Journal of Agricultural, Food and Environmental Sciences, Vol 73 No 3 (2019)
DOI: https://doi.org/10.55302/JAFES19733001m

TIMING OF INOCULATION WITH SELECTED WINE BACTERIA ON THE KINETICS OF MALOLACTIC FERMENTATION AND SENSORY PROPERTIES OF VRANEC WINE FROM TIKVESH WINE REGION

Goran Milanov*, Dushko Nedekovski, Milena Taseski-Gjorgjijevski

Institute of Agriculture, Ss. Cyril and Methodius University in Skopje, Republic of North Macedonia

*Corresponding author: milanovg@yahoo.com

ABSTRACT
The aim of this investigation is to determine the influence of timing of inoculation of four different lactic bacterias by co inoculation during the fermentation and post-fermentation of Vranec variety, to obtain more balanced wines with refined aromas. VP 41, ML Prime, O-Mega and PN4 are the types of commercial available lactic bacterias produced by Lallemand producer, used in malolactic fermentation (MLF) of wine Vranec. The kinetic of transformation of malic into lactic acid was measured. Also, chemical composition of wine (alcohol strength, specific gravity, total extract, total acids, volatile acids, pH, free and total SO_2) was performed. The sensory descriptive analysis was performed. Seven wine experts were involved for the descriptive evaluation of the investigated wines.

Keywords: Vranec, lactic bacteria, MLF, sensory evaluation.

INTRODUCTION
Selected wine bacteria possess the ability to impact the wine aroma profile in a positive way, both texturally and sensorial. Of the many fermentation-derived volatile compounds that contribute to wine aroma and flavor, the ester profile is of major importance for fruity sensory properties and overall quality of wine. In particular, certain ethyl and acetate esters have been shown to be key contributors to the berry fruit character of red wine. Ester hydrolysis and synthesis can be catalyzed by esterases and many wine bacteria have been shown to produce esterases with high activity towards water-soluble short-chain esters (Sumby et al., 2012). Many volatile compounds in wine can be released from their flavorless glycoconjugate precursors by enzymatic hydrolysis (glycosidases).
Four genera of lactic acid bacteria have been identified as the principal micro-organisms involved in MLF: *Lactobacillus*, *Leuconostoc*, *Oenococcus* and *Pediococcus* (du Toit et al., 2011). *Oenococcus oeni* is the predominant species, and is the most effective for overcoming difficult wine conditions. Today's very reliable malolactic starter cultures mainly consist of *O. oeni* strains. A few *Lactobacillus* spp. have also shown that they can perform very efficiently under winemaking conditions and that they possess many favorable characteristics to conduct MLF, especially in high pH wines, as it has been described in a patented application (EP1631657).
Wine pH has been increasing gradually for the last several years. Red wines with pHs over 3.5–3.6 are becoming more frequent. At these pH levels, very fast growth of various indigenous microorganisms is common, some of which are spoilage bacteria that can cause loss of wine quality. The pH of the wine determines which species of LAB are present. Values above pH 3.5 favor the growth of *Lactobacillus* and *Pediococcus* spp., whereas O. *oeni* tends to dominate at lower pH values (Henick-Kling 1993, 1994).
Some selected *L. plantarum* strains have the capacity to induce MLF under high pH conditions, and unlike *Oenococcus oeni*, *L. plantarum* has a facultative heterofermentative metabolism that prevents acetic acid production from hexose sugars. Strains within this species also have a complex enzymatic pool which could positively impact on the fruity sensory profile of wines.
The selection of a "good" oenological *Lactobacillus plantarum* is not easy. As early as 1988, *L. plantarum* was proposed as starter culture, albeit with mixed success. In 2005, *L. plantarum* strains isolated at University Catolica del Sacro Cuore, Italy, highlighting that this species can be used to induce efficient MLF in high pH wines. More recently a highly active pure *L. plantaru*m starter culture, called ML Prime™ has been released to the market. As a result of its specific optimized production process, ML Prime™ promptly expresses a very high malolactic enzyme activity. ML prime is inoculated 24 h after the wine yeast into the fermenting musts, resulting in a fast MLF finishing during alcoholic fermentation. As it converts glucose or fructose only to L-lactic acid, but not into acetic acid, even high pH wines will have very low volatile acidity at the end of MLF.

MATERIALS AND METHODS

Materials

For this investigation Vranec variety grape from Tikvesh wine region was used. Mature and healthy grapes from this variety were harvested by hand.

