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Luca Rolle, Vasileios Englezos, Fabrizio Torchio, Francesco Cravero, Susana Río Segade, Kalliopi Rantsiou, Simone Giacosa, Angelita Gambuti, Vincenzo Gerbi, Luca Cocolin

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Alcohol reduction in red wines by technological and microbiological approaches: a comparative study

L. ROLLE[1,a], V. ENGLEZOS[1,a], F. TORCHIO[2], F. CRAVERO[1], S. RÍO SEGADE[1], K. RANTSIOU[1], S. GIACOSA[1], A. GAMBUTI[3], V. GERBI[1] and L. COCOLIN[1,*]

[1]Università di Torino, Dipartimento di Scienze Agrarie, Forestali e Alimentari, Largo Paolo Braccini 2, 10095 Grugliasco, Torino, Italy.

[2]Istituto di Enologia e Ingegneria Agro-Alimentare, Università Cattolica del Sacro Cuore, Via Emilia Parmense 84, 29122 Piacenza, Italy.

[3]Università degli Studi di Napoli Federico II, Facoltà di Agraria, Dipartimento di Scienza degli Alimenti, 80055 Portici, Napoli, Italy.

[a]Luca Rolle and Vasileios Englezos contributed equally to this article

[*]Corresponding author: Phone: +39-011-6708553, Fax: +39-011-6708549, E-mail address: lucasimone.cocolin@unito.it (L. Cocolin)

Abstract

Background and Aims: The aim of this work was to assess and compare the chemical composition and color characteristics of Barbera red wines obtained after partial ethanol reduction using three promising methodologies for implementing at the industrial level.

Methods and Results: Ethanol reduction was achieved by pre-fermentation addition of grape must-derived liquid (reverse osmosis by-product), mixed fermentations with *Starmerella bacillaris* and *Saccharomyces cerevisiae* yeast strains and post-fermentation wine dealcoholization using a polypropylene membrane. The results showed that the microbiological approach allowed the production of wines with a slightly lower alcohol content (-0.2-0.3% v/v), while facilitating the release of total anthocyanins and some esters of fatty acids (ethyl hexanoate and ethyl dodecanoate) that could contribute positively to wine aroma with pleasant nuances. The low impact of the partial replacement of grape juice on the chemical composition and chromatic characteristics of Barbera wines makes this technique a good option for reducing the ethanol content by up to 1.0-2.0% v/v. Instead, the use of a polypropylene membrane influenced negatively the composition of red wines by reducing significantly the contents of esters (-60%) and anthocyanins (-17%), independently on the dealcoholization level used (up to -2% v/v).

Conclusions: The alcohol reduction strategies can greatly influence the volatile and phenolic composition of the wine. The choice of technological or microbiological approaches in the wine industry is dependent on the ethanol reduction required.

Significance of the Study: This is the first comparative study that uses different strategies to reduce the ethanol content on the same must/wine batch.

Keywords: *Wine ethanol reduction, Mixed fermentations, Grape juice replacement, Polypropylene membrane, Volatile compounds, Phenolic compounds*

Introduction

Wine is one of the most consumed alcoholic beverages in the world, and its moderate intake, especially for red wine, has been found to have health benefits mostly related to the presence of phenolic compounds with antioxidant and cardiovascular protective effects (Lecour et al. 2006, Assunção et al. 2007). The alcohol content in the wine has increased in the last decades, probably in response to current consumer preferences for well-structured and full-bodied wines rich in ripe fruit flavors. Increased sugar accumulation in the grape, due to the late harvest required to assure the aromatic and phenolic maturity, leads to the production of wines with elevated ethanol contents. Furthermore, the improvement of agronomic conditions and climate change over the past 20 years, as a result of global warming, have accentuated the imbalance between the sugar content and phenolic maturity of grape berries, increasing significantly the ethanol content in the wine about 2% v/v (Jones et al. 2005, Mira de Orduña 2010).

Nevertheless, alcohol interacts with other wine components, and its excess can alter the sensorial profile of wine by increasing the perception of bitterness, astringency, hotness and roughness while decreasing fruity aroma (Goldner et al. 2009, King et al. 2013). Furthermore, higher alcohol contents concern to wine consumers because of ethanol-induced health damages and social responsibilities (Assunção et al. 2007). From the commercial point of view, wines with high alcohol content are more taxed in many countries, increasing the cost to consumers (Sharma et al. 2014). In addition, high ethanol levels produced during primary fermentation may be toxic for yeast cells by altering their membrane fluidity, and this in turn can result in arrested or sluggish sugar-to-ethanol conversion (Henderson and Block 2014). Similarly, high ethanol contents could inhibit the malolactic fermentation (MLF) causing several technical difficulties to winemakers (Boulton et al. 1996).

Many efforts in the wine industry are focused on the production of low alcohol wines including viticultural, pre-fermentation and fermentation practices, as well as post-fermentation approaches (Varela et al. 2015). Although taking control of ethanol can be surprisingly difficult, new strategies are being investigated to reduce the ethanol content without compromising the organoleptic quality of wines (Longo et al. 2016). One strategy is the development of viticulture approaches in order to decrease the sugar accumulation in the grapes, which are mainly based on the reduction of leaf area, pre-harvest irrigation, application of growth regulators, selection of grapevine clones and harvest date management

(Novello and De Palma 2013). Pre-fermentation strategies include the blending of the grape juice with that from early-harvested low sugar grapes, or the removal of a portion of fermentable sugars by the use of membranes (García-Martín et al. 2010) or by the addition of glucose oxidase (GOX) enzyme (Pickering et al. 2001). However, these pre-fermentation procedures alter the sensory profile of wines, with added drawbacks to the use of membranes such as technical difficulties and the cost of the equipment required. Kontoudakis et al. (2011) proposed the partial replacement of the high sugar grape juice by an odorless and colorless low ethanol wine, resulting from the treatment with charcoal and bentonite of a wine made from bunch-thinned grapes (early harvested), for lowering ethanol content. This strategy provides promising results since it keeps the phenolic composition and sensory properties of the wine when ethanol is reduced by up to 2% v/v.

Fine tuning of wine yeast metabolism during fermentation is gaining attention in recent years due to it is a low cost and easy to implement strategy without the need for specialized equipment (Ciani et al. 2016). The trends in this field are the development of *Saccharomyces cerevisiae* yeast strains with reduced ethanol yield using metabolic engineering (Heux et al. 2006, Rossouw et al. 2013, Tilloy et al. 2015), and the combination of non-*Saccharomyces* yeasts, able to divert the carbon flux towards multiple metabolites rather than ethanol, with the high fermentative ability of *S. cerevisiae* strains (Giaramida et al. 2013, Contreras et al. 2014, Contreras et al. 2015, Englezos et al. 2015, Canonico et al. 2016, Varela et al. 2016). This strategy seems to be promising, but studies on the effect on the composition and sensory profile of the resulting wines are necessary.

Post-fermentation strategies, already used by a large number of wineries all over the world, involve the addition of low ethanol wine or ethanol removal using membrane-based technologies, such as reverse osmosis, osmotic distillation and pervaporation, spinning cone column distillation and supercritical CO_2 extraction combined with vacuum distillation (Schmidtke et al. 2012). Even these post-fermentation technological strategies have drawbacks because they are difficult to perform, have high cost and affect the composition and sensory attributes of the wine. Nevertheless, the effects are acceptable in partially dealcoholized wines when the ethanol content is reduced by 2% v/v (Diban et al. 2013, Lisanti et al. 2013).

The market is increasingly demanding the production of reduced ethanol wines without compromising the organoleptic quality and few studies have been performed on the impact of the ethanol reduction processes on the chemical composition of wines. The aim of the present study was to evaluate and compare the effect of three ethanol reduction techniques at different levels of alcohol removal on the phenolic and volatile composition of cv. Barbera red wines produced using the same grape batch. To our knowledge, this is the first comparative study among strategies to reduce the ethanol content of wines at different steps of the winemaking process, specifically pre-fermentation by the addition of grape must-derived liquid obtained as a by-product of reverse osmosis, fermentation using mixed cultures of *Starmerella bacillaris* (Synonym *Candida zemplinina*) and *Saccharomyces cerevisiae* and post-fermentation using a polypropylene hollow fiber membrane contactor technique.

