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ORIGINAL PAPER

Effects of different vinification procedures and aging containers on phenolic and volatile composition of Greco white wines

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Abstract Two wines, obtained respectively from *Greco* grapes submitted to (*a*) a traditional vinification in white and (*b*) a vinification including cryomaceration under reductive conditions, were aged for 12 months in glass container and in 3 types of amphorae (raw, glazed, and engobe). The wines obtained by cryomaceration under reductive conditions showed the highest phenolic contents both at racking and after aging. The phenolic contents decreased with aging and proanthocyanidins exhibited the greatest decrease. Antiradical/antioxidant activity at racking and after aging was higher in cryomacerated than in traditional wines. Antioxidant activity was not affected by the type of container. Volatile acids and esters decreased with aging, while alcohols, hydrocarbons, and aromatics increased. According to the principal component analysis (PCA) of results from conventional analyses and antioxidants, the wines were homogeneously grouped as a function of the vinification method applied. The PCA of volatile compounds allows to better emphasize the differences, grouping the data into three clusters according to the type of containers.

Keywords Antioxidant · Container · Cryomaceration · Phenolic · Vinification · Volatile compounds

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Introduction

Grapes contain a large amount of different chemical compounds in skins, pulps, and seeds, which are partially extracted during winemaking. These compounds, together with those deriving from the chemical reactions occurring during winemaking and aging, play a fundamental role in development of some sensory properties of grapes and wines, such as color, flavor, and taste [1].

Phenolic and volatile composition is a very important factor affecting wine quality. Some compounds originate from the grapes where they are synthesized, others are formed during the process of grape must fermentation and afterwards during the storage of wines.

During the production of white wines, the oxidation of phenolics and the loss of flavor compounds can be minimized using a prefermentative cold maceration step, also known as cryomaceration. The maceration of skins in their own juice under controlled conditions (time, temperature, contact with oxygen) prior to fermentation stage improves the quality of white wine due to an increase in the flavor extraction from the skins. On the other hand, skin contact process also causes a greater extraction of phenolic components responsible for some of the major organoleptic properties of wines, in particular color and astringency [2].

Baiano et al. [3] compared the effects of traditional white vinification and vinification in reductive conditions including a cryomaceration step on chemical and physical indices and on antioxidant compounds of *Falanghina* and *Bombino bianco* wines. They observed that the vinification under reductive conditions combined with a cryomaceration step enhances the oenological potential of the starting grapes by increasing the extraction of compounds located into the skins and protecting them from oxidation.

During wine aging, phenolic and volatile profiles change due to disappearance/appearance of compounds. Aging usually takes place in barrels, bottles, and stainless steel tanks though in the last years some manufacturers adopted earthenware containers for wine aging. The aging in wood barrels temporary increases the content of phenolics [4] because wood acts as an extraction support for various phenolic compounds [5]. Bottle storage is important for the improvement of red wine quality but, in the case of white wine, it can lead to color alteration (browning) and eventually deterioration of the overall quality and marketability [6]. Baiano et al. [7] evaluated the effects of aging in raw, glazed, and engobe amphorae, and in steel tanks on chemical and physical indices and on antioxidant compounds of *Falanghina* wines. They found that: flavonoids decreased by about 85% in all the containers; the decrease of flavans reactive with vanilline ranged from 100% of raw and glazed amphorae to 23% of in engobe amphorae; hydroxycinnamoyl tartaric acids decreased by about 11% in raw and engobe amphorae and by ~22% in glazed amphorae and in stainless steel tanks. Concerning the aging of Minutolo white wine, Baiano et al. [8] observed the highest concentrations of aromatics, alcohols, and esters, the lowest contents of terpenic alcohols and terpenes and the highest decrease of flavonoids, hydroxycinnamoyl tartaric acids, and procyanidins in wines aged in glass containers. Instead, among the wines aged in amphorae, wines in glazed amphorae were characterized by the lowest concentrations of volatile acids, alcohols, acetic esters, and ethyl esters.

The purpose of the present study was to investigate the interactive effect of vinification procedures and aging systems on quality, phenolic profile, and volatile composition of an Italian white wine made from *Greco cv*. The study was performed by conventional analysis and by gas and liquid chromatography combined with multivariate statistical analyses.

Materials and methods

Wine samples

Greco 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 winepresses. At harvesting, grapes had the following characteristics: sugar content 15.90 ± 0.20 °Brix; titratable acidity 6.1 ± 0.1 g tartaric acid/L and pH 3.55 ± 0.07.

The following two winemaking procedures were applied: a traditional white vinification (T) with addition of potassium metabisulphite (10 g/100 kg) at the beginning of crusher–destemmer, clarification at 7–8 °C for 7–8 h, fermentation performed at 18 °C by S. *cerevisiae* (10 g/100 kg Fermol Plus, AEB, Brescia, Italy and 10 g/100 kg Ceriferm, Chemical oils Italia SAS, Cerignola, Italy); a reductive vinification including a 24-h skin cryomaceration (C + R) with addition of potassium metabisulphite (5 g/100 Kg) at the beginning of crusher–destemmer, and the obtained must was submitted to the following operations: 24-h skin cryomaceration at 2–4 °C (the temperature of the must, which was around 25 °C was lowered to 2–4 °C by addition of solid carbon dioxide), addition of a mixture of potassium metabisulphite–ascorbic acid (20 g/100 kg Aromax, AEB, Brescia, Italy), separation of skins, clarification at 7–8 °C for 7–8 h, and fermentation at 18 °C by S. *cerevisiae* (10 g/100 kg Fermol Plus, AEB, Brescia, Italy and 10 g/100 kg Ceriferm, Chemical oils Italia SAS, Cerignola, Italy).

After fermentation, the wines were submitted to a first racking and, after 4 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.

Wines were stored for 12 months in three types of earthenware amphorae: raw, glazed, and engobe. The three types of amphorae (Ceramiche Cataldo, Terlizzi, Italy) were made of pure clay treated in three different ways: the raw type was fired once at 1100 °C; the glazed type was fired first at 1100 °C and then at 970 °C and, between the two firing, amphorae were submitted to an internal coating with vitrous particles suspended in water that turn to glass when fired; the engobe type was fired first at 970 °C and then at 1100 °C. All the amphorae were for food use. The wine stored in glass containers was used as a control.

Conventional analyses of wine

Wines were analyzed before the transfer into the aging containers and 12 months after racking. 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 sulfur dioxide (mg/L) were determined according to the EEC Regulation 2676/1990 [9]. 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 using an 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, The Netherlands) equipped with an encapsulated Ag/AgCl electrode (Crison, Lainate, MI, Italy). The EH was expressed in mV. pH values were measured with a CyberScan pH 510 (Eutec

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

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 [10]. 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 (flavonoids, flavans reactive with vanillin, hydroxycinnamoyl-tartaric acids, proanthocyanidins) and the total phenolics were analyzed according to the methods of Di Stefano et al. [11] and Di Stefano and Cravero [12]. When necessary, the extracts were opportunely diluted with aliquots of the extraction solution. The results were expressed as mg per L of wine.

The evaluation of the antioxidant activity was made through the 1,1-diphenyl-2-picrylhydrazyl (DPPH) [13] and 2,2-azinobis-(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS) [14] assays and the results were expressed as mmol of Trolox equivalents/L of wine.

Determination of volatile composition

The volatile composition of the wines produced through cryomaceration under reductive conditions was analyzed by head-space solid phase microextraction hyphenated with gas chromatography–mass spectrometry (HS-SPME–GC–MS). The chromatographic analyses were performed according to the method described by Canuti et al. [15] and Tao et al. [16], 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 (I.S.) solution (83 mg/L in ethanol) was 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 an 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 an HP-INNOWax column (60 m × 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, 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 the 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 peak areas were calculated from the area of the major MS fragment (m/z) and the relevant value was corrected by normalization factor obtained by the $I.S._{ref.}$/$I.S._{sample}$ ratio. Blank runs were made with empty glass vial before each analysis.

