amphorae. Ferulic acid and the corresponding hydroxycinnamic ester (fertaric acid) coeluted and their overall concentrations increased with aging (with the exception of the wines in glass container), probably as a consequence of the decrease of the precursor and of the increase of the ferulic acid. Flavonols such as quercetin were under their detection limits.

The phenolic profiles of grapes and wines can be used as a fingerprint. Using cluster analysis and principal components analysis, Figueiredo-González et al. (2012) found that Gran Negro was characterised by its content of isorhamnetin-3-O-glucoside and syringetin-3-O-glucoside and, along with Mouratón, by its myricetin conjugates while flavonol profile could be unsuitable as fingerprint of Brancellao variety. Concerning Brancellao and Gran Negro berries, no differences were observed in anthocyanin and flavonol contents collected from tips and shoulders thus indicating that is not necessary to harvest them separately to obtain a high quality red wine while, in the case of Mouratón, grapes located inside the shoulder bunch receive less sunlight radiation than those located inside the tip bunch and this fact could explain the lower flavonol and anthocyanin concentrations of the shoulders (Figueiredo-González et al. (2012a,b, 2013)). The phenolic composition determined by means of UV–Vis spectrophotometry was also used to discriminate within different red Garnacha Tintorera-based wines (a dry base wine, a naturally sweet wine, and a fortified sweet wine). In particular, fortified wine exhibited the lowest content because the maceration-alcoholic fermentation was stopped through the addition of alcohol before the complete diffusion of red pigments from skins to must. Instead, the naturally sweet wine showed the highest phenolic content due to the evaporation of water from the grapes (Figueiredo-González, Cancho-Grande, & Simal-Gándara, 2013b). The detailed phenolic composition of the same three types of wine highlighted that: the highest concentration of total proanthocyanidins was detected in the dry wine, followed by the naturally sweet wine, and finally by the fortified sweet wine; no difference in the mean degree of polymerisation was found between dry and naturally sweet wine whereas a slightly lower value was obtained for the fortified wine; the total anthocyanin concentration showed the highest values in the dry wine, followed by the naturally sweet wine, and by the fortified sweet wine (Figueiredo-González, Regueiro, Cancho-Grande, & Simal-Gándara, 2014). The evolution of the phenolic compounds in red Garnacha Tintorera-based wines was used to discriminate among young and aged wines. Compounds such as anthocyanins, esters of hydroxycinnamic acids, flavan-3-ols monomers, oligomers, polymers, and mean degree of polymerisation of proanthocyanidins decreased while hydroxybenzoic and

Table 3
Phenolic composition (expressed as mg per L of wine) of *Minutolo* wines aged in three types of amphorae and in glass containers at 0 and 12 months.

Aging time (months)	Caftaric ac.	Procyanidin B3	Procyanidin B4	Procyanidin B2	Catechin	Cis-Coutaric ac.	Trans-Coutaric ac.	Fertaric ac + Ferulic ac.	Epicatechin	Coumaric ac.	Astilbin
In raw amphorae											
0	187.2 ± 7.6 b ¹	42.2 ± 1.4 a	1.1 b	2.0 ± 0.1 a	13.8 ± 0.9 a	56.7 ± 0.9 b	140.7 ± 3.3 a	13.8 ± 0.6 a	1.8 b	63.5 ± 1.7 a	18.8 ± 0.1 b
12	167.7 ± 4.7 a	37.9 ± 0.2 a	n.d. a	8.7 ± 0.1 b	22.0 ± 0.1 b	48.7 ± 0.3 a	136.4 ± 1.0 a	16.0 ± 0.3 b	1.0 ± 0.2 a	78.6 ± 3.4 b	n.d. a
In glazed amphorae											
0	187.2 ± 7.6 a	42.2 ± 1.4 a	1.1 b	2.0 ± 0.1 a	13.8 ± 0.9 a	56.7 ± 0.9 a	140.7 ± 3.3 a	13.8 ± 0.6 a	1.8 b	63.5 ± 1.7 a	18.8 ± 0.1 b
12	170.8 ± 12.9 a	40.0 ± 0.9 a	n.d. a	9.8 b	20.6 ± 1.4 b	47.8 ± 4.3 a	146.7 ± 5.1 a	16.3 ± 1.7 b	1.5 a	80.1 ± 5.8 b	n.d. a
In engobe amphorae											
0	187.2 ± 7.6 a	42.2 ± 1.4 a	1.1 b	2.0 ± 0.1 a	13.8 ± 0.9 a	56.7 ± 0.9 b	140.7 ± 3.3 a	13.8 ± 0.6 a	1.8 b	63.5 ± 1.7 a	18.8 ± 0.1 b
12	165.1 ± 5.2 a	41.2 ± 2.4 a	n.d. a	8.8 ± 0.4 b	20.7 ± 0.3 b	47.7 ± 1.0 a	143.5 ± 6.5 a	16.1 ± 0.1 b	1.1 ± 0.1 a	85.4 ± 3.0 b	n.d. a
In glass containers											
0	187.2 ± 7.6 b	42.2 ± 1.4 a	1.1 b	2.0 ± 0.1 a	13.8 ± 0.9 a	56.7 ± 0.9 b	140.7 ± 3.3 a	13.8 ± 0.6 a	1.8 a	63.5 ± 1.7 a	18.8 ± 0.1 b
12	153.4 ± 10.4 a	36.7 ± 0.7 a	n.d. a ²	8.3 ± 0.1 b	23.7 ± 0.8 b	41.7 ± 4.1 a	144.0 ± 2.9 a	13.9 ± 0.3 a	1.6 ± 0.4 a	81.9 ± 0.5 b	n.d. a

¹ In column, for each type of container, different letters indicate significant differences at p < 0.05 by LSD multiple range test.

