INFLUENCE OF DIFFERENT STRAINS OF LAB ON QUALITY OF CATARRATTO WINE PRODUCED IN SICILY

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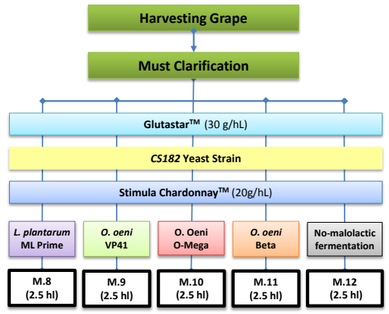
INTRODUCTION: During alcoholic fermentation (AF), the natural development of lactic acid bacteria (LAB) correlates with their sensitivity to increasing ethanol concentrations and resistance to low pH values [1]. The survival of LAB plays a significant role in winemaking, thus guiding a secondary biological process known as malolactic fermentation (MLF). This process converts L-malic acid to L-lactic acid and CO_2 and is carried out by one or more LAB species [2, 3]. MLF improves the microbial stability of wine [4], by the removal of L-malic acid as a possible carbon substrate, and leads to the modification of the wine aroma profile, which is linked to different enzymatic activities. The aim of the present work was to investigate the microbiological, chemical, and sensory characteristics of white wine subjected to malolactic fermentation through the inoculum of ML Prime™ (Lallemand) and 3 different strains of *O.oeni* (VP41®, O-Mega® and Beta) compared to spontaneous malolactic fermentation. For this purpose, Catarratto grapes was used as a case study.

MATERIALS AND METHODS: **Experimental winemaking** The experimental winemaking was carried out by white wine vinification (**Fig.1**).



Microbiological counts of yeast and LAB Must samples collected during fermentation 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 72 h to determine *Saccharomyces* and non-*Saccharomyces* yeast counts. *Lactiplantibacillus plantarum* (ML Prime™) population was determined on De Man, Rogosa and Sharpe (MRS) agar plates (Biogenetics) after aerobic incubation at 28 °C for 3–5 days. *Oenococcus oeni* VP41, O-Mega and PN4 population were monitored using double-layer MRS agar medium (Biogenetics), supplemented with 10 g/L malic acid (Sigma-Aldrich), adjusted to pH 5.2 with 1 mol/L NaOH and incubated under anaerobic conditions at 30 °C for 10 to 14 days [5].

Persistence of Yeast and LAB In order to verify the dominance of the starter CS182 during AF, all isolates were characterized by Interdelta analysis. Genetic diversity within *Saccharomyces* isolates was assessed by Interdelta analysis [6]. Interdelta patterns were analysed using the GelCompar II software (v. 6.1, Applied Maths NV, Sint-Martens-Latem, Belgium) and similarities among patterns were assessed. Profiles showing more than 95% of similarity were considered identical. Dominance of LAB strains, added in the trials, has been verified during fermentation by random amplification of polymorphic DNA-PCR (RAPD-PCR) analysis in 25µL reaction mix using primer M13 [7]. All strains belonging to the *L. plantarum* group were subjected to the recA gene based multiplex PCR described by [8] to distinguish unequivocally among *L. plantarum*, *L. paraplantarum* and *L. pentosus*.

Physic-chemical analysis Glucose, fructose, ethanol, glycerol, acetic acid, malic acid and lactic acid were determined by the enzymatic analyser iCubio iMagic M9 (Shenzhen iCubio Biomedical Technology Co., Ltd., Shenzhen, China). The parameters used in the automated photometric systems were temperature, 37 °C; wavelengths, 340 nm and 415 nm (bichromatic); and optical path, 1 cm. All reagents and standards were purchased from R-Biopharm AG (Darmstadt, Germany). All samples have been diluted until the optimal concentration with respect to the calibration curve of the apparatus.The pH was determined by OIV-MA-AS313-15 method [9], while, total acidity was determined by methodology described by OIV-MA-AS313-01 [10].

Volatile organic compound composition To determinate the volatile components the protocol proposed by [9] was performed. The individual peaks were analysed using the GCMSolution package, Version 2.72. Identification of compounds was carried out using Adams, NIST 11, Wiley 9 and FFNSC 2 mass spectral database. These identifications were also confirmed by other published mass spectra and linear retention indices (LRI). The LRI were calculated using a series of *n*-alkanes (C8-220 C40). In addition. Some of the compounds were confirmed by comparison of mass spectra and retention times with standard compounds available at the Department STEBICEF – University of Palermo.

Sensory evaluation The designed sensory evaluation of experimental wines consisted of two steps: (*i*) sensory acceptance tests performed by consumers and (*ii*) quantitative descriptive analyses were carried out by panelists to define aroma and sensory profiles.

RESULTS: Microbial count of yeasts during fermentation are shown in **Fig. 2**. Microbial count of LAB populations is represented in **Fig. 3**. The trend in malic acid and lactic acid concentrations is shown in **Fig. 4.** All results from GC analysis were conducted in two separate laboratory to confirm identification of compounds. Very high differences were found among trials both of Cataratto wines (**Fig. 5**).

