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ASSESSMENT OF PHENOLIC COMPOUNDS IN WINES FROM LOCAL MOLDOVAN GRAPE VARIETIES BY HPLC–DAD-MS-ESI TECHNIQUE

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Abstract: Grapes (*Vitis vinifera*) are one of the major fruit sources of phenolic compounds. Grape's skin and seeds contain high amounts of bioactive phenolic compounds; polyphenols and wine quality are closely interrelated. This study presents the composition of individual polyphenols, estimated by HPLC–DAD-MS-ESI, the total polyphenolic content and the antioxidant activity of 5 local grapes varieties (Feteasca Alba, Feteasca Regala, Viorica, Feteasca Neagra and Rara Neagra) and one European variety (Cabernet Petit). The value of total polyphenolic content of the studied local red wines (Rara Neagra and Feteasca Neagra) varied between 604 and 1354 $mg \cdot L^{-1}$ and 250 and 300 $mg \cdot L^{-1}$ for white ones (Feteasca Alba, Feteasca Regala and Viorica). The average of antioxidant activities determined by DPPH • (expressed in mmol $Trolox \cdot L^{-1}$) in dry red wines from Feteasca Neagra and Rara Neagra samples was about 500 - 620 mmol Trolox $equivalent \cdot L^{-1}$, which indicates a significant antioxidant effect of these wines. According to the obtained results and the chemometric tools, it was concluded that wines obtained from Moldovan grape varieties are a valuable source of bioactive compounds.

Keywords: *antioxidant activity, bioactive compounds, Moldovan wine, polyphenols*

INTRODUCTION

Recently, consumers have become increasingly concerned about a correct and balanced diet, including proteins, fats, carbohydrates and biologically active compounds. Biologically active compounds are needed by the human body in moderate amounts for optimal functioning, and, since few of them are synthesized in the body, a continuous flow of food products is necessary to satisfy these needs [1]. A diet poor in biologically active compounds contributes to the amplification of stress and the appearance of health deficiencies [2]. The consumption of fruits and vegetables and foods enriched in bioactive compounds and nutraceuticals has increased due to consumers' interest in the relevance of food composition for human health [3].

One of the important sources of phenolic compounds among fruits and vegetables is grapes of the genus *Vitis vinifera*. High levels of phenolic compounds are found in the skin and seeds of grapes, and wine is considered a major source of phenolic compounds from grapes due to the high levels of bioactive polyphenols [4].

Compositionally, the wine includes alcohol, sugars, tannins, mineral and organic acids, proteins, minerals, volatile and phenolic compounds [5]. Polyphenols present anti-aging, anti-obesity, antioxidant, cardioprotective, anti-inflammatory, neuro-protective, antifungal, antiproliferative, antibacterial, antiviral, anti-allergic, anti-hypertensive and anti-thrombotic properties and positive effects on the function of the human microbiota (so-called *French paradox*) [1, 2]. These effects of polyphenols depend on the consumed amount and their bioavailability in the products [6]. Compared to other consumed beverages, these health benefits, which are linked to polyphenols, primarily resveratrol and quercetin, give the wine additional benefits [4].

Polyphenols, vitamins, minerals, terpenes, and enzymes represent a class of compounds with variable structures that exhibit multiple antioxidant effects [5]. The term "phenolic compounds" refers to a class of substances that have phenolic properties, including volatile phenols, flavones and flavanols, anthocyanins and proanthocyanidols, microphenols, phenolic acids, and catechinic tannins (stilbenes, shikimic acid) [4].

According to Hunyadi the mechanism of antioxidant actions of polyphenols was described by the elimination of free radicals given that the single electron transfer process involves high energies, via the hydrogen atom transfer mechanism. Calculations of transition states and intermediates for various reaction steps could provide a stronger foundation for these conclusions. Due to having a hydroxyl group bound to position 3 of the C ring, they have a stronger antiradical effect than their corresponding flavones. The pyrogallol group, an extra hydroxyl group in the B - ring, seems to "enhance" the antioxidant capacity even more. Conversely, the antioxidant capacity is reduced when the B ring contains a single hydroxyl [7]. The phenolic ring's catechol structure contains quercetin and catechin gallates, thus anthocyanidins and their glycosides can be equipotent with these compounds [8].

