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EXPERIMENTAL ASPECTS REGARDING THE MALOLACTIC FERMENTATION OF SOME RED AND WHITE WINES

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ABSTRACT – This scientific paper presents some aspects typical of the process of malolactic fermentation in some wines, respectively, the variation of total, volatile and real acidity (pH), malic acid metabolization and formation of lactic acid, as well as the changes of some composition characteristics (tartaric and citric acids, potassium, calcium, reducing sugars and phenolic compounds). Trials were conducted on seven different wines from the harvest of year 2007: two white wines obtained from Rkațiteli and Zghihară varieties and five red varieties (Burgund mare, Băbească neagră, Cabernet Sauvignon, Merlot and Fetească neagră). We have studied some wines according to their pH values (3.085, 3.135, 3.264, 3.352, 3.422, 3.470 and 3.505) and total acidity (9.96, 8.84, 7.88, 7.56, 7.25, 6.94, 6.63 g/L $C_4H_6O_6$), which are significant in the development of the malolactic fermentation. The investigations were carried out under laboratory conditions, for 40 days. For starting the malolactic fermentation, at the end of wine alcoholic fermentation (after 7-15 days), selected malolactic bacteria (SMB) from the Biolact assortment *(Oenococcus oeni* class) were added in wines as leaven. By the action of malolactic bacteria, we found in all the studied wines a differentiated diminution (according to the initial values of total acidity and of pH) in the concentration of malic acid, correlated to an increase in the lactic acid content. The modifications of the other composition characteristics sustained the evolution of malolactic fermentation, realised by the great diminution of total acidity and the pH increase, after a certain period (about 16-24 days).

Key words: composition characteristics, malolactic bacteria, malolactic fermentation, white and red wines

REZUMAT - Aspecte experimentale privind fermentația malolactică la unele

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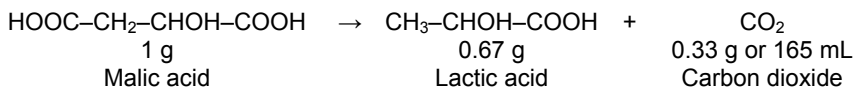
vinuri roşii şi albe. Lucrarea prezintă o serie de aspecte caracteristice procesului de fermentaţie malolactică, respectiv, variaţia acidităţii totale, volatile şi reale (pH), metabolizarea acidului malic şi formarea acidului lactic, precum şi modificarea unor caracteristici de compoziţie (acizii tartric şi citric, potasiu, calciu, zaharuri reducătoare şi compuşi fenolici). Experimentările s-au efectuat pe vinurile din recolta anului 2007: vinurile albe, provenite din soiurile Rkaţiteli şi Zghihară şi vinurile roşii, provenite din soiurile Burgund mare, Băbească neagră, Cabernet Sauvignon, Merlot şi Fetească neagră. S-au luat în studiu vinurile diferenţiate după valorile de pH (3.085, 3.135, 3.264, 3.352, 3.422, 3.470, 3.505) şi de aciditate totală (9.96, 8.84, 7.88, 7.56, 7.25, 6.94, 6.63 g/L $C_4H_6O_6$), parametrii semnificativi pentru desfăşurarea fermentaţiei malolactice. Cercetările au fost efectuate în condiţii de laborator. Pentru declanşarea fermentaţiei malolactice, la sfârşitul fermentaţiei alcoolice, după 7-15 zile, vinurile au fost însămânţate cu bacterii malolactice selecţionate (BMS) din gama biolact aclimatee (clasa *Oenococcus oeni*), administrate sub formă de maia. Prin acţiunea bacteriilor malolactice, la toate vinurile analizate s-a constatat o scădere diferenţiată (în funcţie de valorile iniţiale ale acidităţii totale şi ale pH-ului) a concentraţiei acidului malic, corelată cu o creştere a conţintului de acid lactic. De asemenea, modificările celorlalte caracteristici de compoziţie susţin evoluţia fermentaţiei malolactice, concretizată prin scăderea pronunţată a acidităţii totale şi creşterea valorii pH-ului, după o anumita perioadă de timp (circa 16-24 de zile).

Cuvinte cheie: bacterii malolactice, caracteristici de compoziţie, fermentaţie malolactică, vinuri albe şi roşii

INTRODUCTION

The malolactic fermentation or the biological deacidification represents the change of malic acid from wine into lactic acid and carbon dioxide. This change takes place according to the global reaction:



This reaction shows that by the decarboxylation of malic acid, which is a dicarboxylic acid, and the formation of lactic acid, which is monocarboxylic acid, one of the acid functions disappears. Thus, during the malolactic fermentation, almost half of the acidity determined by malic acid disappears, because it is changed in CO_2, which is released, resulting in the diminution of total acidity (Cotea, 1985).

