Volatile, phenolic and sensory profile of in-amphorae Chardonnay wine by mass spectrometry and chemometric analysis

Fabrizio Rossetti\textsuperscript{a}, Vakare Merkyte\textsuperscript{b}, Edoardo Longo\textsuperscript{b,\ast}, Branimir Pavlic\textsuperscript{c}, Michael Jourdes\textsuperscript{d,e}, Pierre-Louis Teissedre\textsuperscript{d,e}, Emanuele Boselli\textsuperscript{b}

\textsuperscript{a} Università Politecnica delle Marche, Department of Agricultural, Food and Environmental Sciences, Via Brecce Bianche 10, 60131 Ancona, Italy

\textsuperscript{b} Free University of Bozen-Bolzano, Faculty of Science and Technology, Piazza Università 5, 39100 Bozen-Bolzano, Italy

\textsuperscript{c} University of Novi Sad, Faculty of Technology, Bulevar Cara Lazar 1, 21000 Novi Sad, Serbia

\textsuperscript{d} University of Bordeaux, ISVV, EA 4577, Œnologie, 210 Chemin de Leysotte, F-33140 Villenave d’Ornon, France.

\textsuperscript{e} INRA, ISVV, USC 1366 INRA, IPB, 210 Chemin de Leysotte, F-33140 Villenave d’Ornon, France.

Correspondence to:
Dr Edoardo Longo (ORCID: orcid.org/0000-0002-0594-6722)
Address: Faculty of Science and Technology, Free University of Bozen-Bolzano
Piazza Università 5,
39100 Bolzano (Italy)
Phone: +39 0471 017691
edoardo.longo@unibz.it

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Abstract

The sensory properties, the phenolic composition and the volatile profile of Chardonnay wine made in-amphorae were compared with the wine obtained in large wooden barrels (2000 L) and small toasted barrels (225 L). Hierarchical Cluster Analysis and Principal Component Analysis built on the phenolics and volatiles variables allowed to group effectively the samples according to the winemaking material employed. In-amphorae wines showed more abundant catechin and caffeic acid, and less abundant caftaric acid and trans-coutaric acid. Condensation reactions proceeded in the wood containers leading to esterification of organic acids with ethanol and alcohols, whereas in-amphorae wines were characterized by a higher content of free phenolic acids and higher volatile alcohols. Among the volatile compounds, ramified ethyl esters contributed mostly in samples made in small toasted barrels, whereas non-branched ethyl esters contributed more for the samples made in large wooden tanks; higher alcohols contributed more for the in-amphorae wine. The sensory analysis showed negligible differences induced by the in-amphorae vinification with respect to the wooden one. Four variables could distinguish wines made in-amphorae compared to the other containers: solvent and acetone (SA), astringent/pungency (AP), fruity (FR) and color intensity (CI). The overall approach proposed here is promising for future developments of innovative types of Chardonnay wine blends.

Keywords: chemometrics, Chardonnay, amphorae, white wine, aroma, phenolics, sensory analysis
Introduction

Chardonnay is one of the most cultivated grape varieties because it adapts to the various climates and soils of the main viticultural areas in the world, such as Spain, Portugal, USA, Australia, New Zealand, France, Italy, Argentina, South Africa and Chile.\(^1\) The phenolics content of Chardonnay wine has been described in the literature.\(^2\) Chardonnay wines enriched with phenolic compounds have been associated with antioxidant capacities comparable to those of red wines; in addition, antioxidant phenolic compounds have been also related to beneficial health effects in-vivo.\(^3,4\)

Chardonnay wine has been associated with more than 140 identified volatile compounds.\(^5\) However, only few compounds have been designated as the major contributors to the Chardonnay varietal wine aroma, such as ethyl cinnamate (cherry pits), ethyl hexanoate (green grass), ethyl 2-methylbutanoate (apple), ethyl butanoate (fruity), and other unidentified compounds characterized as \textit{burnt sugar}, \textit{wet ashes} and honey aromas.\(^6\) The profile of aroma compounds is heavily influenced by the winemaking practice employed. The alcoholic fermentation and the wine ageing are commonly performed in stainless steel tanks or in oak barrels in order to enhance aroma and obtain high quality wines.\(^7\) Wooden tanks (small toasted barrels or large non toasted barrels) are used because they improve micro-oxygenation. Besides, aroma compounds from wood (toasted or non-toasted) are released into the wine modifying its sensory properties.