² n.d.: not detect.

hydroxycinnamic acids and vitisins increased with aging (Figueiredo-González et al., 2014). The phenolic concentration can be used as fingerprint in order to highlight the influence of grape variety, vine system and enological treatments, as observed by Pérez-Lamela, García-Falcón, Simal-Gándara, and Orriols-Fernández (2007) in the red grape varieties Sousón, Mencía and Brancellao. Figueiredo-González, Cancho-Grande, and Simal-Gándara (2013c) also highlighted the influence on phenolic composition of sugar concentration processes in dried-on- (botrytized, late harvested, frozen) and dried-off-vine (sun-dried, natural dried under cover, chamber-dried) grapes and their aged or fortified sweet wines. The liquid chromatographic separation with ultraviolet detection of phenolic acids such as caffeic acid, p-coumaric acid, gallic acid, gentisic acid, ferulic acid and salicylic acid, flavonols such as quercetin,

and flavanols such as (+)-catechin and (–)-epicatechin was presented as an analytical tool useful in quality control of alcohol-free beers (Alonso García, Cancho-Grande, & Simal-Gándara, 2004). The flavanolic composition of Tempranillo red wines was used to predict the sensory-determined astringency. In particular, epicatechin subunits in extension positions and galocatechin subunits in terminal positions were positively correlated with astringency, while epigallocatechin in both extension and terminal positions was negatively correlated with it (Quijada-Morín et al., 2012). In the present study, the Principal Component Analysis applied to results of the conventional analyses, concentration of phenolics, and antioxidant activity values allowed to highlight the differences between wines at racking and those after 12 months of aging (Fig. 1a) and, after the 12-months aging, among the wines

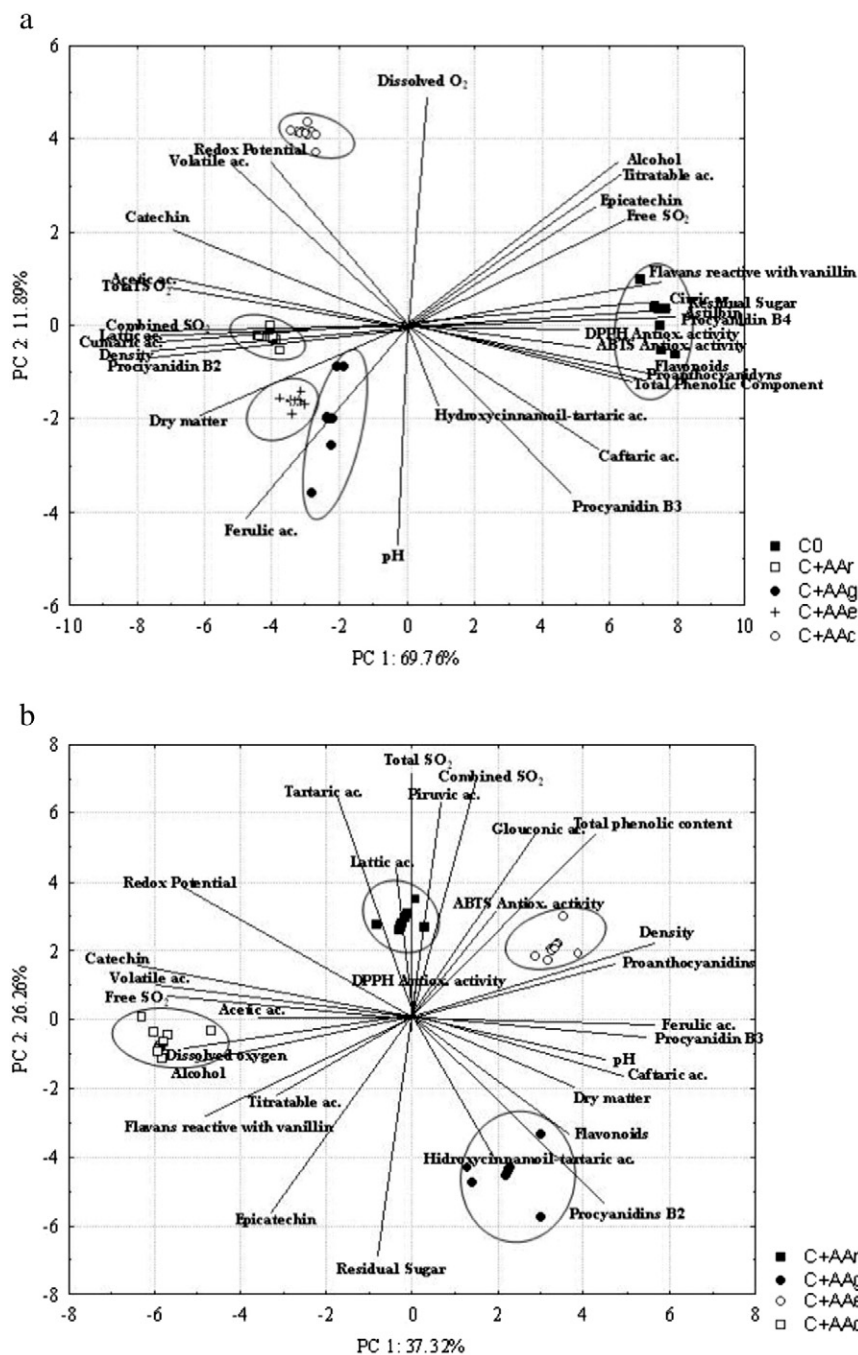


Fig. 1. PCA scatter plots based on results of the conventional analyses, concentration of phenolics, and antioxidant activity values. Projection on the factor plane of a) wines analyzed at racking and after 12 months of storage b) wines analyzed 12 months after racking. C0: wine at racking; C + AAg: wine aged in glazed amphora; C + AAr: wine aged in raw amphorae; C + AAe: wine aged in engobe amphorae; C + AAC: wine aged in glass containers.

Volatile compounds (AU x105) detected into the wine at racking and after 12 months-aging in the 4 different types of containers.

