EFFECTS OF IN-AMPHORAE WINEMAKING ON THE CHEMICAL AND SENSORY PROFILE OF CHARDONNAY WINE

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The high value of dry extract and volatile acidity could statistically differentiate ($P < 0.05$) the Chardonnay wine obtained in amphorae with maceration and stored for six months from the wine obtained in barrels and barriques, as evidenced by the analysis of variance. Similarly, the principal component analysis showed that all the amphorae wines sampled between November and March (not the wine analyzed immediately after alcoholic fermentation) were clearly differentiated from the analogue barrel and barrique wines due to the high volatile acidity, straw colour, and tannic perception. The Chardonnay wine produced in amphorae was characterized by a spicy flavour, pleasant tannic and a less 'green' character than wines from barrels and barriques, but showed a weak varietal aroma. Thus, the commercial offer of finished wines based on Chardonnay grapes can be potentially extended by including a product processed in amphorae.

white wine, earthenware, sensory analysis, chemometrics, barrel, barrique

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INTRODUCTION

Chardonnay is one of the most cultivated grape varieties in the world. Its origin is supposed to be in Burgundy (Calò et al., 2006), but is now spread in different countries, such as France, New Zealand, Australia, Chile, Argentina and, last but not least, Italy. In fact, Chardonnay belongs to that quite rare group of varieties, which can acclimatize in many wine regions, and everywhere provides a wine with sensory characteristics recognizable and fairly constant (Amerine, Roessler, 1976).

More than 140 volatile compounds have been identified in Chardonnay by headspace analysis (Simpson, Miller, 1984; Sefton et al., 1993). Of these, only 11 compounds were identified as key-compounds responsible for the typical aroma of the Chardonnay wine: vanillin, diacetyl, 4-vinylguaiacol, ethyl cinnamate, ethyl hexanoate, ethyl 2-methylbutanoate, ethyl butanoate, guaiacol, plus three unidentified compounds described as 'burnt sugar', 'wet ashes', and 'honey' (Moio et al., 1994; Schlich, Moio 1994).

Further studies performed by Ballester et al. (2005) on 29 Chardonnay wines and 19 other single-varietal white wines revealed that 29 compounds characterize the typical aroma of Chardonnay wine. In order to preserve these compounds and to keep the sensory quality and peculiarity of the grape variety, a very important aspect to consider in winemaking is the permeability of the tank, which affects the process of micro-oxygenation during the storage and thus the final wine quality. Earthenware is a very porous material which has been used for the storage of wine and olive oils since ancient times (Pecci et al., 2013). In Italy, there is evidence of the production of amphorae destined to wine dated to the 4–5th century A.D.

However, making wine in a permeable container such as amphorae led to excessive oxidation of the final products. Often, wine was added with honey and spices in order to mask the acetic off-flavour and could not be stored in amphorae for long periods. So, with the end of antiquity, the use of ceramic containers disappeared definitively from the Italian peninsula.

The recent resumption of using modern earthenware amphorae for winemaking comes from the desire of vintners to rediscover old processing and storage techniques, by adopting traditions disappeared for several centuries (B a i a n o et al., 2014, 2015).

Although the historical tradition of winemaking in-amphorae is well known, there has been only a few scientific information regarding the quality characteristics of wines produced with this technique nowadays.

B a i a n o et al. (2014, 2015) performed some researches on white wines produced with modern amphorae. The results obtained for the Falanghina wine showed that the wines aged in glazed and engobe amphorae had a similar evolution of physico-chemical indices. Engobe amphorae allowed the best retention of phenolic compounds, especially flavans reactive with vanillin compared to raw and glazed amphorae. Other studies performed on Minutolo wine showed the dramatic decrease of flavonoids and flavans reactive with vanillin in the case of raw amphorae. The highest antioxidant activity was exhibited by wines in engobe amphorae, whereas the lowest values were showed by the wines in glazed amphorae.

The aim of this work was to compare the effects of the winemaking process on Chardonnay wine with three different storage systems (barrel, barrique, and amphorae). The chemical determinations and sensory results were statistically elaborated in order to point out significant differences and/or similarities between the three different Chardonnay wines, which were analyzed from the fermentation until bottling after a six-month storage period.

