ORIGINAL PAPER

CHARACTERIZATION OF SEVERAL ROMANIAN WINES FROM THE DOBRUDJA HILLS REGION

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Abstract. *Wine is a polydisperse hydroalcoholic solution with a physical-chemical composition depending on the variety, ecological factors, the degree of maturation and the health status of the grapes, the conditions of production, storage and age. As the purpose of this paper is to provide the physical and chemical characterization of different types of wines and, as such, the alcohol concentration, the total and volatile acidity, the total content of phenolic compounds and the Fe (II) concentrations have been determined. The iron content of wines was obtained using molecular absorption spectrometry in UV-VIS applying the standard addition method. The results of iron content range from 1.66 mg/L to 2.36 mg/L. To verify the veracity of the results, control charts were applied: diagram X and R. The analysis of the control diagrams shows that the quality control conditions of the method to determine iron in wine, reagents, reference materials and results are reliable.*

Keywords: *wines, phenolic compounds, iron.*

1. INTRODUCTION

The Romanian wine sector is one of the largest in the EU as Romania has an ancestral tradition in grape harvesting and wine production due to appropriate weather conditions, relief, and soils. In Romania, the area cultivated with vines occupies about 180,000 hectares, while our country holds the 5th place at the level of the European Union and the 11th place in the world as wine-growing area and the 6th for the production of grapes and wine. Romanian wines are appreciated in the country and abroad. In recent years there has been a revival of Romanian viticulture and winemaking due to the conversion program and the massive investments in this sector [1].

In 2018, Romania's wine exports registered a 24% increase, exceeding 30 million €, according to data presented by Eurostat [2]. Most of the exports went to the EU Member States, while exports to non-EU countries were down.

At a national level, Romania encompasses eight wine-growing regions named in accordance with their geographical position: Crișana and Maramureș Hills, Banat Hills Wine Region, the Wine Region of the Transylvanian Plateau, the Wine Region of the Muntenia and

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Oltenia Hills, the Winery Region of the Sands, the Wine Region of the Dobrudja Hills, the Wine Region of the Moldavian Hills and the Wine Region of the Danube Terraces.

The analysis applied to wines follows the knowledge of their chemical composition or of important chemical and physico-chemical indices to guide the evolution of the wine and the quality of the product. Investigations of physico-chemical indices of wines such as density, alcohol concentration, acidity, extract, glycerin content, and ash may provide some information on the authenticity of wines and the existence of possible forgeries.

The total acidity of wines has two components: fixed acidity and volatile acidity. The former is represented by non-volatile acids (tartaric, citric, etc.) and comes from the raw material and to a lesser extent from the normal fermentative processes. The volatile acidity consists of the sum of the volatile acids, it comes partly from the raw material, from the alcoholic fermentation of the must and from the alteration processes.

In recent years, polyphenols, classes of natural compounds, secondary plant metabolites, have been known for their color (anthocyanins) as well as for their positive influence on human health due to their antioxidant activity [3-9].

As the purpose of the paper is to provide the physical and chemical characterization of different types of wines, the total and volatile acidity, the alcohol concentration, the total content of phenolic compounds and the Fe(II) concentrations have been determined. The iron was determined by molecular absorption spectrometry in UV-VIS using the standard addition method.

To verify the veracity of the results, control diagrams were applied: the X and R diagrams. The concentration of Fe(II) in the control sample was measured for 14 days. Iron content in wines is an important parameter in controlling the quality and stability of wines. The total iron concentration should not exceed 5 mg/L according to the European legislation. Iron is a natural component in wines and at low concentrations is involved in the metabolism as an enzyme and solvent activator. However, increased amounts of iron are responsible for the formation of the so-called “cases” or “hazes” in wines - continuous oxidation processes, loss of aromatic freshness and turbidity. In most cases, the precipitates formed are complexes of Fe(III) with tannins, phytates and proteins or precipitates different from Fe(III) - phosphates of wines. Therefore, the routine control of the iron content is provided through the entire manufacturing process.

2. MATERIALS AND METHODS

2.1. STUDIED SAMPLES

The studied wine samples were from the Region of the Dobrudja Hills: white wine, red wine and rosé wine from a ten years old vineyard. The white wine was sampled from Sauvignon Blanc grapes, the red wine from Merlot grapes and the rosé wine from Cabernet Sauvignon grapes.

2.2. METHODS

Determination of the alcohol concentration

The alcoholic concentration was measured using the picnometer method and the calculation of alcoholic concentration was based on the relative density determination and reading from tables of the alcoholic concentration values at 20ºC, expressed in % vol. function relative density of obtained distillate.