It is well known that the content of polyphenols in must and wine, in addition to its positive effect on the human body, has a significant impact on its organoleptic characteristics, having an important effect on the taste and, therefore, on their quality [9]. Numerous internal and external factors affect the final wine's total phenolic compound concentration which varies from one harvest year to another [10]. By controlling or modeling these variables, it is possible to increase and stabilize the content of polyphenols as well as the organoleptic properties associated with them [6, 9]. These factors are

classified into 3 hierarchical levels by which phenolic compounds can be modulated in wine samples, including: vineyard, winemaking techniques and conditioning and aging methods [11 – 15].
The quantification of biologically active substances in grapes, must and wine is very revealing because it provides important technological information such as the resistance of wines to oxidation [16], and the quantitative contribution of antioxidant activity within the human body after ingestion/consumption [17].
Globally, the originality and typicality of the products currently on the market are crucial deciding factors in the consumer's behavior, therefore, to make Moldovan wine production more competitive on global markets, the placement of wines obtained from local varieties specific to the wine-growing areas of the Republic of Moldova is a required new direction of wine industry [18, 19].
Different techniques can be used to analyze the polyphenolic compound contents in samples (must and wine). These techniques can be summarized as [20 – 25]: spectroscopic methods, NIRs and UV absorbance by Folin-Ciocalteu index and polyphenolic index, etc.), chromatographic methods: gas chromatography, high-performance liquid chromatography, ultra-high performance liquid chromatography and other analytical alternatives (electronic nose and tongue). The most accurate method is ultra-high-performance liquid chromatography (UHPLC), while spectroscopic techniques are the simplest but less selective.
This article summarized the research about the composition and the antioxidant properties of the biologically active compounds in 6 wines: 3 whites and 2 reds of autochthonous grape varieties and 1 red European variety (Cabernet Petit).

MATERIALS AND METHODS

The object of the research were wines made from local red grape varieties (Rara Neagra, Feteasca Neagra), white grape varieties (Feteasca Alba, Feteasca Regala and Viorica) and 1 red European variety (Cabernet Petit), harvest year 2023, produced in the micro-winery section of the Center of Excellence in Oenology, Technical University of Moldova (TUM). Healthy ripe grapes of *Vitis vinifera* varieties Feteasca Alba, Feteasca Regala, Viorica, Rara Neagra, Feteasca Neagra and Cabernet Petit were destemmed, crushed and fermented with the addition of commercial yeasts VINOFERM PR, at controlled temperature between 18 - 20 °C for the white wine technology and 25 - 28 °C for the red wines, during 5 to 7 days of fermentation period.
After the alcoholic fermentation process, the obtained wines were clarified, treated with sulfur dioxide (SO_2 total content 120 - 150 $mg \cdot L^{-1}$) to preserve the samples from microbiological damage and oxidation. All the studied wine samples were analyzed to establish the physicochemical indices: alcoholic strength, sulphur dioxide content, mass concentration of reducing sugars, mass concentration of titratable and volatile acids, acidity, *p*H and sensorial proprieties. The analytical analyses were performed according to OIV methods and the specific literature [26, 27]. Afterward, each wine sample was transferred into 20 sterile glass bottles of 750 mL using a semi-automatic bottling device.

Chemical reagents and materials

Acetonitrile, Folin-Ciocalteu reagent, HPLC-gradient, provided by Merck (Germany) and water was purified with a Direct-Q UV system by Millipore (USA). Quercetin (> 95 %), gallic acid and rutin (98 %), methanol (> 99.9 %), tert-butyl methyl ether (> 99.9 %), hydrochloric acid (37 %), ethyl acetate (> 99.5 %), sodium hydroxide (97 %), phenolphthalein, 2,2-diphenyl-1-picrylhydrazyl (DPPH radical), sulfuric acid (> 98.0 %), etc. were purchased from Sigma (USA). The UV-visible spectrophotometer (T80 series) was used for spectrophotometric measurements.

