ANTIOXIDANT CONTENT OF THREE DIFFERENT VARIETIES OF WINE GRAPES

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ABSTRACT

The antioxidant contents of wines, seeds and skin ethanolic extracts of 'Cabernet Sauvignon', 'Merlot' and 'Pinot Noir' cultivated grape varieties from Recas winery were used in order to verify the influence of the analyzed material (seeds and skins extracts, wine) and of the grape variety on the antioxidant content of samples and to estimate the relationships between different grape varieties based on their antioxidant content. The results showed that the antioxidant content of samples depends on the analyzed material and on the grape variety. The results also show that 'Cabernet Sauvignon' and 'Merlot' grapes varieties are very different in terms of antioxidant content.

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Introduction

In recent years, a high interest on bioactive ingredients such as vitamins, antioxidant compounds, minerals, pro-biotic compounds contained in fruits, vegetables, grains, medicinal and aromatic plants and in their derived products (juices, teas, wines, etc.) (2) is found. Antioxidant compounds are a special category of bioactive ingredients due to their active role in the prevention and improvement of various pathologies associated with stress, aging and unhealthy nutrition (9). This role is done by compounds antioxidant ability to react with free radicals and is usually correlated with the antioxidant capacity. Wines and extracts from grape seeds and skin, especially those obtained from red grapes, often seem to be the basic ingredients in diets for both patients with diseases and healthy people, because of their high antioxidant content.

Different studies showed that for the same class of samples (e.g. wine, extracts, etc.), the antioxidant activity is influenced by the sort of plant (17), the climatic conditions (15), the material used for sample preparation (e.g. seeds, skin), the conditions of obtaining the samples, etc. (4).

Romania is one of the major European countries in terms of wine, the variety of produced wines being remarkable in terms of quality. With an area of about 200000 ha cultivated with wines (approximately 1.5% of the agricultural area of the country and 2.6% of arable land), Romania occupies the 5th position in Europe- after Spain, France, Italy and Portugal. The annual production of wine is about 5 million hectolitres and the annual consumption is around 90-95% of this production. Consumption per person is approximately 27 L, with increasing tendency, which is still relatively small compared to the traditional wine countries like France, Italy and Portugal and about equal to that of Spain, Greece and Austria. There are different varieties of red grapes that are cultivated in Romania, including Feteasca Neagra, Cabernet Sauvignon, Merlot, Burgund Mare, Cadarca, Pinot Noir, etc. (3).

Burgund Mare belongs to the same group of grapes as Pinot Noir being regarded as a bud variation thereof. It exists in Romania for over a century, but its culture was greatly expanded after 1975. The bunches of grapes are uniaxial, cylindrical-conical shaped, with grape berries placed close to each other in clusters, weighing 210-215 g each. Grape berries are spherical, medium size, dark purple-red colored. Skins are thick, covered with persistent pruine. The flesh is juicy and the grape must is colourless. Grapes have a middle vegetation period (165-175 days), great growth force and lower fertility than Pinot Noir, but much higher productivity due to the size of grapes (15-18 t/ha). In terms of technology, Burgund Mare does not reach the quality level of Pinot Noir. Sugar accumulations are less than 200 g/L, and total acidity is about 4.5-5.0 g/L H_2SO_4. Compared with Pinot Noir, Burgund Mare has weaker resistance to frost and is more resistant to drought and gray mold (14).

Cabernet Sauvignon is one of the world's most widely recognized red wine grape variety, being grown in America, Australia, Asia and Europe. Grape bunches are tronconical or conical shaped, with rare grains rachides. The average weight of the bunches is 100-140 g. The grapes have spherical shape, franc taste and thick skins, colored in dark red-purple, with intense pruine. The grapes have a long vegetation period (180-190 days) and the climate of the growing season affects how early the grapes will be harvested (in Romania, the grapes ripen usually in September). The sugar concentration and the total acidity can reach 240 g/L, and 5.0-5.5 g/L H_2SO_4, respectively. Cabernet Sauvignon can be grown in a variety of climates, being resistant to frost, drought and gray mold, but is affected by rot (14).