	Wines (mean concentration expressed as AU x 10 ⁵)					
Compounds	At racking	In glazed amphorae	In raw amphorae	In engobe amphorae	In glass containers	Odour description
<i>Acids</i>						
Acetic acid	18.7 ± 1.6	21.6 ± 1.0	27.92 ± 0.76	22.6 ± 8.8	42.8 ± 2.0	Vinegar
Hexanoic acid	63.94 ± 0.45	45 ± 15	49 ± 83	72.6 ± 3.5	68 ± 15	Cheese, fatty, sour
Octanoic acid	659 ± 12	105 ± 11	131.54 ± 0.64	152 ± 13	170 ± 14	Fatty acid, dry, dairy
Nonanoic acid	77 ± 43	100 ± 60	85 ± 25	74 ± 43	60 ± 53	Cheese, waxy
N-decanoic acid	470 ± 120	31 ± 11	21.06 ± 0.53	47.57 ± 0.18	27 ± 12	Fatty acid, dry, woody
9-decenoic acid	30.2 ± 8.6	n.d. ¹	n.d.	n.d.	n.d.	
Dodecanoic acid	13.6 ± 1.6	5.9 ± 1.7	4.82 ± 0.36	6.67 ± 0.97	4.2 ± 5.7	Fatty
Total	1334	308	319	375	372	
Percentage (%)	4.6	3.0	2.6	1.1	0.6	
<i>Alcohols</i>						
1-propanol, 2-methyl-	220.3 ± 3.2	170 ± 35	214 ± 68	269.8 ± 6.7	371 ± 13	Alcohol, solvent
1-butanol	11.9 ± 2.4	19.8 ± 5.7	17.0 ± 1.5	16.9 ± 2.1	13.22 ± 0.91	Medicinal, phenolic
1-butanol, 3-methyl-	5014 ± 52	3687 ± 250	4700 ± 110	4600 ± 170	6302 ± 39	Fusel, alcohol, sweet, fruity
1-butanol, 2-ethyl-	n.d.	14.8 ± 2.6	6.56 ± 0.18	24.69 ± 0.75	n.d.	
1-pentanol, 3-methyl	19.33 ± 0.37	17.5 ± 1.7	21.9 ± 1.4	17.7 ± 2.4	22.03 ± 0.48	
1 hexanol	90.87 ± 0.76	70.6 ± 4.8	92.7 ± 3.5	2.11 ± 0.35	113.0 ± 4.9	Herbaceous
3-hexen-1-ol [Z]	16.30 ± 0.75	14.80 ± 0.30	22.89 ± 0.71	15.33 ± 0.96	20.00 ± 0.15	Plant, fruity, aromatic
1-hexanol, 2-ethyl	n.d.	103.2 ± 9.1	105.6 ± 3.2	84.6 ± 2.3	39 ± 14	
1-octanol	30.24 ± 0.07	n.d.	n.d.	n.d.	n.d.	Orange, floral
P-menth-1-en-8-ol	150.8 ± 2.6	376 ± 41	521.3 ± 4.8	455 ± 27	300 ± 100	
6-octen-1-ol, 3,7-dimethyl-	71.89 ± 0.87	87 ± 17	37.5 ± 2.2	n.d.	n.d.	
Phenylethyl Alcohol	1493.5 ± 1.2	1568 ± 120	1963.3 ± 5.9	1521 ± 20	1809 ± 22	Flowery, rose, honey
Total	7120	6129	7684	6970	8986	
Percentage (%)	24.3	59.6	61.7	60.9	63.2	
<i>Acetic esters</i>						
Isobutyl acetate	34.6 ± 2.8	n.d.	n.d.	n.d.	n.d.	Apple, banana, pear, sweet
Isoamyl acetate	3900 ± 290	130 ± 11	313.5 ± 3.8	297 ± 10	780 ± 140	Banana, pear
Isobornyl acetate	90.8 ± 2.8	n.d.	n.d.	n.d.	n.d.	
2-phenethyl acetate	692.4 ± 3.4	215 ± 16	239.3 ± 2.9	216 ± 17	174 ± 17	Floral, pineapple, rose, sweet, citrus
Total	4749	345	553	514	951	
Percentage (%)	16.2	3.4	4.4	4.5	6.7	
<i>Ethyl esters</i>						
Ethyl butyrate	91.4 ± 5.2	44.9 ± 5.4	40.9 ± 3.1	55.2 ± 7.2	77.5 ± 8.1	Banana, pineapple, sweet, ethereal
Ethyl 2-methylbutyrate	11.4 ± 2.1	5.2 ± 2.7	9.2 ± 1.1	6.410 ± 0.05	9.85 ± 0.31	
Ethyl 3-methylbutyrate	15.3 ± 1.2	n.d.	8.28 ± 0.07	16.0 ± 1.9	22.4 ± 2.4	
Caproic acid ethyl ester	1500 ± 140	292 ± 11	280.86 ± 0.51	299 ± 15	263 ± 36	Apple, banana, wine-like
Ethyl 3-hexenoate	3.00 ± 0.04	n.d.	n.d.	n.d.	n.d.	
Ethyl heptanoate	9.50 ± 0.98	7.000 ± 0.060	7.09 ± 0.12	6.14 ± 0.12	13.05 ± 0.77	Fruity
Ethyl octanoate	7100 ± 450	937 ± 23	1024 ± 15	1205 ± 22	918 ± 21	Banana, floral, pear, pineapple, wine-like
Ethyl nonanoate	19.4 ± 0.7	13.1 ± 1.9	12.19 ± 0.71	12.09 ± 0.09	17.63 ± 0.68	Oily, fruity, nutty
Ethyl decanoate	3364 ± 29	313 ± 26	338 ± 43	436 ± 11	306.9 ± 7.6	Grap, oily, wine-like
Diethyl succinate	72.6 ± 2.8	448 ± 49	513.3 ± 5.8	445 ± 8	543 ± 29	Apple, apricot, chocolate, cranberry
Ethyl 9-decenoate	839.3 ± 4.8	n.d.	n.d.	n.d.	n.d.	
Ethyl benzoate	n.d.	14.6 ± 6.2	n.d.	n.d.	n.d.	Anise, balsam, cherry, grape, floral
Ethyl laurate	347 ± 48	27.0 ± 2.4	18.8 ± 4.5	26.9 ± 2.5	15.8 ± 1.6	Green, fruity, floral
Ethyl myristate	34.9 ± 7.3	n.d.	n.d.	n.d.	n.d.	Waxy, soapy
Ethyl palmitate	136 ± 24	n.d.	60.7 ± 8.5	n.d.	n.d.	Waxy

Table 4 (continued)