Nowadays, in-amphorae winemaking is becoming a valuable alternative to the traditional wooden containers. The practice of employing earthenware for storage is very ancient. It was used since ancient Rome and it was also adopted in the Middle East.\(^8\) However, it had a major drawback with respect to wooden containers: clay is usually more porous than wood and it allowed a faster oxygen diffusion, which had negative effects on the preservation of the wine. For this reason, in ancient times the wine was usually mixed with other ingredients (such as honey and spices) to cover the off-flavors and off-taste, which were due to such excessive oxygenation. Eventually, wooden containers replaced the earthenware for good and they have been the main vinification tanks adopted until the modern age. Nowadays, the processing technology of earthenware has much improved and it allows slower oxygenation rates than in the past. Consequently, earthenware has been recently re-adopted by several wineries in the attempt of attracting new groups of consumers by proposing revisited traditional winemaking methods. The main aim of in-amphorae winemaking is to provide a beneficial oxygen micro-diffusion without the transfer of wooden aroma compounds (such as
vanillin, tannins and toasted flavors) common to vinification in wood tanks.\[^9\] There is only little scientific information regarding the effects of in-amphorae winemaking on the quality, the chemical profile and the sensory properties of wines obtained with this technique. According to Lanati et al.,\[^10\] Georgian white wines aged in amphorae had a darker -almost orange color, which is uncommon for white wines. Recently, in-amphorae winemaking has been successfully applied for the production of Fiano Passito,\[^11\] Falanghina,\[^12\] Minutolo\[^13\] and Chardonnay\[^14\] white wines.

The aim of this report is to compare the effects of in-amphorae winemaking with classical white wine winemaking process and to determine its impact on the phenolic and volatile profile on the obtained Chardonnay wine. The use of mass spectrometry coupled to UHPLC and GC was a powerful tool to detect, identify and quantify the characterized phenolics and volatile compounds of three winemaking methods (earthenware, large wooden tanks and small toasted barrels). The results are compared with the sensory preferences to provide a better analytical and technological knowledge potentially applicable to the winemaking strategies of a winery.

**Experimental**

**Winemaking**

The winemaking procedures were presented earlier.\[^14\] Briefly, Chardonnay grapes were harvested manually in a single vineyard located in Italy at the end of August. Of the 8 t harvested grapes, an aliquot was destined to three different vinification procedures: two large wooden non-toasted barrels (samples T1 and T2) (2000 L each), two wooden toasted barrels (samples B1 and B2) (225 L each) and two clay amphorae (225 L each) (samples A1 and A2). Thus, six wine samples were used for the chemical and sensory analysis. The chemical quality and sensory profile of the wine was monitored in the first six months of the winemaking process.

**Winemaking in amphorae**

An aliquot of Chardonnay grapes (about 800 Kg) was manually selected and destemmed under nitrogen. Destemmed berries were practically undamaged. Then, the berries were introduced manually into two amphorae. The amphorae (225 L) were made of earthenware and were obtained from Tava srl, Mori (Italy). Earthenware had a porosity lower than 6%, a
water absorption of about 3.5%, with a pore diameter equal to about 0.05 µm, corresponding to a flow of O$_2$ of 0.4 mL/L/month.

A yeast culture of *Saccharomyces cerevisiae* (premium Chardonnay, VASON, Italy) was inoculated at 20 g/100 Kg. Because of the strong development of carbon dioxide during the in-amphorae fermentation and to avoid the contamination by insects or other sources, a “cap” of brushed cotton was applied at the top of container. Anyway, the cap was permeable to the fermentation gases. After a week, the alcoholic fermentation had ended, and the malolactic fermentation was induced by inoculating (1g/hL) strains of lactic acid bacteria (*Oenococcus oeni*). After the malolactic fermentation was over and the gas production stopped, dry ice was placed on the top layer of pomace to prevent oxidation. The amphorae were closed and sealed with covers by means of a silicone gasket suitable for food use. A bunghole allowed dry ice sublimation. Then, carbon dioxide was fluxed through the bunghole with a flexible tube for 4-5 days after sealing to maintain an inert head space. The in-amphorae maceration lasted until next year in March. The wine was racked and then maintained in a reduced atmosphere into a steel tank until manual bottling (which took place in May). Nitrogen was used to displace oxygen inside the bottles and in the headspace between cork and wine.

