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RESEARCH REGARDING THE POSSIBILITIES OF STARTING AND CONTROLLING OF MLF IN RED WINES FROM CERNAVODA VITI-VINICOL CENTRE SITUATED IN MURFATLAR VINEYARD

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Abstract

The malolactic fermentation (MLF) is a biologically desacidification process of the wines especially red wines, made by lactic bacteria that convert malic acid (more aggressive for the taste buds) into lactic acid (pleasant taste buds impress) and CO_2. Between 2009-2011, were made studies reffering to the possibilities of starting and controlling of malolactic fermentation (MLF) in the red wines from Cernavoda Viti-Vinicol Centre, Murfatlar vineyard, as well as on the conditions of this biochemical process. The degradation of malic acid has three effects upon the wines: reduced acidity, microbial stabilisation and a some modification of the organoleptic properties. In the same time we noticed that through malolactic fermentation, the red wines winning in quality. The starting of MLF can be easy initiated, if for this are used wines in full MLF. For induce MLF is necessary an initial titre between $3x10^4$ till $5,0\ x10^4$ cells/mm^3. After inoculation the bacteria cells passed through an adaptation period because their functions were limited by the alcohol content and pH of the wine, and therefore in the medium remain a bacteria number of $1x10^4$ cells/mm^3. For MLF we can recommend some strains of lactic bacteria, isolated from vineyard microflora.

Key words: *malic acid; lactic acid; organoleptic properties, bacteria strain; inoculation.*

INTRODUCTION

In grapes, must and wine, malic acid there is in a big quantity like acid L (-)-malic. During grapes transformation proccess, malic acid and its salts reach in must (Cotea D.V. et al., 2005, 2009). Many researchers have studied the evolution of malic acid during maturation of grapes, alcoholic fermentation, malolactic fermentation and maturation of wines from vessel (Blouin J. et al., 2003). In the process of fermentation and wine storage period, the acidity decreases continuously through:

-the natural insolubilisation and precipitation of potassium hydrogen tartrate, under the influence of alcohol and low winter temperatures;

-through biological degradation of malic acid in must and wine by yeasts and malolactic bacteria (Târdea C., 2007). In the most cases malic acid from the wines is reduced by the biological way through MLF. Malolactic bacteria completely metabolize malic acid from the must and wine, with the formation of lactic acid and CO_2, according to the reaction: $HOOC\text{-}CH_2\text{-}CHOH$+malolactic bacteria -> $CH_3\text{-}CHOH\text{-}COOH + CO_2$. This proccess is important for the wines quality, having in view the effect upon the wines: reduced acidity, microbial stabilisation and an organoleptic properties modification (Baduca-Câmpeanu et al., 2008; Kontek A. et al., 1977). By this study are established the ways for starting and controlling of malolactic fermentation in the red wines from Cernavoda viti-vinicol Centre situated in Murfatlar vineyard.

MATERIALS AND METHODS

The researches were made in SC VINEX MURFATLAR SRL, a private Viti-Vinicol Company, situated in Cernavoda Viti-Vinicol Centre from Murfatlar vineyard, between 2009-2011, having in view:

- spontan starting of malolactic fermentation;
- using wine in full malolactic fermentation;
- using bacterial concentrate from spontaneous microflora;

- using selected bacteria from Leuconostoc oenos specie, for malolactic fermentation starting (Prahl C. et al.,1995).
The spontaneous starting of malolactic fermentation was followed in the vessels with 1000 and 10.000 liters capacity. For to obtain the wines with a big malolactic bacteria density, were used wines produced by carbonic maceration proccess.
Strains of malolactic bacteria used in researchs (10 strains) for inoculation, were selected in laboratories of SC VINEX MURFATLAR SRL by specialized personnel, from red wines with low pH and with the finished spontaneous malolactic fermentation. For lactic bacteria's study were used the techniques and the medium, indicated by Peynaud and Domerq (1959), and for their classification was used Bergey's Manual of Determinative Bacteriology-Ninth Edition (2004).
The bacterial concentrate was produced from Merlot wine in full malolactic fermentation, by the tangential microfiltration method.
The use of bacterial concentrate of spontaneous microflora is recommended for the starting and controlling of MLF in red wines.
The evolution of total number of lactic bacteria was made by counting on Thoma mount, and the number of propagation cells, by filter membrane.
The evolution of lactic bacteria in red wines of Cernavoda Viti-Vinicol Centre was put in evidence by chromatography on paper (the Kunkee method – 1968), and by the evolution of the total number of malolactic bacteria in wine.

