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Aroma profile and composition of Barbera wines obtained by mixed fermentations of *Starmerella bacillaris* (synonym *Candida zemplinina*) and *Saccharomyces cerevisiae*

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ABSTRACT

In recent years there is an increasing global interest for the use of selected non-*Saccharomyces* yeasts by the winemaking industry, mainly due to their positive contribution to the wine complexity. In this study, *Starmerella bacillaris* (synonym *Candida zemplinina*) and *Saccharomyces cerevisiae* were evaluated in mixed (co-inoculated and sequentially) inoculated fermentations with the aim of improving the aroma profile of Barbera wine. The different inoculation protocols and combination of strains tested, influenced the interactions and the fermentation behaviour of the two yeast species. The wines produced with mixed cultures contained higher amounts of glycerol and pleasant esters compared to the wine fermented with *S. cerevisiae* alone. The use of mixed culture fermentations with selected yeast strains and appropriate inoculation strategies could be considered as a tool to enhance the aroma profile of wines produced from non-floral grape varieties like Barbera.

1. Introduction

The Barbera grape is Piedmont's most widely planted red variety of *Vitis vinifera* L. Barbera vineyards are located mainly in two big areas, which produce the most outstanding wines of this grape variety, the region near the town of Alba (Barbera d'Alba) and Asti (Barbera d'Asti). Even if the Nebbiolo-based wines (Barolo and Barbaresco) are considered as the most renowned red wines of this region, Barbera is the quintessential "wine of the people". It is meant to be enjoyed young during the meals. The sensory quality of young fresh wines, produced from non-aromatic grape varieties, like Barbera, depends greatly on numerous chemical constituents, mainly extracted during the pre-fermentation and fermentation process (Delfini et al., 2001).

Among other parameters, the volatile aroma compounds need special attention since it has a substantial influence on the wine quality and its acceptance by the wine consumers (Bruwer, Saliba, & Miller, 2011; Swiegers, Bartowksy, Henschke, & Pretorius, 2005). Aroma is considered as one of the main parameters that is affected by innumerable variations during wine production, ranging from viticulture to winemaking. Particularly the nature and amount of the volatile compounds can be influenced by environmental factors, cultivar and vineyard management, fermentation conditions and lastly by the microbial community consisting of non-*Saccharomyces* and *Saccharomyces cerevisiae* yeast species which take over the fermentation (Fleet, 2003; González-Barreiro, Rial-Ortero, Cancho-Grande, & Simal-Gándara, 2015; Lambrechts & Pretorius, 2000; Swiegers, Francis, Herderich, & Pretorius, 2006).

Wine yeasts found on grapes and consequently in the grape juice, have a strong impact on the wine quality and composition, since are responsible for the production of hundreds of secondary products, which contribute collectively, or individually, to the wine character and composition (Fleet, 2003; Lambrechts & Pretorius, 2000; Romano, Fiore, Paraggio, Caruso, & Capece, 2003).

Wine production is based on spontaneous or inoculated fermentation and in both cases, the dominance of *S. cerevisiae*, either indigenous or inoculated, is desired in order to ensure a complete consumption of sugars. However, the presence of non-

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Saccharomyces yeast has been documented (Fleet, 2008), at significant levels (up to 10^7–10^8 CFU/mL), during fermentation progress and for longer periods than previously thought (Bokulich, Swadener, Sakamoto, Mills, & Bisson, 2015; Cocolin & Mills, 2003). Few years ago, it was believed that the presence of non-*Saccharomyces* yeasts, could make the wine defective due to the production of metabolites of unpleasant origin (Romano, Suzzi, Comi, & Zironi, 1993). Nowadays, this trend is changing and the inoculation of mixed cultures of selected non-*Saccharomyces* yeasts in combination with highly fermentative *S. cerevisiae* strains able to ensure the complete consumption of sugars, is gaining attention and considered as an up-to-date inoculation strategy to enhance wine complexity and avoid unwanted compounds to be produced (Ciani & Comitini, 2015; Fleet, 2008; Jolly, Varela, & Pretorius, 2013) In this context, over the last years there has been an increasing interest regarding non-*Saccharomyces* yeasts and in order to improve the chemical composition and sensory aspect of the wines (Andorrà, Berradre, Mas, Esteve-Zarzoso, & Guillamón, 2012; Gobbi et al., 2013; Sadoudi et al., 2012; Soden, Francis, Oakey, & Henschke, 2000).

The increasing interest of winemakers in improving the complexity of young fresh wines produced from non-aromatic grape varieties requires further effort into understanding the metabolic profiles of specific non-*Saccharomyces* yeast species. To gain an insight into the contribution of these species to wine aroma, the aim of this work was to evaluate the use of controlled multi-starter fermentation cultures of *Starmerella bacillaris* and *S. cerevisiae* to enhance the analytical composition of Barbera wine. Two inoculation protocols were investigated: i) inoculation of both species at the beginning of the fermentation process (co-inoculation), and ii) inoculation of *S. cerevisiae* two days after *Starm. bacillaris* inoculation (sequential inoculation). Control wines were also produced by fermenting the same must with each of the *S. cerevisiae* and *Starm. bacillaris* strains in pure culture. Metabolic profiles of wines produced were compared, in order to highlight the effect of the inoculation strategy and strain selection on the final product.

