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STUDY OF THE CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF SOME VARIETIES OF YOUNG RED WINES FROM DIFFERENT VINEYARDS IN SOUTHERN ROMANIA

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Abstract

Red wine samples were taken from the Stefanesti-Argeș, Babadag and Dragasani vineyard. The study was done on young red wine varieties from 2019 vintage. The chemical parameters were determined from the following varieties of wine: 'Feteasca Neagra', 'Merlot' and 'Pinot Noir'. For the wines studied was observed a higher concentration of total polyphenols and anthocyanins from Dragasani vineyard as compared to the wines from the other two vineyards. The concentration of alcohol in Dobrogea vineyard wines is higher than in the other vineyards studied for 'Merlot' varieties (15.55%) and 'Feteasca Neagra' (15.45%). For the 'Pinot Noir' variety (15.5%) the alcoholic concentration is higher in the Stefanesti vineyard centre. The total acidity at the 'Merlot' (5.93 g/l) and 'Feteasca Neagra' (5.67g/l) varieties from Dragasani vineyard are the highest compared to the other wine varieties, and their pH is the lowest (3.22) compared to the pH of 'Pinot Noir' (3.72) from Stefanesti. Antioxidant activity has been studied by the DPPH radical scavenging assay method and the highest values for are found in Dobrogea, 'Feteasca Neagra' ((EC50)7.19 mg GAE/l) and 'Merlot' ((EC50) 6.56 mg GAE/l).

Key words*: red wine, varieties, antioxidant activity, chemical composition.*

INTRODUCTION

"Wine is the healthiest and most hygienic drink" (Louis Pasteur). It is considered as one of the major sources of energy in human nutrition, it can be counted as a source rich in potassium (K = 0.5-1.2 g/l), or as a vitamin. (Mihalca Al., 2010). Vitamins don't come as a source of energy. They are generally activated in the traces status, their synthesis is not carried out in the body and thus must be consumed with food. Wine contains in variable doses all soluble vitamins (Ouranac A., 1970). Well-developed wine contains vitamin C 10 mg/l from grape pulp. Vitamin B1 in wine is 7-10 mg/l. Vitamin B2 (riboflavin), which plays an important role in protein metabolism and energy production, is found up to 0.5 mg/l. Other vitamins in lower doses are vitamins B3, B5, B6, B12, H and vitamin factors P(C2). (Mihalca Al., 2010).

Since ancient times, wine was considered a food, but also a good medicine. But simultaneously, the wine has its detractors, known to excessive consumption. So if Hippocrates recommends wine to his patients, Pythagoras condemns him. (Mihalca Al., 2010). Numerous studies done by Masquelier and Bourzeix in 1989, devoted to the effects of moderate wine consumption on health, have highlighted a multitude of properties: vascular protection, anticancer factors, antihistaminic factors, antiviral agents, protection against ionizing radiation, sun protection, free radical captors, dietetic supplement etc. (Masquelier J., 1982, 1987).

Studies conducted at Davis University in California have established that phenolic compounds in wine, considered as antioxidants, or other constituents in wine can significantly reduce cancer. (Health Qviz,1996; Watson R., 2018). Camargo's study highlighted the protective effect of consuming 1-2 glasses of wine per day for ischemic brain injuries. (Dejeu L., 2000). Prof. Orgogozo considers that the protective action of wine in relation to strokes accidents is explained by: - inhibition of the aggregation of cells responsible for the formation of atheroma plaque; - decrease dining of blood content in low-density lipoprotein (LDL); - increase in high-density lipoprotein (HDL) blood content (Orgogozo J.M., 1995 and 1997).

The authenticity and the typicality of the wine are basic attributes that guarantee the quality of a wine. First of all, the wine must meet the requirements of authenticity and typicality, because wines with false identity cannot be offered to consumers. (Giosanu D., 2011). The notions are closely linked, without being identical. The authenticity and the typicality of the wines are highly appreciated by the consumers and rigorously controlled, given that the consumption of wine has decreased in all countries, and the consumers increasingly prefer "the authentic wines of quality". The most important parameters used in the typicality of wines are the anthocyanin spectrum in red wines, the content in shikimic acid in white wines, the imprint of amino acids in wine, the isotopic composition of alcohol and water in wine. The quality of the wine is given by the physico-chemical composition of the wine and its organoleptic, taste-olfactory properties. In the case of wine that has a very complex composition, the quality remains a random feature, difficult to define and to establish (Geana E.M., 2016). However, the quality is very severely controlled in all wine producing and importing countries.