RESULTS AND DISCUSSIONS

I. The spontaneous starting of malolactic fermentation in the SC VINEX MURFATLAR Cernavoda red wines, is depending of SO_2 quantity from the wines and of the tank size. In the red wines with pH-3,4 produced by maceration in big tank the degradation of malic acid is beginning early, without influence of free SO_2 doses.
In the red wines produced in small tank capacity (1000 liters) at 18^o temperature, the necessary time for malolactic fermentation starting is between 10-50 days, in this case being influenced of wine SO_2 free content.
The starting of malic acid degradation is produce when in the wine mass there is a bacteria density of $3x10^4 – 4.2x10^4$ cells/mm^3, degradation time for 1.4 g/l malic acid being between 7-8 days.
It has been found that the starting of the metabolisation of malic acid coincides with the moment of acquiring in the wine mass of a bacterial density of 4.2 x 10^4cells/mm^3 and at the end of the metabolisation of malic acid the bacterial density is $7.1x10^4$ cells/mm^3 (Figure 1).

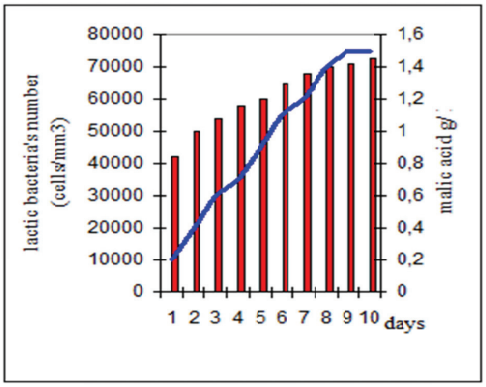

Figure 1. The speed of malic acid degradation and bacteria's number evolution

II. The research found that at the end of alcoholic fermentation in red wines obtained by carbonic maceration process was only half malic acid metabolized. When these wine storage conditions (temperature and SO_2) are adequate these wines completes its malolactic fermentation in 5 days. Blending these wines with other wines in which the malolactic fermentation is desired, is a good solution. The research has shown that if the wine-yeast quantity is higher, the metabolising time of malic acid decreases. Using a proportion of 10% wine-yeasts was achived in the wine inoculated a titre of $5.0x10^4$ cells/mm^3 of lactic bacteria (Figure 2).
From the figure 2, it can see that, after inoculation, malolactic bacteria went through a period of adjustment when their number decreased till $3.8x10^4$ cells/ mm^3 and then increased reaching $8.0x10^4$ cells/mm^3.
III. The using of concentrate bacterial from spontaneous microflora is another way for starting malolactic fermentation proccess.
This concentrate bacterial was obtained by tangential microflitration method from an unsulphitated but malolactic fermented young

wine of Merlot type, the characteristics of which are shown in table 1.

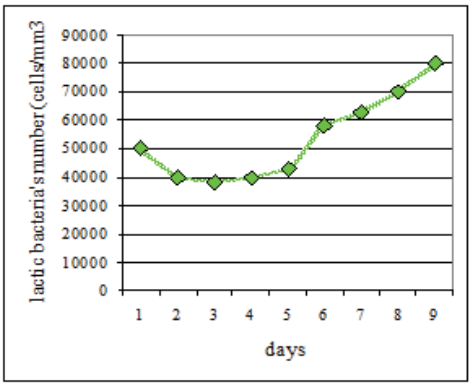

Figure 2. Lactic bacteria number evolution in the wines inoculated with 10% wine-yeasts in full MLF

Table 1. The phisico-chemical and microbiological characteristics of the malolactic fermented Merlot

Type of wine	Wine characteristics		
Merlot	Alcohol% vol		11.7
	Sugars g/l		5.4
	Tot. ac. g/l H2SO4		4.20
	Volatile acidity g/l CH3COOH		0.38
	SO2	total	28.2
		free	0.0
	pH		3.30
	Bacteria no./mm^3		$7.0x10^4$

From the table 1, we can show that at the end of metabolized period of malic acid, the bacterial density was $7,0x10^4$ cells/mm^3. In table 2, are shown the results of the microbiological tests, content of anthocyans and total polyphenols in the concentrate bacterial and in the filtrate obtained.

