LACTOBACILLUS PLANTARUM SEQUENTIAL INOCULATION: MALOLACTIC FERMENTATION AND BIOGENIC AMINES OCCURRENCE IN WINE.

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INTRODUCTION

In recent years co-inoculum of yeast and lactic acid bacteria during alcoholic fermentation (AF) has been proposed as a strategy to improve malolactic fermentation (MLF) in wine with hostile chemical composition i.e. high acidity and ethanol. Usually *Oenococcus oeni* strains are used in simultaneous and sequential MLF in must/wine with low pH.

The current climate changes affected grape/must pH value, sugar concentration and as a consequence a higher ethanol content is in wines causing additional problem for MLF and microbial stability of wine.

Recently strains of *Lactobacillus plantarum* have shown good results for their ability to carried out MLF in high pH (3.5÷3.8) must/wine (Fumi *et al*., 2010). Moreover the homo-fermentative character of *L. plantarum* avoids the risk of an excessive acetic acid production as a consequence of the hetero-fermentative sugar metabolism that could occur when *O. oeni* is inoculated in high pH musts.

MLF is an important and critical step for wine quality and safety. Many researches are carried out about the risk of the production of biogenic amines during MLF (Lonvaud-Funel, 2001). Some researcher (Massera *et al*., 2009) showed that no statistical differences, in the biogenic amine level, result between the timings of inoculation in Malbec wine using commercial strains of *Saccharomyces cerevisiae* and *O. oeni*.

In previous studies *L. plantarum* V22 strain, selected at the Università Cattolica del Sacro Cuore of Piacenza, showed a fast and reliable MLF, a positive impact on chemical and sensory characters of wine, as well as no histamine and tyramine production, when used in simultaneous or sequential inoculum in several must/wine variety with high pH (3.5÷3.8).

The aims of this work were:to test the performance of *L. plantarum* V22 strain when inoculated in low pH (pH 3.3) must/wine during and sequentially the AF and to follow the trend of amines from must to wines at bottling after AF/MLF and after one year of storage into the bottle.

MATERIALS AND METHODS

The trials were made using Cornalin, an autochthonous *Vitis vinifera* cultivar of the Valle d'Aosta. The *S. cerevisiae* strain QD145 was used to ferment the must. The LAB *L. plantarum* V22 was selected because of its good capacity to induce MLF and for its inability to produce histamine and tyramine. The use of QD145 and V22 was chosen based on a previous study that showed the good compatibility between these microorganisms in co-inoculation trials (Fumi *et al.*, 2010).

The nutritional supplement added during the trials were: GO-FERM (30 g/hL during yeast rehydratation), FERMAID (30 g/hL at 1/3rd of AF) and Opti'Malo plus (20 g/hL at the end of AF). The grapes were destemmed and crushed. Skins and juice were equally sub-divided into demijohns of 20 L, picked with 40 mg/L total SO_2 and inoculated at different times.

The thesis consist in:
thesis 1 bacteria inoculated in the must and the yeast 24 h later,
thesis 2 bacteria inoculated 24 h after yeast; thesis 3 bacteria inoculated at 30% of AF;
thesis 4 bacteria inoculated at 60% of AF;
thesis 5-bacteria inoculated at end of AF;
thesis 6 control not inoculated with bacteria.

AF and maceration were conducted at the range temperature between 17 and 23℃, with three punching the cap to day. At the end of MLF the wine was cleared, filtered and bottled. Chemical analysis of must and wine regards: reducing sugars, titratable acidity, pH, alcohol, volatile acidity, total and free SO_2, tartaric acid, L-malic acid, L-lactic acid, citric acid (Compendium methods - OIV 2012); biogenic amines as ethanolamine, histamine, ethylamine, tyramine, phenylethylamine, cadaverine (resolution OIV/OENO 346/2009).

RESULTS AND DISCUSSION

The chemical characterization of the must is shown in **Table 1**. The reducing sugar contents were high while the pH value was low.

