USE OF *LACTIPLANTIBACILLUS PLANTARUM* (ML PRIME™) TO IMPROVE MALOLACTIC FERMENTATION OF CATARRATTO WINE SUBJECTED TO LONG POST-FERMENTATIVE MACERATION

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INTRODUCTION During alcoholic fermentation (AF) the presence of lactic acid bacteria (LAB) correlates with their sensitivity to increasing ethanol concentrations and resistance to low pH values [1]. Once AF is completed, yeast activity diminishes and only LAB resistant to the stressing conditions are able to survive [2]. The survival of these LAB plays a significant role in winemaking, because they drive a secondary biological process known as malolactic fermentation (MLF). This process converts L-malic acid into L-lactic acid and CO_2 and is carried out by one or more LAB species [3, 4]. MLF improves the microbiological stability of wine [5] through the removal of L-malic acid as a possible carbon substrate and determines a series of positive modifications concerning the wine aroma profile. MLF might occur spontaneously or driven by malolactic starter cultures added during vinification [6]. Wine LAB endougenously present in must can perform MLF spontaneously after growing up to a critical population that is necessary to start and achieve malic acid degradation [7]. Their activities depend on the physicochemical characteristics of wine [8]. The aim of the present work was to investigate the microbiological, chemical, and sensory characteristics of white wines using Catarratto grape variety, inoculating ML Prime™ (Lallemand) to perform MLF. All trials were also subjected to a post-fermentation maceration that lasted 60 d.

MATERIALS AND METHODS Experimental design and sample collection The experimental plan of the winemaking process with Cataratto extralucido (white grape variety) is reported in Fig.1. Samples of fermenting musts were collected before inoculation, after inoculation of *S. cerevisiae* CS182, after inoculation of ML Prime™ (*L. plantarum*), at day 1, 2, 3, 4, 5, 8 and at the end of AF. In addition, sampling was carried out during post-fermentation maceration, aging in steel vat and at bottling

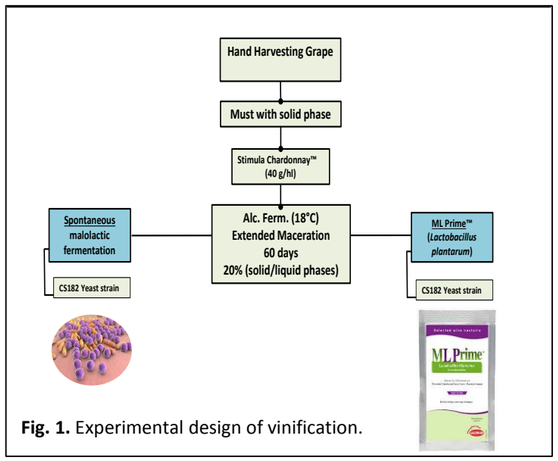


Fig. 1. Experimental design of vinification.

Microbiological counts of yeast and LAB and strain typing Must samples collected during fermentation was subjected to microbiological analysis following the protocol described by [9]. In order to verify the dominance of the starter CS182 during AF, all isolates were characterized by Interdelta analysis [10]. Dominance of LAB strains, added in the trials, has been verified during fermentation by random amplification of polymorphic DNA-PCR (RAPD-PCR) analysis using primer M13 [11].

Physicochemical 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 pH was determined by OIV-MA-AS313-15 method [12], while, total acidity was determined by methodology described by OIV-MA-AS313-01 [13].

Volatile organic compound composition by GC-MS To determinate the volatile components the protocol proposed by [14] 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.

Sensory evaluation The designed sensory evaluation of the experimental wines consisted of two steps: (i) sensory acceptance tests performed by consumers and experts following the methodology applied by [15]; (ii) quantitative descriptive analyses carried out by panelists to define aroma and sensory profiles [16].