Statistical analysis

Before the transfer into the aging containers, 6 samples were withdrawn for each type of vinification (3 from the first repetition, 3 from the second repetition). Each sample was submitted to the aforementioned analyses, which were performed in triplicate. Since the coefficient variation within the three replicates performed on each repetition was similar to that calculated within the 6 total replicates, the volumes of wine corresponding to the 2 repetitions of each vinification type were mixed together and then transferred into the 3 types of amphorae and in the glass container (3 units for each type of amphorae and glass container). After 12 months of aging, 3 samples were withdrawn from each unit and performed in triplicates. The standard deviation values reported in the following tables are those calculated on the total replications.

The averages and the standard deviations were calculated using Excel software V. 11.5.1 (Microsoft, Redmond, WA). The statistical treatment was performed using the package Statistica for Windows V. 8.0. (Statsoft Inc., Tulsa, OK), with the exception of data concerning the volatile compounds, which were treated through the SCAN software from Minitab Inc. (State College, PA, USA). The one-way analysis of variance (ANOVA, performed at racking), the two-way ANOVA (performed after 12 months of aging, $p < 0.05$), and the least significant difference (LSD) test ($p < 0.05$, used also when the ANOVA was not significant) were applied to determine the main effects of vinifications and containers used on the chemical composition of wines. Principal component analysis (PCA), performed using the package Statistica for Windows V. 8.0. (Statsoft Inc., Tulsa,

OK), was applied to the data sets to check the possibility to discriminate the wines aged in different containers.

Results and discussion

Conventional analysis of wine

The wine samples were analyzed at racking and 12 months after racking for parameters having an oenological meaning (Table 1).

The data collected at racking show that, except for density, dry extract, and sugar content, all the characteristics were affected by the method of vinification. In comparison with the wines from the traditional vinification, those obtained through the cryomaceration under reductive conditions had: lower alcohol content, redox potential, and dissolved oxygen (due to the inhibitory effects of both low temperature and reductive conditions); lower volatile acidity (due to the antiseptic effect of sulfur dioxide); lower titratable acidity and consequently higher pH (due to the low temperatures of maceration that caused the precipitation of tartaric acid as potassium bitartrate) [17, 18]. The cryomacerated wines also showed lower free-to-total SO_2 ratio than the traditional wines (34 vs. 42%), probably due to the increase of the SO_2 bound to the acetaldehyde produced at the beginning of fermentation [17]. The lower alcohol content of the wines obtained through cryomaceration under reductive conditions conflicted with the results previously obtained by Baiano et al. [3, 17], who found higher alcohol content in Sauvignon blanc, Bombino, and Falanghina, and also with the findings of Piombino et al. [19] on the 'Malvasia delle Lipari', Antonelli et al. [20] on 'Sauvignon Blanc' and 'Trebbiano Romagnolo' wines, and by Carillo et al. [21] on 'Bianchello del Metauro', who didn't highlight statistically significant differences. These results could be due to the different yeasts used in fermentation.

After 12 months, the single effects of type of vinification can be observed only for titratable acidity (higher in traditional wines), pH (lower in traditional wines), free and total SO_2 and dissolved O_2 (higher in cryomacerated wines). The higher dissolved O_2 of cryomacerated wines could be due to its lower consumption. The oxidation of phenolics was inhibited or slowed down by the protective effects of SO_2. Concerning the single effect of aging, the wines from glass container and glazed amphorae showed the highest titratable acidity and the highest redox potential values and dissolved oxygen concentration, consistent with the lowest free and total SO_2 and the free-to-total SO_2 ratio due to the sulfur dioxide consumed during storage to neutralize the reactive forms of oxygen, and to the increase of the SO_2 bound to acetaldehyde. The interactive effects of vinification and aging were significant for redox potential (higher in wines obtained through the traditional procedure and aged in glass containers), titratable acidity and pH (respectively, lower and higher in wines obtained through the cryomaceration under reductive conditions and aged in raw amphorae), free SO_2 (higher in wines obtained through cryomaceration under reductive conditions and aged in engobe amphorae), total SO_2 (higher in wines obtained through cryomaceration under reductive conditions and aged in raw amphorae), and dissolved oxygen (lower in wines obtained through the traditional procedure and aged in raw amphorae).

Titratable acidity and pH were in agreement with the results previously obtained by Baiano et al. [7]. These results probably depended on the matter that raw amphorae are not inert containers, since clays react with acids and alkalis. In the presence of acid solutions, a cation exchange occurs, with the removal of small amounts of compounds, such as SiO_2, Al_2O_3, and Fe_2O_3, and the formation of H-clays. The results concerning the total SO_2 could be due to the formation of acetaldehyde (produced by a coupled auto-oxidation of certain phenolic compounds), the most important SO_2-binding compound, which contributed to increase the concentrations of the combined SO_2 [7].

Phenolic content and antioxidant activity of wines

Table 2 shows the evolution of several parameters related to the wines antioxidants (concentration of various phenolic classes, concentration of total phenolics, antioxidant activity) at racking and during aging in amphorae and in glass containers.

At racking the wine obtained through cryomaceration under reductive conditions showed the highest concentrations of all the phenolic classes and, consequently, the highest antioxidant activity. These results are due to a series of phenomena: the direct contact between the cryogen carbon dioxide and the must induced a partial solidification of the cellular water inside the berry skins and, as a consequence, the collapse of the cells and the rupture of the cell walls, thus promoting the diffusion of the phenolics; the protective effects of the low temperatures during cryomaceration, which inhibit oxidative enzymes; and the increase of polyphenol solubility and extraction from berry skins due to the higher content in free sulfur dioxide under reductive conditions [17, 22]. Although in white wines, a higher extraction of phenolics is generally undesired because their oxidation is responsible for browning phenomena, the maceration performed at low temperatures and under reductive conditions inhibited oxidative phenomena.

Almost all the phenolic classes exhibited changes of the concentrations during aging. In particular, after 12 months, flavonoids decreased by about 13–20% in wines aged in

Table 1 Quality parameters of *Greco* wines produced by traditional white vinification or by cryomaceration under reductive conditions and aged in three types of amphorae and in glass containers

