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THE MALOLACTIC FERMENTATION CONDUCTION ON RED WINES, USING STARTER CULTURES OF MALOLACTIC BACTERIA

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Abstract

The first selected lactic bacteria appeared at the beginning of the '90's and needed an intense stage of reactivation and acclimation, in the wine in which they followed to "work". The adaptability problem of bacteria to the environment appears to be outdated today, by inventing some bacterial concoctions with direct inoculation, which doesn't need reactivation and who proves to be efficient for most of the wines. The wine composition the alcoholic concentration in SO_2, total acidity, pH), temperature, the modality of preparation and use of the starter cultures, as well as the moment of inoculation, can influence the vitality and the malolactic activity of the lactic bacteria. This paper proposes to verify the efficiency of a commercial concoction of malolactic bacteria, to induce and develop the spontaneous malolactic fermentation, assessing with success in modern wine preparation.
***Keywords**: malolactic fermentation, inoculation, starter cultures, commercial concoction*

Introduction

The malolactic fermentation can be definable as a secondary fermentation leaded by the lactic bacteria, who transform the L-malic acid in L-lactic acid and carbonic anhydride. The "secondary" term refers to the fact that this fermentation starts normally in wines, when the primary fermentation or alcoholic fermentation is finished. The lactic bacteria involved in development of the malolactic fermentation belong to Leuconostoc, Lactobacillus and Pediococcus genders (Popa, 2004).

The malolactic fermentation has three important effects: (Rossi, 1998):

- reduces the wine acidity by transforming the malic acid in lactic acid and CO_2, and, at the same time, determining a slight increase of pH;
- increases the biological stability of the wine, assuring that an unwished malolactic fermentation won't take place in bottled wines;
- modifies the flavor and taste of the wine, increasing its complexity.

The spontaneous malolactic fermentation is unpredictable and can start, only after long periods of delay. This waste of time can represent a considerable cost for the producers. The delays amplify on the other hand, the development risks of contaminated microorganisms that can generate an abnormal taste and smell. These risks are also high, by maintaining the wine in conditions to advantage the growth of contaminated lactic bacteria (high temperatures, little sulphitation, moderate pH).

In order to control the malolactic fermentation, selected lactic bacteria cultures were in train, of whom use, can assure a fast development of the process without difficulties (Hanick-Kling, 1998).

Among the lactic bacteria, the most important species in malolactic fermentation is *Leuconostoc oenos*, who is available in many bacterial solutions.

Still, even at small scale (pilot station), the available bacterial solution cannot be successfully used, but only after a reactivation phase (Lafon-Lafourcade, 1983; Krieger, 1990).

Introduction of lactic bacteria directly in wines makes an important mortality and that is not efficient. The leaven "pied de cuve malo" is an indispensable phase, because the anhydrous bacteria continue their growth in wine and to guarantee sowing success. The more efficient the use of this leaven is, the sooner is developed after the alcoholic fermentation, obtaining an energy economy, in so far as the wines keep a temperature between 16-20ºC (Valade, 1995, Gheorghita, 2006).

Experimental

In this paper we experimentally evaluate the efficiency of the commercial concoction INOFLORE R (which contains the *Leuconostoc oenos* species), for introduction and development of the malolactic fermentation in Oporto, Burgund, Cadarca, Pinot Noir and Merlot wines, obtained from Minis-Maderat vineyard, harvest 2006.

The commercial concoction INOFLORE R has biotechnological properties as follows:

- the bacterial population size $2 \cdot 10^{11}$ UFC/g powder;
- minimal quality keep: 12 months at 4ºC, 18 months at -18ºC;
- optimal inoculation temperature 18-22ºC;
- pH tolerance 3.2 for an inferior pH;
- resistance to SO_2: free < 10 mg/l, total < 60 mg/l;
- ethanol tolerance, maximum concentration 14% vol.