2. Materials and methods

2.1. Yeast strains

Four *Starm. bacillaris* (FC54, BC60, EFR3B and C.z 02) and two *S. cerevisiae* (ScBa49 and ScBa50) strains from the yeast culture collection of the Department of Agricultural, Forest and Food Science (DISAFA, University of Turin, Italy) were used in this study. *Starm. bacillaris* strains were isolated from grape and musts of different varieties and were selected for their oenological attributes in laboratory scale fermentations (Englezos et al., 2015).

2.2. Must preparation

Barbera grapes were harvested, destemmed and crushed. The must with grape skins was heated to 60 °C for 1 h to promote color extraction in a process called *thermovinification* (Boulton, Singleton, Bisson, & Kunkee, 1996, chap. 12) and to deactivate indigenous yeast populations already present in the must. The grape juice was then separated using a stainless steel sieve, cooled down and frozen at −20 °C until use. The efficiency of the pasteurization was checked by plating 100 μL of the treated must on Wallerstein laboratory nutrient (WLN) medium (Biogenetics, Milan, Italy) and then incubated at 28 °C for 5 days. The unfermented must had the following composition: pH 3.20; titratable acidity 5.39 (expressed as g/L of tartaric acid); sugar concentration 244.4 g/L.

2.3. Inoculation procedure

For each strain, an aliquot of a stock in YPD broth (10 g/L yeast extract, 20 g/L peptone, 20 g/L dextrose with 20% glycerol; all from Biogenetics), stored at − 80 °C was streaked onto a WLN medium, 48 h before the preparation of inoculum. Afterwards, one fresh single colony was selected to inoculate 10 mL of sterile must. After 24 h of incubation at 25 °C, 30 mL of sterile must were added to the activated inoculum and then incubated for another 24 h at the same temperature. Finally, the preadapted inoculum was added in 360 mL of fresh sterile must.

2.4. Microfermentation trials

Three sets of fermentations were performed: inoculation of each *Starm. bacillaris* and *S. cerevisiae* strains in pure culture fermentations, simultaneous inoculation of both yeast species (co-inoculation) and inoculation of *S. cerevisiae* after 48 h from the *Starm. bacillaris* inoculation (sequential inoculation). Mixed fermentations were carried out, using 8 different combinations of *Starm. bacillaris* and *S. cerevisiae strains*, according to the experimental plan reported in Table 1. Fermentations were carried in 500 mL sterile glass bottles, containing 400 mL of sterile must per bottle. Pure and mixed culture fermentations were inoculated with the above-mentioned preadapted cultures, to achieve an initial cell population of about 1.0×10^6 cells/mL which was determined through plate counts on WLN medium. The bottles were equipped with sterile glass air locks containing sterile paraffin oil, to allow the carbon dioxide evolved during the fermentation process to escape from the fermenting juice. Fermentations were performed twice, under static conditions at 25 ± 1 °C (semi-anaerobic conditions). Fermentations were stopped when the weight loss remained stable for two days. Wines from both pure and mixed fermentations were then refrigerated for two days at 4 °C to remove solid parts. Afterwards, a solution of potassium metabisulfite was added to the wines, to achieve a total sulphur dioxide concentration of 50 mg/L, which were stored at −4 °C until analysis.

2.5. Microbiological analyses

From each bottle, 1 mL samples were collected in duplicate at 0, 1, 2, 4, 7, 14 and 21 days from the beginning of fermentation to evaluate the viable cell populations. One hundred microliter aliquots of serial dilutions were plated on WLN medium, which allows the visual differentiation of the two yeast species. Plates were incubated at 28 °C and the two types colonies were visually differentiated as described previously by Rantsiou et al. (2012) and subsequently counted.

Table 1
Experimental plan used in this study.

Pure fermentations[a]	Mixed fermentations[a,b]	
	Co-inoculation	Sequential inoculations
Strains	Couples	Couples
S. cerevisiae	FC54 and ScBa49	FC54 and ScBa49
ScBa49	FC54 and ScBa50	FC54 and ScBa50
ScBa50	EFR3B and ScBa49	EFR3B and ScBa49
Starm. bacillaris	EFR3B and ScBa50	EFR3B and ScBa50
FC54	C.z 02 and ScBa49	C.z 02 and ScBa49
EFR3B	C.z 02 and ScBa50	C.z 02 and ScBa50
C.z 02	BC60 and ScBa49	BC60 and ScBa49
BC60	BC60 and ScBa50	BC60 and ScBa50

[a] Inoculum size: 1.0×10^6 cells/mL.
[b] Inoculum ratio: 1:1.