Compounds	Wines (mean concentration expressed as AU x 10 ⁵)					Odour description
	At racking	In glazed amphorae	In raw amphorae	In engobe amphorae	In glass containers	
Nonanal	5.35 ± 0.62	n.d.	n.d.	n.d.	n.d.	Apple, coconut, grapefruit, lemon
B-damascenone	33.8 ± 2.8	n.d.	n.d.	n.d.	n.d.	
Total	39.12	n.d.	n.d.	n.d.	n.d.	
Percentage (%)	0.1	0.0	0.0	0.0	0.0	
<i>Enols</i>						
Linalool	1249.6 ± 7.5	751 ± 56	957.5 ± 2.9	814 ± 90	591 ± 260	Lemon, orange, citrus, floral, sweet
Hotrienol	30.89 ± 0.87	24.3 ± 4.2	39.1 ± 6.2	30.47 ± 0.48	31 ± 19	
Geraniol (E)	32.73 ± 0.18	39.2 ± 7.7	47 ± 11	38.7 ± 9.9	70.1 ± 3.3	
Nerol	73.5 ± 2.3	123 ± 39	168 ± 15	127 ± 11	125 ± 20	
Nerolidol (Z)	24.1 ± 2.5	n.d.	n.d.	n.d.	n.d.	Fruity, rose, sweet Apple, green, woody, citrus, rose
Total	1411	938	1212	1011	818	
Percentage (%)	4.8	9.1	9.7	8.8	5.8	
<i>Terpenes</i>						
Terpinolene	62.7 ± 2.7	5.0 ± 1.6	6.79 ± 0.13	2.85 ± 0.01	7.0 ± 1.3	Plastic Herbaceous, minty, camphoraceous
D-limonene	38.27 ± 0.92	13.2 ± 0.8	22.71 ± 0.42	19.2 ± 9.5	7.90 ± 0.47	
Total	101	18	29	22	15	
Percentage (%)	0.3	0.2	0.2	0.2	0.1	
<i>Ethers</i>						
Geranyl ethyl ether	115.5 ± 5.7	n.d.	n.d.	n.d.	n.d.	
Percentage (%)	0.4	0.0	0.0	0.0	0.0	
<i>Aromatics</i>						
Benzene, 1,3-bis[1,1-dimethylethyl]-	24.07 ± 0.31	167.2 ± 1.8	129.4 ± 3.5	80.82 ± 0.45	43.89 ± 0.21	Almond, cherry, spicy, sweet Musty, vanilla
2,4-dimethylbenzaldehyde	49.8 ± 5.4	46.8 ± 4.0	87.2 ± 6.8	38.1 ± 3.8	930 ± 140	
Butylate hydroxytoluene	15.64 ± 0.90	n.d.	n.d.	n.d.	n.d.	
Phenol, 2,4-bis(1,1-dimethylethyl)-	174 ± 23	112.6 ± 9.9	69 ± 21	55.1 ± 9.8	17.6 ± 1.9	
Indol	20.59 ± 0.51	n.d.	n.d.	n.d.	n.d.	Butter, cheese, chocolate, grape, honey
Total	284	327	285	174	991	
Percentage (%)	1.0	3.2	2.3	1.5	7.0	

The odour descriptors were obtained from Capone et al.⁴⁰ and SAFC “Flavors and Fragrances, European Ed. Catalogue 2007–2008”.

Percentages are referred to the volatile compounds respect to each chemical group in the specific containers.

n.d.: not detect.

aged in the 4 different types of containers (Fig. 1b). Fig. 1a clearly shows that the first two principal components accounted for 69.8 and 11.9% of the total variation, respectively, and that the points representing wines at racking are placed in the half-plane identified by positive values of the first component while those representing the wines after 12 months are all located in the part of the plane identified by the negative values of the same component. After 12 months, with the first two principal components accounted for 37.2 and 26.3% of the total variation, the wines aged in the various types of container were well separated from each other (Fig. 1b).

3.3. Volatile composition of wines

Literature teaches that the levels of volatile components can strongly change by the effect of agronomic and technological practices. According to González-Rodríguez, Noguerol-Pato, González-Barreiro, Cancho-Grande, and Simal-Gándara (2011), residual levels of new generation fungicides such as benalaxyl, iprovalicarb and piraclostrobin used in vineyard reduced the varietal aroma of Godello white wines attributed to geraniol but increased the fruity aroma due to ethyl esters (ethyl hexanoate, ethyl octanoate, and ethyl decanoate) and acetates (3-methyl-1-butyl acetate and 2-phenylethyl acetate) as a consequence of the increase of the Odour Activity Values of these compounds. The stepwise discriminant analysis of volatile compounds in wines produced from Godello grapes treated with the fungicides cyazofamid, famoxadone, mandipropamid, and valifenalate highlighted that the concentrations of 2-phenylethyl acetate, ethyl butanoate, ethyl octanoate, 4-vinylguaiacol, 3-methylbutanoic acid and methionol would be suitable to discriminate between different types of treatments (González-Álvarez, González-Barreiro, Cancho-Grande, & Simal-Gándara, 2012). The treatments also affected the sensory

properties of wines as evaluated by a trained sensorial testing panel determining increases of typical fermentative odours associated with esters and of ripe fruit tastes (González-Álvarez, Noguerol-Pato, González-Barreiro, Cancho-Grande, & Simal-Gándara, 2012). The treatment of Mencía red grapes with a triazole fungicide called tebuconazole didn't exert effects on terpene and higher-alcohol concentrations in the corresponding wines but significantly affected C6-alcohol, ester, and aldehyde concentrations (Noguerol-Pato, González-Rodríguez, González-Barreiro, Cancho-Grande, & Simal-Gándara, 2011). Volatile compounds can represent secondary products as in the case of those produced by *Debaryomyces hansenii* NRRL Y-7426 during the fermentation of detoxified concentrated distilled grape marc hemicellulosic hydrolysates. Compounds such as terpenes, higher alcohols, C6 alcohols, aldehydes, volatile acids, acetates, ethyl esters, volatile phenols, sulphur compounds and hydrocarbons were identified, although only few of them showed Odour Activity Values higher than 1 (Salgado et al., 2012). The volatile composition can be used as wine fingerprint as a function of variety, agricultural and oenological practices. A study of Noguerol-Pato et al. (2012) on grapes of Brancellao variety, highlighted that there was variability for their aromatic composition. For the berries from the tips of the clusters most of volatiles were found in the flesh (except aldehydes). For the berries from the shoulders of the clusters, most of volatiles were found in the skin (monoterpenes, norisoprenoids, aldehydes, and C6 alcohols), while the flesh was slightly richer in aromatic alcohols, volatile phenols and pantolactone. Similar results were obtained for Mouratón grapes (Noguerol-Pato et al., 2012). Differences between shoulders and tips into the cluster were also found in Gran Negro variety (Noguerol-Pato et al., 2012). The simultaneous determination of 32 volatile compounds was used to characterize Mencía monovarietal red wines from the Galician designation of origin “Valdeorras DO”. (Noguerol-Pato, González-Barreiro, Cancho-Grande,