Albeit the concoction is MBR type (capable of direct sowing) 3 work ways were tested: direct inoculation (without reactivation), wine reactivation and must reactivation. In reactivation on wine, the inoculation of concoction INOFLORE R is made after the scheme from figure 1.

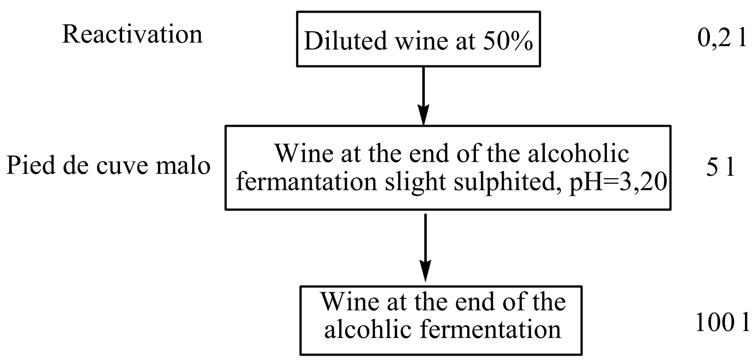

Figure 1. The simplified scheme of preparation of "pied de cuve malo" with reactivation on wine (Valade, 1994)

The INOFLORE R concoction inoculation, in reactivation on must is made in conformity with scheme from figure 2.

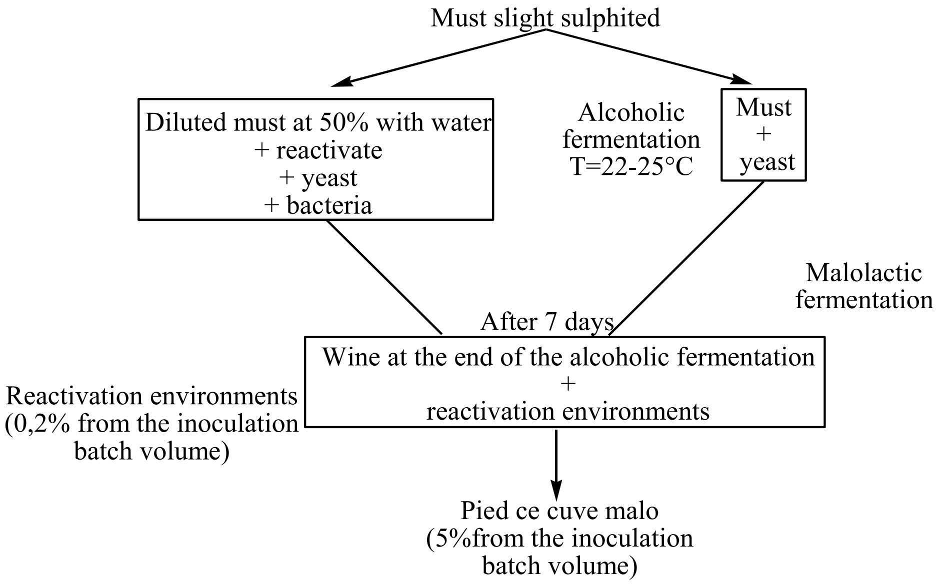

Figure 2. The simplified scheme of preparation of "a pied de cuve malo" with reactivation on must (Valade, 1994)

Results and Discussions

Before the malolactic initiation, the main physico-chemical properties of musts and wines malolactic fermented were determined (table 1). The must determination viewed: sugars concentration, total acidity, pH, malic and lactic acids concentrations, free and total SO_2. The wines determination viewed: alcohol concentration, sugars concentration, total and volatile acidity, pH, malic and lactic acids concentrations, free and total SO_2. The same physico-chemical properties were determined on wines after the malolactic fermentation.