MATERIAL AND METHODS

The Chardonnay grapes were harvested on August 31, 2014 in a single vineyard of 7 ha located in S. Venanzio di Fossombrone (PU, Italy). The vineyard was planted in 2007 and the vine training system was Guyot. An amount of 80 q of grapes was harvested manually in a single day and was destined to three different types of vinification: 2 barrels (2000 l each), 3 barriques (225 l each), and 2 amphorae (225 l each). The quality profile of the wines was monitored in the first six months of the winemaking process from the chemical and sensory point of view. In the area of Fossombrone, the average minimum and maximum temperatures of the last 30 years in June and August are about 15–18°C and 23 –27°C, respectively. In 2014, the average minimum and maximum temperatures were 16.2–18°C and 24.9–26.6°C in the same months, thus they were in average with the climatic values. The average relative humidity was ca. 60% and there were about ten rainy days for each month.

Winemaking in amphorae

The earthenware amphorae (225 l) were obtained from Tava s.r.l., Mori, Italy (Fig. 1) and had a porosity lower than 6%, water absorption of about 3.5%, pore

Fig. 1. Earthenware amphorae (a), a cap of brushed cotton applied at the top of the amphorae (b)

diameter equal to about 0.05 µm, corresponding to a flow of O_2 of 0.4 ml/l/month, according to the producer.

An aliquot of Chardonnay grapes (about 8 q) was manually selected and sent to the destemmer where the air was replaced by nitrogen gas. The berries were separated from the stems and remained practically undamaged. Then, the berries were put manually into the two amphorae. A yeast culture of *Saccharomyces cerevisiae* (premium Chardonnay; VASON, Verona, Italy) was inoculated at 20 g/hl. Because of the strong development of carbon dioxide during the fermentation in amphorae and to avoid the contamination by insects or other sources, a 'cap' of brushed cotton was applied at the top of the container. Anyway, the cap was permeable to the fermentation gas.

The progress of the fermentation was daily monitored using a Baumè hydrometer (Exacta+Optech, San Prospero, Italy). After about a week, the alcoholic fermentation ended and the malolactic fermentation was induced by inoculating lactic acid bacteria strains (*Oenococcus oeni*) at 1 g/hl (Viniflora; VASON). When the malolactic fermentation was over and the gas production stopped, dry ice was deposited on the top layer of pomace inside the amphorae in order to prevent oxidation. The amphorae were then closed and sealed with their cover through a silicone gasket for food use. The dry ice sublimated through the bunghole. Successively, carbon dioxide was flown through the bunghole 4–5 days after closing the amphorae with a flexible tube in order to assure an inert head space. The in-amphorae maceration lasted until March of 2015. Then, the whole mass of pomace was extracted from the top of the container with the aid of pipes and a pump and poured on a grill. The wine was maintained in a reduced atmosphere and was then transferred into a steel tank until bottling, which was carried out in May. Bottling was manual. However, nitrogen gas was used to displace oxygen inside the bottle and in the headspace between the cork and the wine.

Winemaking in barrels and barriques

The remaining 70 q of grapes were used for the other two vinifications, in barrels and in barriques, respectively. The berries were separated from the stems and crushed, still replacing the air with nitrogen, and were cooled up to 10°C through a concentric tubes heat exchanger of about 60 m length. The cooled berries were softly pressed by using a pneumatic press Velvet 50 (Diemme S.p.A., Lugo, Italy), with steps at increasing pressure values, each of about 0.2 bar, until a value of wine-to-grape yield of about 72% was reached. Then, the juice was moved into an underlying tank and was continuously maintained in inert atmosphere conditions with nitrogen gas. Afterwards, the juice was transferred into a steel tank of 80 hl capacity equipped with a cooling system. The juice was decanted for about 34 h at a temperature of 12°C. After decantation, the juice was further clarified through flotation and subsequent removal of the liquid from the lower valve of the tank with a pump. The clarified must was transferred to another steel tank of the same capacity. Afterwards, the tank was heated to 18°C and inoculated with *Saccharomyces cerevisiae* (20 g/hl) (Premium Chardonnay; VASON) and inactivated yeast (30 g/hl) (B-vitality; HTS, Marsala, Italy) was added as nutrients. The must was then divided into two large non-toasted oak barrels, each 20 hl, and in 3 toasted oak barriques of 225 l.