Materials and methods

General winemaking procedure

Vinifications were carried out at pilot-scale in the experimental winery of the University of Torino in 2014. cv. Barbera grapes (variety number VIVC 974 in the *Vitis* International Variety Catalogue (www.vivc.de) (*Vitis vinifera* L.) were harvested in a commercial vineyard located in the Piedmont region (North-West Italy) and crushed. All harvested mass was homogenized in the same tank in order to reduce differences in solid/liquid ratios and then distributed in sixteen 2-hL stainless steel tanks (8 trials x 2 replicates). The initial grape juice had the following composition: 24.9 ºBrix, pH of 3.09, titratable acidity of 10.1 g/L as tartaric acid, 145 mg/L of yeast assimilable nitrogen (YAN) composed by 90 mg/L of organic nitrogen and 55 mg/L of inorganic nitrogen. The unpasteurized grape juice was supplemented with 20 mg/L of potassium metabisulphite prior to inoculation because this dose does not inhibit *Starm. bacillaris* growth (Englezos et al. 2015). Control fermentations (CW) were carried out inoculating 1.0 x 10^6 cells/mL of the commercial *S. cerevisiae* strain Uvaferm BC® (Lallemand Inc., Montreal, Canada) and maintained at 25 ºC until the end of fermentation. The cap was punched down twice daily by hand. When residual sugars achieved contents lower than 2 g/L, the resulting wines (free-run and pressed wines) were transferred to 1-hL stainless steel tanks. Afterwards, the wines were inoculated with 100 mg/L of the *Oenococcus oeni* Lalvin VP41® strain (Lallemand Inc.) to encourage MLF. Once MLF was completed, the wines were racked to remove lees, and free SO_2 content was adjusted to 50 mg/L.

Pre-fermentation approach

For lowering ethanol content about 1.0 and 2.0% v/v (DW1 and DW2, respectively), a given volume of grape juice was removed and replaced with the same volume of a grape must-derived liquid. This liquid was obtained by reverse osmosis of a part of the initial grape juice using a LF-60 equipment (Enomeccanica Bosio s.r.l, Monticello d'Alba, Italy). The volume was calculated according to the potential degree of alcohol of the grape juice (14.7% v/v). The trials were performed in duplicate. After the replacement, two grape juices were obtained. The first grape juice (DW1) had 23.6 ºBrix, pH of 3.09 and titratable acidity of 10.09 g/L of tartaric acid, and the lowest sugar juice (DW2) had 22.6 ºBrix, pH of 3.10 and titratable acidity of 9.71 g/L of tartaric acid.

Microbiological approach

A commercial *S. cerevisiae* strain Uvaferm BC® and two *Starm. bacillaris* strains (FC54 and C.z 03) from the yeast culture collection of DISAFA (Department of Agricultural, Forest and Food Sciences, University of Torino, Italy) were selected based on the results of a previous study (Englezos et al. 2016a). All strains were grown at 25 ºC in YPD medium plates (1% w/v of yeast extract, 2% w/v of bacteriological peptone, 2% w/v of dextrose and 2% w/v of agar, all purchased from Biogenetics, Milan, Italy).

Two sets of mixed fermentations were performed, inoculating 1.0 x 10^6 cells/mL of *Starm. bacillaris* (either FC54 or C.z 03 for each set, resulting in SFW1 and SFW2 wines, respectively) with the addition of 1.0 x 10^6 cells/mL of *S. cerevisiae* Uvaferm BC® after two days of fermentation for each strain combination-replicate (2 trials x 2 replicates). The ferments were inoculated with fresh cultures. Before inoculation, the viable yeast cells were determined through microscopical counts. Briefly, 1 mL of each inoculum was diluted in Ringer's solution (Oxoid, Milan, Italy) and stained with methylene blue to distinguish alive from dead cells. Viable cell populations were counted using a Thoma hemocytometer chamber (BRAND GMBH + CO KG, Wertheim, Germany) under a microscope at 400x magnification.

Microbiological and molecular analysis

The yeast dynamics during the fermentation process was monitored in terms of viable plate counts. Samples were collected at 0, 2, 4, 7 and 14 days after inoculation. Serial dilutions

were performed in sterile quarter-strength Ringer's solution, and the number of colony forming units (CFU)/mL was determined by plating 100 µL of the last three dilutions on two specific media, namely lysine agar and Wallerstein laboratory nutrient agar (WLN). Non-*Saccharomyces* yeasts were counted on lysine agar (Oxoid) containing L-lysine as the sole nitrogen source, which is not assimilated by the *Saccharomyces* spp. (Angelo and Siebert 1987). Putative *Starm. bacillaris* and *S. cerevisiae* were grown on WLN (Biogenetics), which allows their concurrent enumeration (Rantsiou et al. 2012). After plating, the plates were incubated at 30 ºC for 5 days and subsequently counted as described by Rantsiou et al. (2012). To verify the presence and dominance of the inoculated strains, 5 putative colonies of *Starm. bacillaris* and *S. cerevisiae* from each sampling point (25 for each tank) were isolated for further characterization. *Starm. bacillaris* and *S. cerevisiae* strain characterization was performed using Rep and interdelta-PCR, following the protocols described by Englezos et al. (2015) and Charpentier et al. (2009), respectively.

Post-fermentation approach

The wine, after MLF, was partially dealcoholized at three levels (-0.5, 1.0, 2.0% v/v ethanol reduction compared to CW, corresponding to DE0.5, DE1 and DE2, respectively) using the protocol described by Lisanti et al. (2013). All trials were performed in duplicate. An industrial-scale apparatus ALCOLESS PRIMO (Enolife s.r.l. Montemesola, Taranto, Italy) was used, which basically consisted of a polypropylene hollow fibre membrane contactor and two centrifugal pumps to feed wine and stripping water to the system. The membrane contactor was a Liqui-Cel® 4×28 Extra-flow module supplied by CELGARD LLC (Charlotte, USA), equipped with a microporous polypropylene hollow fibre membrane Celgard® ×50. The stripping water was collected at the output of polypropylene membrane.

Chemical analysis

Standard parameters

In the wines obtained, pH was determined by potentiometry using an InoLab 730 pH meter (WTW, Weilheim, DE), and titratable acidity (g/L of tartaric acid, as TA) was estimated using the International Organization of Vine and Wine method (OIV 2008). Reducing sugars, glycerol, tartaric acid, malic acid, lactic acid, citric acid, acetic acid and succinic acid (g/L) and ethanol (% v/v) were determined by high-performance liquid chromatography (HPLC) with a refractive index detector and a diode array detector (DAD) set to 210 nm (Giordano et al. 2009).

Free volatile compounds

Free volatile compounds were extracted and determined by head space solid phase microextraction (HS-SPME) coupled with gas chromatography-mass spectrometry (GC-MS), following the procedure reported by Rolle et al. (2015). The ethanol interference was overcome by the dilution of wine samples as follows. Five mL of each wine sample were placed into a 20 mL glass headspace sampling vial containing 5 mL of deionized water (Purelab Classic system, Elga Labwater, Marlow, United Kingdom) and 2 g of sodium chloride. 1-Heptanol (Sigma-Aldrich, Milan, Italy) was then added as internal standard (200 µL of 1.55 mg/L solution in 10% v/v ethanol). Once the vials were sealed and shaken, a 50/30 µm DVB/CAR/PDMS fibre (Supelco, Bellefonte, PA, USA) was exposed to the headspace of the capped vial for 20 min at 40 ºC. The injection parameters, GC–MS system and chromatographic conditions were previously adapted by Rolle et al. (2015) from those reported by Sánchez-Palomo et al. (2005). A DB-WAXETR capillary column (30 m x 0.25 mm, 0.25 µm, J&W Scientific Inc., Folsom, CA, USA) was used. The compounds were identified using pure standards (Sigma, Milan, Italy) when available and/or the NIST database (http://webbook.nist.gov/chemistry/). Quantitative determinations (µg/L) were performed by the external standard calibration method, with some exceptions. Semi-quantitative values were only reported for 3,7-dimethyl-2,6-octadien-1-ol, ethyl-3-methylbutyl succinate and ethyl hexadecanoate because pure standards were not available.

Phenolic compounds

To assess the phenolic composition of the wines, different spectrophotometric indices were determined (Rolle et al. 2012, 2015): absorbance at 280 nm (as A_{280}), total phenols (mg/L of (+)-catechin, as TP), total flavonoids (mg/L of (+)-catechin, as TF), total anthocyanins (mg/L of malvidin-3-glucoside chloride, as TAI), proanthocyanidins (mg/L of cyanidin chloride, as PRO) and flavanols reactive to vanillin (mg/L of (+)-catechin, as FRV). TP were assessed by the reduction of phosphotungstic and phosphomolybdic acids (Folin-Ciocalteu reagent) to blue pigments by phenolic compounds in alkaline solution. TF and TAI were determined after dilution with ethanol/water/37% HCl (70:30:1). PRO were determined after acid hydrolysis with heating (Bate–Smith reaction) using a ferrous salt ($FeSO_4$) as a catalyst. FRV were determined using vanillin as a reagent in HCl medium.