Red wine is a tannic drink, so it contains polyphenols, which is not the case with other alcoholic beverages. Polyphenols are, in fact, very important constituents and characteristics of wine. A greater interest is in studies is the antioxidant action of some chemical compounds in wine, respectively polyphenols. Polyphenols taste (tart) and colour (anthocyanins) of wine. The colour of the wine can be determined by the shade of the wine and by the colour intensity of the wine. Wine polyphenols form a diverse family, including phenolic acids, monoacids, anthocyanins, flavonili, leucoanthocyans and tannins, which are made up of phenolic compounds. The most sustained beneficial action of polyphenols in wine is the antioxidant one. It is achieved by the capture by polyphenols of free radicals circulated through the blood (Woraratphoka J., 2007; Valkom J., 2007; Yang J., 2009; Visnja K., 2010; Bunea C.I., 2012). Consequently, a vigorous research activity was initiated regarding the analysis of these antioxidants in different grape products and the preparation of extracts and wines with a high content of phenolic antioxidants (Heinonen I.M., 1998).

MATERIALS AND METHODS

For this experiment, 3 wine-growing areas in Romania were studied with 3 varieties of young red wine from 2019 of the grape varieties: 'Feteasca Neagra', 'Merlot' and 'Pinot Noir'. Climate data recording and physico-chemical determinations were made for each vineyard.

For climate records, weather stations were used for each area studied: the one in Stefanesti is an automatic station in the research pilot station with the coordinates 44°51' N and 24°57' E, 300m; Babadag (Dobrogea) weather station has the coordinates 44°53' N and 28°58' E 80 m; respectively Dragasani weather stations has the coordinates 44°317' N and 23°678' E, 195 m.

Our studies were made on many sorts of wines, from different areas, but in this paper we present just only three of them from 2019: 'Merlot', 'Pinot Noir' and 'Feteasca Neagra' - Stefanesti area, important vine area for red wine production, Dobrogea vineyard and Dragasani. We started the wine analysis with an important stage: preliminary examination of samples. This includes colour analysis and microbiological stability. Usually, shade and intensity of wine colour are calculated by optical methods (Niskanen I., 2009).

For each wine variety we determined: minimum alcohol content (alcoholic concentration), relative density, total acidity, contents of SO_2 and anthocyanins. The alcoholic concentration, one of the most important parameters of wine quality, was determined by indirect method, (pycnometer method), after prior separation of alcohol from wine by distillation. Acidity provides physico-chemical stability of wine, gives colour, brightness and freshness of taste. To characterize acidity of wine the following types of acidity are taken into consideration: total acidity, volatile acidity, fixed acidity and ionic/ real acidity of wine. The determination of total