At the end of the alcoholic fermentation, the malolactic fermentation was induced with the inoculation of lactic acid bacteria of the type *Oenococcus oeni* (1 g/hl) (Viniflora; VASON) before closing the bunghole. For the first three months, *bâtonnage* was carried out once a week; then, the frequency of mixing was halved in the next two months. Sulfur dioxide was added to the wine contained in barrels and barriques (25 mg/l) where the wine continued its aging in wood until May. Then the wine was transferred from the oak barriques and barrels into steel tanks, at a temperature of –3°C for ten days. After this period, the wine was immediately transferred in adjacent tanks at low temperature. The filtration was carried out through a filter press before bottling. The bottles were previously rinsed with sterile water and then dried with compressed nitrogen gas at 2 atm. The air was eliminated through a vacuum pump and the insufflation of nitrogen (99.8%), by filling with a slight depression and automatic leveling. The head space of the bottle was saturated with nitrogen gas prior to insertion of the cork stoppers.

Analytical and sensory determinations

The analytical determinations (Brix, titratable acidity, malic acid, and pH) were performed both in the Chardonnay grapes during the maturation (data not reported) and at the day of the harvest.

The chemical determinations carried out in the wine were alcohol content (% vol.), total sulfur dioxide (mg/l), titratable acidity (g/l), volatile acidity (g/l), pH, malic acid (g/l), lactic acid (g/l), and dry extract (g/l). The wine was sampled during the winemaking process at four different time intervals before bottling: 17/9/2014, 28/11/2014, 17/2/2015, 13/3/2015. Bottling took place in May. The chemical analyses (except total sulfur dioxide) were conducted with a WineScan™ (FOSS, Padova, Italy) interferometer, which is based on the Fourier transform infrared spectroscopy (FTIR). The sulfites were determined by using an automatic SO_2 titrator (SO2-Matic 23; Crison Instruments, S.A., Barcelona, Spain), based on the Ripper method (an automatized iodine titration). Iodide 0.01M, sulfuric acid (H_2SO_4) 25%, sodium hydroxide (NaOH) 4M, and solid potassium iodide (KI) were of analytical grade.

Table 1. Chemical and sensory data of the three different wines monitored during the winemaking process and storage (amphorae (n = 2), barrels (n = 2), barriques (n = 3))

	Alcohol (% vol.)	SO_2 tot (mg/l)	TA (g/l)	VA (g/l)	Malic acid (g/l)	Lactic acid (g/l)	Dry extract (g/l)	Vanilla	Tannic	Straw colour
September 17, 2014										
Amphorae	12.5	9.0	6.4	0.23	1.27	0.66	26.2	1	1	2
Barrique	12.7	18	6.5	0.22	1.42	0.48	22.4	1	1	2
Barrel	12.6	18	6.6	0.23	1.71	0.31	21.4	1	1	1
Average ± SD	12.6 ± 0.08	15 ± 5.2	6.5 ± 0.1	0.23 ± 0.0	1.48 ± 0.22	0.48 ± 0.17	23.3 ± 2.5	1 ± 0	1 ± 0	1.7 ± 0.6
November 28, 2014										
Amphorae	12.6	7.0	5.35	0.40	0.02	1.35	23.8	1	4	2
Barrique	12.7	36	5.90	0.25	1.10	0.77	21.3	2	1	2
Barrel	12.7	34	5.85	0.23	0.95	0.82	21.3	1	1	2
Average ± SD	12.7 ± 0.1	25.7 ± 16	5.7 ± 0.3	0.29 ± 0.1	0.60 ± 0.6	0.98 ± 0.32	22.1 ± 1.4	1.3 ± 0.6	2 ± 1.7	2 ± 0
February 17, 2015										
Amphorae	12.5	9.0	5.20	0.37	0.16	1.53	23.1	1	2	3
Barrique	12.7	40	5.30	0.20	0.25	1.45	21.6	3	2	2
Barrel	12.7	40	5.45	0.19	0.12	1.58	21.1	2	1	2
Average ± SD	12.6 ± 0.1	30 ± 18	5.3 ± 0.1	0.25 ± 0.1	0.18 ± 0.1	1.47 ± 0.1	21.75 ± 1.0	1 ± 1	1.7 ± 0.6	2.3 ± 0.6
March 13, 2015										
Amphorae	12.5	35	5.10	0.38	0.17	1.47	21.7	1	2	3
Barrique	12.7	64	5.35	0.28	0.13	1.52	20.2	4	2	2
Barrel	12.7	65	5.35	0.24	0.02	1.49	20.3	2	2	2
Average ± SD	12.6 ± 0.1	55 ± 17	5.23 ± 0.1	0.30 ± 0.1	0.11 ± 0.08	1.49 ± 0.02	20.77 ± 0.8	2.33 ± 1.5	2 ± 0	2.3 ± 0.6