The lactic bacteria number from the concentrate bacterial was $2,1x10^6$ cells/mm^3, while the filtrate was sterile. The content of anthocyans and total polyphenols were greater values in the filtrate than in the concentrate bacterial.

Table 2. The microbiological and phisico-chemical characteristics of the concentrate bacterial and the filtrate obtained from Merlot wine

	Number of lactic bacteria /mm^3	Anthocianins mg/l	PFT g/l
Concentrate bacterial	$2.1x\ 10^6$	210.1	1850
Filtrate	0	320.0	1990

IV. In our researchs, we selected 10 strains of lactic bacteria from young red wines (Pinot noir, Merlot, Cabernet sauvignon, Feteasca neagra, Blawer). In the selection process were had in view criteria that they must have, namely: to possess the capability to increase at low pH, to grow in the presence of alcohol, to produce low amounts of volatile acids, do not degrade certain compounds in wine, as pentoze, glycerol, tartaric acid, etc. Of the 10 strains isolated by us were tested morphologically, physiologically and oenological point of view, it noticed that strains registered with code 1, 2, 5, 7, belonging to the species Leuconostoc oenos, the codes 3 and 6 the species Leuconostoc gracile, and the codes 4, 8 and 10, to Pediococcus cerevisiae species. For verification in production conditions were chosen strains 1, 2 and 5 of the species Leuconostoc oenos. In our experiments, the starting of malolactic fermentation took place when the amount of inoculum was $2.5x10^4$ cells/mm^3 on condition that at least 1.2×10^4 cells/mm can form colonies. After inoculation of lactic bacteria in the wine, the bacteria passed through an adaptation period because their function was limited by the alcohol content and pH of the wine. Three selected bacteria strains verified in wines, has realised the malic acid degradation in 5-7 days. Volatile acidity of the wines have had different growing-up, the smaller was in the sample inoculated with the strain code 2 (0.08 g/l CH_3COOH), and the biggest at the sample inoculated with strain code 1 (0.16 g/l CH_3COOH) (Table 3).

Table 3. The changes in the wine composition, inoculated with different bacteria strains after malolactic fermentation

The strain code	Free SO_2 mg/l	Acetaldehyde mg/l	Totale acidity g/l H_2SO_4	pH	Vol acidity g/l CH_3COOH	Increase of vol. acidity with:
Initial wine	12,2	16,1	4,00	3,60	0,34	-
Malolactic bacteria Code 1	10,5	12,6	3,42	3,67	0,50	0,16
Malolactic bacteria Code 2	10,5	15,8	3,20	3,59	0,42	0,08
Malolactic bacteria Code 5	11,1	29,9	3,30	3,70	3,45	0,11

To organoleptic analysis of the wines obtained, shown there are differences between samples: sample where MLF was carried out with bacteria strain under code 2, was balanced, harmonious, soft and velvety, compared with samples obtained with bacteria strains under code 1 and code 5 which were nice but not with the same harmony. Therefore bacteria under code 2, has been recommended for use in the production process in SC VINEX MURFATLAR SRL from Cernavoda Viti-Vinicol Centre.

CONCLUSIONS

In the red wines obtained by maceration process in tanks of big capacity, the starting of malolactic fermentation was spontaneous produced at the end of alcoholic fermentation. In the red wines produced in small tanks, the malolactic fermentation was later starting, due of SO_2 high-up level;

The quantity of inoculum used for initiation of MLF was $3x10^4 - 4{,}2x10^4$ cells/mm^3;

In production conditions the best results given the Leuconostoc oenos strain 2, which was recommended in production process.

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