RESULTS Plate counts of yeasts during fermentation and post-fermentation maceration are shown in **Fig. 2.** Dynamics of LAB population determined during AF and post-fermentation maceration is depicted in **Fig. 3.**

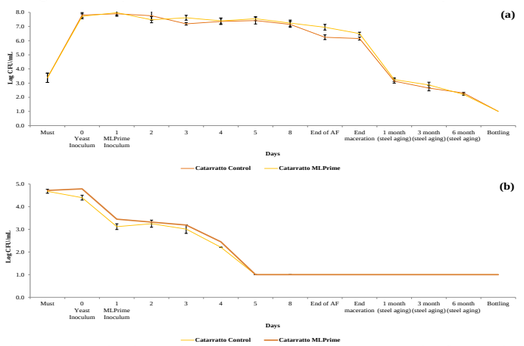


Fig. 2. Microbiological concentration (Log CFU/mL) of samples during AF and post-fermentation maceration: **(a)** Presumptive *Saccharomyces*; **(b)** non-*Saccharomyces.*

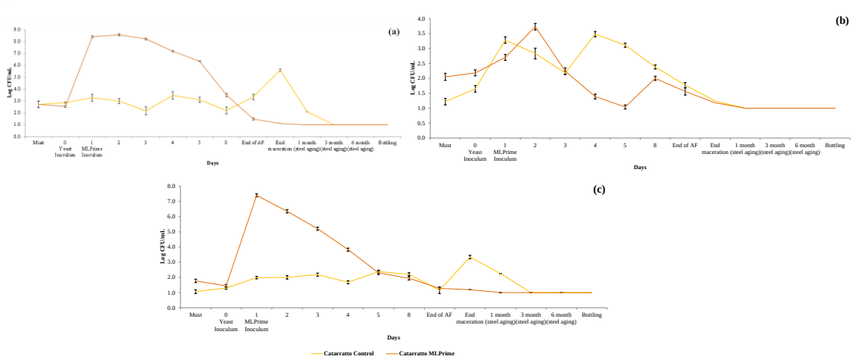
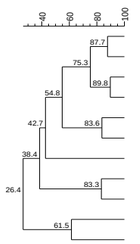


Fig. 3. Microbiological concentration (Log CFU/mL) of LAB during fermentation AF and post-fermentation maceration: **(a)** MRS; **(b)** M17; **(c)** MLO.

The resulting patterns (n = 356) were used to construct a dendrogram (Fig. 4). The analysis revealed 11 main clusters. Cluster G was the most numerous cluster consisting of 319 isolates with the same RAPD profile confirming that the starter strain dominance was 89.61%. In the control trial, that did not receive the *L. plantarum* ML Prime™ addition, no polymorphic profiles comparable to the starter strain ML Prime™ were detected.



Rapd Profile	n. Isolates	Species Blast	% Similarity (Blast)	Accession number (Blast)	Species Eztaxon	% Similarity (Ez taxon)	Accession number (Ez taxon)	Species by Multiplex
A	3	*L. plantarum*	99.52	KX261527.1	*L. pentosus*	99.25	DSM 20314	*L. plantarum*
B	7	*L. plantarum*	100.0	MK774602.1	*L. pentosus*	99.45	DSM 20314	*L. plantarum*
C	4	*L. pentosus*	99.59	KX057507.1	*L. pentosus*	99.38	DSM 20314	*L. plantarum*
D	5	*L. plantarum*	99.66	KX261527.1	*L. pentosus*	99.25	DSM 20314	*L. plantarum*
E	1	*L. pentosus*	99.46	KX057491.1	*L. pentosus*	99.73	DSM 20314	*L. plantarum*
F	3	*L. plantarum*	99.59	KX261527.1	*L. pentosus*	99.52	DSM 20314	*L. plantarum*
G	319	*L. plantarum*	99.36	KX261527.1	*L. pentosus*	98.63	DSM 20314	*L. plantarum*
H	5	*L. plantarum*	99.80	KX261527.1	*L. pentosus*	99.45	DSM 20314	*L. plantarum*
I	2	*L. plantarum*	99.39	KM497500.1	*L. pentosus*	99.25	DSM 20314	*L. plantarum*
J	4	*L. paraplantarum*	98.44	KU315098.1	*L. pentosus*	98.83	DSM 20314	*L. plantarum*
K	3	*L. plantarum*	99.52	KX057688.1	*L. pentosus*	99.45	DSM 20314	*L. plantarum*

Fig. 4. Dendrograms obtained from combined RAPD-PCR patterns of LAB strains from must Catarratto samples generated with M13 primer.

