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Researches concerning the malic acid content of wines during storage period

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Abstract

In conditions of the harvest year 2005 (low real thermal balance, high amount of precipitations in vegetation period, low number of sunstroke hours in vegetation period), grapes possess at maturity low contents in sugars and high in acidity. In these conditions, wines with lower concentrations in alcohol, a high total acidity and low pH are obtained, negatively influencing the start and development of the spontaneous malolactic fermentation.

The wines obtained from Burgund mare and Cabernet Sauvignon grapes varieties a „stabilization" of the malic acid degradation dynamics was produced, by blocking the malolactic fermentation so that doesn't take place any significant diminuation of malic acid during storage period.

Keywords: malic acid, malolactic fermentation, climatic conditions, grapes, red wines

1. Introduction

The predominant acids in grapes at maturity are tartaric and malic acids.

From the studies made in our country results that at the beginning of maturation, in grapes is found more malic acid than tartaric acid. After acidity reduction made by the end of maturation, grapes contain at full maturity 2-7 g/l tartaric acid and 2-3 g/l malic acid [1].

The must content in malic acid vary depending on variety and climatic conditions of the year. In years with cool and moist summers, the grapes and the must resulted from grapes contain more malic acid, than in warm and dry years, when the malic acid is intensely metabolized [2].

The malic acid has as main forming way the combustion respiratory process of sugars. The organic acids formations represents in fact intermediary steps of the respiratory process, releasing a part of the stored energy in sugars molecules [3]. As other organic acids of grapes, the malic acid is formed in the combustion respiratory process of sugars, in order that the vine to get a part of the necessary energy and by the same process is decomposed to final products.

The two steps of the combustion respiratory process are:

1. 1.glucose + oxygen → oxaloacetic acid + cabon dioxide + water + energy
2. oxaloacetic acid + oxygen → malic acid (intermediary compound) → carbon dioxide + water + energy

During grapes maturation, the second step is made as rapid as the climate is more warm and dry. In this way is explained the fact that on the sands in south Oltenia, in very warm autumms, the malic acid from some variety grapes exists in insignificant contents or it disapears [4].

The climatic conditions influence the organic acids and sugars acummulation in grapes determining in the end the physico-chemical characteristics of wines (sugar concentration, alcohol concentration, total

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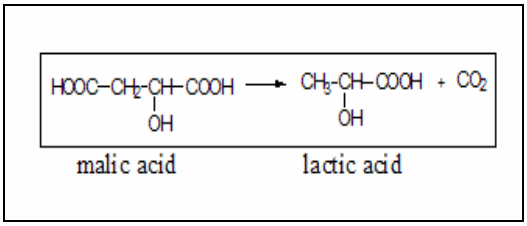
acidity, pH); the physico-chemical characteristics of wines further influence the malolactic fermentation especially when the malolactic fermentation is leaded exclusively by lactic bacteria present in inner micro flora [5].

The malolactic fermentation is a biological deacidifiation process of wine which takes place in the presence of lactic bacteria and consists in the malic acid degradation from wine untill lactic acid and carbon dioxide are formed.



The quality plus that appears after malolactic fermentation is not only due the acidity reduction but also due the lactic acid formation. The lactic acid impresses nicely the papacy taste and assembly better with the astringence given by the phenolic compounds, unlike malic acid, taht gives an immature acidity, an astringent taste and a certain green shade.

So, the rough, bitter taste given by acidity and tannins is gradually erased, being replaced by a delicate and softly taste. Overall, the wines become more harmonious more full and evolved.

In order to determine the evolution of malic acid in grapes and wines can be used more methods [6]:

a) malic acid estimation through paper chromatography (Michaud method);
b) malic acid determination by colorimetric method;
c) malic acid determination through RP-HPLC (high performance liquid chromatography);
d) malic acid determination through enzymatic methods;
e) malic acid determination by using the amperometric biosensor.

In our country, the wine and grapes study from the malic acid content point of view was made in Moldova by dr. Eng. **Ţârdea C. (1966),** in the PhD paper with the title: *"The malolactic fermentation study of wines from Moldova vineyard*" [7].