The anthocyanin profile was also determined using the methodology proposed by Rolle et al. (2012), which involves reverse-phase solid-phase extraction (RP-SPE) with a 1 g Sep-Pak C-18 cartridge (Waters Corporation, Milford, MA, USA) and then analysis of the methanolic extract obtained by HPLC-DAD at 520 nm. The chromatographic system and conditions were previously reported by Rolle et al. (2012). A LiChroCART analytical column (250 mm × 4 mm i.d.) purchased from Merck (Darmstadt, Germany), which was packed with LiChrospher 100 RP-18 (5 μm) particles supplied by Alltech (Deerfield, IL, USA), was used. The mobile phases were: A = formic acid/water (10:90, v/v); B = formic acid/methanol/water (10:50:40, v/v), working at 1 mL/min flow-rate. The amounts of individual anthocyanins were expressed as percentages. Solvents of HPLC-gradient grade and standards were supplied from Sigma (Milan, Italy) and Extrasynthèse (Genay, France).

Color characteristics

The wine color was assessed by color intensity and hue, and by the parameters that define the CIELab space, including clarity (as L*), red/green color coordinate (as a*) and yellow/blue color coordinate (as b*), from which chroma (as C*) and hue angle (as H*) were calculated (OIV 2008). A UV-1800 spectrophotometer (Shimazdu Corporation, Kyoto, Japan) and a 2 mm path length cuvette were used. The CIELab color difference parameter (ΔE*) among the wines was calculated as: $\Delta E^* = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{0.5}$ (OIV 2008).

Statistical analysis

Statistical analyses were performed with the SPSS Statistics software package (version 19.0, IBM Corporation, Armonk, NY, USA). The Tukey-b test ($p < 0.05$) was used when one-way analysis of variance (ANOVA) showed significant differences in the results obtained from the chemical analyses. Principal component analysis (PCA) was also used to differentiate samples taking into account all chemical parameters determined with the exception of the ethanol content.

Results and discussion

Yeast dynamics during fermentation

Figure 1 shows yeast growth for the control fermentation and each of the two trials of mixed fermentations. In control fermentation, *S. cerevisiae* Uvaferm BC® achieved the stationary phase with a population of about 10^7 CFU/mL, which was maintained until the end of the

fermentation period (Figure 1A). Wild non-*Saccharomyces* and *Starm. bacillaris* strains were detected at populations of 10^5-10^6 CFU/mL during the first four days. Afterwards, the viable population exhibited a remarkable drop to undetectable levels at day 7.

In sequential fermentations (SFW1 and SFW2), in which *Starm. bacillaris* was inoculated two days before *S. cerevisiae* inoculation, a completely different picture emerged (Figures 1B and 1C). The two *Starm. bacillaris* strains showed comparable growth dynamics over the first 7 days of fermentation. *Starm. bacillaris* FC54 practically maintained the initial cell population throughout fermentation (> 10^5 CFU/mL), while the population of *Starm. bacillaris* C.z 03 dramatically decreased (< 10 CFU/mL) at the end of fermentation (day 14). Moreover, FC54 and C.z 03 strains affected Uvaferm BC® growth, which reached slightly lower populations compared to the control fermentation. Sugar consumption was slower in sequential fermentations than in Uvaferm BC® pure culture (Figure 2), probably due to the inhibitory effect of *Starm. bacillaris* upon *S. cerevisiae* growth, as previously reported by Englezos et al. (2016a). Therefore, glucose was consumed faster in control compared to mixed culture fermentations (Figure 2).

The dominance of inoculated over indigenous strains during the fermentation process was confirmed using Rep and interdelta-PCR fingerprinting analysis. Cluster analysis with a similarity coefficient of 90% revealed low variability among the recovered strains from the must isolates, excluding the determinant contribution of indigenous *Starm. bacillaris* and *S. cerevisiae* strains on the chemical composition of wines (data not shown).

Chemical analysis

Standard parameters

Table 1 shows the standard parameters of control wines and reduced ethanol wines using the different strategies. The pre-fermentation addition of grape must-derived liquid obtained from reverse osmosis reduced significantly the ethanol content in the final wines (DW1 and DW2). On the other hand, the concentrated must after the reverse osmosis process can be used in food industry, in particular to increase the nutritional quality of fruit juices replacing water by grape juice (Chiusano et al. 2015).

The microbiological approach, which is based on the initial inoculation of *Starm. bacillaris* FC54 (SFW1) or C.z 03 (SFW2), resulted in wines with an ethanol content reduced by about

0.3% v/v in relation to control wines (CW). The production of glycerol was significantly higher for SFW1 and SFW2 wines (increased by more than 1.0 g/L), whereas the acetic acid content was significantly lower only in SFW2 compared to CW. These changes agreed with those previously observed in Barbera wines produced by mixed fermentations with *Starm. bacillaris* and *S. cerevisiae* yeasts (Englezos et al. 2016a). Other researchers also reported the production of higher glycerol amounts using the same couple of yeast species (Andorrà et al. 2010, Giaramida et al. 2013). The higher production of glycerol could be advantageous because it contributes positively to the perceived quality of red wines by providing body, structure and sweetness sensory attributes (Noble and Bursick 1984).

The use of a polypropylene membrane to dealcoholize the wine (DE0.5, DE1 and DE2) caused some changes in the content of organic acids in relation to CW, particularly in tartaric acid and lactic acid, while the differences in titratable acidity were not significant. Using the same technique to reduce the ethanol content up to 0.2% v/v, Liguori et al. (2013a,b) also reported no significant differences in pH, total acidity and the composition of organic acids among the dealcoholized and control Aglianico red wines.

When wines with an ethanol reduction less than 0.5% v/v (SFW1, SFW2 and DE0.5) were compared to each other, DE0.5 wines showed a significantly lower tartaric acid content and therefore a lower titratable acidity value, but these values were quite similar in the three mentioned wines. Instead, SFW1 and SFW2 wines contained a significantly higher glycerol amount. Among wines with an ethanol content reduced by about 1.0% v/v (DW1 and DE1) or 2.0% v/v (DW2 and DE2), the use of a polypropylene membrane led to wines with a slightly higher titratable acidity value (probably due to higher citric acid and succinic acid contents), higher glycerol content and lower acetic acid content, although the differences in titratable acidity and succinic acid were not significant for the wines with an ethanol content reduced by 1.0% v/v.

Free volatile compounds

The free volatile composition of CW and reduced ethanol wines is shown in Table 2. A total of 35 compounds were identified and quantified. In all the Barbera wines analyzed, the volatile profile was mainly composed of n-alcohols, although the predominant volatile compound was an aromatic alcohol (2-phenyl ethanol), followed by 2-methyl-1-butanol. The three ethanol reduction strategies investigated in this study caused a significant decrease in

the total content of alcohols in relation to CW wines (-11.4-27.9%), except for DE2. Particularly for DW1 and DW2 wines, this decrease was mainly associated with the lower 2-phenyl ethanol content (-36.1-37.1%). This pre-fermentation strategy resulted in wines with a significantly lower isobutanol content than CW, while the 1-hexanol content was higher. Other alcohols, such as 2-nonanol and (*R,R-levo*)-2,3-butanediol, were also produced in significantly higher amounts in DW1 wines. Ester and terpene contents in DW wines were not significantly different to those of CW, except for ethyl lactate in DW1 and for diethyl succinate and 2-phenyl acetate in DW2.

During fermentation, the microbiological approach to reduce the ethanol content produced wines (SFW1 and SFW2) with a volatile profile quite similar to that of CW. Nevertheless, the *Starm. bacillaris* yeast strain was a low producer of higher alcohols, which agreed with the results previously published by Sadoudi et al. (2012). Although the reduced ethanol wines had a significantly higher isobutanol content, the 3,7-dimethyl-6-octen-1-ol content was lower compared to CW. Other studies have also reported that *Starm. bacillaris* is a strong producer of isobutanol (Andorrà et al. 2012, Englezos et al. 2016b). In the present work, the use of different yeast strains of *Starm. bacillaris* influenced the content of some higher alcohols. SFW1 wines had a significantly lower 2-octanol content. Instead, SFW2 wines showed significantly higher 1-hexanol and 3,7-dimethyl-2,6-octadien-1-ol contents but lower 1-octanol content than CW.