	Alcohol (vol%)	Density (g/mL)	Redox Pot. (mV)	Volatile Ac. (g acetic ac./L)	Titratable Ac. (g tartaric ac./L)	Dry extract (g/L)	Free SO2 (mg/L)	Total SO2 (mg/L)	Sugar (g/L)	pH	Dissolved O2 (mg/L)
At racking											
T	10.5 ± 0.2b	0.995	195.5 ± 0.2b	0.17 ± 0.03b	6.4 ± 0.1b	24.7 ± 0.5	64.0 ± 1.6b	151.0 ± 21.6a	6.3	3.12 ± 0.03a	5.66 ± 0.11b
C+AA	10.1 ± 0.1a	0.996	154.5 ± 0.1a	0.14 ± 0.02a	5.9 ± 0.1a	24.7 ± 0.6	55.0 ± 5.1a	161.7 ± 8.3b	6.2	3.24 ± 0.02b	5.33 ± 0.24a
Significance	*	ns	*	*	*	ns	*	*	ns	*	*
After 12 months of aging											
Effects of vinification											
T	10.0 ± 0.5	0.997	176.2 ± 21.9	0.26 ± 0.03	6.4 ± 0.3b	25.9 ± 2.9	15.2 ± 4.4a	87.3 ± 4.4a	7.3 ± 0.7a	3.13 ± 0.11a	1.34 ± 0.11a
C+AA	9.9 ± 0.2	0.997	173.8 ± 6.1	0.25 ± 0.02	5.8 ± 0.2a	26.0 ± 1.1	34.3 ± 8.4b	160.5 ± 15.7b	6.7 ± 0.2a	3.23 ± 0.07b	1.99 ± 0.07b
Significance	ns	ns	ns	ns	*	ns	*	*	ns	*	*
Effects of type of aging											
g	9.9 ± 0.6	0.997	167.3 ± 16.7a	0.26 ± 0.03	5.8 ± 0.3a	26.5 ± 3.3	26.6 ± 5.1b	146.3 ± 51.2b	6.7 ± 0.6	3.23 ± 0.10	1.07 ± 0.10a
v	10.0 ± 0.2	0.997	173.8 ± 15.4ab	0.25 ± 0.01	6.2 ± 0.4b	26.5 ± 1.9	19.0 ± 8.1a	122.9 ± 37.7a	10.4 ± 3.1	3.17 ± 0.08	2.15 ± 0.08c
i	9.8 ± 0.3	0.997	171.3 ± 14.9a	0.26 ± 0.02	5.9 ± 0.4a	25.6 ± 1.4	30.1 ± 18.0c	122.2 ± 38.0a	9.2 ± 2.1	3.18 ± 0.08	1.61 ± 0.08b
c	10.2 ± 0.3	0.996	187.5 ± 10.6b	0.25 ± 0.03	6.3 ± 0.3b	25.1 ± 1.6	21.4 ± 10.0a	129.3 ± 31.7a	10.5. ± 2.2	3.15 ± 0.15	2.00 ± 0.15c
	Ns	ns	*	ns	*	ns	*	*	ns	ns	*
Effects of vinification* type of aging											
T_g	9.7 ± 0.8	0.998	166.0 ± 24.9a	0.26 ± 0.03	6.0 ± 0.3c	27.0 ± 5.0	22.4 ± 1.3b	83.2 ± 5.4a	6.1 ± 0.7	3.15 ± 0.09ab	0.80 ± 0.02a
T_v	10.1 ± 0.2	0.997	175.1 ± 22.6ab	0.25 ± 0.01	6.6 ± 0.3d	27.3 ± 2.6	12.8a	87.7 ± 3.2a	7.5 ± 0.3	3.10 ± 0.01a	2.32 ± 0.13e
T_i	9.8 ± 0.3	0.997	170.4 ± 22.7a	0.26 ± 0.03	6.2c	24.8 ± 1.6	13.4 ± 1.3a	87.7 ± 5.7a	7.4 ± 0.5	3.11 ± 0.03a	0.90b
T_c	10.4 ± 0.2	0.996	193.4 ± 12.9b	0.25 ± 0.01	6.6 ± 0.01d	24.4 ± 0.7	12.2 ± 1.3a	89.6 ± 3.6a	7.8 ± 0.2	3.18 ± 0.22ab	2.20 ± 0.24e
C+AAg	10.2 ± 0.1	0.997	168.7 ± 4.7a	0.26 ± 0.02	5.6a	26.1 ± 0.4	30.7 ± 3.6c	177.9 ± 19.3c	6.7 ± 0.2	3.30 ± 0.01c	1.31 ± 0.01c
C+AAv	9.8 ± 0.1	0.997	172.6 ± 5.7ab	0.25 ± 0.03	5.8b	25.8 ± 0.4	27.3 ± 3.9c	158.1 ± 2.5b	7.2 ± 0.6	3.25 ± 0.03bc	1.98 ± 0.02d
C+AAi	9.8 ± 0.3	0.997	172.2 ± 1.8ab	0.26 ± 0.03	5.6 ± 0.1ab	26.3 ± 0.8	46.7 ± 3.8d	156.8 ± 12.6b	6.8 ± 0.4	3.25 ± 0.01bc	1.89d
C+ AAc	10.0 ± 0.2	0.997	181.6 ± 2.3ab	0.25 ± 0.01	6.1 ± 0.1c	25.8 ± 2.1	30.7 ± 2.1c	149.1 ± 9.9b	6.8 ± 0.9	3.12 ± 0.01a	2.24 ± 0.07e
Significance	ns	ns	*	ns	*	ns	*	*	ns	*	*

Single and interactive effects of vinification and type of aging

C+AA, Cryomaceration in reductive vinification, *v* wine aged in glazed amphorae, *g* wine aged in raw amphorae, *i* wine aged in engobe amphorae, *c* wine aged in glass containers, *ns* not significant

In column, different letters indicate significant differences at $p < 0.05$ by LSD multiple range test

Table 2 Phenolic classes and antioxidant activity of *Greco* wines produced by traditional white vinification or by cryomaceration under reductive conditions and aged in three types of amphorae and in glass containers

	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)
At racking							
T	244.6 ± 27.0a	100.9 ± 15.2a	83.0 ± 8.4a	580.2 ± 38.3a	348.1 ± 68.4a	0.05a	1.58 ± 0.27a
C + AA	296.1 ± 31.5b	134.3 ± 7.6b	120.7 ± 8.9b	727.4 ± 18.0b	544.9b	0.07b	2.17 ± 0.20b
Significance	*	*	*	*	*	*	*
After 12 months of aging							
Effects of vinification							
T	202.8 ± 24.7a	86.0 ± 14.1a	65.4 ± 4.5a	434.7 ± 50.0a	204.3 ± 40.6a	0.05 ± 0.01	1.58 ± 0.24a
C + AA	251.1 ± 22.1b	134.4 ± 17.1b	84.9 ± 2.5b	615.4 ± 36.9b	329.2 ± 45.4b	0.06 ± 0.01	2.10 ± 0.19b
Significance	*	*	*	*	*	ns	*
Effects of type of aging							
g	244.6 ± 13.8	120.4 ± 19.8c	74.0 ± 11.6	597.0 ± 91.3b	249.8 ± 80.5a	0.06 ± 0.01	1.81 ± 0.34
v	222.1 ± 30.6	105.4 ± 34.5ab	76.0 ± 10.4	526.5 ± 97.1ab	249.8 ± 80.5a	0.06 ± 0.01	1.86 ± 0.38
i	241.4 ± 43.4	115.4 ± 33.5bc	74.5 ± 10.5	520.6 ± 128.4ab	272.5 ± 97.1ab	0.06 ± 0.01	1.87 ± 0.40
c	218.9 ± 30.8	99.5 ± 23.4a	76.2 ± 11.5	506.4 ± 92.5a	295.2 ± 42.0b	0.05 ± 0.01	1.81 ± 0.25
Significance	ns	*	ns	*	*	ns	ns
Effects of vinification* type of aging							
T_g	212.4 ± 38.6abc	100.9 ± 6.8b	63.9 ± 5.3a	466.2 ± 41.8b	181.6a	0.05 ± 0.01	1.55 ± 0.27a
T_v	199.6 ± 12.9a	74.3 ± 7.4a	67.2 ± 6.4a	439.1 ± 45.4ab	181.6a	0.05 ± 0.01	1.57 ± 0.27a
T_i	206.0 ± 29.7ab	87.8 ± 12.2ab	64.9 ± 3.0a	410.0 ± 70.7a	181.6a	0.05 ± 0.01	1.57 ± 0.24a
T_c	193.1 ± 14.9a	83.4 ± 15.6	65.8 ± 4.3a	423.5 ± 24.5ab	272.5b	0.05 ± 0.01	1.62 ± 0.19a
C + AAg	238.2 ± 12.9cd	136.6 ± 6.7d	84.1 ± 4.1b	627.8 ± 30.5c	317.9 ± 52.4bc	0.06 ± 0.01	2.08 ± 0.09b
C + AAv	244.6 ± 25.8de	136.4 ± 15.8d	84.8 ± 2.2b	613.9 ± 19.3c	317.9 ± 52.4bc	0.06 ± 0.01	2.16 ± 0.18b
C + AAi	276.8 ± 12.9e	143.1 ± 22.2d	84.1 ± 1.5b	631.2 ± 43.9c	363.3c	0.06	2.17 ± 0.30b
C + AAc	244.6 ± 14.9de	118.9 ± 14.3c	86.6 ± 1.3b	589.3 ± 41.4c	317.9 ± 52.4bc	0.06 ± 0.01	2.01 ± 0.12b
Significance	*	*	*	*	*	ns	*