**Winemaking in barrels and barriques**

The remaining 7 tons of grapes were used for winemaking in oak large tanks and toasted oak barrels. The berries were separated from the stems and crushed under nitrogen. Then they were cooled down to 10 °C through a concentric tube heat exchanger of about 60 m length. Cooled berries were softly pressed by using a pneumatic press (Velvet 50, DIEMME, Lugo, Italy) until about 72% wine-to-grape yield was reached. Then, the juice was transferred into a tank and was continuously blanketed with nitrogen gas. Afterwards, the juice was transferred into an 80-hL steel tank, which was equipped with a cooling system. The juice was decanted for about 34 h at 12 °C. After decantation, the juice was further clarified by flotation. Afterwards, the clarified must was heated to 18 °C and inoculated with yeasts in the same conditions used for in-amphorae winemaking. Inactivated yeast (30 g/hL) (B-vitality, HTS, Marsala, Italy) was added as nutrient. Then, the must was divided into two large, non-toasted oak tanks, 20 hL each, and in 2 toasted oak barrels of 225 L.

At the end of the alcoholic fermentation, the malolactic fermentation was induced as in the in-amphorae winemaking. For the first three months, *batonnage* was carried out once a week; then, the frequency of mixing was halved in the next two months. Sulfur dioxide was added to wine in large tanks and toasted barrels (25 mg/L) where the wine continued its aging until
May. Then, the wine was transferred from wood into steel tanks and was kept at a temperature of -3 °C for ten days. After this period, the wine was immediately transferred into other steel tanks. The filtration was carried out through a filter press before bottling. Bottles were previously rinsed with 0.2 µm micro-filtered sterile water and then dried with compressed nitrogen gas at 2 atm. Air was replaced by nitrogen (99.8%) in the head space of the bottles.

**Analytical and Sensory Determinations**

**Chemicals**

Water, methanol and formic acid (all Optima LC/MS grade) for the UHPLC-MS analysis were obtained from Fisher Scientific (Geel, Belgium). Standard compounds (gallic acid, caffeic acid, (+)-catechin, p-coumaric acid) used to confirm the identification of phenolics in wine were purchased from Sigma–Aldrich (Saint Quentin Fallavier, France).

**GC-MS determination of volatile compounds**

The GC-MS determination of volatile compounds was performed according to a published procedure,[15] with slight modifications reported as follows. The wine was introduced into a 10-mL vial and closed with a screw cap equipped with an elastomeric septum. The vial was placed in a heating bath at 40°C for 10 min. Then, a SPME fibre (Divinylbenzene / Carboxen / Polydimethylsiloxane, 1 cm, 50/30 µm) from Supelco/Sigma-Aldrich (Milan, Italy) was introduced into the vial and exposed to the sample headspace for 15 min. The thermal desorption took place in the GC injector at 220 °C for 15 min. The splitless injection (splitless time 0.3 min) was performed in a Varian 3900 gas-chromatograph coupled to a Saturn 2100T (Varian, Walnut Creek, CA, USA) ion trap mass spectrometer. The chromatographic separation was obtained with a ZB-5 capillary column (Phenomenex, 30 m × 0.25 mm I.D., film thickness 0.25 µm). The temperature program of the GC oven started at 40 °C during 10 min, then was raised to 180 °C at 3 °C/min and reached 250 °C at 15 °C/min. The MS transfer line and trap temperatures were set at 200 °C. The ion trap emission current was 10 µA. The mass spectra were recorded in the full scan mode (mass range 31-250 m/z) at 1 scan/sec.

Data were analysed with the Varian Workstation software. The identification of volatile compounds was confirmed (1) with the GC retention index, (2) comparison with the NIST library mass spectra (Version: 2.0; 2002), (3) injection of pure standard substances when available and (4) with the aid of earlier reports.[16,17,18] Samples were analysed in duplicate.
(two different containers); peak areas were normalized over the total ion current (TIC) of each sample and were reported as percentages.