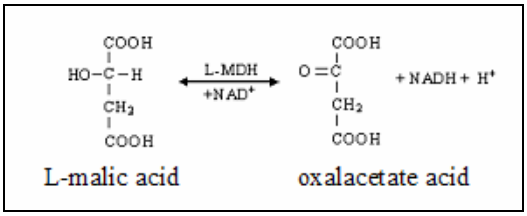
Also, studies concerning the malic acid evolution in the maturation period of grapes were made by **Alexandra Alexiu and colab. (1966)**, the content in malic acid being determined by paper chromatography (Michaud method) [8,9].

At 40 years from theese experiments i porposed myself, using a modern technique to monitor the malic acid evolution during wine storage period in Minis-Maderat vineyards (Arad county) and Recas (Timis county).

The results of this paper work show the fact that there still is an interesting predisposition of certain varieties (Cabernet Sauvignon and Cadarca in Minis vineyard, Burgund Mare and Cabernet Sauvignon in Recas vineyard) to accumulate a larger quantity of organic acids at full maturity, having as consequence obtaining wines with very low pH, difficult to start and finish the malolactic fermentation.

Determining the L-malic acid from wines using enzymatic kits

L-malic acid is oxidised to oxalacetate acid, by NAD (nicotine-adenine-dinucleotide), in the presence of L-malate-dehidrogenase (L-MDH):



The balance of this reaction is on the L-malic acid side. The oxalacetate removal from the reaction leads to the balance movement to oxalacetate acid. In the reaction catalyzed by the enzyme glutamate-oxaloacetate-transaminase (GOT), the oxalacetate acid is transformed into L-aspartame acid in presence of L-glutamic acid.

The formed NADH quantity is stoechiometic with the L-malic acid quantity. The NADH variations are

measured spectrophotometricly in the self absorbence basis at 334, 340 or 365 nm.

Preparing sample for analysis

The present L-malic acid quantity must be between 1 µg and 35 µg (measured at 365 nm) or between 0.5 µg and 20 µg (measured at 340 nm, 334 nm).To obtain a significant difference of absorbence, the sample must be diluted in order to obtain a concentration of L-malic acid of 0.08 and 0.35 g/l or 0.04 and 0.2 g/l.

Table 1. Wine samples dillution before determining the L-malic acid (after Diamedix Diagnostica documentation, 2006)

The estimated quantity of L-malic acid/l		Water dilution	Dilution factor
340 nm, 334 nm	365 nm		
<0.02 g	<0.35 g	-	1
0.2 – 2.0 g	0.35 – 3.5 g	1+9	10
2.0 – 20 g	3.5 – 35 g	1+99	100
>20 g	>35 g	1+999	1000

To determine the content of total L–malic acid (sum of free and esterificated L-malic acid), the white and red wine are submitted to the following treatment: 20 ml of wine are wormed with 6 ml NaOH (2M) during 30 minutes under condensation reflux, stirring from time to time. It is left to cool at 20-25°C, and is neutralized with H_2SO_4 1M, using pH indicator paper. All is transfered in a 50 ml baloon and water is added untill the sign.

Reagents

Kit's composition:

1. Bottle 1 with 30 ml formed from: glicil-glicine buffer, pH 10; L-glutamic acid 440 mg;
2. Bottle 2 with 210 mg NAD, lyophilized;
3. Bottle 3 with 0.4 ml glutamate-oxalacetate-transaminase suspension (GOT), 160 U;
4. Bottle 4 with 0.4 ml solution L-malate dehidrogenase, 2400 Y;
5. Control solution to analyze the L-malic acid.

Apparatus

- spectrophotometer with lenght waves λ = 340-365 nm;
- glass vats with length of 1 cm.