Determination of the total acidity

The total acidity of the wine was determined by the titrimetric method, STAS 6182-1:2008 [10]. The principle of this method consists in the titration or neutralization of the acids from wines with a sodium hydroxide solution with known normality and factor, in the presence of phenolphthalein as an indicator, after the removal of carbon dioxide.

Results were calculated using the formula:

$$\text{Total acidity (tartric acid)} = V_1 \cdot 0.0075 / V \cdot 1000 \text{ [g/L]} \quad (1)$$

V_1 = volume of NaOH 0.1N,
V= volume of wine
0.0075 - corresponding amount of tartaric acid 1ml of NaOH 0.1N

Determination of the volatile acidity with the GlassChem oenological system

The steam was blown through a wine sample to heat it to the boiling point of the water, converting volatile acids (formic, acetic, butyric acid) into vapors. These vapors were entrained in bubbling steam through the sample and transported through the distillation tube. Volatile acids and steam entered the top of the refrigerant and flew into the Erlenmeyer flask.

The total volatile acid content of the condensed liquid was determined by titration with sodium hydroxide, using phenophthalein as an indicator. The volatile acidity was calculated with the following relation:

$$VA = \frac{(V_2 - V_1) * 0{,}006 * 1000}{V_p} \quad (2)$$

where:
V_1 - represents the volume of 0.1N NaOH consumed when titrating distilled water;
V_2 - represents the volume of 0.1N NaOH consumed when titrating the distillate;
0.006 - represents the amount of acetic acid (g) corresponding to 1mL 0.1N NaOH;
V_p - volume of wine sample (mL).

Determination of the polyphenolic compounds content

The total polyphenolic compounds content was measured using molecular absorption spectrometry in the visible range. A double-beam Thermo Spectronic UV–Vis spectrophotometer from Helyos, connected to computer with UV-PC software was used for absorption spectra registration. For intermediate precision study, a different Thermo Spectronic UV–Vis spectrophotometer connected to computer with UV-PC software was

used. Both the instruments have an automatic wavelength accuracy of 0.1 nm and matched quartz cells of 10mm (1.0 cm) cell path length. The total polyphenolic compounds have been measured using Folin Ciocalteu method. The method was tested and validated in a previous paper [11].

Determination of Fe (II) with 1,10 phenanthroline by the standard addition method

To analyze Fe (II), the samples were digested using the Digesdahl apparatus provided by HACH. 1,10-phenanthroline (*o*-phenanthroline) is a very sensitive reagent being used especially in the analysis of iron in the form of traces. Iron, which can be found in the sample in two oxidation states: II and III, must be quantitatively converted to oxidation state Fe(II); this species is the only one that forms an intense complex colored red with the *o*-phenanthroline reagent. The oxidation state is adjusted with hydroxylamine hydrochloride. Although there are other methods of reducing Fe(III) to Fe(II), hydroxylamine hydrochloride is the best because it does not interfere with the absorbance measurements.

Solutions A and B were prepared; solution A contained only the unknown sample, and solution B the unknown sample and a measured volume of standard Fe(II) solution.

Solution A. In a 50 mL volumetric flask, 2 mL of the test sample (Vnec) was pipetted, 5 mL of sodium acetate, 5 mL of hydroxylamine hydrochloride solution and stirred for homogenization. It was waited for 5 minutes for stabilization, then added 5 mL of *o*-phenanthrol reagent. Then we waited for 10 minutes for color stabilization. It was diluted to volume with distilled water and read the absorbance (Anec) at 510 nm, on the spectrometer, against a reference solution (distilled water).

Solution B. In a volumetric flask, with the same volume, 2 mL of sample to be analyzed (Vnec) and 2 mL of standard solution of Fe(II) (Vst) were added, so that the resulting concentration was maximum 1.5 Cx. The reagents were then added in the order shown in solution A. The absorbance of solution B (Anec + st) was read against distilled water.

The concentration of iron in the studied sample was calculated with relation:

$$C_{nec} = \frac{A_{nec} * V_{st} * c_{st}}{V_t * A_{nec+st} - A_{nec} * V_{nec}} \quad (3)$$

where:
c_{st} - standard concentration, 10^{-3} M;
Vst - volume of standard solution, 2 mL;
Vt - total volume, 4 mL;
Vnec - volume of solution to be analyzed, 2 mL.