The physico-chemical characteristics of the wines presented in table 2 (alcoholic concentration, total and volatile acidity, pH) do not satisfy the nutritional requisitions that must have an optimal culture environment for the growth and development of lactic bacteria. On the other hand, the musts, by their chemical composition, represent a propitious environment for the growth and development for the lactic bacteria. Still, the content in malic acid and total acidity is higher in musts, comparative to wines. Also, the content in lactic acid in musts is totally missing, while in wines, the contents in lactic acid vary between 0.2-0.4 g/l. This is accountable, by the fact that the malolactic fermentation starts at the end of the alcoholic fermentation before obtaining the wine.

Table 1. Physico-chemical characteristics of musts before the INOFLORE R concoction inoculation (reactivation on must method)

Types of must	Sugars	Total acidity g/l	pH	Malic acid	Lactic acid	Free SO_2 mg/l	Total SO_2 mg/l
Oporto	207	3.33	3.40	1.9	trace	7	28
Burgund	207	5.48	3.15	3.1	trace	4	27
Pinot noir	205	4.45	3.28	2	trace	5	28
Merlot	208	5.48	3.15	2.5	trace	7	25
Cadarca	178	6.37	2.89	4.2	trace	5	28

Table 2. Physico-chemical characteristics of the wines before the INOFLORE R concoction inoculation

Types of wines	Alco-hol % vol	Sugars g\l	Total acidit y g/l	Volatile acidity g/l	pH	Malic acid g/l	Lactic acid g/l	Free SO_2 mg/l	Total SO_2 mg/l
Oporto	12.1	1.27	3.21	0.28	3.4	1.5	0.34	7	28
Burgund	11.3	1.04	5.25	0.29	3.2	2.7	0.4	4	27
Pinot noir	11.5	1.05	4.40	0.29	3.3	1.5	0.28	7	25
Merlot	12.7	1.04	5.35	0.29	3.2	2	0.3	4	25
Cadarca	10.9	1.04	6.28	0.35	2.9	4.2	trace	5	28

From the data presented in table 3, it is noticed that the Cadarca wine did not have a malolactic fermentation, with the help of the lactic bacteria from inner flora, probably because of a higher total acidity (6 g/l) and because of a too low pH (2.9), By using the INOFLORE R concoction (which contains the Leuconostoc oenos species), a total degradation of the malic acid takes place, concomitantly with the lactic acid accumulation. Albeit, according to the biotechnological characteristics, the INOFLORE R concoction, has as minimum pH tolerance the value of 3.2, he succeeds he starts the malolactic fermentation and to finish it at the value of 2.9.

Table 3. The physico-chemical characteristics of wines after the spontaneous malolactic fermentation

Types of wines	Alco-hol % vol	Sugars g\l	Total acidity g/l	Volatil acidity g/l	pH	Malic acid g/l	Lactic acid g/l	Free SO_2 mg/l	Total SO_2 mg/l
Oporto	12.0	1.27	3.15	0.65	3.5	trace	1,5	7	28
Burgund	11.3	1.04	4.8	0.55	3.3	trace	2	4	27
Pinot noir	11.5	1.05	4.05	0.47	3.3	trace	1,5	5	28
Merlot	12.7	1.04	4.9	0.41	3.3	trace	2	7	25
Cadarca	10.9	1.04	6.0	0.45	2.9	4,0	trace	5	28

The values of the volatile acidity are higher with 0.2-0.3 g/l in case of spontaneous malolactic fermentation, comparative with the conducted malolactic fermentation (table 4). Even in case of conducted malolactic fermentation, there are small differences between the values of the total acidity, dependent on the modality of work: direct inoculation (without inoculation), reactivation on wine and reactivation on must. The higher values of the volatile acidity are recorded in case of direct inoculation of the INFLORE R concoction, nigh values by those recorded in case of spontaneous malolactic fermentation. The lowest values of the volatile acidity are recorded in case of must reactivation of the INFLORE R concoction.