The production of esters was strain-dependent. The total content of esters in SFW1 wines did not differ significantly from that found in CW (Englezos et al. 2016b), and only the diethyl succinate content was significantly higher in SFW1. Nevertheless, increased total content of esters was observed in SFW2 wines (+27.1%), mainly due to the significantly higher contents of ethyl hexanoate and ethyl dodecanoate in relation to CW. Other studies also evidenced the significantly higher production of ethyl esters in wines produced from mixed fermentations with the same couple of species (Englezos et al. 2016b), whereas the differences were not significant for acetate esters (Andorrà et al. 2010). More recently, Andorrà et al. (2012) confirmed that the composition of grape must is a key factor in the production of the metabolites contributing to the wine aroma because of complex interactions between the different yeast species and strains and the grape must constituents during fermentation. Terpene and C_{13}-norisoprenoid contents in SFW wines were close to those of CW. Sadoudi et al. (2012) showed that *C. zemplinina* is a greater producer of terpenols, lactones and

norisoprenoids than *S. cerevisiae* yeast strains. However, the volatile profile for the *C. zemplinina/ S. cerevisiae* co-culture was close to that for the *S. cerevisiae* pure culture probably due to the negative interaction among yeasts. In the case of Barbera variety, the relatively low abundance of terpenols could contribute to the small effects observed.

The wines produced using the post-fermentation approach showed significantly lower contents of total alcohols in relation to CW as a consequence of the decrease of isobutanol, 2-nonanol, 1-octanol and 3,7-dimethyl-6-octen-1-ol contents. The greater level of ethanol reduction (DE2 wine) affected significantly also other higher alcohols, decreasing 3-hexen-1-ol and 1-nonanol but increasing (*R,S-meso*)-2,3-butanediol contents. A strong loss of isobutanol was also observed during red wine dealcoholization using a polypropylene membrane (Liguori et al. 2013a, Motta et al. 2017). Other researchers reported decreased contents of same alcohols in wines dealcoholized using this technological approach (Lisanti et al. 2013).

A strong and significant reduction was observed in the total content of esters (-55.0-65.9%) for DE0.5, DE1 and DE2 wines, as well as in the contents of many individual esters (3-methyl-1-butyl acetate, ethyl heptanoate, ethyl octanoate, ethyl nonanoate, ethyl decanoate, 2-phenyl acetate, ethyl dodecanoate), in relation to CW. These losses increased with increasing the ethanol reduction level (from DE0.5 to DE2 wines). The greater losses of ethyl esters of fatty acids are related to their higher hydrophobic character (affinity to the membrane) and their higher volatility (Diban et al. 2008, Liguori et al. 2013a). Nevertheless, the effect was grape variety dependent because of the differences in the matrix composition of initial wines (Rodríguez-Bencomo et al. 2011, Fedrizzi et al. 2014). In studies performed using the membrane contactor technology for decreasing wine ethanol content, esters were also dramatically reduced already at 2.0% v/v of ethanol removal (Diban et al. 2008, Lisanti et al. 2013). In the present work, ethyl lactate and ethyl hexadecanoate contents in DE wines increased significantly compared to CW and did progressively with increasing the ethanol reduction level. These changes caused important differences in the volatile profile. The predominant esters (representing about 77% of total free esters) in CW wines were ethyl octanoate, 3-methyl-1-butyl acetate and ethyl decanoate, whereas they were ethyl lactate, ethyl octanoate, ethyl hexanoate and 3-methyl-1-butyl acetate in DE0.5, and ethyl lactate, 3-methyl-1-butyl acetate, ethyl hexanoate and diethyl succinate in DE1 and DE2.

Terpenes, such as linalool and 1,4-terpineol, were partially lost with the post-fermentation strategy for ethanol reduction. Diban et al. (2008) also found linalool losses when the wine was subjected to an ethanol reduction of 2.0% v/v using a polypropylene hollow fiber membrane contactor. According to the hydrophobicity of terpenes, their losses should be similar to those of esters, although some authors confirmed that the matrix composition of the initial red wine could facilitate the retention of terpenes in the wine (Rodríguez-Bencomo et al. 2011). In the present work, a significant loss of C_{13}-norisoprenoids, such as *β*-damascenone, was observed for DE0.5, DE1 and DE2 wines compared to CW. This approach decreased significantly the content of benzaldehyde, as reported by Lisanti et al. (2013) for the partial dealcoholization of red wines by the membrane contactor technique. Instead, independently on the ethanol reduction approach used, *γ*-butyrolactone remained almost unchanged in agreement with other studies (Liguori et al. 2013a, Lisanti et al. 2013).

Some important differences were found in the volatile profile among wines with similar ethanol content. DE wines usually showed significantly lower contents of most free volatile compounds detected, with some exceptions. For a reduction of the ethanol content up to 0.5% v/v, DE0.5 wines were characterized by significantly higher contents of 2-phenyl ethanol and ethyl lactate in relation to SFW1 and SFW2. When the ethanol content was reduced by about 1.0% v/v, DE1 wines showed significantly higher contents of isobutanol and 2-phenyl ethanol than DW1. In the case of wines with an ethanol reduction of 2.0% v/v, 2-phenyl ethanol, ethyl lactate and *γ*-butyrolactone were significantly more abundant in DE2 wines than DW2.

Not all free volatile compounds influenced the wine aroma. According to the odor threshold, aliphatic and aromatic alcohols cannot contribute actively to the aroma of Barbera wines despite the high contents of these compounds. Nevertheless, esters of fatty acids and acetates, such as 3-methyl-1-butyl acetate, ethyl hexanoate and ethyl octanoate, which are synthesized by yeast during fermentation and provide a pleasant fruity aroma, were found in contents above their olfactive thresholds in all the wines obtained (30, 14 and 5 μg/L, respectively; Ferreira et al. 2000). The content of ethyl decanoate in DE0.5. DE1 and DE2 wines was below its odor threshold (200 μg/L; Ferreira et al. 2000), and therefore this compound only can contribute to the aroma of CW, DW1, DW2, SFW1 and SFW2 wines. Moreover, the aromatic complexity of SFW2 wines was favored by the significant role of linalool providing floral nuances (odor threshold of 25.2 μg/L; Ferreira et al. 2000). *β*-Damascenone can also

contribute actively to the floral aroma of all the wines studied (odor threshold of 0.05 µg/L; Ferreira et al. 2000), whereas γ-butyrolactone can have a sensorial contribution with sweet notes (odor threshold of 35 µg/L; Ferreira et al. 2000). Nevertheless, sensory analysis would be necessary to confirm the impact on wine aroma.

In two Aglianico red wines with different initial alcohol contents (15.37 and 13.28% v/v), trained panelists did not distinguish in a triangular test wines partially dealcoholized by 2% v/v from initial wines, and overall quality ranking was significantly different only after an alcohol reduction of 5% v/v due to the decrease of important olfactory notes for red wines such as red fruits, cherry and spicy (Lisanti et al. 2013). Nevertheless, the effect of partial dealcoholization on the sensory perception of red wines was variety dependent (Meillon et al. 2009) in agreement with free volatile composition.

Phenolic compounds

Table 3 shows the phenolic composition of CW and reduced ethanol wines. The phenolic composition of CW wines agreed with that previously published for Barbera, although there were some small differences as a result of annual variations (Cagnasso et al. 2008, Bosso et al. 2011). When each reduced ethanol wine was compared to the CW wine, some significant differences were found. In DW wines, high molecular mass flavanols (PRO) decreased (-4.0-9.8%), particularly when increasing the amount of grape juice replaced by the reverse-osmosis liquid. The lower ethanol content could hinder the extraction of high polymerized flavanols from grapes during fermentation (Canals et al. 2005). This approach to reduce the alcohol content also decreased the relative abundance of di-substituted anthocyanins, even though total content of anthocyanins (TAI) remained practically unchanged. A lower content of total anthocyanins would be expected because a portion of grape juice was removed. The replacement of this juice does not necessarily involve anthocyanins losses as this operation is performed previously to maceration. However, in the case of ripe berries, these red pigments are easier extracted from skins during the crushing process and the short time of skin contact, and therefore the removed fraction could contain an important amount of anthocyanins (Canals et al. 2005). The absence of significant differences among DW and CW wines seems to be linked to the enhanced release of copigments during fermentation, which protect anthocyanins against oxidation (Boulton 2001). In agreement with these results, Kontoudakis et al. (2011) found that anthocyanins remained almost unchanged when lowering 3.0% v/v of the ethanol content by replacing a part of the total volume of the grape juice with the same

volume of a low ethanol wine. These authors reported that proanthocyanidins were less abundant in the reduced ethanol wines with respect to control wines, although the differences were significant only for Merlot wines.