Merlot is an old variety of red wine grape from Gironde-Bordeaux wine-growing region. The name Merlot is thought to derive from the “Old French” word for young blackbird, merlot, a diminutive of merle, the “blackbird” (*Turdus merula*), probably from the colour of the grape. Beyond France it is also grown in Italy, Eastern Europe and New World, especially California. It grows in many regions that also grow Cabernet Sauvignon but tends to be cultivated in the cooler parts of those areas. In areas that are too warm, Merlot will ripen too early. Merlot grapes are identified by their loose bunches of large spherical berries. The colour has less of a blue/black hue than Cabernet Sauvignon grapes and with a thinner skin and fewer types of tannin. Grapes have a middle vegetation period (170-180 days), a large force of growth and develop rich foliage. A characteristic of the Merlot grape is the propensity to quickly overripe. It normally ripens up to two weeks earlier than Cabernet Sauvignon. Compared to Cabernet, Merlot grapes tend to have a higher sugar content 205-240 g/L, and total acidity of 4.5-5.5 g/L H_2SO_4. Merlot thrives in cold soil, particularly ferrous clay. The grapes tend to bud early which gives it some risk to cold frost and its thin skin increases its susceptibility to rot. If bad weather occurs during flowering, the Merlot wine is prone to develop colure (14).

The aim of this paper was to verify the influence of the seeds and skins extracts, wine and grape variety on the antioxidant content of samples and to estimate statistically the relationships between grape varieties based on their antioxidant content.

Materials and Methods

Seeds and skins from three different varieties of *Vitis vinifera sp.*: Cabernet Sauvignon - commercial name “Cabernet Sauvignon” (CS), Pinot Noir - commercial name “Burgund Mare” (PN) and Merlot - commercial name “Merlot” (TM), obtained from 2005 harvest from Recas vineyard (Romania) were studied in terms of their antioxidant capacity. For this purpose, alcoholic extracts were prepared by macerating for ten days 5g grape seeds/skin in 50ml of extraction solvent (87% ethanol in distilled water). After filtering, the antioxidant capacity of five samples from each extract was determined by electron spin resonance spectroscopy (EPR), using 2,2,6,6-tetramethyl-4- hydroxypiperidine-N-oxyl (Tempol) (Fluka) free radicals (4). Samples of wines from the same grape varieties as extracts, produced in 2005 by Recas vineyard were analyzed in the same condition, without any preparation step.

The number of free radical molecules decreases in time, with different rates, depending on the concentration of antioxidant compounds when samples containing antioxidants react with Tempol. The antioxidant capacity of extracts was measured through the decrease in time of the relative concentration of the paramagnetic species obtained by double integration of EPR signals (7).

The antioxidant content was determined in each experiment, considering the values obtained by double integration of the initial EPR signal of the free radicals (S_0), and those determined after 20 minutes after adding the extracts or wines (S_{20}), using the relation:

antioxidant content (%)=$[(S_0-S_{20})/S_0]$ 100

Statistical analysis of grape seeds and skins extracts and wines

The assumption of normality of the observations was tested using Statistica Application (v.8.0) and EasyFit (v.5.2), applying the statistical tests of Kolmogorov-Smirnov (K-S) (8, 12), Wilk-Shapiro (W-S) (11), Anderson-Darling (A-D) (1) and Jarque-Bera (J-B) (5, 6).

The analysis of the variance was conducted using Statistica software (v.8.0), to estimate the values of the antioxidant content of seed and skin extracts and wines (based on the assumption of normality).

Estimations of the relative antioxidant content of the extracts compared to wines from the same grape variety, as well as of the samples of the same type but of different grape varieties were done calculating the confidence interval of the ratio of two means, using the GraphPad software.