3. RESULTS AND DISCUSSION

Table 1 presents the results obtained from the determination of the alcohol concentration, the total and volatile acidity, and the total content of phenolic compounds in the studied wines.

Table 1. Results of alcohol concentration, the total and volatile acidity, the total content of phenolic compounds in the studied wines

Studied parameters	Type of wines		
	Red	White	Rosé
Alcohol concentration [%]	13.2	12.5	13.5
Total acidity [g / L $C_4H_6O_6$]	6.5	7.45	6.0
Volatile acidity [g/L CH_3COOH]	0.43	0.38	0.37
Total content of phenolic compounds [mg GAE/L]	51.28	41.85	45.23

From variety to variety, the alcohol content of wine can differ quite wildly. Some wines can have a 12 percent ABV (alcohol by volume), which is generally what most people expect from wine, but others can have an ABV as high as 20 percent – that is almost like drinking straight whiskey. The alcohol concentration of studied wines was between 12.5 and 13.5%, in concordance with the wine legislation [12].

If all the individual acids in a wine are expressed as tartaric acid equivalents and summed, the value for the total acid concentration will be greater than the value for the titratable acidity concentration. This is because the total acidity is the sum of all the organic acid anions in solution, while the titratable acidity measures the total available hydrogen ions in solution. The titratable acidity will always be less than would be expected from the organic acid concentration [13]. This is because total acidity analysis measures both the dissociated and undissociated forms of each individual acid. The total acidity of the studied wines was 6.5 g/L $C_4H_6O_6$ for red wine, 7.45 g/L $C_4H_6O_6$ for white wine and 6 g/L $C_4H_6O_6$ for rose wine in the concordance with the wine's legislation that provide a minimum of 3,5 g/L $C_4H_6O_6$ for wines.

Volatile acidity refers to the steam distillable acids present in wine, primarily acetic acid but also lactic, formic, butyric, and propionic acids. In the legislation of our country [6] it is provided that the volatile acidity of the wines should not exceed 1.08 g /L $C_2H_4O_2$ for white and rosé wines and 1.2 g/L $C_2H_4O_2$ for red wine. Compared with the experimental results, all values were not exceeded the limits imposed.

The results obtained for the determination of the total content of polyphenolic compounds in studied wines, analyzed by the Folin-Ciocalteau method, recorded values for red wine of 51.28 mg GAE/L, white wine 41.85 mg GAE/L and rose wine 45.23 mg GAE/L. These differences between the samples could be the result of a better extraction of the phenolic compounds, due to the longer contact time with the skin and the grape seeds, the fermentation conditions and the temperature, for the red wines, as opposed to the white wines. Also, the amounts of total phenolic compounds vary considerably in different types of wines, depending on the grape variety, environmental factors in the vineyard and the wine processing techniques [14-17].

Fe (II) analysis of wine samples

In order to determine the Fe(II) concentration, 6 measurements were made for the same wine sample. In Table 2 the means of the results obtained for the wine samples analyzed are presented.

Table 2. Mean values of Fe (II) concentrations from the analyzed samples

Samples	C [mg/L]
White wine	1.66
Red wine	1.37
Rose wine	2.36

The results range from 1.66 mg/L to 2.36 mg/L. The iron concentration in the analyzed samples is within the limits of the European legislation (5 mg/L), the rosé wine having the highest concentration of Fe(II).

Control diagram

A control chart is a way of presenting data in which control values are graphically represented in relation to a number of results (or time). The diagrams are used in quality control. They describe individual measures that relate to the quality of individual samples or batches of samples.

The results of the control sample were calculated, this calculation being carried out as for an ordinary sample (using the usual units of measurement). For the determination of iron the standard addition method was used: *solution A* of 0.5 mM concentration and *solution B* concentration 1.02 mM.

The iron concentration was determined for 14 days for the control chart. The results are passed on the control chart and checked if they are reliable. Two control charts were applied: the X-chart in which the control value is the actual result, the iron concentration and the control diagram of the domain in which the control value is the concentration range in which the control sample results are located. Table 3 presents the control values for the two diagrams.

Table 3. The control values for the X and R diagrams

No.	Xi	R’	No.	Xi	R’
1	42.46	---	8	38.55	0.95
2	41.62	0.84	9	40.83	2.28
3	42.79	1.17	10	38.55	2.28
4	40.63	2.16	11	38.44	0.11
5	39.61	1.02	12	38.22	0.22
6	39.00	0.61	13	38.38	0.16
7	39.50	0.50	14	38.11	0.27

For the purpose of interpreting the control diagrams, the significance of each limit in the construction of a control diagram is presented. The control diagrams have a centerline, upper and lower warning limits, upper and lower action limit (Figs. 1-2).