It is important to highlight that SFW2 wines were significantly richer in TAI (+13.8%), particularly in delphinidin-3-glucoside, with respect to CW. Instead, the use of the polypropylene membrane caused the decrease of the TAI content independently on the dealcoholization level (-16.0-17.5%). Ulbricht et al. (2009) reported that phenolic compounds are only marginally adsorbed on the polypropylene membrane surface. Therefore, no significant change in total anthocyanins was observed for red wines of different initial alcohol contents (from 13.67 to 15.46% v/v) and produced from three grape varieties (Merlot, Aglianico and Piedirosso) after a dealcoholization of 2, 3 or 5% v/v (Gambuti et al. 2011). However, a loss of monomeric anthocyanins was noticed after the ethanol reduction of 2.0% v/v, which was independent on the chemical nature of these red pigments. In fact, their oxidation can occur when the wine is in contact with air during the process. On the contrary, Motta et al. (2017) observed a concentration effect on total anthocyanins after dealcoholization at 5.0% v/v alcohol probably due to the greater extent of ethanol removal from wine or to different operative conditions. The findings of the present work are also in agreement with unaffected both the content of PRO and their mean degree of polymerization after the partial dealcoholization (-1 and -2% v/v of ethanol) of red wines made from Cabernet Sauvignon, Grenache and Carignan grapes using other membrane technologies like reverse osmosis (Gil et al. 2013).

According to Table 3, the trends found in the PRO content for DW wines and in the TAI content for SFW and DE wines, with respect to CW, were also observed in total phenols (TP) and total flavonoids (TF), although the differences were not always significant. Furthermore, for each of the three techniques, the content of low polymerized flavanols (FRV) in the wines obtained was unaffected by the dealcoholization process in agreement with other studies (Gambuti et al. 2011).

Also some differences were found in the phenolic composition among wines of the same alcohol content (Table 3). In fact, the post-fermentation approach via the use of a polypropylene membrane affected negatively TAI and TF contents for 0.5, 1.0 and 2.0% v/v of ethanol removal, although the decrease was more accused at the beginning of the

dealcoholization process. The percentage of di-substituted anthocyanins was also higher in these wines, particularly at 2.0% v/v of ethanol removal in relation to the dilution strategy. Instead, the polypropylene membrane technology increased the PRO content for wines dealcoholized by 0.5% v/v compared to microbiological approaches.

Regarding anthocyanin profile, it is important to take into account that the alcohol reduction did not change the proportion of monomeric anthocyanins, with some exceptions, and that the variations observed were not always for the same anthocyanidins using different approaches. In relation to control wines, the maximum differences in reduced ethanol wines were about 1.9, 1.2, 0.8, 1.8 and 1.3% for delphinidin-, cyanidin-, petunidin-, peonidin- and malvidin-3-glucoside, respectively.

Color characteristics

The color parameters of CW wines (Table 4) agreed with those published by Cagnasso et al. (2008) despite some small differences most likely resulting from the vintage effect. Table 4 shows that an ethanol reduction of 0.2-0.5, 1.0 and 2.0% v/v did not affect significantly the color characteristics of the wines obtained using any of the three approaches studied, even when changes were observed in the anthocyanin content of SFW and DE wines.

In the case of the pre-fermentation replacement of grape juice by the reverse-osmosis liquid, the lack of significant differences in the color parameters of wines was related to the anthocyanin content unaffected by the alcohol reduction process. For the microbiological approach, a possible justification is the contribution of copigments to wine color. Copigmentation is an important phenomenon occurring in young red wines. Ethanol has a dissociating role on copigmentation complexes as a consequence of the weakening of hydrophobic interactions (Hermosín Gutiérrez 2003). Nevertheless, the solubility of some copigments could increase with the higher production of ethanol, which would compensate the disruption effect. The wine matrix conditions the effect of ethanol on copigmentation and color because of the key role of the ratio between anthocyanins and related copigments (Boulton 2001).

The limited changes in wine color were also confirmed by the ΔE* parameter. In relation to CW wines, only SFW1 showed chromatic differences hardly perceptible by the human eye (ΔE* parameter = 4.8). However, SFW1 wines were chromatically different from the other

wines with similar ethanol content (SFW2 and DE0.5) in terms of ΔE* (8.5 and 7.4, respectively). Ortega-Heras and González-Sanjosé (2009) reported a perceptibility threshold of 5 for ΔE* parameter.

In agreement with the results obtained in the present study for DE wines, other previously published works reported no significant differences in the color intensity of three red wines (different grape varieties and initial alcohol contents) after an alcohol reduction of 2, 3 or 5% v/v using a polypropylene hollow fibre membrane contactor (Gambuti et al. 2011). Nevertheless, color intensity increased in wines with an ethanol reduction higher than 10% v/v probably because the lower alcohol content, oxygen intake and SO_2 loss during the dealcoholization process promote the formation of more colored pigments (Liguori et al. 2013a).

Multivariate analysis

Principal Component Analysis (PCA) was performed for a global evaluation of the results obtained and better understanding of the chemical differences among Barbera reduced ethanol wines (-0.2-0.5, -1.0 and -2.0% v/v of ethanol removal) obtained using different strategies. Excluding ethanol, the two first principal components explained 53.8% of the variability in the original data. Figure 3 shows the distribution of CW and reduced ethanol wines (Figure 3A) and the projection of each variable (Figure 3B) in the plane defined by the two first principal components. The first principal component accounted for 34.2% of the total variance, and it was mainly associated with the contents of free volatile compounds, such as esters (ethyl octanoate, ethyl nonanoate, 3-methyl-1-butyl acetate, ethyl decanoate, ethyl heptanoate and ethyl dodecanoate), linalool, 1-nonanol and *β*-damascenone, but the coefficients were also high for tartaric acid and total anthocyanins (coefficients higher than 0.87). This component permitted a good separation of DE wines (located in the left side), showing increasingly negative values when increasing the ethanol removal. The other wines were separated in two groups: SFW2 with the more positive values of the first principal component, and CW, DW1, DW2, SFW1 with intermediate values. The second principal component permitted to differentiate SFW1 from the other wines, showing more positive values. This component explained 19.6% of the total variance, it being mainly related to citric acid, titratable acidity, glycerol and CIELab parameters (L*, a*, b*, C* and H*) with coefficients higher than 0.74. The more negative values of this second component corresponded to DW wines.

Therefore, the advantages of the microbiological approach were the higher presence of pleasant volatile compounds and total anthocyanins in the wines obtained, which could be related to the potential of non-*Saccharomyces* yeast species to produce and secrete extracellular hydrolytic enzymes capable of liberating these substances in the wine during fermentation (Strauss et al. 2001). DW wines had a similar global chemical composition to CW. However, DE wines were more negatively influenced by the ethanol reduction process.

Conclusions

In wine industry, partial dealcoholization is becoming a common practice in those regions with an increasing trend to produce high ethanol wines. Wine is a complex matrix whose fragile balance between its components makes it vulnerable to possible changes. Taking into account the important interactions of ethanol with other wine components, alcohol reduction strategies can greatly influence the volatile and phenolic composition of the wine. The present work highlighted that a microbiological approach based on the mixed cultures of non-*Saccharomyces* and *S. cerevisiae* yeasts is very useful for low ethanol reduction levels (-0.2-0.3% v/v) of red wines due to the release of higher amounts of some volatile compounds and anthocyanins contributing positively to wine quality. Nevertheless, the effect was strain-dependent. For a higher ethanol reduction (-1.0 and -2.0% v/v), the pre-fermentation technological approach based on the replacement of a portion of grape juice by the reverse-osmosis liquid hardly altered the composition and chromatic characteristics of the wine, and therefore it could be also an acceptable strategy. The use of a polypropylene hollow fiber membrane with dealcoholization purposes impacted more negatively on the volatile and phenolic composition of the wine compared to the other two approaches studied. However, it is important to consider that the interaction of the wine with the membrane is strongly dependent on the wine matrix and the ethanol reduction required, this technique being effective as remedial treatment for high alcohol wines.

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Table 1. Standard parameters of Barbera red wine before and after ethanol reduction using different approaches.