In order to evaluate the influence of the analyzed material (seeds and skins extracts, wine) and of the grape variety on the antioxidant content of samples, the analysis of variance was performed, applying the method described by Fischer and Mackenzie.

The relationships between the analyzed grape varieties were estimated based on the antioxidant content of wines and extracts from the skin and seeds. To reach this objective, the index of diversity and Simpson (13) and Shannon (10) entropies were used.

Results and Discussions

The antioxidant content (%) calculated for each sample is presented in **Table 1**.

TABLE 1

The antioxidant content of extracts and wines

AC%	Samples				
	1	2	3	4	5
PNP	24.88	24.89	24.76	24.85	24.80
CSP	49.13	49.08	49.11	49.13	49.09
TMP	43.12	43.10	43.08	43.11	43.12
PNS	57.65	57.55	57.52	57.66	57.68
CSS	38.57	38.59	38.57	38.50	38.48
TMS	51.95	51.90	51.92	51.87	51.87
PNV	28.13	28.42	28.40	29.09	28.42
CSV	18.85	18.79	18.83	18.98	18.87
TMV	35.68	35.93	35.79	35.92	35.70

AC%: Antioxidant content (%); PN: Burgund Mare;
CS: Cabernet Sauvignon; TM: Merlot;
P: Skins; S: Seeds; V: Wine;

The assumption of normality of the observations was tested for all 9 considered observables. The results are presented in **Table 2**.

TABLE 2

The analysis of normality of data

Samples		K-S	p_{K-S}	W-S	p_{W-S}	A-D	p_{A-D}	J-B	p_{J-B}
PN	P	0.20	0.96	0.92	0.54	0.26	0.73	0.80	0.67
CS	P	0.23	0.89	0.88	0.33	0.32	0.68	1.35	0.51
TM	P	0.20	0.96	0.88	0.31	0.34	0.66	1.05	0.60
PN	S	0.30	0.65	0.85	0.21	0.42	0.60	1.73	0.42
CS	S	0.32	0.60	0.86	0.21	0.44	0.59	1.64	0.44
TM	S	0.23	0.91	0.91	0.46	0.28	0.71	0.51	0.78
PN	V	0.38	0.37	0.81	0.11	0.59	0.49	4.16	0.13
CS	V	0.27	0.79	0.91	0.46	0.33	0.67	2.46	0.29
TM	V	0.24	0.88	0.86	0.24	0.36	0.64	1.81	0.41

p_X: probability from test "X", where X:
K-S: Kolmogorov-Smirnov; W-S: Wikls-Shapiro;
A-D: Anderson-Darling; J-B: Jarque-Bera

The results showed that the calculated probability from all of the statistical tests, for all observables considered, were higher than 0.05. This indicates that the assumption of normality of the observations could not be rejected for any series of five observations, which allows continuing the application of statistical analysis of the results, based on the assumption of normality of data. The estimated values of antioxidant content of the seed and skin extracts and wines (**Table 2**) are presented in **Table 3**.

TABLE 3

Analysis of variance of data

Sample		Mean	StD	CV%	CI95%	Value
PN	P	24.84	0.06	0.22	0.07	24.84±0.07
CS	P	49.11	0.02	0.05	0.03	49.11±0.03
TM	P	43.11	0.02	0.04	0.02	43.11±0.02
PN	S	57.61	0.07	0.12	0.09	57.61±0.09
CS	S	38.54	0.05	0.13	0.06	38.54±0.06
TM	S	51.90	0.03	0.07	0.04	51.90±0.04
PN	V	28.49	0.36	1.25	0.44	28.49±0.44
CS	V	18.86	0.07	0.38	0.09	18.86±0.09
TM	V	35.80	0.12	0.33	0.15	35.80±0.15

The values in **Table 3** indicate that the coefficients of variation (CV %) are less than 1%, excepting for the sample of PN wine (1.25%). These show a low variability of the results from repeated measurements, which indicate a good agreement between them.