**UHPLC-DAD-ESI-QToF/MS analysis of phenolics**

The phenolic profile of the wines was obtained through a UHPLC system (Agilent 1290 Infinity) equipped with a UV-Vis diode array detector (1290 Infinity DAD) and connected to a ESI-QToF/MS mass spectrometer (Agilent 6530 Accurate Mass) run in negative ionization. The chromatographic separation was carried out with a C18 UHPLC column (2.1 × 100 mm, 1.8 µm, Agilent). Water (Eluent A) and methanol (Eluent B) were used as mobile phases both acidified with 0.1% formic acid. The gradient program was as follows: 0% B for 0.5 min; 0 to 35% B for 19.5 min; 35 to 95% B for 4 min; 95% B for 3 min; 95 to 10% B for 1 min; 10% B for 2 min. The UHPLC flow rate was 0.3 mL/min, the injection volume was 2.0 µL and the column temperature was set at 25°C. The mass spectrometer was operated in extended dynamic range of 2 GHz (m/z 3200 Th). The nebulizer pressure and flow rate were set at 25 psi and 9 L/min, respectively. The drying gas temperature was 300 °C. The sheath gas flow and temperature were set at 11 L/min and 350 °C. The fragmentation, skimmer, OCT and capillary voltage were at 150 V, 65 V, 750 V and 4000 V, respectively. All the analyses were performed in negative mode. Data were analyzed with the Mass Hunter Qualitative Analysis software. The chromatogram was recorded at the wavelength of 280 nm (quantitation wavelength). The identification of phenolics was achieved by comparison of their retention times and exact masses with those of the injected standard compounds. The quantitation was achieved using the DAD calibration curves of pure standard substances. When reference compounds were not available, a calibration with structurally related standard substances was used (gallic acid for protocatechuic acid; caffeic acid for caftaric acid, ethylcaffeate and GRP; (+)-catechin for (-)-epicatechin; p-coumaric acid for cis-coutaric acid and trans-coutaric acid). The integration of the peaks allowed to obtain the concentrations of the identified compounds. Concentrations are expressed in mg/L of standard or of the structurally related standard.

**Sensory evaluation**

The sensory characteristics of the wines stored for one year were evaluated by a panel formed by eight trained judges (professors, researchers and students) at the University Department of Ancona. The wine was served at 12 °C in ISO type tasting glasses (height 155 mm, glass diameter 65 mm, capacity, 215 mL) from Bormioli (Parma, Italy). The glasses were filled
with 50 mL wine. The sensory descriptors evaluated by the panel were identified during the first session with the procedure of the round table.[12] Limpidity, color intensity, olfactory intensity, alcohol/liquor, vinegar, caramel/toasted/cookie, herbaceous/green, fruity, tropical fruits, acid/citrus, alcoholic, sweet/honey, salty, wood/oak, herbaceous/unripe, solvent/acetone, astringent/pungency, burning and wine ‘body’ perception were the sensory descriptors. Each sample was evaluated by using a scale of ten points (1 = no perception, 10 = the highest intensity). The panel also formulated a final judgement for the three different wines.

**Statistical Analysis**

The chemical data (volatile and phenolic compounds) were analyzed by univariate analysis of variance (ANOVA) to determine those variables statistically significant to differentiate samples by the Tukey's Multiple Comparison Test and an α = 0.05 criteria using GraphPad Instat v.1.0 software (San Diego, CA, USA). Principal Component Analysis (PCA) was carried out to highlight the differences and groupings among the wines made in amphorae, in large wooden tanks and in smaller toasted barrels. Data were expressed as single measurement performed on each different bottle for the three typologies of containers (amphorae A1, A2; large wooden non-toasted barrels T1, T2; and wooden toasted barrels B1, B2). PCA was performed using The Unscrambler software (Camo Inc., Corvallis, OR). Hierarchical Cluster Analysis (HCA) was carried out employing a Single Linkage Algorithm and Euclidean Distance with PAST software V 3.18 (Hammer & Harper, Oslo, Norway).

**Results and discussion**

**Volatile compounds**

The chemical profiles of the volatile compounds observed in the Chardonnay wine samples obtained in large non-toasted oak barrels, smaller toasted oak barrels and in amphorae are presented in Table 1.