acidity was made by titration with bromothymol blue. The method consists of wine sample titration (acid neutralization) with a solution of sodium hydroxide in the presence of bromothymol blue, after prior removal of carbon dioxide (Giosanu D., 2011).
The total remaining unfermented sugars in wine were evaluated by refractometric method, by measuring the percentage of soluble solids or refractive index, after prior removal of alcohol and volatile compounds from wine (which changes the refractive index value). Sulphur dioxide is the only antiseptic allowed in wine conservation. The determination of free SO_2, combined SO_2 and total SO_2 is rapidly made by using iodometric oxidation. The anthocyanins are visible phenolic compounds (pigments), which are getting accumulated in grapes and give red colour to wine. They represent 38% of the total phenolic compounds present in wine. The quantitative determination of anthocyanins is made by visible spectrophotometry and is based on the change of anthocyanins colour depending on the pH. We measured the absorbance variation of anthocyanins colour at two pH values, 0.6, 3.5, and compared with distilled water. The measurements were made at 520 nm, the absorbance of the samples being proportional to the anthocyanins content (Giosanu D., 2011).
The total content of polyphenolic compounds (CTCF) was determined by the Folin - Ciocalteu method and by the enzyme method with the BS200 analyser. Tannins are chains of flavonols (promainidines) more or less polymerized in leaves and strings. The method of leucoantocyanids for the determination of tannins is based on the property of tannins to turn hot and into the strong acidic environment (Concentrated HCl) into cyanidine, which is red in colour.
The samples were analyzed according to the technique reported by Brand-Williams and others (1995), with some modifications. Briefly, 1 volume of sample [5 μL for red wine (1: 1, v/v with methanol)] was added to 1 volume of 2,2-diphenyl-1picrylhydrazyl (DPPH) (Sigma) 0.094 mM in methanol up to completing 1 mL. The free radical scavenging activity using the free radical DPPH• reaction was evaluated by measuring the absorbance at 515 nm after 60 min of reaction at 20°C in a spectrophotometer (Specord 205).
Antioxidant activity represented by the amount of antioxidant needed to decrease the initial DPPH radical concentration by 50% (Efficient concentration = EC50 mg GAE/l). (Woraratphoka J., 2007; Rockenbach I. I., 2011)
For the statistical interpretation of the results, the data were included in an Excel database and then statistically interpreted with the SPSS 14.0 program, which uses the Duncan test (multiple t test) for a 5% statistical assurance.

RESULTS AND DISCUSSIONS

The values recorded in Stefanesti are influenced by the climatic data of the 2019 wine year and presented a number of peculiarities whose effects were found both in the course of the vegetative cycle and especially in the level and quality of the grape harvest.
Among these specific features we mention the most significant: low temperatures in the period of April - May leading to a delay of the flowering of the vine by one week (3ºC/April 1st, 5.9ºC/May 1st, 6.1ºC/May 7th), June does not exceed the temperature of 35ºC until 2 days (37ºC June 25 and 35.6ºC June 27), the same in July, there were no reports of very high temperatures (maximum 37.8ºC July 21 and July 26), but in the two months the temperatures during the night were quite low (14ºC/June 03 - June 10, 8.2ºC/July 11) led to the extension of the ripening period of the grapes. (Table 1)
The climatic data in Dragasani do not showed any peculiarities in terms of temperatures, they are kept within the normal limits of the Dragasani wine area (Table 1).

Table 1. Temperature dynamics in 2019 in the ripening stage of the three vineyards studied

Month	Air temperature			Rainfall (mm)	Huglin Index	No days with precipitation > 10 mm	Σ ºt global (ºC)	Σ ºt active (ºC)	Σ ºt useful (ºC)
	T med (ºC)	Average T min (ºC)	Average T max (ºC)						
Dragasani									
April	11.92	6.53	17.83	39.9	146.25	1	357.5	314.5	64.5
May	14.08	9.42	19.06	14.9	203.67	-	436.5	436.5	166.6
June	22.81	17	29.23	139.2	480.6	7	684.5	684.5	384.5
July	22.82	15.84	30.16	63.8	511.19	3	707.5	707.5	397.5
August	25.13	17.39	32.87	1	589	-	779	779	469
Babadag									
April	13.3	8.43	17.97	810.2	163.05	8	401	354.5	124.5
May	17.5	13.1	22.29	518	241.65	4	542.5	542.5	232.5
June	20.5	16.2	24.73	707.1	378.45	7	614.5	614.5	314.5
July	27.3	21.45	32.42	602.7	615.66	6	845	614.7	304.7
August	27.35	20.58	31.94	199.2	609	3	848	848	538
Stefanesti									
April	11.2	5.4	19.1	42.2	171	1	338.1	244.4	53.4
May	16.3	9.8	24.6	93.6	323.95	2	504.1	494.5	194.5
June	22.1	15.5	32.0	193.6	511.5	6	664.6	664.6	364.6
July	22.2	14.5	32.2	70.6	531.95	1	687.1	687.1	377.1
August	24.6	15.7	35.8	6.6	626.2	0	762.1	762.1	452.1

Huglin Index =[(T_{med} – 10)+(T_{max} - 10)]/2 x no.days of the month.
Σºt global = sum of average positive daily temperatures.
Σºt activate = sum of average daily temperatures > 10 ºC.
Σºt useful = sum of differences between average daily temperature > 10ºC and the biological threshold for starting in the vegetation of the vine (10ºC).