Based on the estimated values of the antioxidant content of samples, the relative antioxidant content of the extracts compared to wines from the same grape variety, as well as of the samples of the same type but of different grape varieties, were calculated (**Table 4**).

TABLE 4

The ratios between antioxidant contents

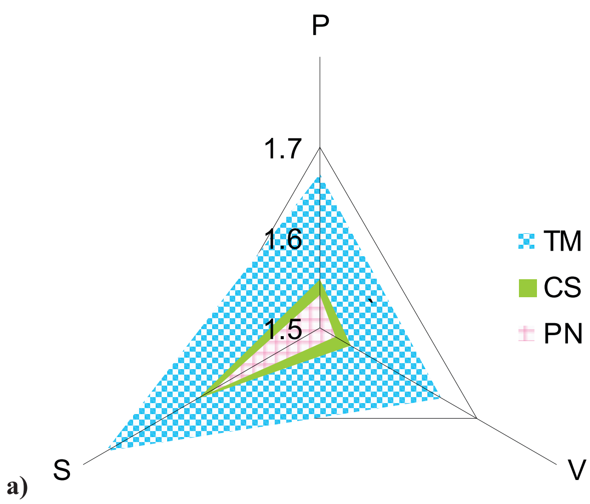
A vs. B	A/B Ratio
CSP vs. CSS	1.274±0.002
CSP vs. CSV	0.603±0.010
CSS vs. CSV	2.043±0.008
PNP vs. PNS	0.431±0.001
PNP vs. PNV	0.872±0.011
PNS vs. PNV	2.022±0.026
TMP vs. TMS	0.831±0.001
TMP vs. TMV	1.204±0.004
TMS vs. TMV	1.450±0.005
CSP vs. PNP	1.977±0.005
CSP vs. TMP	1.139±0.001
PNP vs. TMP	0.576±0.001
CSS vs. BMS	0.669±0.001
CSS vs. TMS	0.743±0.001
PNS vs. TMS	1.110±0.002
CSV vs. PNV	0.662±0.009
CSV vs. TMV	0.527±0.003
PNV vs. TMV	0.796±0.010

A, B: observables; A/B Ratio: with 95% confidence

The results showed that the antioxidant content of seeds for all grape varieties was higher than the antioxidant content of wine. The antioxidant content of wines is higher than the antioxidant content of skin extracts, excepting the case of TM variety. These results could suggest that TM grape variety is the most diverse in terms of the contribution of seeds and skin antioxidants to the antioxidant content of wine. Comparing the antioxidant content of the extracts and wines, one can group the grape varieties. It can be observed that the wine, the seeds and skins extracts of PN variety contain the smallest amount of antioxidants, followed by CS variety. Wine and extracts from seeds and skin of TM grapes have the highest content of antioxidants. This could be better revealed using the representation in logarithmic scale (**Fig. 1**, log scale of sum of data presented in **Table 1**).

From **Fig. 1a)** it is also indicated that the surface of the triangle defining the content of antioxidants from seeds, skins and wine of TM variety includes surfaces defining the antioxidants from CS and PN, showing that TM is better than CS and PN in terms of the antioxidant content of seeds, skins and wine.

The antioxidant content of seeds and skins were reported to the antioxidant content of the wine. The obtained ratios are showed in **Fig. 2**.