Quantitative determination

Total polyphenolic content

The Folin-Ciocalteu method uses reducing capacity, which is expressed as phenolic content, through electron transfer [23]. The Folin-Ciocalteu reagent (Sigma-Aldrich, Germany) was used to estimate the total polyphenolic content at the absorbance of 765 nm using a UV-visible spectrophotometer (T80 series) in triplicate. Total polyphenolic content was calculated using gallic acid from the calibration curve (0 - 120 $\mu g \cdot mL^{-1}$, $y = 33.624x + 30.68$, $R^2 = 0.9978$) and pointed out as gallic acid equivalents per milliliter of sample.

Identification of individual polyphenols by HPLC-DAD-MS-ESI+ analysis

Quantification of the individual phenolic compounds in studied wines, after a prior preparation, was performed using the HPLC-DAD-MS-ESI method [28]. The wine samples were filtered through a 0.45 µm Chromafil Xtra nylon filter and 20 µL were injected into the HPLC system. Analysis was carried out using an HP - 1200 liquid chromatograph equipped with a quaternary pump, autosampler, DAD detector and MS-6110 single quadrupole API-electrospray detector (Agilent-Technologies, USA). The positive ionization mode was applied to detect the phenolic compounds; a different fragmentor, in the range of 50-100 V, was applied. The column was a Kinetex XB-C18 (5 µm; 4.5 x 150 mm i.d.) from Phenomenex, USA. The mobile phase was (A) water acidified by 0.1 % acetic acid and (B) acetonitrile acidified by 0.1 % acetic acid. The following multistep linear gradient was applied: start with 5 % B for 2 min; from 5 % to 90 % of B in 20 min, hold for 4 min at 90 % B, then 6 min to arrive at 5 % B. Total time of analysis was 30 min, flow rate 0.5 $mL \cdot min^{-1}$ and oven temperature 25 ± 0.5 °C.

The spectral values were recorded in the 200 - 600 nm range for all peaks. The UV-VIS spectra were recorded at the wavelength λ = 280, 340 and 520 nm. The wavelength λ = 280 nm is specific for all subclasses of phenolic compounds (hydroxybenzoic acids, hydroxycinnamic acids, flavanols, flavonols and anthocyanins), λ = 340 nm is specific to hydroxycinnamic acids and flavonols and the wavelength λ = 520 nm is specific to anthocyanins.

The comparison of each polyphenol's retention duration and spectral information to the real standards listed in Table 1 allowed for the identification of each polyphenol.

The Scan mode was used for the mass spectrometric detection of positively charged ions. The applied experimental conditions were: gas temperature 350 ºC, nitrogen flow 7 $L \cdot min^{-1}$, nebulizer pressure 241.33 Pa, capillary voltage 3000 V, fragmentor 100 V and

m/z 120 - 1500. Data acquisition and interpretation of results was done using Agilent ChemStation software.

Table 1. *Spectral characteristics, retention times and m/z ratios of the $[M+H]^+$ polyphenolic compounds identified by HPLC- DAD-MS-ESI analysis*