In the wines from the Romanian vineyards, the process of malolactic fermentation was studied for the first time by C. Ţârdea (1966)[*]. The malolactic fermentation is a beneficial biological process, especially for red wines, because it leads to a proper deacidification that confers flexibility to red wines, diminishing their astringent trait. Most of the times, it intensifies the wine colour, although

[*] **Ţârdea C., 1966** - *Fermentaţia malolactică la vinurile roşii din podgoriile Moldovei (Malolactic Fermentation in Red Wines from the Moldavian Vineyards)*. PhD Thesis, Institutul Agronomic Bucureşti

diminutions were also found in the anthocyans and tannin content. It ensures the biological stability to lactic bacteria. For white wines, the malolactic fermentation is generally less preferred because, by diminishing the acidity, it determines the loss of some organoleptic qualities like freshness and fructuosity. In this case, it is however accepted in colder areas, especially in the years when malic acid from wine exceeds the quantity of tartaric acid (Cotea, 1985; Ţârdea et. al., 2007).

In a previous work (Odăgeriu, 2008), we have shown some aspects during the malolactic fermentation of some red wines, respectively, the variation of some physical and chemical indices according to the modification of their total and real acidity. We have continued the previous investigations in this study by presenting new aspects typical of the malolactic fermentation in some white sand red wines, respectively, the variation of total, volatile and real acidity (pH), the metabolization of malic acid and the formation of lactic acid, as well as the change of other composition characteristics (tartaric and citric acids, potassium and calcium) during the studied process.

MATERIALS AND METHODS

Trials were conducted under laboratory conditions in wines from the harvest of year 2007: two white wines from Rkaţiteli (Stăuceni Viticulture Centre from the Republic of Moldova) and Zghihară varieties (Avereşti-Huşi Viticulture Centre); five red wines, of which Burgund mare, Băbească neagră and Feteasсă neagră are from Uricani-Iaşi Viticulture Centre and Cabernet Sauvignon and Merlot, from Ialoveni Viticulture Centre from Republic of Moldova.

Wines were chosen according to the composition parameters that condition the malolactic fermentation: pH and total acidity (*Tables 1-7*).

At the end of alcoholic fermentation (after 7-15 days), wines were inoculated with selected malolactic bacteria (SMB) of *Oenococcus oeni* Class (made by *PASCAL BIOTECH* and purchased by the intermediary of Sodinal LTD), which were added in wines as leaven, as rehydrated biological material (RBM). The leaven was prepared as described below. In red wines, after the deacidification of 5 L Merlot wine with 10 g $CaCO_3$ (when pH increased from 3.470 to 4.450), 25 g nutrient of *Fermoplus Malolactique* and 5 g selected malolactic bacteria (with concentration of 100 g/hL) were added, and the leaven was kept at the temperature of 24ºC for 24 h in order to acclimate bacteria to the limitative conditions of wine and to begin the cellular multiplication. Under similar conditions, in white wines we have used the Zghihară Variety, then, a mixture in equal volumes (1:1) of uninoculated wine (0.5 L from each wine) with inoculated wine (0.5 L prepared leaven) was realised, afterwards the mixture of obtained wine was left for 48 h at a temperature of 20ºC, for the metabolization of 2/3 - 3/4 of the initial content of malic acid from wine.

At the initial moment and at intervals of 0, 4, 8, 12, 16, 24, 32 and 40 days, we took samples from each wine. Thus, from wines obtained from the Rkaţiteli Variety, we took R_1, R_2, R_3, R_4, R_5, R_6, R_7 and R_8 samples; from the Zghihară Variety, we took Z_1, Z_2, Z_3, Z_4, Z_5, Z_6, Z_7 and Z_8 samples; from the

Burgund mare Variety, we took BM_1, BM_2, BM_3, BM_4, BM_5, BM_6, BM_7 and BM_8 samples; from the Băbească neagră Variety, we took BN_1, BN_2, BN_3, BN_4, BN_5, BN_6, BN_7 and BN_8 samples; from Cabernet Sauvignon Variety, we took CS_1, CS_2, CS_3, CS_4, CS_5, CS_6, CS_7 and CS_8, samples; from Merlot Variety, we took M_1, M_2, M_3, M_4, M_5, M_6, M_7 and M_8 samples and from Fetească neagră Variety, we took FN_1, FN_2, FN_3, FN_4, FN_5, FN_6, FN_7, FN_8 samples.

At the end of the malolactic fermentation, when we found that the malic acid reached the recommended quantities, the bacterial activity of wines was stopped by their extraction from deposit and treatment with sulphur dioxide and gelatine, at rates determined in laboratory microprobes.

For each sample taken after filtering and decarbonation, we carried out physical- chemical analyses. Before, during and after the malolactic fermentation, we carried out, from October until December 2007, some analyses concerning the main composition characteristics (total acidity, volatile acidity, pH, tartaric, malic, lactic and citric acids, free and total sulphur dioxide, potassium, calcium, total phenolic compounds, reducing sugars and non-reducing extract). These analyses were done according to present standards (*** 1998; *** 2005) and to the literature (Delfini Cl, 1995; Flanzy Cl., 1998; Ribereau -Gayon et al., 1972; Ţârdea C., 2007; Würdig and Woller, 1989).

Based on the obtained results, we have also calculated the relative deviation (δ_r) in (%), by which were changed total acidity, real acidity (pH), malic and lactic acids from studied wines, during the malolactic fermentation.