Parameters	Control	Pre-fermentation		Fermentation		Post-fermentation					
	CW[a]	DW1	DW2	SFW1	SFW2	DE0.5	DE1	DE2	Sign[b]	Sign[c]	Sign[d]
Ethanol (% v/v)	15.4±0.1	14.6±0.1*	13.7±0.1***	15.1±0.1*	15.2±0.1*	15.0±0.1***	14.5±0.1***	13.6±0.1***	ns	ns	ns
Reducing sugars (g/L)	0.71±0.05	0.85±0.28	0.59±0.02	0.70±0.01b	0.65±0.01a	0.84±0.01c	0.76±0.07	0.85±0.01	***	ns	**
pH	3.38±0.01	3.39±0.02	3.37±0.02	3.32±0.01*,a	3.38±0.01b	3.35±0.01*,ab	3.35±0.01	3.34±0.01	*	ns	ns
TA (g/L)	6.81±0.04	6.68±0.25	6.62±0.01*	7.31±0.16b	7.08±0.01**,ab	6.80±0.01a	6.82±0.13	6.87±0.02	*	ns	**
Tartaric acid (g/L)	1.99±0.05	2.08±0.22	2.09±0.10	2.07±0.02b	2.12±0.06b	1.78±0.02*,a	1.81±0.01*	1.80±0.01*	**	ns	ns
Malic acid (g/L)	nd	nd	nd	0.04±0.06	0.04±0.06	nd	0.11±0.10	nd	-	-	-
Lactic acid (g/L)	2.91±0.01	3.07±0.09	2.92±0.13	3.06±0.17	2.92±0.07	2.99±0.01**	3.02±0.01**	3.04±0.01***	ns	ns	ns
Citric acid (g/L)	0.05±0.08	nd	nd	0.14±0.01	0.10±0.06	0.06±0.01	0.06±0.01	0.07±0.01	ns	-	-
Acetic acid (g/L)	0.40±0.03	0.44±0.01	0.36±0.01	0.33±0.08	0.26±0.03*	0.34±0.01	0.33±0.01	0.33±0.01	ns	**	*
Succinic acid (g/L)	1.43±0.06	1.25±0.04	1.30±0.02	1.40±0.01a	1.52±0.03b	1.38±0.01a	1.39±0.01	1.40±0.01	**	ns	*
Glycerol (g/L)	12.0±0.4	12.1±0.1	11.6±0.2	13.8±0.2*,b	13.5±0.1*,b	12.2±0.1a	12.4±0.1	12.4±0.1	***	***	*

Data are expressed as mean value ± standard deviation. (n = 2). [a,b,c,d]Sign: *, **, *** and ns indicate significance at $p < 0.05$, $p < 0.01$, $p < 0.001$ and not significant, respectively, for differences among each reduced ethanol wine and the control wine ([a]), wines with reduced ethanol content of about 15.0% v/v ([b]), wines with reduced ethanol content of about 14.5% v/v ([c]), wines with reduced ethanol content of about 13.5% v/v ([d]). [b]Different Latin letters within the same row indicate significant differences according to the Tukey-b test ($p < 0.05$) among wines with ethanol content of about 15.0% v/v. TA, titratable acidity expressed as g/L of tartaric acid; nd, not detected.

Table 2. Free volatile compounds of Barbera red wine before and after ethanol reduction using different approaches.

	Control	Pre-fermentation		Fermentation		Post-fermentation					
Compounds (µg/L)	**CW[a]**	**DW1**	**DW2**	**SFW1**	**SFW2**	**DE0.5**	**DE1**	**DE2**	**Sign[b]**	**Sign[c]**	**Sign[d]**
Alcohols											
Isobutanol	328±3	115±3***	186±5***	359±9*,b	411±21*,c	196±5***,a	212±1***	203±19*	**	***	ns
1-Butanol	11.5±0.5	10.3±2.1	9.3±1.9	9.2±1.9	10.0±2.0	11.6±1.1	12.3±0.2	12.7±1.1	ns	ns	ns
2-Methyl-1-butanol	3639±498	3130±256	3005±245	3361±275	3479±450	2406±156	2626±180	2649±248	ns	ns	ns
1-Hexanol	317±13	459±33*	460±33*	407±30ab	471±6**,b	353±2a	365±8*	360±26	*	ns	ns
3-Hexen-1-ol	4.1±0.5	7.2±8.6	5.7±6.8	5.5±6.5	7.0±6.3	2.4±1.1	5.6±0.4	1.8±0.2*	ns	ns	ns
2-Octanol	9.4±1.8	10.5±1.2	10.1±1.2	0.8±0.1*,a	10.0±0.8c	6.2±0.2b	3.1±3.9	2.7±2.8	***	ns	ns
2-Ethyl-1-hexanol	9.0±0.8	9.8±6.1	6.9±4.3	8.6±5.4	7.5±1.4	10.4±1.0	9.2±0.4	10.0±0.3	ns	ns	ns
2-Nonanol	22.3±1.8	53.4±9.8*	48.5±8.9	20.8±3.8	22.7±0.7	14.0±0.2*	12.0±0.5*	9.6±0.3*	ns	*	*
(*R,R-levo*)-2,3-Butanediol	327±32	623±60*	345±33	342±33	408±21	369±9	358±18	403±18	ns	*	ns
1-Octanol	76.5±1.0	99.7±12.2	107.2±13.1	65.5±8.0	63.3±2.9*	63.3±1.5**	55.7±0.3**	48.6±3.8**	ns	*	*
3-Ethyl-2-pentanol	44.4±3.4	41.8±4.7	46.5±5.3	50.2±5.7	44.6±1.8	48.9±1.0	52.0±2.0	49.8±3.6	ns	ns	ns
(*R,S-meso*)-2,3-Butanediol	91.8±7.7	228.3±49.8	126.2±27.5	107.2±23.4	146.7±35.6	124.3±15.1	119.5±5.0	138.1±9.5*	ns	ns	ns
1-Nonanol	22.2±5.4	20.9±2.5	19.7±2.4	17.7±2.1ab	20.4±1.5b	12.3±0.4a	9.9±1.2	3.3±0.3*	*	*	*
Methionol	12.7±2.3	9.3±0.6	9.7±0.6	13.0±0.8b	13.8±0.4b	10.2±0.2a	9.9±0.9	10.2±0.6	*	ns	ns
3,7-Dimethyl-6-octen-1-ol	27.0±0.3	23.3±1.9	22.0±1.8	18.7±1.5*,ab	21.4±0.1**,b	17.1±0.1***,a	14.8±0.1***	13.6±0.7**	*	*	*
3,7-Dimethyl-2,6-octadien-1-ol[e]	0.4±0.3	5.4±1.9	5.4±1.8	4.8±1.6	6.0±1.1*	1.4±0.1	0.9±0.4	1.2±0.1	ns	ns	ns
2-Phenyl ethanol	10409±755	6545±232*	6651±236*	8188±290a	8433±115a	9361±64b	9467±343	9684±767	*	**	*
ΣAlcohols	15352±278	11392±31**	11064±10**	12979±22**	13574±302*	13006±75**	13333±181*	13601±1082	ns	**	ns
Esters											
3-Methyl-1-butyl acetate	1405±105	1522±265	1178±205	1058±184ab	1427±127b	632±28**,a	697±26*	473±65**	*	*	*
Ethyl hexanoate	463±1	1388±378	410±112	592±161a	1447±109**,b	776±29**,a	522±1***	455±13	**	ns	ns
Hexyl acetate	27.1±6.6	22.7±5.7	37.8±9.4	21.4±5.3a	43.2±2.0b	20.9±0.5a	12.0±1.5	10.6±0.4	*	ns	ns
Ethyl heptanoate	74.3±6.7	50.5±13.0	64.7±16.6	54.0±13.9a	92.0±8.5b	21.1±1.0**,a	10.6±0.5**	10.0±0.5**	*	*	*
Ethyl lactate	685±20	836±30*	731±26	789±28a	737±21a	882±12**,b	936±14**	1002±54*	*	ns	*
Ethyl octanoate	4217±325	4744±1500	3295±1042	3211±1015b	5250±319c	822±25**,a	400±15**	330±14**	*	ns	ns
Ethyl nonanoate	26.6±1.3	30.8±8.7	18.6±5.3	18.3±5.2b	34.5±2.4c	3.4±0.1**,a	1.8±0.1**	1.7±0.3**	**	*	*
Ethyl decanoate	1249±130	1344±412	713±219	819±251b	1446±152c	126±7**,a	77.0±4.0**	66.1±2.1**	*	*	ns
Diethyl succinate	413±41	451±19	615±26*	741±32*,b	401±14a	459±8a	503±25	445±39	***	ns	*
2-Phenylethyl acetate	254±12	208±21	185±18*	232±23ab	256±7b	190±2*,a	184±5*	167±12*	*	ns	ns
Ethyl dodecanoate	57.0±1.8	68.2±4.0	53.5±3.2	61.9±3.7b	126.6±3.0**,c	19.3±0.2**,a	15.4±0.2***	14.7±1.1**	***	**	**
Ethyl-3-methylbutyl succinate[e]	29.6±1.1	27.5±4.7	38.3±6.5	45.9±7.8	28.6±1.8	29.4±0.9	30.1±0.6	25.3±2.4	ns	ns	ns
Ethyl hexadecanoate[e]	7.8±2.1	11.7±6.1	12.2±6.3	21.3±11.0	36.0±12.9	28.4±5.1*	28.3±3.8*	32.6±5.5*	ns	ns	ns
ΣEsters	8907±308	10704±2578	7351±1593	7664±1617ab	11325±705*,b	4009±84**,a	3417±29**	3033±198**	*	ns	ns
Other compounds											
Linalool	23.0±0.5	22.7±4.1	23.4±4.2	23.5±4.2ab	29.7±2.7b	14.8±0.7**,a	13.8±0.1**	11.4±0.6**	*	ns	ns
1,4-Terpineol	51.1±1.4	46.2±7.7	45.8±7.6	40.7±6.8	41.1±3.1	35.5±1.3**	32.9±0.4**	30.2±2.1**	ns	ns	ns
Benzaldehyde	95.8±3.4	84.3±4.3	84.0±4.3	71.4±3.7*,ab	65.7±3.6*,a	81.2±2.3*,b	80.5±1.4*	72.8±5.0*	*	ns	ns
γ-Butyrolactone	40.8±5.3	36.1±4.3	31.7±3.8	39.3±4.7	38.5±2.6	39.9±1.4	42.0±2.7	47.7±2.6	ns	ns	*
β-Damascenone	11.5±0.7	10.3±1.4	9.8±1.4	8.2±1.1a	11.3±0.7b	6.9±0.2*,a	5.9±0.2**	5.2±0.3**	*	*	*