VP 41, ML Prime, O-Mega and PN4, as the types of commercial available lactic bacterias produced by Lallemand producer, were used for the malolactic fermentation (MLF) of the wine Vranec. The fermentation of the grapes was followed by Lalvin ICV D-254™ yeast and Fermaid E, yeast nutrient and Optimalo D, nutrient for MLF, from the same producer. For better color, stability and higher phenolic composition, EX-V for Lallemand producer was used.

Method

On a small electric crusher the grapes were immediately destemmed and dosage of 30 mg/L SO_2 was added. Then, the grape must was divided into 5 stainless steel tanks, with capacity of 30 kg each. Dosage of 1 g/hL EX-V enzyme was added and each variant was inoculated with selected active yeast Lalvin ICV D-254™, dosage 25g/hL. 24 hours after yeast addition, four lots were inoculated with different wine bacteria strains, as it is outlined below. The control variant is without bacteria inoculation.

- Control (Lalvin ICV D254™)
- Variant 1 co-inoculation (Lalvin ICV D254™ + Lalvin VP 41™)
- Variant 2 co-inoculation (Lalvin ICV D254™ + O-MEGA™)
- Variant 3 co-inoculation (Lalvin ICV D254™ + ML Prime™)
- Variant 4 co-inoculation (Lalvin ICV D254™ + PN4™)

During the alcoholic fermentation (AF) the cap was plunged 3 times daily. During the fermentation, 15 g/hL nutrient Fermaid E™ was added. The temperature during the alcoholic fermentation was in the range from 24 to 26°C. The transformation from malic to lactic acid was analyzed every 3 days. After 14 days of fermentation, the wine was separated, than the pomace was gently pressed to extract the remaining juice. After that the wine was left to settle for 2 days. After racking the wine a complete chemical analysis was conducted. At the end of alcoholic fermentation the control wine was divided in 5 equal parts and sequentially inoculated with the same bacteria strains previously used in the co-inoculation trial. Along with the different strains of wine bacteria, a bacteria nutrition addition was also made with Opti'Malo™ 20 g/hL

- Control
- Variant 1 sequent. Lalvin VP 41™ + Opti'Malo™
- Variant 2 sequent. O-MEGA™ + Opti'Malo™
- Variant 3 sequent. ML Prime™ + Opti'Malo™
- Variant 4 sequent. PN4™ + Opti'Malo™

Analysis of the samples

L-malic and L-lactic acid concentrations were determined using Oenolab enzymatic kit on an Agilent 8453 UV-vis spectrophotometer.

Various OIV-methods were used for determination of chemical composition (alcohol strength, specific gravity, total extract, total acids, volatile acids, pH, free and total SO_2) of the samples.

RESULTS AND DISCUSSION

The climate condition in Macedonia is very suited for cultivation of high quality red wines. The good climatic conditions are the key factor for grapes with good quality. Thus, they are used for the production of high quality premium wines from this variety. The obtained level of malic acid in Vranec grape juice was 1.15 g/L and the sugar content in Vranec grapes was 24.5 Brix.

For this study one yeast and four different strains of wine LAB were used. The co-inoculation treatment was compared with post alcoholic fermentation inoculation of LAB. The alcoholic fermentation was completed after a period of approximately 9 days. For higher extraction of phenolic components the wine was racked after 14 days. The obtain results from chemical analysis of the wines after alcoholic and malolactic fermentation are shown in Table 1.

Table 1. Chemical composition of Vranec wine produced with different malolactic bacteria

Variants	Sp. Gravity 20/20	Alcohol vol%	Total extract g/L	Total acids g/L	Volatile acids g/L	pH	Free SO_2 g/L	Total SO_2 g/L
Control	0.9947	14.24	32.3	6.4	0.55	3.45	32.00	55.12
co-VP 41	0.9943	14.29	32.8	5.8	0.51	3.38	28.00	52.32
co-ML Prime	0.9946	14.11	33.6	5.7	0.48	3.40	28.00	48.52
co-OMEGA	0.9947	14.11	33.9	5.5	0.46	3.38	25.60	4852
co-PN 4	0.9942	14.38	32.8	5.6	0.53	3.39	25.60	52.32
post-VP 41	0.9944	14.20	32.8	5.8	0.46	3.40	21.76	45.25
post-ML Prime	0.9947	14.47	33.9	5.8	0.45	3.39	32.00	51.25
post-OMEGA	0.9945	14.38	33.6	5.7	0.49	3.38	21.76	51.25
post-PN 4	0.9944	14.29	32.8	5.6	0.50	3.41	25.60	48.52

As can be seen in Table 1, the total acidity (TA) in the Control is higher than the other treatments because there was no transformation from malic to lactic acid. The level of malic acid in the control sample remains the same 1.15 g/L. All other co-inoculated samples had undergone a complete malic acid transformation. The level of volatile acids is in normal range which indicates that the alcoholic fermentation is normal without unwonted microbiological interactions. The obtained results for alcohol and total extract in all variants, as shown in Table 1 are in the same range with control sample. It indicates that the MLF didn't have influence on these parameters.