RESULTS AND DISCUSSION

The main composition characteristics of the analysed wines (eight samples for each wine) are shown in *Tables 1-7*. Thus, the alcohol content (% vol.) from the studied control wines had the following values: 11.10 (Rkaţiteli), 11.50 (Zghihară) 10.80 (Burgund mare), 11.10 (Băbească neagră), 12.00 (Cabernet Sauvignon), 12.20 (Merlot) and 12.50 (Fetească neagră). The other values of the control samples, which were less modified, referred to the succinic acid that had values of 0.38, 0.42, 0.47, 0.52, 0.60, 0.72 , respectively, 0.64 g/L, and to the content of total free sulphur dioxide, which was found between 9.3 (32.0) and 13.3 (35.0) mg/L.

The main composition characteristics (total and volatile acidity, pH, malic, lactic and citric acids) of these wines, involved in the typical processes of the malolactic fermentation according to the wine type, are shown in the above-mentioned tables.

The total acidity, expressed in g/L $C_4H_6O_6$ has decreased as it follows: in wines obtained from the Rkaţiteli Variety, from 9.96 (R_1 sample) to 7.86 (R_8 sample), at a rate of 21.1% (2.10 g/L); in wines obtained from the Zghihară Variety, from 8.84 (Z_1 sample) to 6.83 (Z_8 sample), at a rate of 22.7 % (2.01 g/L); in wines obtained from the Burgund mare Variety, from 7.88 (BM_1 sample) to 6.14 (BM_8 sample), at a rate of 22.1% (1.74 g/L); in wines

obtained from the Băbească neagră Variety, from 7.56 (BN_1 sample) to 6.02 (BN_8 sample) at a rate of 20.4% (1.54 g/L); in wines obtained from the Cabernet Sauvignon Variety, from 7.25 (CS_1 sample) to 5.80 (CS_8 sample) at a rate of 20.0% (1.45 g/L); in wines obtained from the Merlot Variety, from 6.94 (M_1 sample) to 5.78 (M_8 sample) at a rate of 16.7% (1.16 g/L); in wines obtained from the Fetească neagră Variety, from 6.63 (FN_1 sample) to 5.36 (FN_8 sample), at a rate of 19.2% (1.27 g/L).

The malic acid (g/L), which was degraded by lactic bacteria during the malolactic fermentation, has decreased as it follows: in wines obtained from the Rkațiteli Variety, from 4.32 (R_1 sample) to 0.76 (R_8 sample), at a rate of 82.4% (3.56 g/L); in wines obtained from the Zghihară Variety, from 3.93 (Z_1 sample) to 0.65 (Z_8 sample), at a rate of 83.5% (3.28 g/L); in wines obtained from the Burgund mare Variety, from 3.41 (BM_1 sample) to 0.54 (BM_8 sample) at a rate of 84.2% (2.87 g/L); in wines obtained from the Băbească neagră Variety, from 2.82 (BN_1 sample) to 0.38 (BN_8 sample) at a rate of 86.5% (2.44 g/L); in wines obtained from the Cabernet Sauvignon Variety, from 2.52 (CS_1 sample) to 0.35 (CS_8 sample) at a rate of 86.1% (2.17 g/L); in wines obtained from the Merlot Variety, from 2.22 (M_1 sample) to 0.38 (M_8 sample) at a rate of 82.9% (1.84 g/L) and in wines obtained from the Fetească neagră, from 2.30 (FN_1 sample) to 0.40 (FN_8 sample), at a rate of 82.6% (1.90 g/L).

The lactic acid (g/L), as main product of the malolactic fermentation and as result of the metabolization of some sugars (pentoses and hexoses) or of the transformation of some acids (citric and succinic), has increased as it follows: in wines obtained from the Rkațiteli Variety, from 0.29 (R_1 sample) to 2.78 (R_8 sample), at a rate of 10×85.9% (2.49 g/L); in wines obtained from the Zghihară Variety, from 0.30 (Z_1 sample) to 2.61 (Z_8 sample), at a rate of 10×77.0% (2.31 g/L); in wines obtained from the Burgund mare Variety, from 0.38 (BM_1 sample) to 2.40 (BM_8 sample), at a rate of 10×53.2% (2.02 g/L); in wines obtained from the Băbească neagră Variety, from 0.41 (BN_1 sample) to 2.15 (BN_8 sample), at a rate of 10×42.4% (1.74 g/L); in wines obtained from the Cabernet Sauvignon Variety, from 0.40 (CS_1 sample) to 1.96 (CS_8 sample), at a rate of 10×39.0% (1.56 g/L); in wines obtained from the Merlot Variety, from 0.50 (M_1 sample) to 1.85 (M_8 sample), at a rate of 10×27.0% (1.35 g/L) and in wines obtained from the Fetească neagră Variety, from 0.45 (FN_1 sample) to 1.83 (FN_8 sample), at a rate of 10×30.7% (1.38 g/L).

During the trial, in all the studied wines we noticed a differentiated decrease (according to the initial values of total acidity and pH) in the concentration of malic acid, correlated to a corresponding increase in the content of lactic acid.

