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Evolution of microbiological and chemical parameters during red wine making with extended post-fermentation maceration

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ABSTRACT

The aim of the present work was to investigate the microbiological, chemical, and sensory characteristics of red wine subjected to post-fermentation maceration that was extended to 90 days. For this purpose, the 'Aglianico di Taurasi' grape was used as a case study. The total yeast concentration increased until day 40 of maceration and decreased thereafter, whereas the concentration of lactic acid bacteria slightly increased. *Dekkera/Brettanomyces* spp. and acetic acid bacteria were not detected. The yeast community was composed of *Saccharomyces cerevisiae*, *Zygosaccharomyces bisporus*, *Metschnikowia pulcherrima*, *Hanseniaspora guilliermondii*, *Hanseniaspora uvarum*, *Pichia guilliermondii*, *Aureobasidium pullulans* and *Debaryomyces carsonii*. Nine *S. cerevisiae* strains were detected at high levels at different times of maceration.

The results of all the conventional chemical analyses of the wines were in agreement with the regulations of commercial production and, interestingly, the changes in terms of concentration demonstrated the presence of yeast and LAB populations that were not only alive but also in a metabolically active state until day 90 of maceration. The alcohol and glycerol contents slightly increased until day 90. The concentrations of malic acid decreased, whereas those of lactic acid slightly increased throughout the maceration process.

Furthermore, different durations of maceration resulted in significant differences in the total polyphenol content, which was higher at 40–50 days. The main phenolic compounds were benzoic and cinnamic acids and catechins. Interestingly, the highest ratio between (+)-catechin and (−)-epicatechin was found on day 40. In addition, the highest antioxidant activity was observed between days 40 and 50. The concentration of volatile organic compounds, which were mainly represented by alcohols, increased until the end of the maceration process. Sensory analysis revealed that samples that were subjected to maceration for a long period of time showed the highest odour and taste complexity and no off-odours and/or off-flavours were detected. These data confirmed that extending post-fermentation maceration to 90 days has no negative impact on the microbiological, chemical and sensory composition of wines, but affects the polyphenol content and potential health benefits of the resulting wine.

1. Introduction

The 'Aglianico di Taurasi' grapevine is one of the economically most important cultivars of the Campania region (Pomarici et al., 2004). The quality of the resulting wine—the highest category is 'Taurasi D.O.C.G.'—is well recognised (Piombino et al., 2004) and its composition varies significantly depending on several factors, from agronomic practices to the technology of vinification (Mazzei et al., 2010).

Rate, kinetics, and duration of fermentation strictly depend on the yeast population present in the must (Zambonelli, 1998). The metabolic activities of yeast on must components determine the production of several compounds that significantly contribute to the aroma of wines (Pretorius, 2000). An important step in the production of red wine is represented by the maceration process, whose effects influence the quality of the wine (Bautista-Ortín et al., 2005). The main purpose of maceration is the extraction of colour compounds (anthocyanins and phenolic substances) from the solid components of the grape. However, this process also affects the sensory profile of the resulting wines because other compounds such as aromatic substances and precursors, nitrogen compounds, polysaccharides, and minerals are released during the maceration process (De Beer et al., 2006). The transfer of these compounds from grape skins and seeds to the must is influenced by several factors, including temperature, contact duration, alcohol content, SO_2 concentration, grape variety, maturation degree, and microbial populations (Romero-Cascales et al., 2005).

Four maceration techniques may be applied during wine production: conventional maceration, consisting of moving the must from the bottom of the vat to the top or immersing the floating layer of skins in the

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fermenting bulk or by transferring the entire liquid phase of the must from one vat to another one (délestage); carbonic maceration, which is carried out with whole grapes fermented in a CO_2-rich environment; pre-fermentation maceration or "cold soaking", in which grape skins and seeds are put in contact with the liquid must at low temperatures one or two days before fermentation begins and the must is moved a few times per day; and post-fermentation maceration of variable duration, i.e. a few days to 21 days (Ivanova et al., 2011; Gambuti et al., 2004).

The prolonged contact between grape skins and seeds with the must allows a higher extraction of polyphenolic compounds, especially catechins and proanthocyanidins (or condensed tannins) that are more concentrated in the pulp of grape berries (Ivanova et al., 2011). Longer macerations also provide stability of the colour compounds (Gómez-Plaza et al., 2002). Among these, polyphenols are gaining interest due to their positive effects (antioxidant, anticancer, cardioprotective, antimicrobial, antiviral, and neuroprotective) on the consumers' health (En-Qin et al., 2010).

The main objective of the present work was the evaluation of the influence of long post-fermentation maceration on the evolution of yeast and lactic acid bacteria (LAB) populations, release of polyphenol compounds from skins and seeds, volatile organic compounds (VOCs), and antioxidant activity of red wine. The 'Aglianico di Taurasi' cultivar was used as model system.

2. Materials and methods

2.1. Experimental winemaking and sample collection

The experimental winemaking was carried out by prolonging the contact between solid (grape skins) and liquid (wine) phases of grape must to 90 days after the tumultuous phase of alcoholic fermentation. This was considered an extension of the post-fermentation maceration period.

The grapes of the 'Aglianico di Taurasi' grapevine were subjected to the experimental vinification process that took place at the winery 'Azienda Agricola Contrade di Taurasi' located in Taurasi (Avellino, Campania, Italy) (41°00′11.94″N; 14°58′25.82″E).