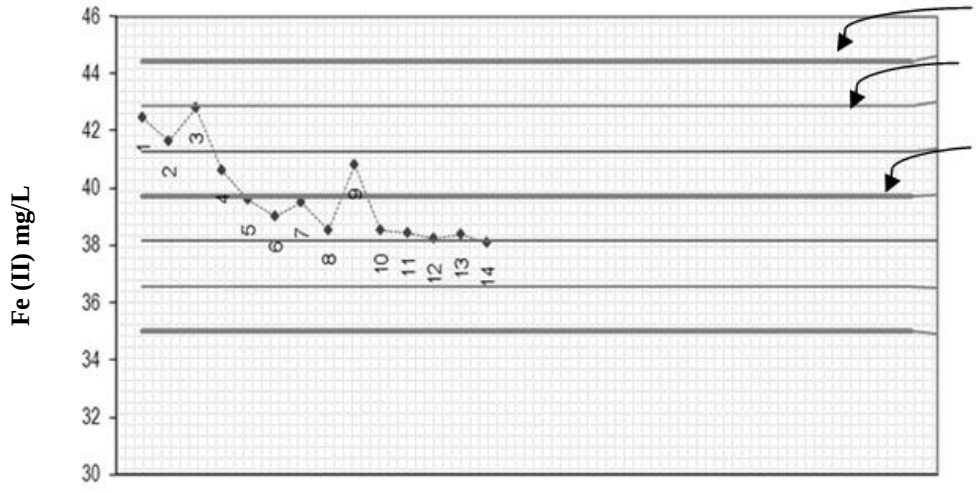

Figure 1. X control diagram.

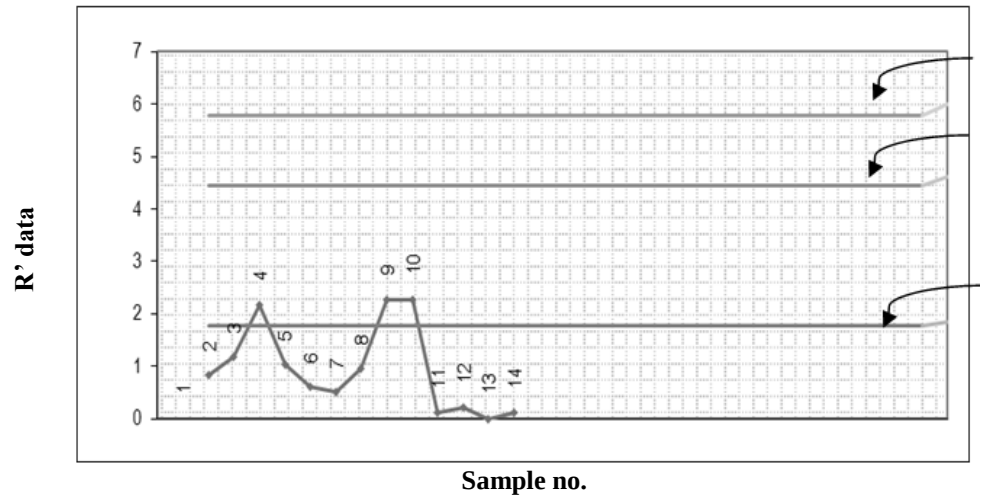

Figure 2. R control diagram.

The centerline (mean value) defines the best estimated value of the concentration of the graphically represented parameter; often the average is calculated from 10-20 results, but the estimated or "nominal" value can be that given by a control sample in which an MRC is used. The warning limit defines an interval in which there is a 95% probability of finding a control value (mean ± 2 σ). The action limit defines an interval in which the majority (99.7%) of the control values is expected to be located (mean ± 3 σ).

The control charts in which experimental results are represented were constructed using a special program for constructing control charts in Excel. In Table 4 the calculation program of the limits of the control diagram is presented.

Table 4. Calculation of warning and action limits for X and R diagram in Fe (II) analysis of wine samples.