Single and interactive effects of vinification and type of aging

C + AA cryomaceration in reductive vinification, *v* wine aged in glazed amphorae, *g* wine aged in raw amphorae, *i* wine aged in engobe amphorae, *c* wine aged in glass containers, *ns* not significant

In column, different letters indicate significant differences at $p < 0.05$ by LSD multiple range test

raw amphorae, by about 18% in wines aged in glazed, by 7–16% in engobe amphorae, and by 18–21% in glass containers. The concentrations of flavans reactive with vanillin dropped by 13, 17, and 26% in traditional wines aged in engobe amphorae, glass containers, and glazed amphorae, respectively, while it remained unchanged in all the cryomacerated wines with the exception of those aged in glass containers, which decreased by 12%. Hydroxycinnamoyl tartaric acids greatly decreased most in cryomacerated wines than in the traditional ones. The ranges of reduction were 24–30, 19–30, 24–28%, for raw and engobe amphorae, glazed amphorae, and glass containers, respectively. Proanthocyanidins exhibited the greatest changes, mainly in the case of the traditional wines, which showed the following decreases: −48% for wines in raw, glazed and engobe amphorae, and −22% in glass containers. Concerning the cryomacerated wines, proanthocyanidins decreased by about 42% in raw and glazed amphorae, and in glass containers, and by 33% in engobe amphorae. The high loss of proanthocyanidins during aging was due to their strong antiperoxidative activity [23]. Also after 12 months of aging, the vinification technology exhibited a strong effect on phenolic content, which was higher in cryomacerated wines. The single effect of type of aging was significant only for the concentrations of flavans reactive with vanilline (higher in raw amphorae) and proanthocyanidins (higher in glass containers).

The antioxidant activity of wines was measured through the DPPH and ABTS assays, which give information on the radical scavenging or antiradical activity. According to the data reported in Table 2, the antiradical/antioxidant activity at racking was higher in cryomacerated than

in traditional wines. After aging, the antioxidant activity measured by the DPPH assay was not affected by the vinification method or by the type of container, while the ABTS assay still highlighted higher values in cryomacerated. Data also highlighted the absence of correlations between DPPH and ABTS results and values of antioxidant activity higher when measured through the ABTS assay. These results are in agreement with the finding of Martysiak-Zurowska and Wenta [24] and confirm that the DPPH method is characterized by a lower sensitivity than ABTS assay as a consequence of the slower reactions with most antioxidant present in wines. Furthermore, DPPH can fail in determining antioxidant activity because some compounds have spectra that overlap with those of DPPH.

The interactive effects of vinification and aging containers were significant for all the phenolic classes and for the results of the ABTS assay. The highest concentrations of the various classes of phenolic compounds and the highest antioxidant activity measured through the ABTS assay were always found in wines obtained through cryomaceration under reductive conditions, independently on the type of aging containers. These results are only partially in agreement with the findings of Baiano et al. [7], who found no significant differences among wines aged in different types of containers only for flavonoids and hydroxycinnamoyl tartaric acids, while flavans reactive with vanillin and total phenolics were always higher in the inert containers than in the amphorae.

The PCA applied to results of the conventional analyses, concentration of phenolics, and antioxidant activity values was applied to highlight the differences between wines produced through the two types of vinification. Furthermore, the PCA was applied to the standardized analytical indices measured after 12 months of aging to discriminate wines as a function of the type of container. Figure 1a shows that the first two principal components accounted for 76.7 and 6.8% of the total variation, respectively, and that the points representing the traditional wines at racking were placed in the half-plane identified by positive values of the first component while those representing the cryomacerated wines are all located in the part of the plane identified by the negative values of the same component. Figure 1b represents the projection of the variables on the factor plane and indicates that cryomacerated wines were mainly grouped for their higher antioxidant concentrations. The projections of the wines samples and of the variables on the factor planes after 12 months of aging are shown in Fig. 1c, d, respectively. The first two principal components accounted for 47.7 and 19.9% of the total variation. Also after the aging time, the wines were mainly grouped based on the vinification procedures. Furthermore, the traditional wines were homogeneously grouped as a function of the aging container, with the exception of those aged in engobe amphorae. In particular, the wines aged in glazed amphorae and glass containers were characterized by their higher redox potential and antioxidant activity, while those aged in raw amphorae were characterized by their higher concentration of free SO_2. Within the group of cryomacerated wines, those aged in raw and glazed amphorae were overlapped The low percentage of the explained variation and the closeness of the samples indicate that, conversely to the finding highlighted by Baiano et al. for *Falanghina* wines [7], the aging in different type of containers was unable to give specific characteristics to the wines.

The loadings of each original variable (eigenvectors) in the PCA were determined, to highlight the analytical indices significantly related to the first two PCs. The eigenvectors for the wines analyzed at racking and after 12 months of aging are reported in Table 3. As can be inferred from the eigenvectors at racking, most variables related to the vinification procedures concentrations of antioxidants, density, volatile acidity, pH, dissolved oxygen, redox potential were associated with PC1. After 12 months, most variables were associated with PC1, but alcohol, density, volatile acidity, dry extracts, pH, redox potential, and DPPH were associated with PC2.

Volatile composition of wines

Changes in the volatile components of *Greco* wine produced through cryomaceration under reductive conditions were investigated after 1 year of aging in different types of containers. As reported in Table 4, the eighty-nine volatile compounds detected were grouped into 8 different classes: acids (4 compounds), alcohols (15), ethyl esters (23), other esters (12), carbonyl compounds (5), hydrocarbons (13), terpenes (13) and aromatics (4). The odour description of most of all was reported from Capone et al. and SAFC [25, 26].

Table 4 shows the trend of each specific compound. At racking, the composition was in a decreasing order: ethyl esters (~56%), alcohols (~27%), other esters (~13%), acids (4%), hydrocarbons (~1%), terpene derivatives (0.5%), aromatics (0.2%), and carbonyl compounds. During 12 months of aging, acids and esters dramatically decreased probably due to autoxidation that yields to carbonyl compounds [27], while alcohols, hydrocarbons, and aromatics increased. In particular, at 12 months of ripening the following substances were not detected because their content was under the detection limits: hexanoic acid (note of cheese, fatty and sour), octanoic acid and n-decanoic acid (fatty acid and dry), octanoic acid- 2-phenylethyl ester, octadecanoic acid ethyl ester, linoleic acid ethyl ester (fruity, green and floral flavor), n-capric acid isobutyl ester, 3-octanone (banana, berry, butter and cheese note), 2-bornene, β-damascenone