The absorbences differences are determined ($A_1 – A_2$) for standard and for sample. The obtained difference for standard is decreased from the difference obtained for the sample and we obtain ΔA. Depending on ΔA, the malic acid concentration from samples is calculated.

$$\Delta A = (A_2\text{-}A_1)_{sample} - (A_2\text{-}A_1)_{standard}$$

Table 2. Work protocol to determine the L-malic acid (after Diamedix Diagnostica documentation, 2006)

Pipetting	Standard	Sample
Solution 1	1.000 ml	1.000 ml
Solution 2	0.200 ml	0.200 ml
Suspension 3	0.010 ml	0.010 ml
Sample	-	0.100 ml
Bidistilled water	1.000	0.900 ml
Are mixted and the solutions absorbences are read after 3 minutes (A_1). The reaction is started by adding:		
Solution 4	0.010 ml	0.010 ml
Are mixed, we wait 10 minutes untill the reaction is finished and the absorbences of standard and sample are read(A_2)		

3. Results and discussions

In this paper work , monitoring the malic acid evolution during storage period was made for the year 2005 harvest, this year prooving to be difficult to start and finish the spontaneous malolactic fermentation for wines obtained in Minis vineyard and for thode obtained in Recas vineyard.

The centralized data from table 3 and 4 show us that in harvest years 2004, 2005 and 2006, years very different from the climatic point of view, the sugars and acidity content is significantly modified.

The sugars content for the same grape variety is higher in the climatic conditions of harvest year 2004, when the real thermal balance is more than 3700°C, the number of insolation hours is over 1370 hours, and the preciptation level is under 350 mm Hg col.,

comparative with harvest year conditions 2005 when the real thermal balance hardly reaches 3100°C, number of insolation hours does not cross 1300 hours and the precipitations level is way cross 500 mm Hg col.

From the 7 black variety grapes analyzed only to Pinot noir variety, the content in sugars is maintained in a high concentration (202 g/l) in harvest year conditions 2005. All other varieties have modest sugars content comparative with those registered in harvest year 2004, when almost all analyzed varieties have around 200 g/l at full maturity.

Except Pinot noir variety, at all other varieties can be noticed a sugars cintent that decreases in harvest year 2005, comparative with harvest year 2004, which must be put in correlation with less favorable climatic conditions.In harvest year 2006 all grapes varieties except Pinot noir present higher sugars contents comparative with those registered in 2005, but lower with those registered in harvest year 2004.

The acidity content for the same grapes varieties is lower in harvest year 2004, when the thermal balance and number of insolation hours is higher, comparative with the same parameters registered in harvest years conditions 2005 and 2006.

Table 3. Climatic conditions in the vegetation period (1 IV – 30 IX), years 2004, 2005 and 2006 in Minis-Maderat Vineyard

Month	Real thermal balance (°C)		
	2004	2005	2006
April	458.7	175.9	417.3
May	532.1	397.8	501.8
June	621.0	572.1	577.5
July	745.8	688.0	797.8
August	759.1	669.5	692.1
September	625.0	600.7	522.6
Total	3741.7	3104	3509.1

Note: The data were given by the Meteorological Station of Cercetation-Development Resort for Viticulture and Vinification Minis.

From the 7 black grapes varieties analyzed only the Pinot noir and Cadarca varieties are noticed higher total acidity contents in harvest year 2004 conditions comparative with harvest year 2005. All other varieties present higher total acidity values in harvest year 2005 comparative with harvest year 2004. In 2006, are noticed the highest values of total acidity, only the Oporto and Pinot noir are exceptions, the acidity being maintained at reasonable limits.

Table 4. Total acidity (g/l sulphuric acid) at full maturity of black grapes, Minis-Maderat vineyard, harves years 2004, 2005 and 2006

Variety	Total acidity (g/l sulphuric acid)		
	2004	2005	2006
Burgund mare	5.78	6.44	6.81
Cadarcă	6.07	5.88	6.9
Cabernet Sauvignon	6.22	6.75	7.74
Merlot	4.31	5.83	6.12
Pinot noir	5.48	5.39	5.25
Oporto	4.90	4.95	3.92
Blauerzweigelt	5.14	5.29	5.88

Note: The determinations were made in the wine chemistry laboratory from Resort of Cercetation-Development for Viticulture and Vinification Minis.