Ethyl esters, such as ethyl hexanoate, diethyl succinate, ethyl octanoate and ethyl decanoate were the most relatively abundant compounds in all samples. Other esters detected in all investigated samples were: propyl butanoate; ethyl propionate; propanoic acid, 2-methyl-, ethyl ester; butanoic acid ethyl ester; butanoic acid, 2-methyl-ethylester; butanoic acid, 3-methyl-ethylester; ethyl 4-decanoate and ethyl dodecanoate. The alcohols present in all samples were 1-hexanol, 2-ethyl-1-hexanol and phenylethyl alcohol. Other volatiles present
in all samples were three furan compounds (compound 9, 10 and 20 of Table 1). Other ubiquitous compounds were: ionone and 1,2-dihydro-1,1,6-trimethyl-naphthalene. These results were in accordance with data reported by Cejudo-Bastante et al.,\textsuperscript{[16]} in which it was shown that diethyl succinate, phenylethyl alcohol and ethyl hexanoate were the most dominant compounds in Chardonnay wine stored for 1 year. Furthermore, Ivanova et al.,\textsuperscript{[17]} reported that Chardonnay wines from Macedonia and Hungary possess the highest amount of total esters in comparison to other red and white wines from the same regions. This is also in agreement with the relatively high esters content in this work (Table 1). Hopfer et al.,\textsuperscript{[18]} also reported a similar volatile profile in Chardonnay wines.

The identification of the more volatile compounds with retention times (Rt) below 2.5 min was difficult due to the overlapping of several peaks. Therefore, these compounds were not suitable variables in the further statistical analysis.

**Phenolic compounds**

The phenolic profile has been characterized by means of UHPLC-ESI(-)-QToF/MS. A typical chromatogram (in-amphorae wine sample, single wavelength monitoring at 280 nm) is shown in Fig. 1. In all the three storage systems, eleven compounds were identified and were listed in Table 2. The peaks were numbered according to the elution order.

The compounds identified were mainly hydroxycinnamic acids and their esters, namely caffeic acid (CF), p-coumaric acid (PC), cis- and trans-coutaric acid (CC and TC, respectively), caftaric acid (CT), glutathionyl caftaric acid (grape reaction product - GRP) and ethylcaffeate (ET). The identification of these compounds was achieved based on their retention times the experimental masses (m/z) of the deprotonated molecules. Gallic acid (GA), protocatechuic acid (PR), (+)-catechin (CA) and (-)-epicatechin (EC) were also detected, assigned and quantified.

Figure 1 presents a chromatogram of a Chardonnay wine produced in-amphorae. In-amphorae wines (A1 and A2) showed more abundant (+)-catechin and caffeic acid, and less abundant caftaric acid and trans-coutaric acid. Besides, there was a broad peak between 21 and 23 min, which could not be associated to any specific chemical component.

**Statistical analysis of the chemical data**

Previous reports showed that volatile compounds are suitable variables to differentiate white wines stored under different conditions in raw, glazed and engobe amphorae\textsuperscript{[9,13]}. They were also influenced by the different level of toasting in wooden vinifications.\textsuperscript{[19]}
Herein, statistical analysis was applied to the chemical data to identify markers for the different vinifications employed. For exploratory data analysis, data pre-treatment is a useful practice to avoid trivial conclusions. ANOVA was used to assess if some differences in the aroma and phenolic compositions were statistically significant to discriminate the samples (six samples: A1, A2, B1, B2, T1, T2) according to the three different storage materials employed. The chemical variables evaluated with ANOVA were the phenolic compounds listed in Table 2 (11 compounds) and the aroma compounds reported in Table 1 (44 compounds), with the exception of five unidentified compounds eluted in the first 2.5 min of the chromatogram. These compounds were excluded from the analysis due to their too high volatility and since they overlapped in a short elution interval in the early chromatogram.

ANOVA showed that only one volatile (ethyl decanoate, 41) and one phenol (trans-coutaric acid, TC) were able to discriminate completely each one of the three classes from the other two. Hence, to give a more comprehensive perspective of the results of the chemical analysis in association with the three different vinifications, multivariate statistical analysis was performed over the entire dataset (39 volatile compounds plus 11 phenols). Hierarchical Cluster Analysis (HCA) employing Euclidean Distances and Principal Components Analysis (PCA) were applied. A neat clustering of the three different winemaking procedures was obtained with HCA (Fig. 2). The HCA dendrogram shows that the similarities between the two wooden containers were much higher than those between any wooden container and the amphorae. This difference may be also the result of the long maceration time between the wine and the solid parts of the berries (i.e., seed and skin) in the in-amphorae winemaking process (however, the discussion of the PCA reported below showed that the phenolic variables were not so effective in describing the sample variance as the volatile compounds were).