Peak no	Retention time [min]	Max Absorption λ_{max} [nm]	$[M+H]^+$ (m/z)	Compound	Subclass
1	3.38	319	313	*Trans*-Caftaric acid (*t*-Caffeoyl-tartaric acid)	Hydroxycinnamic acid
2	4.02	275	171	Gallic acid	Hydroxybenzoic acid
3	5.64	280	199	Ethyl gallate	Hydroxybenzoic acid
4	9.05	280	139	*p*-Hydroxybenzoic acid	Hydroxybenzoic acid
5	9.66	295	155	Protocatechuic acid	Hydroxybenzoic acid
6	10.17	321	327	Fertaric acid (Feruloyl-tartaric acid)	Hydroxycinnamic acid
7	10.54	280	229	Resveratrol	Stilbene
8	10.78	322	297	Coutaric acid (*p*-Coumaroyl-tartaric acid)	Hydroxycinnamic acid
9	11.37	280	579	Procyanidin dimmer	Flavanol
10	12.01	530, 280	463 493	Peonidin-glucoside Malvidin-glucoside	Anthocyanin Anthocyanin
11	12.43	280	291	Catechin	Flavanol
12	13.14	322	181	Caffeic acid	Hydroxycinnamic acid
13	13.56	531, 279	519	Pelargonidin-malonyl-glucoside	Anthocyanin
14	13.75	322	209	Caffeic acid ethyl ester	Hydroxycinnamic acid
15	13.87	280	291	Epicatechin	Flavanol
16	14.62	530, 280	535	Malvidin-acethyl-glucoside	Anthocyanin
17	14.92	531, 279	434	Pelargonidin-glucoside	Anthocyanin
18	15.18	531, 280	505	Peonidin-acethyl-glucoside	Anthocyanin
19	15.67	531, 280	609	Peonidin-coumaroyl-glucoside	Anthocyanin
20	15.72	280	199	Syringic acid	Hydroxybenzoic acid
21	16.32	360, 255	465	Quercetin-glucoside	Flavonol
22	16.99	360, 255	479	Quercetin-glucuronide	Flavonol
23	17.06	530, 280	639	Malvidin-coumaroyl-glucoside	Anthocyanin
24	17.51	360, 255	435	Quercetin-arabinoside	Flavonol
25	21.51	360, 255	303	Quercetin	Flavonol

Antioxidant activity, DPPH Assay

$$AA = \frac{A_c - A_f}{A_c} 100\ \% \quad (1)$$

where: AA - % of antioxidant activity, A_c - control reaction absorbance (initially) and A_f - testing sample absorbance (after 30 min).
The results were expressed as mmol Trolox·L^{-1} sample using the calibration curve with Trolox (0 - 250 mmol·L^{-1}). The quantification of hydroxybenzoic acids and flavanols was carried out using the calibration curve with gallic acid (y = 33.624x + 30.68, R^2 = 0.9978), hydroxycinnamic acids were quantified a s chlorogenic acid equivalent (y = 22.585x - 36.78, R^2 = 0.9937), flavonols as rutin equivalent (y = 26.935x – 33.784, R^2 = 0.9981) and for anthocyanins a calibration curve was made with cyanidin equivalent (y = 55.789x - 143.21, R^2 = 0.9951).

Sensory analysis
The wine samples were assessed for aroma and taste characteristics by 12 tasters (academic staff, students and wine industry experts) according to the OIV evaluation method and the specific literature [26]. The tasters evaluated specific parameters of the wines within ranges from 0 to 10. The mean of all the results, considering each taster's evaluation, was used to determine the final score.

Statistical Analysis
The experimental data were statistically processed using Microsoft Office Excel 2007 (Microsoft, Redmond, WA, USA) to determine the mean values along the standard error. Using a significance level of $p < 0.05$, the ANOVA and ACP statistical tests were used to analyze multiple variances following the Tukey test.

RESULTS AND DISCUSSION

Dry red and white experimental wines obtained from local varieties and processed in the micro-winery section at the Department of Oenology and Chemistry were submitted to physicochemical analyses and the obtained results are presented in Table 2.
Table 2 shows that after the post-fermentation process, alcoholic degrees vary between 10.71 - 13.22 % vol., and the titratable acidity and volatile acidity were within the permissible limits for dry white and red wines. The organoleptic characteristics of the wines obtained from the local grape varieties were also performed and they corresponded to the typicality of these local wines: with characteristic aromas and without off-odors [18].
The alcoholic fermentation in the experimental wine samples lasted between 5 to 11 days, the temperature during fermentation was between 16 - 21 °C and the density values decreased from 1100 g·L^{-1} to 950 g·L^{-1}.