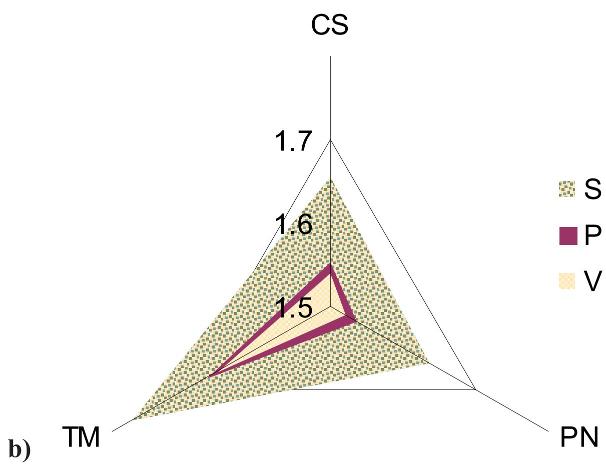


Fig. 1. a). Content of antioxidants (log scale) in seeds (S), skins (P) and wine (V) of three (TM, CS, and PN) grape varieties; **b)**. The contribution of seeds (S), skins (P) to wine (V) antioxidants in the three varieties of grape (TM, CS, and PN)

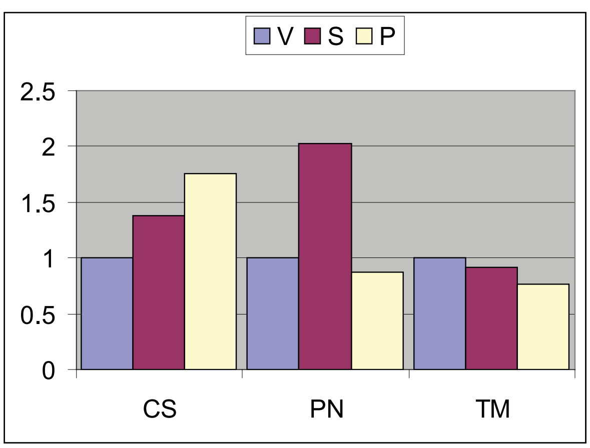


Fig. 2. The ratios between seeds (S) and skins (P) antioxidants and antioxidant content of wines (V) for three varieties of grapes (CS, PN and TM)

As Fig. 2 clearly indicates the antioxidants from seeds and skin compared with the antioxidant content of wines depends on the grape variety. In the case of CS variety, the antioxidant content of seeds and skins are about 100% and respectively 50% higher than the antioxidant content of wine. The anioxidant content of PN seeds is higher than 2 folds than the antioxidant content of wine, whereas the antioxidant content of seeds is lower than that of wine. In TM variety, the antioxidant content of seeds and skins is lower than the antioxidant content of wine.

In order to assess the influence of the analyzed material on antioxidant effect of a grape variety, principal component analysis (PCA) was applied. As it can be seen from **Fig. 3** the content of antioxidants depends on the analyzed material (seeds and skin extracts, wine), and the contributions of the antioxidants from seeds, skins and wine to the antioxidant content of a grape variety are very different.

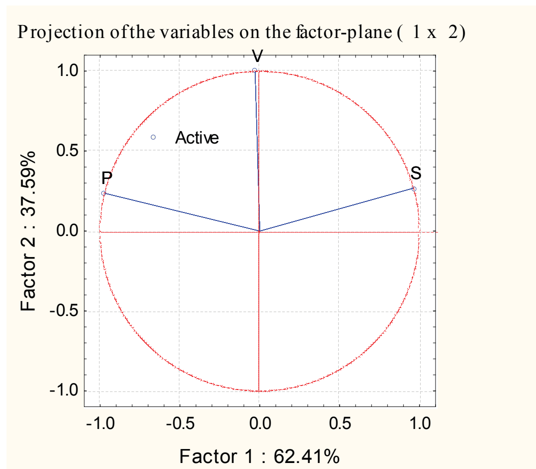


Fig. 3. Principal factors in antioxidants from seeds (S), skins (P) and wine (V) using the observations from all three grape varieties

As **Fig. 3** shows, two components could be identified from the three observable (S, P and V) being significant to built an accurate profile of the antioxidant content of wines based on Factor 1 and Factor 2.

It was also found that, in terms of antioxidant content, grape varieties CS and TM are very different (**Fig. 4**).

