EXPERIMENTAL ASPECTS REGARDING THE MALOLACTIC FERMENTATION USING THE FREEZE-DRIED CULTURE OF *OENOCOCCUS OENI* FOR RED WINES

ASPECTE EXPERIMENTALE PRIVIND FOLOSIREA UNUI PREPARAT DE *OENOCOCCUS OENI* LA FERMENTAŢIA MALOLACTICĂ A UNOR VINURI ROŞII

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Abstract. *This paper presents some typical aspects of the malolactic fermentation process, respectively, the variation of total acidity, real acidity (pH) and volatile acidity, malic acid metabolization and formation of lactic acid. Also, other composition characteristics like tartaric and citric acids, potassium, calcium, reducing sugars and phenolic compounds were studied. The experiment was conducted both in laboratory conditions as well as in industrial conditions, on Feteasca neagra and Cabernet sauvignon wines. In order to start the malolactic fermentation, after 7-15 from the end of alcoholic fermentation, a freeze-dried culture of Oenococcus oeni (commercialized under the name of FD-DVS Viniflora CH11) were inoculated directly into wine. In all samples except the control sample we found a decrease of total acidity and malic acid content, correlated to an increase of the pH and lactic acid content. Regarding the potassium and calcium cations, there was a decrease. Also, the content of phenolic compounds, showed differences from the control sample.*
Key words: red wines, composition characteristics, malolactic fermentation, *Oenococus oeni* FD-DVS Viniflora CH11.

Rezumat. *Lucrarea prezintă o serie de aspecte caracteristice procesului de fermentaţie malolactică, respectiv modificarea aciditătii totale, a aciditătii reale (pH) şi volatile, precum şi metabolizarea acidului malic şi formarea acidului lactic. Totodată, s-a urmărit şi modificarea unor caracteristici de compoziţie, precum acizii tartric şi citric, cationii de potasiu şi calciu, respectiv zaharuri reducătoare şi compuşi fenolici. Experimentele s-au realizat atât în condiţii de laborator cât şi în condiţii industriale, pe vinuri obţinute din soiurile Feteasca neagră şi Cabernet Sauvignon. Pentru a declanşa fermentaţia malolactică, la 7-15 zile după sfârşitul fermentaţiei alcoolice, vinurile au fost însămânţate cu preparatul pe baza de Oenococcus oeni comercializat sub denumirea de FD-DVS Viniflora CH11, administrat sub formă de granule, direct în vin. La toate probele analizate, cu excepţia probei martor, s-a*

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constatat o scădere diferențiată a concentrației acidului malic, corelată cu creşterea conținutului de acid lactic. Realizarea fermentației malolactice a fost concretizată şi prin scăderea pronunțată a acidității totale şi creşterea valorii pH-ului. În ce priveşte cationii de potasiu şi calciu, s-a constatat o diminuare a acestora. De asemenea, conținutul de compuşi fenolici a înregistrat diferențe față de proba martor.

Cuvinte cheie: vinuri roşii, caracteristici de compoziție, fermentație malolactică, preparat FD-DVS Viniflora CH11.

INTRODUCTION

Malolactic fermentation is a beneficial biological process, especially for red wines, for the following reasons: it leads to convenient deacidification, which gives the red wines qualities of suppleness, diminishing their astringency, often intensifies the color of the wines, although there are decreases of content in anthocyanins and tannins; provides biological stability to lactic bacteria (Cotea, 1985; Delfini, 1995; Vodošek Vrščaj *et al.,* 2008).

In a previous paper (Odăgeriu *et al.,* 2009), aspects of malolactic fermentation of red and white wines, the variation of some compositional characteristics and physical-chemical indices, depending on the modification of their total and actual acidity, were presented. Continuing the previous research, the present paper presents a series of new aspects characteristic of the malolactic fermentation process of some red wines, explicitly the use of a lactic bacteria preparation which allowed to be added directly in to the wine in the form of grains, much more accessible to the oenologist in its preoccupations to initiate this biochemical process. At the same time, changes in total acidity, volatile and real (pH), metabolism of malic acid and lactic acid formation, as well as modification of other compositional characteristics (tartaric and citric, potassium, calcium) during the studied process were followed.

MATERIAL AND METHOD

The experiments were carried out both under laboratory and industrial conditions, between October and December 2016, on wines obtained from the Fetească Neagră and Cabernet Sauvignon varieties cultivated in the Bohotin zone of the Husi vineyard. Thus, the above mentioned wines were obtained from the grapes harvested in the 2016 at the S.C. Agroindustriala Bucium Iaşi Winery and were analyzed in the laboratory of the Research Center for Oenology Iaşi.

To activate the malolactic fermentation, 7-15 days after the end of the alcoholic fermentation, the wines were seeded with the FD-DVS Viniflora® CH11 (a pure lyophilized culture of Oenococcus oeni). This is a heterofermentative malolactic bacterium that ensures rapid and safe malolactic fermentation after inoculation directly into the wine. It is especially suited for inoculation of low pH and high levels of alcohol and does not produce biogenic amines (histamine, thyramine, putrescine, phenylethylamine, isoamilamine, cadaverine) as opposed to indigenous bacteria of wine.