Table 2. *Physicochemical indices of experimental wines*

Indices	Feteasca Alba	Feteasca Regala	Viorica	Feteasca Neagra	Rara Neagra	Cabernet Petit
Alcohol content, [% vol.]	10.71±0.14	11.34±0.21	12.45±0.15	13.22±0.25	12.87±0.20	11.25±0.21
Mass concentration of reducing sugars, [g·L^{-1}]	3.8±0.1	2.8±0.2	3.5±0.4	4.0±0.1	2.5±0.20	3.8±0.2
Mass concentration of titratable acids, [g·L^{-1}] tartaric acid	6.4±0.3	6.8±0.2	6.2±0.4	7.5±0.1	6.7±0.20	6.6±0.3
Mass concentration of volatile acids, [g·L^{-1}] acetic acid	0.38±0.05	0.42±0.1	0.32±0.15	0.44±0.22	0.52±0.17	0.63±0.20
Active acidity (*p*H)	3.21±0.01	3.42±0.01	3.15±0.01	3.22±0.01	3.46±0.01	3.24±0.01

The sensory analysis of the experimental samples revealed the predominance of fruity tones in Feteasca Regala: ripe fruit, green fruit, green hay and higher acidity that contributes to the sensation of freshness, texture and taste persistence. The honey tone was better expressed in the Feteasca Alba sample, a slight increase in unctuousness was felt in the red varieties due to the balance between tannins, fatty acids and glycerol. Overall, the organoleptic palette specific to the ampelographic autochthonous grape varieties was found in the studied wine samples, without off-odors and unpleasant taste.

The total antioxidant capacity in the studied wine samples was determined and expressed in µg of gallic acid/quercetin and mmol Trolox·L^{-1} of wine (Table 3).

The total polyphenol content (TPC) of red wines exceeded that of white wines by up to ten times. These differences from red wines' superior extraction of phenolic compounds result not only from longer contact time with grape skins and seeds, as well as different fermentation and temperature conditions than for white wines, but also because of anthocyanin presence in the red grape skin.

Among the red wines, no significant differences were obtained between the TPC values, the phenolic content of the Feteasca Neagra wine being higher by a few units than that of the Rara Neagra wine. TPC varied amongst wine samples based on grape variety, vineyard environmental factors and wine processing methods (Table 3).

Studies suggest that almost 60 % of the polyphenols present in grapes remain in waste after their processing. These polyphenols can be used in another field, namely pharmaceutical or food industry, because they are a source of biological active compounds. The red pigments found in grapes skin, for example, anthocyanins, degrade easily, so optimal technological operations and other ingredients that can influence the antioxidant capacity and sensory properties must be taken into account [8, 29].

Flavonoids and procyanidins are generally considered to be the two main groups of active constituents in grapes, thus time and temperature are very important factors that influence their content in wines. It can be observed that total phenolic compounds, expressed in mg GAE·L^{-1} in the local grape varieties Feteasca Neagra and Rara Neagra were relatively lower than in the Cabernet Petit variety.

Table 3. *The average content of total polyphenols* of studied wine samples, expressed in different equivalents (mg GAE·L^{-1}, mg quercetin·L^{-1} and mmol Trolox·L^{-1})*

Indices	Rara Neagra	Feteasca Neagra	Cabernet Petit	Feteasca Alba	Feteasca Regala	Viorica
Total phenolic compounds, [mg·L^{-1}]	1354.96	791.86	1248.95	249.59	259.85	295.69
Total phenolic compounds, expressed in [mg GAE·L^{-1}]	517.12	536.82	503.07	167.54	143.21	186.76
Total phenolic compounds, expressed in [mg quercetin·L^{-1}]	22.07	28.54	51.62	7.54	8.68	5.24
Total phenolic compounds, expressed in [mmol Trolox·L^{-1}]	564.25	607.25	914.28	46.42	52.14	50.28

**p ≤ 0.05, analyses were performed in triplicate*

The link between the antioxidant activity and polyphenols of grape pomace and the lyophilization process does not affect the composition and antioxidant activity of the extracts from this raw material [13]. It was found that the antioxidant capacity of the wines from Feteasca Neagra and Rara Neagra local varieties, although lower than that of Cabernet Petit wines, is about 564-607 mmol Trolox equivalent, which indicates a significant antioxidant effect of these wines. However, the antioxidant capacity of local white varieties wines is considerably lower. There is a good correlation between total flavonoid content and antioxidant capacity. The total polyphenol contents of the wines under investigation were comparable to other studies, which revealed that the Feteasca Regala variety had a total polyphenolic content of about 230 mg GAE·L^{-1} [30] and an antioxidant activity of 12.43 mmol Trolox·L^{-1} [11].
A summary of polyphenolic compounds: phenolic acids and their derivatives, stilbenes, flavanols and anthocyanins are shown in Table 4.

