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Preservation of acidity in traditional wine

Application of *L. thermotolerans* at the production of "Vino Santo"

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INTRODUCTION

The production of Vino Santo is traditional in some Italian regions: grapes are dried in ventilated room called "Vinsantaia" for 3 - 4 months, reaching a sugars concentration of 35 – 40%. Spontaneous alcoholic fermentation (AF) take place after grape pressing by a consortium of *Saccharomyces* and *non-Saccharomyces* yeasts that colonizes Caratelli, the traditional little barrels used for this wine. After alcoholic fermentation, Vino Santo ages up to 10 years in barrels in oxidative conditions, because the Caratelli are not filled. The organoleptic equilibrium and the durability of Vino Santo are based on the maintenance of an adequate acidity that leads to regulating the microbial evolution both during grape drying and through the winemaking process. The acidity of "Vino Santo" cannot only be represented by acetic acid accumulated by yeast due to osmotic stress, because the wine would be unbalanced. To obtain a high-quality Vino Santo' it is necessary to maintain a composite acid profile based on the contribution of tartaric and lactic and. In this sense a relevant contribution can be given using *non-Saccharomyces* yeasts capable to convert sugars in organic acids.

L. thermotolerans (LT) is a yeast able to survive up to 13% (v/v) of ethanol and it is able to produce lactic acid from sugars, already during alcoholic fermentation. In condition of oxygen deficiency, typical of alcoholic fermentation, LT activates genes involved in the pentose phosphate pathway and in the citric acid cycle but, in opposition to that occurs in *S. cerevisiae*, is also observed that LT hyperactivates genes encoding for enzymes of the lactate dehydrogenase (LDH) class, allowing the production of lactic acid form hexose sugars. Other interesting aspects of LT activity during AF are the increase of the concentration of glycerol, the decrease in the acetic acid content in wine, and a peculiar modulation of the volatile compounds of the wine.

Until today LT is applied in the production of red wines. In this work we evaluated the use of this yeast in the production of Vino Santo. The evolution of sequential AF made between LT and *S. cerevisiae* was followed by microbiological and chemical determinations, evaluating the aromatic profile of obtained wines. Among the different technological variables, a peculiar attention was paid on the nutrition protocol furnished at the yeasts, considering the need to increasing the knowledge regarding the nutritional requirements of *non-Saccharomyces* yeasts used in oenology.

METHODOLOGY

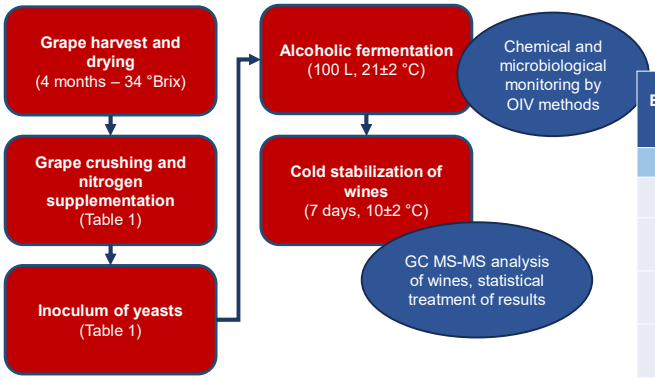

Table 1. Experimental plan performed to evaluate the potential of *L. thermotolerans* in the acidification of Vino Santo during alcoholic fermentation, in function of different nitrogen supplementation. Each trial was performed in 3 replicates. Organic nitrogen: Natuferm Bright, Oenobrands; DAP: diammonium phosphate salt.

Experimental trial	*S. cerevisiae* (PDM, Oenobrands)	*L. thermotolerans* (Laktia, Lallemand)	Nitrogen supplementation (Each dose 300 mg L^{-1})				
			Day 1	Day 5	Day 7	Day 10	Day14
SC	Inoculum at Day 0	NO	Organic N	Organic N	-	-	DAP
LTDAP	Inoculum at Day 5	Inoculum Day 0	DAP	DAP	-	-	DAP
LTNO	Inoculum at Day 5	Inoculum Day 0	Organic N	Organic N	-	-	DAP
LTNO+	Inoculum at Day 5	Inoculum Day 0	Organic N ×3	Organic N ×3	Organic N	Organic N	DAP

RESULTS

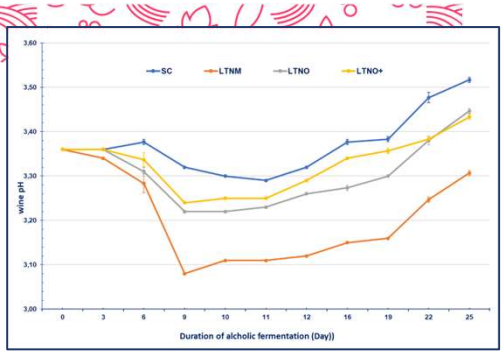

Figure 1. Evolution of pH of Vino Santo during AF (Mean data ± SD, n=3). *L. thermotolerans* (LT trials) resulted in a cellular concentration in wine over the 7 log units for 14 days ensuring the lowering of Vino Santo pH. The best result in terms of pH lowering was obtained by mineral nitrogen supplementation (LTNM trial).

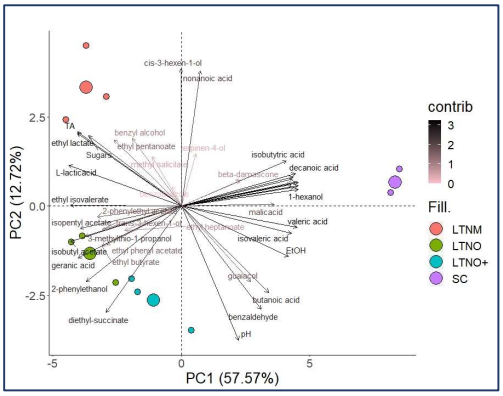

Figure 2. Principal component analysis (PCA) of compositive profile of Vino Santo obtained by different yeast inoculum and nitrogen supplementation protocol. Projection in the plane of first two factors of variables (Compositive data) and cases (Experimental trials). The presence of *L. thermotolerans* (LT) clearly differentiated wines from the control (SC). Also, slight differences were found changing nitrogen supplementation (mineral or organic).

CONCLUSIONS

Early inoculum of *LT, before S. cerevisiae,* ensured the regular evolution of activation of alcoholic fermentation AF. Both yeasts showed good tolerance to the wine limiting factors, maintaining a concentration up to 7 log units for the entire duration of alcoholic fermentation.

Wines produced by the two yeasts showed a higher content of lactic acid (+1,57 ÷ 2,50 respect to the control) that resulted in a pH below 3.50 (**Fig. 1**) . The ethanol accumulation in presence of LT was lower that that of control (- 2% vol/vol), The volatile profile of wines (**Fig. 2**) was influenced both by the yeast specie and the nutrition protocol in LT wines. LT produced a higher concentration of acetate and esters, compared to *S. cerevisiae.*

In conclusion, *L. thermotolerans* showed promising potentiality in restoring an adequate acidic profile in wines made from dried grapes, also giving peculiar character due to the synthesis of specific volatile metabolites. These results are in accordance with previous studies on different wines however, further investigations into the nutrition management of *L. thermotolerans* and volatile metabolites would be interesting as a confirmation of the present work.

ACKNOWLEDGEMENTS

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MORE DETAILS ABOUT THIS RESEARCH ARE PUBLISHED ON OENO ONE

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