Table 1 - Variation of main composition characteristics during the malolactic fermentation in Rkațiteli white wine

Specification	Unit of measure	Wine sample							
		R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8
		Time (days)							
		0	4	8	12	16	24	32	40
Total acidity	g/L $C_4H_6O_6$	**9.96**	**9.93**	**9.77**	**9.30**	**8.89**	**8.14**	**8.01**	**7.86**
	δ_r (%)	0.0	-0.3	-1.9	-6.6	-10.7	-18.3	-19.6	-21.1
Volatile acidity	g/L $C_2H_4O_2$	0.38	0.38	0.39	0.40	0.45	0.47	0.48	0.49
Real acidity (pH)		**3.077**	**3.078**	**3.084**	**3.088**	**3.098**	**3.141**	**3.182**	**3.204**
	δ_r (%)	0.0	0.0	0.2	0.4	0.7	2.1	3.4	4.1
Malic acid	g/L	**4.32**	**4.26**	**4.01**	**3.18**	**2.42**	**1.14**	**0.98**	**0.76**
	δ_r (%)	0.0	-1.4	-7.2	-26.4	-44.0	-73.6	-77.3	-82.4
Lactic acid	g/L	**0.29**	**0.34**	**0.51**	**1.08**	**1.61**	**2.49**	**2.62**	**2.78**
	$\delta_r \times 10^{-1}$ (%)	0.0	1.7	7.6	27.2	45.5	75.9	80.3	85.9
Malic acid/ lactic acid		14.90	12.53	7.86	2.94	1.50	0.46	0.37	0.27
Citric acid	g/L	0.12	0.12	0.11	0.10	0.06	0.04	0.03	0.02
Total tartaric acid	g/L	**3.87**	**3.86**	**3.84**	**3.83**	**3.81**	**3.78**	**3.73**	**3.70**
Potassium	mg/L	**890**	**887**	**882**	**880**	**875**	**868**	**860**	**853**
Calcium	mg/L	**74**	**74**	**74**	**74**	**74**	**72**	**71**	**71**
Total phenolic compounds	g/L	0.25	0.25	0.25	0.24	0.24	0.24	0.23	0.23
Reducing sugars	g/L	1.45	1.44	1.44	1.42	1.40	1.38	1.36	1.34
Non-reducing extract	g/L	19.16	19.14	19.02	18.73	18.44	17.98	17.87	17.76

Table 2 - Variation of main composition characteristics during the malolactic fermentation in Zghihară white wine

Specification	Unit of measure	Wine sample							
		Z_1	Z_2	Z_3	Z_4	Z_5	Z_6	Z_7	Z_8
		Time (days)							
		0	4	8	12	16	24	32	40
Total acidity	g/L $C_4H_6O_6$	**8.84**	**8.75**	**8.54**	**8.01**	**7.34**	**7.02**	**6.90**	**6.83**
	δ_r (%)	0.0	-1.0	-3.4	-9.4	-17.0	-20.6	-21.9	-22.7
Volatile acidity	g/L $C_2H_4O_2$	0.44	0.44	0.45	0.47	0.51	0.52	0.53	0.55
Real acidity (pH)		**3.178**	**3.180**	**3.187**	**3.202**	**3.240**	**3.272**	**3.308**	**3.340**
	δ_r (%)	0.0	0.1	0.3	0.8	2.0	3.0	4.1	5.1
Malic acid	g/L	**3.93**	**3.81**	**3.46**	**2.53**	**1.42**	**0.87**	**0.72**	**0.65**
	δ_r (%)	0.0	-3.1	-12.0	-35.6	-63.9	-77.9	-81.7	-83.5
Lactic acid	g/L	**0.30**	**0.38**	**0.62**	**1.27**	**2.04**	**2.43**	**2.54**	**2.61**
	$\delta_r \times 10^{-1}$ (%)	0.0	2.7	10.7	32.3	58.0	71.0	74.7	77.0
Malic acid/ lactic acid		13.10	10.03	5.58	1.99	0.70	0.36	0.28	0.25
Citric acid	g/L	0.13	0.13	0.12	0.11	0.07	0.06	0.05	0.03
Total tartaric acid	g/L	3.04	3.02	3.00	2.96	2.91	2.88	2.86	2.82
Potassium	mg/L	798	793	789	781	772	764	758	752
Calcium	mg/L	78	78	77	76	74	74	73	72
Total phenolic compounds	g/L	0.28	0.28	0.28	0.27	0.27	0.26	0.25	0.25
Reducing sugars	g/L	1.76	1.76	1.75	1.73	1.70	1.68	1.66	1.64
Non-reducing extract	g/L	19.86	19.80	19.65	19.30	18.86	18.64	18.54	18.49

Table 3 - Variation of main composition characteristics during the malolactic fermentation in Burgund mare red wine