Table 4. *Phenolic compound quantification* in red wines expressed in mg·L^{-1}*

Chemical group of phenolic compounds	Rara Neagra	Feteasca Neagra	Cabernet Petit
Phenolic acid	723.81	458.82	621.39
Anthocyanin	180.89	121.93	184.98
Flavan	319.83	139.62	316.4
Flavanol	118.18	51.39	120.57
Micro-phenolic compound (stilbene)	12.26	20.11	5.63

**p ≤ 0.05, analyses were performed in triplicate*

The investigated red wine varieties' total polyphenolic content was following the literature's data, 450.9 - 724.1 mg GAE·L^{-1} [4, 11, 30].
The wine made from the local grape variety Rara Neagra is emphasized by the presence of an impressive number of phenolic compounds (λ = 280 nm) and flavanol compounds (λ = 340 nm), responsible for the antioxidant activity of biologically active substances.

Rara Neagra is a variety particularly rich in anthocyanins (λ = 520 nm), the total content of polyphenolic compounds being 1355 mg·L^{-1}.
The comparison of the wine produced from local varieties with the wine made from the Cabernet Petit variety showed close amounts of total polyphenols (Rara Neagra - 1355 mg·L^{-1} compared to Cabernet Petit - 1249 mg·L^{-1}). Concerning the Feteasca Neagra variety, the total content of polyphenols is lower - 792 mg·L^{-1}. All quantified phenolic compounds in red local wines lead to the production of fine wines, with a pleasant ruby-red color and aroma (truffles, cherries, vanilla, raspberries, etc.) [9].
Other studies described similar contents of total phenolic compounds in ranges of 1120 - 2250 mg·L^{-1} for variety Feteasca Neagra and 1460 - 1950 mg·L^{-1} respectively for Rara Neagra immediately after the alcoholic fermentation. Higher contents are obtained by roto-maceration and thermo-maceration of the must, due to the long-term maceration-fermentation in the presence of the berry skin and high temperature (thermo-maceration) which advantaged the maximal extraction of the components studied from the grape skin [31].
Similar values of total phenolic compound content in wines were found by other researchers [32], ranging from 646.05 mg·L^{-1} for Feteasca Neagra to 678.04 mg·L^{-1} for Rara Neagra.
Additionally, the polyphenolic complex composition of wines made from regional white grape varieties was determined (Table 5).

Table 5. *Phenolic compound quantification* in white wines expressed in mg·L^{-1}*

Chemical group of phenolic compounds	Feteasca Alba	Feteasca Regala	Viorica
Phenolic acid	204.95	227.29	247.51
Anthocyanin	19.01	16.66	20.74
Flavan	16.76	9.84	12.33
Flavonol	7.36	6.08	11.46
Microphenolic compound (stilbene)	1.50	n/d	1.85

**p ≤ 0.05, analyses were performed in triplicate*

The total content of polyphenols varies between 250 and 296 mg·L^{-1}, being attested to the same compounds as in the studied red wines, with the obvious exception of anthocyanins. The phenolic derivatives and the flavans are found in the range of 4.7 - 9.14 mg·L^{-1} for white wines, estimated as a significative element of the potential taste and aroma of wines and an antioxidant compound important for the health of the human body [33].
The phenolic acids are also responsible for the formation of the individual aromatic palette of grape varieties and wines [14]. These results demonstrate that Moldovan wines are extremely rich sources of bioactive compounds.
The presence of micro phenolic compound - resveratrol was found in Feteasca Alba and Viorica, but the amounts did not exceed 1.85 mg·L^{-1}. This stilbene participates facultatively in wine flavor but has important implications for human health with well-known characteristics and is very appreciated by wine consumers. Resveratrol has a

strong anti-inflammatory and antibacterial effect, maintains the elasticity and firmness of the skin and prevents its premature aging, it has a good anti-allergic effect, is recommended for people suffering from bronchial asthma, also a form of allergy [1 – 4]. Resveratrol offers stronger antioxidant protection compared to vitamins E and C, especially due to its hydrophilic and lipophilic properties. Two phenolic rings joined by a double bond make up its structure, which is characterized by planarity, conjugation, and electronic delocalization. The reciprocal position of the hydroxyls does not allow the formation of intramolecular hydrogen bonds, though radicalization of OH groups, generates extremely stable radicals [34].