The wine samples from the two studied varieties consisted of several experimental variants. For the Fetească neagră variety, three variants were

differentiated between them as follows: V1 divided into V1.1. (the initial control wine), i.e. wine (1000 L) before malolactic fermentation and V1.2. (final) representing the wine (1000 L) seeded with malolactic bacteria at the end of malolactic fermentation; V2 divided into V2.1. (initially), i.e. wine (1000 L) prior to malolactic fermentation, and previously malic acid at 1 g / L and V2.2 was added. (final) wine sample (1000 L) similar to V2.1. (seeded with malolactic bacteria) at the end of malolactic fermentation; V3 divided into V3.1. (initially), i.e., wine (500 L) before malolactic fermentation, and previously malic acid was added at 2 g / L and V3.2. (final) similar to V3.1. i.e. wine (500 L) seeded with malolactic bacteria at the end of malolactic fermentation.

The wine samples obtained from the Cabernet Sauvignon variety, three other variants were differentiated between them as follows: V4 divided into V4.1. (the initial control wine), i.e. wine (1000 L) before malolactic fermentation and V4.2. (final) representing wine (1000 L) seeded with malolactic bacteria at the end of malolactic fermentation; V5 divided into V5.1. (initially), i.e. wine (1000 L) before malolactic fermentation and in which malic acid was previously added at a dose of 1 g / L and V5.2. (final) wine (1000 L) similar to V5.1. (seeded with malolactic bacteria) at the end of malolactic fermentation; V6 divided into V3.1. (initially), i.e. wine (500 L) before malolactic fermentation, and previously malic acid was added at a dose of 2 g / L and V6.2. (final) similar to V6.1. i.e. wine (500 L) seeded with malolactic bacteria at the end of malolactic fermentation.

The addition of malic acid was conditioned by the fact that wines from the 2 varieties with a content of about 2.10 g / L (but which required strictly malolactic fermentation) did not cover a larger range of malic acid (3-4 g / L) needed to verify the specific predilection of the bacteria studied for this acid.

Thus, the content of a packet (for 25 hL) for the wines of each variety was given as granules directly into the wine (initially in 5 L of wine and then 2.0 L of it in 1000 L and 1.0 L respectively in 500 L vessels).

At the start and the end of the malolactic fermentation process, the samples were collected for each distinct wine. Thus, from samples of the Fetească Neagră variety, the samples were: V1.1, V1.2, V2.1, V2.2, V3.1, V3.2, and samples from the Cabernet Sauvignon wine were: V4 .1, V4.2, V5.1, V5.2, V6.1, V6.2. The wine samples were stored in a area where the temperature ranged between 14 and 18 ° C. These were mixed by hand 4-5 times a day.

At the end of the malolactic fermentation, when it was found that the expected quantities of malic acid had been reached, the bacterial activity of the wines was stopped by removing from the deposit and treating them with sulfur dioxide and gelatin, with established doses based on laboratory microprobes.

For each sample taken after filtration and decarbonation, the physico-chemical analyzes were performed. Analyzes of the main compositional characteristics (total acidity, volatile acidity, pH, tartaric, malic, lactic, citric, free and total sulfur dioxide, potassium, calcium, total phenolic compounds, reducing sugars) during and after the malolactic fermentation acquired between October and December 2016. These were done according to the current standard methods (*** 2012; *** 2015) and the specialized literature (Bauer *et al.,* 2004; Bartowsky, 2005; Croitoru, 2005; Flanzy, 1998; Lepădatu *et al.,* 1975; Odăgeriu *et al.,* 2008; Ribereau-Gayon *et al.,* 1972; Ţârdea, 2007; Vodošek Vrščaj *et al.,* 2008; Würdig and Woller, 1989).

RESULTS AND DISCUSSIONS

The main compositional characteristics of the tested wines (eight samples per wine) are shown in tables 1 and 2.

Thus, the alcoholic strength (expressed in % vol.) of the control wines studied had the following values, between 12.85 and 12.95 for Fetească Neagră and between 13.42 and 13.50 for Cabernet Sauvignon.

Other values of the control samples, less modified, refers to succinic acid (which had values from 0.58 and 0.67 g / L respectively) and free (total) sulfur dioxide content, which was between 2.9 (39.1) and 8.3 (49.5) mg / L.

The main compositional characteristics (total and volatile acidity, pH, malic, lactic, citric acids) involved in specific malolactic fermentation processes depending on the type of wine, are presented in the abovementioned tables.