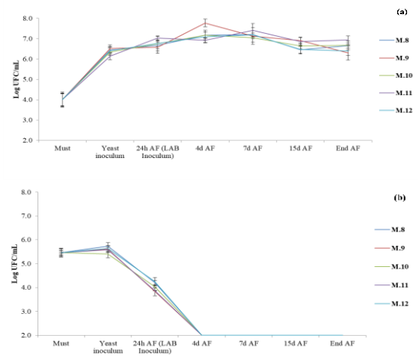


Fig. 2. Microbiological concentration (Log CFU/mL) of samples during alcoholic fermentation of Catarratto: **(a)** Presumptive *Saccharomyces*; **(b)** non-*Saccharomyces.*

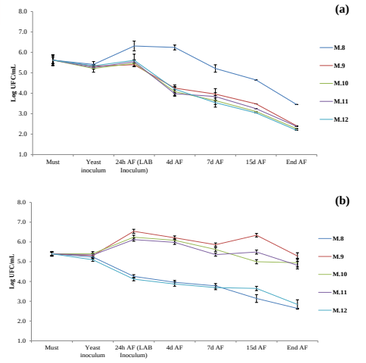


Fig. 3. Microbiological concentration (Log CFU/mL) of samples during alcoholic fermentation of Catarratto: **(a)**presumptive *Lactiplantibacillus* spp. on MRS medium; *Oenococcus* spp. on MRS+malic acid **(b).**

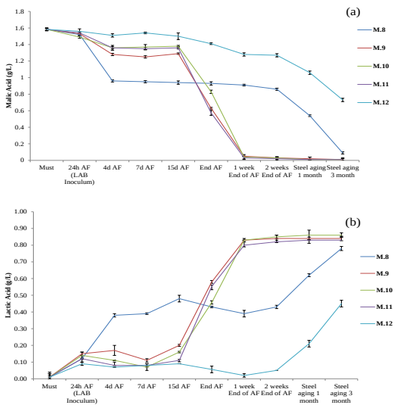


Fig. 4. Trend of malic acid **(a)** and lactic acid **(b)** during the different stages of Catarratto vinification.

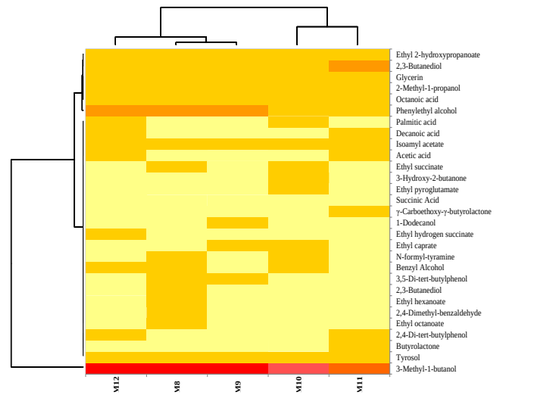
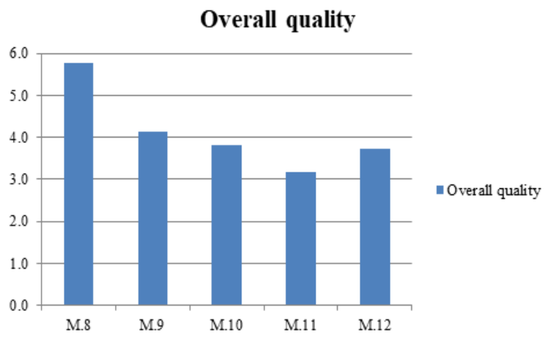


Fig. 5. Heat-map analysis of VOCs resulted from GC-MS analysis of Catarratto wines.

All results from sensory analysis are reported in **Fig. 6**. Very high differences were found among trials. Wines produced by inoculum of ML Prime™ showed the best sensory characteristics.



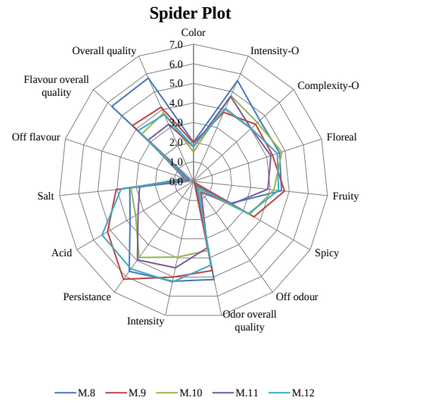


Fig. 6. Sensory analysis of Cataratto wines.

CONCLUSIONS: Catarratto wines subjected to guided malolactic fermentation by using *L. plantarum* (ML Prime™) and *O. oeni* (VP41®, O-Mega® and Beta®) showed differences both in terms of volatile organic compounds and in sensorial aspects. VOCs, determined through GC-MS analysis showed very similar *L. plantarum* and *O. oeni* VP41® profiles. The M8 wine showed the highest flavor and odor overall quality scores and the MLPrime™ malolactic fermented wine was the most appreciated by the panelists. *L. plantarum* represents a valid alternative to *O. oeni* as a starter for malolactic fermentation in Catarratto wines.

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