In March having small peculiarities represented by the minimum of -2°C in March 15 and the maximum of 25°C March 18 and poor rainfall, only in March 18 were recorded of 18.7 mm. April with minimum temperatures of 2°C/April 3 and 21 and maximum of 25°C/April 27, with poor precipitation, with rain in April 18 of 15.4 mm. In May, climate data remained normal (22-28°C) of this rain-free area, only on May 31 were recorded the maximum temperature of 20°C. In the grapes ripening time of June - August there were deficient months in terms of precipitation, there were no particular manifestations of temperatures, only in August there were higher temperatures (36°C/August 10 and 11).

Climate data from the Dobrogea vineyard showed peculiarities such as low temperature in March (T max 5°C/March 25) and high rainfall (99.5mm/March 16 and 17, 99.8 mm/March 21 and 22). The April month was evidenced by low maximum temperatures (7-18°C), the highest was 25°C/April 29 and 30 and heavy rainfall (99.8 mm/April 25, 26 and 29). May is not a particular manifestation of temperatures, only heavy rainfall (99.5 mm/May 4, 10, 24 and 31). June recorded high temperatures above 30°C and heavy rainfall due to storms (99.3 mm/June 4, 8, 13, 20 and 28). The months of July and August had no specific features in terms of temperatures, instead there were storms with high precipitation (99.5 mm/July 2, 13 and 24; 99.0 mm/August 6 and 26) (Table 1).

From a chemical point of view, wine is a polydisperse hydroalcoholic solution, in which several organic and mineral substances are dissolved or present in colloidal state.

The chemical analysis of the wine aims to establish the composition parameters that define the quality of the wine.

Table 2 show the correlations between the indicators studied, of which we highlight the following: It is noted that pH correlates positively with alcoholic concentration, density, volatile acidity, and antioxidant activity (r = 0.237, r = 0.128, r = 0.185, respectively r = 0.064) and significantly positive with sugar (r = 0.432*), which explains that there is a balanced ratio between these indicators.

Table 2. Matrix of correlations between biometric and biomedical indicators studied in wine

		Alcohol concen tration % vol	Dry extract, g/l	Dens at 20 degreesC	Total acidity, g/l AT	Volatile acidity, g/l AA	Sugar g/l	pH	Total anthocyanins content (mg/l)	Total polyphenol content (mg GA/g)	Tannins (mg/l)	Antioxidant activity, mg GAE/l
Alcohol concentration % vol	Pearson Correlation	1	,628(**)	-,284	-,298	-,088	,088	,237	,171	,106	,249	,260
Dry extract, g/l	Pearson Correlation	,628(**)	1	,067	,059	-,532(**)	-,048	-,217	,538(**)	,253	,251	-,089
	Sig. (2-tailed)	,000		,711	,744	,001	,792	,226	,001	,156	,158	,621
Dens at 20 degreesC	Pearson Correlation	-,284	,067	1	-,054	,148	,814(**)	,128	,116	-,439(*)	-,416(*)	-,072
	Sig. (2-tailed)	,110	,711		,765	,411	,000	,479	,519	,011	,016	,691
Total acidity, g/l AT	Pearson Correlation	-,298	,059	-,054	1	-,145	-,281	-,667(**)	,338	,051	,179	-,232
	Sig. (2-tailed)	,093	,744	,765		,422	,114	,000	,054	,778	,318	,193
Volatile acidity, g/l AA	Pearson Correlation	-,088	-,532(**)	,148	-,145	1	,477(**)	,185	-,196	-,084	,083	,643(**)
	Sig. (2-tailed)	,627	,001	,411	,422		,005	,304	,274	,640	,644	,000
Sugar g/l	Pearson Correlation	,088	-,048	,814(**)	-,281	,477(**)	1	,423(*)	-,034	-,557(**)	-,368(*)	,250
	Sig. (2-tailed)	,626	,792	,000	,114	,005		,014	,850	,001	,035	,161
pH	Pearson Correlation	,237	-,217	,128	-,667(**)	,185	,423(*)	1	-,348(*)	-,431(*)	-,352(*)	,064
	Sig. (2-tailed)	,184	,226	,479	,000	,304	,014		,047	,012	,045	,722
Total anthocyanins content (mg/l)	Pearson Correlation	,171	,538(**)	,116	,338	-,196	-,034	-,348(*)	1	,474(**)	,606(**)	,116
	Sig. (2-tailed)	,341	,001	,519	,054	,274	,850	,047		,005	,000	,521
Total polyphenol content (mg GA/g)	Pearson Correlation	,106	,253	-,439(*)	,051	-,084	-,557(**)	-,431(*)	,474(**)	1	,659(**)	,385(*)
	Sig. (2-tailed)	,558	,156	,011	,778	,640	,001	,012	,005		,000	,027
Tannins (mg/l)	Pearson Correlation	,249	,251	-,416(*)	,179	,083	-,368(*)	-,352(*)	,606(**)	,659(**)	1	,169
	Sig. (2-tailed)	,163	,158	,016	,318	,644	,035	,045	,000	,000		,348
Antioxidant activity , mg GAE/l	Pearson Correlation	,260	-,089	-,072	-,232	,643(**)	,250	,064	,116	,385(*)	,169	1
	Sig. (2-tailed)	,145	,621	,691	,193	,000	,161	,722	,521	,027	,348	