X-Chart (Individuals)			R'-Chart (Moving Range)		
	Current Values	**Future Values**		**Current Values**	**Future Values**
Xm	39.72	39.75	**R'm**	20.1	0.942
S	1.57	1.632	**[S]**	17.82	0.835
CV	4%	4%			
		N=14			N=13
	Current Limits	**Future Limits**		**Current Limits**	**Future Limits**
Upper Control	44.43	44.65	**Upper Control**	5.787	6.017
Upper Warning	42.86	43.02	**Upper Warning**	4.449	4.626
+1 sd	41.29	41.38			
Xm=central	39.72	39.75	**R'm central**	1.771	1.841
-1 sd	38.15	38.12			
Lower Warning	36.58	36.49			
Lower Control	35.01	34.86			

In the case of diagram X, the method is not under control when:
• 1 result is outside the limits of action;
• 2 consecutive results are located between the warning and action limits;
• no more than 3 up to maximum 5 points are not on the same side of the mean value;
• 7 consecutive results show an increasing or decreasing trend;
• 10 out of 11 results are located on the same side of the centerline.

In the case of the R diagram, the method is not under control when: 1 result is outside the limits of action; 7 consecutive results are located above the centerline. From Figs. 1-2 the

correctness and confidence of the results can be observed. It can be seen that the values alternate up and down, as they are not consecutive. Also, rules 2 and 3 are abided by, so the obtained results fall within the upper and lower warning limits. The analysis of the control diagrams shows that all the rules described above are observed, so the quality control conditions of the method, reagents, reference materials and results are met.

4. CONCLUSION

The alcohol concentration of studied wines was between 12.5 and 13.5 %, in accordance with the wine legislation. The total acidity of the studied wines was 6.5 g/L $C_4H_6O_6$ for red wine, 7.45 g/L $C_4H_6O_6$ for white wine and 6 g/L $C_4H_6O_6$ for rosé wine in compliance with the wine legislation that provides a minimum of 3.5 g/L $C_4H_6O_6$ for wines. The total content of polyphenolic compounds in studied wines recorded values of 51.28 mg GAE/L for the red wine, 41.85 mg GAE/L for the white wine and 45.23 mg GAE/L for the rosé wine. The results confirmed that the Romanian wines tested are a good source of antioxidants and, therefore, moderate consumption can have a beneficial influence on human health.

REFERENCES

[1] *****European Commission, *Evaluation of the CAP measures applicable to the wine sector*, Case study report: Romania, 2018.
[2] *****Eurostat, Wine production and trade in the EU, 2019 https://ec.europa.eu/eurostat/web/products-eurostat-news/-/EDN-20191121-1
[3] Levaj, B., Dragovi-Uzelac, V., Delonga, K., Ganic, K.K., Banovic, M., Kovacevic, D.B., *Food Technol. Biotechnol*, **48**(4), 538, 2011.
[4] Radulescu, C., Hossu, A.M., Ionita, I., *Dyes and Pigments*, **65**(2), 175, 2005.
[5] Buruleanu, L., Radulescu, C., *et. al.*, *Analytical Letters*, **52**(8), 1195, 2019.
[6] Buruleanu, L., Radulescu, C., *et. al.*, *Analytical Letters*, **51**(7), 1039, 2018.
[7] Radulescu, C., Stihi, C., *et. al.*, *Analytical Letters*, **50**(17), 2839, 2017.
[8] Radulescu, C., Olteanu, R.L., *et. al.*, *Analytical Letters*, **52**(15), 2393, 2019.
[9] Radulescu, C, Hossu, A.M., *Rev. Chim. (Bucharest)*, 56(7), 742, 2005
[10] *****STAS 6182-1:2008 - *The total acidity of the wine*
[11] Dobrinas, S., Soceanu, A., Dragan Artem, V., *Environmental Engineering and Management Journal*, **14**(4), 863, 2015.
[12] *****HG. 512/20.07.2016, *Methodological norms for application of the law of vine and wine in the system of common organization of the wine market* no. 164/2015
[13] Boulton, R.B., Singleton, V.L., Bisson, L.F., Kunkee, R.E., *Juice and Wine Acidity* in: *Principles and Practices of Winemaking*, Chapman & Hall, New York, 1996.
[14] Vrček, I.V., Bojić, M., Žuntar, I., Mendaš, G., Medić-Šarić, M., *Food Chemistry*, **124**(1), 354, 2011.
[15] David, M., Serban, A., Radulescu, C., *et. al.*, *Bioelectrochemistry*, **129**, 124, 2019.
[16] Vilas-Boas, A., Valderrama, P., Fontes, N., Geraldo, D., Bento, F., *Food Chemistry*, **276**, 719, 2019.
[17] Minnaar, P., Nyobo, L., Jolly, N., Ntushelo, N., Meiring, S., *Food Chemistry*, **268**, 287, 2018.