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EVOLUTION OF CONTENT IN ORGANIC ACIDS OF RED WINE IN RELATIONSHIP WITH THE VARIETY AND PEDOCLIMATIC CONDITIONS FROM VALEA CĂLUGĂREASCĂ VINEYARD

Elena COARFĂ, Mona Elena POPA

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăşti Blvd., District 1, Bucharest, Romania

Corresponding author email: elena.coarfa@gmail.com

Abstract

The acidity of grapevine products has a major influence on several aspects. It is involved in the microbiological, tartan and protein stability, determines the color of wine and the speed of maturation and ageing, but in particular, determines the taste balance. This paper aimed to present the evolution of content in organic acids during the period 2017-2019 in the Valea Călugărească vineyard from three grapes cultivars: Cabernet Sauvignon, Merlot and Fetească Neagră which were analysed through Ion Chromatography (IC-MS) method. The experimental measurements established that the ripe grapes and the must contains in the beginning higher amounts of malic acid: for example in 2017, 1120 mg/l malic acid in November and only 324 mg/l in March 2018 comparing with next harvest when the malic acid content was in November 2018 - 1389 mg/l and decreased to 310 mg/l in March 2019. In terms of ecoclimatic conditions 2017 was characterized by a moderate heliothermal regime, especially in April and May, when the average annual values were overcome compared to 2018 when the heliothermic regime was high and based on relatively low water resources.

***Key words**: malolactic fermentation, organic acids, red wine.*

INTRODUCTION

The agricultural sector, including grape and wine, is sensitive to climate change and the associated extreme weather. The global wine industry is already experiencing impacts, including earlier growing seasons, changes in precipitation patterns, and increased frequency and intensity of extreme weather events, all of which are affecting wine quality. To adapt to the challenges and opportunities created by climate change industry stakeholders must develop and sustain a level of adaptive capacity (Pickering et al., 2014).

Vineyards in Romania grow under temperate-continental climate, with frequent occurrences of extreme climatic conditions and with some regional variations. Prolonged high temperature during the maturation of grapes determined a high level of sugar accumulation, the loss of acids through respiration (Bucur & Dejeu, 2016).

Romania is well known and appreciated for her wines, occupying 13th in world rankings of winemakers, with approximate 5 million hl of annual wine product (Visan et al., 2017). Grapevine (*Vitis vinifera* L.) is one of the most important crops in Romania. According to the national statistical database published by the National Institute of Statistics, Romania has cultivated 177,000 hectares of grapevine in 2017 (NIS 2018, published in 2019) (Chireceanu et al., 2019).

The determination of organic acids in foods and beverages provides relevant information from the standpoint of monitoring the fermentation process, checking product stability, validating the authenticity of juices or concentrates and studying the organoleptic properties of fermented products (Castineira et al., 2002).

The primary varietal flavours are accumulated in the skin and grape through specific processes of the metabolism. They are determined by the genetic nature of the varieties, and by the specific pedological and climatic factors (Palade & Popa, 2015).

The character of wine is affected by range of factors in which we could include the location of vineyard, grapes varieties, agricultural engineering, the time of harvesting and the production technology. In case of lack of sun and also cold summer weather in our geographical conditions we might encounter the production of lower quality grapes, which results in a lower content of fermentable saccharides and a higher content of organic acids, mostly of malic acid which presence is not desired. However, there is a possibility to decrease the excessive content of acids. First of all, it is related to biodegradation of acids when lactic fermentation bacteria transform malic acid into lactic acid (Kučerová & Široký, 2011).

The quality of red wine and their organoleptic characteristics are nowadays well defined when considering the effects of malo-lactic fermentation (Alonso et al., 1998).

Organic acids are important for the wine stability. Therefore, their analysis in wines is required for quality control as well as to check the evolution of acidity during the different stages of winemaking (starting from the grapes juices, continuing to the alcoholic fermentation and wine stabilization processes), since important changes in wine would be detected by alterations in the acid content (Robles et al., 2019).