From the table above results that at winrs obtained in Minis vineyard from harvest 2005, monitored from october untill may next year, the quantities of malic acid were significantly reduced. Also from the data above, results that the malolactic fermentation didn't take place in wines obtained from Burgund mare, Cabernet Sauvignon and Cadarca varieties, at the end of storage period, finding again quantities of 2.5-4.0 g/l malic acid.

The obstruction of malic acid degradation during storage period and implicitly the malolactic fermentation must be put in connection with low pH's of theese wines (pH 2.8-2.88) and contents in sulphuric anhidride quite high (total SO_2 32-48 mg/l).

From wines with malolactic fermentation, the most significant variation of malic acid content during storage period took place at the wine obtained from Blauerzweigelt variety (the concentration of malic acid decreased from 2.1 g/l in october to full degradation at the end of november).From wines that did not have a malolactic fermentation, the most significant variation of malic acid content took place at the wine obtained from Cabernet Sauvignon variety (the concentration in malic acid decreased

from 4.75 g/l in october to 3.5 g/l in may next year). The most insignificant variation of the malic acid content during storage period had the wine obtained from Cadarca variety at which the malic acid concentration not only that did not decrease but registered a light increase from 3.75 g/l in november to 4.0 g/l in may. This increase may be explained by the new transition of salificated acids in free forms, during storage.

Table 5. Wines content in malic acid, during storage, harvest 2005, Miniș vineyard

Variety	Determination*)	Harvest 2005 – basement storage			
		Analyze date (2006)			
		Oct.	Nov.	March	May
1.Burgund mare**	Acidity	5.95	5.20	5.0	4.8
	Malic acid	3.5	3.1	2.5	2.5
	pH	2.84	2.9	2.9	2.9
	Total SO_2	32	28	25	25
2. Merlot	Acidity	4.60	4.20	4.2	4.2
	Malic acid	1.0	0.5	-	-
	pH	2,89	3,12	3,2	-
	Total SO_2	24	20	18	18
3. Cabernet** Sauvignon	Acidity	6.25	6.15	5.5	5.2
	Malic acid	4.75	4.5	3.9	3.5
	pH	2.82	2.9	2.9	2.9
	Total SO_2	45	40	40	-
4. Oporto	Acidity	4.10	3.90	3.7	3.7
	Malic acid	0.5	traces	-	-
	pH	3	3.25	3.4	3.4
	Total SO_2	20	18	18	16
5. Pinot noir	Acidity	4.55	4.0	3.5	3.5
	Malic acid	1.0	traces	-	-
	pH	2.9	3.16	3.2	-
	Total SO_2	20	18	16	16
6. Cadarcă**	Acidity	5.70	5.50	5.1	5.0
	Malic acid	4.0	3.75	3.7	4.0
	pH	2.88	2.9	2.9	2.9
	Total SO_2	49	40	35	35
7. Blauerzweigelt	Acidity	4.80	3.90	3,8	3,6
	Malic acid	2.1	traces	-	-
	pH	3.1	3.3	3.3	-
	Total SO_2	25	23	20	20

*) Acidity is expressed in g/l sulphuric acid, malic acid is expressed in g/l, and total SO_2 in mg/l
**) Wines with unmade malolactic fermentation

Table 6. Wines content in malic acid, during storage, harvest 2005, Recaș vineyard

Variety	Determination*)	Harvest 2005 – basement storage			
		Analyze date (2006)			
		Oct.	Nov.	March	May
1. Burgund mare**	Acidity	7.1	6.627	6.5	6.5
	Malic acid	3.25		2.5	2.5
	pH	2.84	2.9	2.9	2.9
	Total SO_2	45	42	40	40
2. Merlot	Acidity	6.0	6.0	5.3	5.2
	Malic acid	2.7	2.0	traces	-
	pH	3.0	3.1	3.26	3.25
	Total SO_2	27	23	20	20
3. Cabernet** Sauvignon	Acidity	6.0	6.0	5.9	5.9
	Malic acid	3.96	2.8	2.5	2.5
	pH	2.82	2.9	2.9	2.9
	Total SO_2	45	40	40	40
4. Oporto	Acidity	4.27	4.0	3.7	3.7
	Malic acid	0.6	traces	-	-
	pH	3.29	3.3	3.4	3.4
	Total SO_2	27	24	22	22
5. Pinot noir	Acidity	5.88	5.3	5.12	5.12
	Malic acid	0.73	traces	-	-
	pH	3.0	3.1	3.2	3.2
	Total SO_2	27	25	23	23