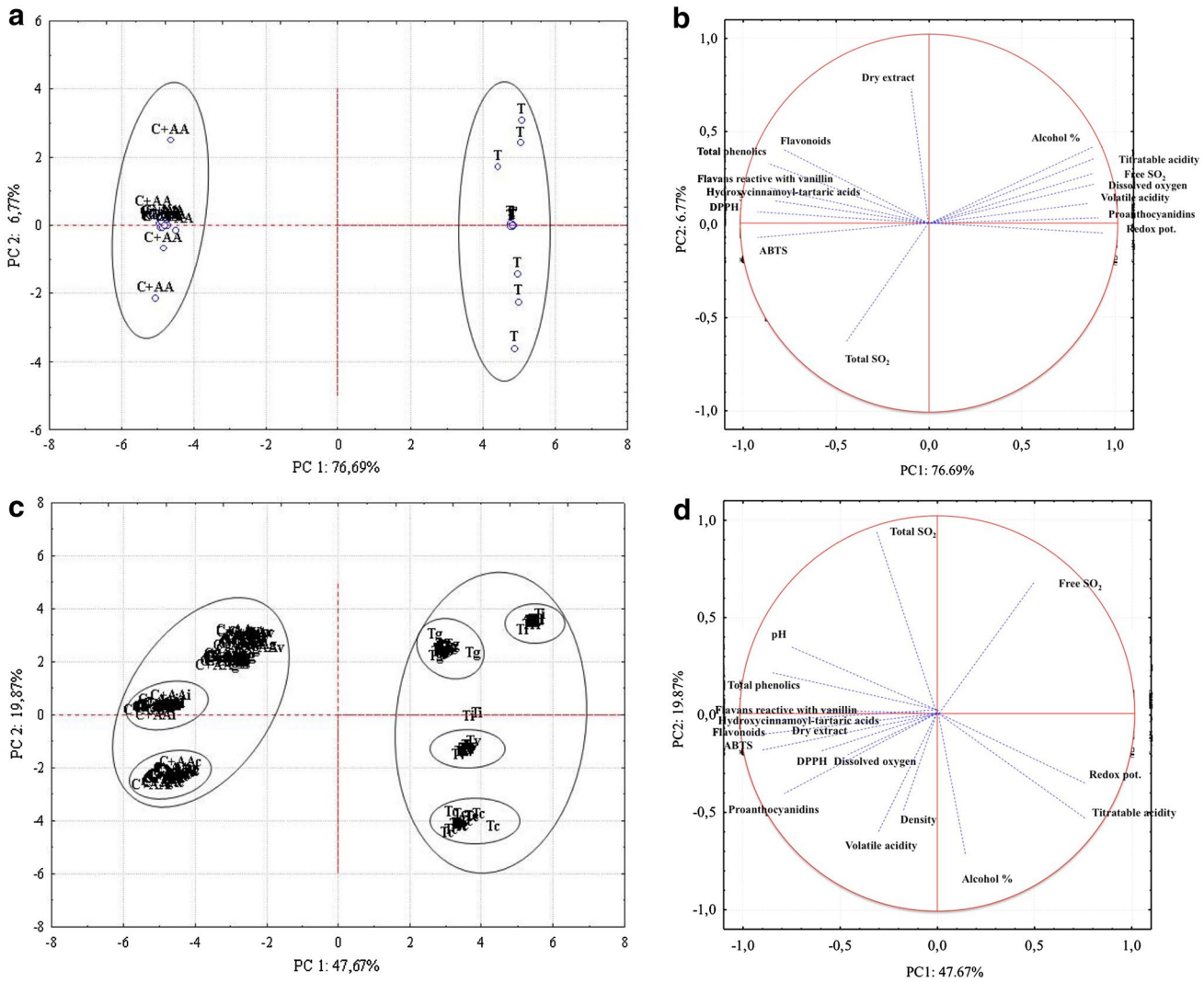

Fig. 1 PCA scatter plots based on results of the conventional analyses, concentration of phenolics, and antioxidant activity values. Projection on the factor plane of wines and variables analyzed at racking (**a, b**, respectively) and 12 months after racking (**c, d** respectively). *T* traditional white vinification, *C + AA* cryomaceration in reductive vinification, *v* wine aged in glazed amphora, *g* wine aged in raw amphorae, *i* wine aged in engobe amphorae, *c* wine aged in glass containers

(apple, herbaceous, woody flavor) and indole (butter, cheese, chocolate and grape note).

During aging, fatty acid ethyl esters, acetate esters and a lot of other esters significantly decreased, due to hydrolysis processes [28]. In particular, some of these esters (i.e., octanoic acid- 2-phenylethyl ester, octadecanoic acid ethyl ester, linoleic acid ethyl ester, and n-capric acid isobutyl ester) were not detected in the aged wines. On the other hand, according to other authors [28–30], the ethyl esters of some organic acids, such as propanoic acid- 2-hydroxy-ethyl ester (ethyl lactate) and butanedioic acid-diethyl ester (diethyl succinate), increased during wine storage. These results are not surprising, and can be explained by different hydrolysis esterification equilibria [31]. Furthermore, the dependence of these processes from the micro-oxygenation explains the different behavior of diethyl succinate content in glass containers samples. In fact, in this type of container, the concentrations of diethyl succinate were not statistically different from those of the samples at racking. Another example that clearly demonstrates the role of micro-oxygenation in the formation equilibria is the different behavior that n-caproic acid isobutyl ester showed depending on aging conditions: its content was higher in raw amphora samples, if compared with the other.

Terpenes were also affected by the oxidative conditions, and thus from the type of amphoras used. In particular, at 12 months, 2-bornene and β-damascenone disappeared in all types of containers, while the concentration

Table 3 Eigenvector values of the variables resulting from the PCA of *Greco* wines produced by traditional white vinification or by cryomaceration under reductive conditions as analyzed after racking and after 12 months of aging

Variables	At racking		After 12 months of aging	
	PC1	PC2	PC1	PC2
Total phenolic compounds	−0.195829	0.136938	−0.207960	0.040631
Flavans reactive with vanillin	−0.178607	0.117261	−0.200387	0.080294
Hydroxycinnamoyl-tartaric acids	−0.189554	0.106850	−0.210323	−0.028518
Flavonoids	−0.162719	0.206141	−0.190778	0.112914
Proanthocyanidins	0.192480	0.000436	−0.194225	−0.128350
Alcohol (vol%)	0.187224	0.226618	0.012621	−0.329775
Density (g/cm^3)	−0.185974	0.076775	0.000614	0.434614
Volatile acidity	0.157302	0.012247	0.042016	0.365374
Titratable acidity	0.186577	0.130785	0.183073	−0.170113
Dry extract	−0.010227	0.497164	−0.014181	0.203738
Free SO_2	0.180042	0.032103	−0.191259	0.150959
Total SO_2	−0.086047	−0.487304	−0.212682	−0.045760
pH	−0.196549	0.113815	−0.079696	−0.392600
Dissolved O_2	0.180214	0.030334	−0.138963	−0.066087
Redox potential	0.201748	−0.003678	−0.080567	0.236709
DPPH	−0.151088	0.064288	0.025949	−0.296060
ABTS	−0.159045	−0.049593	−0.147615	0.022884

of α-terpineol and β-citronellol increased in the wine aged in glazed amphora. This outcome is not surprising, and it is supported by the data reported by other authors, who highlighted that such compounds initially increase and then decrease, depending on the presence of oxygen. This behavior is strictly related to the formation of terpenes, originating from the oxidation of other terpenols [32].

The wines aged in raw amphorae highlighted the highest values of 1-propanol while those aged in glazed amphora and in glass containers have the highest value of 1-butanol and ethyl lactate, respectively. These substances have high perception thresholds (equal to or higher than 10 mg/l) [33], and contribute to lactic, alcohol, medicinal, and phenolic notes.

Hydrocarbons increased their content during the time in all samples studied with a similar trend, while, in aromatic class, benzene-1,3-bis[1,1-dimethylethyl] and phenol-2,4-bis(1,1-dimethylethyl) showed higher contents in wine aged in raw amphorae, most probably because in these containers there was a higher oxygen concentration. Indole disappeared in all samples after 12 months.

After 12 months, the obtained PCA (Fig. 2, with the first two principal components accounting for 46.8 and 17.5% of the total variation, respectively) allows to better emphasize the separation among samples at the end of aging process grouping the data into three clusters. In particular, the wine aged in glass containers and in engobe amphorae was placed in the part of the plane identified by negative values of both the components and exhibited a similar behavior showing a volatile component relatively rich in hydrocarbons and poor in aromatics, terpene derivates, and carbonyl compounds. The wines aged in raw amphorae, placed in the quadrant identified by positive values of both the components, revealed higher contents in terpenes and carbonyl compounds. The wine aged in glazed amphorae, placed in the quadrant identified by positive values of the first component and negative values of the second component, was characterized by a relatively poor volatile profile in alcohols and esters.