PCA (Fig. 3) offers a tool for visualizing the data structure by reducing the data dimensionality while retaining as much as possible the information present. The PCA plot in Fig. 3 (biplot) shows the loadings plot and scores plot in the space defined by the two first principal components PC-1 vs PC-2. The first two principal components using all the variables accounted for 68% of the total variance (PC-1 46 % and PC-2 22%) with this model. The distribution of the samples and variables were strongly influenced by the storage conditions, since wine in non-toasted tanks (T1, T2) and toasted barrels (B1, B2) were grouped in the upper right and in the lower right quadrants respectively, while in-amphorae samples were distributed in the central-left zone. Overall, the PC-1 differentiated successfully the wooden from the in-amphorae samples, whereas PC-2 differentiated effectively the large
tanks from the toasted barrels. 1-hexanol (17), limonene (24), 2-ethyl-1-hexanol (25), linalool (31) correlated along PC-1 with in-amphorae samples (A). Ethyl propionate (8), ethyl hexanoate (23), ethyl 2-ethyl-hexanoate (26), ethyl 2-furancarboxylate (27), nonanone (29), ethyl octanoate (35), ethyl decanoate (41) and ethyl dodecanoate (44) clustered closer to the non-toasted wooden tank (T). Samples obtained in toasted oak containers (B) correlated instead with ethyl 2-methyl-propanoate (11), ethyl 2-methyl-butanoate (15), ethyl 3-methyl-butanoate (16), ionone (37), ethyl nonanoate (38) and sesquiterpene (43). Generally, the alcohols were clustered nearer to the in-amphorae samples (A1 and A2). Non-branched ethyl esters clustered closer to the samples obtained in non-toasted wooden tanks (T1 and T2). Branched esters were clustered preferentially nearer to the wines obtained in toasted barrels (B1 and B2). Among the phenols, catechin (CA), caffeic acid (CF), p-coumaric acid (PC), epicatechin (EC) and protocatechuic acid (PR) were clustered closer to the in-amphorae wines. Gallic acid (GA), ethyl caffeate (ET), glutathionyl caftaric acid (GRP), caftaric acid (CT), cis-coutaric (CC) and trans-coutaric acid (TC) were positively correlated with wood containers. Notably, non-toasted wooden tank wines (T1, T2) contained a much higher amount of gallic acid compared to the other wines.

**Sensory panel**

The sensory evaluation was less accurate than the chemical analysis in describing the samples variance. The radar plot in Fig. 4 gives a schematic representation of the results. Due to the higher phenolic acids content (protocatechuic, p-coumaric, caffeic) in-amphorae wines were characterized by a high pungent (AP) taste. Moreover, the presence of 2-ethyl-1-hexanol and 1-hexanol was possibly related in the in-amphorae wines to higher herbaceous/green (HG) and solvent (SA) characters, as already reported.[6,21] Four variables could be used to differentiate in-amphorae wines compared to the other containers: solvent and acetone (SA); astringent/pungency (AP); fruity (FR) and color intensity (CI). In particular for CI, wines that scored 4.5 or less were made in-amphorae and the ones with 4.6-6.6 score were made in wood.

**Conclusion**

Three Chardonnay wines were obtained using three different winemaking containers (non-toasted wood tanks, toasted barrels and clay amphorae). The nature of the containers is considered to be a potential factor differentiating wines obtained from the same Chardonnay grapes. Sensory and chemical properties (volatile profile and phenolic composition) were
analyzed and the data were processed by univariate and multivariate statistical analysis. Chardonnay wines produced under different conditions can be classified according to their chemical properties (volatile profile and phenolic composition) by multivariate statistical analysis of their mass spectrometric data. Condensation reactions proceeding in the wood containers lead to esterification of linear (2000-L oak tanks) or branched (225-L toasted oak barrels) organic acids with ethanol and other alcohols, whereas the in-amphorae wines were characterized by a high contents of free phenolic acids and of higher volatile alcohols. The sensory and phenolic profiles were less effective than the volatiles in differentiating between earthenware and the wooden samples. The results of this work demonstrate the possibility of obtaining wines with peculiar chemical and sensory characteristics using different materials for winemaking containers. A first insight on the chemical properties of a modern in-amphorae wine obtained from a grape variety of international importance is provided.

Acknowledgements
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References


Table 1. Volatile compounds determined by GC-MS in the wines produced in toasted wooden barrels (B1, B2), non-toasted wooden tanks (T1, T2) and clay amphorae (A1, A2) (relative percentages).