Soon after harvest, the grapes were subjected to stemmer-crushing. After placing the must into steel vats and adding potassium metabisulphite (6 g/hL), it was inoculated with the autochthonous starter strain *Saccharomyces cerevisiae* NF66 (culture collection of the Department of Agricultural and Forest Science — University of Palermo, Italy) (15 g/hl). The starter (used as paste) was characterised by a viable cell concentration of 7.6×10^{12}-colony forming units (CFU)/g. Diammonium phosphate and diammonium sulphate salts (1:1) (15 g/hL) were also added as activators of the fermentation process. Eighty hectolitres of must were transferred into two stainless steel vats (40 hL each) where the fermentation (8 days at 26 °C) took place. During the tumultuous phase of alcoholic fermentation but only after raising the cap, the content of each vat was mixed in order to facilitate the contact between the solid and liquid phases of the must. In particular, this was done three times per day in order to remove the liquid phase from the bottom of a single vat to the top of the same vat. Furthermore, from day 4 until day 8 of alcoholic fermentation, each vat was subjected to one délestage per day. This was done by transferring the entire liquid phase of the must of each vat into empty stainless steel vats, letting them stand for 4 h, and transferring the liquid phase back into the original vat. The scope of this action was to facilitate the contact between the liquid phase of the must with oxygen.

At the end of the tumultuous phase of alcoholic fermentation (day 8), the bulk content (both liquid and solid phases) of each vat was transferred into steel vats with a capacity of 5 hL. All vats were filled until a final solid-to-liquid ratio of 1:3 was reached and closed to avoid contact with oxygen. They were cooled at 16 ± 1 °C and subjected to different durations of post-fermentation maceration: 13 d, which represented the control of post-fermentation maceration, 20, 40, 50, 60, 70 and 90 d. The production of commercial 'Aglianico di Taurasi' wine after the tumultuous phase of alcoholic fermentation is, in general, based on a maceration period of 13 d, which is considered to be the minimal duration of maceration for this wine. Thus, in this study, post-fermentation maceration of 13 d was used as control trial. The vinification process, i.e., from grape must until day 90 of post-fermentation maceration, was performed in duplicate.

2.2. Microbiological analysis

Must samples collected from grape must until the end of maceration were serially diluted in Ringer's solution (Sigma-Aldrich, Milan, Italy). Decimal dilutions were spread-plated (0.1 mL) onto Wallerstein Laboratory (WL) nutrient agar (Oxoid, Basingstoke, UK) and incubated at 28 °C for 48–72 h to determine total yeast (TY) counts. The sample dilutions were also spread-plated onto *Dekkera*/*Brettanomyces* differential medium (Rodrigues et al., 2001) and incubated at 25 °C for 14 d to detect presumptive *Dekkera*/*Brettanomyces* spp.

The *Dekkera*/*Brettanomyces* population was also counted by filtering (0.45-μm pore size filter, Sartorius, AubagneCedex, France) the samples using the same media and incubation conditions reported above. To count the lactic acid bacteria (LAB), the sample dilutions were pour-plated onto Man, Rogosa, and Sharpe (MRS) agar (Oxoid) and incubated at 28 °C for 48–72 h, and onto medium for *Leuconostoc oenos* (MLO) agar (Caspritz and Radler, 1983) and incubated at 28 °C for 5 d. The latter medium was used for the enumeration of acidophilic LAB. The acetic acid bacteria (AAB) population was enumerated onto Kneifel agar medium (OIV, 2010) and incubated at 25 °C for 10 d. All analyses were carried out in duplicate.

2.3. Yeast isolation and identification

Yeasts were isolated only from WL differential medium. At least five colonies per morphology were randomly collected from the agar plates, purified to homogeneity after several sub-culturing steps onto WL, and at least three isolates (from each sample) sharing the same morphology were subjected to genetic characterisation.

DNA extraction was performed using the InstaGene Matrix kit (Bio-Rad Laboratories, Hercules, CA) according to the manufacturer's instructions. In order to perform a first differentiation of yeasts, all selected isolates were subjected to restriction fragment length

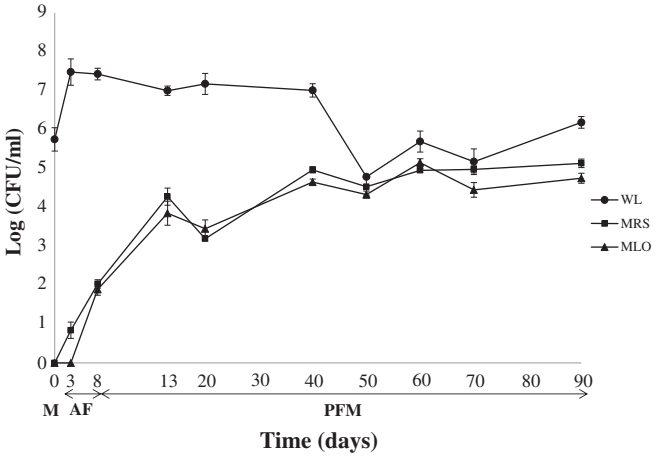

Fig. 1. Microbial loads of wine samples collected during experimental vinification of "Aglianico di Taurasi" wine. Symbols: ●, TY on WL; ■, LAB on MRS; ▲, LAB on MLO. Data represent the mean of four replicates of two independent experiments. Bars represent standard deviation of the mean. Vertical bars not visible are smaller than symbol size. Abbreviations: AF, alcoholic fermentation; PFM, post-fermentation maceration.