The anthocyanin content was correlated positively with dry extract, total polyphenols and tannins, (r = 0.538**, r = 0.474**, r = 0.606**) having in all three cases distinctly significant and negative correlations with, sugar and volatile acidity, (r = -0.034 and r = 0.196 respectively), which explains that anthocyanins are formed in the presence of sugar and acids in grapes (Table 2). Their presence in grapes leads to an increase content in anthocyanins. Some acids (tartaric acid) may increase the content of anthocyanin pigments and the biochemical quality of grape (Lee, 1996).

Figure 2 showed the differences between the Dobrogea vineyards and the other two (Drăgăşani vineyard and Ştefăneşti vineyard centre) regarding alcoholic concentrations, both as concern 'Feteasca Neagră' from Dobrogea vineyard, which is around 15.45% vol., and the one from Ştefăneşti it reaches max. 13.2% vol., respectively from Drăgăşani vineyard to 14.77% vol.

By considering the influence of the area on the alcohol strength on the average of the varieties, it can be observed that the highest values were recorded in the vineyards of Dragasani and Dobrogea, and the lowest in Stefanesti vineyard centre (Figure 1). As regards the alcoholic strength of the three varieties analysed in the three geographical areas, the highest alcohol content was recorded at the 'Pinot Noir' wine in Stefanesti vineyard centre.

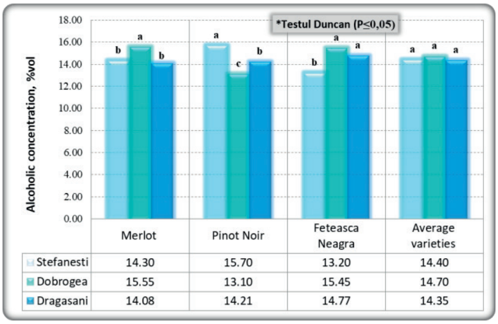

Figure 1. Influence of the geographical area on the alcoholic concentration of wine, depending on the variety

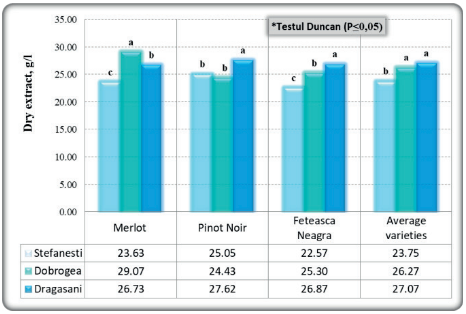

Figure 2. The influence of the geographical area on the dry extract of the wine, depending on the variety

Figure 2 shows the influence of the area on the dry extract of wines, on the average of the varieties, it can be observed that the highest value of extracts were recorded at the Dragasani and Dobrogea vineyards, and the lowest at Stefanesti vineyard centre, and the differences between the two classes of homogeneity are statistically assured.