The obtained results for co-inoculation of malic acid in Figures 1 & 2 show that in all the treatments, the L-malic acid was metabolized into L-lactic acid except in the control sample, where L-malic acid was unchanged. The MLF kinetics show that ML Prime™ was very effective and able to degrade the L-malic acid faster than the other bacteria, followed by PN4™ strain. The other two strains were slightly slower but still very efficient in degrading L-malic acid.

Following the traditional sequential inoculation technique with selected LAB after alcoholic fermentation, kinetics of malic acid degradation had been almost identical between the four investigated LAB strains. Although not recommended for sequential inoculation in red wines, the ML Prime strain start faster than the others, but all LAB strains degraded malic acid within 3 weeks.

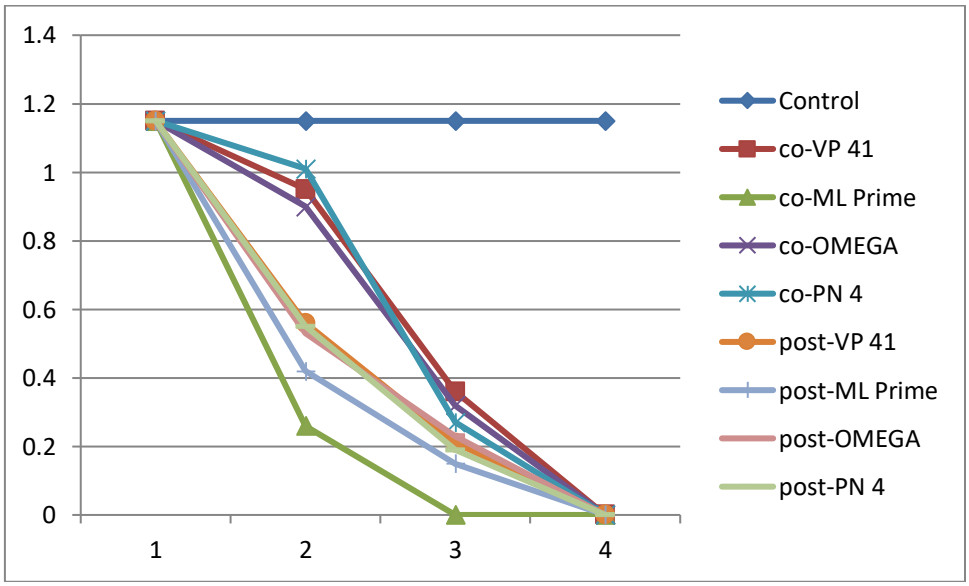

Figure 1. Decrease of L-malic acid with different malolactic strains

Sensory analyses of the wine

The sensory descriptive analysis was performed according to the method of Ubigli (2004). Seven wine experts were involved for the descriptive evaluation of the investigated wines. The panel proposed 11 descriptors for the

final evaluation. All wine samples were evaluated during one tasting session. All the results of the tasting were statistically analyzed.