SO_2 tot = total sulfur dioxide, TA = titratable acidity, VA = volatile acidity, SD = standard deviation

The sensory characteristics of the wines were evaluated through a panel formed by twelve trained judges (professors, researchers, and students). 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 judges were identified during the first session with the procedure of the round table: 'straw colour', 'vanilla flavour', and astringent (tannin) perception. Each sample was evaluated by using a scale of four points (1 = no perception, 4 = highest intensity). The panel also formulated a final judgement of the three different finished wines.

Statistical analysis

All the data were analyzed by the univariate analysis of variance (ANOVA, $P < 0.05$) to determine which variables were statistically significant in order to differentiate the samples by using the Tukey's Multiple Comparison Test and an $\alpha = 0.05$ criterion. In addition, also the correlation coefficients among the variables and the related P-values were calculated. The GraphPad Instat software (Version 1.0, 2005) was used for the ANOVA and the correlation matrix.

Principal component analysis (PCA) was carried out to point out differences or groupings between the wines obtained in amphorae, barrels, and barriques and analyzed during a 6-month storage period. PCA was performed using The Unscrambler software (Camo Inc., Corvallis, USA).

RESULTS

For the entire mass of Chardonnay grapes used in the experiment, the mean values of sugar content, titratable acidity, malic acid, and pH were 21.9°Brix, 7.60 g/l, 2.25 g/l, and 3.17 l, respectively. From these results, the Chardonnay grapes were already mature and suitable for the harvest at the end of August because the potential alcohol content was 12.50% vol. and the titratable acidity was not too high for a correct winemaking process (it should be usually less than 10 g/l for still wines). This resulted in a moderate content of malic acid.

However, very different wines were obtained from the same raw material, consisting of a batch of Chardonnay grapes, harvested in the same vineyard and in the same day but processed in different ways. Table 1 shows the chemical and sensory results of the three different wines monitored during the winemaking process and stored until the next May.

Analysis of variance

The univariate ANOVA performed using all the data reported in Table 1 (including all the sampling times) showed that only the dry extract and volatile acidity could statistically differentiate the wine samples according to the container at $P < 0.05$. The dry extract (Fig. 2a) was significantly different between amphorae and barrel ($P = 0.0258$). The volatile acidity (Fig. 2b) was significantly different between amphorae and barrique or barrel ($P = 0.0152$).

Although other chemical and sensory parameters between the three types of wines were numerically different, no other significant differences were registered. However, the univariate approach is not completely suitable to describe a multivariable model. In fact, the chemical or sensory variables which could well describe the variance of the samples at the beginning of the ageing (September 2014) might not be able to differentiate the wines during or at the end of the sampling period (March 2015), or *vice versa*. Thus, a multivariate approach was studied, such as the model elaborated by using the PCA.