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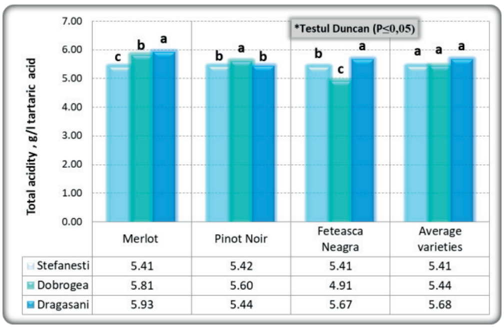

Figure 3. Influence of the geographical area on total acidity in wine, depending on the variety

total acidity (expressed in g/l tartaric acid) recorded the high value at different variety depending on the area. Thus, the highest value of total acidity was registered to the Merlot and 'Feteasca Neagra' variety at Dragasani vineyards, respectively at Dobrogea in the case of 'Pinot Noir' variety (Figure 3).
The differences of total acidity depend on the pedological composition of the soil in each vineyard.

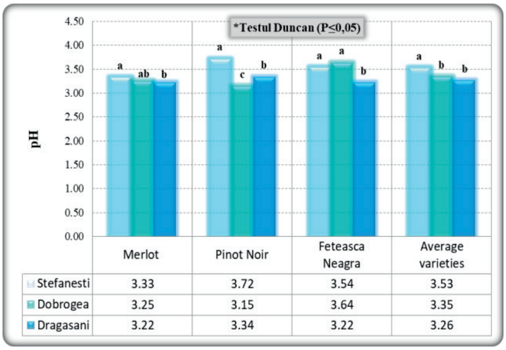

Figure 4. The influence of the geographical area on the pH of the wine, depending on the variety

The pH depends on the variety of wine and the geographical area, for example at 'Pinot Noir' from Stefanesti is 3.72, and for the variety from Dobrogea it is 3.15 (Figure 4).
On average of the varieties, the highest pH was recorded to the Stefanesti vineyard centre.

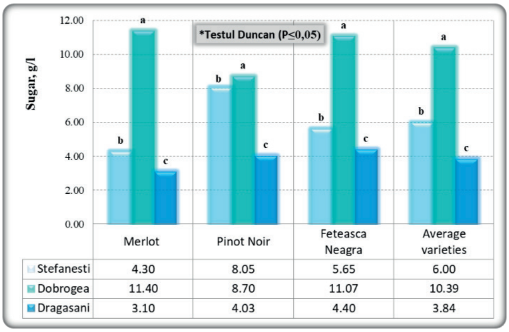

Figure 5. Influence of geographical area on wine sugar, by variety

In all three cultivars studied, due to the climate conditions the highest sugar content was induced at Dobrogea vineyard and the values are ensured statistically compared to Stefanesti and Dragasani areas, respectively (Figure 5). On average of the varieties studied, sugar level recorded at Dobrogea was 2.7 times more than Dragasani area.

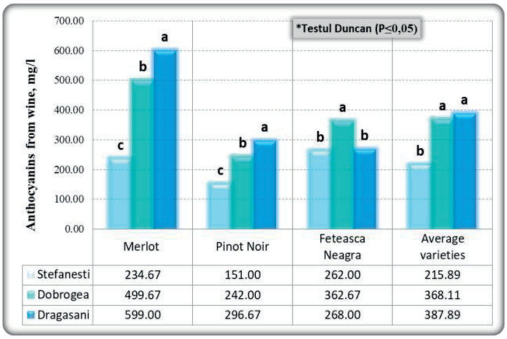

Figure 6. Influence of the geographical area on the anthocyanins from wine, depending on the variety

The highest anthocyanin content is at 'Merlot' wine (599 mg/l) from Dobrogea vineyard and the lowest is at 'Pinot Noir' (151 mg/l) from Stefanesti (Figure 6). On average of the varieties studied, at Dragasani and Dobrogea areas, anthocyanin levels recorded high value than Stefanesti area. The content of anthocyanin is given primarily by the winemaking technology (the time of contact of the must with marc) and to a lesser extent by the soil pH.