& Simal-Gándara, 2009). GC-MS analysis was used to obtain the aromatic fingerprint of Godello wines and its correlation with the wine sensory properties (González-Álvarez, González-Barreiro, Cancho-Grande, & Simal-Gándara, 2011). The compounds that mainly contributed to the flavour of Godello wines were those conferring fruity (ethyl esters and acetates), spicy (fatty acids), or floral (terpenes) aromas. Similar studies were performed on three Garnacha Tintorera-based wines: a base wine, a naturally sweet wine, and a mixture of naturally sweet wine with other sweet wine obtained by fortification with spirits (González-Álvarez, Noguerol-Pato, González-Barreiro, Cancho-Grande, & Simal-Gándara, 2013; Noguerol-Pato, González-Álvarez, González-Barreiro, Cancho-Grande, & Simal-Gándara, 2012). Sotolon and acetoin were the main compounds in naturally sweet wine. The odorant series most dominant in Garnacha Tintorera base wine were floral, fruity and spicy. The most marked odorant series affected by off-vine drying of the grapes were floral, caramelized and vegetal-wood. The odorant series affected by the switch-off of alcoholic fermentation with ethanol followed by oak barrel aging were caramelized and vegetal-wood. Noguerol-Pato, González-Álvarez, González-Barreiro, Cancho-Grande, and Simal-Gándara (2013) studied the volatile composition of Garnacha Tintorera grapes during raisining. The major free volatile compounds were isoamyl alcohols, benzaldehyde, and guaiacol while the main bound volatile compounds were isoamyl alcohols, ethyl vanillate and benzoic acid. The aromatic profile of the raisins were caramelised, floral, phenolic and burned. Analogous studies were performed to obtain the fingerprint of extra-virgin olive oil (Reboredo-Rodríguez, González-Barreiro, Cancho-Grande, & Simal-Gándara, 2012, 2013), and the effects of processing on pepper Oruña-Concha et al., 1998). In the present experiment, changes in the volatile components of *Minutolo* wine were investigated during one year of aging in different types of containers. The 65 volatile compounds found by means of HS-SPME-GC-MS were grouped into the following 9 different classes (see also Table 4) (Capone et al., 2013): acids (7 compounds), alcohols (12), acetic esters (4), ethyl esters (19), other esters (8), carbonyl compounds (2), enols (5), terpenes (2), ethers (1), and aromatics (5). The more representative compounds of each class were: octanoic acid (acids); 1-butano 1,3-methyl alcohol and phenylethyl alcohol (alcohols); isoamyl acetate (acetic esters); ethyl octanoate at racking and diethyl succinate after 12 months (ethyl esters); hexyl acetate and isoamyl caprylate at racking and hexyl acetate and 1-O-hexyl 2-O-octadecyl oxalate after 12 months (other esters); β -damascenone at racking (carbonyl compounds); linalool and nerol at racking and after 12 months (terpenes); geranyl ethyl ether at racking (ethers).

Carbonyl compounds and ethers were under the detection limit in all the aged samples and dramatic decreases were measured in acetic (87–93%), ethyl (81–84%) and other (84–90%) esters, terpenes (71–85%), acids (72–77%), and enols (42–14%). This behaviour was independent on the type of containers, and was already highlighted by other authors (Lambropoulos and Roussis, 2007). Instead, aromatic volatile compounds decreased in engobe amphorae (–39%), remained unchanged in raw amphorae, and increased in glass containers (+249%) and glazed amphorae (+15%). Alcohols decreased in engobe (–3.5%) and glazed (14%) amphorae and increased in raw amphorae and glass containers (8 and 26%, respectively). The wines aged into the glass containers were characterized by the highest concentrations of aromatics, alcohols, acetic ester, other esters, and by the lowest contents of enols and terpenes. Among the wines aged in amphorae, wines in glazed amphorae were characterized by a relatively poor volatile profile, with the lowest concentrations of volatile acids, alcohols, acetic esters, and ethyl esters.

Also in the case of volatile profiles, the first differences occurred between wines at racking and those after 12 months of aging. The Principal Component Analysis (Fig. 2a) shows that the first two principal components accounted for 74.1 and 19.7% of the total variation, respectively, and that the points representing wines at racking are placed in the half-plane identified by negative values of the first component while those representing the wines after 12 months are located in the

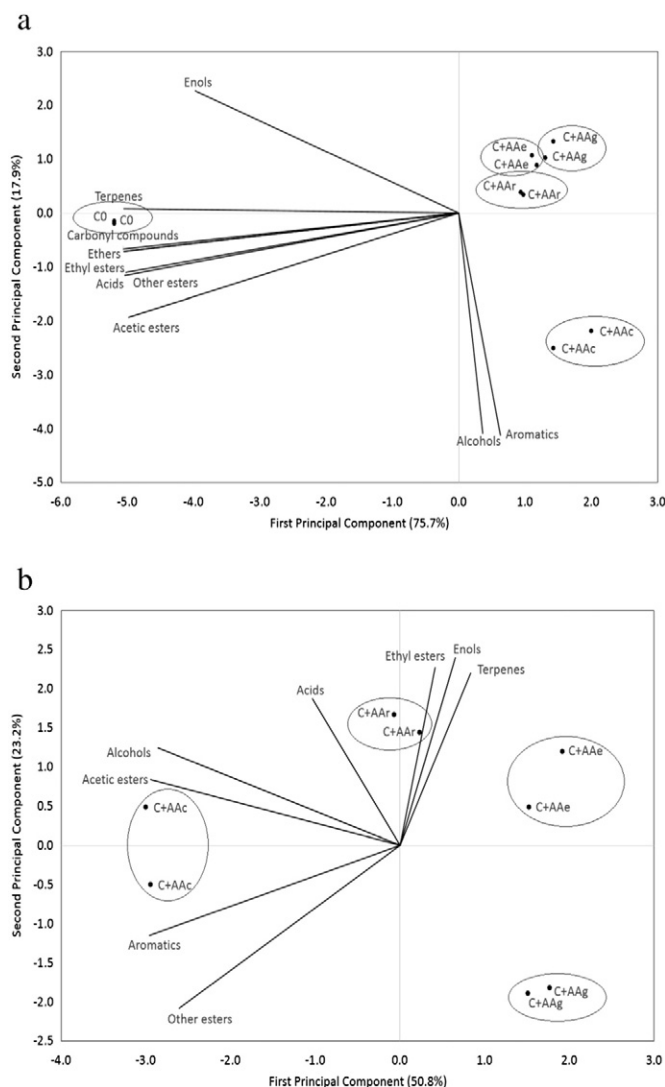


Fig. 2. PCA scatter plots based on the concentration of 10 classes of volatile compounds. Projection on the factor plane of a) wines analyzed at racking and after 12 months of storage b) wines analyzed 12 months after racking. CO: wine at racking; C + AAr: wine aged in glazed amphorae; C + AAe: wine aged in raw amphorae; C + AAg: wine aged in engobe amphorae; C + AAC: wine aged in glass containers.

part of the plane identified by positive values of the same component. After 12 months, with the first two components explaining 78% of the total variation, the wines appeared well grouped as a function of the types of container in which were aged (Fig. 2b), particularly in the case of those aged in the glass containers.