Principal component analysis

The PCA was carried out in order to get a better overview of the effects of different winemaking procedures on the quality of semi-finished and finished Chardonnay wines in relationship with the ageing period. The data (samples and variables) used for the multivariate analysis are a subpopulation of those reported in Table 1. In fact, the variables which remained unchanged or showed a negligible variation (alcohol, pH) were excluded from the model. Sulfur dioxide was also excluded because it is a variable more related to the winery practices rather than to the storage conditions.

The bi-plots reporting PC1 vs PC2 and PC1 vs PC3 are displayed in Fig. 3. The first two principal components (PC1 and PC2) accounted for 79% of the total variance of the model (Fig. 3a). PC1 was positively correlated with volatile acidity, tannin, lactic acid, and straw colour and negatively correlated with titratable acidity and malic acid. PC2 was positively correlated with the dry extract and negatively correlated with the vanilla flavour (Fig. 3b).

The distribution of the samples was strongly influenced by the storage time: all the samples 1 and 2 (except a2) were located in the left part of the plot, whereas the samples 3 and 4 were gradually located in the right quadrants. All the ‘a’ samples (amphorae) showed a peculiar distribution. The amphorae wines 2–4 were clearly differentiated from the analogue barrel and barrique wines due to the high volatile acidity, straw colour, and tannic perception. Barrel and barrique wines showed a higher vanilla flavour than the similar amphorae wines, presumably due to the storage in wood, as reported by Herrero et al. (2016). It was confirmed that the high dry extract characterized all the amphorae wines due to the pomace maceration. The main components of the dry extract of wine include colouring substances, tannins, organic acids, salts, glycerol, and colloids.

As expected, the malic acid content and titratable acidity decreased during the storage of all the wines. Malic acid was replaced by lactic acid due to the malolactic fermentation.

The amphorae wine showed lower titratable acidity (even if this difference was not significant) than the other two types of wines all through the storage. Presumably, a higher potassium extraction took place from the pomace during the maceration resulting in higher tartaric precipitation. Barrels and barriques showed almost the same values all through the storage period.

Potassium metabisulphite was added immediately after the malolactic fermentation in barrels and barriques. Initially, the volatile acidity increased with a peak, then it was stabilized at values of around 0.4 g/l. The barrels maintained almost constant values, while barriques showed higher values than barrels. The volatile acidity was presumably influenced by the

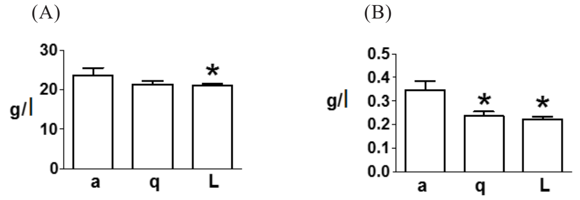

Fig. 2. Dry extract comparison between amphorae, barrique and barrel (A), volatile acidity comparison between amphorae (a), barrique (q), and barrel (L) (B)

*significant difference between amphorae wine samples and selected samples low sulfite content and permeability of the container, which enabled the wine contact with oxygen, resulting in higher production of acetic acid.

The malolactic fermentation was closely dependent on the inoculation of *Oenococcus oeni*. The transformation of malic acid into lactic was much faster in amphorae than barrels and barriques. This trend could be due to the contact of the wine with the pomace during fermentation in amphorae, compared to barrels and barriques; the skins contain wild species of lactic acid bacteria, capable of completing the malolactic fermentation. The barriques needed more time for the conversion of malic acid into lactic acid, probably because of the bacteriostatic effect of the tannins present in the wood. However, the values of lactic acid detected in wines obtained from the three winemaking techniques did not vary very much at the end of the process.

Sensory analysis

Apart from the average sensory results reported in Table 1 and in Fig. 3, the sensory panel also expressed a final judgement on the three different Chardonnay wines. According to the panel, the wine produced in amphorae resulted to have a mature scent, a less 'green' character than wines from barrels and barriques, but a weak varietal aroma probably due to excessive maceration. The tannic content of amphorae Chardonnay was remarkable: it was made of elegant tannins, a pleasing taste which was higher than in the other wines. The panelists perceived a spicy scent, which was the index of a good maturation of the wine and did not resemble vanilla notes.