Data are expressed as mean value ± standard deviation. (n = 2). [a,b,c,d]Sign: *, **, *** and ns indicate significance at $p < 0.05$, $p < 0.01$, $p < 0.001$ and not significant, respectively, for differences among each reduced ethanol wine and the control wine ([a]), wines with reduced ethanol content of about 15.0% v/v ([b]), wines with reduced ethanol content of about 14.5% v/v ([c]), wines with reduced ethanol content of about 13.5% v/v ([d]). [b]Different Latin letters within the same row indicate significant differences according to the Tukey-b test ($p < 0.05$) among wines with ethanol content of about 15.0% v/v. [e]Semi-quantitative determination.

Table 3. Phenolic composition of Barbera red wine before and after ethanol reduction using different approaches.

	Control	Pre-fermentation		Fermentation		Post-fermentation					
Parameters	**CW[a]**	**DW1**	**DW2**	**SFW1**	**SFW2**	**DE0.5**	**DE1**	**DE2**	**Sign[b]**	**Sign[c]**	**Sign[d]**
A_{280}	44.1±2.0	41.8±0.4	39.6±0.1	40.8±1.1	42.7±1.4	43.6±1.1	43.3±1.4	43.7±0.6	ns	ns	*
TP (mg (+)-catechin/L)	1789±18	1651±6**	1538±153	1815±67	1854±98	1659±18*	1698±61	1703±31	ns	ns	ns
TF (mg (+)-catechin/L)	1197±9	1162±17	1082±15*	1176±15b	1205±32b	1063±6**,a	1059±17**	1067±6**	*	*	ns
PRO (mg cyanidin chloride/L)	1037±26	995±59	935±1*	944±26a	1019±40ab	1066±13b	1080±20	1084±1	*	ns	ns
FRV (mg (+)-catechin/L)	279±22	264±9	239±36	224±7	221±7	201±36	202±29	228±33	ns	ns	ns
FRV/PRO	0.27±0.01	0.27±0.01	0.26±0.04	0.24±0.01	0.22±0.02	0.19±0.04	0.19±0.03	0.21±0.03	ns	ns	ns
TAI (mg malvidin-3-glucoside chloride/L)	325±14	328±5	308±9	353±2b	370±3*,c	268±7*,a	273±11*	271±1*	***	*	*
∑ Delphinidin-3-glucoside (%)	8.8±0.5	9.7±0.5	9.7±0.1	9.2±1.0	10.7±0.3*	9.1±0.1	9.3±0.1	9.1±0.1	ns	ns	**
∑ Cyanidin-3-glucoside (%)	4.2±0.4	3.1±0.2	3.0±0.1*	4.4±0.3	3.6±0.3	4.6±0.1	4.5±0.1	4.6±0.1	ns	*	***
∑ Petunidin-3-glucoside (%)	11.1±0.4	11.7±0.4	11.6±0.2	11.1±0.9	11.9±0.1	11.2±0.1	11.3±0.1	11.1±0.1	ns	ns	ns
∑ Peonidin-3-glucoside (%)	9.4±0.5	8.4±0.5	7.6±0.1*	8.0±0.5	8.0±0.2	8.4±0.1	8.5±0.1	8.4±0.1	ns	ns	**
∑ Malvidin-3-glucoside (%)	49.8±0.5	49.6±0.7	50.4±0.1	50.3±2.4	48.5±0.2	50.2±0.2	50.0±0.1	50.1±0.2	ns	ns	ns
∑ Acetyl glucosides (%)	12.7±0.4	13.5±0.1	13.6±0.1	12.4±0.2	12.8±0.1	12.6±0.3	12.5±0.3	12.7±0.3	ns	ns	ns
∑ Cinnamoyl glucosides (%)	4.0±0.1	4.0±0.1	4.1±0.1	4.6±0.2	4.5±0.3	3.9±0.1	4.0±0.1	4.0±0.1	ns	ns	ns

Data are expressed as mean value ± standard deviation. (n = 2). [a,b,c,d]Sign: *, **, *** and ns indicate significance at $p < 0.05$, $p < 0.01$, $p < 0.001$ and not significant, respectively, for differences among each reduced ethanol wine and the control wine ([a]), wines with reduced ethanol content of about 15.0% v/v ([b]), wines with reduced ethanol content of about 14.5% v/v ([c]), wines with reduced ethanol content of about 13.5% v/v ([d]). [b]Different Latin letters within the same row indicate significant differences according to the Tukey-b test ($p < 0.05$) among wines with ethanol content of about 15.0% v/v. A_{280}, absorbance at 280 nm; TP, total phenols; TF, total flavonoids; PRO, proanthocyanidins; FRV, flavanols reactive to vanillin; TAI, total anthocyanins.

Table 4. Chromatic characteristics of Barbera red wine before and after ethanol reduction using different approaches.

	Control	Pre-fermentation		Fermentation		Post-fermentation					
Parameters	**CW[a]**	**DW1**	**DW2**	**SFW1**	**SFW2**	**DE0.5**	**DE1**	**DE2**	**Sign[b]**	**Sign[c]**	**Sign[d]**
L*	14.8±1.8	13.8±2.5	13.5±0.3	17.0±0.2	13.2±1.9	13.7±0.1	13.4±0.1	13.5±0.1	ns	ns	ns
a*	46.7±2.0	45.5±3.0	45.2±0.3	49.1±0.2	44.8±2.3	45.3±0.2	45.0±0.1	45.2±0.1	ns	ns	ns
b*	33.7±3.0	31.9±4.1	31.5±0.4	37.2±0.3	30.9±3.2	31.8±0.2	31.3±0.1	31.6±0.1	ns	ns	ns
C*	57.6±3.4	55.6±4.8	55.1±0.5	61.6±0.3	54.4±3.7	55.4±0.2	54.8±0.1	55.2±0.1	ns	ns	ns
H* (rad)	0.63±0.02	0.61±0.03	0.61±0.01	0.65±0.01	0.61±0.02	0.61±0.01	0.61±0.01	0.61±0.01	ns	ns	ns
Color intensity (AU, OP 10 mm)	11.0±0.8	11.4±1.9	11.5±0.2	9.9±0.2	11.9±1.4	11.8±0.1	12.1±0.1	12.0±0.1	ns	ns	ns
Color hue	0.64±0.01	0.61±0.05	0.59±0.01*	0.65±0.01b	0.58±0.02a	0.65±0.01b	0.64±0.01	0.65±0.01	*	ns	**
ΔE*	-	2.38	2.96	4.78	3.74	2.60	3.26	2.89			

Data are expressed as mean value ± standard deviation. (n = 2). [a,b,c,d]Sign: *, ** and ns indicate significance at $p < 0.05$, $p < 0.01$ and not significant, respectively, for differences among each reduced ethanol wine and the control wine ([a]), wines with reduced ethanol content of about 15.0% v/v ([b]), wines with reduced ethanol content of about 14.5% v/v ([c]), wines with reduced ethanol content of about 13.5% v/v ([d]). [b]Different Latin letters within the same row indicate significant differences according to the Tukey-b test ($p < 0.05$) among wines with ethanol content of about 15.0% v/v. L*, clarity; a*, red/green color coordinate; b*, yellow/blue color coordinate; C*, chroma; H*, hue angle; ΔE*, average CIELab color difference with respect to control.