Conclusions

The experimental results demonstrated that vinification methods and types of aging are useful tools in diversification of monovarietal white wines.

Cryomaceration under reductive conditions allowed to increase and protect against oxidation the phenolic compounds in addition to the already well-known ability to preserve and enhance the volatile fraction. The prevention of phenolic oxidation has a fundamental importance in white wines to avoid unpleasant color changes caused by the formation of quinones.

The in-amphorae aging was able to enhance the grape varietal characteristics, to guarantee microxygenation without the contribution of phenolics and volatile compounds transferred to wine from the wood of the barrels, and to give rise to wines with different phenolic and volatile composition depending on the type of amphora.

Table 4 Volatile compounds (AU × 10^5) isolated from *Greco* wines produced through cryomaceration under reductive conditions and aged for 12 months in three types of amphorae and in glass containers

Compounds (m/z)	tr (min)	At racking	After 12 months of aging				Odour description
			C + AAc	C + AAg	C + AAi	C + AAv	
Acids							
Furan-2-carboxylic acid, 5-(1-hexynyl) (192)	31.5	1.6 ± 0.3c	0.2ab	0.3 ± 0.1ab	n.d.a	0.5 ± 0.1ab	
Hexanoic acid (60)	35.0	6.2 ± 2.0b	n.d.a	n.d.a	n.d.a	n.d.a	Cheese; fatty; sour
Octanoic Acid (60)	40.3	148 ± 54b	n.d.a	n.d.a	n.d.a	n.d.a	Fatty acid; dry; dairy
n-Decanoic acid (60)	46.2	65.8 ± 9.6b	n.d.a	n.d.a	n.d.a	n.d.a	Fatty acid; dry; woody
Total		222	0	0	0	1	
Percentage (%)		4.0	0.01	0.01	0	0.02	
Alcohols							
1-Propanol (59)	9.7	1.4 ± 0.1a	1.6 ± 0.1a	2.9 ± 0.3b	1.8 ± 0.8ab	2.4 ± 0.3ab	Alcohol; ripe fruit
1-Propanol, 2-methyl- (43)	11.3	22.2 ± 1.6a	25.6 ± 4.3a	29.5 ± 4.7a	28.0 ± 5.2a	27.7 ± 3.7a	Alcohol; solvent
1-Butanol (56)	13.0	0.8 ± 0.1a	1.1 ± 0.2ab	2.6 ± 0.3c	1.4 ± 0.2b	3.0 ± 0.2c	Medicinal; phenolic
1-Butanol, 2-methyl- (57)	15.0	296 ± 19a	370 ± 48a	335 ± 53a	376 ± 18a	301 ± 30a	
1-Butanol, 3-methyl- (55)	15.2	537 ± 35a	673 ± 80a	600 ± 92a	682 ± 31a	541 ± 57a	Fusel; alcohol; sweet; fruity
1-Butanol, 2-ethyl- (70)	18.4	n.d.a	0.1 ± 0.1a	1.2 ± 0.1b	0.2a	2.2 ± 0.4c	
1-Pentanol, 4-methyl (56)	18.6	1.6 ± 0.2a	1.7 ± 0.2a	1.3 ± 0.2a	1.6 ± 0.1a	1.2 ± 0.2a	
1-Pentanol, 3-methyl (56)	19.1	2.8 ± 0.3a	2.9 ± 0.4a	3.2 ± 0.2a	2.9 ± 0.1a	3.1 ± 0.5a	Chocolate; wine-like; green
1 Hexanol (56)	20.0	19.3 ± 2.8d	17.9 ± 2.4cd	11.9 ± 0.8ab	14.4 ± 0.2bc	8.8 ± 1.1a	Herbaceous
3-Hexen-1-ol [E] (41)	20.3	0.3 ± 0.1a	0.8 ± 0.1c	0.8 ± 0.1c	0.8c	0.6 ± 0.1b	Plant; fruity; aromatic
1-Heptanol (70)	23.3	0.4 ± 0.1a	0.9 ± 0.1d	0.7 ± 0.1bc	0.7c	0.6b	
1-Hexanol, 2-ethyl (57)	24.4	0.4 ± 0.1a	10.3 ± 0.8b	15.7 ± 1.1c	19.2 ± 2.3c	15.0 ± 2.6c	Rose; sweet
1-Octanol (56)	26.5	2.14 ± 0.3c	1 ± 0.1b	0.5 a	0.6 ± 0.1a	0.3 ± 0.1a	Orange; floral
1-propanol, 3-(methylthio) (106)	31.4	0.7 ± 0.1a	0.7 ± 0.2a	0.6 ± 0.2a	0.6 ± 0.1a	0.6a	
Phenylethyl Alcohol (91)	36.8	632 ± 25a	573 ± 23a	683 ± 89a	633 ± 43a	640 ± 100a	Flowery; rose; honey
Total		1516	1682	1688	1765	1549	
Percentage (%)		27.4	55.15	54.1	60.06	55.53	
Ethyl esters							
Butanoic acid, ethyl ester (71)	9.8	11.0 ± 8.1a	10.8 ± 1.3a	28.4 ± 2.6b	18.1 ± 0.4ab	26.0 ± 8.8b	Banana; pineapple; sweet; ethereal
Butanoic acid, 2-methyl-, ethyl ester (57)	10.2	1.3 ± 0.9a	2.9 ± 0.3ab	3.9 ± 0.1ab	2.9 ± 0.1ab	4.7 ± 1.3b	Fruity; green
Butanoic acid, 3-methyl, ethyl ester (88)	10.7	1.6 ± 0.2a	3.0 ± 0.5a	3.7 ± 0.6a	2.6 ± 0.1a	4.3 ± 1.8a	Apple
Hexanoic acid, ethyl ester (88)	16.0	416.8 ± 4.4e	119 ± 12c	167 ± 15d	85 ± 11b	41 ± 15a	Apple; banana; wine-like
Heptanoic acid, ethyl ester (88)	19.4	1.4 ± 0.3b	0.6a	1.8 ± 0.2b	0.5 ± 0.1a	0.6 ± 0.1a	Fruity
Propanoic acid, 2-hydroxy, ethyl ester (S) (45)	19.7	2.3 ± 0.1a	8.0 ± 0.9b	4.2 ± 1.3b	7.3b	2.9 ± 0.3a	Lactic
Ethyl 2-hexenoate (97)	19.8	1.6 ± 0.7b	0.4a	0.4a	0.3 ± 0.1a	0.3a	Fuity; green apple
Octanoic acid, ethyl ester (88)	22.7	1440 ± 286b	389.0 ± 9.0a	364 ± 54a	249.2 ± 7.2a	240 ± 33a	Banana, floral, pear, pineapple, wine-like
Nonanoic acid, ethyl ester (88)	25.9	3.4 ± 0.9b	1.1 ± 0.1a	2.9 ± 0.1b	1.6 b	2.7 ± 0.3b	Oily; fruity; nutty
Decanoic acid, ethyl ester (88)	29.0	650 ± 90b	155.0 ± 9.4a	125 ± 12a	160.3 ± 0.7a	225 ± 28a	Grape; oily; wine-like
Butanedioic acid, diethyl ester (101)	30.3	15.7 ± 2.7a	166 ± 24b	173 ± 18b	149 ± 11b	158 ± 33b	Apple; apricot; chocolate; cranberry; grape
Ethyl 9-decenoate (88)	30.6	33.7 ± 6.1b	3.4 ± 0.7a	2.8 ± 0.1a	2.3 ± 1.6a	6.1 ± 0.4a	
Undecanoic acid, ethyl ester (88)	31.9	0.4 ± 0.2b	n.d.a	0.2ab	0.1 0a	0.2ab	Coconut