The wine produced in barrique was characterized by an aromatic profile with 'vanilla notes'. However, the flavour profile easily evoked a wine obtained from

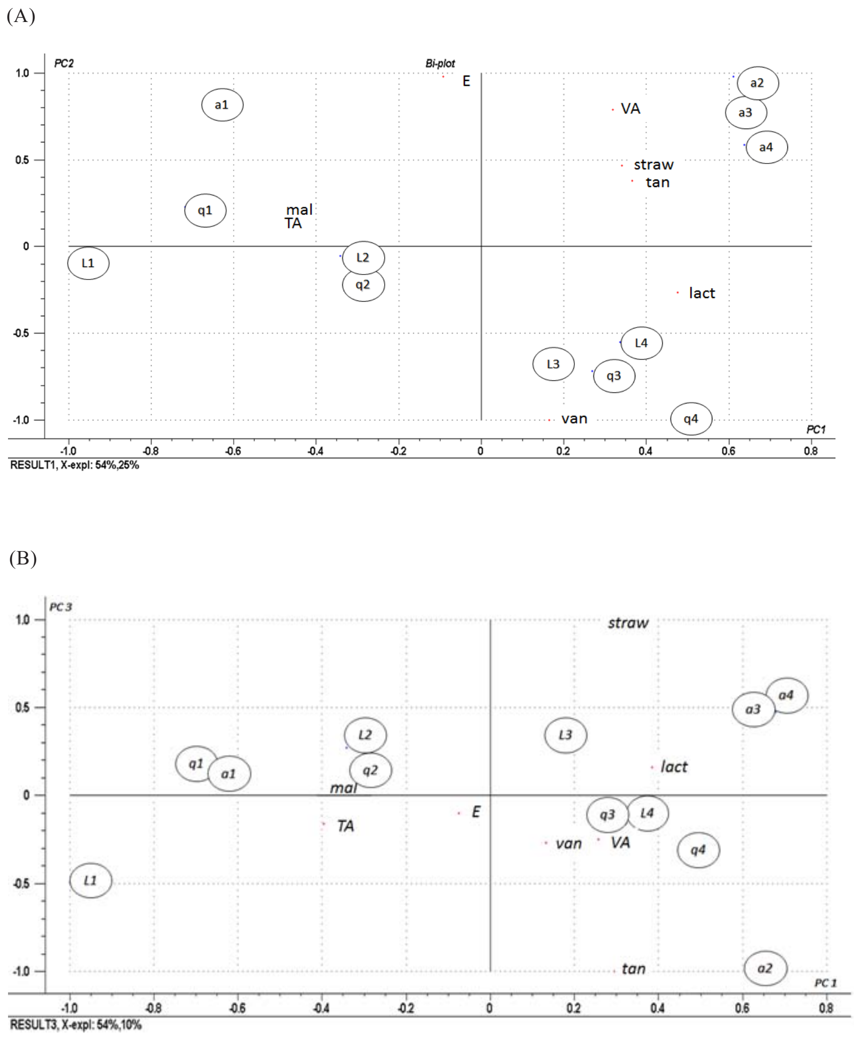

Fig. 3. Principal components analysis (PCA) of the Chardonnay wines: PC 1 vs PC 2 (A), PC 1 vs PC 3 (B)

a = amphorae, q = barrique, L = barrel, tan = tannin, mal = malic acid, van = vanilla flavour, VA = volatile acidity, lact = lactic acid, E = dry extract, straw = 'straw' colour, TA = titratable acidity; 1, 2, 3, 4 = time of sampling (respectively: 17/9/2014, 28/11/2014, 17/2/2015, 13/3/2015)

Chardonnay grapes. This wine revealed characteristics of freshness, harmony, and a remarkable woody flavour. The panelists suggested to blend the barrique wine with other types of wine in order to reduce its high woody and vanilla notes. The barrel characterized the wine with fruity sensations corresponding to the Chardonnay grape variety. Among the three winemaking techniques, wine aged in barrel resulted in the most balanced product, with spicy and light woody and vanilla notes. It was characterized by a full, balanced, fruity, and persistent flavour. The panelists considered this wine as a good product to be potentially blended with the other two experimental Chardonnay wines. From the sensory point of view, the wine obtained in barrels resulted to be the most 'complete' among the wines obtained from the three vinifications.