3.4. NMR measurements

In Fig. 3, for descriptive purposes, it is possible to observe the ^1H NMR spectrum obtained for a *Minutolo* wine sample after 12 months of aging. In the figure caption, some of the most characteristic signals were assigned to the relevant compounds, according to Baiano et al. (2012) and to Son et al. (2009).

Nevertheless, in this work the ^1H NMR was used as a non-targeted fingerprinting technique, i.e. the spectra were acquired as a whole (fingerprint) without focusing the attention on the assignments of particular resonances to specific metabolites and thus obtaining a comprehensive and rapidly informative description of the analyzed wine samples (Baiano et al., 2012; Longobardi et al., 2012). In fact, although the compounds identified from their NMR signals are easily determined by other less expensive methods, the aim of the NMR application in this

- Baiano, A., Varva, G., De Gianni, A., Viggiani, I., Terracone, C. Del, & Nobile, M.A. (2013c). Differences in physical-chemical characteristics and antioxidant profiles of 'Falanghina' white wines during aging in amphorae. *Food Chemistry*, 146, 226–233.
- Benítez, P., Castro, R., Sánchez Pazo, J.A., & Barroso, C.G. (2002). Influence of metallic content of fino sherry wine on its susceptibility to browning. *Food Research International*, 35, 785–791.
- Brand-Williams, W., Cuvelier, M.E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food Science Technology*, 28, 25–30.
- Budic-Leto, I., & Lovric, T. (2002). Identification of phenolic acids and changes in their content during fermentation and aging of white wines Posip and Rukatac. *Food Technology and Biotechnology*, 40, 221–225.
- Callegón, R.M., Morales, M.L., Silva Ferreira, A.C., & Troncoso, A.M. (2008). Defining Sherry vinegar typicity: Sensory and chemical approach. *Journal of Agriculture and Food Chemistry*, 56, 8086–8095.
- Camara, J.S., Alves, M.A., & Marques, J.C. (2006). Evolution of oak-related volatile compounds in a Spanish red wine during 2 years bottled, after aging in barrels made of Spanish, French and American oak wood. *Analítica Chimica Acta*, 563, 189–203.
- Canuti, V., Conversano, M., Li Calzi, M., Heymann, H., Matthews, M.A., & Ebeler, S.E. (2009). Headspace solid-phase microextraction–gas chromatography–mass spectrometry for profiling free volatile compounds in Cabernet Sauvignon grapes and wines. *Journal of Chromatography A*, 1216, 3012–3022.
- Capone, S., Tufariello, M., Francioso, L., Montagna, G., Casino, F., Leone, A., & Siciliano, P. (2013). Aroma analysis by GC/MS and electronic nose dedicated to *Negroamaro* and *Primitivo* typical Italian Apulian wines. *Sensors and Actuators B-Chemical*, 179, 259–269.
- Carrol, D., & Starkey, H. (1971). Reactivity of clay minerals with acids and alkalis. *Clays and Clay Mineral*, 19, 321–333.
- Cheyrier, V., Atanasova, V., Fulcrand, H., Mazauric, J.P., & Moutounet, M. (2002). Oxygen in wine and its role in phenolic reactions during aging. In M. Allen, S. Bell, N. Rowe, & G. Wall (Eds.), *Use of gases in winemaking. Proceedings of Seminar held in Adelaide. 10 October 2002, Adelaide* (pp. 23–27). Adelaide: Australian Society of Viticulture and Oenology.
- Crupi, M., Coletta, A., Milella, R.A., Perniola, R., Gasparro, M., Genchi, R., & Antonacci, D. (2012). HPLC-DAD-ESI-MS Analysis of Flavonoid Compounds in 5 Seedless Table Grapes Grown in Apulian region. *Journal of Food Science*, 77, 174–181.
- Dallas, C., Ricardo Da Silva, J.M., & Laureano, O. (1995). Degradation of oligomeric procyanidins and anthocyanins in a Tinta Roriz red wine during maturation. *Vitis*, 34, 51–56.
- Di Stefano, R., & Cravero, M.C. (1991). Metodi per lo studio dei polifenoli delle uve. *Rivista di Viticoltura ed Enologia*, 2, 37–43.
- Di Stefano, R., Cravero, M.C., & Gentilizi, N. (1989). Metodi per lo studio dei polifenoli dei vini. *L'Enotecnico*, 5, 83–89.
- EEC Regulation 2676 (1990). Community methods for the analysis of wine. *Official Journal of European Communities*, L272, 1–192.
- Figueiredo-González, M., Cancho-Grande, B., Boso, S., Santiago, J.L., Martínez, M.C., & Simal-Gándara, J. (2013a). Evolution of flavonoids in Mouratón berries taken from both bunch halves. *Food Chemistry*, 138, 1868–1877.
- Figueiredo-González, M., Cancho-Grande, B., & Simal-Gándara, J. (2013b). Evolution of colour and phenolic compounds during Garnacha Tintorera grape raisining. *Food Chemistry*, 141, 3230–3240.
- Figueiredo-González, M., Cancho-Grande, B., & Simal-Gándara, J. (2013c). Garnacha Tintorera-based sweet wines: Chromatic properties and global phenolic composition by means of UV-Vis spectrophotometry. *Food Chemistry*, 140, 217–224.
- Figueiredo-González, M., Cancho-Grande, B., & Simal-Gándara, J. (2013d). Effects on colour and phenolic composition of sugar concentration processes in dried-on- or dried-off-vine grapes and their aged or not natural sweet wines. *Trends in Food Science and Technology*, 31, 36–54.
- Figueiredo-González, M., Cancho-Grande, B., Simal-Gándara, J., Teixeira, N., Mateus, N., & De Freitas, V. (2014a). The phenolic chemistry and spectrochemistry of red sweet wine-making and oak-aging. *Food Chemistry*, 152, 522–530.
- Figueiredo-González, M., Martínez-Carballo, E., Cancho-Grande, B., Santiago, J.L., Martínez, M.C., & Simal-Gándara, J. (2012a). Pattern recognition of three *Vitis vinifera* L. red grapes varieties based on anthocyanin and flavonol fingerprints, with correlations between their biosynthesis pathways. *Food Chemistry*, 130, 9–19.
- Figueiredo-González, M., Regueiro, G., Cancho-Grande, B., & Simal-Gándara, J. (2014b). Garnacha Tintorera-based sweet wines: Detailed phenolic composition by HPLC/DAD-ESI/MS analysis. *Food Chemistry*, 143, 282–292.
- Figueiredo-González, M., Simal-Gándara, J., Boso, S., Martínez, M.C., Santiago, J.L., & Cancho-Grande, B. (2012b). Anthocyanins and flavonols berries from *Vitis vinifera* L. cv. Brancellao separately collected from two different positions within the cluster. *Food Chemistry*, 135, 47–56.
- Figueiredo-González, M., Simal-Gándara, J., Boso, S., Martínez, M.C., Santiago, J.L., & Cancho-Grande, B. (2012c). Flavonoids in Gran Negro berries collected from shoulders and tips within the cluster, and comparison with Brancellao and Mouratón varieties. *Food Chemistry*, 133, 806–815.
- Gemma, M., Clark, K.S., Barnett, N.W., Niere, J.O., & Adams, M.J. (2008). Generalised 2D-correlation NMR analysis of a wine fermentation. *Analytica Chimica Acta*, 629, 128–135.
- González-Álvarez, M., González-Barreiro, C., Cancho-Grande, B., & Simal-Gándara, J. (2011). Relationships between Godello white wine sensory properties and its aromatic fingerprinting obtained by GC-MS. *Food Chemistry*, 129, 890–898.