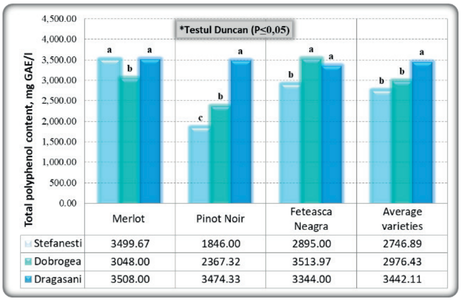

Figure 7. Influence of the geographical area on the content of total polyphenols in wine, depending on the variety

In Figure 7 is showed influence of the geographical area on the content of total polyphenols in wine, depending on the variety and the highest content of total polyphenols was recorded at 'Merlot' (3508 mg GAE/l) from Dragasani vineyard, while 'Pinot Noir' from the Stefanesti vineyard center had the lowest total polyphenols content (1846 mg GAE/l). Polyphenols have a fundamental property they are antioxidant compounds.

Antioxidant activity was influenced by the climate and pedological characteristics. For all studied varieties, the highest values of this chemical parameters content were recorded at Dobrogea vineyard (Figure 8).

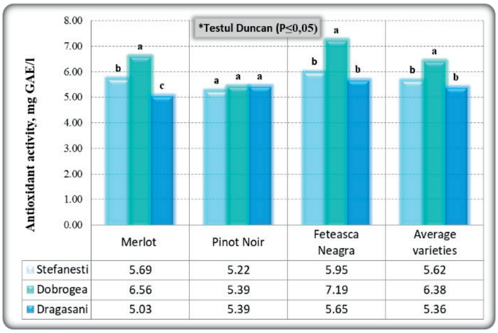

Figure 8. Influence of the geographical area on antioxidant activity, depending on the variety

Antioxidant activity is also influenced by the variety a higher antioxidant activity is observed at Dobrogea vineyard at 'Feteasca Neagra' variety (EC(50) = 7.19 mg GAE/L) and the lowest value at 'Merlot' variety (EC(50) = 5.03 mg GAE/L) from Dragasani vineyard.

CONCLUSIONS

There are differences of concentration of alcohol in the same variety of wine in example for 'Feteasca Neagra' variety from all three vineyards, due to the differences of soil, climatic conditions and winemaking technology.

Among the correlations between the studied indicators we highlight the following: It is noted that pH correlates positively with alcoholic concentration, density, volatile acidity, and antioxidant activity, (r = 0.237, r = 0.128, r = 0.185, respectively r = 0.064) and significantly positive with sugar (r = 0.432*) which explains that there is a balanced ratio between these indicators.

The anthocyanins content was correlated positively with dry extract, total polyphenols and tannins, (r = 0.538**, r = 0.474**, r = 0.606**), having in all three cases distinctly significant. Also, the anthocyanins content had a negative correlations with sugar, volatile acidity and pH (r = -0.034, r = -0.196 and r = -0.348*, respectively). That explains that anthocyanins are formed in the presence of sugar and acids from grapes, their presence in grapes leads to an increase of the content in anthocyanins.

Total acidity recorded the high value at different variety depending on the area. The total acidity at the 'Merlot' (5.93 g/l) and 'Feteasca Neagra' (5.67 g/l) varieties from Dragasani vineyard are the highest compared to the other wine varieties from Dobrogea and Stefanesti area.

In general, the pH of the wines depends mainly on the area. The highest pH value was recorded in Stefanesti for most of studied varieties (3.33-3.72 depending by the variety).

In all three cultivars studied, due to the climate conditions, the highest sugar content was induced at Dobrogea vineyard and the values are ensured statistically compared to Stefanesti and Dragasani areas, respectively. On average of the studied varieties, sugar level recorded at Dobrogea was 2.7 times more than Dragasani area.

On average of the varieties studied, at Dragasani, Dobrogea and Stefanesti areas, anthocyanin levels recorded high value at Stefanesti area. The content of anthocyanin is given primarily by the winemaking technology (the time of contact of the must with marc) and to a lesser extent by the soil pH.

Antioxidant activity was influenced by the variety, geographical area and winemaking technology, for all varieties studied, the highest values of this parameter was recoded at Dobrogea vineyard.

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