The analysis of correlations was performed using the data reported in Table 1 in order to point out the correlations among sensory and chemical variables. The alcohol content (not significantly different in the samples) and the total sulfites (which were directly added to the wine) were excluded from the elaboration. The colour intensity of the wine was directly related to the volatile acidity ($R = 0.607$, $P = 0.036$) and the lactic acid content ($R = 0.588$, $P = 0.044$) and was inversely correlated with the titratable acidity ($R = -0.653$, $P = 0.021$). The tannic perception was linked to the extent of the malolactic fermentation, in fact it was directly related to the volatile acidity ($R = 0.732$, $P = 0.0068$), and inversely related to the malic acid content ($R = -0.666$, $P = 0.018$) and to the titratable acidity ($R = -0.611$, $P = 0.035$). The flavour of vanilla was not significantly correlated with the chemical variables, because the type of tank had a stronger influence than the chemical variables.

DISCUSSION

The Chardonnay wine composition may vary to a large extent according to the maturity stage and hygienic state of the grapes, the geographical origin, and the winemaking practices. Cozzolino et al. (2003) and Stummer et al. (2005) reported a pH range of 3.0–3.4, a titratable acidity of 6.6–7.1 (g/l as tartaric acid), a volatile acidity of 0.20–0.40 (g/l as acetic acid), and a dry extract of 25.3 g/l for Chardonnay wines which were not processed in amphorae. These data are compatible with the results reported in Table 1, taking into account the different geographical origin and processing technology of the wines.

So far, only few previous studies have been carried out on winemaking in amphorae, probably due to a very recent rediscovery of amphorae as a suitable container in enology.

A couple of research works were focused on the influence of vinification and ageing of wine in amphorae compared to glass (Baiano et al., 2015) and to stainless steel tanks (Baiano et al., 2014) on the physico-chemical parameters and antioxidant activity of wine. According to Baiano et al. (2014) the organic acids like tartaric acid and malic acid showed no differences between the different types of Falanghina wine at the beginning and at the end of the storage. In the present work carried out on Chardonnay, the main difference was the higher rate of conversion of malic acid into lactic acid observed in amphorae with respect to barrels and barriques, presumably due to the maceration with the pomace.

The dry extract of Chardonnay wines decreased from the first sampling carried out on September 17, 2014 to the last sampling on March 13, 2015, differently from Baiano et al. (2014), where the dry extract remained constant up to 6 months. However, the Chardonnay wine obtained in amphorae with maceration showed a remarkably higher final dry extract than the wine obtained from barrels and barriques. This was presumably due to the contact with the pomace, which led to the diffusion of extractable components.

CONCLUSION

Using a single variety of grape, such as Chardonnay, three chemically and sensorically different wines were obtained by using amphorae, barrels, and barriques.

According to the trained panel, the amphorae wine resulted to have less sensory characters typical of the Chardonnay grape. Moreover, aromatic compounds typical of the aging in wood containers were not present in amphorae Chardonnay wine. The tannin content of this wine was appreciated, due to the maceration with the pomace, which was not related to the storage in oak wood.

For these reasons, the blend of different types of Chardonnay wines (made in barrique, barrel or amphorae) in predefined quantities could lead to new potentially marketable types of wine, rather than a 100% Chardonnay obtained in amphorae.

Each container can influence the chemical and sensory quality of the final wine due to its peculiar geometry and material characteristics. By using the modern amphorae, winemakers may extend their commercial wine offer by exalting the characteristics of the grape in a different and innovative way, not related to the ageing in wood. However, further research with a larger data set as well as with wines obtained from other grape varieties will be useful to deepen the study of earthenware in the contemporary enology.

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