Specification	Unit of measure	Wine sample							
		BM_1	BM_2	BM_3	BM_4	BM_5	BM_6	BM_7	BM_8
		Time (days)							
		0	4	8	12	16	24	32	40
Total acidity	g/L $C_4H_6O_6$	**7.88**	**7.80**	**7.57**	**7.26**	**6.82**	**6.43**	**6.25**	**6.14**
	δ_r (%)	0.0	-1.0	-3.9	-7.9	-13.5	-18.4	-20.7	-22.1
Volatile acidity	g/L $C_2H_4O_2$	0.49	0.49	0.51	0.54	0.57	0.60	0.61	0.62
Real acidity (pH)		**3.264**	**3.267**	**3.272**	**3.306**	**3.358**	**3.402**	**3.440**	**3.468**
	δ_r (%)	0.0	0.1	0.2	1.3	2.9	4.2	5.4	6.3
Malic acid	g/L	**3.41**	**3.29**	**2.90**	**2.36**	**1.63**	**0.98**	**0.72**	**0.54**
	δ_r (%)	0.0	-3.5	-15.0	-30.8	-52.2	-71.3	-78.9	-84.2
Lactic acid	g/L	**0.38**	**0.46**	**0.73**	**1.12**	**1.63**	**2.08**	**2.26**	**2.40**
	$\delta_r \times 10^{-1}$ (%)	0.0	2.1	9.2	19.5	32.9	44.7	49.5	53.2
Malic acid/ lactic acid		8.97	7.15	3.97	2.11	1.00	0.47	0.32	0.23
Citric acid	g/L	0.15	0.15	0.13	0.11	0.08	0.06	0.05	0.04
Total tartaric acid	g/L	**2.47**	**2.46**	**2.44**	**2.40**	**2.37**	**2.34**	**2.32**	**2.30**
Potassium	mg/L	**940**	**937**	**933**	**924**	**920**	**914**	**909**	**906**
Calcium	mg/L	**82**	**82**	**82**	**81**	**79**	**78**	**77**	**77**
Total phenolic compounds	g/L	1.35	1.35	1.35	1.33	1.33	1.32	1.32	1.31
Reducing sugars	g/L	2.36	2.36	2.35	2.32	2.30	2.28	2.27	2.25
Non-reducing extract	g/L	21.25	21.20	21.02	20.78	20.49	20.21	20.09	20.01

Table 4 - Variation of main composition characteristics during the malolactic fermentation in Băbească neagră red wine

Specification	Unit of measure	Wine sample							
		BN_1	BN_2	BN_3	BN_4	BN_5	BN_6	BN_7	BN_8
		Time (days)							
		0	4	8	12	16	24	32	40
Total acidity	g/L $C_4H_6O_6$	**7.56**	**7.50**	**7.22**	**6.97**	**6.48**	**6.21**	**6.07**	**6.02**
	δ_r (%)	0.0	-0.8	-4.5	-7.8	-14.3	-17.9	-19.7	-20.4
Volatile acidity	g/L $C_2H_4O_2$	0.53	0.53	0.55	0.59	0.62	0.65	0.66	0.68
Real acidity (pH)		**3.352**	**3.356**	**3.390**	**3.429**	**3.470**	**3.515**	**3.572**	**3.593**
	δ_r (%)	0.0	0.1	1.1	2.3	3.5	4.9	6.6	7.2
Malic acid	g/L	**2.82**	**2.72**	**2.31**	**1.76**	**1.06**	**0.60**	**0.44**	**0.38**
	δ_r (%)	0.0	-3.5	-18.1	-37.6	-62.4	-78.7	-84.4	-86.5
Lactic acid	g/L	**0.41**	**0.49**	**0.78**	**1.18**	**1.67**	**1.99**	**2.10**	**2.15**
	$\delta_r \times 10^{-1}$ (%)	0.0	2.0	9.0	18.8	30.7	38.5	41.2	42.4
Malic acid/ lactic acid		6.88	5.55	2.96	1.49	0.63	0.30	0.21	0.18
Citric acid	g/L	0.17	0.17	0.15	0.12	0.09	0.07	0.06	0.04
Total tartaric acid	g/L	2.70	2.68	2.64	2.60	2.57	2.55	2.53	2.52
Potassium	mg/L	1010	1005	995	988	986	985	983	983
Calcium	mg/L	93	93	92	91	90	89	88	88
Total phenolic compounds	g/L	1.56	1.56	1.55	1.53	1.52	1.51	1.50	1.48
Reducing sugars	g/L	2.75	2.74	2.72	2.69	2.66	2.65	2.65	2.64
Non-reducing extract	g/L	22.13	22.09	21.87	21.63	21.33	21.14	21.05	20.99

Table 5 - Variation of main composition characteristics during the malolactic fermentation in Cabernet Sauvignon red wine