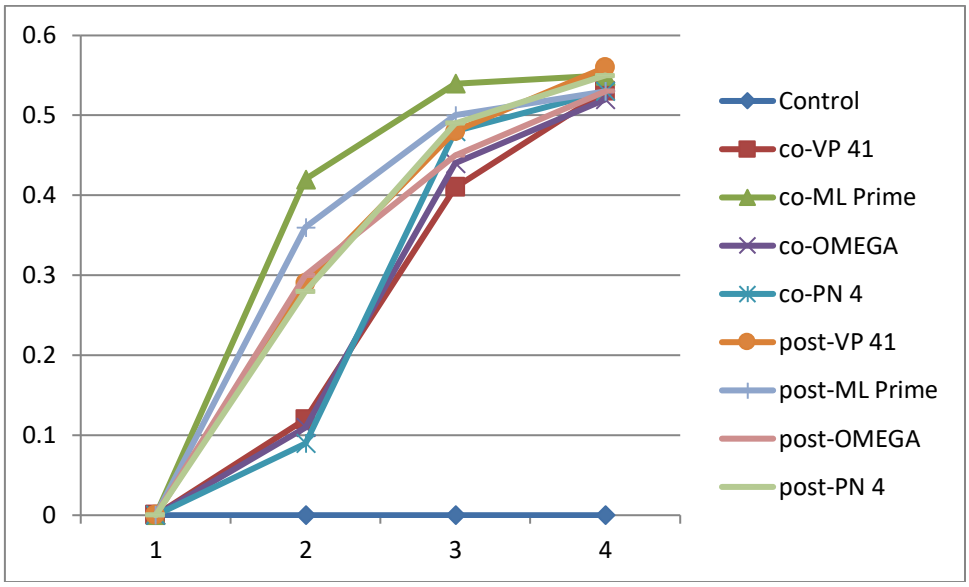

Figure 2. Increase of L-lactic acid with different malolactic strains

The 2017 Vranec wine from Tikvesh wine region were analyzed by sensory panelists (Figures 3 & 4) which highlights how these wines have been shaped during MLF driven by different selected wine bacteria strains. Figure 3 shows the wines, which have been co-inoculated with lactic acid bacteria 24 hours after the yeast inoculation in the grape mash. Positive sensorial impact of the wine lactic acid bacteria strain O-MEGA has been noticed at the co-inoculated sample: the obtained wine had higher body and acidity, more red and black fruit balanced aromas compared to the other samples (Figure 3). It shows the potential of O-MEGA™ for keeping varietal aromas and increasing intensity of the same, as well as providing freshness on the Vranec wine made from high maturity grapes. The results were quite different as opposed to the control wine in which astringency and bitterness were the dominant characteristics. In general, the co-inoculated wines have fruiter sensorial impact on the panelists due to the higher concentrations of fruity esters in the wines, which was observed and from other authors (Abrahamse and Bartowsky, 2012).

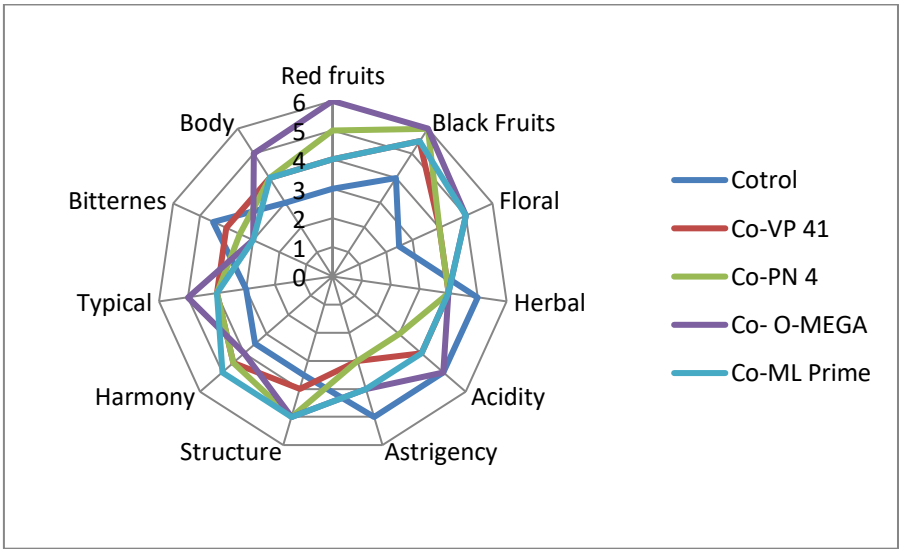

Figure 3. Sensory description of Vranec wine Co-inoculated
with 4 selected wine lactic acid bacteria strains compared to a control wine without MLF

From the sensorial description for sequential inoculation (Figure 4) it can be noticed that the lactic acid bacteria strain PN 4 brings more body, creaminess and more back fruit sensation to the wine. Again the

control wine showed more astringency and bitterness than the wine sequentially inoculated with lactic acid bacteria. Overall the LAB strains showed positive sensory impact on the wines body, structure and harmony and lowered the herbal notes, thus improved the overall aroma of the wines.