Organic acids and total acidity play a pivotal role in wine sensory perception, and directly influence the overall organoleptic character of wines (Chidi et al., 2018).

Organic acids play a major role in the microbiological and physicochemical stability and sensory properties. Monitoring of multiple organic acids simultaneously is often essential and is typically conducted with ion/liquid chromatography (Ohira et al., 2014).

The main organic acids found in grapes are tartaric, malic and in a smaller extent, citric acid. Other organic acids such as, succinic, acetic, lactic, fumaric acids can also be present (Cosme et al., 2017).

In grapes, organic acids are involved in glycolytic and shikimic pathway, but can also result from the Krebs cycle or the glyoxylic pathway, where mostly they remained unchanged (compared to those from the grapes) (Niculaua et al., 2014).

Organic acids belong to the most important components that complete the overall character and taste of wine. Ripe grapes contain major amounts of tartaric and malic acids, in a minor extent, citric acid is present, too. In addition to the above-mentioned organic acids, the wines contain the products of yeast and malolactic fermentation such as acetic, lactic and succinic acids (Zeravik et al., 2015).

MATERIALS AND METHODS

Wine is an alcoholic beverage obtained exclusively from total or partial alcoholic fermentation of crushed fresh grapes or grape must and continues to surprise consumers with its incredible flavors and aromas (Raducu et al., 2019).

Experiments were conducted during the period 2017-2019 in the Valea Călugărească vineyard from three grapes cultivars: Cabernet Sauvignon, Merlot and Fetească Neagră which were analysed through Ion Chromatography (IC-MS) method.

Ion chromatography (IC) is a standard method for wine analysis. This technique is dedicated to develop selective, sensitive, robust and as fast as possible applications for the detection of organic acids in matrix-loaded samples. Only small sample volumes in the µl range are necessary. Automatic sample preparation (e.g. Inline Ultrafiltration, Dilution or Dialysis) guarantees reproducible results and minimum time for lab work.

The advantage of ion chromatography is that chemically similar substances can be determined simultaneously in a single analysis. Acids are an important component in wine. They preserve the wine and give it expression and character. Wine contains several kinds of acids. The most important ones are tartaric acid, malic acid, and lactic acids.

Organic acids are omnipresent in winemaking. Some of them are present already in the grape while others appear during fermentation. The sum of organic acids and their composition have a direct influence on the taste of the respective wine.

Settings of the mass spectrometer were optimized for small measurements. By an adapted tuning, low concentration levels of ions can be detected. A diverter valve between IC and MS was used to only switch the flow to the MS when analytes of interest are expected. The conductivity signal is also a good tool to monitor the status of the system. During instrument calibration, automated sample preparation or the elution of matrix components, the flow is switched to the waste in order to avoid contamination of the MS detector. Metrohm IC and Waters MS are easily operated under just one software. Filtration is generally

recommended in IC to avoid blockages in the injection valve, in the capillary connection, and in the column. It is indispensable for samples containing particles. Inline Ultrafiltration combines sample injection directly with filtration. The two parts of the ultrafiltration cell are separated by a filter membrane. On one side, the sample is carried through the cell at a high flow rate. On the other side, some of the sample is drawn off through the membrane and transported to the injection valve.
The formation of filter cake is prevented by continuous flushing of particles out of the cell at a high flow rate. For organic acid analysis in wine we used: column: Metrosep A Supp 5 - 100/4.0; eluent: 5.0 mmol/l Na_2CO_3 + 5.0 mmol/l $NaHCO_3$, 1.0 ml/min; sample volume: 20 µl, inline dilution: 1:10.