Table 1 – *Chemical parameters of must. n.d. not detected*

Reducing sugars g/L	217	Ethanolamine mg/L	10.6
Titratable acidity g/L	6.47	Histamine mg/L	0.1
pH	3.27	Ethylamine mg/L	0.5
Tartaric acid g/L	5.7	Tyramine mg/L	n.d.
Malic acid g/L	1.43	Phenylethylamine mg/L	0.9
Citric acid g/L	0.12	Cadaverine mg/L	0.1

The timing of the bacteria inoculation do not affect on AF, the sugar degradation is comparable among the theses (**Figure 1**); the level of acetic acid not showed differences among the theses (**Table 2**).

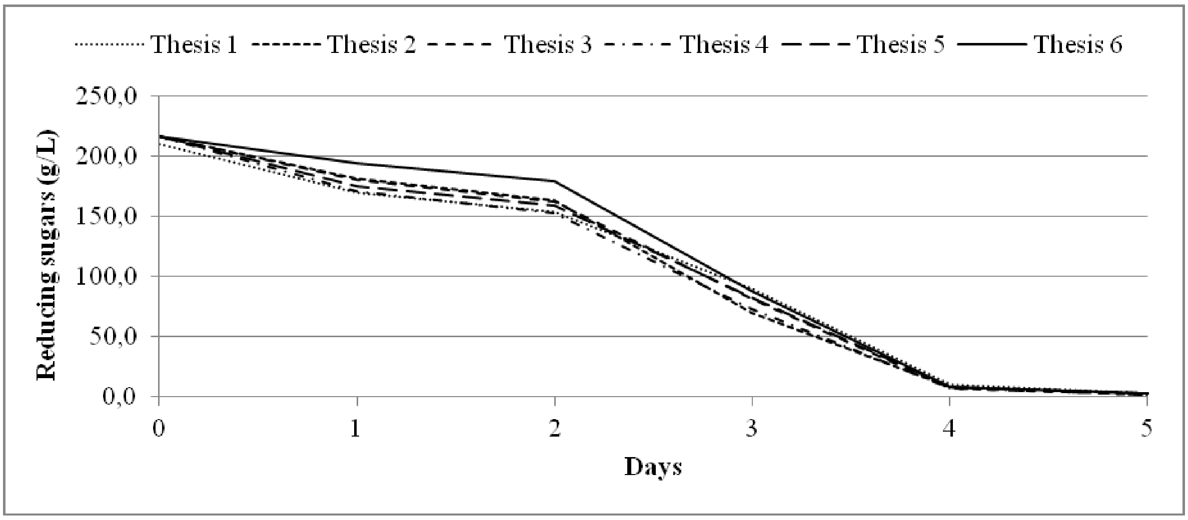

Figure 1 - *AF trends in theses with different co-inoculation and sequential inoculation time*

Malolactic bacteria complete the degradation of the L-malic acid in 15-16 days and there were no substantial differences among the inoculated theses (**Figure 2**). In the control (thesis 6) the malic acid content didn't change during this period.