Table 4 (continued)

Compounds (m/z)	tr (min)	At racking	After 12 months of aging				Odour description
			C + AAc	C + AAg	C + AAi	C + AAv	
Benzeneacetic acid, ethyl ester (91)	33.4	1.8 ± 0.2a	3.1 ± 0.3ab	6.7 ± 0.3b	4.1 ± 0.7ab	12.8 ± 2.5c	
Acetic acid, 2-phenylethyl ester (104)	34.3	227 ± 30b	49.7 ± 4.8a	58.7 ± 5.5a	51.0 ± 5.7a	50.7 ± 9.4a	
Dodecanoic acid, ethyl ester (88)	34. 8	83 ± 18b	10.0 ± 0.9a	16.0 ± 1a	12.2 ± 1.9a	20.1 ± 1.6a	Green; floral; fruity
Butanedioic acid, diethyl ester (101)	36.4	10.3 ± 0.5a	16.4 ± 5.0ab	29.0 ± 7.3b	21.3 ± 3.5b	23.1 ± 6.3b	Spicy
Tetradecanoic acid, ethyl ester (88)	40,0	8.1 ± 3.2b	0.4 ± 0.1a	1.2 ± 0.1a	0.5 ± 0.1a	0.8 ± 0.1a	Waxy; soapy
Hexadecanoic acid, ethyl ester (88)	45.6	39.7 ± 4.5b	0.5 ± 0.1a	1.0 ± 0.1a	0.5 ± 0.2a	0.9 ± 0.0a	Waxy
Ethyl 9-hexadecenoate (88)	46.5	0.8 ± 0.8ab	0.6 ± 0.3a	1.2 ± 0.1ab	0.2 ± 0.2a	2. ± 0.5b	
Octanoic acid, 2-phenylethyl ester (104)	50.6	13.2 ± 2.3b	n.d.a	n.d.a	n.d.a	n.d.a	Fruity; green; floral
Octadecanoic acid, ethyl ester (88)	54.1	0.8 ± 0.1b	n.d.a	n.d.a	n.d.a	n.d.a	Waxy
Linoleic acid, ethyl ester (67)	58.2	1.5 ± 1.0b	n.d.a	n.d.a	n.d..a	n.d.a	
Total		2964	941	991	769	822	
Percentage (%)		53.6	30.8	31.7	26.2	29.5	
Other esters							
1-Butanol, 3-methyl-, acetate (70)	12.3	330 ± 79b	61.4 ± 6.1a	49.6 ± 5.2a	49.0 ± 3.4a	30 ± 15a	Banana
1-Butanol, 3-methyl-, propanoate (57)	14.5	1.3 ± 0.2ab	0.3a	1.7 ± 0.2b	1.1 ± 0.3ab	1.9 ± 0.7b	Apple; apricot; banana; citrus; pineapple; tomato; wine-like
Acetic acid, hexyl ester (43)	17.3	29.5 ± 5.4b	3.9 ± 0.5a	2.7 ± 0.5a	3.0 ± 0.2a	1.8 ± 0.5a	Apple, cherry, fruity; green; citrus; sweet
n-Caproic acid isobutyl ester (99)	20.0	0.8 a	0.6 ± 0.1a	1.0 ± 0.1b	0.7 a	0.7 ± 0.1a	Apple; chocolate; pineapple; sweet
Octanoic acid, methyl ester (74)	21.3	2.3 ± 0.3b	0.2 ± a	0.4a	0.1 ± 0.1a	0.3 a	Fruity; green; citrus
Isopentyl hexanoate (70)	23.5	12.1 ± 3.1a	3.6a	9.5 ± 0.2a	4.3 ± 0.a	4.7 ± 0.5a	
n-Caprylic acid isobutyl ester (127)	26.4	1.50 ± 1a	0.6a	0.8a	0.8 ± 0.2a	1.0 ± 0.1a	
Octanoic acid, 3-methylbutyl ester (70)	29,6	68 ± 13b	5.0 ± 0.5a	8.6 ± 0.6a	6.1 ± 0.5a	9.8 ± 0.9a	Apple; coconut; green; waxy; fruity; pineapple; sweet
n-Capric acid isobutyl ester (155)	32.4	0.6 ± 0.1b	n.d.a	n.d.a	n.d.a	n.d.a	
Pentadecanoic acid, 3-methylbutyl ester (70)	35.3	26.8 ± 8.2b	n.d.a	n.d.a	1.0 ± 0.2a	0.8 ± 0.1a	
Propanoic acid, 2-methyl-, 1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester (71)	35.8	255 ± 66b	22.0 ± 4.4a	16.1 ± 5.6a	8.8 ± 1.4a	16.8 ± 5.3a	
β-Phenylethyl butyrate (104)	38.2	3.0 ± 0.6c	0.1 ± 0.1a	0.7 ± 0.1ab	0.4 ± 0.1ab	1.1 ± 0.3b	Grape; floral; strawberry; sweet
Total		733	98	91	75	69	
Percentage (%)		13.2	3.2	2.9	2.5	2.5	
Carbonyl compounds							
Carbonyl like compound (101)	13.5	n.d.a	1.8 ± 0.3b	4.0 ± 0.3c	1.6 ± 0.4b	4.3 ± 0.7c	
3-Octanone (99)	16.8	0.4 ± 0.1b	n.d.a	n.d.a	n.d.a	n.d.a	Banana; berry; butter; cheese; musty; spicy; herbaceous; vegetable; earthy; green
Benzaldehyde (106)	25.8	0.5 ± 0.1b	0.3 a	0.5 ± 0.1a	0.4a	0.6 ± 0.1b	Almond; cherry; sweet

Table 4 (continued)