<table>
<thead>
<tr>
<th>No.</th>
<th>Compounds</th>
<th>Rt (min)</th>
<th>Barrels</th>
<th>Tanks</th>
<th>Amphorae</th>
<th>Base peak (m/z)</th>
<th>Fragments (m/z)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>B1</td>
<td>B2</td>
<td>T1</td>
<td>T2</td>
<td>A1</td>
</tr>
<tr>
<td>1</td>
<td>n.i.</td>
<td>1.7</td>
<td>5.23</td>
<td>4.47</td>
<td>4.29</td>
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<td>2</td>
<td>n.i.</td>
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<td>5.98</td>
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<td>1.18</td>
<td>1.61</td>
<td>1.94</td>
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<tr>
<td>3</td>
<td>n.i.</td>
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<td>2.08</td>
<td>2.50</td>
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<td>1.46</td>
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<tr>
<td>4</td>
<td>n.i.</td>
<td>1.9</td>
<td></td>
<td>1.98</td>
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<td></td>
<td></td>
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<tr>
<td>5</td>
<td>n.i.</td>
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<td>2.35</td>
<td>7.75</td>
<td>6.90</td>
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<td>6</td>
<td>Propyl butanoate</td>
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<td>8</td>
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<td>0.06</td>
<td>2.27</td>
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<td>0.04</td>
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<td>2-Butyltetrahydrofuran</td>
<td>4.7</td>
<td>29.22</td>
<td>18.87</td>
<td>13.23</td>
<td>14.67</td>
<td>31.79</td>
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<td>2(3H)-Furanone, dihydro-3-hydroxy-4,4-dimethyl</td>
<td>5.1</td>
<td>0.01</td>
<td>2.86</td>
<td>3.18</td>
<td>3.17</td>
<td>2.78</td>
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<tr>
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<td>Propanoic acid, 2-methyl-, ethyl ester</td>
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<td>0.22</td>
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<td>0.22</td>
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<td>Compounds</td>
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<tr>
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<td>Terpinolene</td>
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<td>Isoeugenyl hexanoate</td>
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<td>Naphthalene,1,2-dihydro-1,1,6-trimethyl</td>
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<td>Ethyl dodecanoate</td>
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</tbody>
</table>

Rt, retention time (min); n.i., not identified; % = average concentration of the compounds; t.i., tentative identification.
Table 2. Phenolic compounds analyzed by UHPLC-DAD-ESI(-)-QToF/MS in the wines produced in toasted barrels (B1, B2), non-toasted wooden tanks (T1, T2) and clay amphorae (A1, A2).

<table>
<thead>
<tr>
<th>Peak no.</th>
<th>Compound</th>
<th>Elemental composition (ion)</th>
<th>Rt (min)</th>
<th>ESI(-)-QToF/MS [M-H] (m/z)</th>
<th>Exp. Acc. Mass [M-H] (m/z)</th>
<th>Fragments (m/z)</th>
<th>Error (mDa)</th>
<th>Relative Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>gallic acid (GA)</td>
<td>[C7H5O5]</td>
<td>4.7</td>
<td>169.0195</td>
<td>169.0142</td>
<td>125.0266</td>
<td>5</td>
<td>12.7</td>
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<tr>
<td>2</td>
<td>protocatechuic acid (PR)</td>
<td>[C7H5O4]</td>
<td>7.3</td>
<td>153.0210</td>
<td>153.0193</td>
<td>109.0308</td>
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<tr>
<td>3</td>
<td>caftaric acid (CT)</td>
<td>[C13H11O9]</td>
<td>8.9</td>
<td>311.0458</td>
<td>311.0409</td>
<td>179.0380 - 149.0118</td>
<td>5</td>
<td>2.4</td>
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<tr>
<td>4</td>
<td>glutathionyl caftaric acid (GRP)</td>
<td>[C23H26N3O15S]</td>
<td>9.7</td>
<td>616.1190</td>
<td>616.1090</td>
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<tr>
<td>5</td>
<td>cis-coutaric acid (CC)</td>
<td>[C13H11O8]</td>
<td>10.3</td>
<td>295.0495</td>
<td>295.0459</td>
<td>163.0424 - 119.0528</td>
<td>4</td>
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<tr>
<td>6</td>
<td>trans-coutaric acid (TC)</td>
<td>[C13H11O8]</td>
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<td>295.0494</td>
<td>295.0459</td>
<td>163.0428 - 119.0521</td>
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<td>7</td>
<td>(+)-catechin (CA)</td>
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<td>289.0760</td>
<td>289.0718</td>
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<td>4</td>
<td>10.4</td>
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<tr>
<td>8</td>
<td>caffeic acid (CF)</td>
<td>[C9H7O4]</td>
<td>13.4</td>
<td>179.0381</td>
<td>179.0350</td>
<td>135.0476</td>
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<tr>
<td>9</td>
<td>(-)-epicatechin (EC)</td>
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<td>245.0896</td>
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<td>p-coumaric acid (PC)</td>
<td>[C9H7O3]</td>
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<td>119.0524</td>
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<tr>
<td>11</td>
<td>ethylcaffeate (ET)</td>
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<td>207.0663</td>
<td>179.0373 - 161.0268</td>
<td>3</td>
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</tbody>
</table>