- González-Álvarez, M., González-Barreiro, C., Cancho-Grande, B., & Simal-Gándara, J. (2012a). Impact of phytosanitary treatments with new fungicides (cyazofamid, famoxadone, mandipropamid and valifenalate) to control downy mildew on the volatile profile of Godello white wines. *Food Chemistry*, 131, 826–836.
- González-Álvarez, M., Nogueiro-Pato, R., González-Barreiro, C., Cancho-Grande, B., & Simal-Gándara, J. (2012b). Changes on the sensorial attributes of white wines with the application of new anti-mildew fungicides under critical agricultural practices. *Food Chemistry*, 130, 139–146.
- González-Álvarez, M., Nogueiro-Pato, R., González-Barreiro, C., Cancho-Grande, B., & Simal-Gándara, J. (2013). Sensory Quality Control of Young vs. Aged Sweet Wines Obtained by the Techniques of Both Postharvest Natural Grape Dehydration and Fortification with Spirits During Vinification. *Food Analytical Methods*, 6, 289–300.
- González-Rodríguez, R.M., Nogueiro-Pato, R., González-Barreiro, C., Cancho-Grande, B., & Simal-Gándara, J. (2011). Application of new fungicides under good agricultural practices and their effects on the volatile profile of white wines. *Food Research International*, 44, 397–403.
- Lambropoulos, I., & Roussis, I.G. (2007). Inhibition of the decrease of volatile esters and terpenes during storage of a white wine and a model wine medium by caffeic acid and gallic acid. *Food Research International*, 40, 176–181.
- Lanati, D., Marchi, D., & Mazza, G. (2001). Georgian white wines in amphoras. Organoleptic and analytical properties of wines obtained with different winemaking Techniques. *L'Enologo*, 40, 111–117.
- Lee, C., & Jaworsky, A. (1987). Phenolic compounds in white grapes in New York. *American Journal of Enology and Viticulture*, 38, 277–281.
- Longobardi, F., Ventrella, A., Napoli, C., Humpfer, E., Schütz, B., Schäfer, H., Kontominas, M.G., & Sacco, A. (2012). Classification of geographical origin of olive oil by ¹H NMR fingerprinting combined with multivariate analysis. *Food Chemistry*, 130, 177–183.
- Martysiak-Zurowska, D., & Went, W. (2012). A comparison of ABTS and DPPH methods for assessing the total antioxidant capacity of human milk. *Acta Scientiarum Polonica Technologia Alimentaria*, 11, 83–89.
- Nogueiro-Pato, R., González-Álvarez, M., González-Barreiro, C., Cancho-Grande, B., & Simal-Gándara, J. (2012a). Aroma profile of Garnacha Tintorera-based sweet wines by chromatographic and sensorial analyses. *Food Chemistry*, 134, 2313–2325.
- Nogueiro-Pato, R., González-Álvarez, M., González-Barreiro, C., Cancho-Grande, B., & Simal-Gándara, J. (2013). Evolution of the aromatic profile in Garnacha Tintorera grapes during raisining and comparison with that of the naturally sweet wine obtained. *Food Chemistry*, 139, 1052–1061.
- Nogueiro-Pato, R., González-Barreiro, C., Cancho-Grande, B., Martínez, M.C., Santiago, J.L., & Simal-Gándara, J. (2012b). Floral, spicy and herbaceous active odorants in Gran Negro berries from shoulders and tips into the cluster, and comparison with Brancellao and Mouratón varieties. *Food Chemistry*, 135, 2771–2782.
- Nogueiro-Pato, R., González-Barreiro, C., Cancho-Grande, B., Santiago, J.L., & Simal-Gándara, M.C. (2012c). Aroma potential of Brancellao grapes from different cluster positions. *Food Chemistry*, 132, 112–124.
- Nogueiro-Pato, R., González-Barreiro, C., Cancho-Grande, B., & Simal-Gándara, J. (2009). Quantitative determination and characterization of the main odorants of Mencia monovarietal red wines. *Food Chemistry*, 117, 473–484.
- Nogueiro-Pato, R., González-Barreiro, C., Simal-Gándara, J., Martínez, M.C., Santiago, J.L., & Cancho-Grande, B. (2012d). Active odorants in Mouratón grapes from shoulders and tips into the bunch. *Food Chemistry*, 133, 1362–1372.
- Nogueiro-Pato, R., González-Rodríguez, R.M., González-Barreiro, C., Cancho-Grande, B., & Simal-Gándara, J. (2011). Influence of tebuconazole residues on the aroma composition of Mencia red wines. *Food Chemistry*, 124, 1525–1532.
- Oliveira, C.M., Silva Ferreir, A.C., Guedes de Pinho, P., & Hogg, T.A. (2002). Development of a potentiometric method to measure the resistance to oxidation of white wines and the antioxidant power of their constituents. *Journal of Agriculture and Food Chemistry*, 50, 2121–2124.
- Ortega-Heras, M., González-Sanjósé, M.L., & González-Huerta, C. (2007). Consideration of the influence of aging process, type of wine and oenological classic parameters on the levels of wood volatile compounds present in red wines. *Food Chemistry*, 103, 1434–1448.
- Oruña-Concha, M.J., López-Hernández, J., Simal-Lozano, J.A., Simal-Gándara, J., González-Castro, M.J., & de la Cruz García, C. (1998). Determination of volatile components in fresh, frozen and freeze-dried "Padrón-type" peppers by GC-MS using dynamic headspace sampling and microwave desorption. *Journal of Chromatographic Science*, 36, 583–588.
- Peña-Neira, A., Hernández, T., García-Vallejo, C., Estrella, I., & Suarez, J.A. (2000). A Survey of phenolic compounds in Spanish wines of different geographical origin. *European Food Research and Technology*, 210, 445–448.
- Peng, Z., Duncan, B., Pocock, K.F., & Sefton, M.A. (1998). The effect of ascorbic acid on oxidative browning of white wines and model wines. *Australian Journal of Grape and Wine Research*, 4, 127–135.
- Pérez-Lamela, C., García-Falcón, M.S., Simal-Gándara, J., & Orriols-Fernández, I. (2007). Influence of grape variety, vine system and enological treatments on the colour stability of young red wines. *Food Chemistry*, 101, 601–606.
- Poláková, P., Herszage, J., & Ebeler, S.E. (2008). Wine flavor: Chemistry in a glass. *Chemical Society Reviews*, 37, 2478.
- Quijada-Morín, N., Regueiro, J., Simal-Gándara, J., Tomás, E., Rivas-Gonzalo, J.C., & Escribano-Bailón, M.T. (2012). Relationship between the sensory-determined astringency and the flavanolic composition of red wines. *Journal of Agricultural and Food Chemistry*, 60, 12355–12361.
- Re, R., Pellegrini, N., Progettante, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an imprecise ABTS radical cation decolorization assay. *Free Radical Biology & Medicine*, 26, 1231–1237.
- Reboredo-Rodríguez, P., González-Barreiro, C., Cancho-Grande, B., & Simal-Gándara, J. (2012). Dynamic headspace/GC-MS to control the aroma fingerprint of extra-virgin olive oil from the same and different olive varieties. *Food Control*, 25, 684–695.
- Reboredo-Rodríguez, P., González-Barreiro, C., Cancho-Grande, B., & Simal-Gándara, J. (2013). Effects of sedimentation plus racking process in the extra virgin olive oil