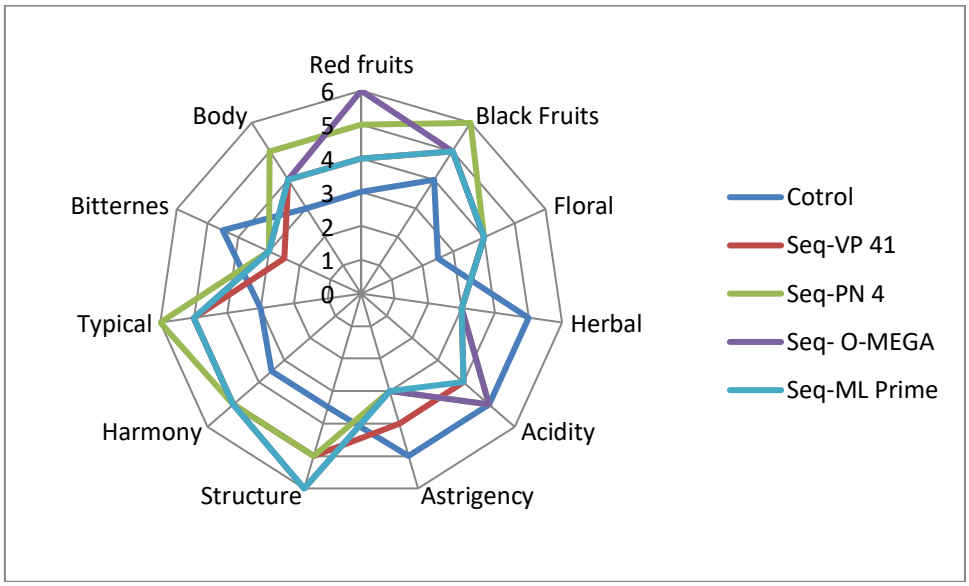

Figure 4. Sensory description of Vranec wine inoculated
with 4 selected wine lactic acid bacteria strains after the alcoholic fermentation (sequentional) compared to a control wine without MLF

CONCLUSIONS

In this investigation the influence of timing of inoculation of four different lactic bacterias by co-inoculation during the fermentation and post-fermentation of Vranec variety was determined. VP 41, ML Prime, O-Mega and PN4 from Lallemand producer are commercially available lactic bacterias, used in malolactic fermentation (MLF) of wine Vranec. The obtained results for Vranec wine from Tikvesh wine region have shown that the use of selected wine LAB strain can assure a fast and completed malolactic fermentation regardless of both MLF inoculation strategy. Co-inoculation resulted in higher aromatic profile, higher fruit intensity and lower herbal notes.

REFERENCES

Abrahamse, C. and Bartowsky, E. 2012. Inoculation for MLF reduces overall vinification time: New research based on laboratory and winery trials in Shiraz delivers potential time and cost benefits. Aust. N.Z. Grapegrower Winemaker 578, 41-46.

Abrahamse, C.E., Bartowsky, E.J., 2012. Timing of malolactic fermentation inoculation in Shiraz grape must and wine: influence on chemical composition. World J. Microbiol. Biotechnol. 28 (1), 255-265.

Bartowsky, E.J. and Henschke, P.A. 2004. The buttery' attribute of wine – diacetyl – desirability, spoilage and beyond. Int J Fd Microbiol 96, 235-252.

Costello P, Francis IL, Bartowsky EJ., 2012. Variations in the effect of malolactic fermentation on the chemical and sensory properties of Cabernet Sauvignon wine: interactive influences of *Oenococcus oeni* strain and wine matrix composition. Aust J Grape Wine R.18, 287–301.

Du Toit, M., Engelbrecht. L., Lerm, E. & Krieger-Weber, S., 2011. *Lactobacillus*: The Next Generation of Malolactic Fermentation Starter Cultures – An Overview. Food Bioprocess Technol.4, 876-906.

Henick-Kling, T, 1994 (2nd ed.) Malolactic fermentation. In Wine Microbiology and Biotechnology. Harwood Academic Publishers, Australia. pp. 289-323.

Henick-Kling, T., 1993. Malolactic Fermentation. Wine Microbiology and Biotechnology. Fleet G.H. ed. Switzerland: Harwood Academic Publishers. pp. 289-326.

Mario Ubigli (2004) Edagricole – Edizioni Agricole de II Sole 24 ORE Edagricole S.r.l.. via Goito, 13-40126 Bologna.

Martineau B, Henick Klining T, 1995. Formation and degradation of diacetyl in wine during alcoholic fermentation with *Sacharomyces cerevisiae* strain EC11 and malolactic fermentation with *Leuconostoc oenos* strain MCW. Am J Enol Vitic 46(4), 442-448.

Sumby K.M., Jiranek V. and Grbin P.R., 2013. Ester synthesis and hydrolysis in an aqueous environment and strain specific changes during malolactic fermentation in wine with *Oenococcus oeni*. Food Chem 141, 1673-1680.

Sumby KM, Grbin PR, Jiranek V, 2012. Characterization of EstCOo8 and EstC34, intracelular esterase, from the wine-associated lactic acid bacteria *Oenococcus oeni* and *Lactobacillus hilgardii*. Journal of Applied Microbiology 114, 413-422.