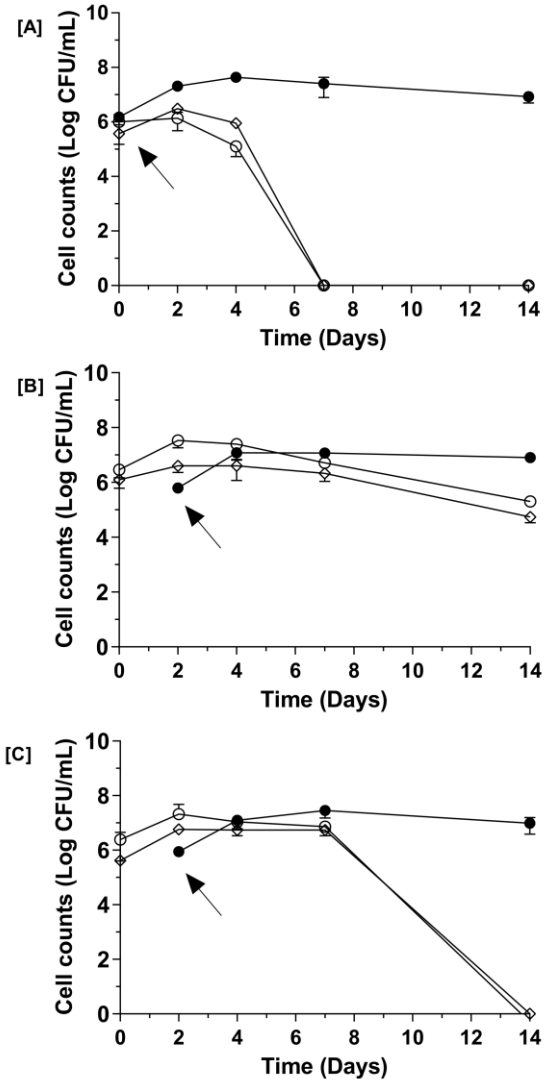

Fig. 1 Growth dynamics of yeast during pilot scale fermentations. Control fermentation (A), mixed fermentations with FC54 (B) and C.z 03 (C). *S. cerevisiae* (-●-), *Starm. bacillaris* (-○-) and indigenous non-*Saccharomyces* yeasts (-◊-). Counts are the mean CFU/mL values ± standard deviations of two independent experiments. The arrow indicates the *S. cerevisiae* inoculation.

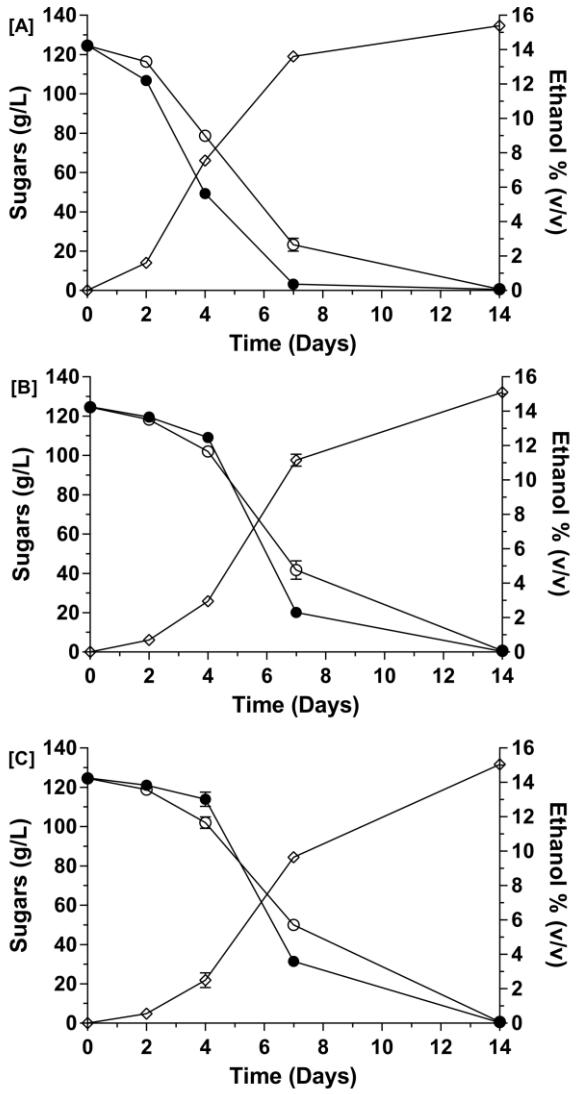

Fig. 2 Evolution of metabolites during control fermentation (A), mixed fermentations with FC54 (B) and C.z 03 (C). Glucose (-●-), fructose (-○-) and ethanol (-◊-). Data are expressed as mean value ± standard deviation of two independent experiments.

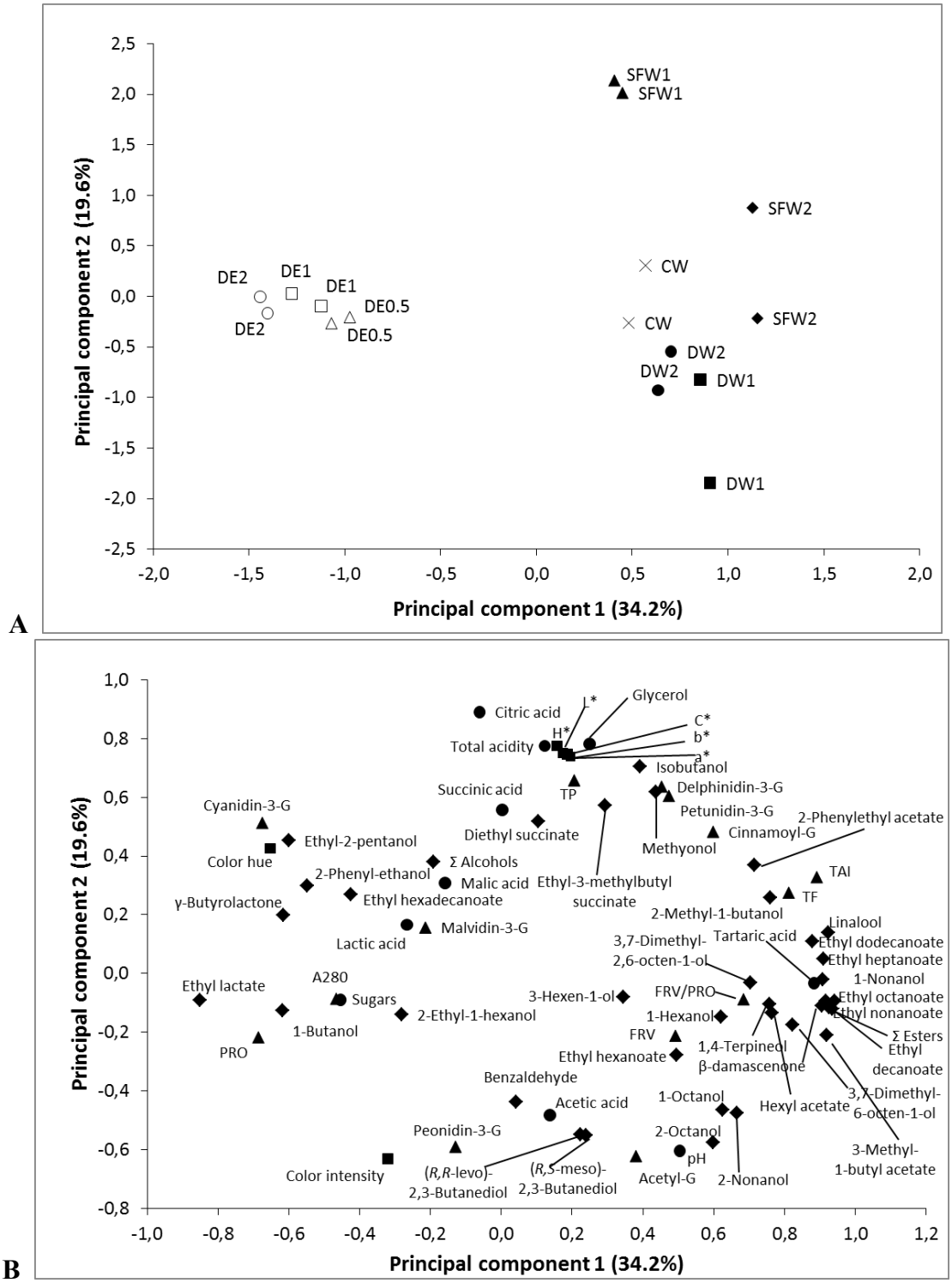

Fig. 3 Score plot (A) and loading plot (B) of the first and second principal components corresponding to PCA of the standard parameters (●), volatile compounds (♦), phenolic composition (▲) and chromatic characteristics (■) of Barbera wines: CW (×), SFW1 (▲), SFW2 (♦), DW1 (■), DW2 (●), DE0.5 (△), DE1 (□) and DE2 (○). G, glucoside.