Specification	Unit of measure	Wine sample							
		CS_1	CS_2	CS_3	CS_4	CS_5	CS_6	CS_7	CS_8
		Time (days)							
		0	4	8	12	16	24	32	40
Total acidity	g/L $C_4H_6O_6$	**7.25**	**7.18**	**6.94**	**6.56**	**6.19**	**5.94**	**5.84**	**5.80**
	δ_r (%)	0.0	-1.0	-4.3	-9.5	-14.6	-18.1	-19.4	-20.0
Volatile acidity	g/L $C_2H_4O_2$	0.42	0.43	0.44	0.47	0.49	0.53	0.56	0.58
Real acidity (pH)		**3.422**	**3.429**	**3.438**	**3.492**	**3.514**	**3.585**	**3.678**	**3.695**
	δ_r (%)	0.0	0.2	0.5	2.0	2.7	4.8	7.5	8.0
Malic acid	g/L	**2.52**	**2.40**	**1.96**	**1.36**	**0.72**	**0.40**	**0.38**	**0.35**
	δ_r (%)	0.0	-4.8	-22.2	-46.0	-71.4	-84.1	-84.9	-86.1
Lactic acid	g/L	**0.40**	**0.49**	**0.82**	**1.24**	**1.68**	**1.90**	**1.93**	**1.96**
	$\delta_r \times 10^{-1}$ (%)	0.0	2.3	10.5	21.0	32.0	37.5	38.3	39.0
Malic acid/ lactic acid		6.30	4.90	2.39	1.10	0.43	0.21	0.20	0.18
Citric acid	g/L	0.28	0.27	0.26	0.24	0.22	0.19	0.16	0.14
Total tartaric acid	g/L	2.62	2.61	2.59	2.55	2.53	2.49	2.46	2.44
Potassium	mg/L	592	589	585	577	572	562	556	550
Calcium	mg/L	52	52	52	51	51	50	49	49
Total phenolic compounds	g/L	2.26	2.25	2.24	2.23	2.21	2.20	2.18	2.16
Reducing sugars	g/L	2.86	2.85	2.81	2.79	2.78	2.78	2.76	2.75
Non-reducing extract	g/L	25.60	25.54	25.37	25.11	24.84	24.64	24.55	24.48

Table 6 - Variation of main composition characteristics during the malolactic fermentation in Merlot red wine

Specification	Unit of measure	Wine sample							
		M_1	M_2	M_3	M_4	M_5	M_6	M_7	M_8
		Time (days)							
		0	4	8	12	16	24	32	40
Total acidity	g/L $C_4H_6O_6$	**6.94**	**6.89**	**6.75**	**6.56**	**6.19**	**5.84**	**5.82**	**5.78**
	δ_r (%)	0.0	-0.7	-2.7	-5.5	-10.8	-15.9	-16.1	-16.7
Volatile acidity	g/L $C_2H_4O_2$	**0.50**	**0.54**	**0.57**	**0.61**	**0.63**	**0.65**	**0.66**	**0.66**
Real acidity (pH)		**3.470**	**3.475**	**3.533**	**3.552**	**3.629**	**3.645**	**3.652**	**3.665**
	δ_r (%)	0.0	0.1	1.8	2.4	4.6	5.0	5.2	5.6
Malic acid	g/L	**2.22**	**2.12**	**1.96**	**1.57**	**1.08**	**0.44**	**0.42**	**0.38**
	δ_r (%)	0.0	-4.5	-11.7	-29.3	-51.4	-80.2	-81.1	-82.9
Lactic acid	g/L	**0.50**	**0.58**	**0.70**	**1.01**	**1.34**	**1.78**	**1.81**	**1.85**
	$\delta_r \times 10^{-1}$ (%)	0.0	1.6	4.0	10.2	16.8	25.6	26.2	27.0
Malic acid/ lactic acid		4.44	3.66	2.80	1.55	0.81	0.25	0.23	0.21
Citric acid	g/L	0.25	0.22	0.19	0.16	0.14	0.13	0.12	0.11
Total tartaric acid	g/L	2.36	2.34	2.30	2.28	2.24	2.22	2.21	2.20
Potassium	mg/L	670	665	657	650	643	640	636	634
Calcium	mg/L	58	58	57	57	56	56	56	56
Total phenolic compounds	g/L	2.01	2.01	2.00	1.98	1.97	1.95	1.94	1.92
Reducing sugars	g/L	2.60	2.59	2.57	2.52	2.52	2.51	2.49	2.47
Non-reducing extract	g/L	23.50	23.43	23.30	23.13	22.89	22.63	22.59	22.55

Table 7 - Variation of main composition characteristics during the malolactic fermentation in Fetească neagră red wine

Specification	Unit of measure	Wine sample							
		FN_1	FN_2	FN_3	FN_4	FN_5	FN_6	FN_7	FN_8
		Time (days)							
		0	4	8	12	16	24	32	40
Total acidity	g/L $C_4H_6O_6$	**6.63**	**6.58**	**6.42**	**6.09**	**5.73**	**5.44**	**5.39**	**5.36**
	δ_r (%)	0.0	-0.8	-3.2	-8.1	-13.6	-17.9	-18.7	-19.2
Volatile acidity	g/L $C_2H_4O_2$	0.57	0.59	0.63	0.66	0.69	0.71	0.73	0.75
Real acidity (pH)		**3.505**	**3.514**	**3.530**	**3.654**	**3.743**	**3.782**	**3.796**	**3.806**
	δ_r (%)	0.0	0.3	0.7	4.3	6.8	7.9	8.3	8.6
Malic acid	g/L	**2.30**	**2.20**	**1.90**	**1.52**	**0.98**	**0.51**	**0.46**	**0.40**
	δ_r (%)	0.0	-4.3	-17.4	-33.9	-57.4	-77.8	-80.0	-82.6
Lactic acid	g/L	**0.45**	**0.54**	**0.76**	**1.03**	**1.41**	**1.74**	**1.78**	**1.83**
	$\delta_r \times 10^{-1}$ (%)	0.0	2.0	6.9	12.9	21.3	28.7	29.6	30.7
Malic acid/ lactic acid		5.11	4.07	2.50	1.48	0.70	0.29	0.26	0.22
Citric acid	g/L	0.20	0.18	0.15	0.12	0.10	0.08	0.06	0.05
Total tartaric acid	g/L	2.08	2.06	2.05	2.00	1.98	1.96	1.94	1.92
Potassium	mg/L	1040	1035	1032	1023	1019	1014	1010	1004
Calcium	mg/L	92	92	92	90	89	89	89	89
Total phenolic compounds	g/L	1.80	1.80	1.79	1.77	1.76	1.75	1.74	1.72
Reducing sugars	g/L	2.94	2.92	2.90	2.88	2.86	2.84	2.84	2.83
Non-reducing extract	g/L	24.20	24.15	24.00	23.78	23.55	23.34	23.27	23.20