RESULTS AND DISCUSSIONS

In order to find out the correlation of organic acid content with climatic conditions, a picture for the experimented years is presented below. 2017 was characterized by a moderate heliothermic regime against a background of rich water resources, especially in April and May, when it was overcome multiannual values. Pluviometric data indicate the average annual amount of precipitation being between 510-590 mm, with large variations in them from one year to another. The vegetation period (April), it started with temperatures lower than the normal (11.7ºC), and a higher water regime, confronted with multiannual values.
As shown in Table 1, the thermal regime is as follows:

Table 1. Thermal regime from April-November 2018/ April-October 2017

Month	Medium temp. ºC Apr.-Nov. 2018	Medium temp. ºC Apr.-Oct.2017	Precipitation mm Apr.-Nov. 2018	No. Precipitation days - 2018	Precipitation mm Apr.-Oct. 2017	No. Precipitation days – 2017
April	16.4	11.7	2.0	3	44.8	9
May	19.7	17.5	16.8	6	67.3	9
June	22.3	21.5	79.4	17	81.5	6
July	22.2	23.6	111.8	8	75.8	11
August	24.1	23.3	28.2	3	62.7	6
September	18.2	18.1	39.2	6	54.4	9
October	14.3	12.2	20.2	2	46.2	4
November	4.8	-	19.2	10	-	-

Compared with 2017 the year of 2018, was characterized in general by a high heliothermal regime amid relatively low water resources. Low water registered regime in April led to an uneven start to vine vegetation, there was a delay in the development of shoots in the first stages of vegetation. Low thermal registered regime in April resulted in a late start (about 10 days) and the uneven growth of the vine, which has been recovered late in May and June.
Determination of organic acids plays an important role in the evaluation of the development of wine in terms of chemistry and biochemistry.
Grapes and wine contain a variety of organic acids, in concentrations ranging depending on the variety, climate, soil, technological process applied. The organic acids from must are presented in concentrations of 3-12 g/l and the most common are: malic, tartaric, citric, ascorbic, succinic, lactic, glutaric, fumaric, pyruvic. It can be see the changes in organic acids content from Tables 2 to 5.
Tartaric and malic acid account for 70-90% of the total acids present in the grape berry, existing at roughly a 1:1 to 1:3 ratio of tartaric to malic acid (Kliewer, 1966; Lamikarna et al., 1995). For this reason, citric acid with acids like tartaric and malic are considered major products of wine-making. Tartaric acid concentration in the grapes is determined by genetic factors, but also environmental, varying between 5 and 10 g/l. In the year of 2017 the results obtained for organic acid content, before malolactic fermentation, in our experimental settings are presented in Table 2.

Table 2. Acid content in red wines before malolactic fermentation

November 2017 IC-DVV Valea Călugărească

No.	Grape variety	Citric acid (mg/l)	Tartaric acid (mg/l)	Malic acid (mg/l)	Succinic acid (mg/l)	Acetic acid (mg/l)	Lactic acid (mg/l)
0	**1**	**2**	**3**	**4**	**5**	**6**	**7**
1	**Cabernet Sauvignon (CS)**	150	4328	1120	623	354	245
2	**Merlot (M)**	120	4102	1015	597	395	210
3	**Fetească Neagră (FN)**	176	3975	1235	629	401	238

Table 3. Acid content in red wines after malolactic fermentation

March 2018 IC-DVV Valea Călugărească

No.	Grape variety	Citric acid (mg/l)	Tartaric acid (mg/l)	Malic acid (mg/l)	Succinic acid (mg/l)	Acetic acid (mg/l)	Lactic acid (mg/l)
0	**1**	**2**	**3**	**4**	**5**	**6**	**7**
1	**Cabernet Sauvignon (CS)**	130	4010	324	604	525	625
2	**Merlot (M)**	115	3980	296	585	530	710
3	**Fetească Neagră (FN)**	158	3725	308	630	525	735

Tartaric acid decreases during ripening of the grapes because of the dilution effects resulting from the growth of the grape berry (Table 2 and Table 3). Tartaric acid, naturally present in grape, must and wine is the L(+)-isomer, resulting from the biochemical conversion of glucose. In must the tartaric acid is founded in concentrations of 1-7 g/l. It is the best of the mash acid. The value of tartaric acid in Feteasca neagra was the smallest in march 2018 - 3725 mg/l and decreased from 3975 mg/l. Feteasca neagra is an authentic grape and wine specific to Valea Călugărească vineyard and reddish-brown soil.