Compounds (m/z)	tr (min)	At racking	After 12 months of aging				Odour description
			C + AAc	C + AAg	C + AAi	C + AAv	
Benzeneacetaldehyde (91)	29.0	n.d.a	n.d.a	1.6 ± 0.2b	0.3 ± 0.1a	3.1 ± 0.3c	
Benzaldehyde, 3,4-dimethyl (133)	34.3	1.4 ± 0.1a	2.4 ± 0.2b	5.0bc	2.5 ± 0.3b	6.9 ± 0.8c	
Total		2	5	11	5	15	
Percentage (%)		0.04	0.1	0.3	0.2	0.5	
Hydrocarbons							
Pentane, 1-(1-ethoxyethoxy)- (73)	11.8	n.d.a	45.8 ± 9.3b	40 ± 12b	44.3 ± 4.3b	31.6 ± 5.6b	
Dodecene (57)	14.7	0.3a	10.0 ± 1.3b	13.7 ± 2.3b	10.6 ± 1.2b	8.9 ± 2.8b	
1,4 hexadiene (67)	18.5	0.7 ± 0.1b	n.d.a	n.d.a	n.d.a	0.1 a	
Tetradecane (71)	21.5	0.4 ± 0.1a	14.1 ± 0.9b	18.5 ± 1.8c	15.0 ± 0.2b	15.4 ± 1.8bc	
Hexadecane (57)	27.7	1.5 ± 0.6a	53.6 ± 3.0bc	46.6 ± 2.6c	55.0 ± 2.0b	51.5 ± 3.5bc	
Heptadecane (57)	30.7	5.9 ± 0.8a	57 ± 3c	45.4 ± 1.5b	57.0 ± 3.3c	55.2 ± 4.3c	
Naphthalene, 1,2-dihydro-1,1,6-trimethyl- (157)	32.4	10.9 ± 4.4b	0.4 ± 0.1a	1.0 ± 0.4a	0.1 ± 0.1a	1.5 ± 0.7a	
Hexadecane, 2,6,10,14-tetramethyl- (57)	32.8	7.3 ± 1.3a	20 ± 1b	18.0 ± 0.7b	19.7 ± 2.0b	20.6 ± 0.7b	
Octadecane (57)	33.5	6.0 ± 2.4a	48.6 ± 2.7b	41.0 ± 2.1b	48.6 ± 3.4b	48.5 ± 2.2b	
Heneicosane (57)	34.9	4.2 ± 0.2a	8.5 ± 0.5b	5.7 ± 1.2ab	7.5 ± 1.9b	5.8 ± 0.2ab	
Pentadecane (57)	36.2	10.8 ± 3.4a	20.0 ± 1.6ab	24.5 ± 1.0ab	26.0 ± 2.ab	27.5 ± 1.4b	
Eicosane (57)	39.0	5.8 ± 0.9a	8.6 ± 0.5b	7.9 ± 0.5b	8.8 ± 0.8b	9.2 ± 0.4b	
Heneicosane (57)	41.2	1.3 ± 0.3a	2.0 ± 0.2b	1.7 ± 0.1ab	1.4 ± 0.1b	2.1 ± 0.1ab	
Total		55	288	264	294	278	
Percentage (%)		1	9.45	8.44	10.00	9.96	
Terpene derivatives							
Limonene (68)	14.8	0.6 ± 0.1a	0.6 ± 0.3a	0.9 ± 0.1a	0.9 ± 0.1a	1.0 ± 0.1a	Herbaceous; minty
4-Carene (93)	17.7	0.3 ± 0.2b	n.d.a	0.2 ab	n.d.a	0.4 ± 0.1b	Lemon
Nerol oxide (68)	24.0	0.6 ± 0.1b	0.3a	0.2a	0.2a	0.6 ± 0.1b	
2-Bornene (121)	25.4	0.8 ± 0.1b	n.d.a	n.d.a	n.d.a	n.d.a	
Terpene like componds (192, 93, 177)	25.9	3.0 ± 0.8b	0.3 a	0.7 ± 0.2a	n.d.a	1.1 ± 0.5 a	
β-Ionene (116)	26.0	0.7 ± 0.1a	0.4a	0.2a	0.3a	0.2a	Berry; cherry; woody; violet
beta.Myrcene (93)	26.2	1.2 ± 0.1a	1.1 ± 0.1a	1.4 ± 0.2a	1.0 ± 0.2a	3.9 ± 0.3a	Anise; grape; fruity; herbaceous
Hotrienol (71)	28.2	0.9 ± 0.1b	0.3 ± 0.3ab	0.4 ± 0.3ab	0.1 ± 0.2a	0.9 ± 0.2b	
α-Terpineol (59)	30.8	0.4 ± 0.1a	0.7 ± 0.1a	0.8 ± 0.1a	0.5 ± 0.1a	2.3 ± 0.7b	Lilac
β-Citronellol (69)	32.6	0.6 ± 0.1a	0.6 ± 0.1a	1.0 ± 0.2a	0.9 a	1.7 ± 0.2b	Geranium; rose
β-Damascenone (69)	34.4	14.6 ± 1.2b	n.d.a	n.d.a	n.d.a	n.d.a	Apple, herbaceus, woody
Nerolidol (69)	39. 8	3.6 ± 0.7b	0.5 ± 0.9a	0.8 ± 0.1a	0.5 ± 0.2a	1.3 ± 0.1a	Apple; green; woody; citrus; rose
Farnesol (69)	39.9	3.1 ± 0.8b	n.d.a	n.d	0.1a	0.4 ± 0.1a	Anise; apricot; balsam
Total		31	5	7	5	14	
Percentage (%)		0.5	0.2	0.2	0.1	0.5	
Aromatics							
Benzene, 1.3-bis[1.1-dimethylethyl]- (175)	22.6	0.4 ± 0.1a	14.9 ± 1.0b	31.3 ± 3.9c	14.4 ± 0.2b	18.2 ± 2.9b	
Butylated hydroxytoluene (205)	41.2	1.3 ± 0.4b	0.3 a	0.5 ± 0.1a	0.2 ± 0.2a	0.7 ± 0.2a	
Phenol. 2.4-bis(1.1-dimethylethyl)- (191)	47.5	8.4 ± 2.2a	16.0 ± 3.2ab	38.5 ± 4.9c	12.7 ± 4.6a	23.7 ± 3.3b	
Indole (117)	53.8	1.3 ± 0.6b	n.d.a	n.d.a	n.d.a	n.d.a	Butter; cheese; chocolate; grape

Table 4 (continued)

Compounds (m/z)	tr (min)	At racking	After 12 months of aging				Odour description
			C + AAc	C + AAg	C + AAi	C + AAv	
Total		11	31	70	27	43	
Percentage (%)		0.2	1.0	2.2	0.9	1.5	

C+AA Cryomaceration in reductive vinification, *v* wine aged in glazed amphorae, *g* wine aged in raw amphorae, *i* wine aged in engobe amphorae, *c* wine aged in glass containers, *nd* not determined

In line, different letters indicate significant differences at $p < 0.05$ by LSD multiple range test

* Peak areas of the major MS fragments (m/z, indicated after the compound name) were corrected by normalization factor, as described in the experimental section

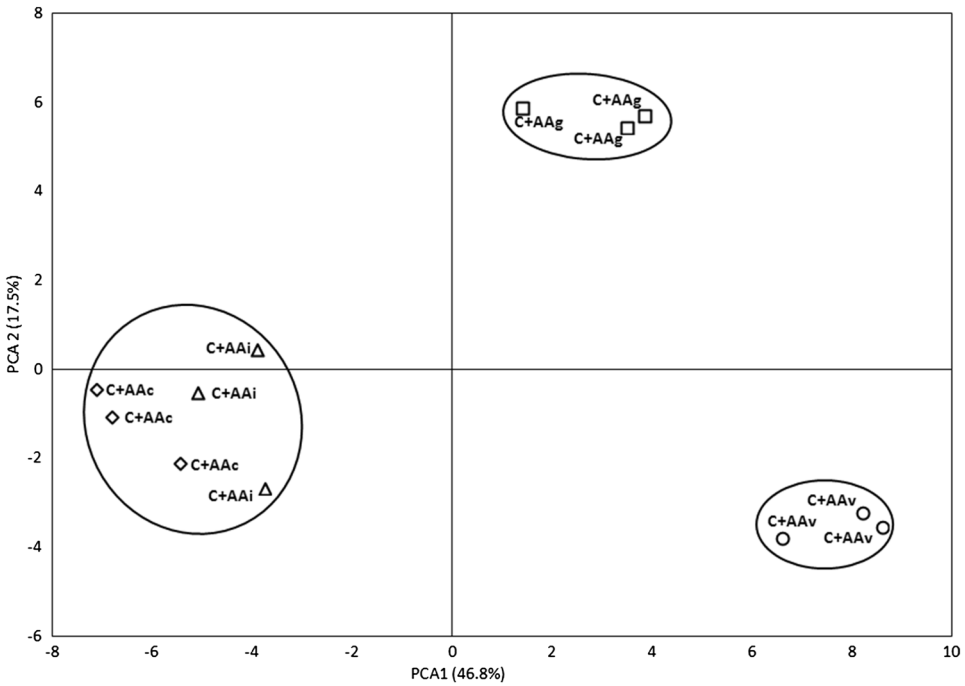

Fig. 2 PCA scatter plots based on the concentration of 8 classes of volatile compounds. Projection on the factor plane of wines produced through cryomaceration under reductive conditions and analyzed 12 months after racking. *C+AA* cryomaceration in reductive vinification, *v* wine aged in glazed amphorae, *g* wine aged in raw amphorae, *i* wine aged in engobe amphorae, *c* wine aged in glass containers

Compliance with Ethical Standards

Compliance with ethics requirements This article does not contain any studies with human or animal subjects.

Conflict of interest The author has no conflict of interest to declare.

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