The trend in malic acid and lactic acid concentrations is shown in **Fig. 4**. All other parameters monitored (sugars, ethanol, volatile acidity, total acidity, glycerol) showed a trend typical of regular AF. The composition of the VOCs generated by the wines is reported in **Fig. 5**.

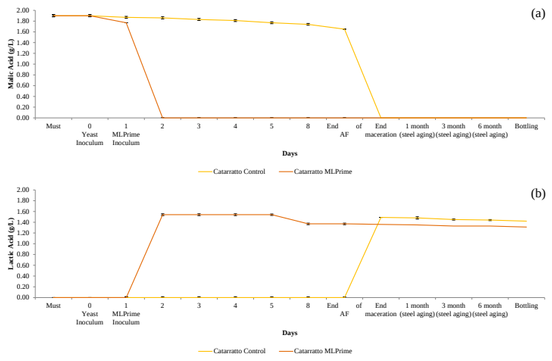


Fig. 4. Trend of malic acid **(a)** and lactic acid **(b)** during the different stages of Catarratto vinifications.

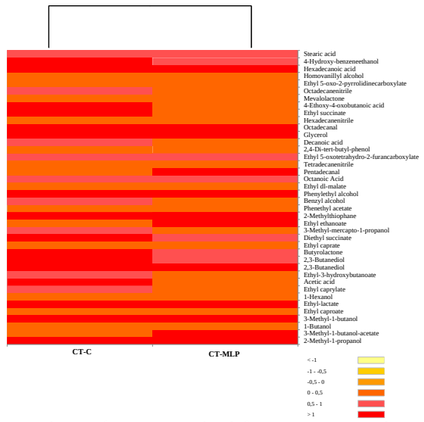


Fig. 5. Heat-map analysis of VOCs resulted from GC-MS analysis of Catarratto wines.

Wine consumers gave the highest scores for Catarratto wine produced with the addition of ML Prime™ (Fig. **6a**). PCA analysis based on the values of the descriptors is represented in Fig. **6b** through biplot graph.

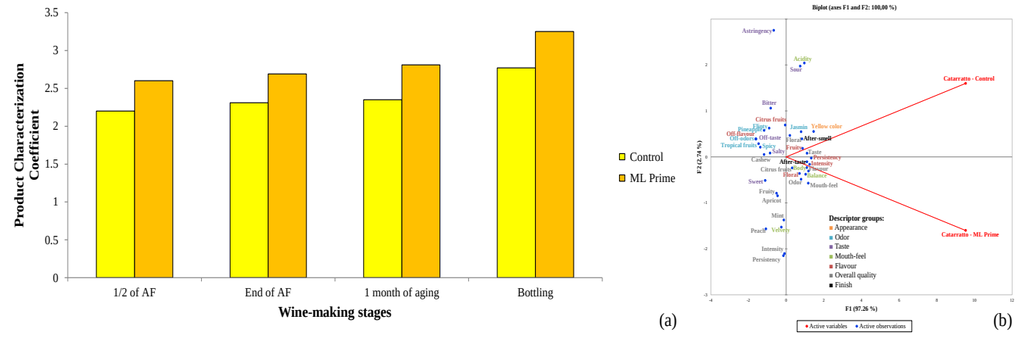


Fig. 6. Sensorial evaluation of wines: **(a)** product characterization for overall acceptability of experimental wine ; **(b)** Biplot graphs show relationships among factors, variables and trials.

CONCLUSIONS The wines obtained with the MLF starter were extremely different when compared to the respective controls (spontaneous malolactic fermentation). The differences obtained are related to different descriptors regarding appearance, odor, taste, mouth-feel, flavour, overall quality and finish.

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