The malic acid peak concentrations of 20 g/l in the grapes at the time they give the berry of. The amount of malic acid decreases with increasing temperature, so that the time of harvesting down malic acid concentration somewhere between 1-7 g/l.

Malic acid is a very popular substrate for yeast, especially for the malolactic bacteria. Therefore, depending on the chosen wine-making technology the concentration of malic acid in wine can greatly decrease (Table 2 and Table 3). The malic acid is the most important metab olized acid by lactic acid bacteria. For Cabernet Sauvignon we can see that from 1120 mg/l in November 2017 the concentration decreased at 324 mg/l in march 2018. Same happened with values of Fetească Neagră and Merlot.

Citric acid is found in low concentrations in grapes, 0.3-0.5 g/l, in general in the skin berry, and in the pulp, and its concentration remains low throughout the different stages of maturation. Citric acid in the wine is found in relatively low concentrations, ranging from 150-300 mg/l., rarely exceeding 700 mg/l. The Merlot variety had the smallest value of citric acid 158 mg/l in march 2018.

As we can see in the Figure 1 after malolactic fermentation is a slight decrease in acidity, because malic acid in the presence of lactic acid bacteria is transformed into lactic acid, wine becomes soft, smooth and non-invasive.

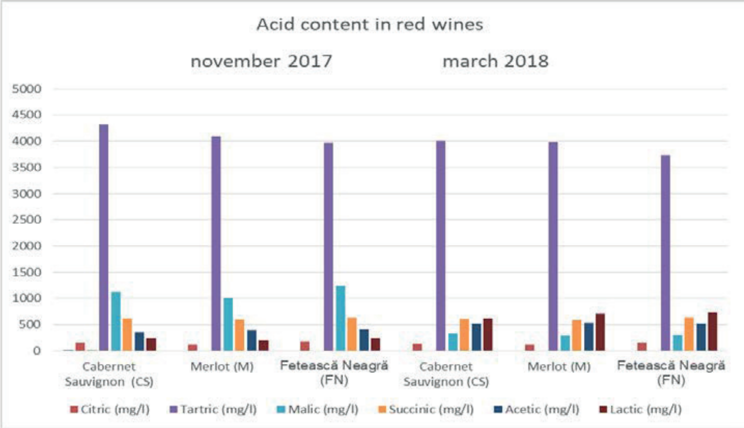

Figure 1. The evolution of acid content in red wines before and after malolactic fermentation

Malic acid is presented more in wines obtained from grapes insufficient development. Lactic acid is a secondary-product of the alcoholic fermentation and a principal-product of malolactic fermentation. As we can see the values of lactic acid rise as a sign of passing wine through malolactic fermentation. For example, the largest increase was recorded for Merlot and Fetească Neagră, almost 500 mg/l, from 210 mg/l to 710 mg/l for Merlot. The correlation value is correlated with the strength of succinic acid alcohol, succinic acid, representing 10% of alcoholic strength in terms of volume. Acetic acid is formed during the alcoholic fermentation and has a particular importance for the organoleptic characteristics of the wine, in the wine is the main volatile acid. For Cabernet Sauvignon variety we can observed an increase from 354 mg/l in November 2017 to 525 mg/l in march 2018, under the effect of yeast strain. The year of 2018 comparative with 2017 - experimental measurements established that the ripe grapes and the must contains in the beginning higher amounts of malic acid: for example in 2017, 1120 mg/l malic acid in November and only 324 mg/l in March 2018 comparing with next harvest when the malic acid content was in November 2018 - 1389 mg/l and decreased to 310 mg/l in March 2019. In 2018 we can notice a higher value of malic acid for all the three varieties: Cabernet Sauvignon, Merlot and Fetească Neagră, before malolactic fermentation caused by heliothermal regime high in July, August and September, against a normal rainfall and very low in September, who led to a better accumulation of sugars in grapes (Table 4 and Table 5). We can say that the pedoclimatic conditions and the soil influences the characteristics of grapes, musts and finally the acid content of wines before and after malolactic fermentation and the quality of red wines. From the Tables 4 and 5 representing the acids content before and after malolactic fermentation, it seems:

- for Cabernet Sauvignon the citric acid had a smaller value in November 2017 - 150 mg/l compered to November 2018 - 160 mg/l and it fell after malolactic fermentation in March 2019 - 157 mg/l; 3 mg/l difference in 2018 to 20 mg/l difference in 2017; for Merlot the difference remained the same, but for Fetească Neagră the differences was from 18 mg/l in 2018 to 1 mg/l in 2019;
- the values of tartaric acid in 2017 decreases during ripening of the grapes, for all the three varieties: Cabernet Sauvignon, Merlot and Fetească Neagră, because of the dilution effects resulting from the growth of the grape berry. Comparing with 2018 when the berries was little, but reach in sugars, because of the low thermal regime registered in April the differences between values were for example only 322 mg/l for Fetească Neagră in 2018-2019 to 250 mg/l for Fetească Neagră in 2017-2018;

Table 4. Acid content in red wines before malolactic fermentation

November 2018 IC-DVV Valea Călugărească

No.	Grape variety	Citric acid (mg/l)	Tartaric acid (mg/l)	Malic acid (mg/l)	Succinic acid (mg/l)	Acetic acid (mg/l)	Lactic acid (mg/l)
0	1	2	3	4	5	6	7
1	**Cabernet Sauvignon (CS)**	160	4475	1389	678	370	290
2	**Merlot (M)**	155	4120	1186	650	330	273
3	**Fetească Neagră (FN)**	148	4022	1015	608	315	249

Table 5. Acid content in red wines after malolactic fermentation

March 2019 IC-DVV Valea Călugărească

No.	Grape variety	Citric acid (mg/l)	Tartaric acid (mg/l)	Malic acid (mg/l)	Succinic acid (mg/l)	Acetic acid (mg/l)	Lactic acid (mg/l)
0	1	2	3	4	5	6	7
1	**Cabernet Sauvignon (CS)**	157	4008	310	650	548	810
2	**Merlot (M)**	150	3980	302	639	527	798
3	**Fetească Neagră (FN)**	147	3700	296	610	502	790

- the succinic acid values it remains almost unchanged during the aging step and aging of wine, for example for Merlot from 650 mg/l in November 2018 to 639 mg/l in march 2019, a difference of 11 mg/l;
- for Fetească Neagră the value of acetic acid increase from 315 mg/l in November 2018 to 502 mg/l in march 2019, a difference of 187 mg/l with 63 mg/l more than 2017-2018 values;
- the values of lactic acid rise as a sign of passing wine through malolactic fermentation. For example, the largest increase was recorded for Feteasca neagra from 249 mg/l in November 2018 to 790 mg/l in march 2019, a difference of 541 mg/l, more than 2017-2018.

In Figure 2 compared to Figure 1 we can notice difference, but in the same time similarities of the acids values from the three varieties: Cabernet Sauvignon, Merlot and Fetească Neagră, based on different factors such as temperature, light, fertilization, water supply, acidity and technological process applied that affect the malolactic fermentation who is almost finished, the ultimate goal is getting a quality red wine. The tartaric acid has the major values decreases during maturation.