Table 1

Variation of main compositional characteristics during malolactic fermentation - Fetească neagră

Element/ Parameters	U.M.	Sample 1		Sample 2		Sample 3	
		initially sample $V_{1.1}$	final sample $V_{1.2}$	initially sample $V_{2.1}$	final sample $V_{2.2}$	initially sample $V_{3.1}$	final sample $V_{3.2}$
Alcohol	% vol.	12.85	12.95	12.85	12.90	12.85	12.85
TA	g/L $C_4H_6O_6$	**6.40**	**5.28**	**7.55**	**5.63**	**8.55**	**5.93**
	δ_r (%)	0.00	- 17.50	0.00	- 25.43	0.00	- 30.48
VA	g/L $C_2H_4O_2$	**0.46**	**0.62**	**0.49**	**0.65**	**0.48**	**0.69**
pH		**3.58**	**3.80**	**3.37**	**3.81**	**3.23**	**3.78**
	δ_r (%)	0.00	6.11	0.00	13.01	0.00	17.16
Malic acid	g/L	**2.18**	**0.34**	**3.08**	**0.43**	**3.91**	**0.48**
	δ_r (%)	0.00	- 84.40	0.00	- 86.04	0.00	- 87.72
Lactic acid	g/L	**0.46**	**1.75**	**0.43**	**2.14**	**0.44**	**2.43**
	$\delta_r \times 10^{-1}$ (%)	0.00	28.04	0.00	39.76	0.00	45.22
Malic acid / Lactic acid		6.37	0.27	9.62	0.27	11.94	0.27
Citric acid	g/L	0.33	0.28	0.31	0.22	0.32	0.25
Tartaric acid	g/L	2.12	1.96	2.10	1.88	2.12	1.78
Potassium	mg/L	1031	997	1030	986	1028	963
Calcium	mg/L	95	91	95	88	95	83
Phenolic compounds	g/L	2.05	2.00	2.05	1.97	2.05	1.23
Sugars	g/L	3.51	3.29	3.51	3.32	3.51	3.51
NE	g/L	22.71	21.86	23.70	22.31	24.64	22.59

Table 2

Variation of main compositional characteristics during malolactic fermentation - Cabernet Sauvignon

Element/ Parameters	U.M.	Sample 4		Sample 5		Sample 6	
		initially sample $V_{4.1}$	final sample $V_{4.2}$	initially sample $V_{5.1}$	final sample $V_{5.2}$	initially sample $V_{6.1}$	final sample $V_{6.2}$
Alcohol	% vol.	13.42	13.46	13.42	13.50	13.42	13.47
TA	g/L $C_4H_6O_6$	**6.79**	**5.68**	**7.99**	**6.04**	**8.96**	**6.31**
	δ_r (%)	0.00	- 16.35	0.00	- 24.41	0.00	- 29.58
VA	g/L $C_2H_4O_2$	**0.51**	**0.65**	**0.53**	**0.64**	**0.52**	**0.70**
pH		**3.52**	**3.80**	**3.33**	**3.73**	**3.20**	**3.69**
	δ_r (%)	0.00	8.01	0.00	12.09	0.00	15.31
Malic acid	g/L	**2.07**	**0.34**	**3.01**	**0.39**	**3.83**	**0.54**
	δ_r (%)	0.00	- 83.57	0.00	- 87.04	0.00	- 85.90
Lactic acid	g/L	**0.57**	**1.88**	**0.56**	**2.25**	**0.58**	**2.31**
	$\delta_r \times 10^{-1}$ (%)	0.00	22.98	0.00	30.18	0.00	29.83
Malic acid / Lactic acid		4.88	0.24	7.22	0.23	8.87	0.321
Citric acid	g/L	0.39	0.34	0.38	0.32	0.38	0.35
Tartaric acid	g/L	2.31	2.06	2.34	2.15	2.31	2.01
Potassium	mg/L	1202	1148	1200	1158	1202	1140
Calcium	mg/L	104	98	104	100	104	96
Phenolic compounds	g/L	2.62	2.58	2.62	2.54	2.62	2.50
Sugars	g/L	3.82	3.78	3.82	3.74	3.82	3.76
NE	g/L	25.22	24.39	26.12	24.80	27.20	25.11

VA - (volatile acidity); TA – (total acidity); NE- (non-reducing extract)

CONCLUSIONS

Spreading with Selected Malolactic Bacteria (BMS) of the biolact aclimatee series (Oenococcus oeni class), used in the maize form is recommended in oenological practice as it provides optimum conditions for the biological deacidification of wines. From this point of view, there is an effective metabolism of malic acid to lactic acid, which gives appreciable organoleptic qualities to the wines undergoing this process.

Changes in other compositional features support an optimal evolution of malolactic fermentation, resulting in increased pH and a pronounced decrease in total acidity over a period of time (approximately 20-34 days).

Malolactic fermentation is a beneficial process for red wines because it ensures their biological stability against lactic bacteria. In connection with this

aspect it is appreciated that this leads to a convenient deacidification, which gives the red wines the qualities of suppleness by reducing astringency (decreasing the tannin content) and, at the same time, increasing their color.

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