*) Acidity is expressed in g/l sulphuric acid, malic acid is expressed in g/l, and total SO_2 in mg/l
**) Wines with unmade malolactic fermentation

In conclusion, at wines obtained from Burgund mare, Cabernet Sauvignon and Cadarca varieties a „stabilization" of the malic acid degradation dynamics was produced, by blocking the malolactic fermentation so that doesn't take place any significant diminuation of malic acid during storage period.

From the data exposed in the table above results that the malolactic fermentation didn't take place in wines obtained Burgund mare, Cabernet Sauvignon at the end of storage period, finding again quantities of 2.5 g/l malic acid.

The obstruction of malic acid degradation during storage period and implicitly the malolactic fermentation must be put in connection with low pH's of theese wines (pH 2.9) and contents in sulphuric anhidride quite high (total SO_2 40-45 mg/l).

From wines with malolactic fermentation, the most significant variation of malic acid content during storage period took place at the wine obtained from Merlot variety (the concentration of malic acid decreased from 2.7 g/l in october to full degradation at the end of march).

From wines that did not have a malolactic fermentation, the most significant variation of malic acid content took place at the wine obtained from Cabernet Sauvignon variety (the concentration in malic acid decreased from 3.96 g/l in october to 2.5 g/l in may next year).

The most insignificant variation of the malic acid content during storage period had the wine obtained from Burgund mare variety which the concentration in malic acid decreased from 3.25 g/l in october to 2.5 g/l in may next year.In conclusion, at wines obtained from Burgund mare and Cabernet Sauvignon grapes varieties varieties a „stabilization" of the malic acid degradation dynamics was produced, by blocking the malolactic fermentation so that doesn't take place any significant diminuation of malic acid during storage period.

4. Conclusion

The variation of the malic acid content depends on variety and climatic conditions (harvest year). From the results obtained in years 2004, 2005 and 2006 is noticed that the grapes of Cabernet Sauvignon at Minis and Burgund mare at Recas present, at aturity, high contents in malic acid and during the storage period do not take place significant decreases of malic acid, through a bioconversion in lactic acid. We could conclude that exists a direct connection between the variation of malic acid content and grapes varieties, the same vrieties (Cabernet Sauvignon at Minis and Burgund mare at Recas) manifesting in different climatic years a high specificity for accumulating high quantities of malic acid at maturity and for a insignificant reduction of malic acid during wine storage. From the made experiments to follow the malic acid evolution in grapes and wines obtained in Minis and Recas vineyards we can conclude:

- climatic conditions influence the malic acid accumulation in grapes and it's evolution during maturation; in harvest years 2005 and 2006 (rich in precipitations, with a real thermal balance relatively low, low number of insolation hours during vegetation period) a large amount of malic acid is accumulated at all studied varieties comparative with harvest year 2004;
- the massive accumulation of malic acid in grapes, in conditions of high acidity and low pH, determines an obstruction of malic acid degradation through malolactic fermentation, during vinification and conservation period of wines; Un-degraded quantities may present risk factors to wine stability;
- there is a direct correlation between variety and content in malic acid of grapes; grapes from Oporto and Pinot noir variety accumulate at maturity, reduced quantities of malic acid, that can be degraded easily during the malolactic fermentation, while grapes from Cabernet Sauvignon and Burgund mare varieties are the first in what

concerns the malic acid content of grapes in maturity phase, the malic acid reduction being insignificant not even during conservation period.

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