The studied samples were quantified in contents of t-resveratrol, with values between 1.5-1.85 $mg \cdot L^{-1}$ in white wines, while higher amounts were quantified in red wines (12.26-20.11 $mg \cdot L^{-1}$). The studied white and red wines had higher levels of t-resveratrol than those reported in the literature for white wines within the range of 1.37 - 1.7 $mg \cdot L^{-1}$ [35] and 14.74 - 17.15 $mg \cdot L^{-1}$, respectively for red wines [36].

Radicalization of caffeic acid can generate two radicals, specifically the 3-OH and 4-OH radicals. The former radical's energetic stability is caused by the –CH=CH–COOH group's electron delocalization effect. Resonance and conjugation effects are enhanced by the -CH=CH-bridge that exists between the carboxyl group and benzene in caffeic acid. Hydrogen bonds are formed in part by the OH groups and these radicals are key intermediates in caffeic acid's antioxidant activity. [8].

A significant role in the quality of wines, their physicochemical and organoleptic qualities, in the evolution during winemaking and aging of wine belongs to tannins [37]. The concentration in white wines varies between 0.1 - 0.5 $g \cdot L^{-1}$ and in the range of 2 - 5 $g \cdot L^{-1}$ respectively for red ones [12, 20].

Red wines are more stable against oxidation during all technological operations, including bottle aging, because of the antioxidant and conservative role of tannins. Thereby, caffeic acid and its derivatives show significant antioxidant properties. Catechin and epicatechin act as antioxidants and help neutralize free radicals, unstable chemical compounds that can destroy cell membranes and DNA, causing cell death [38]. The justification lies in the fact that the radicalization of catechin and epicatechin molecules leads to the formation of extremely stable isoenergetic radical species [37, 38]. Several clinical studies have highlighted the fact that the antioxidant action of quercetin is much stronger than that of vitamins C and E, but also of beta-carotene. Theoretical calculations indicate that quercetin can exist in two conformational states, which can easily interconvert [39].

CONCLUSIONS

The local Moldovan wines made from autochthonous red grape varieties (Rara Neagra, Feteasca Neagra) and white grape varieties (Feteasca Alba, Feteasca Regala, Viorica) are defined as genuine sources of bioactive compounds, with an important antioxidant capacity and moderate consumption may have a multiple health benefits and disease prevention. The total polyphenol content of red wines was up to 10 times higher than that of white wines. These differences are the result of better extraction of phenolic compounds due to longer contact time with grape skin and seeds, fermentation conditions and temperature, for red wines compared to white wines. Among the important

polyphenols, the major compounds determined in all samples were gallic acid, resveratrol, caffeic acid, catechin, epicatechin and procyanidin.
The average of antioxidant activities determined by DPPH• (expressed in mmol Trolox·L^{-1}) in wines from Feteasca Neagra and Rara Neagra (local varieties), although lower than that of Cabernet Petit (European variety) wine, was about 500 - 620 mmol Trolox equivalent·L^{-1} samples, which indicates a significant antioxidant effect of these wines.
Given that high energies are required for the electron transfer process, the majority of polyphenols seem to scavenge free radicals via the hydrogen atom transfer mechanism. As in the cases of tocopherol, resveratrol, and quercetin, the optimal compounds within the electron transfer mechanism are those that exhibit planar conformation and electronic delocalization, resulting in ionization potential values that are lower than those of the reference phenol. According to the obtained results and the use of chemometric tools, it was concluded that wines obtained from Moldavian grapes varieties are a valuable source of bioactive compounds which confirms the so-called *French paradox*.

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