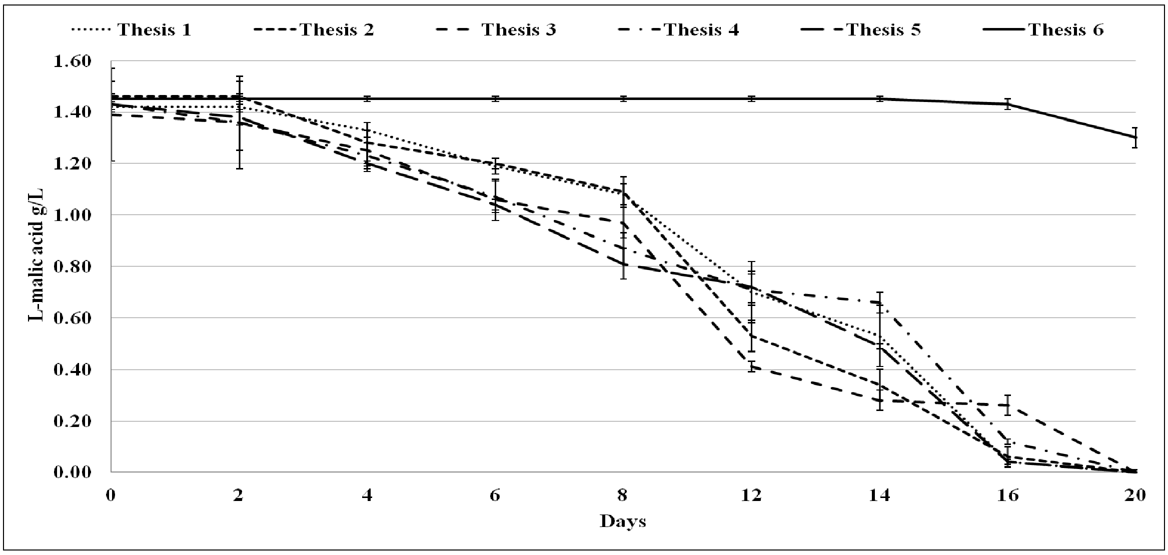

Figure 2 - *Trend of L-malic acid degradation in theses with different co-inoculation and sequential inoculation time*

After one year of aging in bottle histamine, ethylamine and phenylethylamine values are similar in all theses and slightly lower respect to bottling step data, the theses are significantly different in ethanolamine content (**Figure 3**).

	Thesis 1	Thesis 2	Thesis 3	Thesis 4	Thesis 5	Thesis 6
Ethyl alcohol % v/v	12,53 ± 0,01	12,72 ± 0,01	12,67 ± 0,01	12,73 ± 0,01	12,6 ± 0,01	12,32 ± 0,01
Reducing sugars g/L	0,73 ± 0,13	0,79 ± 0,00	0,71 ± 0,33	0,77 ± 0,10	0,68 ± 0,23	0,82 ± 0,05
Total SO_2 mg/L	29,44 ± 0,01	30,08 ± 0,01	30,08 ± 0,01	30,72 ± 0,01	28,8 ± 0,01	26,88 ± 0,01
pH	3,36 ± 0,06	3,4 ± 0,04	3,44 ± 0,01	3,36 ± 0,08	3,43 ± 0,04	3,5 ± 0,01
Titratable acidity g/L	4,22 ± 0,03	3,78 ± 0,21	4,17 ± 0,50	3,83 ± 0,01	4,18 ± 0,43	3,77 ± 0,09
Acetic acid g/L	0,26 ± 0,01	0,29 ± 0,05	0,30 ± 0,06	0,31 ± 0,08	0,41 ± 0,01	0,39 ± 0,15
Malic acid g/L	0,05 ± 0,01	0,09 ± 0,04	0,02 ± 0,03	0,11 ± 0,01	0,03 ± 0,04	0,1 ± 0,05
L-lattic acid g/L	0,89 ± 0,01	0,88 ± 0,00	0,88 ± 0,04	0,87 ± 0,02	0,79 ± 0,00	0,85 ± 0,02
Citric acid g/L	0,15 ± 0,01	0,01 ± 0,01	n.d.	n.d.	0,03 ± 0,02	n.d.
Ethanolamine mg/L	7,70 ± 1,84	8,15 ± 0,35	7,65 ± 0,78	7,35 ± 0,07	8,35 ± 2,76	7,95 ± 0,35
Histamine mg/L	0,20 ± 0,14	0,35 ± 0,07	0,35 ± 0,07	0,30 ± 0,01	0,20 ± 0,14	0,40 ± 0,01
Ethylamine mg/L	1,65 ± 0,64	2,25 ± 0,64	1,95 ± 0,78	2,05 ± 0,78	1,70 ± 0,42	1,65 ± 0,35
Tyramine mg/L	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Phenylethylamine mg/L	1,35 ± 0,21	1,35 ± 0,07	1,25 ± 0,07	1,25 ± 0,07	1,30 ± 0,28	1,35 ± 0,21
Cadaverine mg/L	n.d.	n.d.	n.d.	n.d.	n.d.	0,05 ± 0,07

Table 2 - *Chemical parameters of wine at the end of malolactic fermentation. n.d. = not detected*