In all the studied wines, we found that the content of malic acid has slightly decreased in the first 16 days and then (during the next 8 days), it generally had the greatest diminution and, subsequently, during 40 days, it diminished slowly, reaching, at the end, values comprised between 0.65 and 0.76 g/L in white wines and, respectively, between 0.35 and 0.54 g/L in red wines. In all the studied wines, we have also noticed that the content of lactic acid has slightly increased in the first 16 days and then (during the next 8 days), it had the greatest increase and, subsequently, during 40 days, it has slowly increased, reaching, at the end, the value of 2.78 g/L in white wines and values comprised between 1.83 and 2.40 g/L in red wines.

The citric acid (g/L), which, during the malolactic fermentation, was degraded by lactic bacteria, has decreased as it follows: in wines obtained from the Rkațiteli Variety, from 0.12 (R_1 sample) to 0.02 (R_8 sample) at a rate of 83.3% (0.10 g/L); in wines obtained from the Zghihară Variety, from 0.13 (Z_1 sample) to 0.03 (Z_8 sample), at a rate of 76.9% (0.10 g/L); in wines obtained from the Burgund mare Variety, from 0.15 (BM_1 sample) to 0.04 (BM_8 sample), at a rate of 73.3% (0.11 g/L); in wines obtained from the Băbească neagră Variety, from 0.17 (BN_1 sample) to 0.04 (BN_8 sample), at a rate of 76.5% (0.13 g/L); in wines obtained from the Cabernet Sauvignon Variety, from 0.28 (CS_1 sample) to 0.14 (CS_8 sample), at a rate of 50.0% (0.14 g/L); in wines obtained from the Merlot Variety, from 0.25 (M_1 sample) to 0.11 (M_8 sample) at a rate of 56.0% (0.14 g/L) and in wines obtained from the Fetească neagră Variety, from 0.20 (FN_1 sample) to 0.05 (FN_8 sample), at a rate of 75.0% (0.15 g/L).

The volatile acidity (volatile acids), as a direct result of the metabolization of citric acid and as by-product of other secondary reactions, typical of the malolactic fermentation, expressed as g/L $C_2H_4O_2$, has increased as it follows: in wines obtained from the Rkațiteli Variety, from 0.38 (R_1 sample) to 0.49 (R_8 sample), at a rate of 28.9% (0.11 g/L); in wines obtained from the Zghihară Variety, from 0.44 (Z_1 sample) to 0.55 (Z_8 sample), at a rate of 25.0% (0.11); in wines obtained from the Burgund mare Variety, from 0.49 (BM_1 sample) to 0.62 (BM_8 sample), at a rate of 26.5% (0.13); in wines obtained from the Băbească neagră Variety, from 0.53 (BN_1 sample) to 0.68 (BN_8 sample), at a rate of 28.3% (0.15); in wines obtained from the Cabernet Sauvignon Variety, from 0.42 (CS_1 sample) to 0.58 (CS_8 sample), at a rate of 38.1% (0.16); in wines obtained from the Merlot Variety, from 0.50 (M_1 sample) to 0.66 (M_8 sample), at a rate of 32.0% (0.16) and in wines obtained from the Fetească neagră Variety, from 0.57 (FN_1 sample) to 0.75 (FN_8 sample), at a rate of 31.6% (0.18). The values of volatile acidity did not exceed the limit of 0.20 g/L $C_2H_4O_2$, which is shown in the literature (Bauer and Dicks, 2004; Cotea, 1985; Odăgeriu et al., 2008; Țârdea et al., 2000; Țârdea, 2007;

Würdig and Woller, 1989) pointing out the best development of the malolactic fermentation.

At the same time, there was found a diminution in the main components, which influenced the solubility of tartaric compounds, respectively, tartaric acid, potassium and calcium. At its turn, this diminution was also influenced by the increase in the pH value.