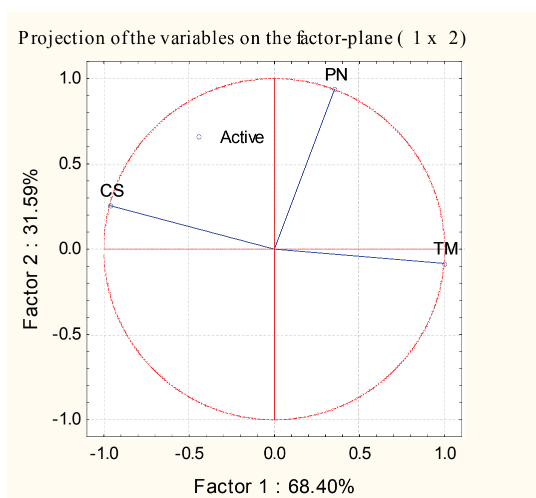
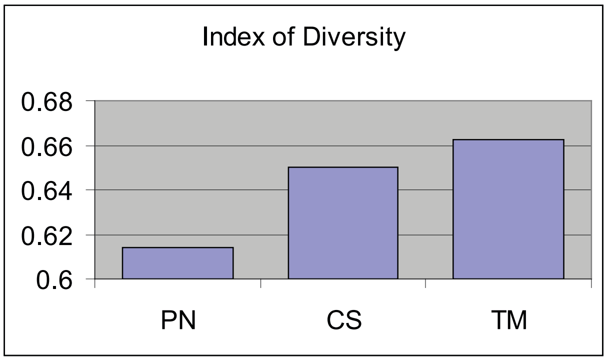


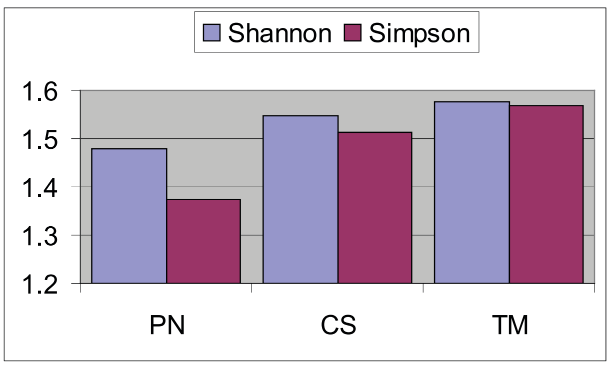
Fig. 4. Principal factors in antioxidants of CS, PN and TM grape varieties using the observations from seeds, skins, and wines

Also **Fig. 4** shows that the behaviour of CS is converse to that of TM variety, in terms of their antioxidant content. This observation is also supported by **Fig. 2**.

Another important aspect is the diversity of antioxidant distribution in seeds, skins and wine- the diversity of antioxidant content of grapes varieties can be expressed using the index of diversity or Simpson & Shannon entropies (**Fig. 5**).



a): Index of diversity



b): Simpson and Shannon entropies

Fig. 5. Diversity of antioxidant content of grape varieties

Both measurements (diversity - **Fig. 5a)** and entropy - **Fig. 5b**) show that the diversity of antioxidant content increases from PN to CS and to TM varieties. The highest diversity of TM is explained by the fact that the antioxidants are almost uniformly distributed (**Fig. 2**) in seeds, skins and wine, respectively. The lowest diversity of PN indicates the fact that the majority of antioxidants are found in seeds (in percentage of the content) - as **Fig. 2** depicts for PN relative to wine.

Conclusions

This study was conducted on three varieties of wine grapes (Cabernet Sauvignon, Pinot Noir and Merlot) and was in regard to their antioxidant content in three parts of the wine making process. The existence of two principal factors on the distribution of the antioxidants in different parts of grapes was shown. The Merlot variety was found to have the highest diversity of antioxidants in the grape, having in same time the highest content of antioxidants. In the opposite case, there were the Pinot Noir variety, for which diversity was found to be the lowest, and antioxidants were twice more frequent in seeds than in skins and in wine.

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