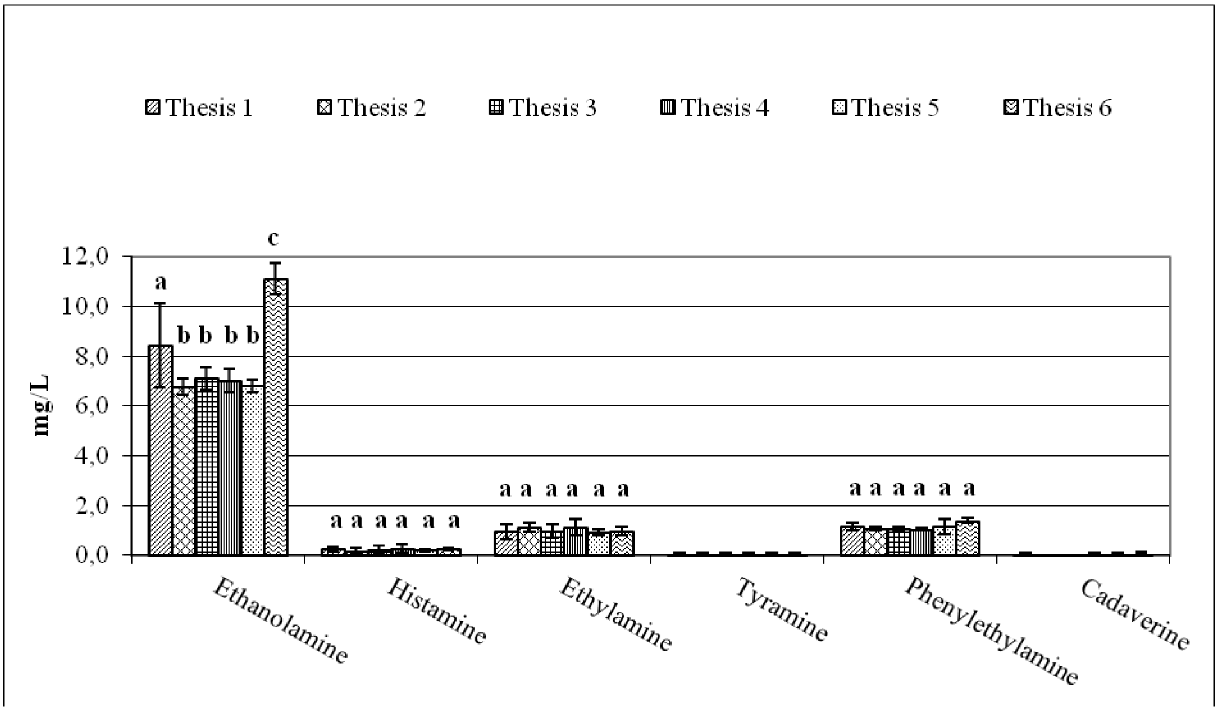

Figure 3 – *Biogenic amines in wine after 1 year in bottle. Error bars: standard deviation. Means followed by the same letter are not significantly different (P≤0.05)*

Conclusions

In conclusion the *L. plantarum* V22 was able to degrade the L-malic acid also to low pH (~3.3), nevertheless the MLF was completed slowly compared to pH>3.5 (Fumi *et al.*, 2010); the amines in wines are not related to *L. plantarum* V22 activity; *L. plantarum* V22 could be used as MLF starter in winemaking because it is able to perform MLF in different conditions of inoculation and with different pH values; moreover, the risk of the presence of unwanted biogenic amines in wine, as histamine and tyramine, is minimized.

Reference

- Fumi M. D., Silva A., Krieger-Weber S., Deleris-Bou M., Du Toit M. (2010). A new generation of malo-lactic starter cultures for high-ph wine. In: Proceedings Symposium "Intervitis Interfructa 2010" - Quality Sustainability Marketing Impact on Innovation. Stuttgart, 24-26 March 2010, 35-44, Stuttgart T:FDW - Association of German Wine-scientists.
- Lonvaud-Funel A. (2001). Biogenic amines in wines: role of lactic acid bacteria. *FEMS Microbiology Letters*, **199**, 9-13.
- Massera A., Soria A., Catania C., Krieger S., Combina M. (2009). Simultaneous inoculation of Malbec (*Vitis vinifera*) musts with yeast and bacteria: effects on fermentation performance, sensory and sanitary attributes of wines. *Food Technology and Biotechnology*, **47** (2), 192-201.