Table 4. The physico-chemical characteristics of wines after the conducted malolactic fermentation

Types of wines	Alco-hol % vol	Sugars g\l	Total acidity g/l	Volatil acidity g/l	pH	Malic acid g/l	Lactic acid g/l	Free SO_2 mg/l	Total SO_2 mg/l
Oporto	12.1	1.07	3.15	0.51	3.5	trace	1,5	7	28
Burgund	11.3	1.04	4.6	0.43	3.3	trace	2	4	27
Pinot noir	11.5	1.05	3.9	0.4	3.3	trace	1,5	7	25
Merlot	12.8	1.03	4.6	0.4	3.3	trace	2	7	25
Cadarca	11.0	1.03	4.8	0.55	3.2	trace	3,5	5	28

Comparative to wine sowing, the sowing technique of musts, present some advantages: simplification of the reactivation protocol by decreasing the inoculate volume; an absolute adaptation of the lactic bacteria, seeing the lack of alcohol from the environment; standardization of the inoculation moment; low values of the volatile acidity and the assurance that undesirable compounds are not obtained (biogenic amines, biacetyl, acetoine).

In what concerns the duration of the malolactic fermentation, we have to mention that the Oporto and Pinot noir wines malolacticly yeast faster comparative with the Burgund and Merlot wines. The conducted malolactic fermentation is developing much faster, comparative with the spontaneous malolactic fermentation. Even within the conducted malolactic fermentation, there are differences dependent on the modality of work: direct inoculation (without reactivation), reactivation on wine and reactivation on must. The lower duration takes place in case of reactivation on must, and the higher duration is registered in case of direct inoculation (without reactivation). According to physico-chemical characteristics of wines, the duration of the conducted malolactic fermentation vary between 5 and 18 days, being dependent on the reactivation version of the bacterial concoction, while in case of spontaneous malolactic fermentation (with inner flora), the duration extends with 20-40 days (figure 3). It is worthy of note that the Cadarca wine does not succeed to malolacticly yeast in "spontaneous" conditions, not even in 120 days, because of a markedly low pH (2.9) for initiating the malolactic fermentation.

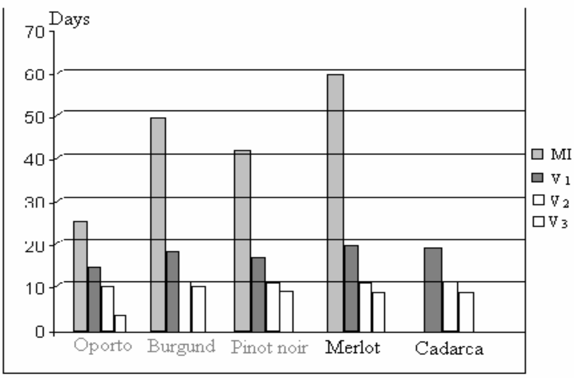

Figure 3. The influence of the bacterial sowing on the malolactic fermentation duration.

MI - indigene micro flora; V_1 - direct inoculation variant (without reactivation); V_2 - reactivation on wine variant; V_3 -reactivation on must variant.

Conclusions

The wine composition (alcoholic concentration, SO_2 concentration, total acidity, pH), the way of preparation and use of bacterial cultures, as well as the inoculation moment, influence the start and duration of the malolactic fermentation. The lactic bacteria sowed in must, gave promising results (benefit of time of approximately 7 days in proportion to wine sowing and approximately 30 days in proportion to unsowed samples). The conducted malolactic fermentation by sowings of selected lactic bacteria, present noticeable advantages, comparative with the spontaneous malolactic fermentation, enjoined in modern vivification, as: reduces the wine acidity, in a short time, having a superior degradation kinetic of the malic acid; controls the detrimental bacteria from wine, barricading the wine morbidity and contributing to increasing its biological stability; allows the limitation of the indesirable characters of the wine, given by the volatile compounds, reduces aroma substances, biogenic amines; improves the organoleptic profile of the wine, increasing its complexity by attributing some lubricity and fructose flavors.

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