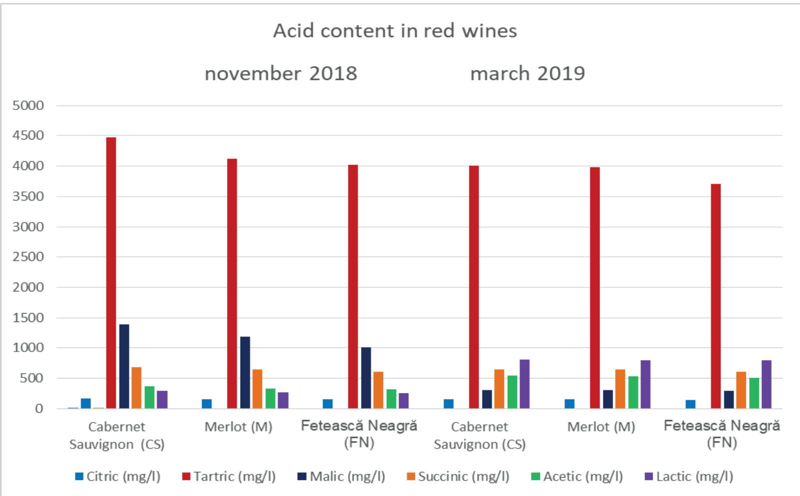

Figure 2. The evolution of acid content in red wines before and after malolactic fermentation

Organic acids in wine derive either directly from the grape, or are the result of microbiological activities that take place before, during or after alcoholic and malolactic fermentation. While the most commonly measured feature of wine acidity is the total acidity (TA) and pH, some organic acids are important markers for fermentation management and wine flavour and aroma. Malic acid is monitored to measure The progress of malolactic fermentation, acetic acid is monitored as an indicator of fermentation problems or of spoilage, and citric acid may be added to adjust acidity and chelate metal ions to prevent nutrients from precipitation resulting from the interaction of nutrients with metal ions, such as iron precipitating with phosphorus (Fowles,1992).

Tartaric acid is not metabolised by grape berry cells via respiration in the same manner as malic acid, and the level of tartaric acid in the grapes remains relatively consistent throughout the ripening process. The concentration of tartaric acid in grapes depends largely on The grape variety and soil composition of the vineyard (Ribéreau-Gayon et al., 2006).

CONCLUSIONS

The meteorological data recorded on a 2-year study, for Valea Călugărească vineyard centre, shows that the average annual temperature is around 10.8°C. Winters are relatively short and cold becomes more intense in January and the first half of February. Frost-free period is an average of 202 days. The average temperature of the hottest (July) is +22.4°C, showing variations between 20.7°C and +25.6°C. The number of days with an average temperature of over 10°C range between 175-226. The amount of active temperature is between 3300 and 4040. The total number of hours of sunshine is 2146. The phase of maturation of the grapes (the months of August-September) rainfall is

lower, favoring the ripening.

The organic acids content in wine is related to the region and it is also associated with climate during the growth and ripening of grapes. In Valea Călugărească vineyard were the climate is warmer than in the others regions and the soil helps to obtain good quality red wines, the values of tartaric acid in 2017 decreases during ripening of the grapes, because of the dilution effects resulting from the growth of the grape berry for all the three varieties, comparing with 2018 when the berries was little, but reach in sugars, because of the low thermal regime registered in April the differences between values were for example only 322 mg/l for Fetească Neagră in 2018-2019 to 250 mg/l for Fetească Neagră in 2017-2018. The excess of malic acid gives wine a distinctly more sour character, the values of malic acid were a little bigger in 2018 than in 2017, before malolactic fermentation caused by heliothermal regime high in July, August and September, against a normal rainfall and very low in September, who led to a better accumulation of sugars in grapes. The most common process is malolactic fermentation; the malic acid becomes converted to softer lactic acid, the values of lactic acid rise, in 2018 more than in 2017, as a sign of passing wine through malolactic fermentation. Succinic acid is stable and durable in time, it remains unchanged during the aging step and aging of wine, for example in Fetească Neagră from 629 mg/l in November 2017 to 630 mg/l in march 2018.

In conclusion, the content of organic acids depends by factors like: ecoclimatic conditions, region, soil, the time of harvesting and the production technology, because they determined the quality of red wine and their organoleptic characteristics, determines the taste balance.

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