- aroma fingerprint obtained by DHS-TD/GC-MS. *Food Bioprocess Technology*, 6, 1290–1301.
- Riedl, K.M., Carando, S., Alessio, H.M., McCarthy, M., & Hagerman, A.E. (2002). Antioxidant activity of tannins and tannin-protein complexes: assessment in vitro and in vivo. In M.J. Morello, F. Shahidi, & C.-T. Ho (Eds.), *Free Radicals in Food - Chemistry, nutrition, and health effects*. ACS Symposium Series, 807. (pp. 188–200).
- Rigaud, J., Cheynier, V., Souquet, J.M., & Moutounet, M. (1991). Influence of must composition on phenolic oxidation kinetics. *Journal of the Science of Food and Agriculture*, 57, 55–63.
- Saija, A., Scalese, M., Lanza, M., Marzullo, D., Bonina, F., & Castelli, F. (1995). Flavonoids as antioxidant agents: importance of their interaction with biomembranes. *Free Radical Biology & Medicine*, 19, 481–486.
- Salgado, J.M., González-Barreiro, C., Rodríguez-Solana, R., Simal-Gándara, J., Domínguez, J.M., & Cortés, S. (2012). Study of the volatile compounds produced by *Debaryomyces hansenii* NRRL Y-7426 during the fermentation of detoxified concentrated distilled grape marc hemicellulosic hydrolysates. *World Journal of Microbiology and Biotechnology*, 28, 3123–3134.
- Singleton, V.L. (1987). Oxygen with phenols and related reactions in musts, wines, and model systems: observations and practical applications. *American Journal of Enology and Viticulture*, 38, 69–77.
- Singleton, V.L., & Rossi, J.A. (1965). Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, 16, 144–158.
- Son, H.S., Hwang, G.S., Ahn, H.J., Park, W.M., Lee, C.H., & Hong, Y.S. (2009). Characterization of wines from grape varieties through multivariate statistical analysis of ¹H NMR spectroscopic data. *Food Research International*, 42, 1483–1491.
- Tao, Y., Li, H., Wang, H., & Zhang, Li (2008). Volatile compounds of young Cabernet Sauvignon red wine from Changli County (China). *Journal of Food Composition and Analysis*, 21, 689–694.
- Tesfaye, W., Morales, M.L., García-Parrilla, M.C., & Troncoso, A.M. (2002). Evolution of phenolic compounds during an experimental aging in wood of Sherry vinegar. *Journal of Agriculture and Food Chemistry*, 50, 7043–7061.
- Vivas, N., & Glories, Y. (1996). Role of oak wood ellagitannins in the oxidation process of red wines during aging. *American Journal of Enology and Viticulture*, 47, 103–107.
- Wildenrad, H.L., & Singleton, V.L. (1974). The production of aldehydes as a result of oxidation of polyphenolic compounds and its relation to wine aging. *American Journal of Enology and Viticulture*, 25, 119–126.
- Zafrilla, P., Morillas, J., Mulero, J., Cayuelas, J.M., Martínez-Cachá, A., Pardo, F., & Lopez Nicolás, J.M. (2003). Changes during storage in conventional and ecological wine: phenolic content and antioxidant activity. *Journal of Agriculture and Food Chemistry*, 51, 4694–4700.
- Zhang, Q.F., Fu, Y.J., Huang, Z.W., Shangguang, X.C., & Guo, Y.X. (2013). Aqueous stability of astilbin: effects of pH, temperature, and solvent. *Journal of Agriculture and Food Chemistry*, 61, 12085–12091.