The tartaric acid (g/L), as result of the insolubilization of tartaric compounds during the malolactic fermentation, has decreased as it follows: in wines obtained from the Rkațiteli Variety, from 3.87 (R_1 sample) to 3.70 (R_8 sample), at a rate of 4.4% (0.17 g/L); in wines obtained from the Zghihară Variety, from 3.04 (Z_1 sample) to 2.82 (Z_8 sample), at a rate of 7.2% (0.22 g/L); in wines obtained from the Burgund mare Variety, from 2.47 (BM_1 sample) to 2.28 (BM_8 sample), at a rate of 7.7% (0.19 g/L); in wines obtained from the Băbească neagră Variety, from 2.70 (BN_1 sample) to 2.52 (BN_8 sample), at a rate of 6.7% (0.18 g/L); in wines obtained from the Cabernet Sauvignon Variety, from 2.62 (CS_1 sample) to 2.44 (CS_8 sample), at a rate of 6.9% (0.18 g/L); in wines obtained from the Merlot Variety, from 2.36 (M_1 sample) to 2.20 (M_8 sample), at a rate of 6.8% (0.16 g/L) and in wines obtained from the Fetească neagră Variety, from 2.08 (FN_1 sample) to 1.92 (FN_8 sample), at a rate of 7.7% (0.16 g/L).

Potassium and calcium contents have also decreased because of the insolubilization of tartaric compounds during the malolactic fermentation, at the same time with the diminution in tartaric acid content. Thus, the potassium content (mg/L) has decreased as it follows: from 890 (R_1 sample) to 853 (R_8 sample) in wines obtained from the Rkațiteli Variety; from 798 (Z_1 sample) to 752 (Z_8 sample) in Zghihară wine; from 940 (BM_1 sample) to 906 (BM_8 sample) in Burgund mare wine; from 1010 (BN_1 sample) to 983 (BN_8 sample) in Băbească neagră wine; from 592 (CS_1 sample) to 550 (CS_8 sample) in wines obtained from the Cabernet Sauvignon Variety; from 670 (M_1 sample) to 634 (M_8 sample) in wines obtained from the Merlot Variety and from 1040 (FN_1 sample) to 1004 (FN_8 sample) in wines obtained from the Fetească neagră Variety. The calcium content (mg/L) has shown a lower diminution than the potassium content and decreased as it follows: from 74 (R_1 sample) to 71 (R_8 sample) in wines obtained from the Rkațiteli Variety; from 78 (Z_1 sample) to 72 (Z_8 sample) in Zghihară wine; from 82 (BM_1 sample) to 77 (BM_8 sample) in Burgund mare wine; from 93 (BN_1 sample) to 88 (BN_8 sample) in Băbească neagră wine; from 52 (CS_1 sample) to 49 (CS_8 sample) in wines obtained from the Cabernet Sauvignon Variety; from 58 (M_1 sample) to 56 (M_8 sample) in wines obtained from the Merlot Variety and from 92 (FN_1 sample) to 89 (FN_8 sample) in wines obtained from the Fetească neagră Variety.

During this trial, we found in all the analysed wines a differentiated diminution of total acidity, correlated

to similar increases of the pH value, according to the decrease in malic acid content and the increase in lactic acid content. Thus, the greatest diminution in g/L $C_4H_6O_6$ (2.10, 2.01) of total acidity was found in white wines (Rkațiteli and Zghihară), which had the highest initial values of malic acid (4.32 and 3.93 g/L), followed by the acidity diminution in the two red wines (Burgund mare and Băbească neagră), which were more acid (1.74 and 1.54 g/L $C_4H_6O_6$) and had initial contents of malic acid of 3.41 and 2.82 g/L, and by the diminution found in the last three red wines (Cabernet Sauvignon, Merlot and Fetească neagră) of 1.45, 1.16 and 1.27 g/L $C_4H_6O_6$, at which the initial content of malic acid was of 2.52, 2.22 and 2.30 g/L.

The experimental data have also shown that the duration of the malic acid metabolization was differentiated according to wine type (white or red), initial total acidity (given by the contents of malic, tartaric, succinic, lactic, citric and volatile acids), pH value and to the content of total free sulphur dioxide, temperature and whole composition of the studied wines. We also mention that in these trials we preferred a temperature of 16-18ºC, closer to the technological conditions from the oenological practice.

CONCLUSIONS

The inoculation with selected malolactic bacteria (SMB) from the Biolact assortment (*Oenococcus oeni* Class), added in wines as leaven, is recommended in the oenological practice, because it offers the best conditions for the biological deacidification of wines. Therefore, we found an efficient metabolization of malic acid to lactic acid, which gives high organoleptic qualities to the wines submitted to this process.

The modifications of the other composition characteristics sustain the best evolution of malolactic fermentation, materialized by the increase in pH value and the great diminution in total acidity for a certain period (about 16-24 days).

The malolactic fermentation is a beneficial process, both for red and white wines, because it ensures their biological stability towards the lactic bacteria. Correlated to this aspect, we assess that it leads to a proper deacidification, which gives flexibility to red wines, by diminishing the astringency (tannin content decrease) and intensifying their colours.

In white wines, the malolactic fermentation is indicated only for the diminution of total acidity within the limits of 1.0-2.0 g/L $C_4H_6O_6$, because the organoleptic qualities are affected by greater decreases. However, this process can be successfully used in white and red wines, with greater contents than 4.5 g/L malic acid (total acidity of 9.0-12.0 g/L $C_4H_6O_6$), on the condition that deacidification should be done only in part of wine and then it should be blended with the initial wine, as fast as possible, after ceasing the malolactic fermentation and wine clearing with specific treatments.

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