1referring to the chromatographic trace of Fig. 1. 2(eq. mg/L of the relative standard). Rt, retention time (min).
Figure 1. UV chromatogram ($\lambda = 280$ nm) of a Chardonnay wine with the identified peaks indicated and numbered. Peaks assignments are reported in Table 2.
Figure 2. HCA of the Chardonnay wines obtained in toasted barrels (B1, B2), non-toasted tanks (T2, T2) and amphorae (A1, A2).
Figure 3. Principal Components Analysis (PCA) of Chardonnay wines (PC 1 versus PC 2). 

A1, A2: wines made in amphorae; T1, T2: wines made in non-toasted oak 2000-L tanks; B1, B2: wines made in toasted oak 225-L barrels. Volatile compounds: 6 = propyl butanoate, 7 = acetic acid, 8 = ethyl propionate, 9 = 2-Butyltetrahydrofuran, 10 = 2(3H)-furanone, dihydro-3-hydroxy-4,4-dimethyl, 11 = Propanoic acid, 2-methyl-, ethyl ester, 12 = 2-pentanol, 13 = Ethyl butanoate, 14 = Propanoic acid, 2-hydroxy-, 15 = butanoic acid, 2-methyl-ethylester, 16 = Butanoic acid, 3-methyl-ethylester, 17 = 1-hexanol, 18 = isoamyl acetate, 19 = pentyl acetate, 20 = furan, 2.2’-[oxybis(methylene)]bis-, 21 = 4-ethylbenzoic acid, 2-methylpropylester, 22 = n.i., 23 = ethyl hexanoate, 24 = limonene, 25 = 2-ethyl-1-hexanol, 26 = ethyl 2-ethylhexanoate, 27 = ethyl 2-furancarboxylate, 28 = penty l isobutyrate (amyl isobutyrate), 29 = nonanone, 30 = terpinolene, 31 = linalool, 32 = phenylethanol, 33 = diethyl succinate, 34 = octanoic acid, 35 = ethyl octanoate, 36 = isopentyl hexanoate, 37 = ionone, 38 = ethyl nonanoate, 39 = napthalene, 1,2-dihydro-1,1,6-trimethyl, 40 = 4-decenolic acid, ethylester, 41 = ethyl decanoate, 42 = octanoic acid, 3-methylbutylester, 43 = sesquiterpene, 44 = ethylester of dodecanoic acid. Phenolic compounds: GA = gallic acid, PR = protocatechuic acid, CT = caftaric acid, GRP = glutathionyl caftaric acid, CC = cis-caftaric acid, TC = trans-caftaric acid, CA = catechin, CF = caffeic acid, PC = p-coumaric acid, EC = epicatechin, ET = ethylcaffetae.
Figure 4. Sensory profile of Chardonnay wines obtained in toasted oak barrels (B1, B2), non-toasted oak tanks (T1, T2) and in clay amphorae (A1, A2). LI, limpidity; CI, color intensity; OL, olfactory intensity; AL, alcohol/liquor; VI, vinegar; CTC, caramel/toasted/cookie; HG, herbaceous/green; FR, fruity; TF, tropical fruits; AC, acid/citrus; A%, alcoholic; SW, sweet/honey; SL, salty; WO, wood/oak; HU, herbaceous/unripe; SA, solvent/acetone, AP, astringent/pungency, BU, burning; BO, wine ‘body’ perception.